Neuroscience of Social Stress
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Neuroscience of Social Stress
The socially stressed individual has emerged as an important research target in investigations on anxiety, depression, PTSD, psychotic outbursts, and alcohol and drug use disorders. During the last decade, preclinical and clinical models have been refined enabling translation from work with animals to human subjects and vice versa. In the current volume, innovative and informative work on neural and biobehavioral mechanisms of coping with social challenges have been assembled in order to survey the current state of knowledge and point into fruitful new directions. Beginning with studies implicating the canonical biogenic amines, several chapters discuss neuropeptides, neurosteroids, endocannabinoids, and neuroinflammatory processes during exposure to social stress. Brief episodes of social stress recruit well-characterized adaptations in peripheral and neural processes, whereas the cascade of neural mechanisms that result from persistent exposure to early adversity, interpersonal violence, childhood social stress, and also chronic social stress only begin to be delineated. Higher order neural and biobehavioral processes underlying complex social behaviors such as social bonding, engaging, and interacting with others versus disengagement, isolation, and salience of alternate non-social rewards are being identified in children and adults. The role of caregiving and parenting on the developing brain and the impact of early social stress on socioemotional neurodevelopment are beginning to specify neurodevelopmental pathways of stress illness risk versus resilience.

Treatment of the so-called stress disorders began with a focus on the HPA axis and negative feedback from glucocorticoids to receptor subtypes in hypothalamic and extra-hypothalamic regions. Clinical success with CRF antagonists awaits temporally and regionally more specific molecules. Repeated and chronic social stress exposure significantly changes the blood brain barrier increasing risk of neuroinflammatory along with peripheral inflammatory changes that may drive vulnerability for specific stress illnesses with specific immune links. This highlighted research further opens up additional stress-inflammatory targets for intervention to be tested in the clinic.
An important development during the last decade is the study of sex-specific neural mechanisms in most psychiatric disorders, among them stress disorders. The emerging evidence points not only to sex differences in the vulnerability and resilience to social stress, but also to sex-specific coping mechanisms. Several of the papers included in this volume elucidate these sex differences and point to sex-specific coping, highlighting the need for sex-specific treatments.

The current collection of contributions emphasize that exposure to various specific types of social stressors involves distinctive neural mechanisms. Epigenetic evidence is beginning to highlight how exposure to these different social stressors in discrete pre- and post-natal sensitive periods and in repeated and often chronic ways impacts adult social life. This important area of inquiry is bound to identify an array of stressor-specific molecules in discrete projections from brain stem to subcortical and cortical targets and reciprocal. Contrary to the classic view of stressor-general responses, multi-level neural networks for various social and other types of environmental stressors reveal marked specificity in responses thereby pointing to differential risk pathways for specific stress illnesses.

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Part I
Basic Research
Neuroendocrine Mechanisms of Social Bonds and Separation Stress in Rodents, Dogs, and Other Species

Miho Nagasawa and Takefumi Kikusui

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Abstract Mammalian species form unique bonds between mothers and infants. Maternal care, including suckling, is necessary for infant survival, and the mother and, sometimes, the father require a lot of effort in nurturing infants. An infant’s probability of survival depends on the extent of the investment of care by the mother. In parallel, mothers must identify their offspring and invest only in those who possess their genes to achieve evolutionary benefits. Therefore, they need to recognize their offspring and show a strong preference for them. For this reason, bond formation between mothers and infants is important. The mother monitors her offspring’s physical condition and stays close to them. The offspring also form strong bonds with their mothers. Therefore, a separation between the mother and infant causes severe stress for both parties. Although it was initially thought that such bonds between mother and infant are limited to the same species, we have also
observed a similar phenomenon in the human–dog relationship. In this article, we discuss the neuroendocrine mechanisms that underlie bond formation and separation based on findings of neurobiological research in mice and the relationship between humans and dogs.

**Keywords** Bonding · Glucocorticoid · Human–dog relationship · Oxytocin · Separation stress

### 1 Introduction

In the process of mammalian evolution, animals have developed class-specific adaptations for pre- and postnatal development, including placentation and milk provisioning. Interestingly, only females have the unique ability to provide perinatal resources through the placenta and produce milk for their offspring after delivery. These differences in investments for offspring by the sexes are thought to largely reflect the lifestyle strategies of each sex. The reproductive strategy of female mammals is to produce relatively fewer offspring than egg-laying vertebrates, and its success is determined by the ability to ensure infant survival beyond the weaning age. On the other hand, reproductive success in males is generally determined by competing with other males to mate with as many females as possible. Therefore, intensive infant care has mainly evolved in females, and it is hardly surprising that females and their offspring form special relationships, such as mother–infant bonding. Intensive maternal care has evolved, and it has been preserved uniquely in mammals; it is highly probable that mother–infant bonding is universal to all mammalian species.

When a dyad, such as a mother and an infant, forms a bond, separation causes severe stress for both parties. There are obvious behavioral and physiological stress symptoms, such as separation vocalization, seeking the counterpart, vigilance toward the environment, depressive-like behavior, increase of glucocorticoid secretion, and heightened autonomic activities. In humans, bereavement of family members and divorce, among others, are known to cause severe stress (Cacioppo et al. 2015). Several studies have demonstrated similar effects of maternal separation on neurobehavioral changes in rats and mice. This behavior is not only exhibited toward the same species, but also between different species; it has been found that the death of a pet can cause great stress for its owner.

Interestingly, the formation of bonds and separation stress are regulated by neuroendocrine systems. This means that bond formation and separation are not only cognitive; they involve the entire body responses. In this chapter, we introduce the neuroendocrine mechanisms that underlie bond formation and subsequent separation stress. We emphasize the significance of the conserved use of the oxytocin (OT) neural system in bond formation (Young et al. 2001; Young and Wang 2004; Kendrick 2004). We also review the effect of separation and deprivation after...
bonding by studying the effect of maternal separation and early weaning on neurobehavioral development in rodent offspring and human–dog separation stress. These provide insights into the biological significance of bonding and separation in mammals. An understanding of the underlying mechanisms, even in rodents and other mammals, may help to treat or prevent disorders related to stress, especially from child abuse or childhood neglect (Heim et al. 1997; Agid et al. 1999).

2 Forming Bonds

Social bonding is hypothetical constructs that cannot be measured directly. However, several behavioral and physiological measures have been used as indices of social bonding, which include behavioral distress, or elevated corticosteroid levels following separation from the bonding partner (DeVries 2002), and positive interactions, including increased proximity after separation (Hennessy 1997). Social bonding has not yet been clearly defined. However, it has been proposed that social bonding can be distinguished from social affiliation, in which corticosteroid elevation does not occur following separation (DeVries et al. 2003). Further, a subsequent reunion with a bonding partner ameliorates separation distress or aversive experiences. This phenomenon is termed as “social buffering” (Kikusui et al. 2006b); its effect depends on the degree of affiliation with the partner, and it is strongest with the bonding partner, as seen in the dyad of a mother and an infant.

Mother–infant bonding is special, given its influence on the neurophysiological development of the offspring. This was first suggested in humans by Bowlby’s attachment theory (Bowlby 1969). The significance of bonds in infants and during juvenile periods has been demonstrated by the sequelae of disruption/separation. Several psychological and animal research studies have reported that maternal/parental stress during the juvenile period, such as child abuse or childhood neglect, is correlated with severe deleterious long-term effects on the cognitive, socio-emotional, and behavioral development (Hildyard and Wolfe 2002).

Hereafter, we summarize the neurochemical and endocrinological mechanisms underlying the social bonding in rodents (Fig. 1), especially focusing on OT.

3 Neuroendocrine Regulation of Bonding in Mothers

Several studies have demonstrated that oxytocin plays a key role in mother–infant interaction. Orchestrated OT effects have been demonstrated in maternal behavior. Some exteroceptive social cues from infants, such as infants’ odors, physical touch, or vocalizations, mainly drive mothers to seek, as well as facilitate the recognition of their infants. In rodent mothers, these social stimuli from pups can initiate and maintain maternal behaviors. Suckling of the nipple is a representative stimulus in lactating females, because suckling has been demonstrated to stimulate OT release

neurons are in the PVN and SON in the hypothalamus and their fibers innervate to the nucleus accumbens and PFC, similar to DA. In the nucleus accumbens, DA and OT are involved in the social reward. These neurocircuitry are involved in social bonding: when a dyad forms bond, these neurochemical pathways are activated. The HPA axis comprises the paraventricular nucleus of the hypothalamus (PVN), the anterior pituitary gland (AP), and the adrenal cortex (AC). Stressful stimuli activate the PVN, which releases CRF (green line) in the pituitary and stimulates adrenocorticotropic hormone (ACTH) secretion into the bloodstream. In turn, ACTH reaches the adrenal to induce release of glucocorticoids. Acute and chronic separation stress lead to activate HPA axis. When the pair is reunited, OT released in the brain and pituitary, resulting in the inhibition of HPA axis.

Some researchers have extensively investigated how neuroendocrine changes during parturition, such as those in the medial preoptic area (mPOA) in the hypothalamus, enhance the neural mechanisms responsible for maternal behavior (Numan and Sheehan 1997). Significant evidence suggests that OT neuroendocrine systems play a key role in the initiation of maternal behavior after birth. It is now clear that OT, which is synthesized in the neurons of the hypothalamus (paraventricular nucleus [PVN] and supraoptic nucleus), is not released into the peripheral circulation, causing milk ejection, but also into the brain (Neumann et al. 1993). If new mothers are separated from their pups and not permitted to interact with them at all, their maternal responsiveness declines over the first postpartum week (Orpen and Fleming 1987). Among various pup stimuli, physical contact is thought to play an important role in the maintenance of maternal behavior. Without any relation to the physiology of lactation, several types of tactile stimuli such as touch or massage-like stroking and warmth have also been shown to stimulate OT release in rats (Uvnas-Moberg et al. 1993).
general circulation from the posterior pituitary, but also centrally to act upon OT receptors widely expressed in the central nervous system (Barberis and Tribollet 1996; Gimpl and Fahrenholz 2001) during vaginal stimulation and parturition (Forsling 1986). In addition, maternal behavior is greatly impaired in post-parturient rats that received PVN lesions during pregnancy (Insel and Harbaugh 1989) and in female rats administered with an OT antagonist immediately after parturition (van Leengoed et al. 1987). Furthermore, OT receptors proliferate in several forebrain areas including the mPOA, ventromedial hypothalamus, and bed nucleus of the stria terminalis of female rats at the time of parturition (Jirikowski et al. 1989; Pedersen et al. 1994). These changes in OT receptor expression may be related to the induction of maternal behavior because intraventricular and mPOA infusions of OT induce a rapid onset of maternal behavior in virgin female rats (Pedersen et al. 1994).

We demonstrated that MOPA oxytocin receptor-expressing neurons are responsible for the induction of allo-maternal behavior in virgin female mice when they are repeatedly exposed to the alien pups (Okabe et al. 2017). Initially, they ignored the pups; however, after the sensitization, they showed maternal-like behavior toward the pups. If the oxytocin receptor-expressing neurons in the MOPA are blocked, this sensitized allo-maternal behavior is suppressed. In addition, the OT receptor-expressing neurons in the MOPA are activated during the pup sensitization phase (Okabe et al. 2017). These data suggest that experience-dependent allo-maternal care is also regulated by central OT systems.

Exteroceptive social cues from pups, such as infant odors or vocalizations, may not have as strong an effect on the development of nurturance as physical stimuli, which play an important role in making mothers seek pups out and maintain proximity (Okabe et al. 2013). Infant odors are not only used as cues to attract mothers, but also for the recognition of the infants (Ostermeyer and Elwood 1983). In ewes, oxytocin is released into the general circulation at birth to facilitate parturition and milk let-down. It also acts in the olfactory bulb to facilitate the recognition of their own lamb (Lévy et al. 1995). In rats, the odors of infants are aversive for females before parturition and lactation, while on approach of parturition, these odors become very potent stimuli (Lévy et al. 2004). This change in the responsiveness of the mothers is governed by hormonal changes during parturition, and it is suggested that the OT neuroendocrine system participates in this process.

Pregnancy and parturition enhance the sensitivity and neural firing in the olfactory bulb in rats, and OT infusion directly into the olfactory bulb increases the firing frequency and spontaneous excitatory postsynaptic currents in granular cells (Yu et al. 1996) by both pre- and postsynaptic mechanisms, a process integral to olfactory recognition memory (Engelmann et al. 1998; Dluzen et al. 2000). Similar to the responsiveness to pup odors, the response to pup separation calls has also been shown to be more pronounced in mothers than naïve females (Okabe et al. 2010). It has been shown that OT is required for plastic changes in the auditory neural circuit to allow mother mice to respond to the separation calls of their pups in an experience-dependent manner. OT acts on the inhibitory neurons in the auditory cortex and fine-tunes the calls of the pups (Marlin et al. 2015; Schiavo et al. 2020). We also observed that the responsiveness to pup USVs was elicited by mating
experience (Okabe et al. 2010), although the neural mechanism through which mating increases responsiveness is currently unknown.

4 Infants’ Side in Bond Formation and Neuroendocrine System

It has been suggested that the OT neuroendocrine system is involved in these behaviors in infants. The central administration of OT has been found to reduce the frequency of isolation-induced USV in young rat pups (Insel and Winslow 1991), suggesting a possible role of central OT in the effective “calm” response infants display during social contact. Central OT also induces paw sucking in neonatal rats (Nelson and Alberts 1997). These innate attachments regulated by OT neuroendocrine systems ensure the infant’s physical development and the subsequent development of adequate attachment behaviors accompanying pup growth. In return, adequate attachments maintain maternal behaviors in response to pup stimuli.

These proximity behaviors of pups toward the mother appear to be mediated by thermo-tactile sensory domains. In the rat, neonatal pups spend as much time huddling with a warm fur-covered tube as with littermates (Alberts and Brunjes 1978). The olfactory system also mediates infant attachment behaviors. Newborn rats fail to locate the nipple if their mother’s ventrum is washed (Hofer et al. 1976), indicating that smell contributes to nipple localization. It has been shown that rat pups achieve initial contact with a nipple by recognizing the amniotic fluid that their mother has deposited on her ventrum (Teicher and Blass 1977). In the rabbit, a volatile pheromone is released from the milk for moving pups to guide inexperienced infants through a head-searching pattern to find and grasp the nipple (Schaal et al. 2003). It has also been suggested that OT is involved in milk sucking because central OT induces paw sucking in neonatal rats (Nelson and Alberts 1997). These innate attachments ensure that an infant’s physical development and the subsequent development of adequate attachment behaviors accompany pup growth. In return, adequate attachments facilitate maternal behaviors in response to pup stimuli. It is conceivable that infant bonding to the mother is formed through mutual interaction with the mother.

Panksepp and colleagues have proposed the intriguing hypothesis that the neural mechanisms underlying bonding are organized into a socially directed motivational system within the brain during infancy, which continues to modulate affiliative behaviors throughout the animal’s life span (Nelson and Panksepp 1998). However, it is not well understood how such neural mechanisms, if they exist, contribute to the neural dynamics involved in bond formation by the infant. We tested whether the oxytocin levels of pups were pharmacologically manipulated and investigated the consequences of their social behavior during adulthood. Pups treated with oxytocin antagonists showed impairment of the social novelty approach (Mogi et al. 2014).
Adults treated with OT when they were pups retrieved significantly more pups than those that received 3 μg of an OT antagonist when they were adults (Mogi et al. 2014). These results support Paksepp’s hypothesis that OT-related bonding mechanisms during infancy can modulate the social interactions in adulthood involved in OT systems.

5 Separation Stress in Pups

Infant mammals, including humans, emit specific acoustic signals when separated from the mother (Stark 1981). In rodents, pup ultrasonic vocalizations (USVs) are thought to represent these signals. It has been shown that the highest rates of calling are elicited by separating the pup from the dam and the littermates and placing it alone in novel surroundings with a cool temperature (Dirks et al. 2002). For the isolated pups, contact with the dam, littermates, or home cage shavings markedly reduces the calling rate (Hofer et al. 1993). These isolation calls, together with pup odor, elicit direct maternal search, pup carrying, and retrieval (Esposito et al. 2013). We demonstrated that there were individual differences in pup calls, and the mother memorized their pup calls and demonstrated unique behavior to their pup calls (Mogi et al. 2017). Isolation-induced USVs have often been called “distress vocalization” and extensively used as markers for distress in infant rodents (Winslow and Insel 1991).

The actions of anxiolytic and anxiogenic compounds, such as drugs acting on benzodiazepine-GABA receptor complex or 5-HT receptors, on USVs are generally compatible with this explanation (Miczek et al. 1995). OT has been suggested to modulate these calls, but the direction of its effect remains unclear. Central OT administration attenuates the production of USVs in isolated rats (Winslow and Insel 1991). On the other hand, OT knockout (OTKO) and OT receptor knockout (OTRKO) mouse pups emit fewer isolation-induced USVs than wild-type pups (Takayanagi et al. 2005). The reason for this contradiction is unclear, but OTKO and OTRKO pups may be less sensitive to maternal separation than wild-type pups. Interestingly, Hofer et al. (1994) found that after infant rats had brief contact with the dam, they produced USVs at a rate significantly higher than that during isolation not preceded by dam contact (Hofer et al. 1994). Maternal potentiation is not species-specific for rats, since it also occurs in mice and guinea pigs (Moles et al. 2004; Hennessy et al. 2006). Given the above findings, it has been theorized that maternal potentiation observed in rodents is a reflection of the infant’s bonding to the mother (Shair 2014). It has been demonstrated that the expression of maternal potentiation is regulated by dopamine activity in the nucleus accumbens (Muller et al. 2008) or opioid receptors (Moles et al. 2004; Hennessy et al. 2006). These observations underscore the possibility that the neural system known to be involved in the reward process may be important for infant bonding.
6 Social Buffering: Relief from Stress and Anxiety

The separation of infants from attachment figures, such as mothers, causes severe stress responses as mentioned above, and the stress responses are eliminated by their reunion with their counterparts. In guinea pigs, it has been found that an unfamiliar adult female reduces plasma cortisol levels and vocalization responses in pre- and post-weaning guinea pigs during brief exposure to a novel environment. However, the presence of the mother has a greater effect on the cortisol levels of young pups, at least during the pre-weaning period (Graves and Hennessy 2000). It is plausible that this strong “social buffering” is due to bond formation between the infant and the mother.

Recently, we found that the presence of the mother had marked effects on reducing corticosterone levels and anxiety-like behavioral responses of pre-weaning mouse pups (postnatal day 14) during exposure to a novel environment. In addition, we compared the OT contents of several nuclei of pups with and without their mother and found that the OT content of the cingulate cortex was lower in pups without their mother than in those with their mother, despite the absent differences in the other nuclei examined. Our preliminary data suggest that central OT release may be distinguished by the nucleus and regulated by the presence of the mother. Interestingly, the expression of OT receptors has been shown to differ between infant and adult rodents. There is a transient, but marked, overexpression of OT receptors in the infant relative to the adult, particularly in the cingulate cortex (Tribollet et al. 1989). In humans, the cingulate cortex is an important neural site for emotional or empathic responses (Singer et al. 2006). The infant-specific OT neural system may be regulated by the mother’s presence and related to infant bonding.

The mother’s effect on the reduction in HPA axis activity has been confirmed in the context of olfactory aversion learning by odor-shock conditioning (Moriceau and Sullivan 2006). Neonatal rat pups (postnatal day 8) learned to approach an odor even after pairing that odor with a painful stimulus. In contrast, aversion learning was evident to the same extent as that observed in adult mice in rats during postnatal days 12–15, as the HPA axis and the amygdala were developing and glucocorticoid secretion begins in response to electric shock. However, the absence of mothers was required for the completion of aversion learning during this period because the glucocorticoid response to electric shock was inhibited by the presence of the mothers. Because mothers were effective even when they were anesthetized, it is thought that the stimuli from the mother that are responsible for this effect are not tactile. In domestic animals, such as pigs, dogs, and horses, maternal odor can reduce stress and anxiety in conspecifics. Collectively, the presence of a mother can reduce fear and stress responses in pups, which can be brought about by odorant information from the mother.
7 Maternal Separation Stress in Infancy

The developmental effects of mother–infant bonding have also been demonstrated experimentally in non-human primates. For example, in a study by Winslow et al. (2003), mother-reared or human nursery-reared monkeys were subjected to a novel environment with or without a cage mate. The monkeys reared by their mothers exhibited a reduction in cortisol response when a social partner was available, while nursery-reared monkeys did not. In nursery-reared monkeys, social contact, such as allogrooming and intermale mounting, was drastically reduced. These findings suggest that because nursery-reared monkeys have less social contact in a novel environment, the social buffering effect is impaired. Thus, separation stress during developmental periods strongly influences offspring sociality in human and non-human primates (Heim et al. 1997; Agid et al. 1999), although the underlying mechanism is not fully understood. If maternal interaction is prevented by early weaning, socio-emotional and behavioral development are severely impaired in rodents, suggesting the biological significance of mother–infant bonding in mammals (Kikusui and Mori 2009; Mogi et al. 2011). A similar phenomenon was observed in domestic animals. Early-weaned piglets show low growth performance (Leibbrandt et al. 1975), increased aggressive behavior (McGlone and Curtis 1985), and the development of abnormal behavior patterns (Dybkjaer 1992).

Maternal separation during early lactation, when the bond is established, has been well characterized, and it is known to have a great effect on the developing infant, particularly in altricial species. For example, maternal deprivation (180 min/day) in rats during the first 10 days of the neonatal period has been shown to have serious consequences, such as increased anxiety (Plotsky and Meaney 1993; Caldji et al. 2000; Liu et al. 2000), reduced resistance against cancer (LaBarba and White 1971), and enhanced neuroendocrine responses to stressors (Ogawa et al. 1994; Ladd et al. 1996; Francis et al. 1999) accompanied by epigenetic changes in the central nervous system (Weaver et al. 2004), later in life.

During this period, tactile stimuli to pups are significant for the development of their sociality. Meaney and colleagues have shown that offspring nurtured by mothers with more frequent licking and grooming behaviors during the first week postpartum exhibit reduced anxiety and behavioral fearfulness or corticosterone levels in response to stress during adulthood, compared with offspring with mothers exhibiting less frequent licking/grooming (Liu et al. 1997; Caldi et al. 1998). The reception of tactile stimuli decreases DNA methylation in the glucocorticoid receptor (GR) gene in the hippocampus, thereby increasing the expression of these receptors after maturation (Weaver et al. 2004). This enhances glucocorticoid feedback sensitivity in the hippocampus, which results in decreased reactivity to stress in offspring nurtured by good mothers (Liu et al. 1997; Caldi et al. 1998).
8 Early Weaning Stress in Juvenile Animals

In addition, the disruption of mother–infant bonding later during the lactation period, such as early weaning, greatly affects the infant’s neurobehavioral development. Here, weaning is defined as breaking the bond between the dam and her offspring, which includes the cessation of suckling and physical separation from the dam, with the cessation of social protection by the dam. Undoubtedly, early and sudden weaning is usually observed in farm animals (Weary et al. 1999), and it has been demonstrated that they cause physiological and behavioral maladaptive phenotypes in adulthood in rodent models, in addition to other mammalian species such as dogs and pigs (Kikusui and Mori 2009).

We have revealed the neurobehavioral effects of early weaning in laboratory rodents. Early weaning appears to have long-lasting effects on emotional behaviors, leading to increased anxiety-like behavior in response to challenging events in novel environments (Kanari et al. 2005; Ito et al. 2006; Kikusui et al. 2008). In mice, early weaning has been shown to affect the neuroendocrine stress response. Baseline levels of corticosterone, the corticosterone response to a novel cage, and hippocampal GR mRNA expression levels were assayed at the age of 3, 5, and 8 weeks (Kikusui et al. 2009). Basal corticosterone levels in early-weaned male mice, but not in females, were higher than those in normally weaned mice. When the mice were subjected to the elevated plus-maze test, those that were early-weaned had greater anxiety and secreted more corticosterone than normally weaned mice. In addition, GR expression in early-weaned mice was higher at the age of 3 weeks, but lower at the age of 8 weeks, compared with normally weaned mice (Kikusui et al. 2006a). Decreased GR expression in the hippocampus lowers feedback inhibition by circulating glucocorticoids, resulting in higher corticosterone secretion after stress exposure, which is consistent with our endocrine data. We also examined the effect of early weaning on the mRNA expression of 5-hydroxytryptamine (5HT) receptors in the hippocampus, particularly 5HT1A and 5HT1B because these receptors are reported to be involved in anxiety behavior (Gross et al. 2002). Early-weaned mice had lower 5HT1B expression levels in the hippocampus than the normally weaned mice; no effect was found with regard to 5HT1A expression. These results suggest that the manipulation of the weaning time modulates adult anxiety behavior, and this change may involve the 5HT system in the hippocampus (Nakamura et al. 2003).

In rats, the effects of early weaning on autonomic stress responses have been studied. When rats were introduced to a novel environment, early-weaned males, but not females, showed an increase in stress-induced hyperthermia (a transient rise in core temperature), compared with normally weaned rats (Ito et al. 2006). Therefore, both separation and early weaning appear to affect autonomic stress responses. These results suggest that, in rodents, early weaning increases negative emotions and enhances physiological responses to stress.

Novakova (1975) first reported the effects of early weaning on subsequent maternal behavior in rats and found that early weaning decreased nest building
and the acceptance of fostered pups (Novakova 1975). Because maternal licking and grooming of the pups play a critical role in non-genomic transmission, we assessed these behaviors as well as nursing behaviors, from day 15 to 21 postpartum, to describe the type of maternal care associated with deprivation in early-weaned mice (Kikusui et al. 2005). It was shown that the mothers spent 3% of their time in licking/grooming or arched-back nursing their pups on postpartum day 15, and the duration of these behaviors gradually decreased until day 21 postpartum. Taking all maternal behaviors together, mothers spent 50% of their time attending to their pups during this period. Early-weaned female mice performed less licking/grooming and arched-back nursing of their pups than the normally weaned mice, although the time spent away from or attending to pups did not differ in the groups (Kikusui et al. 2005). These findings suggest that early weaning deprives offspring of maternal care, and these offspring consequently show less maternal behaviors as adults.

9 Human–Dog Bonding

Dogs are the oldest domesticated animals, and they may have started to live with humans 35,000 years ago. Since domestication has been achieved, humans have developed a cooperative relationship with dogs in activities such as hunting, guarding, and herding (Coppinger and Schneider 1995). In recent times, dogs have become more closely involved with human activities; there are service dogs, drug-sniffing dogs, and alert dogs. They have developed a close relationship with humans. The importance of human–dog bonding has attracted increased attention owing to its psychological aspects. The investigations into the human–dog relationship began after Levinson (1962) reported a case in which a child who had communication problems clinically benefited from a dog’s company (Levinson 1962).

However, the reason why dogs have mental and physical effects on humans remains unclear, although there are some hypotheses (Kruger and Serpell 2006). One hypothesis suggests that humans develop positive feelings and behavior while caring for dogs because an attachment similar to the human mother–infant relationship can be formed between humans and dogs (Serpell 1996). Attachment or social bonding can positively influence the psychological and physiological aspects of human beings. To measure this human–dog “attachment relationship,” some psychological parameters for the degree of attachment humans have toward companion animals have been developed. As a result, it has been reported that the degree of attachment that humans have toward dogs, in addition to owning them, is related to human health, wellbeing, and the development of positive feelings (Ory and Goldberg 1983; Garrity et al. 1989; Triebenbacher 1998). However, most of these studies evaluated only the feelings of humans using psychological scales or questionnaires, and the concept of human–dog attachment is still not clearly defined. Therefore, these studies failed to decipher the human–dog relationship from a biological perspective.
On the other hand, the attachment of dogs to humans has been examined using a behavioral test, the Strange Situation Test, which was originally developed for human infants. In this test, dogs showed types of attachment behavior toward their owners, which was similar to that of human infants toward their mothers (Topál et al. 1998). Moreover, we found the emotional contagiousness between dogs and their owners by the analysis of heart rate variability, and the time shared in the same environment is a key factor for emotional contagiousness (Katayama et al. 2019). These results imply that biological mechanisms underlie human–dog bonding.

10 Oxytocin and Human–Dog Bonding

Visual cognitive ability, especially gazing, is the most fundamental and important social cue in humans. Visual communication abilities have been considered to have developed in dogs as a by-product of their domestication and the need to develop behavior that is required for a symbiotic relationship with humans (Hare et al. 2002; Hare and Tomasello 2005). Dogs may utilize gaze as a social attachment cue like humans (Miklósi et al. 2003). We focused on the dogs’ gaze and constructed a hypothesis that the concentrations of oxytocin in the urine of dog owners are affected by their dogs’ gaze, which functions as an attachment behavior. To test this hypothesis, the urinary oxytocin concentrations of dog owners were measured before and after 30-min interactions with their dogs (Nagasawa et al. 2009). The participants were allowed to freely interact with their dogs. Before and after the interactions, the urine samples of the participants were collected. In the control experiment, the participants were forbidden from looking at their dogs directly by instructing them to face the wall to inhibit the gaze stimuli from the dog to the owner.

Using cluster analysis, the owners were divided into two groups: one received a longer gaze duration from their dogs and reported a higher degree of relationship with their dogs (LG), whereas the other received a shorter gaze duration and reported a lower degree of relationship (SG). Urinary oxytocin levels were higher in the LG than in the SG after normal interaction with their dogs (interaction experiment) but not in the control experiment. In the interaction experiment, a high correlation was found between the frequency of behavioral exchanges initiated by the dog’s gaze and the increase in urinary oxytocin levels, suggesting that the dog’s gaze stimulated the owner’s oxytocinergic system (Nagasawa et al. 2009). In addition, the owners received eye gaze from the dog showed higher care-taking behavior to LG, as the LG showed an increase in OT after the interaction (Nagasawa et al. 2015). This revealed that there is an OT-mediated positive loop between dogs and owners.

These results indicate a strong possibility that oxytocin played an important role in human–dog bonding. It is still unclear whether a dog’s gaze at its owner is innate or learned, and whether the dog’s gaze has different functional meanings to humans and conspecifics. We can assume that such behavior is very similar to that observed in human infants (Robson 1967); hence, owners gazed by their dogs speculate their
dogs’ emotional statuses and consider the gaze as an attachment behavior. Therefore, a dog’s gaze is considered a significant cue for social contact in humans.

The results of this study suggest that of all the interactions observed between a dog and its owner, the dog’s gaze, as a factor that contributes to social bonding, has a particularly strong effect on the owner’s neuroendocrine system. It is not yet clear whether oxytocin concentrations in the urine reflect the activity of the central nervous system; however, it has been shown that there is a strong positive relationship between the oxytocin concentration in the urine and that in plasma (Amico et al. 1987); plasma oxytocin is related to hypothalamic activity (Ludwig et al. 1994). Based on these data, it is likely that urinary oxytocin, at least to some extent, reflects hypothalamic oxytocinergic activity.

This study provides a clue for the neural mechanisms through which interaction with dogs affects physical and mental health in humans. It is said that animals have species-specific attachment styles. This study suggests that humans and dogs may have a common attachment style, which may partially explain why dogs can adapt to human society.

11 Separation Stress Between Dog and Human

As mentioned above, there is a neuroendocrine regulation in human–dog bonding, which means that separation between them can cause severe stress, as described in humans and rodent models. In humans, it is well documented that some pet owners show stress symptoms after the death or loss of their pets. The grief associated with bereavement has long been ignored as disenfranchised grief (Cordaro 2012). However, as the number of pets owned has increased and the awareness that pets are members of the family has increased, the term “pet loss” has become more prevalent, and it has been examined qualitatively and quantitatively in the fields of sociology, psychology, and psychiatry (Kemp et al. 2016). In a comparative study of human- and pet-bereaved people, there was no significant difference in the levels of grief severity (Lavorgna and Hutton 2019). Pet loss could be as painful as the loss of close persons, and the stronger the connection to the animals, the more intense the grief is (Amiot and Bastian 2015). According to a cross-sectional study of grief and PTSD due to pet bereavement, 20% of people experience significant features of grief reactions and 5-12% of people experience major pathological disruption (Adrian et al. 2009). On the other hand, posttraumatic growth has also been reported, where human- or pet-bereavement results in psychological and social growth (Packman et al. 2017). However, most studies have been based on questionnaires and interviews, and the neuroendocrinological effects of separation from pets have rarely been examined.

There is evidence that dogs also show separation stress in the absence of the owner. Dogs’ behavioral problems, known as separation anxiety, are common in modern countries. These dogs show vomiting, barking, urination, and defecation after the owner left. In Finland, 17.2% of dogs show symptoms of separation anxiety
Separation anxiety is an intimate part of the human–dog relationship, and it is also likely dependent on the nature of attachment between the human and the dog (Schwartz 2003). Separation anxiety in puppies is designed to prevent separation from and maintain proximity with their mother and littermates. Young dogs at 5–7 weeks of age, before weaning, showed similar behavioral and endocrine responses to separation from the mother (Nagasawa et al. 2014), indicating that separation anxiety is related to high attachment to the owner. This raises the hypothesis that the process of domestication appears to have resulted from the retention of juvenile behaviors in adults, which is called neoteny (Trut et al. 2004). The dependence of the young on parental figures, such as the owner, is a characteristic of juvenile behavior. However, these behavioral responses sometimes become severe, and the owners and veterinarians recognize them as separation anxiety.

The neural mechanisms of separation anxiety in dogs are poorly understood. However, the clinical and medical treatments for this disorder can shed light on the mechanisms. The use of psychoactive medication is recommended for the treatment of separation anxiety in dogs if behavior modification alone is unsuccessful. Dogs with acute or extreme separation anxiety, such as a reaction to the death of an attachment figure or substantial destruction of property, are treated with medications. Alprazolam and other benzodiazepines are rapidly absorbed (Overall 1997). Tricyclic antidepressants may require several weeks to take effect, and they may be more appropriate for long-term management. Clomipramine has been successful in the treatment of separation anxiety in dogs (Overall 1997). These effective medications are similar to those used in human psychiatry, suggesting that the same neural mechanisms underlie separation anxiety in dogs.

Long-term separation from their owners also affects dogs’ behaviors. Unlike human, it is unclear whether dogs can cognitively process the concept of death. However, they may develop signs of emotional distress simply because of the absence of the attachment figure. In our study, we examined endocrine and behavioral differences in dogs that were forced into separation from their owners due to natural disasters in the Fukushima area (Great East Japan Earthquake in Japan, 2011), and those that were abandoned by the owner. Fukushima dogs exhibited lower trainability and lower attachment to practitioners who were taking care of them. They also showed very high urine cortisol concentrations – approximately five- to ten-fold higher than those in abandoned dogs from another area of Japan (Nagasawa et al. 2012). Impaired learning ability due to oversecretion of glucocorticoids is a core symptom in people who have experienced extreme stress, including those with PTSD (Layton and Krikorian 2002). In addition, PTSD patients have been reported to show impaired ability with respect to attachment and bonding (Charuvastra and Cloitre 2008). The disaster-affected dogs in this study appeared to demonstrate the same behaviors. These results suggest that the dogs from Fukushima suffered an extremely stressful crisis, probably due to separation from their owners and their experiences of the earthquake.

Dogs are unique animals, in terms of their domestication process and social communication skills with humans; it is said that they have acquired these skills
through convergent evolution with humans. Therefore, dogs can be unique models for understanding bond formation and separation stress in humans.

12 Conclusion

We reviewed the behavioral and neuroendocrine mechanisms underlying bond formation and separation stress from rodent models to human–dog relationships. The importance of the mechanisms of bond formation and stress caused by separation is easy to understand when we consider the relationship between mother and infants entrained by the specific reproductive strategies of mammals. For mammals, separation stress is more intense than other forms of stress, and they lead to lifelong negative effects in developing animals. Discovering the detailed mechanisms and how to ameliorate them is important in modern societies, where abandonment and child abuse continue to increase. We have also found that dogs have acquired abilities similar to those of humans through living with humans. They have also formed strong bonds with humans, and separation from humans can lead to severe stress. In the future, our understanding of human social stress biology may also be advanced through studies on dogs.

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Methods and Challenges in Investigating Sex-Specific Consequences of Social Stressors in Adolescence in Rats: Is It the Stress or the Social or the Stage of Development?

Cheryl M. McCormick

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Abstract Adolescence is a time of social learning and social restructuring that is accompanied by changes in both the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-adrenal (HPA) axis. The activation of these axes by puberty...
and stressors, respectively, shapes adolescent development. Models of social stress in rats are used to understand the consequences of perturbations of the social environment for ongoing brain development. This paper reviews the challenges in investigating the sex-specific consequences of social stressors, sex differences in the models of social stress used in rats and the sex-specific effects on behaviour and provides an overview of sex differences in HPA responding to stressors, the variability in pubertal development and in strains of rats that require consideration in conducting such research, and directions for future research.

**Keywords** Adolescence · Puberty · Sex differences · Social behaviour · Social stress

## 1 Introduction

In 2007, when we first reviewed the literature on hypothalamic-pituitary-adrenal (HPA) responses to stressors in adolescence in rodents, the limited available evidence (nine papers) indicated that adolescents and adults did differ in HPA responding to stressors, with adolescents showing higher and/or more prolonged responses to stressors than did adults (McCormick and Mathews 2007). Further, there were only 10 papers we could find, including two from my laboratory, that investigated whether there were any consequences of stressor exposures in adolescence that would be evident in adulthood (McCormick and Mathews 2007). We wrote that given the sex differences in HPA function, sex differences in the developmental trajectory such as attainment of pubertal milestones, sex differences in the nervous system, and with many of these sex differences emerging in adolescence, the consequences of adolescent stress exposures would likely differ for males and females. At the end of 2020, there is much evidence to support this proposition.

The investigation of how stressors affect adolescent development has grown exponentially over the last twenty years, and rats and mice continue to be the main animal models used in such investigations (see McCormick et al. (2017b) for a discussion of the translational relevance of such research). This review focusses on rats rather than mice or other rodents to serve the focus of a discussion of sex differences, which also entails a discussion of strain differences; the added factor of species is beyond the scope of this review (see, for example, differences in the sexual differentiation of rats versus mice (Bonthuis et al. 2010)). Because of the clinical importance of adolescence as a time in which many psychiatric disorders emerge, and with stressful experiences known to be a pre-disposing factor for such disorders, much of the pre-clinical research focussed on endpoints such as anxiety-like and depressive-like behaviour (McCormick and Green 2013) and responses to drugs of abuse (Andersen 2019). Investigations of learning and memory functions were also prevalent based on the well-established effects of chronic stressors on such functions in adulthood (Green and McCormick 2013). More recently, the shift has turned towards a greater attention to effects of adolescent stressors on social
behaviour in both investigations of humans and rats. This shift is in recognition of the importance of social learning and peer relationships in adolescence and of the ongoing development of brain regions implicated in social behaviour (Andrews et al. 2021; Burke et al. 2017). Thus, although the methods by which rats are stressed are numerous and involve physical (e.g., foot shock, injuries), psychological (e.g., restraint), predator cues (e.g., fox urine), and combinations thereof, social stressors may target the developing social systems more readily than other stressors, and social stressors may alter the social systems in adolescence more so than in adulthood (Tzanoulinou and Sandi 2015). The main types of social stressors used with rats are isolation, social defeat, and social instability and are discussed in a later section.

Two non-mutually exclusive means by which social stressors administered in adolescence produce lasting effects on behavioural development are: (1) chronic elevations in glucocorticoids as a consequence of repeated stressor-induced activation of HPA axis; and (2) altered social learning that results from the social stress procedures. Glucocorticoids (notably cortisol in humans and corticosterone in rats) have widespread effects on brain development and neural plasticity through genomic and nongenomic actions, with their effects on remodelling the brain most evident during times of rapid development such as the perinatal period (Maccari et al. 2014; McEwen 2016; McEwen and Akil 2020). Adolescence also is a sensitive period to the consequences of prolonged elevations in glucocorticoids given continued brain development and the re-organization of systems brought about by puberty (Brown and Spencer 2013; Eiland and Romeo 2013). In turn, social experiences contribute to the shaping of the developmental trajectory. The importance of social experiences for tuning adult behaviour and the development of the prefrontal cortex is known in rats (Himmler et al. 2013; Bell et al. 2010; Pellis et al. 2019). In guinea pigs, appropriate social rules were only learned by adolescent males through experiences in large-mix sex colonies, which provided agonistic encounters that were not provided from pair-housing with another male or from single-housing (reviewed in Sachser et al. 2011). Thus, both activation of the HPA axis and variation in social experiences will be considered in discussing the sex-specific effects of social stressors.

An aim of the review is to discuss the challenges of investigating sex differences in the behavioural consequences of social stressors experienced in adolescence in addition to reviewing the main types of social stressors and their effects. I begin by an overview of adolescence and puberty in rats, and then discuss sex differences in social behaviour and in the HPA axis in adolescence, and subsequently describe the main findings of various social stress procedures administered to adolescent rats. I then provide an overview of our investigations of adolescent social instability stress in female and male rats. I conclude with recommendations for future research.
2 Adolescence and Puberty in Rats

2.1 Defining Adolescence and Puberty in Male and Female Rats

One of the factors researchers contend with is at what age to administer a social stress protocol because there are no clear markers for the onset and offset of adolescence. The definition of adolescence in humans provided by the World Health Organization is people who are between the ages of 10 and 19 years, with the onset of puberty marking the transition from childhood to adolescence (http://www.who.int/maternal_child_adolescent/topics/adolescence/dev/en/). In rats, the broadest definition has adolescence extending from postnatal day (P) 21, a typical age at which laboratory rats are weaned, to P60, around the time of sexual maturity (Tirelli et al. 2003). This age span involves a pre-pubertal and a post-pubertal period based on physical markers of puberty, specifically, vaginal opening in females and separation of the prepuce from the glans penis in males. Both of these physical markers are associated with a rise in hypothalamic-pituitary-gonadal function. In female rats, the onset of vaginal opening is highly correlated with the onset of ovulation and first oestrus (Castellano et al. 2011; Ojeda and Urbanski 1994). In male rats, preputial separation coincides with a rise in testosterone concentrations and with sperm in the epididymis (for more review of pubertal markers in rats, see McCormick and Mathews 2010; McCormick et al. 2017b). As in humans, pubertal events occur earlier in female than in male rats. Thus, any investigation of sex differences in adolescence involves comparisons of two groups that are at different developmental points. This conundrum is not resolved, however, by using different ages of females and males to keep pubertal onset constant between the two because the trajectory for other milestones has female rats attaining adult-typical levels in some cases earlier and in some cases later than male rats (e.g., qualitative change in performance on the elevated pus maze later in females than in males, Imhof et al. 1993; increase in hypothalamic expression of leptin receptors plateaus later in adolescence in females than in males, Smith and Waddell 2003). Further, the pattern of developmental change during adolescence differs for female and male rats for many endpoints (e.g., hypothalamic gene expression, Walker et al. 2012).

2.2 Strain Differences in Pubertal Development

A second factor to contend with are the considerable strain differences in the mean day of onset of vaginal opening and of preputial separation. For example, Wistar rats attain preputial separation earlier and have mature sperm motility earlier than do Sprague Dawley males (Campion et al. 2013). Further, there is considerable variation within strains of rats and within litters in the age of attainment of these physical markers. Within-strain variation can be exacerbated by environmental factors such
as diet, illumination, housing, and rearing conditions that can shift pubertal onset (e.g., Vandenbergh 1976). To illustrate the variation in the literature, Table 1 provides a non-systematically gathered sample of papers in which onset of vaginal opening and/or of preputial separation is reported. Evidence from both human (Beltz et al. 2020) and rat (Juraska and Willing 2017) studies indicates that age at pubertal onset is as critical a factor to consider as is postnatal age to understand individual differences in development. Thus, keeping track of such markers may help explain variation within an experiment and across laboratories. In a later section, how gonadal function and pubertal status are relevant to the body’s response to stressors is described.

3 Sex Differences in Social Behaviour in Adolescence in Rats

3.1 Brain Regions Implicated in Social Behaviour

Social behaviour requires coordination among several brain regions that are reciprocally connected (see Fig. 1). The brain regions that are considered to be most critical to the network of social behaviour are the medial amygdala, bed nucleus of the stria terminalis, lateral septum, preoptic area; these brain regions have a high density of androgen and oestrogen receptors, as would be expected given the effects of sex hormones on, and sex differences in, social behaviour. These regions also are important sites of action of neuropeptides such as oxytocin and arginine vasopressin, which also have well-known roles in social behaviour (Dumais and Veenema 2016). Other regions such as the hippocampus, prefrontal cortex, nucleus accumbens, and paraventricular nucleus of the hypothalamus also contribute to social function. Many of these brain regions change significantly across the adolescent period. A review of the neural control of social behaviour and adolescent brain development is beyond the scope of this review. For reviews of these topics, see, Prounis and Ophir (2020), Bludau et al. (2019), Premachandran et al. (2020), and Shaw et al. (2020).

3.2 Sex and Strain Differences in Social Behaviour

Social stressors by definition disrupt social behaviour, and any sex-specific effects may involve sex differences in social behaviour in adolescence. Bolles and Woods (1964) provide a rich description of the emergence of social behaviour and the stimulus value of littermates in Sprague Dawley rats from birth to early adolescence. Social grooming began at ~P13 and developed into “fighting”/social play by P20, and there were no systematic sex differences. Social play is a main difference in the social behaviour of adolescent and adult rats, with more play behaviour in young
## Table 1 A sample of investigations of postnatal day (P) of vaginal opening in females and of preputial separation in males

<table>
<thead>
<tr>
<th>Strain</th>
<th>Experimental group</th>
<th>Mean P ± SEM</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Onset of vaginal opening</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not specified</td>
<td>On low fat diet</td>
<td>32.4 ± 0.6</td>
<td>Frisch et al. (1975)</td>
</tr>
<tr>
<td></td>
<td>On high fat diet</td>
<td>34.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Handled in first week of life</td>
<td>~33</td>
<td>Withuhn et al. (2003)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Nonsurgical controls</td>
<td>41.0 ± 1.0</td>
<td>Kinsey-Jones et al. (2010)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Prenatal vehicle injection controls</td>
<td>~33</td>
<td>Davis et al. (2011)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Juvenile odour stress controls</td>
<td>~39</td>
<td>Li et al. (2019)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>From litters of 4 pups</td>
<td>34.3 ± 0.3</td>
<td>Wu et al. (2016)</td>
</tr>
<tr>
<td></td>
<td>From litters of 12 pups</td>
<td>36.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Sham surgery at P12–P15</td>
<td>50% attain by P30; 100% by P36</td>
<td>Hagenauer et al. (2011)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Diet controls</td>
<td>25% attain by P34, 100% by P37</td>
<td>Iwasa et al. (2010)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Rearing controls</td>
<td>~35.5</td>
<td>Cowan and Richardson (2019)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Diet controls</td>
<td>32 ± 2.16</td>
<td>Matsuzaki et al. (2018)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Controls, several studies</td>
<td>Means of P31–P34</td>
<td>Lewis et al. (2002)</td>
</tr>
<tr>
<td>Lister</td>
<td>Vehicle injection controls</td>
<td>33.9 ± 1.94</td>
<td>Hodgson et al. (2020)</td>
</tr>
<tr>
<td>Long Evans</td>
<td>Unmanipulated</td>
<td>34.9</td>
<td>Drzewiecki and Juraska (2020)</td>
</tr>
<tr>
<td>Long Evans</td>
<td>Rearing controls</td>
<td>37.6 ± 0.7</td>
<td>Davis et al. (2020)</td>
</tr>
<tr>
<td>Long Evans</td>
<td>Rearing controls</td>
<td>50% attain by P32; 100% by P35</td>
<td>Eck et al. (2020)</td>
</tr>
<tr>
<td>Long Evans</td>
<td>Unmanipulated</td>
<td>Within P32–P34</td>
<td>Mohr et al. (2019)</td>
</tr>
<tr>
<td>Holtzmann</td>
<td>Singly housed Group housed</td>
<td>35.9 ± 1.19</td>
<td>Vandenergh (1976)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.6 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>Injected on P25, P27, P29 with saline</td>
<td>50% attain by P32; 100% by P34</td>
<td>Cardoso et al. (2010)</td>
</tr>
<tr>
<td>Wistar</td>
<td>Neonatal vehicle injections</td>
<td>~35</td>
<td>Sominsky et al. (2012)</td>
</tr>
<tr>
<td>Wistar</td>
<td>Prenatal nutrition controls</td>
<td>36.1 ± 1.5</td>
<td>Engelbregt et al. (2000)</td>
</tr>
<tr>
<td><strong>Onset of preputial separation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Unmanipulated</td>
<td>39.14 ± 0.4</td>
<td>Korenbrot et al. (1977)</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>Controls, several studies</td>
<td>Means of P44–P48</td>
<td>Lewis et al. (2002)</td>
</tr>
</tbody>
</table>

(continued)
adolescents than in adults. Social play is maximal before puberty (Thor and Holloway 1984a) and is exhibited by males more frequently than by females (Pellis et al. 1997). Puberty further increases the qualitative differences of play fighting between the sexes, mostly through changes in males (Pellis 2002). In an operant reinforcement task, male rats had a higher preference for nose pokes leading to a

Table 1 (continued)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Experimental group</th>
<th>Mean P ± SEM</th>
<th>Reference</th>
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<tr>
<td>Sprague Dawley</td>
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<td>Schneider et al. (2017)</td>
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<td>Bodensteiner et al. (2014)</td>
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<td>Long Evans</td>
<td>Controls</td>
<td>41.9 ± 0.4</td>
<td>Davis et al. (2020)</td>
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<td>Prenatal gavage controls</td>
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<td>Stanko et al. (2010)</td>
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<td>Long Evans</td>
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<td>Keeley et al. (2015)</td>
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<td>Unmanipulated</td>
<td>42.33</td>
<td>Drzewiecki and Juraska (2020)</td>
</tr>
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</table>

Fig. 1 A cartoon of a sagittal section of a rat brain illustrating the main neural regions underlying social behaviour and their interconnectivity.

OB = olfactory bulb; PFC = prefrontal cortex; HPC = hippocampus; LS = lateral septum; nACC = nucleus accumbens; BNST = bed nucleus of the stria terminalis; POA = preoptic area; PVN = paraventricular nucleus; MeA = Medial amygdala; VTA = Ventral tegmental area

 adolescente than in adults. Social play is maximal before puberty (Thor and Holloway 1984a) and is exhibited by males more frequently than by females (Pellis et al. 1997). Puberty further increases the qualitative differences of play fighting between the sexes, mostly through changes in males (Pellis 2002). In an operant reinforcement task, male rats had a higher preference for nose pokes leading to a
social stimulus than did female rats at P35-P41 (Schatz et al. 2019), which suggests a greater reward value of social interaction in males than in females. The sex differences diminish in adults, although social investigation and social interaction typically remain higher in male than in female rats (e.g., Johnston and File 1991; Carrier and Kabbaj 2012; Lian et al. 2018).

The extent to which strain differences (Ku et al. 2016; Siviy et al. 2003; Himmler et al. 2013, 2014) and/or sex differences (e.g., Northcutt and Nwankwo 2018; Pellis et al. 1997) are evident in social behaviour in rats depends on test conditions. For example, some studies isolate rats for up to 24 h before a social interaction test to increase social behaviour during the test. Such isolation, however, is stressful for rats (discussed in a later section). Sex differences are more readily observed in the home cage without any prior isolation (Auger and Olesen 2009; Thor and Holloway 1984b). At P23, both male and female rats will form a conditioned place preference (CPP) for a chamber paired with a social stimulus, although the social CPP was greater in males than in females when they were housed in isolation and not when housed in pairs (Douglas et al. 2004). In contrast, at P55 there was no sex difference and only those in isolation housing had a social CPP (Douglas et al. 2004). Thus, the procedures involved in a behavioural assessment may moderate the sex-specific effects of social stressors.

Sex differences in social recognition memory (Markham and Juraska 2007) and in the social modulation of learning (Mikosz et al. 2015) are observed in adults; the development of these sex differences in adolescence is under-studied. There were no sex differences in social recognition memory (Dumais and Veenema 2016) or sex differences favouring males (Veenema et al. 2012) in P26-P30 Wistar rats, which may be because of the strain of rat. No sex difference in social recognition memory was found in adult Wistar rats, although the neural mechanisms involved, such as the role of vasopressin in the lateral septum, were age- and sex-specific (Veenema et al. 2012). These results highlight other important considerations for investigating the sex-specific consequences of social stressors in adolescence, that the same behavioural endpoint may be achieved by different neural mechanisms in males and females and that these mechanisms may differ across development.

### 3.3 Sexual Differentiation and Social Development

The role of gonadal hormones in the organization and activation of social behaviour and its underlying neural circuitry is well-established (Auger and Olesen 2009; VanRyzin et al. 2020). Some sex differences in rats are independent of gonadal hormones and involve chromosomal and uterine effects (e.g., Reisert and Pilgrim 1991; Carruth et al. 2002; McCarthy et al. 2009). Appropriate social experience at specific points in development, however, also is required for typical development in rats (e.g., Bell et al. 2010) and may also contribute to sex differences. For example, the sex difference in how rats dodge from a peer to protect food develops in the absence of physical social interaction; physical social interaction is required to be
experienced in pre-pubertal adolescence for males to properly orient the dodge in relation to the opponent (Pellis et al. 1999). Male Wistar rats that were housed singly during the time that social play is most abundant (P22-P35) showed many differences in social behaviour as adults (reduction in social interaction, atypical responding in a resident-intruder test) while there was no evidence of impairments in the non-social behaviour measured (Hol et al. 1999).

By P21, the earliest age that has been used to define adolescence or juvenility in rats, there has been extensive sexual differentiation of the nervous system. Although the main time of sexual differentiation is the perinatal period in rats, there is evidence that puberty is a second period of sexual differentiation that has lasting influence on social development (e.g., Ahmed et al. 2008; Cooke and Woolley 2005; Brown et al. 2015). Thus, gonadal hormones may have organizational effects in adolescence in addition to the known activational effects. The extent to which a stressor in adolescence affects hypothalamic-pituitary-gonadal function may also underlie sex-specific effects of stressors. In addition, dominance hierarchies emerge in male rats after puberty and are less pronounced in female rats (Adams and Boice 1989; Blanchard et al. 1988; Pellis and Pellis 1990). Dominant-submissive pairs differ in their reactivity to stressors (Barnum et al. 2008). In so far as stressor procedures are applied in post-pubertal adolescence, the outcomes of the stressors may depend on the dominant-submissive status of the rat (e.g., McCormick et al. 2017a).

4 Sex Differences in HPA Responding to Stressors in Adolescence

4.1 Stressors and the HPA Axis

The elevated exposure to glucocorticoids from the prolonged and repeated activation of the hypothalamic-pituitary-adrenal (HPA) axis is an important basis for the consequences of many, though not all, stressors (Pacak and Palkovits 2001). The specific physiological responses depend much on the kind of stressor, with most stressors fitting into two broad categories, psychological (e.g., a perceived external threat) and physical (e.g., an injury). Although some have proposed social stressors to be a category distinct from other psychological stressors (Pacak and Palkovits 2001), social and psychological stressors both involve an appraisal process that is key in determining the response to the event. In addition, the systems involved in a stress response depend on a myriad of factors, including the intensity, frequency, controllability, and predictability of the stressor. In brief, neural projections to the paraventricular nucleus (PVN) of the hypothalamus lead to the release of corticotrophin hormone and arginine vasopressin from the PVN when a stressor is perceived as such, causing the release of ACTH from the anterior pituitary into blood circulation, resulting in the release of glucocorticoids from the adrenal cortex. The changes resulting from such activation of the HPA axis are adaptive not only for
confronting a stressor, but also in shaping future responses to stressors. Thus, the genomic and nongenomic actions initiated by glucocorticoids are a basis for the neural plasticity that leads to enduring cognitive and behavioural effects. A rise in glucocorticoids alone does not constitute a stress response; for psychological and social stressors, it is how an event is perceived and hence the state of the neural substrate upon which the glucocorticoids are acting that determine the consequences (Koolhaas et al. 2011).

Sex differences in the HPA axis are found at every level of the axis, and in rodents, females show greater HPA responding to stressors than do males, with oestradiol serving to increase and testosterone serving to dampen HPA function. This sex difference in responding to stressors involves both organizational effects of hormones during the perinatal period and activational effects of sex hormones at later stages of life (Zuloaga et al. 2020; Green and McCormick 2016). (For more extensive review of the HPA axis, see Bartlett et al. 2019; Mifsud and Reul 2018).

4.2 HPA Function and Gonadal Status in Adolescence in Rats

Adolescent rats typically show greater HPA responding to many different stressors than do adults (for some exceptions, see McCormick et al. 2015). For example, both pre-pubertal males and females secrete more corticosterone in response to restraint than do their adult counterparts (e.g., Viau et al. 2005; Bingham et al. 2011; Doremus-Fitzwater et al. 2009; Foilb et al. 2011). Further, pre- and post-pubertal adolescents typically showed greater and/or more prolonged expression of immediate early genes in several hypothalamic and extra-hypothalamic brain regions in response to a stressor (Hodges et al. 2014; Lui et al. 2012; Novak et al. 2007; Viau et al. 2005). Nevertheless, the differences between adolescents and adults in HPA responding to stressors are not readily attributable to the immature gonadal status of adolescents (e.g., Romeo et al. 2004a, b; Green et al. 2016), although they may involve developmental shifts in the regulation of the HPA axis by gonadal hormones in adolescence into adulthood in both female (Evuarherhe et al. 2009) and male (Green et al. 2019) rats. The age differences also likely involve maturation at many levels of the axis as well as to the neurocircuitry that projects to the PVN (Eiland and Romeo 2013; Spear 2000). Further, there is evidence that adolescence is a second window for sexual differentiation of several brain regions involved in regulating HPA function and social behaviour (Cooke and Woolley 2009; De Lorme et al. 2012).
4.3 Sex Differences in the Development of the HPA Axis in Adolescence

There are marked sex differences in the development of the HPA axis evident in the adolescent period. For example, there is a linear increase in the basal concentrations of corticosterone from once detectable in neonates to adult-typical levels in male Wistar rats at P40 and in female Wistar rats at P60 (Pignatelli et al. 2006). Similarly, the volume of the adrenal increases until P60, but more so in females than in males (Pignatelli et al. 2006). In male and female Sprague Dawley rats investigated from birth to 12 weeks of age, *Pomc* expression (one of the end products of which is ACTH) in the anterior pituitary followed a sinusoidal pattern across ages, with peaks at 2, 6, and 12 weeks and no sex difference (Bjelobaba et al. 2015). The developmental change in expression of other anterior pituitary genes (*Gh1, Prl, Cga, Tshb, Fshb, Lhb*, and *Gnrhr*), however, was relatively linear and differed for males and females. At P30, Sprague Dawley males and females did not differ in CRH mRNA in the PVN, although females showed a greater increase in expression than did males after 30 min of restraint (Viau et al. 2005). There was no sex difference in corticosterone concentrations after 20 min of isolation/confine to a small container in P30 Sprague Dawley rats (McCormick et al. 2001) or after 60 min in P26-P30 Sprague Dawley rats (McCormick et al. 2002) and in Long Evans rats (McCormick et al. 2007) or after 30 min (Viau et al. 2005) or 90 min (Doremus-Fitzwater et al. 2009) of restraint in P30 Sprague Dawley rats. In Wistar rats, females had higher corticosterone concentrations at baseline and after 30 min of isolation in a novel cage than did males (Lundberg et al. 2017). At P45, Long Evans females had higher corticosterone concentrations immediately after 15 min on an elevated platform (McCormick et al. 2008) and after a 15 min forced swim (Mathews et al. 2008a, b) than did males. Thus, sex differences in HPA responding to stressors typically are more robust and similar to that in adults in post-pubertal adolescents than in pre-pubertal adolescents in rats. An outstanding consideration, however, is the extent to which the two sexes and different age groups perceive a stressful event in the same way, a point discussed again in the next section.

4.4 Social Context Alters the Response to Stressors in Adolescence

The HPA response to, and recovery from, a stressor is moderated by the social context, and social bonds are an important factor in buffering the effects of stressors (Armario et al. 1983; Kiyokawa et al. 2004). Behavioural effects, however, do not always match the endocrine effects. For example, in P34 Sprague Dawley rats that were isolated for 24 h and then placed in a test arena with either a familiar peer (cagemate since weaning) or an unfamiliar peer for 30 min, corticosterone concentrations were higher than at baseline and did not differ based on peer familiarity (Cirulli et al.
Nevertheless, unfamiliar pairs displayed more social investigation and play than did familiar pairs, with no marked sex differences observed. When the experiment was repeated using a test arena similar in size to the home cage and both a low and high illumination condition, those males returned to familiar partners had lower corticosterone concentrations than did those with unfamiliar peers when tested in low illumination and did not differ under high illumination, although both types of pairings had lower corticosterone than those placed for 30 min in the test arena alone (Terranova et al. 1999). In contrast to their previous report, those with a familiar peer displayed more social interaction than when with an unfamiliar partner, particularly in female pairings. The results, however, highlight the importance of methodological aspects in the results obtained, such as lighting conditions and size of test arenas.

My laboratory investigated adolescent P30 and P45 Long Evans rats in their home cages to gauge the effect of partner familiarity on recovery from stressors. Both age groups of adolescents of both sexes had elevated corticosterone concentrations after 60 min of isolation/confine in small containers (McCormick et al. 2007). Those returned to their original cage partner had lower corticosterone concentrations after 60 min than did those returned to an unfamiliar cage partner after confinement (McCormick et al. 2007; Hodges et al. 2014). Further, whereas males with an unfamiliar cage partner were more active and less social than those returning to a familiar cage partner, the behaviour of females did not differ markedly between the two conditions. Thus, sex differences were more evident in the social behavioural measures after this stressor than in the corticosterone measures.

We also investigated the effect of repeated pairings with new cage partners after daily 1 h isolation/confine from P30 to P45 (our social instability stress (SIS) procedure, discussed more in a later section) in Long Evans rats. Repeated exposures to the isolation and pairings with new cage partners did not affect baseline concentrations of corticosterone when measured on P45 (McCormick et al. 2007). There was evidence of habituation to isolation by P45, such that males and females returned to the familiar cage partner after each daily isolation had reduced corticosterone concentrations than those undergoing a first isolation at P45. Females returned to a new cage partner after each of the isolations showed no evidence of habituation to the isolation in terms of corticosterone concentrations, and although males did, both males and females paired repeatedly with new cage partners also had lower concentrations of plasma corticosteroid binding globulin (CBG, which limits the actions of corticosterone) than did the controls and those returned to a familiar partner. Thus, both SIS males and females had higher concentrations of free (unbound to CBG) corticosterone, which suggests that SIS results in elevated exposure to glucocorticoids in both sexes across the 16 days of exposures. Further, whereas males returned to a familiar cage partner after each isolation did not show the increase in CRH mRNA in the central nucleus of the amygdala after isolation that those exposed to a first isolation did, SIS males did show such an increase to their 16th isolation (there were no group differences in females). We speculated that continued elevation of CRH mRNA in the amygdala in SIS males was because isolation remained a more highly salient stimulus for them because it predicted the pairing of a new cage partner (McCormick et al. 2007). This speculation was
supported in a later study in which blood samples were collected in males (females were not included) at P45 after 1 h with the new cage partner for the 16th time versus the first time, or after 1 h with the familiar cage partner for the 16th time versus the first time (Hodges and McCormick 2015). Although the SIS males had evidence of habituation based on corticosterone concentrations obtained from immediately after isolation (consistent with our previous study), the SIS males showed evidence of sensitization to repeated pairings with new partners, with corticosterone concentrations higher than those placed with a new cage partner for the first time. Notably, males undergoing the SIS procedures in adulthood from P70 to P85 rather than in adolescence did not differ in corticosterone concentrations from those returned after isolation to a new cage partner, suggesting that adults, and not adolescents, habituate to repeated cage partners. Thus, repeated changing of cage partners seems to be a more salient event for adolescent than for adult males. Further, the results indicate that both the social learning environment, particularly in males, and the exposure to elevations in corticosterone differ in adolescence for SIS and control rats.

5 Social Stressors

The three main procedures used as social stressors in rats are isolation housing, social defeat, and social instability. The consequences of social stressors depend not only in terms of these broad qualitatively different categories, they also depend on specific features within a category such as intensity, controllability, duration, predictability, and intermittency. Intensity of a stressor is readily quantified with stressors such as foot shock (i.e., the amperage of the shock), whereas it is less readily quantified for social stressors. Further, glucocorticoid release is not a good indicator of the intensity of a stressor because its release reaches a plateau with increasing intensity (Natelson et al. 1981; Armario et al. 1986). The extent to which the onset and offset of a stressor can be controlled and/or predicted mitigates the effects of stressors (Maier and Watkins 2010), although few social stressor procedures have involved controllability and predictability. Procedures that involve social defeat and social instability typically vary on dimensions such as frequency (how often the social stressor is encountered) and duration (length of time of exposure to the stressor), whereas isolation housing procedures tend to vary only in terms of duration (the onset and offset of the procedure).

5.1 Isolation Housing

Isolation housing is often cast as a social stressor. Nevertheless, it does not lead to the prolonged and repeated elevations in glucocorticoids associated with other stressors such as foot shock, restraint, social defeat, or social instability, which has led to the evaluation that isolation is not a suitable model to investigate chronic stress
effects (Holson et al. 1991; Haller et al. 1999). A common finding is for isolation housing to reduce baseline corticosterone concentrations (e.g., Pisu et al. 2016), although sometimes differences are found in HPA responses to a novel stressor in rats housed in isolation relative to group housed rats. Isolation housing, however, highlights the importance of social interaction in rodents and can be used as a model of psychopathology resulting from minimal stimulation. Adolescents are more susceptible than are adults to the negative consequences of isolation housing (Einon and Morgan 1977; Panksepp and Beatty 1980), although there are exceptions whereby adults showed effects and adolescents did not (e.g., Douglas et al. 2003).

Isolation housing has marked effects on behaviour towards the self and others. For example, male and female Wistar rats isolated from P21 to P70 displayed aberrant self-manipulative behaviour during isolation (Day et al. 1982). Both male and female rats display more aggression when re-exposed to peers after isolating housing (Day et al. 1982; de Moura Oliveira et al. 2019) and may elicit more aggression from peers (Luciano and Lore 1975). Some remediation from the effects of isolation is provided by a return to social housing (Hellemans et al. 2004; Hol et al. 1999). Environmental enrichment during isolation housing attenuated its effects, suggesting that the lack of stimulation is a key factor in the effects of isolation housing (Belz et al. 2003); rats in this study began isolation in late adolescence (P56), however. Whether enrichment is as effective when isolation begins earlier remains to be determined. As mentioned in a previous section, depriving rats of social play under conditions of social housing by separating pairs in the home cage by mesh during adolescence has lasting consequences for social behaviour. Such a condition could provide an important control group for isolation housing.

The importance of the quality of social relationships in the adolescent period is indicated in a study by Arakawa (2007). Wistar males and females housed in isolation from P26 until testing at P37 had reduced shock-prod burying and more crouching-freezing than did rats pair-housed with either a same-sex littermate or a non-littermate peer of the same sex, although they did not differ from rats that were pair-housed with either a non-same-sex peer or an adult female. In a second experiment (Arakawa 2007), male and female rats housed in isolation from P26-P40 were then housed either with a same-sex and same-age littermate or with another same-sex rat from the isolation condition until tested at P77. Rehousing of isolated males did not remediate their performance in the shock-prod test relative to controls. Isolated females showed remediation of their performance only when housed with a control female and not with another isolated female. The performance of the control males and females housed with an isolated peer did not differ from those housed continuously with a littermate. Thus, similar effects to isolation can be found from manipulations of the cage partner, and remediation from isolation housing is moderated by the qualities of the social partner.
5.2 Sex Differences in the Effects of Isolation Housing in Rats

A PubMed search on December 31, 2020 using the terms “social stress and isolation and sex differences” led to a small proportion of studies that involved both males and females and whereby isolation housing began in the adolescent period (see Table 2). This search may not be an exhaustive list of such studies. For the most part, the effects of isolation were the same in male and female rats, although for many of the measures there were also sex differences. When there was an interaction with sex, it was typically an effect of isolation housing in females and not males, which is consistent with studies where isolation was administered to adult rats. The majority of the studies did not involve a social rehousing period after the isolation, and thus the acute effects of isolation on performance cannot be separated from the lasting changes that may have been produced. When social housing did occur, sometimes the socially-housed control group was re-housed, and sometimes it was not. Whether social rehousing would have an effect in the controls when tested soon after is unknown, and such rehousing may contribute to the variability in findings across studies. Further, most studies investigated only one time point after isolation housing. Investigating whether there are immediate effects, lasting effects, delayed effects (i.e., emerge with time) and ideally within the same study is important for understanding the effects on ongoing development in adolescence into adulthood in males and females.

5.3 Social Defeat

Social defeat, also known as the resident-intruder paradigm, was originally developed by Miczek (1979) and Koolhaas et al. (1980) and involves placing an experimental rat in the position of intruder in the cage of a resident aggressive rat. The interaction between the resident and intruder may last for a pre-defined time or until a certain amount of aggression has been displayed by the resident or until a submissive posture is exhibited by the intruder. The interaction may be prolonged as a psychological stressor by keeping the pairs housed separated only by a barrier. The residents typically involve male rats (often retired breeders) because of the reduced aggression in female rats other than when lactating and the reduced aggression of males towards female rats. It can be difficult to obtain reliably aggressive male rats, which can increase the variability in the results obtained (MacKay 2016).

The social defeat procedure increases corticosterone release in both adolescent and adult rats, although the release of corticosterone is potentiated after repeated social defeat in adolescents (Watt et al. 2009), whereas that of adults remains of similar magnitude to the first and the repeated defeat (Covington and Miczek 2005). Social defeat results in the largest increase in corticosterone concentrations of the social stressors, yet the effects of chronic social defeat are attenuated by social
<table>
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<th>Strain</th>
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<th>Social rehousing</th>
<th>Start of test days</th>
<th>Effect of isolation relative to socially housed of the same sex</th>
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<td>P98</td>
<td>= entries to centre of OF ♀ + ♂</td>
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(continued)
Methods and Challenges in Investigating Sex-Specific Consequences of...

Table 2 (continued)

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<th>Start of test days</th>
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<td></td>
<td>P49</td>
<td>P49 ↑ climbing in FST in ♂; = ♂</td>
<td>Hong et al. (2012)</td>
</tr>
<tr>
<td>SD</td>
<td>P30–P60</td>
<td>2</td>
<td>No</td>
<td>P61</td>
<td>↓ sucrose preference ♂; = ♂</td>
<td>Pisu et al. (2016)</td>
</tr>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>2</td>
<td>No</td>
<td>P49</td>
<td>= anxiety on EPM ♂ + ♂ = ethanol preference ♂ + ♂</td>
<td>Roeckner et al. (2017)</td>
</tr>
<tr>
<td>LE</td>
<td>P21–P55</td>
<td>5</td>
<td>No</td>
<td>P56</td>
<td>= anxiety on EPM ♂ + ♂ = amph-induced locomotion ♂ + ♂</td>
<td>Liu et al. (2018)</td>
</tr>
<tr>
<td>LE</td>
<td>LAn</td>
<td>P21–P73</td>
<td>3–4</td>
<td>No</td>
<td>= anxiety on EPM ♂, ♂</td>
<td>de Moura Oliveira et al. (2019)</td>
</tr>
<tr>
<td>SD</td>
<td>P21–P63</td>
<td>3</td>
<td>No</td>
<td>P63</td>
<td>↑ anxiety on EPM ♂; = ♂</td>
<td>Mavrikaki et al. (2019)</td>
</tr>
<tr>
<td>SD</td>
<td>P21–P49</td>
<td>3</td>
<td>Yes, both groups</td>
<td>&gt;P70</td>
<td>↓ sucrose preference ♂ + ♂ = object recognition ♂ + ♂</td>
<td>Begni et al. (2020)</td>
</tr>
</tbody>
</table>

SD Sprague Dawley, LE Long Evans, LAn selectively bred for low-anxiety, HAn selectively bred for high-anxiety, OF open field, IS inescapable shock condition in the Triadic Learned Helplessness Test, CPP conditioned place preference

housing in adult male rats (Koolhaas et al. 2011). The opposite may be true for adolescent males (Burke et al. 2017). Individual differences such as coping style have been used to predict those that will be most vulnerable to the consequences of social defeat (Wood et al. 2010). A challenge in conducting age and sex comparisons is that resident males behave less aggressively towards young adolescent male and
adult female intruders than they do adult males. In turn, both adolescent male (Burke and Miczek 2015) and adolescent female (Ver Hoeve et al. 2013) intruders behave differently towards the residents than do adult intruders, specifically by reducing the frequency of upright defensive behaviour. Thus, the experience of social defeat differs for adolescent and adult rats.

5.4 Sex Differences in the Effects of Social Defeat in Rats

A PubMed search on Jan 1, 2021 using the terms social defeat and sex differences found few studies that involved adolescent rats. There was one study whereby Sprague Dawley male and female juvenile rats that witnessed the social defeat of their dam by a resident male on P21–P27 showed signs of depressive-like behaviour in adulthood in the forced swim test (Liu et al. 2018). Another report indicated that social defeat-induced expression of immediate early genes in the medial amygdala was higher in P28 Long Evans males in the right hemisphere than in that of females; expression in the left hemisphere did not differ between the two sexes (Weathington et al. 2012). There are some cases where there was research on both sexes, however in separate reports. For example, long-lasting effects of social defeat on cognitive performance was found in Sprague Dawley rats when administered in adolescence in males (Snyder et al. 2015b) and not in females (Snyder et al. 2015a) using a resident male for male intruders and a lactating resident female for female intruders. They also reported reduced dendritic branching and spines in the medial prefrontal cortex when the social defeat was applied either to adolescents or adult females, but not to adult males (Urban and Valentino 2017).

There are abundant reports of effects of social defeat on a wide variety of behavioural endpoints and it has been used both as a model of post-traumatic stress disorder and of bullying. Direct comparisons of its effects in females relative to males in rats are lacking. There are more direct comparisons of the effects of social defeat in males and females in mice (e.g., Steinman et al. 2015; Wright et al. 2018) and hamsters (e.g., Huhman et al. 2003). For more extensive reviews of the social defeat model, see Shimamoto (2018), Solomon (2017), and Hammels et al. (2015).

5.5 Social Instability

Social instability procedures in rats also are numerous and varied. In some cases, it has involved disrupting stable colonies by introducing new members (e.g., Taylor et al. 1987). Other procedures have involved a combination of periods of isolation housing (typically 24 h at a time) with periods of crowding in smaller cages in which group membership (mixed-sex groups) was changed (Haller et al. 1999; Herzog et al. 2009; Nowacka-Chmielewska et al. 2017) (for other similar variants of such a
model, see Lemaire et al. 1994; Suchecki and Tufik 2000). This form of social instability is reported to affect female more so than male rats (Blanchard et al. 2001). To the best of my knowledge, this model has been used exclusively in adult rats. Visible burrow systems may also provide social instability (Beery et al. 2020).

5.6 My Model of Social Instability Stress (SIS)

The model of social instability stress (SIS) I developed for use in adolescent rats involves 1 h of isolation after which the rats are paired with a new cage partner that also is undergoing the stress procedure; the isolation and partner exchange occurs daily for 16 days in mid-adolescence, after which rats are returned to their original cage partners. The isolation is different from isolation housing and is better described as confinement in that it is more like a bout of restraint stress than isolation housing; both my isolation containers and restraint apparatuses involve a confined space that limits movement. Confinement to small spaces is considered a psychological stressor, and thus the daily change of cage partners is the social component of the SIS procedure. In contrast to the crowding and isolation housing procedure of social instability (Baranyi et al. 2005), no aggression is observed in our SIS procedure. Non-stressed controls are also pair-housed and unmanipulated except for regular cage maintenance. As described in a previous section, the pattern of corticosterone release during SIS differs from that of rats that undergo daily 1 h isolation with no change of cage partner from P30-P45, and the two groups differ in behaviour in the home cage after isolation, yet more so in males than in females. Adolescent SIS male and female rats differed in corticosterone release from control rats when faced with a new psychological stressor and did not differ from controls when tested several weeks later as adults (elevated platform, McCormick et al. 2008; forced swim, Mathews et al. 2008b; restraint, McCormick et al. 2005). Thus, the increased activation of the HPA axis in adolescence and/or the different social learning involved in the SIS procedure in adolescence are eliciting changes in ongoing brain development rather than a dysregulated HPA axis.

5.7 Effects of the SIS Procedure

Table 3 summarizes our behavioural findings in experiments for which both females and males were included as well as from another laboratory (Roeknner et al. 2017) that used a variant of our procedure. Also included in Table 3 are separate experiments from my laboratory of SIS in females and males with similar procedures. Our initial investigations with adolescent SIS involved behavioural responses to psychostimulants, and greater effects of adolescent SIS were observed in female than in male rats. Further, the effects were evident when tested several weeks after the stress procedure. Effects of SIS on spatial performance were only evident in male
<table>
<thead>
<tr>
<th>Strain</th>
<th>Days of SIS</th>
<th>Start of test days</th>
<th>Relative to non-stress controls</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>P33–P45</td>
<td>P72 ± 4</td>
<td>↑ locomotor sensitization to 0.5 mg/kg nicotine ♀; = ♂</td>
<td>McCormick et al. (2004)</td>
</tr>
<tr>
<td>LE</td>
<td>P33–P45 or</td>
<td>P72 ± 4</td>
<td>= locomotor sensitization to 0.25 mg/kg nicotine ♀ + ♂ ↑ locomotor activity to 0.5 mg/kg amphetamine ♀; = ♂</td>
<td>McCormick et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>P65–80</td>
<td>P107 ± 4</td>
<td>= locomotor sensitization to 0.25 mg/kg nicotine ♀ + ♂ = locomotor activity to 0.5 mg/kg amphetamine ♀; = ♂</td>
<td></td>
</tr>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>P46–P47</td>
<td>↓ locomotor sensitization to 0.5 mg/kg nicotine ♀; = ♂</td>
<td>McCormick and Ibrahim (2007)</td>
</tr>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↑ CPP to 1.0 mg/kg (not 0.25, 0.5) amphetamine ♀ ↑ CPP to 0.25 mg/kg (not 0.5, 1.0) amphetamine ♂ ↑ locomotor sensitization to 1.0 mg/kg amphetamine ♀ + ♂</td>
<td>Mathews et al. (2008b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↓ CPP across doses of amphetamine ♀ + ♂ = locomotor sensitization to 1.0 mg/kg amphetamine ♀ + ♂</td>
<td></td>
</tr>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↑ immobility (depressive-like) in the FST ♀; = ♂</td>
<td>Mathews et al. (2008a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↑ climbing in the FST ♂; = ♀</td>
<td></td>
</tr>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↓ anxiety in the EPM ♀; = ♂</td>
<td>McCormick et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↓ anxiety in the EPM (oestrus, not dioestrus) ♀ ↑ anxiety in EPM ♂</td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↓ anxiety in the EPM ♀; = ♂</td>
<td>Surraka et al. (in preparation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↓ anxiety in the EPM ♀; = ♂</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
Table 3 (continued)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Days of SIS</th>
<th>Start of test days</th>
<th>Relative to non-stress controls</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>P49</td>
<td>↑ anxiety on EPM ♂ = ♀</td>
<td>Roeckner et al. (2017) (daily iso + new peer every 5 days for ♂, 7 days for ♀)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ ethanol preference ♂ = ♀</td>
<td></td>
</tr>
<tr>
<td>LE ♂</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>= spatial location memory</td>
<td>McCormick et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↓ spatial location memory</td>
<td></td>
</tr>
<tr>
<td>LE ♀</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>= spatial location memory</td>
<td>McCormick et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↓ spatial location memory</td>
<td></td>
</tr>
<tr>
<td>LE ♂</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↓ memory for context conditioning</td>
<td>McCormick et al. (2013b)</td>
</tr>
<tr>
<td></td>
<td>or P70–P85</td>
<td>P70</td>
<td>↑ memory for cue conditioning</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P86 or</td>
<td>= memory for context conditioning</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P110</td>
<td>= memory for cue conditioning</td>
<td></td>
</tr>
<tr>
<td>LE ♀</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↓ memory for context conditioning</td>
<td>Morrissey et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>or P70–P85</td>
<td>P70</td>
<td>↓ memory for cue conditioning</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P86 or</td>
<td>= memory for context conditioning</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P110</td>
<td>= memory for cue conditioning</td>
<td></td>
</tr>
</tbody>
</table>

Social behaviour

<table>
<thead>
<tr>
<th>Strain</th>
<th>Days of SIS</th>
<th>Start of test days</th>
<th>Relative to non-stress controls</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↓ social interaction ♂ + ♀</td>
<td>Asgari (2020)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↓ social interaction ♂ + ♀</td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↓ social interaction ♂ + ♀</td>
<td>Surraka et al. (in preparation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P70</td>
<td>↓ social interaction ♂ + ♀</td>
<td></td>
</tr>
<tr>
<td>LE</td>
<td>P30–P45</td>
<td>P46 or</td>
<td>↓ sucrose intake in presence of a peer ♂ = ♀</td>
<td>Herlehy (2019)</td>
</tr>
<tr>
<td></td>
<td>or P70–P85</td>
<td>P70</td>
<td>↓ sucrose intake in presence of a peer ♀ + ♂</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P86 or</td>
<td>↓ sucrose intake in presence of a peer ♀ + ♂</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P110</td>
<td>↓ sucrose intake in presence of a peer ♀ + ♂</td>
<td></td>
</tr>
</tbody>
</table>

and in female rats when tested several weeks after the termination of the SIS. Such a delayed effect in performance on hippocampal-dependent tasks was reported after chronic variable stressors in adolescence in rats (Isgor et al. 2004). Such
time-dependent results suggest that the experience of stressors is shaping how development unfolds (both studies included reports of differences in the hippocampus between stressed and non-stressed rats as adults).

The effects of SIS on anxiety-like and depressive-like behaviour were modest, sex-specific, and depended on whether the rats were tested soon or long after the stress procedure. The decrease in anxiety in the EPM in females and not in males was found in three of our studies and in both Long Evans and Wistar rats (see Table 3). It was not found by Roeckner et al. (2017), perhaps because of strain differences (Sprague Dawley) or because their procedure had fewer changes of cage partners (every 5 days for males and every 7 days for females rather than daily). When Long Evans rats underwent daily 1 h isolation/confinement from P30–P45 without any change in cage partner, no effect on anxiety-like behaviour in the EPM was found (Simone et al. 2018), which suggests that the change of cage partners may be a key factor in the effects of SIS. Another laboratory that used the SIS procedure with 16 days of 1 h isolation and changes of cage partners every 4 days found sex-specific differences in dendritic morphology in the medial prefrontal cortex relative to controls in Sprague Dawley rats (no behavioural measures were included) (Breach et al. 2019). How many days of change of cage partners is necessary and the extent to which variation in the number of days of partner changes influences outcomes remain to be determined. Reducing the number of cage partner changes makes the use of the SIS model more practical because fewer rats are required in a cohort to ensure a new partner for each day of change.

Nevertheless, strain differences likely contribute to variation in the effects observed particularly considering the variation in the anxiety profile (Ramos et al. 1997) and HPA responding to stressors (Konkle et al. 2003) across strains, and because the ages at which the SIS is applied involves a different age of onset of puberty across strains (see Table 1). A study of SIS in adolescence that involved male Wistar rats (Provensi et al. 2019) found a reduction in context conditioning memory as we have in Long Evans males (Morrissey et al. 2011), but whereas they found a decrement in object recognition memory, we found a decrement in spatial location memory (McCormick et al. 2012). We are currently investigating Wistar rats in our laboratory to gauge how the effects of SIS may differ in both sexes from what we have observed with Long Evans rats.

Our results with Long Evans rats indicate that adolescents are more sensitive to the SIS procedure than are adults (McCormick et al. 2005, 2010, 2012; Morrissey et al. 2011; Herlehy 2019). Variations in the SIS procedure may produce greater effects when administered to adult rats. A variant that involved immobilizing adult male Sprague Dawley rats twice while in the presence of a predator and while also undergoing a daily change of cage partners for 31 days increased anxiety-like behaviour and impaired object recognition when tested 4 months after the stress exposures (no females or adolescents were involved) (Zoladz et al. 2015). Another variant involved housing of 5 per cage rather than cage pairs and 5 weeks of 1 h immobilization in a cone-shaped mesh, with 3 of 5 switched per cage after immobilization. When tested immediately after the 5 weeks of SIS, those for which stress began at P28 had reduced fear potentiated startle relative to controls, whereas those
for which stress began at P56 had increased fear potentiated startle relative to controls. The differences, however, were largely driven by differences in the control groups; the control rats tested at ~P65 had greater startle than did those tested at ~P90 (Tsai et al. 2014). The age difference in sensitivity to our SIS procedure may be an advantage in understanding adolescent-specific plasticity and suggests that the procedure is a much milder form of stress than is social defeat or predator stress and not as pathological as isolation housing, procedures for which effects in adults are more readily obtained.

There are few studies of social behaviour in Table 3 because we have only begun to investigate the effects of SIS on social behaviour in females. In a direct comparison of females and males, Asgari (2020) extended our previous findings of an immediate and long-lasting decrease in social interaction after adolescent SIS in Long Evans males (Green et al. 2013; Hodges et al. 2017, 2018, 2019) to show a similar decrease in SIS females relative to controls, although social interaction was lower overall in females than in males. We recently found the same decrease in social interaction after adolescent SIS in Wistar rats (Surraka et al., in preparation). The reduction in social interaction with an unfamiliar rat in SIS rats is in contrast to increases in social interaction that are observed after isolation housing in adolescence (Varlinskaya and Spear 2008). We also found that adolescent SIS male rats had a short-lived reduction in sucrose intake in a social context than did controls, whereas SIS female rats did not differ from controls (Herlehy et al. 2019). Whether SIS in adolescent female rats will have the effects we have observed in male rats on other social measures (increased aggression in a food competition, Cumming et al. (2014); reduced social discrimination memory, Hodges et al. (2018); sexual performance, McCormick et al. (2013a); stimulus quality McCormick et al. (2017a) remains to be determined.

6 Conclusions and Recommendations for Future Research

This overview of adolescent social stressors was limited to the sex-specificity of effects on behaviour. Many of the same studies, however, included neural measures for which there also was sex-specificity. For the most part, the neural effects do not map on readily to the behavioural effects, which is to be expected; although sex differences in behaviour surely involve sex differences in the brain (and also in other systems), the absence of a sex difference on a behavioural measure may have been accomplished through different strategies and different neural mechanisms (McCarthy et al. 2017). More experimental interventions are required to determine the relationships between the behavioural and neural consequences of the stressors.

Further, whether the tests used for behavioural assessment measure the same factors in females and males remains a problem, particularly because most of the tests were developed for use in adult males (Johnston and File 1991; Fernandes et al. 1999). The same concern exists for comparisons of adolescent and adult rats.
Sex-specific effects in adolescence of stressors other than social stressors have been found in rats (e.g., Bourke and Neigh 2011). Environmental enrichment in adolescence also has differential effects in female and male rats (e.g., Peña et al. 2006) and may attenuate the effects of stress experienced in adolescence (e.g., Smith et al. 2017). The main arguments for the use of social stressors are the importance of social learning in adolescence and to model social stress in people. The choice of social stressor to use in adolescence depends on the aims of the research, and there is much variation in the procedures within each type of social stressor. Each type has advantages and disadvantages. Isolation housing has advantages as a model of psychopathology, yet it does not involve prolonged elevations of glucocorticoids as a factor in its effects. It has the advantage of its ease of administration and has been used productively to investigate sex-specific effects in a variety of species (e.g., Drosophila melanogaster (Leech et al. 2017); Syrian hamsters (Ross et al. 2017); prairie voles (McNeal et al. 2017). Social defeat is highly stressful and has been used as an animal model of bullying and PTSD. It is a model that may be easier to use in more aggressive species such as mice given that it can be difficult to obtain reliably aggressive male rats. In addition, there has been success in developing aggressive non-lactating residents in female mice (Newman et al. 2019) whereas in rats, aggressive resident females are dams with pups. Social defeat produces profound effects in both adult and adolescent rats, and thus might obscure sensitivity unique to adolescent sensitivity. Further, aggression towards adolescents differs than that towards adults, which makes interpreting age differences more difficult. Social instability stress, as we have used it, is a far milder social stressor procedure that might highlight sensitivity that is unique to adolescence and allows for direct comparison of the sexes and age groups, and it has also been used effectively in mice (e.g., de Lima and Massoco 2017; Schmidt et al. 2010). Nevertheless, in none of these types of social stressors can the perception of the experience be controlled. For example, we have suggested based on patterns of corticosterone release that change of cage partners is a more salient event for adolescent males than for adult males, and that adolescent males experience changes of cage partners as a homotypic stressor whereas adults experience changes of cage partners as a homotypic stressor (Hodges and McCormick 2015). The consequences of a stressor depend on how it is perceived.

Irrespective of the type of procedure, interpretation of the basis of sex differences of effects of social stressors in adolescence is challenging. To what extent do they depend on the sexual differentiation that has occurred to the point at which the stressors are administered? Are they the result of age comparisons that are confounded by sex differences in the progression of puberty? Is it because of the disruption of sex-specific social experiences that determine the progression to adulthood? Is it because the stressors elicit different physiological responses in females and males that perturb ongoing development? To some extent these question matter most if the aim is to understand sex differences. If the aim is to understand sex-specificity, then comparisons between the sexes are unnecessary; the goal is to understand the mechanism in each sex. The latter point is not to say that the comparison of the sexes does not provide insight. It provides similar insights as do
comparisons of strains or comparisons of species, which can inform research on mechanisms. It does behoove researchers to investigate both sexes, as females continue to be under-studied. The lack of females as subjects of such research is not apparent in the tables herein because they were limited to investigations in both sexes in rats. Males are predominant in investigations of one sex only for many species. In a recent review of the effects of adolescent social stressors on reward-related behaviour in rats and mice, the lack of investigation of females is evident; only one of the six studies using conditioned place preference included females, only two of the six studies of drug-induced locomotion included females, only one of the seven studies of drug self-administration included females, and neither of the studies on food-seeking included females (Watt et al. 2017).

As for future directions, more investigation of the means by which social stressors exert their effects and how to mitigate these effects are required. Two examples of promising approaches are the following. In Wistar male rats, a diet enriched in ω-3 polyunsaturated fatty acids prevented the changes in gut microbiota composition, in cognitive performance, and in hippocampal expression of brain-derived neurotrophic factor that resulted after social instability stress (Provensi et al. 2019), which also suggests that the gut microbiome mediates the consequences of adolescent social instability stress. We have found sex-specific effects of social instability stress on the gut microbiome in LE rats (McCormick et al. 2020), thus it will be of interest to see if such a treatment is effective in females. In another study, the role of glucocorticoid receptor signalling was investigated by pharmacologically disrupting its signalling in a study of adolescent chronic variable stress (combinations of physical and psychological stressors) in SD female and male rats (Cotella et al. 2020). Drug treatment was able to prevent the sex-specific effects of the stress exposures evident 5 weeks later in a forced swim test relative to controls in females and not in males (Cotella et al. 2020). Investigating how disrupting GR signalling influences the effects of social stressors should be explored.

An additional question for future research is to determine under what conditions social stress in adolescence is deleterious or beneficial for females versus males. For example, adolescent male rats that underwent repeated social defeat in adolescence and were pair-housed coped better than did unstressed controls when confronted with an aggressive resident in adulthood (Buwalda et al. 2013). These results are in keeping with the “mis-match hypothesis”, whereby early life experiences (typically neonatal experiences) serve to prepare the individual for its later environment, and negative consequence are more likely to be faced when there is a discrepancy between the early environment and the later environment (Nederhof and Schmidt 2012; Santarelli et al. 2017). Adolescence may also be an additional opportunity for fine-tuning based on the current environment. Thus, how females and males fare in adulthood under different contexts based on the experience of social stress in adolescence requires investigation. Further, given the role of perinatal experiences in shaping development into adolescence and adulthood, how such factors shape responses to social stress in adolescence, as well other predictors of individual differences in the effects of social stressors in both sexes, would add to the understanding of how social relationships help program development across the lifespan.
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Neuroinflammation and Mitochondrial Dysfunction Link Social Stress to Depression

Fiona Hollis, Brittany S. Pope, Erin Gorman-Sandler, and Susan K. Wood

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Abstract Major depressive disorder is a debilitating mental illness and a leading cause of global disease burden. While many etiological factors have been identified, social stress is a highly prevalent causative factor for the onset of depression. Unfortunately, rates of depression continue to increase around the world, and the
recent COVID-19 pandemic has further exacerbated this mental health crisis. Though several therapeutic strategies are available, nearly 50% of patients who receive treatment never reach remission. The exact mechanisms by which social stress exposure promotes the development of depression are unclear, making it challenging to develop novel and more effective therapeutics. However, accumulating evidence points to a role for stress-induced neuroinflammation, particularly in treatment-resistant patients. Moreover, recent evidence has expanded the concept of the pathogenesis of depression to mitochondrial dysfunction, suggesting that the combined effects of social stress on mitochondria and inflammation may synergize to facilitate stress-related depression. In this chapter, we review evidence for neuroinflammation and mitochondrial dysfunction in the pathogenesis of social stress-induced depression and discuss these in the context of novel therapeutic targets for the treatment of depression.

Keywords  Blood brain barrier · Chronic stress · Cytokine · Microglia · ROS

1 Introduction

Depression is a serious mental health illness that afflicts over 4% of the population (WHO 2017). With an estimated 322 million people affected worldwide, depression is listed as the second cause of global disease burden (Smith 2014). Depression is diagnosed as a single disorder, Major Depressive Disorder; however, it encompasses an enormous combination of symptoms (Akil et al. 2018), including low mood, sadness, lack of energy, anhedonia, and alterations in critical activities such as appetite, sleep and activity levels, and cognition (Association and others 2013). At its most severe, depression can lead to suicide, accounting for close to 1.4% of worldwide deaths in 2016 (WHO 2017). Current therapies are less than ideal, as only a third of patients achieve remission under a single therapy, and even fewer achieve remission following more than two therapies (Rush 2007), leaving approximately 50% of patients with inadequate treatment (reviewed in (Akil et al. 2018)). Aside from the human toll, depression induces a stark economic burden, costing an estimated $210.5 billion inflation-related dollars in 2010 in the United States alone (Miller and Raison 2016). As the prevalence of depression increased approximately 18% between 2005 and 2015, and now amidst the COVID-19 pandemic prevalence of depression symptoms in adults in the US alone has tripled (Ettman et al. 2020) the human and economic burden can only worsen. Taken together, these figures highlight the urgent need for greater understanding of the biological factors underlying depression and the identification of new therapeutic targets.
1.1 Etiological Factors in Depression

Humans have suffered from major depressive disorders (first called melancholia) dating back to medieval times. During the mid-twentieth century, it was first recognized that depression could be precipitated by a neurochemical imbalance in the brain. In fact, these findings were inadvertently derived from pharmacological studies in humans that identified that certain drugs, such as reserpine and iproniazid, designed to treat high blood pressure and tuberculosis, respectively, had side effects on one’s affect (West and Dally 1959). Combined with the knowledge that these mood altering drugs also impact monoamine neurotransmitters, the monoamine hypothesis of affective disorders was developed (Schildkraut 1965). Many classes of antidepressant drugs target these neurotransmitter systems, but the long onset of action (weeks to months) suggests that downstream mechanisms, rather than acute changes in neurotransmitters, regulate depressed mood. Moreover, 10–30% of patients are resistant to traditional therapies (Al-Harbi 2012; Joffe et al. 1996), indicating the heterogeneous nature of the disease. As such, research is focused on identifying novel causative mechanisms that can be used as therapeutic targets to treat depressive disorders.

Surprisingly, even before the formation of the monoamine hypothesis, it was initially noted that activation of the immune system could regulate psychiatric functioning when Julius Wagner-Jauregg won the Nobel Prize for this pioneering observation in 1927. Nearly 60 years later, the link between inflammatory cytokines and depression was first reported (Maes et al. 1990). More recently, studies have demonstrated that certain subpopulations of depressed patients exhibit greater levels of inflammatory cytokines such as C reactive protein (CRP) and interleukin (IL)-6 in the plasma (Irwin and Miller 2007) and cerebrospinal fluid (Devorak et al. 2015; Sasayama et al. 2013). In fact, various patient populations that are resistant to traditional antidepressants exhibit increased inflammatory cytokines (Strawbridge et al. 2019). Importantly, clinical studies have utilized chronic administration of the cytokines IL-2 and interferon (INF)-α for use as chemotherapeutics and identified that these treatments induced depression in a large number of patients (Denicoff et al. 1987; Renault et al. 1987). Furthermore, findings from animal models also support inflammation’s causal role in depression. Immune system activation stimulated by injection of pro-inflammatory cytokines, lipopolysaccharide (LPS) injection, or inducing a bacterial infection results in a profound neuroinflammatory response accompanied by depressive-like behavior such as anhedonia or behavioral despair (Ji et al. 2014; Johnson et al. 2005; Kaster et al. 2012; O’Connor et al. 2003; Zhang et al. 2014; Zhu et al. 2015). Taken together, inflammation has emerged as a major target to investigate for its role in the pathogenesis of stress-related disorders such as depression.

In addition to inflammation as a target for depression, recent evidence has expanded the concept of pathogenesis of depression even further. Specifically, accumulation of reactive oxygen species (ROS) or oxidative stress and a reduction in ATP levels are now recognized as potential drivers in depression symptomatology.
While ATP dysregulation was only recently recognized for its role in depression, this should not be particularly surprising given that the brain has high energy demands and as a result is considerably vulnerable to impaired ATP production (Halliwell 1992). Mitochondria play a critical role in cellular energy metabolism and supply the large energy demand required by the brain, especially under stressful conditions and there is mounting evidence that patients with psychiatric disorders demonstrate mitochondrial abnormalities at the functional level and vice versa.

1.2 Social Stress as a Causative Factor in Depression

Despite heroic efforts for nearly a century to understand the root cause of depression, rather than definitive mechanisms known to cause depression in humans, such as is known for other medical disorders, our knowledge is largely limited to a list of factors that increase the risk of developing depression. This is likely a result of the heterogeneous nature of depression, where many different experiences and exposures can induce a depressive phenotype. The World Health Organization identifies that depression can be precipitated by substance use disorders, medications, illnesses, or genetics, and research in animals and evidence in humans also identifies that chronic exposure to stressful experiences, often of a social nature, remains a significant risk factor for the development of major depression (Krishnan and Nestler 2008). In fact, social stressors are the most common form of stress humans experience and include a wide range of stressors stemming from one’s social environment, and exposure to psychosocial stressors such as loss of a loved one, bullying or psychosocial abuse, is one of the most robust predictors of depression onset in humans (Almeida 2005; Miller and Raison 2016). Not surprisingly, victims of bullying and psychosocial abuse are at increased risk for depressive illness (Comencanha et al. 2017; Kaltiala-Heino et al. 2010; Tiwari et al. 2008; Wielaaard et al. 2018).

Experimental animal models exquisitely exploit social stress as a vulnerability factor to reliably reproduce an anhedonic or depressive-like state. It is from these preclinical studies whereby major advances have been made in understanding the role of inflammation and mitochondria in the development of stress-induced depressive-like phenotypes (Table 1). One of the most common forms of social stress used in rats and mice is social defeat (SD). Originally developed by Miczek and Koolhaas, the resident-intruder paradigm capitalizes on the natural social hierarchy of rodents (Koolhaas et al. 1997, Koolhaas et al. 1980; Miczek 1979; Miczek et al. 1982). Since its original description, many labs have adopted some variation of this model to study social stress in which a naïve male “intruder” is placed into the home cage of a more aggressive male “resident” for a specified amount of time, typically 5–60 min (Bhatnagar and Vining 2003; Sgoifo et al. 1996; Wood et al. 2010). This is usually repeated for several days to weeks. This repeated exposure to an acute bout of social stress has been shown to consistently produce sympathetic nervous system (SNS)
<table>
<thead>
<tr>
<th>Model</th>
<th>Stress duration</th>
<th>Behavioral alterations</th>
<th>Biochemical alterations</th>
<th>Treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD, mice</td>
<td>14 days (continuous; 5 min/day physical interaction with 24 h/SC)</td>
<td>↑ Anxiety in LD, ↓ Social interaction</td>
<td>↑ ROS in NAcc, PFC, PVN, HIPP</td>
<td>NAC and PLX5622 lowered ROS and attenuated behavioral effects</td>
<td>Lehmann et al. (2019)</td>
</tr>
<tr>
<td>SD, mice</td>
<td>10 days (continuous; 10 min/day physical interaction with 24 h/SC)</td>
<td>↑ Anxiety in EPM, ↑ Social avoidance</td>
<td>↑ TSPO, ↑ ROS, ↑ Pro-inflammatory cytokines</td>
<td>ONO-2952 suppressed cytokine expression and ROS production, and attenuated behavioral effects</td>
<td>Nozaki et al. (2020)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>7 days (continuous; physical interaction until submission or 10 min followed by sensory exposure for remaining 30 min)</td>
<td>↑ Anxiety in EPM and LD, ↓ SP, ↑ Memory impairment</td>
<td>↑ Oxidative stress, ↑ ERK1/2 and IL-6, ↓ Antioxidant proteins, ↓ CAMK-IV, CREB, and BDNF</td>
<td>N/A</td>
<td>Patki et al. (2013)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>5 days (continuous; physical interaction until submission or 15 min followed by sensory exposure for remaining 30 min)</td>
<td>↓ SP in passive coping rats</td>
<td>↑ IL-1β, TNF-α, GM-CSF in passive coping rats</td>
<td>RSV reduced neuroinflammation and attenuated behavioral effects</td>
<td>Finnell et al. (2017a, b)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>7 days (continuous; physical interaction until submission or 10 min followed by sensory exposure for remaining 30 min)</td>
<td>↑ Anxiety in EPM, LD, OF, and MB, ↑ FST immobility, ↑ Memory impairment</td>
<td>↑ Oxidative stress, ↓ Antioxidants</td>
<td>Grape powder (quercetin and RSV) both reversed and prevented behavioral effects, and normalized oxidative stress markers</td>
<td>Solanki et al. (2017)</td>
</tr>
<tr>
<td>SD, mice</td>
<td>10 days (continuous; 5 min/day physical interaction with 24 h/SC)</td>
<td>↓ SP, ↑ FST immobility, ↓ Social interaction</td>
<td>↑ IL-1β, IL-6, TNF-α, ↑ ROS, ↑ NLRP3, ↑ ASC, Caspase-1</td>
<td>Allicin attenuated neuroinflammation, oxidative stress, and behavioral effects</td>
<td>Gao et al. (2019)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>2 h</td>
<td>N/A</td>
<td>↑ T cell count, ↓ B cell count</td>
<td>N/A</td>
<td>Stefanaki and (continued)</td>
</tr>
<tr>
<td>Model</td>
<td>Stress duration</td>
<td>Behavioral alterations</td>
<td>Biochemical alterations</td>
<td>Treatment</td>
<td>Reference</td>
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<tr>
<td>SD, mice</td>
<td>10 days (continuous; 5 min physical interaction with 24 h/SC)</td>
<td>↓ SP and in susceptible rats</td>
<td>↑ IL-1β, TNF-α, iNOS</td>
<td>N/A</td>
<td>Engler (1998)</td>
</tr>
<tr>
<td>Social disruption, mice</td>
<td>6 days (continuous; 2 h/day)</td>
<td>↑ anxiety in LD</td>
<td>↑ CD14, CD86, and TLR4 on microglia</td>
<td>Propranolol reduced anxiety-like behavior and stress-induced shifts in microglial activation</td>
<td>Tang et al. (2018)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>5 days (continuous; physical interaction until submission or 15 min followed by sensory exposure for remainder of 30 min) + SD challenge prior to sacrifice</td>
<td>N/A</td>
<td>↑ Plasma IL-6, TNF-α, IFN-γ, CeA TNF-α</td>
<td>DSP-4 reduced stress-induced inflammatory priming</td>
<td>Wohleb et al. (2011)</td>
</tr>
<tr>
<td>WS, mice</td>
<td>10 days (continuous; mouse observed SD for 10 min/day)</td>
<td>↑ social avoidance</td>
<td>↑ IL-6</td>
<td>OVX reduced anhedonia, immobility, burying and inflammatory responses</td>
<td>Hodes et al. (2014)</td>
</tr>
<tr>
<td>WS, rats</td>
<td>5 days (continuous; 15 min/day + WS challenge prior to sacrifice)</td>
<td>↓ SP and in susceptible rats</td>
<td>↑ CeA IL-1β, Plasma IL-1β, TNF-α, and IL-6</td>
<td>O VX reduced anhedonia, immobility, burying and inflammatory responses</td>
<td>Finnell et al. (2018)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>5 days (continuous; physical interaction until submission or 15 min followed by sensory exposure for remainder of 30 min)</td>
<td>↓ SP and in susceptible rats</td>
<td>↑ IL-1RA prevented anhedonia in susceptible rats</td>
<td>IL-1RA prevented anhedonia in susceptible rats</td>
<td>Wood et al. (2015)</td>
</tr>
<tr>
<td>SD, mice</td>
<td>6 days (continuous; 2 h/day)</td>
<td>↓ social interaction</td>
<td>↑ Peripheral IL-1β and microglial reactivity following LPS challenge</td>
<td>Imipramine reversed SD-induced social avoidance and reduced neuroinflammation/microglial reactivity</td>
<td>Ramirez et al. (2015)</td>
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<tr>
<td>SD, mice, hamsters</td>
<td>Mice: 1 day with 3 x 2-min physical interaction for a total of 10 min Hamsters: 1 day with 3 x 5 min physical interaction</td>
<td>↑ Social avoidance in susceptible mice Subordinate social status in susceptible hamsters</td>
<td>↑ NAcc cysteine, ↑ vmPFC inosinic acid, AMP in resilient mice ↑ NAcc fumarate, ↑ vmPFC methionine</td>
<td>N/A</td>
<td>Dulka et al. (2017)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>13 days across 3 weeks (5 min physical interaction followed by 55 min sensory exposure/day)</td>
<td>↓ SP, ↑ immobility in FST</td>
<td>↑ Creatine in PFC of defeated rats; ↓ succinic acid in PFC of defeated rats</td>
<td>N/A</td>
<td>Liu et al. (2017)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>1 day (5 min physical interaction, followed by 55 min sensory exposure)</td>
<td>↓ SP, ↑ immobility in FST</td>
<td>↑ HIPP lactic acid, myo-inositol, N-acetyl-L-aspartic acid</td>
<td>N/A</td>
<td>Liu et al. (2018)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>13 days across 3 weeks (5 min physical interaction followed by 55 min sensory exposure/day)</td>
<td>↓ SP, ↑ immobility in FST in susceptible rats</td>
<td>↑ HIPP lactic acid, GABA, aminooxyacetic acid</td>
<td>N/A</td>
<td>Yang et al. (2019)</td>
</tr>
<tr>
<td>SD, mice</td>
<td>10 days (5 min physical interaction/day)</td>
<td>↑ Social avoidance, ↑ Anxiety in LD</td>
<td>Subordinate defeated mice: ↑ phosphocreatine, total creatine, glutamine, taurine, alanine in NAcc</td>
<td>N/A</td>
<td>Larrieu et al. (2017)</td>
</tr>
<tr>
<td>SD, rats</td>
<td>3 weeks (5 days/week; 1 h physical interaction)</td>
<td>↓ SP</td>
<td>↓ Cytochrome c oxidase activity in HIPP, NAcc, midbrain, hypothalamus, thalamus, especially in high sucrose consuming rats</td>
<td>N/A</td>
<td>Kanarik et al. (2011)</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Model, organism</th>
<th>Stress duration</th>
<th>Behavioral alterations</th>
<th>Biochemical alterations</th>
<th>Treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD, mice</td>
<td>28 days (continuous; 5–10 min physical interaction/day)</td>
<td>↑ Social avoidance, ↓ SP, ↑ immobility in FST and TST</td>
<td>↑ Lipid peroxidation, ↓ superoxide dismutase, catalase activity in HIPP</td>
<td>20(S)-protopanaxadiol (PPD) and imipramine reversed SD-induced social avoidance, altered SP, and immobility in FST but not TST; both reversed SD-induced decreases in superoxide and catalase activities. PPD only reversed SD-induced lipid peroxidation</td>
<td>Jiang et al. (2019)</td>
</tr>
<tr>
<td>SD, mice</td>
<td>2 days (continuous; 6 h physical interaction/day)</td>
<td>↑ Anxiety in EPM; Mutation-specific ↓ in 5-HT</td>
<td>N/A</td>
<td>N/A</td>
<td>Gimsa et al. (2009)</td>
</tr>
<tr>
<td>SD, mice</td>
<td>10 days (continuous; 10 min physical interaction followed by 24 h/SC)</td>
<td>↑ Social avoidance, ↑ anxiety in OF, ↑ immobility in TST, ↓ social preference</td>
<td>↑ NAD, NADPH, ↓ citrate and isocitrate in PFC; ↑ succinate, ↓ D-glucose-6-phosphate, oxaloacetate in HIPP; ↓ citrate synthase gene expression in PFC</td>
<td>Dl-3-n-butylphthalide (NBP) reversed SD-induced behavioral alterations; restored levels of HIPP succinate and D-glucose-6-phosphate; did not restore levels in PFC</td>
<td>Wang et al. (2020)</td>
</tr>
</tbody>
</table>

Model type and organism used are listed, as well as duration of stressor, behavioral and biochemical alterations resulting from stress, and treatment used if applicable. 24 h/SC 24-h sensory contact, ASCA Apoptosis-associated Speck-like protein containing a CARD (caspase activation and recruitment domain), BDNF brain-derived neurotrophic factor, CAMK-IV calcium/calmodulin-dependent protein kinase type IV, CORT corticosterone, CREB cAMP response element-binding protein, EPM elevated plus maze, ERK 1/2 extracellular signal-regulated protein kinase, FST forced swim test, GM-CSF granulocyte-macrophage colony-stimulating factor, LD light/dark box, MB marble burying, NAC N-acetylcycteine, OF open field test, ROS reactive oxygen species, RSV resveratrol, SD social defeat, TNF-α tumor necrosis factor alpha, TSPO translocator protein 18 kDa, iNOS inducible nitric oxide synthase, PFC prefrontal cortex, HIPP hippocampus, IL interleukin, SP sucrose preference, WS witness stress, OVX ovariectomy, MCP-1 monocyte chemoattractant protein-1, NAcc nucleus accumbens, PVN paraventricular nucleus

"Indicates females were used in the study
activation, enhanced activation of the HPA, increased levels of pro-inflammatory cytokines, and anhedonia, among disruption of other physiological and behavioral endpoints which are relevant to stress-related depression. More recently, another variation of this model has been established in which either a male or female “witness” is placed behind a clear, perforated partition in a protected region of the resident’s home cage and subjected witnessing the SD encounter between the resident and intruder on the other side of the partition. This witness stress model consistently produces effects similar to those of SD with respect to the SNS, HPA axis, and the inflammatory response in both male and female rats and mice (Finnell et al. 2017b, 2018; Hodes et al. 2014; Iniguez et al. 2018; Patki et al. 2014; Warren et al. 2013). While this chapter will focus on studies using SD or witness stress to elicit depressive-like phenotypes, there are several other useful preclinical models of social stress that should be mentioned (see Box 1 for examples).

Box 1
Rodent models of social isolation stress offer another relevant social stress model (Grippo et al. 2007a, b; Holt-Lunstad et al. 2015; Oh et al. 2019; Steptoe et al. 2004; Wallace et al. 2009). Alternatively, rodents can be housed in hierarchical colonies in which the struggle for dominance creates a chronic, continuous stressful environment for both the dominant and the subordinate animals (Blanchard et al. 1995; Herman and Tamashiro 2017; Larrieu et al. 2017; Melhorn et al. 2017). Related, social instability is yet another model of social stress in which experimental animals are exposed to unstable, ever-changing hierarchies which produce persistent behavioral and neuroendocrine effects (Bartolomucci et al. 2004; Haller et al. 1999; Schmidt et al. 2007; Sterlemann et al. 2008).

Taken together, controlled experiments using social stressors in rodents have proven effective in modeling aspects of depressive-like behavior in a manner that has high face, predictive and construct validity as is discussed throughout this chapter. Major advances in understanding the pathophysiology of depression have pointed toward a link between depressive symptomatology and inflammation or mitochondrial dysfunction. This chapter will focus on SD/witness stress studies that uncover important findings on these burgeoning areas of research with a focus on understanding the historical and current state of knowledge on how these systems become dysregulated and what functional consequences this could pose in the pathogenesis of depression. We will explore the intricate interplay between mitochondrial function and inflammation to highlight potential areas that warrant further study with the overarching goal to indicate putative biomarkers to better target development of antidepressant drugs.
2 Evidence of Social Stress-Induced Inflammatory (Mal)adaptations

Stressors, particularly of a social nature, are well recognized for their ability to impact the immune system. Though a dysregulated neuroinflammatory response is harmful, transient neuroinflammation induced by bouts of social stress is not inherently detrimental, but adaptive in the acute sense. Just 2 h of resident-intruder confrontation resulted in increased T helper cells and decreased B cells, facilitating an increased T/B cell ratio (Stefanski and Engler 1998). Importantly, this activation of the adaptive immune system, specifically cytotoxic CD8+ T cells, is necessary for recovery from immune-induced depressive-like responses (Laumet et al. 2018). Studies identified that depressive-like behaviors were associated with accumulation of Th1 and Th17 cells in the brains of SD mice and further, direct administration of Th17 produced depressive-like behaviors in this study (Beurel et al. 2013). SD studies in rats have also demonstrated dysfunctional changes in the adaptive immune system whereby 5 daily exposures to SD stress rendered passive coping susceptible rats with a sensitized response to concanavalin A stimulation; more CD4+ splenocytes expressed pro-inflammatory cytokines among susceptible rats that developed the depressive phenotype as compared with controls or active coping resilient rats (Finnell et al. 2017b). Additional studies in susceptible mice identified that repeated SD stress resulted in increased proportions of pro-inflammatory Th17 cells and suppression of peripheral regulatory T (Treg) cells which may regulate CNS function and contribute to stress-induced inflammation within the brain, ultimately promoting depressive-like behaviors (Ambree et al. 2019). These peripheral T cells can enter the CNS through leaky regions of the BBB like the choroid plexus (Lewitus et al. 2008; Ransohoff et al. 2003). In support of this, depletion of Treg cells prior to stress exposure resulted in more depressive-like behavior compared with untreated mice (Kim et al. 2012). Further, the transfer of T cells from mice resistant to the depressive-like effects of chronic SD stress to naïve mice had antidepressant-like effects (Miller and Raison 2016), also suggesting that resilience or susceptibility factors may be encoded within Treg cells.

Components of the innate immune system are also activated during stress and are found to interact with several stress-sensitive systems. For example, SD stress leads to an increase in splenic leukocytes and glucocorticoid sensitivity of the immune cells (Engler et al. 2004) and inflammatory cytokines such as IL-6 and TNF-α activate the HPA axis, allowing an individual to adapt to stressful stimuli and optimize survival (Turnbull and Rivier 1995). Under healthy conditions, HPA axis activation leads to cortisol release which subsequently serves to initiate negative feedback on the inflammatory system to prevent a chronic, sustained pro-inflammatory state. Taken together, these findings demonstrate how acute activation of the immune system under stressful circumstances helps regulate the physiological systems necessary to promote survival in the context of a social threat. The point at which these systems become hyperactivated or do not return to baseline...
following cessation of the stressor is our current best understanding of how adaptive processes become maladaptive, as we will discuss throughout this chapter.

### 2.1 Social Stress-Induced Sensitization of Microglia

As the resident immune cells in the brain, microglia play a critical role in the inflammatory response to stress. Generally, upon activation, microglia can be polarized toward two distinct states, either the classical M1 pro-inflammatory phenotype or the alternative M2 anti-inflammatory phenotype (Franco and Fernandez-Suarez 2015). In response to chronic SD stress, there is a shift such that more microglia exhibit the pro-inflammatory M1 phenotype (Tang et al. 2018). This stress-induced shift of microglia toward the M1 state may be facilitated by stress-induced increases in corticosterone given that activation of microglial glucocorticoid receptors stimulates a shift toward the pro-inflammatory state (Barrientos et al. 2015; Finnell and Wood 2018; Ros-Bernal et al. 2011). Further, while a single stress exposure may produce a transient neuroinflammatory response, repeated exposure to social stress results in neuroinflammatory priming, which is largely mediated by microglial sensitization (Frank et al. 2016). For example, the microglia isolated from mice subjected to repeated SD stress exhibited a greater inflammatory response compared with controls, demonstrating that social stress exposure primes the microglial cells such that a subsequent challenge produces an exaggerated inflammatory response (Wohleb et al. 2011). This idea of neuroinflammatory priming is supported in a recent study of repeated SD stress in rats in which only rats with a history of repeated social stress exhibited greater neuroinflammation in the central amygdala compared with that of rats with a history of control (Finnell et al. 2019).

While the exact triggers initiating this neuroinflammatory priming is not clear, the DAMP molecule high mobility group box 1 (HMGB-1) is a key player in this signaling cascade. HMGB-1 is released from microglia and other cell types to promote expression of pro-inflammatory cytokines via activation of the nucleotide-binding oligomerization domain-like receptor (NLRP3) inflammasome (Weber et al. 2015). While the exact mechanisms by which NLRP3 is activated are incredibly complex and not fully understood (Kelley et al. 2019), it is understood that, in a two-step process of priming and activation, NLRP3 inflammasome assembly and activation promotes the synthesis of inflammasome-dependent cytokines (Fleshner et al. 2017; Sutterwala et al. 2014). This complex, considered the NLRP3 inflammasome, cleaves procaspase-1, where mature activated caspase-1 can cleave pro-IL-1β into mature and active IL-1β and IL-18 pro-inflammatory cytokines (Fleshner et al. 2017; Lopez-Armada et al. 2013).

The complex, dual nature of this process may be credited to prevent accidental or uncontrollable hyperactivity of the inflammasome, allowing activation only in the presence of proinflammatory signals (DAMPs or PAMPs) (Bauernfeind et al. 2009). However, several preclinical models of stress have demonstrated that stress-induced increases in HMGB1 are associated with sensitized release of inflammatory...
cytokines such as TNF-α, IL-6, IL-1β, and IFN-γ which further correlate with depressive-like behaviors such as reduced sucrose preference and increased immobility in the forced swim and tail suspension tests (Franklin et al. 2018; Fu et al. 2019; Lian et al. 2017; Liu et al. 2019; Weber et al. 2015; Zhang et al. 2019). Some of these studies went on to show that antidepressant treatments inhibited neuroinflammation through HMGB1-mediated pathways (Fu et al. 2019; Liu et al. 2019). While findings strongly suggest HMGB1 as a key mediator for stress-induced neuroinflammation and depressive-like behavior (as reviewed in (Frank et al. 2015, 2016)), additional studies examining the role for HMGB1-mediated neuroinflammatory priming, specifically in stressors of a social nature, are still needed.

2.2 Social Stress-Induced Breakdown of the Blood Brain Barrier (BBB)

In addition to immune cells within the brain contributing to stress-induced neuroinflammation, cytokines circulating in the periphery also promote inflammation in the brain in the context of social stress. The effect of stress on BBB permeability has been studied most extensively using SD stress. Repeated SD stress exposure produces persistent increases in both central and peripheral cytokines, and many studies associate these findings with the development of a depressive-like phenotype (Finnell et al. 2017a, 2018, 2019; Hodes et al. 2014; Patki et al. 2013; Ramirez et al. 2015; Wood et al. 2015). Interestingly, rats that are susceptible to the depressive-like effects of SD exhibit increased inflammation and increased BBB permeability compared with resilient rats that do not develop depressive-like behaviors following repeated SD stress (Pearson-Leary et al. 2017). Moreover, IL-1β accumulation within the brain causes BBB leakage, and results in infiltration of many different immune cell types including macrophages, neutrophils, T cells, and dendritic cells (Shaftel et al. 2007). Thus, the inflammatory effects of social stress may promote BBB permeability, thereby further enhancing neuroinflammation and facilitating the development of depressive-like behaviors. Social stress has also been shown to disrupt the BBB in a mouse model of SD as indicated by stress-induced reductions in claudin-5, a tight junction protein specific to endothelial cells in the nucleus accumbens (NAcc) and hippocampus and increased BBB permeability (Menard et al. 2017). Moreover, in a chronic SD model in male mice, the BBB is compromised in susceptible mice, but not resilient or control mice and BBB disruption in this model is associated with enhanced inflammation and depressive-like behaviors (Lehmann et al. 2018). Additionally, SD stress results in reduced levels of neuronal cAMP in the NAcc, further contributing to BBB permeability and exacerbating behavioral susceptibility to stress (Zhang et al. 2020).

While inflammation itself may lead to a leaky BBB, there are other mechanisms by which social stress may alter BBB function. For example, as mentioned above,
HMGB-1 is upregulated by SD stress and plays an important role in BBB dysfunction as anti-HMGB-1 monoclonal antibodies protect against BBB dysfunction in both humans and rats (Festoff et al. 2016; Zhang et al. 2011). Furthermore, HMGB-1 may affect the BBB by several mechanisms including upregulation of the zymogen matrix metalloproteinase-9 (MMP-9) from microglia and infiltrating monocytes to contribute to endothelial damage (Crocker et al. 2006; Qiu et al. 2010), causing a leaky BBB (Seo et al. 2013). In fact, MMP-9 protein expression is elevated in response to social defeat stress (Stelzhammer et al. 2015; Wu et al. 2015), supporting the possibility that this may function to contribute to SD-induced BBB disruption. In all, any breakdown of the BBB can enhance transfer of inflammatory molecules from the periphery to the brain and may play a prominent role in the accumulation of SD-induced neuroinflammation.

2.3 Evidence of Maladaptive Accumulation of Neuroinflammation as a Result of Chronic Stress

The conditions under which neuroinflammation renders a maladaptive response is unclear, but chronic rather than acute social stress exposure may be required for these persistent alterations in neuroinflammation. For example, acute exposure to social stress was not effective in producing lasting peripheral or central inflammation (Hueston et al. 2011). However, history of a single acute exposure to social stress can promote enhanced neuroinflammation in response to a subsequent stress exposure (Audet et al. 2011). Importantly, studies that investigate neuromodulations in susceptible versus resilient populations of rodents in the context of social stress have shed light on the permissive role that neuroinflammation plays in susceptibility to social stress. For example, rats characterized as susceptible based on developing depressive-like and anxiety-like behaviors exhibit greater IL-1β expression within stress-sensitive brain regions such as the locus coeruleus (LC) in male rats (Wood et al. 2015), the major noradrenergic nucleus within the brain associated with depression (Ressler and Nemeroff 2000; Serafini 2012; Valentino and Aston-Jones 1995). This increased inflammation was evident as early as 24 h after the final stress and persisted for at least 6 days selectively in the passive coping susceptible cohort (Finnell et al. 2017a; Wood et al. 2015). Other studies have identified that cytokine expression is greater in the striatum and hippocampus of male mice that demonstrate behavioral susceptibility to repeated SD stress when measured 4 days after the final SD, while this effect is lacking in the resilient cohort (Ballestin et al. 2021). Moreover, using the traditional SD paradigm of 10 daily exposures of defeat in mice (Golden et al. 2011), persistent expression of translocator protein 18 kDa (TSPO), a mitochondrial protein expressed in microglia that signals their activation, was persistently upregulated for over a week after the final SD stress in microglia harvested from the basolateral amygdala, lateral habenula, and ventral hippocampus (Nozaki et al. 2020).
Many brain regions in susceptible rodents do respond to SD by increasing neuroinflammation, however these alterations are region specific and thus not exhibited uniformly throughout the brain (see Fig. 1). For example, Nozaki et al. 2020 found elevated TNF-α in the ventral hippocampus of susceptible mice, yet this effect was absent in the dorsal hippocampus. Interestingly, certain stress-sensitive brain regions respond to SD with an anti-inflammatory response. For example, within the dorsal raphe (DR), a major serotonergic center of the brain, cytokine expression was not elevated in susceptible rats; rather, resilient rats exhibited a decrease in DR IL-1β (Finnell et al. 2017a; Wood et al. 2015). Furthermore, elevated cytokines are not always specific to susceptible cohorts; elevated IL-1β mRNA was reported in the hypothalamus of resilient mice after two stress exposures (De Miguel et al. 2011). While seemingly contradictory, these studies highlight the fact that SD stress produces region specific alterations in neuroinflammation.

The inflammatory system is highly complex and while cytokine expression or microglial number can provide a glimpse into the current inflammatory response of a particular brain region, there are plethora factors that can alter the inflammatory potential in a functional manner within the brain. In the LC, for example, where susceptible rats exhibited exaggerated IL-1β mRNA expression and resilient rats exhibit decreased IL-1β and TNF-α, SD was also found to alter the expression of several other immune regulating genes. Genes encoding for receptors of interleukin, beta-adrenergics, and neuropeptide Y, as well as cytochrome P450s, matrix

![Fig. 1 Neurobiological alterations in inflammation and metabolism linked to social defeat stress. Alterations in inflammatory and metabolic markers observed following rodent social defeat reported in the literature are summarized. Open questions regarding the influence of social defeat stress on brain mitochondrial function, the interactions between mitochondrial function and inflammation, as well as effects on cell-specific metabolism are highlighted. PFC prefrontal cortex, NAcc nucleus accumbens, Hipp hippocampus, BLA basolateral amygdala, Htl hypothalamus, DR dorsal raphe, LC locus coeruleus, IL interleukin, TNF tumor necrosis factor, ROS reactive oxygen species, M0 resting state microglia, M1 pro-inflammatory microglial phenotype. Figure was made using Servier Medical Art](image-url)
metallopeptidases, and cellular adhesion molecules were altered by defeat, and in many cases were unique to susceptible rats (Wood et al. 2015). Of considerable interest in the DR, inducible nitric oxide synthase (iNOS), an inflammatory signaling molecule was suppressed more than sixfold in resilient rats (vs. controls) while susceptible rats demonstrated a modest 1.8-fold reduction. This dramatic decrease in iNOS found in resilient rats may serve as a protective mechanism engaged during active coping (Wong et al. 1996) because suppression of iNOS has been shown to promote the M2 (anti-inflammatory) type microglia (Franco and Fernandez-Suarez 2015). While significant changes in gene expression patterns exist when comparing the adaptations that occur in susceptible vs. resilient populations, there are also some commonalities between these groups that may exhibit protective effects. For example, increased expression of the IL-1 receptor type 2 (IL-1r2), a decoy receptor, emerged in the LC (McMahan et al. 1991; Wood et al. 2015) and increased expression of suppressor of cytokine signaling 3 in the ventral tegmental area was identified in both susceptible and resilient groups (Krishnan et al. 2007).

While changes in inflammatory-related molecules are well defined in many brain areas, efforts are focused on identifying the impact that inflammatory cytokines may have on neurons within stress-sensitive brain regions. It has been shown that IL-1β promotes increased firing of noradrenergic cells of the LC (Borsody and Weiss 2002a), a response that is critical for attending to stressful stimuli. Because transient LC activation is a critical adaptive response to stress (Valentino and Van Bockstaele 2008), this is considered yet another way that acute stress-elicited inflammation is a capable of promoting a healthy stress response. Moreover, pro-inflammatory cytokines such as IL-1β may increase norepinephrine (NE) levels in stress-sensitive brain regions such as the amygdala, prefrontal cortex, and LC (Lacosta et al. 2000; Sirivelu et al. 2012). In some cases, social stress has also been shown to increase brain-derived neurotrophic factor (BDNF) in the hippocampus (Duclot and Kabbaj 2013), thus facilitating learning and memory, which may be regulated by cytokines, yet when these cytokines become dysregulated depressive-like behaviors ensue. For example, the presence of elevated cytokines either in the brain or periphery in a social stress-exposed individual will promote overactivation of LC-NE neurons (Borsody and Weiss 2002a, b). Exaggerated LC activity is linked to both depression and anxiety (Weiss et al. 1994) and thus an elevated cytokine milieu could serve as the conduit between social stress and depression. Beyond the LC-NE system, many other neurobiological systems are regulated by cytokines, including serotonin, corticotropin releasing factor, and BDNF. The regulation of these systems by stress-induced cytokines is reviewed in Finnell and Wood (2016) and serves as likely targets through which persistent inflammation promotes behavioral dysfunction.
2.4 Benefits of Anti-inflammatory Treatments in the Face of Chronic Social Stress

Because SD elicits potentially adaptive immune responses in the acute sense, it is challenging to pinpoint a specific transitional period where the adaptive becomes deleterious. However, in addition to the strong association between increased neuroinflammation and SD susceptibility, what has also been clearly defined is that development of depressive-like or anxiety-like behaviors induced by repeated SD can be blocked or reversed by inhibiting the inflammatory system by various methods. For example, administration of resveratrol, a natural anti-inflammatory agent, blocks the development of anhedonia in male rats exposed to SD, but only at doses that are high enough to inhibit neuroinflammation in the LC (Finnell et al. 2017a). More specifically, inhibiting activation of the IL-1 receptor in the brain (ICV infusion of IL-1ra) blocks the development of SD-induced anhedonic behavior in rats (Wood et al. 2015). Furthermore, studies have also utilized IL-6 knockout mice and administration of IL-6 neutralizing antibodies to temper SD-induced inflammatory responses, also concluding that susceptibility to SD is linked to activation of the immune system (Hodes et al. 2014).

Rather than blocking actions of specific cytokines, studies have also proven the importance of the immune system in social stress-induced behavioral dysfunction by depleting microglial expression. Studies in the stress-sensitive BALB/c mice revealed that social defeat-induced shifts in behavior could be inhibited by minocycline treatment, a robust inhibitor of microglial activation, therefore linking microglia to depressive-like behaviors in mice (Ito et al. 2020). Further, using this same method of microglial antagonism, Weber and colleagues demonstrated that elimination of microglia in sensitized rats prevented accumulation of monocytes and stress-induced behavioral deficits following subsequent acute stress (Weber et al. 2019). Utilizing yet another pharmacological method to suppress microglial activation, a TSPO antagonist also inhibited the development of the behavioral phenotype that characterizes socially defeated susceptible mice as measured by the elevated plus maze and social avoidance (Nozaki et al. 2020). Notably in mice, depletion of microglia via colony-stimulating factor 1 receptor antagonist PLX5622 before and during 2 weeks of chronic SD protected against the behavioral deficits induced by SD stress as determined in the light/dark box and social interaction test (Lehmann et al. 2019). In all, exaggerated neuroinflammation in stress susceptible subsets of rodents, combined with the evidence that inhibiting or reversing these neuroinflammatory processes is stress protective provides significant support for the conclusion that accumulation of neuroinflammation in response to repeated social stress is a likely regulatory factor in the pathogenesis of stress-induced depression.
3 The Mitochondrion and Its Involvement in Social Defeat

3.1 Mitochondrial Function at Rest and Under Stress

Mitochondria are dynamic subcellular organelles that are intricately linked to numerous essential cellular and physiological processes, including energy production. The brain is particularly energy demanding, commanding a disproportionate 20% of the body’s energy supply while comprising just 2% of the body (Attwell and Laughlin 2001; Mink et al. 1981). Energy supply is fundamental in the brain, with neurons and glia requiring precise mitochondrial function and distribution. In neurons, mitochondria shoulder the burden of energy production in the form of adenine triphosphate (ATP) to support processes such as neurogenesis, neurotransmission, and synaptic plasticity (Kann and Kovács 2007; Rangaraju et al. 2014). To meet this demand, mitochondria dynamically travel along axons to areas of high demand (reviewed in (Sheng 2017)) where ATP is generated from oxygen and glucose through a cascade of processes beginning with glycolysis, continuing with electron transfer through the electron transport chain (Gray and Winkler 1996), and culminating in the generation of an electrochemical gradient (Mitchell 1961) that provides the potential energy to catalyze the synthesis of ATP from ADP and inorganic phosphate in a process known as oxidative phosphorylation (OXPHOS; (Abrahams et al. 1994).

Stress, including complex behaviors that occur in social interactions, sharply increases energy demand in the brain (Bryan 1990) by setting off acute and long-term changes in cellular composition, structure, and function that underlie behavioral and physiological adaptations according to the available energy supply (Picard et al. 2014). To match the increases in energy demand, a number of physiological processes, including the secretion of glucocorticoids and catecholamines, lead to a mobilization of resources that can be metabolized by mitochondria via the citric acid or TCA cycle (reviewed in (Picard et al. 2018)). Under chronic conditions, this energy demand can lead to allostatic load and maladaptive perturbations that eventually translate to pathologies such as depression (Morava and Kozicz 2013).

3.2 Genetic Evidence for Impact of Social Defeat on Mitochondria

Recent advances in analytical technology have allowed for the study of genes, proteins, and metabolites, in what are more commonly referred to as “omics studies.” In the search for shared biological pathways that are relevant to social stress-induced depression, omics-based approaches are powerful tools to allow unguided identification of the most significant biological pathways associated with particular social stress exposures. At the transcriptomic level, we have benefitted from major advances in next generation RNA sequencing (RNA-seq) technology and accompanying biostatistical tools, including weighted gene correlation network analysis.
Initial studies of mice exposed to chronic SD resulted in little discussion of metabolic changes, though a few mild alterations in mitochondrial gene expression in the ventral tegmental area and NAcc are noted in supplementary tables (Berton et al. 2006; Krishnan et al. 2007). This is unsurprising given that the focus at the time was identifying large expression changes in individual candidate genes such as \( \text{Bdnf} \) and epigenetic enzymes such as \( \text{Hdac5} \). With the emphasis on pathway and pattern analysis, however, recent studies using RNA-seq technologies have highlighted a number of metabolic changes at the transcriptional level following SD, particularly in OXPHOS and TCA cycle genes in several different brain regions (see Table 2: Babenko et al. 2018; McCann et al. 2019; Nasca et al. 2019; Smagin et al. 2016), suggesting that metabolic dysfunctions may generalize to other social stressors in a manner relevant to human depression. Single-cell RNA-seq experiments, while still in their infancy and currently lacking in SD studies, have the potential to improve our cellular resolution and allowed us to dissect heterogenic brain regions to gain specificity for some of these metabolic changes that are occurring.

Importantly, the findings of these transcriptional studies are bolstered by independent evidence of mitochondrial dysfunction following SD exposure at the protein level. Advances in separation techniques such as liquid and gas chromatography
combined with the advances in mass spectroscopy have improved sensitivity, selectivity, and throughput (Shih 2019) of proteomic and metabolomic studies. In particular, the metabolome is often considered to be closest to the phenotype and more predictive of phenotypic changes induced by external stimuli, such as social stress (reviewed in Humer et al. (2020)). Mice and hamsters that were resilient to the effects of SD had higher levels of small molecules that modulate oxidative stress and higher cellular energy consumption (Dulka et al. 2017). Notably, dominant hamsters had higher levels of fumarate in the NAcc than subordinates, suggesting enhanced ATP levels via increased Kreb cycle intermediates (Dulka et al. 2017). Metabolomic analysis of socially defeated rats that exhibit depressive-like behavior found increased levels of lactic acid and other indicators of enhanced glycolysis and mitochondrial dysfunction (Liu et al. 2018), as well as altered lipid, carbohydrate, and amino acid metabolism in the hippocampus (Yang et al. 2019) and decreased prefrontal cortex levels of succinic acid, a key metabolite in the tricarboxylic acid cycle (Liu et al. 2017). Though it should be noted that a recent, separate study of chronic defeated mice found a shift away from glycolysis toward carbon consumption in the tricarboxylic acid (TCA) cycle in the hippocampus and a decrease in TCA cycle intermediates in the prefrontal cortex (Wang et al. 2020). Proton Magnetic Resonance Spectroscopy (1H MRS) studies have similarly highlighted alterations in the metabolism of amino acids, lipids, and neurotransmitters, though notably these findings were evident in the hippocampus, as well as prefrontal cortex and amygdala (Prabhu et al. 2019), and nucleus accumbens (Larrieu et al. 2017). Finally, a recent multi-omics approach combined RNA-seq with proteomic analysis of the bed nucleus of the stria terminalis (BNST) and blood cells of two different strains of mice following chronic SD stress (Misiewicz et al. 2019). Here the authors detected differential expression of mitochondrial-related genes according to the mouse strain and susceptibility to SD stress at both the gene and protein level (Misiewicz et al. 2019). Notably, when compared to gene expression of exposure-induced panic attack human subjects, mitochondrial gene expression was reduced (Misiewicz et al. 2019). Evidence of altered energy metabolism and mitochondrial dysfunction in some of the same OXPHOS pathways have been observed in human depression patients (reviewed in (Zuccoli et al. 2017)), particularly in glutamatergic neurons. Taken together, these findings suggest that SD stress may induce mitochondrial dysfunction at the gene and protein levels that have relevance to human depressive disorders.

3.3 Evidence of Functional Mitochondrial Changes in Stress

With the wealth of evidence pointing to metabolic modifications at the gene and protein levels, it stands to reason that these alterations should have functional consequences. Indeed, early studies did not evaluate mitochondrial changes following SD, but chronic restraint stress inhibits the enzyme activities of mitochondrial OXPHOS complexes in rodent cortex (Madrigal et al. 2001). Separate studies
similarly found decreased OXPHOS enzyme activities following chronic mild stress in the cortex and cerebellum (Rezin et al. 2008), and hippocampus and hypothalamus (Gong et al. 2011). Moreover, chronic mild stress dissipated mitochondrial membrane potential and damaged the mitochondrial structure (Gong et al. 2011). Interestingly, examination of models of mitochondrial dysfunction also exhibits susceptibilities to stress. Mice harboring neuron-specific mutation of Polg1, the gene that encodes the catalytic subunit of the mtDNA polymerase, accumulated mtDNA deletions in the brain (Kasahara et al. 2006). These mice exhibited altered circadian rhythms that resembled symptoms of insomnia that are reported in depression (Kasahara et al. 2006). Furthermore, mice with transplanted mitochondria from a different mouse line also containing an ATPase mutation exhibited enhanced anxiety-like behavior under basal conditions and enhanced response to SD stress (Gimsa et al. 2009). Additionally, genetic manipulation of mitochondrial respiratory subunits in male mice generated distinct body-wide alterations in the response to restraint stress (Picard et al. 2015), suggesting an enhanced susceptibility to depressive-like pathologies. Indeed, in vitro experiments demonstrated that rat brain cells treated with corticosterone exhibited translocation of glucocorticoid receptors into mitochondria and modulation of calcium and oxidation in an inverted U-shape manner (Du et al. 2009). Acute application of corticosterone enhanced mitochondrial calcium and promoted neuroprotective effects, while chronic applications resulted in decreased mitochondrial calcium and membrane potential and enhanced levels of mitochondrial oxidation (Du et al. 2009). This study demonstrated a direct link between the effects of stress and brain mitochondrial function and provided a mechanism of action. More recent studies have identified that individual differences in brain mitochondrial function are associated with high and low anxiety phenotypes (Hollis et al. 2015, 2018) that can render individuals susceptible to chronic stress and subsequent pathologies such as depression (reviewed in Weger and Sandi (2018)). Male rats that exhibited high anxiety-like behavior on the elevated plus maze had lower levels of mitochondrial respiration in the nucleus accumbens (Hollis et al. 2015; van der Kooij et al. 2018) and higher levels in the prefrontal cortex, compared to low-anxious counterparts. Interestingly, the level of mitochondrial function in the nucleus accumbens significantly correlated with the animal’s social status, such that high anxious animals were prone to lose a social competition and become subordinate (Hollis et al. 2015; van der Kooij et al. 2018). Enhancing accumbal mitochondrial function using complex I substrates increased the ability of high anxious animals to become dominant (Hollis et al. 2015). Given the relationship between social status and social defeat, these findings highlight the potential for mitochondrial functional involvement in susceptibility to social defeat.

Unfortunately, there is a dearth of studies investigating functional mitochondrial changes following chronic SD. Based on the evidence from omics studies and the inhibition induced in other chronic stress models, one would speculate that brain mitochondrial respiratory function would be similarly compromised. A study by Kanarik and colleagues support this speculation, as they reported widespread decreases of metabolic activity in the brain, particularly throughout the mesolimbic
dopaminergic circuitry, as measured by cytochrome c oxidase histochemistry following SD (Kanarik et al. 2011). Additionally, as SD can reliably identify susceptible and resilient populations, studies are needed to understand whether individual differences in brain mitochondrial function may underlie these individual responses to SD. ¹H MRS studies suggest that this may be the case. Alterations in metabolites, such as taurine, glutamine, and phosphocreatine in the nucleus accumbens following SD predicted susceptibility to stress and the subsequent development of depressive-like behaviors (Larrieu et al. 2017). Dominant mice subjected to SD were more susceptible to develop depressive-like symptoms that were accompanied by decreased metabolites in the nucleus accumbens (Larrieu et al. 2017). These findings point to the tantalizing possibility that energy shortages in the brain may underlie susceptibility to stress and depression.

### 3.4 Evidence for Involvement of ROS-Related Changes in Social Defeat

Over the last decade, the roles of oxidative stress and ROS have gained attention in stress-related disorders, including depression. Similarly increased oxidative markers have been observed in the brains of rodents exposed to different stress regimens including maternal deprivation (Uysal et al. 2008), social isolation (Serra et al. 2006), and chronic variable stress (Herbet et al. 2017; Rezin et al. 2008). SD has been shown to consistently induce ROS and increase markers of oxidative stress. Male rats exposed to 7 days of SD stress exhibited increased levels of plasma 8-isoprostane (a marker of lipid peroxidation) and hippocampal protein carbonylation (Patki et al. 2013; Solanki et al. 2017). These increased markers of oxidative stress were accompanied by decreased levels of antioxidant enzyme proteins, including glyoxalase1 and glutathione reductase, key antioxidant proteins (Patki et al. 2013; Solanki et al. 2017). A separate study of socially defeated male mice reported significant increases in additional oxidative stress markers such as lipid peroxidation and decreases in antioxidant enzyme activities of superoxide dismutase and catalase (Jiang et al. 2019). While these changes have been reliably observed in the hippocampus, there has been some disagreement with regard to other brain regions such as the amygdala and prefrontal cortex where some studies find no evidence of oxidative stress (Patki et al. 2013), while others noted similar changes in the hippocampus as in amygdala (Solanki et al. 2017), prefrontal cortex (Lehmann et al. 2019; Solanki et al. 2017), as well as nucleus accumbens and hypothalamus (Lehmann et al. 2019). This increase in oxidative stress appears consequential for the behavioral alterations induced by SD as levels of peroxidation and 8-isoprostane significantly correlated with anxiety-like behavior on the elevated plus maze and anhedonic behavior in the sucrose preference test (Patki et al. 2013).
4 Mitochondrial Function as a Link Between Neuroinflammation and Depressive-Like Effects of Social Defeat

So far, we have separately presented evidence for the involvement of neuroinflammation and mitochondrial function in the depressive-like effects of SD. In this section, we will examine the link between mitochondrial involvement in immunity and inflammation under SD stress, and the resulting potential implications for depression. As discussed above, social stress may lead to an inflammatory response that induces depressive-like behaviors. Additionally, mitochondrial function both modulates and is modulated by stress and therefore proposed to have a role in social stress-induced depressive-like behaviors. As numerous reviews have been published describing a comprehensive involvement of mitochondria in innate immunity (Arnoult et al. 2011; Banoth and Cassel 2018; Lopez-Armada et al. 2013; West 2017), here we will discuss only those mechanisms that may be implicated in stress-related depression.

Several studies have elucidated fragments of mitochondrial dysfunction and inflammation in relation to social stress. Notably, the study by Lehmann et al. provides evidence of a possible role for mitochondria in chronic SD stress (Lehmann et al. 2019). Specifically, 14 days of SD in mice led to affective behavioral changes and an increase in ROS production (although this ROS was not explicitly described as mitochondria-derived), which were attributed to microglial activation (Lehmann et al. 2019). When microglia were depleted using PLX5622 during stress, ROS levels remained lower and behavioral changes, measured by light/dark box and social interaction, were not observed after treatment (Lehmann et al. 2019) indicating that microglia act as an essential source of ROS in various brain regions and are required to mediate behavioral changes induced by chronic SD (Lehmann et al. 2019). As mitochondria are a primary source of intracellular ROS, it is tempting to assume their role in these results (Lopez-Armada et al. 2013). Indeed, the findings from Nozaki and colleagues that the TSPO, known to localize to the mitochondrial outer membrane and expressed in activated microglia, is upregulated in limbic brain regions following 10 days of chronic SD would support mitochondrial function as a link between ROS and activated microglia (Nozaki et al. 2020). Chronic SD was also associated with behavioral changes in the elevated plus maze and social avoidance test, corresponding with increased ROS levels (Nozaki et al. 2020). Interestingly, the TSPO antagonist ONO-2952 ameliorated these effects (Nozaki et al. 2020). While no experiments directly concerning mitochondria were performed in this study, it is curious to consider how TSPO mitochondrial localization could be involved with the observed inflammatory response (Nozaki et al. 2020).

Additionally, manipulation of inflammation via antioxidants yielded positive results in SD studies. Grape powder has been proposed to produce anxiolytic and antidepressant effects in stress models, due mainly to its presence of polyphenols such as resveratrol and quercetin, which have anti-inflammatory, antioxidant, and neuroprotective activities (Finnell et al. 2017a; Jardim et al. 2018; Solanki et al. 2017). In their 5-day SD model, Finnell et al. observed that pre-treatment with
resveratrol reduced neuroinflammation and ameliorated depressive-like behaviors in rats (Finnell et al. 2017a). While they attributed neuroinflammatory mechanisms to the observed behavioral changes, the authors also speculated resveratrol’s role in oxidative stress as being an additional possible mechanism (Finnell et al. 2017a). Importantly, a study by Solanki et al. demonstrated the antioxidant effects of grape powder in both preventing and reversing behavioral and biochemical changes induced by 7 days of SD in rats (Solanki et al. 2017). Grape powder administration protected against and reversed SD-induced depressive- and anxiety-like behaviors, short- and long-term memory impairments, and increases in oxidative stress (Solanki et al. 2017). These studies demonstrating inflammatory and oxidative mechanisms in SD-induced behavioral changes could further be linked to mitochondrial involvement, as resveratrol has been shown to modulate mitochondrial function and dynamics (Jardim et al. 2018). Importantly, as few to no studies have directly explored the relationship of mitochondrial-specific-ROS immune interactions and social stress-induced depressive behaviors, research should aim to fill these gaps.

A common link between the mROS and mtDNA in inflammation lies in their roles in NLRP3 inflammasome activation. Importantly, many studies exist indicating NLRP3 involvement in stress-induced depressive behaviors (reviewed in (Kaufmann et al. 2017)). In brief, studies of chronic stress have shown activation of the inflammasome complex, with instances where antidepressants such as fluoxetine can decrease this activation. Further, NLRP3 may act as a peripheral biomarker of depression (Kaufmann et al. 2017). However, research specifically involving SD is sparse. Using a 10-day chronic SD mouse model of depression, Gao et al. provided evidence of antidepressant-like effects of IP-injected allicin (a biologically active compound derived from garlic), possibly through neuroinflammatory mechanisms (Gao et al. 2019). They showed that chronic SD produced depressive-like behaviors (as measured by sucrose preference, forced swim, and social interaction) and significantly increased hippocampal protein levels of NLRP3, ASC, and caspase-1, and IL-1β, all of which were attenuated by allicin (Gao et al. 2019).

Finally, while mitochondria influence the inflammatory response, it is also important to consider the alternative of how inflammation acts to alter mitochondrial function. Pro-inflammatory cytokines are known to induce mitochondrial dysfunction. For example, Doll et al. demonstrated that acute, physiologically relevant administration of the pro-inflammatory cytokine, TNF-α, in both mouse hippocampal neuronal cells (HT-22 cell line) and primary cortical neurons produces rapid mitochondrial dysfunction followed by loss of cell viability (Doll et al. 2015). Specifically, they observed a dose-dependent reduction in basal and maximal mitochondrial respiration and ATP production (Doll et al. 2015). Furthermore, HT-22 cells showed an increase in caspase 8 activation with a correlating decrease in mitochondrial membrane potential, leading to release of cytochrome c by mitochondria (Doll et al. 2015). Similarly, Samavati et al. demonstrated decreased mitochondrial membrane potential and ATP levels in H2.35 murine hepatocytes after administration of TNF-α (Samavati et al. 2008). In addition, they showed that TNF-α inhibited cytochrome c oxidase (Complex IV or CIV) activity in isolated, intact bovine and murine hepatocytes through phosphorylation of CIV subunit I.
Finally, induction of inflammation via LPS injection in adult rats was sufficient to induce significant decreases in mitochondrial respiration in striatal and nigrostriatal brain regions (Hunter et al. 2007). Given the induction of neuroinflammation by SD, these studies suggest the potential for a vicious cycle of inflammation and mitochondrial dysfunction that could lead to depressive pathologies. In fact, patients who demonstrate antidepressant treatment resistance have higher levels of both oxidative stress markers and neuroinflammation compared with individuals who readily respond to antidepressant treatment (Lindqvist et al. 2017; Strawbridge et al. 2015; Vavakova et al. 2015). As no studies to date have directly examined the effects of SD-induced pro-inflammatory cytokine release on brain mitochondrial function, it remains to be seen whether this interplay between cytokines and mitochondrial function is underlying the behavioral effects observed following chronic SD.

5 Future Agenda

Here we have presented evidence for the involvement of both mitochondrial dysfunction and neuroinflammation in the depressive-like behaviors induced by SD. However, a number of open questions remain. Studies so far have highlighted expression changes in metabolic pathways but neglected to examine functional mitochondrial alterations following SD. Measurements of mitochondrial function would be particularly important to understand whether and how these changes might relate to the neuroinflammation observed after SD exposure. It is enticing to consider that the observed results may be attributed to mDAMPS such as mROS or mtDNA, which have been shown to have a role in NLRP3 inflammasome activation. Additionally, cell-type specific studies of mitochondrial function in cells crucial for neuroinflammation such as microglia are necessary to understand exactly their role in the inflammatory process. Pinpointing the causality of both mitochondrial dysfunction and neuroinflammation will help to identify targets that will get at the root of the molecular problem, rather than potentially treating a downstream consequence. Finally, most of the studies discussed in this chapter have been performed in males. As depression has higher incidences in women than men, it will be important to examine sex differences in the mitochondrial and inflammatory responses to SD/witness stress.

References


Neuroinflammation and Mitochondrial Dysfunction Link Social Stress to...


Mean Girls: Social Stress Models for Female Rodents

Jace X. Kuske and Brian C. Trainor

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Abstract Social stressors are known to have strong negative impacts on mental health. There is a long history of preclinical social defeat stress studies in rodents focusing on males that has produced important insights into the neural mechanisms...
that modulate depression- and anxiety-related behavior. Despite these impressive results, a historical weakness of rodent social stress models has been an under-representation of studies in females. This is problematic because rates of depression and anxiety are higher in women versus men. Recently there has been a surge of interest in adapting social stress methods for female rodents. Here we review new rodent models that have investigated numerous facets of social stress in females. The different models have different strengths and weaknesses, with some model systems having stronger ethological validity with other models having better access to molecular tools to manipulate neural circuits. Continued use and refinement of these complementary models will be critical for addressing gaps in understanding the function of neural circuits modulating depression- and anxiety-related behavior in females.

**Keywords** Anhedonia · Hamster · Oxytocin · Peromyscus · Sex differences · Vole · Witness defeat

1 Introduction

Social stressors have become a main focus as risk factors for adverse mental health outcomes. Although treatments are available for mental illnesses such as depression and anxiety, many individuals do not respond to existing therapies (Fava 2003; Akil et al. 2018). A unifying theme in medicine is that understanding the underlying mechanisms of diseases can provide a rational path for the development of novel treatments. Animal models can play a key role in this process because of the power to perform mechanistic experiments that can help us understand results from clinical studies. Social stressors can take several forms, such as social isolation (Weintraub et al. 2010), the loss of a familiar partner (McNeal et al. 2014), or aggressive interactions (Kudryavtseva et al. 1991). Social stress models based on aggressive interactions have proved amenable to study. This is because many of the behavioral and physiological effects of stress exposure are evolutionarily conserved. In particular, the losers of aggressive contests across fish, birds, rodents, and primates generate similar physiological responses such as release of adrenal hormones and long-lasting changes in social behavior. Although social stressors can take several forms, in this chapter we will primarily focus on the utility of social defeat models. Unlike more conventional stress models such as restraint stress (Grissom and Bhatnagar 2009), most individuals do not habituate to social stressors. This may explain why certain social stress phenotypes such as social avoidance can be replicated in different species and labs around the world (Kudryavtseva et al. 1991; Blanchard et al. 1993; Tornatzky and Miczek 1993; Martinez et al. 1998; Huhman 2006). Despite these strengths, social stress models have historically had an important weakness: under-representation of studies on females. This is problematic because of the over-representation of some mental illnesses in women compared to men, such as social anxiety disorder, generalized anxiety disorder, and major
depression (McLean et al. 2011; Bangasser and Valentino 2014; Asher and Aderka 2018). An additional problem is that therapeutic approaches to mental illness can act differently in men and women (Dalla et al. 2010; Williams and Trainor 2018). Thus, even if stress has similar behavioral effects in males and females, the underlying mechanisms could be different.

The over-representation of males has not been unique to studies of stress biology (Beery and Zucker 2011; Prendergast et al. 2014), prompting an increased focus across biomedical research to test hypotheses in both males and females (Tannenbaum et al. 2016; Miller et al. 2017). While there is still progress to be made (Mamlouk et al. 2020), numerous new social stress models have been developed in the past decade that allow for the study of females. Some model systems rely on species with social systems in which females engage in robust aggression. Increased female aggression in these species (hamsters, California mice, prairie voles) differs from the more conventional rat and mouse lines used for behavioral neuroscience research, in which inter-female aggressive interactions are generally muted. However, creative experimental designs, often under more ethologically relevant conditions have been developed in domesticated mice and rats. These methods provide access to powerful molecular tools for studying neural circuits. Here we review female social stress models in behavioral neuroscience (Fig. 1) and break down key findings within each approach. We also consider the strengths and weaknesses of each model with an appreciation that no single model system can fully capture the complexities of stress-induced mental illnesses (Table 1). It is

Fig. 1 Methods for studying social defeat have been developed for several different species. Social stress can be induced via direct physical social defeat or witness defeat protocols. Partner loss in monogamous species is another important form of social stress with translational relevance. Different behavioral assays allow for systematic quantification of the impact of social defeat in different contexts. Social interaction tests assess behaviors in novel or unfamiliar contexts while conditioned defeat and sucrose anhedonia tests are important assessment of behavior in familiar environments.

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important to note that recent developments in chronic variable stress protocols have emerged as an important category of models for studying stress-induced behavioral and neurobiological phenotypes (Hodes et al. 2015; Williams et al. 2020b). However, we will limit the focus to recent developments in social stress models adapted for females.

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<th>Species</th>
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<td>Syrian hamster</td>
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<td>Effects of adolescent stress on behavior are well described in males and females</td>
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2 Syrian Hamsters

The social behavior of Syrian hamsters (*Mesocricetus auratus*) has been studied for decades (Powers and Valenstein 1972; Garrett and Campbell 1980; Kollack-Walker and Newman 1995). This species is known for its relatively intense aggressive behavior (Johnston 1975). Originating in the far western regions of Asia (Siegel 1985) Syrian hamsters are solitary, with females engaging in offspring care alone without interacting with a mate (Gattermann et al. 2001). This social organization likely contributes to a reduced impact of social isolation on behavior in this species (Ross et al. 2017). Unlike many species of rodents, female Syrian hamsters engage in territorial aggression, and females are actually more aggressive than males when not sexually receptive (Payne and Swanson 1970). This trait likely impacts their responses to social stress. The unique social organization provided an excellent opportunity to apply techniques to study social stress in males and females (Huhman et al. 2003).

2.1 Conditioned Defeat

Social defeat methods in hamsters were first developed in males (Potegal et al. 1993). In this paradigm, a resident-intruder test is used in which the focal animal is introduced into the home-cage of an aggressive, same-sex hamster. This can occur once or on consecutive days. The most common behavioral assay is performed a day later when the focal hamster is tested in the home-cage as a resident with a non-aggressive intruder (Faruzzi et al. 2005; Solomon 2017). Typically, an unstressed hamster will engage in aggressive behaviors including chasing, biting, lunging, and upright or side attacks as well as social investigation. In contrast, stressed hamsters are more likely to exhibit defensive behaviors such as fleeing, submissive postures, tail lifts, and upright/side defense. This behavioral phenotype is referred to as “conditioned defeat” and resembles certain phenotypes associated with stress-related mental illness (Huhman 2006). These methods have been used in male hamsters to identify neural circuits involved in the acquisition and expression of these behaviors including the bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAc), and basolateral amygdala (Markham et al. 2009; Cooper and Huhman 2010; Gray et al. 2015). More recently, these methods have been applied to females, resulting in unique phenotypes from males.

Interestingly, the effects of defeat on behavior are less profound in females than in males (Huhman et al. 2003). Males often show greater amounts of submissive behaviors like tail lifts and fleeing, whereas these responses were blunted in females. Stressed females showed more aggressive behaviors, indicating that these territorial behaviors in females are less sensitive to the effects losing aggressive encounters. A major question is how this occurs. Studies examining the effects of defeat on behavior across the 4-day estrous cycle suggest that gonadal hormones are not a
key mechanism. While stressed females showed higher levels of aggression during stages of the estrous cycle when estrogens were low, submissive behaviors were muted across all stages of the cycle (Solomon et al. 2007). In this same study, researchers saw that both defeated and non-defeated females tested on the day of estrus displayed lordosis towards female intruders. The unexpected display of lordosis to another female has been documented previously (Johnston 1977), but its function is unknown. Thus, despite these interesting patterns, gonadal hormones in adults do not appear to be a key causal mechanism. Instead, elevated aggression levels in female hamsters may interfere with the display of submissive behaviors.

In male hamsters, social hierarchy plays a role in resilience to conditioned defeat where defeat stress induces stronger effects on subordinate males than dominant males (Morrison et al. 2012). As females are more aggressive than males (Payne and Swanson 1970), this may explain why they are less sensitive to defeat in resident-intruder tests. In resident-intruder tests, the resident has what is referred to as a “home advantage” or “residence effect” – which describes the increased ability to win an aggressive encounter when an animal is on their own territory which is seen in humans and rodents alike (Carre et al. 2006; Fuxjager et al. 2010). When this home advantage is removed, female Syrian hamsters are more sensitive to defeat stress. In one study, social defeat induced social avoidance in females that were tested in a novel environment with a stimulus hamster confined to a small wire cage (Rosenhauer et al. 2017). Thus, in novel environments, stressed females respond more similarly to males. Further research is needed to understand the underlying mechanisms for these intriguing sex differences. Interestingly, defeat stress has distinct effects on transcription in the basolateral amygdala (BLA).

The BLA is a key brain region for the formation of conditioned defeat phenotypes in males (Jasnow et al. 2005; Dulka et al. 2020). When RNAseq was used to compare effects of defeat on gene expression in males and females, there was little to no overlap in gene expression across social status between the sexes (McCann et al. 2019). Essentially, sex-specific differences in gene expression were observed across dominants, subordinates, or in unstressed controls. These sex-specific transcriptional responses to hierarchy formation are consistent with previous transcriptional analyses in rodents. Sex-specific transcriptional responses to social stress have been observed in the hippocampus (Marrocco et al. 2017), NAc (Hodes et al. 2015) and amygdala (Walker et al. 2020) as well as in humans diagnosed with stress-related mental illnesses such as depression (Labonté et al. 2017). Although each of these studies focuses on different brain regions, the broad pattern of sex-specific transcriptional patterns are consistent. The sex-specific transcriptional responses to defeat in female hamsters suggest that further mechanistic study of this region is warranted. Indeed, sex-specific mechanisms of behavior have been documented for other behaviors in hamsters. For example, inhibition of V1a receptors within the anterior hypothalamus reduces aggression in males but increases aggression in females (Gutzler et al. 2010). These studies are important because they show that even when the sexes are behaving similarly, there can be sex differences in underlying mechanisms.
2.2 Summary

Strengths of the hamster social defeat model include strong ethological validity of aggressive behavior in females and a deep understanding of neural circuits underlying conditioned defeat behavioral phenotypes in males. Currently there are relatively few genetic tools for studying hamsters, although this is beginning to change. Hamsters are an important model species for other biomedical fields such as immunology (Safronetz et al. 2012), which has incentivized the development of genetic tools for this species (Fan et al. 2014). Recently generated V1a receptor knockout hamsters showed that adult males and females were more aggressive than wild types (Taylor et al. 2019). While these results were consistent with previous work in females, the results in males were unexpected as V1a receptors generally promote aggression in males. This suggests that V1a receptors may have important developmental effects on aggressive behaviors. These data highlight the potential for developing new transgenic hamster lines.

Currently, it is unclear how or why females show weaker responses to social defeat in resident-intruder tests. However, stressed females do exhibit social avoidance phenotypes when tested in an unfamiliar environment. While this phenotype is similar to males, stress has sex-specific effects on transcriptional responses within the BLA (McCann et al. 2019), which is known to play a critical role in behavioral responses to stress. This suggests that further study of the neural circuits impacted by social defeat in female Syrian hamsters has the potential to identify sex-specific mechanisms of anxiety- and depression-related behaviors.

3 California Mice

The California mouse (Peromyscus californicus) is another species in which females are aggressive towards other females (Davis and Marler 2003). This species is monogamous (Ribble 1991) and has long been studied as a model for understanding neuroendocrine mechanisms of parental care (Gleason and Marler 2010; Harris et al. 2013; Hyer et al. 2017) and aggression (Oyegbile and Marler 2005; Fuxjager et al. 2010). Besides elevated aggression levels in this species, a clue that California mice could be useful for studying social stress came from the observation that female residents had higher corticosterone levels following an aggressive encounter than males (Trainor et al. 2010). This suggested that social conflict in females might have stronger physiological effects in females versus males. Systematic characterizations of behavior show that the effects of social defeat stress are context-specific with females showing stronger changes in unfamiliar social contexts and males showing stronger deficits in complex cognitive tasks. We will also review the impact of a different form of stress, separation of pair-bonded mice.
3.1 Social Defeat

A standard protocol uses three episodes of defeat across 3 days with only one defeat session per day with a same-sex opponent (Trainor et al. 2011). If each episode is limited to 7 min or stopped after the resident attacks the focal mouse seven times, there is no difference in the number of attacks males and females receive. During the defeat episodes, both female and male mice display equal levels of freezing and defensive behaviors, although escape behaviors are more frequent in females than males (Trainor et al. 2013). This is reminiscent of “darting” behavior in female rats, in which females attempt to escape footshocks instead of freezing (Gruene et al. 2015). Interestingly this more active coping behavior in females is associated with increased corticosterone levels immediately following defeat, an effect that is dependent on ovarian hormones (Trainor et al. 2013). The long-term effects of social defeat on behavior have been examined using a wide range of tests. In general, the effects of defeat on social behavior are stronger in females whereas males exhibit stronger effects of defeat in non-social contexts.

3.2 Social Interaction Test

A social interaction test is the most commonly used behavioral assay for California mice (Greenberg et al. 2014). Alterations in social approach behavior have translational relevance because withdrawal from social situations is a common symptom for both depression and anxiety disorders (Saris et al. 2017). In social defeat models, social interaction tests also have pharmacological validity because stress reduces approach to an unfamiliar stimulus mouse, and this effect is reversed by chronic but not acute antidepressant treatment (Berton et al. 2006). Finally, effects of social defeat on behavior in this assay can last for many weeks, similar to the chronic nature of depression and anxiety. The test used for California mice has three distinct phases: open field, acclimation, and interaction. First the focal mouse is placed in an empty, novel open field arena and the time spent in the center of the arena and total distance traveled are measured. The arena is much larger (almost 1 m long) than typical apparatuses for social behavior. For comparison, a typical three-chambered test for social interaction uses chambers that are 20 cm long (Yang et al. 2011). Next, an empty wire cage is introduced into the open arena against one of the walls. This acclimation phase can serve as a novel object test, as mice will typically investigate the empty cage. Video tracking software is used to track the time spent within 8 cm of the empty cage. Finally, an unfamiliar same-sex conspecific is placed into the wire cage for 3 min. The use of a wire cage where the focal mouse can see, smell, and interact with the target mouse is a key aspect of the test, as the use of a perforated plexiglass cages results in much lower levels of social approach. During the interaction phase, social approach is defined as the time spent within 8 cm of the target mouse. The large arena size facilitates the quantification of a second variable, social
vigilance. Social vigilance is defined as the time the focal mouse spends outside of the interaction zone (within 8 cm of target mouse) with its head oriented towards the unfamiliar mouse. A combination of avoidance and vigilance is characteristic of behavioral inhibition in children (Fox et al. 2005), which is an important risk factor for the development of anxiety disorders in adults (Clauss and Blackford 2012). The effects of social defeat on these behaviors are sex-dependent.

Female California mice are particularly sensitive to the long-term effects of defeat stress. Effects of defeat on social approach are relatively weak 1 day after the last episode of defeat, but become stronger 4 weeks later, as females spend less time interacting with the target mouse compared to males (Trainor et al. 2011). This sex difference does not vary across the ovarian cycle and is not affected by ovariectomy (Trainor et al. 2013). Although chronic stressors have been linked to disrupted ovarian cycles in rodents (Pollard et al. 1975) and humans (Bae et al. 2018), this is not observed in studies of California mice. This is likely due to the use of three relatively brief episodes of social defeat. Thus, it is instructive that phenotypes such as stress-induced social avoidance can be de-coupled from circulating gonadal hormones in adults. Importantly stress-induced decreases in social approach are reversed with 4 weeks of chronic but not acute treatment with an antidepressant (Greenberg et al. 2014), as has been reported in male mice. The social interaction test has proved useful for assessing the possible utility of novel therapeutic approaches. For example, a single systemic dose of a brain-accessible oxytocin receptor (OTR) antagonist administered 30 min before a social interaction test was sufficient to restore social approach and reduce social vigilance in females that had been previously exposed to social defeat (Duque-Wilckens et al. 2018). In contrast, a short acting kappa opioid receptor (KOR) antagonist had no effect on behavior if administered before a social interaction test in stressed females (Williams et al. 2018). However, if KOR antagonists were administered immediately before each of three episodes of defeat, reductions in social approach and increases in social vigilance of females were blocked. These findings suggest that OTR antagonists might have unanticipated utility for reducing social anxiety whereas KOR antagonists might have more utility in prophylactic approaches to reduce the adverse impact of stressful experiences. Adult male California mice do not show a social anxiety phenotype following defeat in the social interaction test. However, pre-stress KOR antagonist treatment blocked defeat-induced sucrose anhedonia in both males and females (Williams et al. 2018). Site-specific manipulations suggest that OTR can have similar effects on social anxiety-related behaviors in both males and females.

For example, the BNST has been identified as a key brain region mediating the effects of social defeat on social approach and social vigilance (Duque-Wilckens et al. 2018, 2020). In stressed females, activation of OTR in the BNST is necessary for increased social vigilance while oxytocin infusions into the BNST of unstressed males or females are sufficient to induce social vigilance. Thus sex differences in the effects of defeat on social approach appear to be driven by differences in the activity oxytocin neurons. Females exposed to defeat 2 weeks prior to testing in the social interaction test have more oxytocin/c-fos colocalizations than control females whereas this difference is absent in males (Steinman et al. 2016). A key result is
that when oxytocin/c-fos colocalizations are examined within 1 h of a third episode of defeat, effects of stress are more robust in males than in females. When stressed males are tested in a social interaction test immediately after defeat exposure, the same combination of reduced social approach and increased social vigilance is observed (Duque-Wilckens et al. 2020). Thus, sex differences in the social interaction test appear to be largely based on the persistence of changes in social approach and vigilance. However, in more familiar environments (the home-cage), males and females show more similar responses to social defeat.

3.3 Context-Dependent Effects of Social Defeat

One of the first signs that effects of social defeat are context-dependent was observed in habituation-dishabituation tests. These tests are conducted in the familiar home-cage, in which mice are presented with diluted urine from unfamiliar same-sex conspecifics as a way to assess investigation of a social odor. Eight weeks after defeat, females spent significantly less time than controls investigating social odors (Trainor et al. 2011). Although stressed males showed more interest in social odors than stressed females, they spent significantly less time investigating these odors than control males. Similar results were observed in resident-intruder tests, where both males and females exhibited a conditioned defeat phenotype similar to male and female hamsters (Steinman et al. 2015). Stressed male and female California mice also exhibit reduced anogenital sniffing of intruders (which typically occurs before initiating aggression) and increased freezing behavior. Effects of stress also extend to sucrose preference, a widely used behavioral assay used to assess an anhedonia (loss of pleasure) phenotype. These tests are also conducted in the familiar home-cage. Both males and females exposed to social defeat show a reduced preference for sucrose (Williams et al. 2018). Together these results suggest that in males, the impact of stressful experiences is blunted in novel environments. This hypothesis is supported by conditioned place preference assays, which are conducted in novel testing arenas. Kappa opioid receptor agonists are generally aversive, and female California mice form a place aversion at lower doses than males (Robles et al. 2014; Laman-Maharg et al. 2017). There is no evidence for sex differences in the pharmacodynamics of KOR ligands (Laman-Maharg et al. 2018) and in general males are more sensitive to the aversive effects of KOR agonists than females (Russell et al. 2014; Chartoff and Mavrikaki 2015). Currently the basis for context-dependent effects of social defeat in California mice is unknown.
3.4 Male-Biased Effects of Stress on Cognitive Flexibility

Interestingly, one domain in which males are more strongly affected by stress than females is cognitive flexibility. When California mice were tested for spatial memory in a Barnes maze, social defeat had no effect on how quickly males and females learned to find an escape chamber (Laredo et al. 2015). However, when the location of the escape chamber was moved, stressed males had significantly longer path lengths to reach the escape chamber and made significantly more errors than controls. On the other hand, females were not affected by defeat stress during this reversal phase. A nearly identical pattern was observed in college students randomly assigned to undergo the Trier Social Stress Test (TSST) or a control condition (Shields et al. 2016). Men assigned to the TSST made more errors on reversal stages of the Wisconsin Card Sort task than controls whereas women were unaffected. Although California mice do not have strong spatial memory performance as other species of *Peromyscus* (Jašarević et al. 2012), the specific impact of defeat stress on reversal (but not acquisition) in males has been replicated in a 4-choice digging task that is less dependent on spatial memory (Wright and Trainor, unpublished). Previous work in rats reported that repeated restraint stress had stronger adverse effects on measures of cognitive flexibility in females than males (Grafe et al. 2017). This study used an operant task to assess reversal learning, so it is unclear whether task differences, forms of stress, or species differences contribute to the different results in California mice and rats.

3.5 Partner Loss Stress

An important alternative model for studying social stress is partner loss, which is associated with numerous adverse health outcomes in humans (Stroebe et al. 2007). California mice are one of the few monogamous species of rodents in which males and females form a pair-bond (Gubernick and Nordby 1993; Pultorak et al. 2015). Separation of a partner can impact the brain and behavior that go beyond social isolation. So far most work has been done in male California mice, but this set a groundwork for future work in females. For example, males that were isolated from a pair-bonded female had higher baseline corticosterone levels and slower healing of skin wounds (Glasper and DeVries 2005), similar to reports women experiencing the stress of a chronically ill spouse (Kiecolt-Glaser et al. 1991). To date, studies using females have yet to be published. In addition to assessing the physiological responses to isolation, it would be highly informative to know observed additional behavioral changes (i.e., signs of anhedonia, anxiety-like behaviors, increased social investigation following social deprivation, etc.).
3.6 Summary

California mice provide several options for studying the impact of social stress in ways that are translationally relevant for understanding behavioral phenotypes associated with anxiety and depression in women and men. Some phenotypes have proved remarkably robust, such as the social interaction test, which provides consistent results across hundreds of tests (Trainor et al. 2013). While effects of defeat in the social interaction test are sex-specific, other behavioral responses such as sucrose anhedonia and conditioned defeat are observed in both sexes. The relatively large size of this species (adults are 40 g) has also made this species amenable to site-specific pharmacological manipulations (Campi et al. 2014; Duque-Wilckens et al. 2016). The long life-span of California mice and prolonged period of adolescent development also makes this species conducive for the study of adolescent development (Wright et al. 2020). Although there has been progress in applying some modern neuroscience approaches to study the neural circuits impacted by stress (Duque-Wilckens et al. 2020), there are fewer molecular tools available compared to more conventional mouse and rat lines. As with hamsters, the growing availability of CRISPR based gene editing techniques may allow for the development of more tools for this species.

4 Prairie Voles and Mandarin Voles

The prairie vole (Microtus ochrogaster) is probably the best studied monogamous species of rodent, which is well known for its ability to form pair-bonds (Carter et al. 1995). Decades of work has identified neuroendocrine mechanisms underlying attachment within pairs (Young and Wang 2004) and parental behavior (Kenkel et al. 2017). One aspect of pair-bonding is selective aggression towards unfamiliar individuals in males (Winslow et al. 1993) and females (Bowler et al. 2002), which has been recently applied to study social defeat in females. Similar methods have also been adapted for Mandarin voles (Microtus mandarinus), a monogamous vole from Asia in which females are aggressive towards other females. In addition to social defeat methods, we will also briefly review studies that have examined partner loss as a form of social stress.

4.1 Social Defeat

For prairie voles, focal voles assigned to defeat undergo a combination of physical interaction with a same-sex resident (15 min) followed by a longer period of threat (45 min) in which the aggressive resident and focal vole are separated by a perforated barrier (Tickerhoof et al. 2019). Under this protocol, both males and females...
vigorously attack intruders, although males attack more frequently than females. Social defeat results in an acute increase in corticosterone levels in female prairie voles (Smith et al. 2013). One week after defeat, both males and females exhibit reduced social approach towards an unfamiliar stimulus vole in a three-chambered test. Stressed males and females also show an anxiogenic phenotype in the elevated plus maze. No differences were observed in a sucrose preference test. These results show the prairie vole has strong potential for comparing social stress-related phenotypes in males and females.

Studies in Mandarin voles use a longer stress protocol, consisting of 2 weeks of daily 10 min episodes of physical social defeat (Wang et al. 2018). After this period of physical interaction, the focal vole is separated from the aggressive resident by a perforated barrier. Females assigned to this defeat protocol show reduced social approach and increased freezing in a social interaction test. Anxiogenic phenotypes are also observed in stressed female Mandarin voles during the elevated plus maze (Wang et al. 2019). Although males and females have not been directly compared using this protocol, these social defeat methods have been used to model stress buffering behaviors in pair-bonded voles (Li et al. 2019). Both males and females that observed a pair-bonded partner exposed to social defeat showed increased allogrooming behavior, suggesting that similar to prairie voles, males and females are highly sensitive to social stress. Together, these findings in prairie voles and Mandarin voles suggest that both species will be very useful for studying the neural circuits impacted by social defeat stress.

### 4.2 Partner Loss Stress

The loss of a pair-bonded partner is also an important form of stressor that can induce strong behavioral and neuroendocrine effects related to depression and anxiety. When males or females were separated from a pair-bonded mate, this induced passive immobility responses in the forced swim test and increased plasma adrenocorticotropic hormone and corticosterone levels in both sexes (Bosch et al. 2009; McNeal et al. 2014). A follow-up study showed that isolated prairie voles had greater corticosterone reactivity to a stressor following isolation from a mate (Grippo et al. 2020). In females, environmental enrichment (running wheel and other items) attenuated the behavioral effects of partner separation (Normann et al. 2018). Many of the behavioral effects of partner loss resemble the effects of social isolation in non-pair bonded prairie voles (Pizzuto and Getz 1998; Grippo et al. 2007, 2008; Ruscio et al. 2007, 2009; Pourmajeﬁ-Nazarloo et al. 2013).
4.3 Summary

Like hamsters and California mice, prairie voles and Mandarin voles can be studied to determine the impact of intra-female aggressive interactions on behavior and brain function. Although the neural circuitry affected by social defeat is only just beginning to be studied, decades of work on the neural mechanisms of pair-bonding in prairie voles provides a strong foundation for neural circuits of interest and methods for studying behavior in this unique species. In addition, the ability to study partner loss in prairie voles and Mandarin voles provides another important line of inquiry to understand how a different form of social stress affects depression and anxiety-related behaviors and corresponding neural circuits. Although currently there are few genetic tools available for voles, this is starting to change. New knockout (Horie et al. 2019) and knockin (Horie et al. 2020) prairie vole lines have been created using the CRISPR/Cas9 system, which demonstrates the feasibility of applying this approach to different rodent species (Donaldson and Manoli 2020). The ability to create voles with promoter-specific Cre recombinase yields the ability to target cell-type specific populations of neurons in a manner that previously was possible only in more conventional mouse and rat lines. In addition, advanced neuroscience techniques such as 1-photon in vivo calcium imaging have been adapted for use in prairie voles (Scribner et al. 2020), which allows for the measurement of neural activity in individual neurons. The development of these tools suggests that prairie voles will be an important model species for studying the impact of social stress in females.

Up to this point, the species reviewed are all ones in which intra-female is readily induced in laboratory settings. However, the majority of neuroscience tools have been developed for domesticated mouse and rat lines. In these species, intra-female aggression is low or absent in standard laboratory aggression tests. The need to study the impact of social stress in females has led to the development of some creative new approaches for studying females in these species.

5 Domestic Mice

Social defeat experiments using different transgenic lines of male C57BL/6J mice have proved to be a powerful tool for identifying circuits and molecular pathways related to depression and anxiety-related behaviors. A widely used protocol in mice (Golden et al. 2011) is based on work from Kudryavtseva and colleagues (Kudryavtseva et al. 1991; Avgustinovich et al. 1997) and involves 10 consecutive days of brief physical interactions with an aggressive resident. These interactions are followed by a period of sensory contact, in which focal mice are separated from aggressive residents by a perforated plexiglass barrier. During this time, residents often make aggressive threats towards focal mice. About half of male mice exposed to social defeat will show reduced social approach in a social interaction test (Krishnan et al. 2007). These mice are usually referred to as “susceptible,” and
this phenotype can be reversed by chronic but not acute antidepressant treatment (Berton et al. 2006). Interestingly, about half of males exposed to social defeat do not show a reduction in social approach and these mice are often referred to as “resilient.” This term is something of a misnomer, as it implies that these mice have “recovered quickly” from a bad experience. In fact, when tested in other behavioral contexts like an elevated plus maze (Krishnan et al. 2007) or fear extinction (Meduri et al. 2013), strong effects of stress are observed. We will refer to this phenotype as “unsusceptible” to refer to the lack of phenotype in the social interaction test with the understanding that these mice still exhibit important stress-induced behavioral phenotypes.

Adapting social defeat methods to female mice has been challenging. In naturalistic contexts, male Mus typically defend territories from other males while females generally move between territories (Crowcroft 1955). Overt aggressive behaviors used by males to defend territories are largely absent in females (Scott 1966), at least under standard laboratory conditions. These aspects of Mus social organization have formed the main challenges to studying social stress in females. To overcome these barriers, three general strategies have emerged: (1) create behavioral contexts in which males will attack females, (2) construct paradigms in which females observe social defeat occurring among males, and (3) produce behavioral contexts in which females will attack females.

5.1 Male Aggression Towards Females

A chemogenetic approach uses designer receptors exclusively activated by designer drugs (DREADD) to enhance neural activity within circuits controlling aggression in male mice (Takahashi et al. 2017). To accomplish this, a virus expressing an excitatory DREADD was infused into the ventrolateral subdivision of the ventro-medial hypothalamus (VMHvl). A systemic injection of clozapine-N-oxide (CNO) is then used to activate the VMHvl to robustly increase aggression towards both male and female intruders (Lin et al. 2011). This effect is mediated primarily by activation of neurons expressing estrogen receptor α (ERα) (Lee et al. 2014), and males attack females more readily when these neurons are selectively activated. When a standard protocol of 10 days of social defeat plus sensory contact was used, effects of defeat on social interaction behavior in females were surprisingly modest. Less than 20% of females showed a susceptible phenotype marked by reduced social approach to an unfamiliar female target mouse. In males, usually about 60% of mice show reduced social approach (Krishnan et al. 2007). The weaker effect in females could be due to the relatively transient nature of CNO activation of the Gq-DREADD in the VMHvl. Indeed, during the later phases of the sensory contact period, males did not make aggressive threats towards females across the barrier as is typically observed for male intruders. Thus, sensory contact between females and resident males might result in some kind of extinction learning. Indeed, social defeat of females by males without the sensory contact produced more robust decreases in
social approach. Similar to California mice, no effects of estrous cycle were observed on behavior in the social interaction test.

A shortened version of this social defeat protocol (only 3 days of social defeat) also induced significant decreases in social approach in females but not males (Issler et al. 2020), similar to typical results in California mice. This protocol was used to show that overexpression of the microRNA transcript LINC00473 in the medial prefrontal cortex (which is downregulated in women diagnosed with depression) blunts the effects of defeat stress on social approach in females. Another study showed that chemogenetic activation of ERα neurons in VMHvl can induce male aggression even when this mouse is introduced into the home-cage of female mice. A 6-day defeat protocol induced robust decreases in social approach as well as anxiety phenotypes in the elevated plus maze (Yin et al. 2019). Thus, it appears that the key to successful chemogenetic-assisted social defeat protocols for females is the elimination of the sensory contact phase rather than altering the number of episodes of social defeat. Chemogenetic activation of ERα neurons within VMHvl in female mice can also increase aggression towards female intruders in about 60% of tests (Hashikawa et al. 2017). This suggests that future refinements could produce a protocol based on female–female aggressive interactions.

A second general approach takes advantage of the role of olfactory cues to promoting aggression in rodents. Aggressive behavior is typically preceded by anogenital sniffing, during which the detection of male pheromones activates neural circuits driving aggression (Hashikawa et al. 2016). Collecting urine from a male CD-1 mouse and applying a sample of it to the base of the tail of a female C57BL/6J can induce aggression from male CD-1 in about 60% of tests (Harris et al. 2018). This approach was used in a 10-day social defeat protocol using sensory contact. After excluding stressed females that experienced fewer than 4 days of aggressive attacks over the 10-day protocol (23% of females), stressed females exhibited reduced social approach. When these females were divided into susceptible and non-susceptible females, susceptible females also exhibited reduced sucrose preferences. Estrous cycles were unaffected by social defeat. The male urine and chemogenetic methods rely on manipulations that strongly activate circuits for male–male aggression. However, these are not the only approaches for generating male–female aggression.

A third general approach is based on the observation that extremely aggressive male mice will often indiscriminately attack males or females. The introduction of focal C57BL/6J male and female mice into the home-cage of an aggressive CD-1 mouse causes the CD-1 resident to robustly attack the male intruder (Yohn et al. 2019). The CD-1 resident also attacks the female intruder, although significantly less (and with longer latency) than the male intruder. Intriguingly, CD-1s attack females throughout the estrous cycle, although rates are highest during proestrus and metestrus. Episodes of social defeat are conducted over a 10-day period with different CD-1s. During the sensory contact period, either the male or the female C57BL/6J intruder was cohoused with the aggressive CD-1. The cages used permitted only one cage divider, and co-housing male and female focal mice could cause a confound due to sexual experience. Thus on half of the days the focal male experienced
sensory contact with the familiar aggressive CD-1 while the female was housed with a novel CD-1 mouse. On alternating days the female was housed with the familiar CD-1 and the male housed with the unfamiliar CD-1. Thus each focal mouse experienced a total of 10 days of physical defeat with the 10 days of sensory contact (5 days spent with a familiar aggressor and 5 with an unfamiliar CD-1). About 70% of females exposed this social defeat regimen showed reduced social approach compared to controls. These females also showed anxiogenic phenotypes in the elevated plus maze and novelty-suppressed feeding test as well as sucrose anhedonia. Males exposed to this stress paradigm showed largely equivalent behavioral responses as females in all behavioral assays. Even though females were exposed to lower levels of physical aggression than males, robust behavioral phenotypes were observed. This is consistent with observations in California mice, where behavioral phenotypes were not strongly associated with the amount of aggression in individual episodes of defeat (Trainor et al. 2013).

5.2 Witness Defeat

The protocols reviewed above all rely on physical interactions between aggressive mice and focal mice, but mice show altered physiological responses when living with aggressive cagemates – even if they are not directly attacked (Henley et al. 1973). Building on these observations, an interesting discovery was that simply observing aggressive interactions can induce anxiety- and depression-like behavioral responses (Sial et al. 2016; Warren et al. 2020). Male C57BL/6J mice that observe other male C57BL/6J mice exposed to an aggressive male CD-1 mouse show similar increases in corticosterone both acutely and chronically (Warren et al. 2013). In fish (Oliveira et al. 2001) and humans (Bernhardt et al. 1998), observing competitions has also shown an increase in testosterone levels, a response that often occurs in individuals that win competitions (Marler and Trainor 2020). An important application of the witness defeat protocol was the demonstration of its utility in female mice (Iñiguez et al. 2018). One day after a female C57BL/6J observes social defeat of another male, corticosterone levels are significantly elevated. During this time, social approach and sucrose preferences are also reduced compared to control females that were handled but did not observe aggressive interactions. Female C57BL/6J mice exposed to witness defeat also show stronger increases in social vigilance than males (Duque-Wilckens et al. 2020). These phenotypes were reversed by acute treatment with low-dose ketamine or the anxiolytic chlordiazepoxide, illustrating pharmacological validity of this approach. Similar to California mice exposed to social defeat stress, witness defeat increased the number of oxytocin positive cells in the BNST but not the paraventricular nucleus of female C57BL/6J.
5.3 Female–Female Aggression

Previous work in mice demonstrated that lactating females will be aggressive towards female intruders, but this tendency is relatively brief (Svare and Gandelman 1973; Rosenson and Asheroff 1975). This is a major obstacle for implementing social defeat protocols. An alternative approach showed that when intact female Swiss Webster (CFW) mice were cohoused in pairs with intact male CFW mice, they reliably exhibited aggressive behaviors towards female C57BL/6J mice (Newman et al. 2019). Female aggressive behavior decreased steadily after females had pups but increased after females were repaired with castrated males. Similar to male C57BL/6J mice, females had increased corticosterone levels if exposed to either a single episode of defeat or after chronic exposure to 10 episodes of defeat. Females exposed to chronic defeat exhibited more defensive behaviors and reduced approach behaviors to a non-aggressive female intruder introduced to the home-cage. In stressed females, social approach was increased by low-dose ketamine treatment. Also, in the home-cage chronic social defeat impaired nest-building behavior, a motivated behavior that is stress sensitive (Otabi et al. 2016). A social interaction test using a large testing arena showed that females exposed to chronic defeat showed increased social vigilance, although curiously there was no effect on social approach. This observation is consistent with recent evidence indicating that neural mechanisms of social approach and social vigilance are distinct (Williams et al. 2020a). Similar to the other stress models reviewed above, estrous cycles did not appear to be impacted by stress and did not have robust effects on behavioral assays.

5.4 Summary

The methods reviewed above are exciting because they provide a mechanism for applying the powerful genetic tools developed for C57BL/6J to understand the molecular pathways and neural circuits contributing to stress-related behavioral phenotypes in females. While each approach has weaknesses, wide adoption of several of these methods will allow the field to rigorously identify stress-induced behavioral phenotypes and their associated neural mechanisms. A common strength of male-induced defeat of female mice is the generation of susceptible and unsusceptible phenotypes, similar to individual variation that has been identified in males. The ability to identify molecular- and circuit-based differences in these phenotypes has strong potential for understanding human resilience. However, common weaknesses are questionable ethological significance and the relative equivalency of phenotypes between males and females. While male aggression in rodents is rarely directed towards females in naturalistic contexts, these interactions clearly induce robust phenotypes. The introduction of new female intra-sexual aggression methods was an important methodological development (Newman et al. 2019) that has great potential. In the future, it will be interesting to assess
whether there are subtle differences between intra-sexual aggression versus inter-
sexual aggression that might affect behavioral phenotypes or underlying neurobi-
ological phenotypes. Regardless, these new behavioral models for transgenic mice
will allow better use of molecular tools to study neural circuits of depression and
anxiety-related behaviors.

6 Domestic Rats

As in mice, social defeat in male rats has proven to be a robust approach for inducing
behavioral and neurobiological phenotypes related to depression and anxiety (Tidey
and Miczek 1996; Wood et al. 2010). Most of the challenges faced by mouse
researchers in adapting social stress protocols to females apply to rats as well.
Two main approaches have been evaluated so far; lactating dams and witness defeat.
In addition to these relatively new methods, there is a strong literature examining the
effects of social instability stress during adolescence (McCormick et al. 2005;
Hodges et al. 2018). In this approach, cagemates of developing rats are regularly
switched, which creates an unstable social environment that has long-lasting effects
on social behavior and brain function in males and females. These studies are
reviewed in another chapter of this volume (McCormick 2021).

6.1 Lactating Dams

Lactating rat dams can be induced to exhibit aggression towards female intruders,
with the peak aggression levels occurring during postnatal days 3–12 (Erskine et al.
1978). This approach has been used to examine effects of stress on anxiety and
depression-related behaviors as well as drugs of abuse. In one study, female Wistar
rats were exposed to a single episode of defeat by a lactating dam and then tested in a
social interaction test 2 h later (Lukas and Neumann 2014). Social defeat did not
reduce social approach to unfamiliar rats or a familiar cagemate, although there was
a slight reduction in social approach if the same lactating dam was used as a stimulus
rat. It is interesting that defeat does not induce robust decreases in social approach at
this time point because in male mice (Bruchas et al. 2011) and California mice
(Duque-Wilckens et al. 2020), social defeat can reduce social approach on this
relatively short time scale. Future studies could examine whether multiple episodes
of defeat might induce a more robust effect on social approach in female rats. It could
also be informative to examine behavior several days after the last episode of defeat,
as effects of defeat on social approach in California mice are stronger several weeks
after the last episode of defeat compared to 1 day after (Trainor et al. 2011).
Interestingly intracerebroventricular infusion of oxytocin in rats did not restore
social approach in this condition, consistent with the hypothesis that oxytocin can
reduce social approach in aversive contexts (Steinman et al. 2019).
Seven episodes of social defeat did not affect passive floating behavior by adult female Sprague-Dawley rats in a forced swim test conducted 2 days or 1 month after the last episode of defeat (Ver Hoeve et al. 2013). A different protocol alternated episodes of social defeat with restraint stress over a 12-day period (Bourke and Neigh 2011). This regimen decreased latency to engage in passive floating in the forced swim test and reduced sucrose preference in both adult and adolescent females. Both social defeat protocols induced defensive behaviors in focal females such as upright posture and boxing, so it’s unclear why social defeat alone is insufficient to induce depression-related behaviors or passive coping responses in females. A procedure consisting of 3 weeks of daily aggressive encounters was used in which focal female rats were introduced into the home-cage of an aggressive dam (Shimamoto et al. 2011). Episodes of aggression occurred twice per day. After 1 week of stress, stressed females showed a significant reduction in saccharin preference. The prolonged stress exposure also disrupted estrous cycles. Stress also blunted dopamine and serotonin release in the NAc in response to cocaine. In contrast, other studies using fewer episodes of defeat show that stressed female rats self-administer cocaine at higher rates (Haney et al. 1995) and enhanced dopamine release in the NAc following a cocaine challenge (Holly et al. 2012).

### 6.2 Witness Defeat

A challenge for implementing social defeat with lactating dams is the need to maintain breeding pairs as aggression levels that occurs shift as pups grow older. An alternative approach includes witness defeat, in which Sprague-Dawley female rats observe another male exposed to defeat on 5 consecutive days (Finnell et al. 2018). During episodes of defeat females threw bedding at the partition separating the female from the males, similar to defensive treading behavior reported in male rats (Cromwell and Berridge 1994). In tests conducted 3–4 days after the last episode of defeat, stressed females had decreased sucrose preference and increased passive floating behavior in a forced swim test. However, the effects of stress on these behaviors were blunted in ovariectomized females, even though there were no differences in behavior across the estrous cycle.

It’s unclear why this witness protocol induced behavioral responses in the forced swim test whereas previous studies using lactating dams as aggressors did not (Ver Hoeve et al. 2013). Both studies included a delay between defeat exposure and behavior testing, although the lactating dam study waited 1 month. Although social defeat-induced decreases in social approach behaviors have been observed to last for 8 weeks (Trainor et al. 2011), it’s possible that passive coping strategies may recover more quickly. Further study is needed to assess the timeline of behavioral phenotypes for lactating dam-induced stress and witness defeat. It would also be interesting to assess the impact of these stress models on social approach or vigilance.
6.3 Summary

To date, the strongest effects of female–female defeat have been on reward-related behaviors such as cocaine self-administration or saccharin preference. While the effects of female–female social defeat on social behavior are not as strong as male–male social defeat, there is still potential to examine longer term effects of stress. Experimenting with female aggression in sexually experienced females (similar to new methods in mice) could be worth investigating to reduce the constant need for lactating dams, which are only aggressive for a short period of time. New witness defeat methods are also an attractive alternative for inducing robust physiological and neurobiological responses to stress. The use of CRISPR has led to rapid growth in the availability of new transgenic rat lines (Bäck et al. 2019), which should facilitate the types of cell-type specific manipulations that are currently available in mice.

7 Conclusions

An important question moving forward is how some of the methods will shed light on underlying mechanisms for sex differences in stress-related psychiatric disorders. For many of the new models reviewed above, investigators focused on establishing novel methods in females and did not directly compare males and females. A few cases have directly compared males and females. In some cases, behavioral phenotypes were similar such as in mice (Yohn et al. 2019) or prairie voles (Tickerhoof et al. 2019). In other cases, such as Syrian hamsters and California mice, the effects of social defeat in males and females are context-specific. In light of the increased prevalence of stress-related mental illnesses in women compared to men, it might be expected that, for at least some behavioral variables, there could be sex differences. A challenge for comparing results across species is that different methodologies are used. Although several studies have compared one versus multiple episodes of social defeat, other parameters such as sensory contact likely play an important role in determining behavioral phenotypes. Systematic evaluation of these parameters across species could provide key insights in understanding the mechanisms of behavioral phenotypes. For example, while a typical chronic mild stress protocol induced depressive-like behaviors in both males and females, an abbreviated subchronic mild stress protocol has stronger effects in females than males (Hodes et al. 2015). It is important to note that studying the same phenotype in both males and females is intrinsically valuable because different neurobiological mechanisms can produce a similar phenotype in males and females (De Vries and Boyle 1998; Shansky 2018). Overall, these innovative methodologies provide a path for investigators to examine how stress alters molecular and neurobiological pathways regulating stress-sensitive behaviors in females.
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Development of Mixed Anxiety/Depression-Like State as a Consequence of Chronic Anxiety: Review of Experimental Data

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Abstract The review presents experimental data considered from the point of view of dynamic changes in the brain neurochemistry, physiology, and behavior of animals during the development of mixed anxiety/depression-like disorder caused by chronic social stress from norm to severe psychopathology. Evidences are presented to support the hypothesis that chronic anxiety rather than social defeat stress is an etiological factor in depression. The consequences of chronic anxiety for human health and social life are discussed.

Keywords Anhedonia · Chronic anxiety · Chronic social defeat stress · Mice · Mixed anxiety/depression-like state · Sensory contact model · Serotonin

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1 Introduction

There are several dozens of methodical techniques to induce depression- or anxiety-like states in animals. The available animal models potentially can be used for studying depression (Willner and Mitchell 2002; Miczek et al. 2008; Hao et al. 2019; Gururajan et al. 2019; Nabizadeha 2019) or anxiety (Griebel 1995; Harro 2018; Réus et al. 2014) in humans. However, preference is to be given the models in which depression is induced by chronic stress, which is recognized as a potent risk factor for the development of depressive pathology in humans (Blanchard et al. 1993; Kessler 1997; Willner 1997). Cold swim or inescapable shock (Weiss et al. 1975), immobilization stress (Curzon and Datla 1993), sequential exposure to various types of severe (Katz 1981) or unpredictable chronic mild stress (Willner 1997) as well as social defeat stress (Miczek et al. 2008, 2011) led to the development of depressive-like symptoms in animals, which, in authors’ opinion, is similar to those in humans: anhedonia-like state, helplessness, reduced stress reactivity, weight loss, etc. However, there has always been a question about the artificiality of experimental settings, since the well-recognized etiological factors of depression in humans are lack of social support or stability, prolonged mental loads and problems of adaptation in conditions of chronic emotional stress, health problems (George et al. 1989; Phifer and Murrell 1986).

In recent years, the Chronic Social Defeat Stress (CSDS) model has been recognized as the most preferred model for studying the mechanisms of depression (Berton et al. 2006; Golden et al. 2011), which is used in many laboratories. This model was based on the Social Model of Depression (Kudryavtseva et al. 1991a), which met all validity criteria proposed for experimental models of depression (McKinney and Bunney 1969): the similarity of etiology, symptoms, sensitivity to antidepressants, and neurochemical changes in the brain similar to those in depressed patients. In a comparative aspect, this model and its shortened and simplified modification of CSDS (Golden et al. 2011) had both advantages and disadvantages (Kudryavtseva 2011a). Over time as a result of numerous studies, the authors of the Social Model of Depression (Kudryavtseva et al. 1991a) had come to a conclusion that chronic anxiety caused by the expectation of an unavoidable psychoemotional negative events leads to develop a mixed anxiety/depression-like disorder in animals. One of the main advantages of this model is the possibility to study dynamic changes in behaviors, brain neurochemistry, physiology from norm to severe pathology over time in animals. This review aims to demonstrate the facts in favor of this assumption and to revise the interpretation of both own and published data of other authors.
2 Experimental Model of Mixed Anxiety/Depression-Like State: Etiology, Symptomatology, Sensitivity to Anxiolytics and Antidepressants

Social Model of Depression (Kudryavtseva et al. 1991a; review, Kudryavtseva and Avgustinovich 1998; Kudryavtseva 2011b; Avgustinovich et al. 2004; Galyamina et al. 2017) was developed at the middle of the 1980s at the Institute of Cytology and Genetics SB RAS under the original name “sensory contact model” (Kudryavtseva 1991) later renamed as “model of chronic social conflicts” (Kudryavtseva 2000, 2011b; Kudryavtseva et al. 2014). This model was identified the most relevant model for studying depression and the consequences of chronic social stress (Keeney and Hogg 1999; Bartolomucci and Leopardi 2009) and was tested in different laboratories (Borodin et al. 2002; Beitia et al. 2005; etc.).

2.1 Behavioral Technique for the Development of Anxiety- and Depression-Like State in Mice

Male mice from different home cages that are maximally aligned by weight are placed in pairs in experimental cages, divided into two equal compartments by a transparent partition with holes that allows the animals to see, hear, and perceive each other’s smells, but prevents physical contact (sensory contact conditions). Testing begins 2 days after the animals have adapted to the new conditions of housing and sensory acquaintance with each other and is carried out in the afternoon (1.00–6.00 p.m. local time). At the time of testing, the cage lid is changed to a transparent one necessary for observing the animals, and, after 5 min (activation period) the partition is removed for 10 min, which leads to agonistic interaction between the mice. The experience of victories or defeats in agonistic interactions with the same partner, manifested in the first tests (within 3 days), is reinforced subsequently with repeated interactions with other partners of the opposite type of social experience (winners or losers). For this, a defeated male is placed in an unfamiliar cage on someone else’s bedding with another aggressive male behind a partition. Aggressive males always remain in their compartments. During the test the partition is returned to its place if intense attacks from the aggressive male last more than 3 min (in some case – less) to prevent damage of the losers. As a result, animals with repeated experience of social defeats (losers, defeated mice) demonstrate postures of submission or avoidance in agonistic interaction with an aggressive partner. In experiments, defeated male mice were studied after three (Losers-T3), ten (Losers-T10), and 20 (Losers-T20) tests (days) of agonistic interactions. Mice without experience of daily agonistic interactions served as controls.

The following tests were used to estimate changes in behavior and psychoemotional states of male mice: the partition test (Kudryavtseva 2003), which assesses the communicativeness (sociability) of animals by their reaction to
a familiar or unfamiliar partner in the adjacent compartment of the cage; the elevated plus maze (EPM) test (Rodgers and Cole 1994), used to assess the level of anxiety in animals, the behavior of which is sensitive to anxiolytics; the Porsolt test (Porsolt et al. 1977), in which behavior is sensitive to the antidepressants and is used to assess the level of depressiveness (Kudryavtseva et al. 1991a); the open-field test (to assess exploratory and motor activity), and others. It was shown previously that the behavioral parameters in the partition test (number of approaches to the partner and total time of reaction to the partner in the neighboring compartment) negatively correlate with the parameters of behavior in the EPM test (Kudryavtseva 2003): the lower communication in the partition test and higher anxiety, estimated by the EPM test in animals and vice versa.

It has been shown that repeated agonistic interactions invoke considerable changes in the behaviors and emotional status of male mice. Comparison of the behavior male mice in intact state or in 2–3 days of agonistic interactions, with mice having passed through 10 and 20 days of agonistic interactions allowed to suggest common diagnostic criteria for the development of behavioral pathology in male mice to confirm the development of psycho- and/or neuropathology in animals (Note 1).

**Note 1 Criteria of Developing Pathology in Experimental Animals**
(Adapted from Kudryavtseva 2006; Kudryavtseva et al. 2008)

Behavioral pathology was acknowledged if several of the criteria are met:

1. Increased (or decreased) expression or and duration of the demonstration of behavioral forms;
2. Emergence of novel behavioral forms that were not demonstrated by the animals before agonistic experience;
3. Inadequacy of behavioral responses to some social or environmental stimuli, uncontrollable behavior;
4. Nonadaptive behavior under environmental conditions or in experimental situations;
5. Generalization of dominating motivations (anxiety or and depression-like states);
6. Expressed multiple neurochemical and neurogenomic alterations in the brain similar to those in depressive patients;
7. Persistence of changed behaviors, emotional states, and neurochemical changes in brain at least 2 weeks after cessation of agonistic interactions (period of relative rest for the defeated mice).
2.2 The Stages of the Developing Anxiety- and Depression-Like States in Male Mice

The entire spectrum of depressive symptoms develops within 20 days of agonistic interactions. Three critical periods were selected during which the changes emerging in male mice were studied in process of development of experimental depression: from the norm to severe behavioral pathology.

Stage-T3 (acute stress) – The initial stage or stage of acute stress typically occurs after 2–3 days of agonistic interactions and is obviously caused by elevated corticosteroids levels in the blood of animals (Serova and Naumenko 1991). This assumption was supported by a large number of hemorrhages and erosions of the gastric mucosa in the Losers-T3 (Kudryavtseva et al. 1991a). In social behavior of defeated males active defense against attacks of an aggressive opponent prevails (flights, counterattacks). On the day after defeat the role reversal from defeater to offender was still possible. During this period the motor and exploratory activities of the Losers-T3 in most of behavioral tests did not differ significantly from those of intact animals.

Stage-T10 (forming pathology) – After 10 days of confrontations the Losers-T10 avoided approaching the partition and demonstrated fear towards the aggressor behind the partition. They were less interested in an unfamiliar partner in the neighboring compartment. During this period active defense (including flight) as responses to the attacks of an aggressive partner was progressively replaced by passive behaviors (Kudryavtseva et al. 1991a). At the moment of an attack by an aggressive partner defeated males often displayed a posture of complete submission – “on their back,” which persisted for a long time after the aggressor stopped attacks and retreated, which was considered as a result of panic reaction. This behavior resembled the previously described behavior of the intruder during the daily attacks of the resident (Miczek et al. 1982): when the resident attacked or threatened, the intruder displayed flight or defensive behavior, standing on its hind legs with his ears pulled in and tracking the movement of the resident. This pose was demonstrated by the intruder even before the attack of the resident.

Stage-T20 (depression) – After 20 days of confrontations the Losers-T20 displayed all the signs described in Table. 1. There are changes in physiology, as well as in social and individual behaviors. A distinctive form of behavior that is not found in animals at other stages is the posture of indifference (“nose to the corner” or “nose to the sawdust”), which the losers demonstrate during the entire testing period and thereafter, regardless of changes in the situation, lighting, presence or absence of the aggressor in the immediate vicinity. It is assumed that together with the entire set of emerging symptoms, this indicates the manifestation of deep depression-like states in animals.
Symptoms of Mixed Anxiety/Depression-Like State in Mice

Table 1 compares the symptoms of depression in humans and their possible analogs in defeated mice. Only depression symptoms with a pronounced physiological and behavioral manifestation in the losers were taken into consideration. It was shown that the Losers-T20 develop total behavioral deficit: they demonstrate decreased motor and exploratory activities in behavioral tests and increased passive swimming time (Kudryavtseva et al. 1991a; Kudryavtseva and Avgustinovich 1998) in the Porsolt test. They never demonstrate aggression in a provoking social situation. The Losers-T20 were found to have impaired communication, as indicated by a lowered interest to a familiar or unfamiliar partner in the neighboring compartment in the partition test (Kudryavtseva 2003), as well as by pronounced anxiety in the EPM and open-field tests (Avgustinovich et al. 1997; Kudryavtseva and Avgustinovich 1998), which indicates the development of generalized anxiety. Defeated mice were completely indifferent to any environmental influences (Kudryavtseva et al. 1991a, b). A decrease in body weight and testosterone levels in the blood, as well as a sustained increase in the basal level of corticosteroids (Kudriavtseva et al. 1994)
and a reduced reproduction (Kaledin et al. 1993) was observed in mice during this period. The development of psychogenic immunodeficiency was convincingly demonstrated, as evidenced by enhanced carcinogenesis (tumor growth) and other parameters of humoral and cellular immunity (Devoino et al. 1993; Kudryavtseva et al. 2007; Kudryavtseva et al. 2019).

Earlier, it has been shown that mice exposed to repeated attacks by other mice showed decreased nociception (Miczek et al. 1982). We also found a decreased pain sensitivity at the stage of deep depression-like state, even on the next day after agonistic interactions (Avgustinovich et al. 2004). Interestingly, along with persisting analgesia, a decrease in stress reactivity, which is the main symptom of depression, has been revealed: the number of hemorrhages and erosions in the gastric mucosa of the Losers-T20 did not increase on the next day after agonistic interaction, in contrast to Losers-T3 and Losers-T10 (Kudryavtseva et al. 1991a).

Cessation of the agonistic interactions and placing of the depressed males in the condition of relative rest without daily confrontations or in a neutral cage in comfortable conditions together with females for 1–2 weeks did not reverse the pathological state. Defeated mice kept displaying marked anxiety, behavioral deficit, decreased communicativeness, and high level of depressiveness, as indicated by the results of behavioral tests (Kudryavtseva et al. 1991a; Avgustinovich et al. 2005). Persistence of the resulting psychoemotional disturbances suggests that the animals have developed the behavioral pathology requiring drug treatment.

2.4 Anhedonia in the Shadow of Chronic Social Defeat Stress

Anhedonia is one of the core symptoms of major depression (DSM-5 2013), which may be broadly understood as an unwillingness to do anymore whatever used to bring pleasure and satisfaction (communicate, eat, do sex, do sports) and, overall, a lack of interest in life. For that reason, one of the main requirements towards experimental depression models is that they be able to demonstrate the development of anhedonia in the animals that have been exposed to stressful events and also exhibit other behavioral changes that are indicative of a depression-like state.

A commonly used hedonic stimulus in the experiments is an aqueous solution of sucrose or saccharin, which animals begin to give preference to after a while, in free choice of two bottles with water and sweet solution. It has been demonstrated that unpredictable physical stress, chronic mild stress, various combined stresses are able to reduce sucrose or saccharine consumption in animals (Katz 1981; Monleon et al. 1995; Willner 1997; Moreau 2002; Pothion et al. 2004; Rygula et al. 2005). Decreased sucrose consumption is considered an indication of anhedonia and, therefore, depression in animals. However, poor repeatability of the results (decrease of sucrose consumption during exposure to chronic stress) even within a single model (in particular, a chronic mild stress model (Willner 1997), and the sensitivity of sweet solution consumption to too many experimental options (animal strain; preliminary deprivation of water or food; slight differences in experimental design)
raised doubt as to the specificity of sucrose preference and whether decrease in sucrose consumption resulted from stress (Hagan and Hatcher 1997; Harris et al. 1997).

Our approach to compare sucrose solution consumption across the CSDS and control groups under the two-bottle free-choice regimen contributed to understanding the cause of variability of this parameter (Bondar et al. 2009). Additionally, supplementation of sucrose solution with small doses of vanillin, which has a scent attractive to mice, was found to be a successful trick, which allowed keeping the time required for the development of sucrose solution preference to a minimum: the highest preference (about 70% of total liquid intake) was observed as early as on day two following the introduction to control mice.

It was shown that experimental context has a strong effect on sucrose solution intake by the losers. In one experiment, the mice long familiar with the vanillin/sucrose solution demonstrated a sucrose preference of about 65–70% when exposed to defeat stress. Similarly, the mice consumed another attractive food, cheese, more willingly than standard pellets (Kudryavtseva et al. 2006a, b). It was concluded (Bondar et al. 2009) that if hedonic consumables become a regular and favored food, under chronic stress their consumption does not decrease but even may increase.

In other experiments, sucrose solution was offered to defeated animals previously unfamiliar with it. In 5–10 days, there was a decreased vanillin/sucrose solution preference by the losers as compared to the controls (10–40% of total liquid intake). It appears as though, unlike intact animals, the losers were not willing to consume vanillin/sucrose solution – they preferred water. This implies that CSDS-evoked depression-like state definitely includes its core symptom, anhedonia. It is possible that decreased sweet solution intake in mice may equally be due to enhanced anxiety or depression. A lowered consumption of sucrose solution may indicate an escape from an unfamiliar food with a strange smell and taste. Equally, this could be evidence of anhedonia or fear of new stimuli, lack of interest in them or indifference with which depressed mice were noted in our previous experimental situations (Kudryavtseva and Avgustinovich 1998). For that reason, absence of decrease in vanillin/sucrose solution intake by animals exposed to stress should not be considered as sufficient evidence of a lack of the depression-like state. Anhedonia is probably manifested by the lack of the post-deprivation effect rather than a decrease in preferred food consumption. As demonstrated in our experiments (Kudryavtseva et al. 2008; Bondar et al. 2009) 3 days of sucrose deprivation did not restore sucrose consumption. Moreover, it has been shown that under free-choice conditions the controls and the losers preferred to eat cheese (80% of total food), but not pellets. After 3 days of cheese deprivation, the least food motivation and the least level of cheese consumption were found in the losers as compared with the controls. Therefore, these observations challenge (at least in animal models) the generally accepted paradigm that anhedonia measured by reduced preferable food intake (sucrose or cheese) is a key symptom of depression.

Thus, in defeated mice anhedonia manifests itself as an abrupt reduction in sucrose solution consumption and by a failure to attain recovery after deprivation. However, it was also demonstrated that vanillin/sucrose solution intake and
preference under the two-bottle free-choice regimen strongly depend on the experimental context with prior acquaintance with the hedonic stimulus or the lack thereof, considered a likely critical factor. Decrease in sucrose solution consumption is evidence of anhedonia only when other symptoms of depression are present. Hedonic solution intake can be decreased over various conditions or diseases, in particular, a high level of anxiety or pathological aggression.

2.5 Etiology: Chronic Anxiety and/or CSDS Effects?

In our model the physical (painful) component of the impact is insignificant, since the experimenter stops the intense attack of the aggressive male by separating the opposing mice with a partition that prevents damage to the losing males. As etiologic factor, chronic social defeat stress is considered as primary etiological factor of the development of depression-like state in defeated male mice. However, agonistic interactions between group-housed males also occur daily.

It should be noted that, during the test, the attacks of the aggressor usually last several seconds. Within the framework of this experimental model, the expectation of an unfavorable development of social events is accompanied by anxiety and fear of being attacked by a strong and aggressive partner throughout the experiment, since the losers are constantly in a cage with different aggressive males behind transparent perforated partitions. They hear and see aggressor’s threats (tail rattling). When the partition returns to its place after agonistic interaction, the aggressors stay near it for a long time, climb on it, poke their nose into the holes in the partition and shake it, demonstrating a pronounced motivation to overcome the partition. The presence of aggressive motivation for such behavior is evidenced by the fact that after the partition is removed, the aggressor immediately attacks the loser or scatters his nest or bedding: total time spent near the partition before agonistic interaction correlates significantly with total time of aggression during agonistic interaction test (Kudryavtseva 2003). The losers usually do not approach the partition, almost all the time being at the opposite wall of the cage demonstrating fear and anxiety. But if they sometimes come close to the partition, the aggressor immediately returns and tries again and again to overcome the partition. Thus, the losers are constantly in a state of threat of attack from an aggressive partner around the clock. Chronic anxiety in defeated mice grows from the first days of agonistic interactions and continues to increase as the depressive state intensifies (Avgustinovich et al. 2004). It is natural to assume that the main psychopathogenic factor causing the development of mixed anxiety/depression-like disorder is chronic anxiety rather than repeated social defeats per se. The chronic anxiety and corresponding genetic background in C57BL/6 mice seems to be the decisive and most adequate etiologic factor for the development of depression-like state.
2.6 Effects of Antidepressants and Anxiolytics Under Preventive and Therapeutic Treatments

The applied pharmacological approaches (Note 2) made it possible to assess the features of the relationship between anxiety and depression in the context of the mixed anxiety/depression-like disorder in mice and to study the mechanisms of comorbidity. The logic was as follows. If the drugs, anxiolytics, or antidepressants reduce the severity of both anxiety and depression-like state, it can be concluded, under an assumption requiring verification, that these diseases have a single origin and could be termed anxious depression. If a drug affects only one symptom of psychoemotional disorder, then there are probably two independent states within mixed anxiety/depression-like disorder, and this disorder will require different pharmacological corrections for anxiety- and for depression-like states.

Note 2 Method for Screening of Psychotropic Drugs

For pharmacological studies an experimental approach for the screening of psychotropic drug effects under simulated clinical conditions was used (Fig. 1) (Kudryavtseva et al. 2008). Under preventive administration, the drugs are chronically administered to animals for 2 weeks in process of behavioral pathology formation, starting from the seventh day of social stress in order to prevent the development of depressive or anxious states. This type of injection allows identifying the protective effect of the drugs. Under therapeut-ic administration, the drugs are injected chronically to animals with formed anxiety/depression-like disorder during a 2-week period of relative rest in the absence of agonistic interactions. The effect of the drugs was studied in various behavioral tests in comparison with behavior of a similar group of saline injected males. The efficacy of the drug was assessed by comparison with the behavior of intact animals.

Fig. 1 Schemes of chronic drug administrations under preventive and therapeutics treatments of defeated mice (Galyamina et al. 2017)
It has been shown that the antidepressants imipramine and clomipramine administered preventively and therapeutically for 2 weeks had antidepressant and anxiogenic effects (Kudryavtseva et al. 1991a; Smagin et al. 2011; Table 2). Fluoxetine, a widely used antidepressant from the class of selective serotonin reuptake inhibitors (Wong et al. 1995), caused a noticeable antidepressant effect and no anxiolytic effects under therapeutic treatment (Kovalenko et al. 2007). Under preventive and therapeutic treatments diazepam had anxiolytic effect only in Losers-T10 in anxious state and did not affect depression-like state or even induced weak pro-depressive effects in mice (Galiamina et al. 2013).

Thus, within the accepted paradigm of interpreting the effects of anxiolytics and antidepressants, the results of the pharmacological study have led to the conclusion that under influence of chronic anxiety, C57BL/6 mice develop the mixed anxiety/depression-like state rather than anxious depression.

**Table 2** Preventive and therapeutic effects of anxiolytics and antidepressants on the anxiety- and depression-like states in defeated mice

<table>
<thead>
<tr>
<th>Drugs, schemes, manipulations</th>
<th>Sociability (partition test)</th>
<th>Anxiety (EPM test)</th>
<th>Depression (Porsolt test)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preventively</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazepam (0.5 mg/kg)</td>
<td>Anxiolytic Weak effect</td>
<td>Anxiolytic Weak effect</td>
<td>Pro-depressive</td>
<td>Galiamina et al. 2013</td>
</tr>
<tr>
<td>Imipramine (10 mg/kg)</td>
<td>Anxiogenic</td>
<td>–</td>
<td>Antidepressive</td>
<td>Kudryavtseva et al. 1991a</td>
</tr>
<tr>
<td>Fluoxetine (15 mg/kg)</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>Kovalenko et al. 2007</td>
</tr>
<tr>
<td><strong>Therapeutically</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine (25 mg/kg)</td>
<td>No effect</td>
<td>No effect</td>
<td>Antidepressive</td>
<td>Kovalenko et al. 2007</td>
</tr>
<tr>
<td>Diazepam (anxious mice after 10 days) (0.5 mg/kg)</td>
<td>Anxiolytic</td>
<td>Anxiolytic</td>
<td>No effect</td>
<td>Kovalenko and Kudryavtseva 2010</td>
</tr>
<tr>
<td>Diazepam (depressed mice after 20 days) CSDS (0.5 mg/kg)</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>Galiamina et al. 2013</td>
</tr>
<tr>
<td>Clomipramine 40 mg/kg</td>
<td>Anxiogenic</td>
<td>Anxiogenic</td>
<td>Antidepressive</td>
<td>Smagin et al. 2011</td>
</tr>
</tbody>
</table>
3 Dynamic Changes in Brain Serotonergic Activity Under the Development of Mixed Anxiety/Depression-Like State in Mice: Retrospective Analysis

It has been argued that the combination of anxiety and depression is quite common and most difficult to treat in humans (Gorman 1996; Lydiard and Brawman-Mintzer 1998; Stossel 2013). The animal model under consideration makes it possible to investigate the dynamic changes in various psychoemotional and brain neurochemical indicators from the norm to severe pathology.

3.1 Retrospective Introduction into the Role of Serotonin in Depression

The central role of the brain serotonergic system in depressive disorders has been confirmed by a large number of clinical and preclinical data. However, despite many decades of research, the role of serotonin (5-HT) in the mechanisms of depression is still not clear and the existing results do not allow one to come to unambiguous conclusions about the mechanisms of serotonergic regulation of this psychopathology (Curzon 1988). Initially, a hypothesis was put forward about the hypofunction of the brain serotonergic system. Some authors have associated depression with a functional brain deficiency of 5-HT (Lapin and Oxenkrug 1969; Van Praag and Korf 1974), as evidenced by a decreased level of its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid in patients with depression (Bowers 1974; Van Praag et al. 1970). Reduced reuptake of 5-HT and changes in postsynaptic serotonin receptors in depressed individuals were considered as indicators of compensatory mechanisms at diminished serotonergic activity (Meltzer and Lowy 1987). However, the subsequent studies did not produce sufficient evidence to reject the assumptions about other serotonergic activity in depression. Normal (Roy et al. 1985) or even elevated levels of 5-HIAA (Reddy et al. 1992) were found in the cerebrospinal fluid of such patients. Studies of the postmortem brains have revealed increased ligand-labeled serotonin 5-HT$_2$ binding (Arango et al. 1992; Mann et al. 1986) and number of 5-HT$_{1A}$ receptors (Matsumura et al. 1991) in the frontal cortex and blood platelets (Arora and Meltzer 1989) of suicide victims. In the postmortem samples of hippocampus of suicides, a reduced number of 5-HT$_{1A}$ receptors and their reduced affinity in the amygdala were found (Cheetham et al. 1990), although there were no differences (Arranz et al. 1994) with respect to subjects who died of accidental causes. In general, the existing studies on the role of 5-HT in depression did not provide a clear picture of the pathological process (Ad Sitsen and Montgomery 1994; Brown et al. 1994; Deakin 1994; Stahl 1992).

In addition, dysregulation of the brain monoaminergic systems is believed to be the cause of depressive illness (Ressler and Nemeroff 2000). Along with the
noradrenergic theory of depression (Schildkraut 1965; Stone 1987), impaired dopamine (DA) metabolism has been implicated in the mechanisms of depression (Fibiger 1995; Randrup and Braestrup 1977). However, as in the case of 5-HIAA, the cerebrospinal fluid of depressed patients with suicidal behavior was found to have decreased (Engstrom et al. 1999) or unchanged (Mann and Malone 1997) levels of DA metabolite, homovanillic acid. Direct measurements of the precursors and metabolites of DA in postmortem brain samples from depressed patients also gave conflicting results (Arranz et al. 1997; Bowden et al. 1997). Since antipsychotics by blocking DA receptors cause depression symptoms, while DA precursors, DA agonists, and DA reuptake inhibitors have a therapeutic effect (Kapur and Mann 1992), it becomes obvious that a decrease in dopaminergic activity in the brain definitely contributes to depression (Meyer et al. 2001). At the same time, it was shown that depression is common in Parkinson’s disease (Tom and Cummings 1998) and schizophrenia (Wong et al. 1997), the mental illnesses associated with lowered or increased activity of the dopaminergic system, respectively. Thus, as in the case of 5-HT, existing clinical and preclinical data indicate both decreased and increased DA metabolism in depression.

3.2 Dynamic Changes of Brain Serotonergic Activity in Depressive Mice

3.2.1 Serotonergic Activity in Brain Regions in Dependence on the Stage of Depression in Defeated Mice

Based on the primary data collected when studying the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), activity of tryptophan hydroxylase (TPH) and monoamine oxidase (MAOA), and binding affinity (K_d) and number (B_max) of 5-HT_1A receptors in the brain of affected animals, it could be assumed (Kudryavtseva and Bakshtanovskaya 1988) that the development of experimental depression is accompanied by dynamic changes in serotonergic activity depending on the duration of agonistic interactions (3, 10, 20 days) (review, Avgustinovich et al. 2004). The contradictory literature data within the framework of this concept may be explained by the fact that, as a rule, the researchers considered depression as an end result, thereby overlooking that it is a process in time with the activity of the brain monoaminergic systems undergoing changing as the pathology worsens.

It was critical that biochemical activity in the brain of animals in our studies was measured on the next day after the last agonistic interactions, when the effects of acute stress resulting from social confrontations were eliminated. This made it possible to record the cumulative changes sustained in the brain during the entire experimental exposure. Further studies of the brain serotonergic activity at different stages of experimental depression (Kudryavtseva and Bakshtanovskaya 1988; review, Avgustinovich et al. 2004) have confirmed this assumption. The data indicated various changes in the parameters of the brain serotonergic activity
throughout the biochemical cascade of events (synthesis, catabolism, or reception) depending on the depth of developing psychopathology, as well as the brain region.

It has been shown that Stage-T3 is accompanied by an increase in 5-HT levels in hypothalamus, amygdala, and dorsal striatum. In the midbrain decrease of 5-HT levels and increase of metabolic coefficient (5-HIAA/5-HT) were found. It was natural to assume that at this stage there is nonspecific activation of the serotonergic system in defeated males in response to acute social stress. Many authors have also shown a stress-induced increase in the level of 5-HT in the brain of animals (Chaoouloff 1993; Kawahara et al. 1995; Malyszko et al. 1994). Increase in the levels of 5-HT and 5-HIAA was found in the midbrain, pons, frontal cortex, and hippocampus of rats 20 h after exposure to electric shock (Adell et al. 1989). In a model with unavoidable electroshock in rats, an increased level of 5-HT was found in the amygdala (Kawahara et al. 1995) and hippocampus (Malyszko et al. 1994).

The Stage-T10 is accompanied by the development of severe anxiety-like in the losers (Avgustinovich et al. 2004). The period of transition from norm to pathology is characterized by maximum changes in the serotonergic parameters compared to the control. The 5-HIAA level, TPH activity, and 5-HT\textsubscript{1A}(B\textsubscript{max}) were decreased in the amygdala, 5-HIAA level and 5-HIAA/5-HT coefficient in the nucleus accumbens, increase of 5-HT level and decrease of 5-HIAA level and 5-HIAA/5-HT and 5-HT\textsubscript{1A} (B\textsubscript{max}) in the hippocampus, decrease of 5-HT level and MAOA activity in the dorsal striatum, and increase of 5-HT level and TPH activity in the hypothalamus and 5-HIAA level and 5-HIAA/5-HT coefficient in the midbrain. It was hypothesized that the reason for pronounced (pathological) anxiety that attends in the losers during this stage is due to an imbalance in the serotonergic activity in different brain regions: hypofunction in the limbic regions containing neuron endings (amygdala, nucleus accumbens, and hippocampus) and activation in the regions containing neuron bodies (hypothalamus and midbrain).

At Stage-T20 of pronounced depression-like state (review, Avgustinovich et al. 2004), in all brain regions of depressed mice (with the exception of hypothalamus) no differences were found in the levels of 5-HT and 5-HIAA as compared with the controls. However, the number of 5-HT\textsubscript{1A}(B\textsubscript{max}) receptors and their binding affinity (K\textsubscript{d}) were increased in the amygdala (Avgustinovich et al. 2001), and the activities of the TPH and MAOA were decreased in the hippocampus. At different stages of developing depression, the level of 5-HT and TPH activity was found to increase in the hypothalamus, which clarifies its role in the mechanisms of anxiety and depression.

The dynamical changes in 5-HT uptake into blood platelets, which has generally been recognized as a peripheral marker of the brain neuronal activity (Grahame-Smith 1988; Von Hahn et al. 1980), are in favor of the hypothesis that in depressive-like mice the functional activity of the serotonergic system decreases: the levels of blood platelet 5-HT at Stage-T3 and Stage-T10 were stable and significantly decreased at Stage-T20 (Avgustinovich et al. 2004).

Finally, it was assumed that the earlier stages of developing depression are accompanied by the activation, and the later stage, apparently, by the exhaustion of the serotonergic system (hypofunction).
3.2.2 Pharmacological Study of Serotonin Receptors Sensitivity

Pharmacological analysis was used to compare the intensity and direction of the behavioral response to the acute administration of \( 5\text{-HT}_{1A} \) receptor agonists in animals from different experimental groups (the controls and losers at Stage-T10, and -T20). In this case, within each corresponding group, the behavior of drug-treated mice was compared with that of saline-treated controls. Losers-T10 and control mice were found to react differently to \( 5\text{-HT}_{1A} \) receptor agonist flesinoxan (0.5 mg/kg): after drug injection the control males demonstrated decreased communication scores, whereas Losers-T10 did not display any changes in their behavior (Kudriavtseva et al. 1996). A similar effect was observed after the acute administration of the \( 5\text{-HT}_{1A} \) receptor agonist ipsapirone (3 mg/kg) to Losers-T20, indicating a decreased pharmacological sensitivity of serotonergic receptors to stimulation (Avgustinovich et al. 2003). Thus, it was assumed that the development of mixed anxiety/depression-like state is accompanied by desensitization \( 5\text{-HT}_{1A} \) receptors to the stimulating action of \( 5\text{-HT} \) (Kudriavtseva et al. 1996). Oppositely, the \( 5\text{-HT}_{2} \) receptor antagonist ritanserin (2.0 mg/kg) was ineffective in the control mice but caused changes in Losers-T10. These data are in agreement with the finding that suicide victims and depressed patients have a reduced number of \( 5\text{-HT}_{1A} \) receptors in the hippocampus or their reduced affinity in the amygdala (Cheetham et al. 1990). A change in the receptor sensitivity following a change in the serotonergic activity supports the hypothesis of dynamic changes during the development of the depression pathology.

The hypothesis of dynamic changes in the activity of brain monoaminergic systems under the influence of chronic psychosocial emotional stress was confirmed by E. Fuchs and colleagues in a series of works (Fuchs and Flugge 2002; Flugge 1995) using a similar behavioral model for tree shrews. In subordinates who were housed in pair-cages with aggressive partners separated by a wire mesh, different types of adrenergic receptors (Flugge et al. 1997) and \( 5\text{-HT}_{1A} \) receptors (Flugge 1995) were down- or up-regulated depending on the duration of social interactions.

3.2.3 Neurogenomics of the Serotonergic System in Depressive Mice

Whole transcriptome analysis (RNA-Seq) was used to identify changes in the expression of serotonergic genes encoding the tryptophan hydroxylase 2 (\( Tph2 \)), serotonin transporter (\( Slc6a4 \)), monoamine oxidase A and B (\( Maoa, Maob \)), DOPA decarboxylase (\( Ddc \)), and different serotonin receptors (\( Htr\)s) involved in the synthesis, inactivation, and reception of \( 5\text{-HT} \) in the brain regions of defeated male mice at Stage-T20 (Kudryavtseva et al. 2017). The most changes in gene expression were found in the midbrain raphe nuclei: The \( Tph2, Ddc, Slc6a4, Htr2a, Htr3a, \) and \( Htr5b \) genes were downregulated and the \( Htr4 \) gene was upregulated. In the ventral tegmental area, the \( Tph2, Ddc, Maob, Htr1a, Htr4, \) and \( Slc6a4 \) genes were upregulated and \( Htr3a \) gene was downregulated. In the hypothalamus of the
defeated mice, the *Maoa* gene was downregulated, and the *Htr6* gene was upregulated. In the dorsal striatum, the *Htr1a* gene was downregulated and the *Htr7* gene was upregulated. The *Htr1b* gene was upregulated in the hippocampus (Kudryavtseva et al. 2017).

Earlier it has been shown that chronic or acute social defeat stress modulates 5-HT$_{1B}$ mRNA in the dorsal and ventral striatum, and daily social defeat stress leads to an increase in 5-HT$_{1B}$ receptor mRNA levels in nucleus accumbens (Furay et al. 2011).

These findings are consistent with the earlier RT-PCR data (Boyarskikh et al. 2013). Moreover, the decreased expression of serotonergic genes in the midbrain raphe nuclei persists for a long time even after two weeks of relative rest. Although the expression patterns of the serotonergic genes in brain regions may differ, it is obvious that in the midbrain raphe nuclei with the densest population of serotonin neuron bodies, decreased expression of serotonergic genes is noted at Stage T20, which may indirectly support the assumption on decreased metabolism of serotonin in mice with depression-like state.

It should be noted that neurogenomics data obtained over the last few years by whole transcriptome analysis revealed changes in the expression of numerous genes in brain regions of mice with mixed anxiety/depression-like state, namely, of mitochondrial (Babenko et al. 2018), ribosomal (Smagin et al. 2016), monoaminergic (Kudryavtseva et al. 2004; Kovalenko et al. 2016; Babenko et al. 2020), autistic (Kudryavtseva et al. 2018) genes and the changes in the expression of neurotrophic transcription factors (Berton et al. 2006; Kudryavtseva et al. 2010) and collagen (Smagin et al. 2019) genes. Thus, it may be assumed that changes in serotonergic activity in brain regions and blood as a result of the development of psychopathology in male mice, over time, lead to changes in cellular, molecular, and neurogenomic processes.

With growing knowledge about the dynamics of neurochemical changes of the brain, the treatments of developing pathology are increasingly discussed in the context of disease progression. So, if a bad mood is accompanied by a complex of somatic abnormalities (decreased body weight, sexual dysfunction, immune suppression, etc.), as well as by low mobility and indifference, indicating an advanced stage of depression, one may assume that the underlying cause for these changes is hypofunction of the monoaminergic systems and can suggest neurotransmission-enhancing drugs as a potential treatment choice. Obviously, the same drugs can worsen the condition of patients with increased serotonergic activity and anxiety. Theoretically, at this stage drugs that temporarily block serotonergic transmission or, for example, relieve anxiety and stress will be more effective, which, in turn, will mitigate the effects of depression-provoking factors. However, we do not know how “to treat” genes, the altered expression of which can cause relapses of the disease even after successful treatment by traditional medicines.
4 Genetic Predisposition to the Development of Anxiety and Depression-Like States

Heredity plays a major role in the etiology of many disorders, in particular depression and schizophrenia (Mackinnon and Mitchell 1994; Sham 1996; Bassett et al. 2002). This follows from the studies of twins or families suffering from these diseases. However, according to many theories (Rosenthal 1971), it is not the disease itself that is inherited, but a predisposition to it is. Environmental factors, often of a psychogenic nature, and stressful situations form a provoking background and determine the likelihood of development of depression in persons predisposed to it.

Traditionally, the experimental genetic approach focuses on the study of behavioral variability in inbred (Trullas and Skolnick 1993; Crawley et al. 1997; Willner and Mitchell 2002) and in genetically modified (reviews, Wood and Toth 2001; Mohammad et al. 2016; Scherma et al. 2019) animals. Numerous studies have demonstrated differences in the manifestation of “trained helplessness” and anxious behavior in mice (Crawley et al. 1997). Some rat lines, such as Wistar-Kyoto (Pare 2000), FSL (Flinders Sensitive Line) (Yadid et al. 2000), HDS (high DPAT-sensitive) (Gonzalez et al. 1998; Overstreet et al. 2003), KHA (Koltushi High Avoidance) (Zhukov and Vinogradova 2002), and different mouse strains (Söderlund and Lindskog 2018; Patel et al. 2019) are regarded as adequate genetic models that can be used to understand the mechanisms of depression.

Effects of CSDS on the behavior of mice were also studied in the CBA/Lac and DBA/2 strains and outbred CD-1 mice after prolonged agonistic interactions using a battery of tests similar to that used for the C57BL/6 J mice. Similarly, in the C57BL/6 J, CBA/Lac, and CD-1 losers, a CSDS induced an increased immobility behavior in all tests, decreased communications in the partition test, and the development of pronounced anxiety as according to EPM scores. However, in some tests the CSDS induced diverse effects which are presumably mediated by heredity differences in psychoemotional state in mice of different strains.

Specific features that could characterize the depressive C57BL/6 J mice are high level of innate anxiety (trait anxiety) (Avgustinovich et al. 2000; Kudryavtseva et al. 2006a, b): in familiar conditions (home cage) they responded to novel stimulus with high levels of anxiety, while in unfamiliar conditions (open-field test) they display an active behavioral strategy, which, however, may be a consequence of panic reaction in the stress condition. These mice respond to prolonged social stress with further increases in anxiety level, leading to the formation of a depression-like state.

The CBA/Lac mice are notable for their propensity to manifest catatonic or cataleptic-like behaviors, freezing in frightening or novel situations (Kudryavtseva and Bakshtanovskaya 1989; Kulikov et al. 1995; Lipina et al. 2003; Kudryavtseva et al. 2006a, b), which were interpreted as the signs of pronounced “state” anxiety (Avgustinovich et al. 2000). The CSDS did not enhance immobility in the Porsolt test. Thus, the different effects of the CSDS on the development of one or another psychoemotional disorder can probably be due to the peculiarities of innate anxiety in animals of different strains.

After 20 days of the CSDS the CD-1 losers demonstrated a unique combination of pronounced anxiety and depressiveness according to the EPM and Porsolt tests,
respectively, and abnormal locomotor activity in the open-field test (Kovalenko et al. 2015). This phenomenon may be viewed as specific for the CD-1 mice. It was suggested that these animals may be potentially used for modeling the key symptoms of bipolar disorder.

The DBA/2J losers were less sensitive to the CSDS than the mice of other strains: under repeated defeats exploratory behavior and locomotor activity in the open-field test did not change, and no signs of depressiveness were detected (Vishnivetskaya et al. 2016).

Interestingly, in a modification of this model (Berton et al. 2006; Golden et al. 2011), some defeated mice of the C57BL/6 strain were shown to be less susceptible to the CSDS: in 40–50% of the defeated males no depression-like behaviors were observed after 10 days of agonistic interactions. Intermittent or daily social defeat stress can produce different effects on many behavioral and neurochemical parameters of animals (Miczek et al. 2011). Thus, genetically defined characteristics and/or susceptibility to stress affecting psychoemotional states as well as experimental context may be responsible for the development of specific symptoms under the CSDS.

5 General Discussion: Chronic Anxiety and Social Consequences

According to a generally accepted definition (DSM-5), anxiety is an emotional state arising in the situations of uncertain danger that is accompanied by anticipation of adverse events. Any instability, violation of the usual course of things can lead to the development of anxiety. Anxiety subsides with the disappearance of the threat.
As a rule, a high level of anxiety is associated with an unfavorable environment, especially if the anxiety is chronic, and there is a consciously or unconsciously perceived threat to a person’s life. In this case, chronic anxiety can become an aggravating factor in the development of many diseases, which increases the severity of anxiety, thus closing a vicious circle. Excessive anxiety is characterized by hypertrophy, tension, lack of motivation, inadequacy, and preservation of the emotional state after the disappearance of the stimulus (DSM-5). Anxiety can take on a generalized form, which is characterized by a feeling of insecurity. Situations that trigger anxiety are varied, and the manifestations of anxiety are individual and multifaceted. Many people suffer from this state (Stossel 2013). It is the degree of severity of anxiety that determines the border between normality and pathology, disease, and health.

Severe anxiety can be a cause and effect of an illness, as well as a harbinger or sign of its development. Clinical data indicate that symptoms of anxiety and/or depression are often observed in many neuropsychiatric diseases, such as obsessive-compulsive disorder, post-traumatic stress disorder, schizophrenia, epilepsy, Alzheimer’s disease, drug addiction, attention deficit and hyperactivity syndrome, autism, Tourette’s syndrome, Huntington’s disease, and others (DSM-5 2013; reviews Goodwin 2015; Remes et al. 2016). Depression and anxiety can also result from medication, or they can be concomitant conditions that develop independently of the underlying disease. Factors inducing manifestation of depression and anxiety may differ.

Thus, there are exogenous factors, such as adverse environmental and social influences leading to the formation of chronically elevated anxiety. Under prolonged exposure, chronic anxiety can provoke the development of various diseases in individuals with genetic predisposition thereto. Increased anxiety may also be of endogenous origin, developing as a result of an imbalance in the neurotransmitter systems involved in the pathogenesis of somatic or psychoneurological disorders or as a result of medication. In this case, the development of anxiety may be secondary to the primary disease.

Comorbidity of generalized anxiety disorder and major depression is regarded in many publications (reviews, Gorwood 2004; Goodwin 2015; Crocq 2017). It was widely recognized that anxiety may be predictor for depression and is a risk to early onset major depression (Parker et al. 1999; Nechita et al. 2018). Clinical studies have shown that classical antidepressants, such as tricyclic antidepressants, selective serotonin reuptake inhibitors, and monoamine oxidase A inhibitor, may also be effective in the treatment of anxiety disorders such as panic, phobias, post-traumatic stress disorder, and generalized anxiety. These findings indirectly support the view that the comorbidity for these disorders has common pathogenic mechanisms affecting separate components of serotonin metabolism. The genes exerting a multiplicity of actions on anxiety and depression are primarily those encoding the proteins involved in the regulation of the serotonergic system, which is implicated in the pathogenesis of both disorders (reviews, Gorwood 2004; Goodwin 2015). The results of our experiments are in compliance with this assumption.
It was also shown in our experiments that plausible candidate genes are the genes encoding tryptophan hydroxylase 2, serotonin transporter, monoamine oxidase A, and serotonin receptors, whose expressions were shown to be changed in animals with mixed anxiety/depression-like disorder (Kudryavtseva et al. 2017). Thus, chronic anxiety is associated with persistent neurochemical changes specific mainly for the brain serotonin pathways. In the first days of unavoidable social stress, this system is activated – the levels of serotonin and its metabolites increase in many brain regions. Ongoing stress and anxiety lead to excessive release of serotonin, and as a result, to depletion of the serotonergic system and, finally, to the development of psychoemotional disorders. These processes lead to a change in the expression of serotonergic genes (Kudryavtseva et al. 2017), which persist for a long time (Boyarskikh et al. 2013). It may be assumed that these persisting changes in gene function can be the cause of relapses of depression.

According to the World Health Organization, anxiety and depression are the most common psychoemotional disorders that require pharmacological correction (DSM-5 2013). In most cases disease causes and risk factors can be traced back to a person’s lifestyle. However, the social background as an etiological factor stimulates the development of chronic anxiety in individuals significantly more often than their personal problems.

Obviously, anxiety and fear have become the main emotions experienced by humanity currently. Global scale events such as climate cataclysms, economic crises, pandemics for a long time plunge into a state of alarm a huge number of people, including those who are not directly affected by these negative events. The anxiety of one person is multiplied by the anxiety of others. Strong social stress causes anxiety experienced by most of the world’s population. The consequences for both individuals and society can be very serious. They are provoked by society, and with prolonged exposure they themselves provoke the development of various psychopathological conditions, which, obviously, are social diseases by nature.

Moreover, constant state of heightened anxiety forms a person’s readiness to defend against real or imagined danger. This creates the basis for an increase in social aggression, which naturally gives rise to reciprocal aggression, which leads to the development of major anxiety. The social negative environment has the maximum effect, contributing to the manifestation of both fear and irritable aggression as defensive reaction. In such conditions and in conditions of social conflict, aggression can develop even in persons who are not inclined to manifest it.

The large-scale phenomena causing the development of anxiety and fear in society are perceived today as threat to life. Taking into account all the numerous changes that occur in the organism under the influence of chronic anxiety, first of all, decrease of reproductive function and compromised immunity, as well as their inevitable consequences – increased morbidity from stress-induced diseases (including cancer), social conflicts provoking military actions and communal riots – one can predict rapid degeneration of human population.
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Unravelling the Neuroinflammatory Mechanisms Underlying the Effects of Social Defeat Stress on Use of Drugs of Abuse

S. Montagud-Romero, J. Miñarro, and M. Rodríguez-Arias

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Abstract The immune system provides the first line of the organism’s defenses, working to maintain homeostasis against external threats and respond also to internal danger signals. There is much evidence to suggest that modifications of inflammatory parameters are related to vulnerability to develop mental illnesses, such as depression, autism, schizophrenia, and substance use disorders. In addition, not only are inflammatory parameters related to these disorders, but stress also induces the activation of the immune system, as recent preclinical research demonstrates. Social stress activates the immune response in the central nervous system through a number of mechanisms; for example, by promoting microglial stimulation, modifying peripheral and brain cytokine levels, and altering the blood brain barrier, which allows monocytes to traffic into the brain. In this chapter, we will first deal with the
most important short- and long-term consequences of social defeat (SD) stress on the neuroinflammatory response. SD experiences (brief episodes of social confrontations during adolescence and adulthood) induce functional modifications in the brain, which are accompanied by an increase in proinflammatory markers. Most importantly, inflammatory mechanisms play a significant role in mediating the process of adaptation in the face of adversity (resilience vs susceptibility), allowing us to understand individual differences in stress responses. Secondly, we will address the role of the immune system in the vulnerability and enhanced sensitivity to drugs of abuse after social stress. We will explore in depth the effects seen in the inflammatory system in response to social stress and how they enhance the rewarding effects of drugs such as alcohol or cocaine. To conclude, we will consider pharmacological and environmental interventions that seek to influence the inflammatory response to social stress and diminish increased drug intake, as well as the translational potential and future directions of this exciting new field of research.

**Keywords**  Cocaine · Ethanol · Immune system · Neuroinflammation · Social stress

**Abbreviations**

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<tr>
<td>BLA</td>
<td>Basolateral amygdala</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CPP</td>
<td>Conditioned place preference</td>
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<tr>
<td>CX3CL1</td>
<td>Chemokine (C-X3-C motif) ligand 1</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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<td>HPC</td>
<td>Hippocampus</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>LHb</td>
<td>Lateral habenula</td>
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<tr>
<td>MDMA</td>
<td>3,4-Methylenedioxyamphetamine</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
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<tr>
<td>NAcc</td>
<td>Nucleus accumbens</td>
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<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
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<tr>
<td>PrL</td>
<td>Prelimbic cortex</td>
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<tr>
<td>SD</td>
<td>Social defeat</td>
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<tr>
<td>STR</td>
<td>Striatum</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptors</td>
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<td>TLR4-KO</td>
<td>TLR4 knockout</td>
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<tr>
<td>TNFa</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>vHPC</td>
<td>Ventral HPC</td>
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<tr>
<td>VWR</td>
<td>Voluntary wheel running</td>
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1 Introduction

Our immune system is composed of a series of defense mechanisms that are activated in response to harmful stimuli so as to maintain the homeostasis of our organism. The immune system is composed of two arms: a non-specific innate arm, which constitutes the first line of defenses against foreign organisms and offers early protection against a large variety of pathogens; and the acquired arm, which acts through specific antibodies that attach to their target pathogen (Delves and Roitt 2000; Nicholson 2016). These subsystems are activated by threats from internal (e.g., damaged cells) or external (bacteria or viruses) environments (Masi et al. 2017). It is now known that innate immunity is a highly sophisticated system for maintaining a healthy tissue microenvironment (Hato and Dagher 2015). Different types of cell, such as macrophages, dendritic and natural killer cells, and epithelial cells, which function as a physical barrier, participate in the immune system. All of these cells are crucial, as they detect and process threat signals by activating pattern-recognition receptors (Chaplin 2010; Roh and Sohn 2018). When activated, these receptors release specific products, like cytokines and chemokines. Cytokines are recruiting proteins that attract cells, molecules, and fluid into the affected site, causing inflammation as a result (Chaplin 2010; Roh and Sohn 2018). Chemokines, on the other hand, are small secreted cytokines commonly described as chemo attractants and can be homeostatic or inflammatory, depending on their function (Koper et al. 2018; Mecca et al. 2018). Subsets of T and B cells also participate in immunity by secreting antibodies (the acquired immune system) when pathogens cannot be neutralized by the activated innate arm. Both branches of the immune system work together in a global, cooperative, and regulated way to maintain the normal functioning of the body (Masi et al. 2017).

The central nervous system (CNS) is protected from external influences and intruders by the blood brain barrier (BBB), but this protection is far from absolute, and varies with age and brain region. Several studies have demonstrated an interaction between immune cells and the CNS, since the immune system includes signals in the CNS that detect the presence of any infection. At the same time, the CNS processes signals and adjusts immune function (Dantzer et al. 2008; Dantzer 2017; Soria et al. 2018). The neuroimmune response relies on microglia, immune cells that reside in the CNS and which are activated when pathogens or damaging substances disrupt homeostasis (Kigerl et al. 2014; Venegas and Heneka 2017). Alterations in how the immune and nervous systems communicate may explain different pathological conditions, including psychiatric disorders and several immune-mediated diseases (Filiano et al. 2015; Lasselin et al. 2018).
1.1 Inflammation and Mental Health

Recent research points to the role of the inflammatory system in the development of mental illnesses such as depression or anxiety (Dantzer et al. 2008; Miller et al. 2009; Soria et al. 2018; Liu et al. 2020). Increases in proinflammatory markers can cause sadness, anhedonia, fatigue, and symptoms related with depressive disorder (Slavich and Irwin 2014). Likewise, autism, schizophrenia, and substance use disorder are associated with modulations of the immune system, suggesting a dysregulation of immune function underlies many psychiatric disorders (Morgan et al. 2012; Frick et al. 2013; Cui et al. 2014; Torres-Platas et al. 2014; Fiedorowicz et al. 2015; Réus et al. 2015; Ménard et al. 2017). For instance, chronic neuroinflammation, oxidative stress, and mitochondrial dysfunction have been reported in autism spectrum disorder (Kumar et al. 2012), and schizophrenia has been linked with cytokine disruption and proinflammatory protein production (Miller et al. 2011; Upthegrove et al. 2014; Khandaker et al. 2015). Furthermore, peripheral immune modulators have been shown to induce psychiatric symptoms in clinical and preclinical models (Dantzer et al. 2008; Harrison et al. 2009; Eisenberger et al. 2010; Haroon et al. 2012). Consequently, risk factors for mental illnesses include medical conditions associated with chronic inflammatory and immunological alterations (Leboyer et al. 2012).

Similarly to depression, intake of drugs of abuse has been related with modulations of the immune system (Harricharan et al. 2017; Nennig and Schank 2017). Acute and chronic exposure to alcohol produces an increase in cytokine release through interaction with the pattern-recognition receptors expressed in immune cells, known as Toll-like receptors (TLR). Human and animal studies (Crews et al. 2006; Mayfield et al. 2013; Coleman and Crews 2018; Guerri and Pascual 2019a) have shown that alcohol triggers signaling pathways by activating innate immune system receptors (e.g. TLR) and (NOD)-like receptors (e.g., inflammasome NLRs) in glial cells, increasing the production of proinflammatory cytokines and chemokines, which in turn leads to neuroinflammation and brain damage (Montesinos et al. 2016a; Guerri and Pascual 2019b). Specifically, TLR4 plays a crucial role by inducing astrogliosis and microgliosis in response to alcohol binge consumption (Pascual et al. 2007; Vetreno and Crews 2015; Montesinos et al. 2015, 2016b). When TLR4 knockout (TLR4-KO) rodents are employed, ethanol-induced activation of cytokines and inflammatory mediators is absent (Montesinos et al. 2015, 2016b). Accordingly, blockade of the alcohol-induced neuroimmune response has been shown to affect ethanol consumption (Robinson et al. 2014). Likewise, psychostimulants such as cocaine or methamphetamine also activate microglia and peripheral immune cells (Moratalla et al. 2017; Xu et al. 2017; Sil et al. 2019). Moreover, studies show that the integrity of the BBB is affected by repeated cocaine use, which increases its permeability (Kousik et al. 2012; Rodríguez-Arias et al. 2017). Cytokines like interleukin (IL)-1β, or chemokines like chemokine (C-X3-C motif) ligand 1 (CX3CL1), have been identified as predictors of cocaine use,
showing a correlation with the severity of cocaine symptoms, according to DSM-IV-TR criteria for cocaine abuse/dependence (Araos et al. 2015).

1.2 Inflammation and Stress

Immune signaling also regulates the hypothalamic-pituitary-adrenal (HPA) axis and brain processes that modulate affective behavior in the face of exposure to a stressor (Haroon et al. 2012). These two systems are bidirectionally related, so exposure to painful and stressful experiences alters the HPA axis, triggers the immune system, and activates proinflammatory mechanisms (Silverman et al. 2004; Tracey 2009; Haroon et al. 2012; Michopoulos et al. 2017). Stress can provoke different behavioral patterns and physiological responses that impact directly on the organism, altering neural and behavioral development, modulating physiology and behavior, and contributing to increased morbidity and earlier mortality across nearly all species studied (Bath et al. 2017). Numerous studies have sought to understand the phenomena of psychological and social stress, as well as its consequences for the body’s systems. The most representative stress paradigm used in animal models is social defeat (SD) stress due to its high translational value (Miczek et al. 2008; Hammels et al. 2015). The SD procedure consists in aggressive confrontation between two conspecific males in a repeated and intermittent manner (Covington and Miczek 2001), imitating the subordination status that can occur in human relationships (Selten et al. 2013). A defeat is defined when the intruder displays a supine posture for five consecutive seconds, a response that typically occurs after three-to-five biting attacks from the resident, at which point the confrontation is terminated. As most studies have been performed in laboratory rodents, the behavioral characteristics of social defeat have been defined very specifically in these species (Miczek et al. 2008). For example, defeated mice adopt an upright posture with limp forearms and head angled upward and emit audible vocal signals. On the other hand, defeated rats present a supine posture with limp extremities and emit loud and frequent ultrasonic vocalizations (Miczek et al. 1982; Tornatzky and Miczek 1993). Social defeat also affects other typical behaviors of the species, with defeated animals engaging in less motor activity and exploration, reduced eating behavior or even less water intake (Meerlo et al. 1996). In addition, defeated animals exhibit social withdrawal (Kudryavtseva et al. 1991; Meerlo et al. 1996; Huhman et al. 2003), less sexual behavior (Yoshimura and Kimura 1991), and respond with submission behaviors and vocalizations (Potegal et al. 1993). Exposure to SD stress induces a wide range of physiological and endocrine responses, with a significant increase in corticosterone levels (Montagud-Romero et al. 2016b; Rodríguez-Arias et al. 2017), and changes in numerous neurotransmitter systems, such as the serotonergic, dopaminergic, or GABAergic systems (Montagud-Romero et al. 2018).

Research has consistently demonstrated the consequences of SD and drug consumption, showing that the rewarding effects of cocaine, amphetamine, 3,4-metilendioxi-metanfetamina (MDMA), or alcohol are increased in defeated
rodents exposed to any one of a diverse set of SD methodologies (Burke et al. 2013; García-Pardo et al. 2015; Montagud-Romero et al. 2017, 2018; Newman et al. 2018). However, although SD has been strongly related with substance use, the exact mechanisms at play have not been clarified. Currently, there is no doubt that social stress activates the inflammatory system within the brain, increasing the number of macrophages, stimulating microglia and leukocytes, and modifying peripheral cells and inflammatory mediators (Stankiewicz et al. 2015; Pfau and Russo 2016; Rodríguez-Arias et al. 2018). All these data suggest that social stress is an activator of the inflammatory system and a factor involved in the increased sensitivity to the reinforcing effects of drug intake.

This chapter will first assess the most important results of the short- and long-term consequences of SD stress on the neuroinflammatory response, highlighting along the way the differences between resilient and susceptible rodents. In a second section, the role of the immune system in the enhanced vulnerability and sensitivity to drugs of abuse after social stress will be addressed. We will also briefly discuss pharmacological and environmental interventions that may influence the inflammatory response to social stress and diminish increased drug intake, as well as the translational potential and future directions of this field of research.

2 Social Defeat Stress Activates the Neuroinflammatory Response

Psychological stress is the origin of the cascade of neuroimmune reactions involving brain-to-immune and immune-to-brain signaling that converge to influence mood and behavior (Wohleb et al. 2015). Initial studies showed that social stress, such as repeated social defeat or social disruption, increases myeloid (CD11b+) cells in the blood, and that these cells maintain an inflammatory and glucocorticoid-insensitive phenotype in mice (Engler et al. 2004, 2005; Avitsur et al. 2005; Powell et al. 2009; Wohleb et al. 2011). The neuroinflammatory process is also mediated by CD11b+ cells activated in the brain (microglia and CNS macrophages), which are the main regulators of the inflammatory response to repeated and acute SD in mice (Stankiewicz et al. 2015; Serrats et al. 2010; Yirmiya and Goshen 2011). These results were extended when it was shown that SD augments circulating monocytes (CD11b_/SSClo/Ly6Chi) and brain macrophages (CD11b_/CD45hi) in a cycle-dependent manner that corresponds with the development of anxiety (Wohleb et al. 2014a). Accordingly, when CD45 cells were labelled, the number of monocytes in the hippocampus (HPC) of mice was found to have increased after six cycles of repeated SD (McKim et al. 2016). Immune cells such as Iba-1+ microglial cells, whose levels have been shown to increase in the HPC and prefrontal cortex (PFC) of defeated mice and rats exposed to chronic SD (Tian et al. 2019; Ferle et al. 2020), would seem to contribute to macrophage recruitment (Wohleb et al. 2013).
Increments in short- and long-term proinflammatory cytokines – e.g. tumor necrosis factor alpha (TNFa), IL-6, and IL-1 β – after chronic and intermittent SD exposure have been reported in numerous brain structures (the striatum (STR)), HPC, and PFC in both mice and rats (Deng et al. 2019; Ferrer-Pérez et al. 2018; Gao et al. 2019a, b; Kopschina Feltes et al. 2019; Tian et al. 2019; Ben-Azu et al. 2020; Ferle et al. 2020; Jiang et al. 2020; Nozaki et al. 2020; Montagud-Romero et al. 2020). Levels of other cytokines, such as IL-7, IL-10, IL-12, IL-17, and IL-18, also increase in limbic areas immediately or several days after the last agonistic encounter when rats and mice undergo repeated and chronic SD (Finnell et al. 2019; Song et al. 2020; Nozaki et al. 2020). Remarkably, in the case of mice, most of the cytokines whose levels rise in brain areas are also increased in serum (Liu et al. 2019; Niraula et al. 2019; Jiang et al. 2020; Zhu et al. 2019) after chronic or intermittent SD.

Immediate and long-lasting changes in chemokine levels after repeated SD have also been described in several mice brain areas, such as the HPC and STR (Montagud-Romero et al. 2020; Reguilón et al. 2020a). Stimulation of the chemokine (C-X3-C motif) ligand receptor of brain microglia may produce anti-inflammatory actions (Cardona et al. 2006; Corona et al. 2010) and promote microglial quiescence under homeostatic conditions (Wolf et al. 2013). Microglial activation following repeated SD has been consistently associated with a reduced expression of mice brain CX3CL1 (Wohleb et al. 2013) and its receptor (Wohleb et al. 2014b). Accordingly, intermittent SD induces a decrease in CX3CL1 striatal levels without changes in the HPC (Montagud-Romero et al. 2020). However, the opposite has been reported by Reguilón and co-workers (2020a), who observed increased protein levels of the chemokines CX3CL1 and CXCL12 in the STR of mice after intermittent SD. It is relevant to point out that these results were obtained in different strains of mice (OF1 vs C57), whose sensitivity to SD varies (for example, the OF1 strain is more reactive). In addition, chronic stress has been shown to up-regulate CXCL1/CXCR2 messenger RNA (mRNA) expression in the HPC (Song et al. 2020) and CXCL1 and CXCL2 in enriched microglia/macrophages (Sawicki et al. 2015) of mice. Together, these data provide evidence of the influence of adhesion molecules and chemokines in facilitating the recruitment of myeloid cells to the brain in response to social stress.

SD also affects the integrity of the BBB. Under non-stress conditions, cytokines in peripheral blood only can cross into the CNS through circumventricular organs and cytokine-specific transporters (Zhu et al. 2019). Mice exposed to intermittent SD during adolescence and evaluated in adulthood show a marked reduction in the expression of the tight junction protein claudin-5 and an increase in basal laminin degradation in the nucleus accumbens (NAcc) and HPC, which, in turn, increases permeability of the BBB (Kernagis and Laskowitz 2012; Rodríguez-Arias et al. 2017). Peripheral immune cells bind to the vascular endothelium of the BBB through adhesion proteins such as E-selectin (Ransohoff et al. 2003). SD increases the quantity of these proteins depending on the number of exposures to social stress, forming very firm unions due to the increase of peripheral chemokines (Sawicki et al. 2015). Once the vascular endothelium has been crossed, the foot of the astrocytes constitutes a second barrier that is damaged by the metalloproteinases.
expressed by macrophages (Bechmann et al. 2007). In addition, the BBB transmits inflammatory signals to the CNS. When cytokine receptors present in endothelial cells are activated, they induce the production of more inflammatory cytokine and other mediators such as prostaglandins (Bebo Jr and Linthicum 1995; Ericsson et al. 1997; Vallières and Rivest 1999). Recent studies by Lehmann et al. (2018, 2020) suggest that, in animals susceptible to SD, the immune response generated in the CNS is mediated by vascular damage and blood leakage across the BBB. Mice susceptible to the effects of SD show specific impairments of the BBB, presenting microhemorrhages and microbleeds (Lehmann et al. 2020). Resilient animals, that do not exhibit depressive behaviors or increased permeability of the BBB, seem to exhibit vascular adaptations that maintain the integrity of the BBB. In this context, it has been hypothesized that permissive epigenetic changes which allow transcriptional activity in the promoter of the CLDK5 gene guarantee the integrity of the BBB despite the stress situation (Dudek et al. 2020).

Long-lasting alterations in microglia activation have also been documented in the NAcc and prelimbic cortex (PrL) of mice 3 weeks after the last intermittent SD (Rodríguez-Arias et al. 2018); a reduction in morphotypes 1 and 2 – associated with a less inflammatory response – and increases in morphotypes 3, 4, and 5 – considered reactive microglia morphotypes and associated with a more inflammatory response – were observed in both structures. Repeated social stress also induced a long-lasting reduction in the number of Glial fibrillary acidic protein + cells in the NAcc, with astrocytes assuming a role as the main supporting glia for neurons (Rodríguez-Arias et al. 2018). Similar results were obtained in the HPC of rats immediately after the last SD of a 5-week period of daily sessions (Araya-Callís et al. 2012). These results suggest that SD induces short- and long-term changes in glial cells.

Activation of TLR, chemotactic receptors like CCR2, and co-stimulator receptors is also enhanced by chronic social disruption stress in mice (Avitsur et al. 2003; Powell et al. 2009; Hanke et al. 2012; Weber et al. 2017). Substantial up-regulation of the transcription factor NFkB p-p65 and proinflammatory proteins such as IL-1β and IL-17A has recently been observed and has been linked with the TLR4 response in the brain of defeated mice in a chronic or intermittent SD paradigm (Ben-Azu et al. 2020; Montagud-Romero et al. 2021). The transcription factor NFkB activates the proinflammatory signal transduction that results in neuroocyte injury and apoptosis and affects the transcription of many proinflammatory cytokines and chemokines. However, this effect is absent in TLR4-KO mice, who do not show increased cocaine or alcohol reward, thus highlighting the critical role of TLR4 in the effects of chronic or intermittent SD. In addition, an increase in the number of CCR2+ macrophages in the brain of chronic SD mice was demonstrated by Wohleb et al. (2012); accordingly, the genetic knockout of CCR2 prevented stress and monocyte recruitment to the brain in response to neuroinflammation (Wohleb et al. 2013). A detailed description of the studies included in this section is provided in Table 1.
### Table 1: Detailed description of the effects produced by social defeat stress on the neuroinflammatory response

<table>
<thead>
<tr>
<th>Authors</th>
<th>Animal</th>
<th>Social defeat</th>
<th>Type of SD</th>
<th>Short or long term</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ben-Azu et al. (2020)</td>
<td>Swiss Albino mice</td>
<td>7 days</td>
<td>Chronic</td>
<td>After Behavioral Tests (Short)</td>
<td>↑TNF-α (STR, HPC, PFC) ↑IL-6 (STR-PFC) ↑NFKB (PFC, HPC) ↑Nox-2 (PFC-HPC) ↑COX-2 (STR, PFC, HPC)</td>
</tr>
<tr>
<td>Deng et al. (2019)</td>
<td>C57BL/6 male adult mice</td>
<td>10 days</td>
<td>Chronic</td>
<td>8 days after the last SD</td>
<td>↑TNF-α, IL-6, IL1β (HPC)</td>
</tr>
<tr>
<td>Ferle et al. (2020)</td>
<td>Wistar rats</td>
<td>5 days per week (3 weeks)</td>
<td>Chronic</td>
<td>2 h after SD</td>
<td>↑Iba-1 cells (BLA) ↑PPARγ immunopositive cells (MOPFC and BLA) ↑PPARγ mRNA levels (AMY and PFC) ↑mRNA IL1b and TNF-α (HPC and AMY)</td>
</tr>
<tr>
<td>Ferrer-Pérez et al. (2018)</td>
<td>OF1 mice</td>
<td>4 days</td>
<td>Intermittent</td>
<td>After the 4 SD (Short)</td>
<td>↑IL6 (STR, PFC, serum)</td>
</tr>
<tr>
<td>Finnell et al. (2019)</td>
<td>Male Sprague-Dawley rats</td>
<td>5 days + (1 acute 6 days later)</td>
<td>Chronic+1</td>
<td>Immediately</td>
<td>↑L-6, TNF-a, INFy (serum) ↑TNF-a, IL7, IL10, IL17, IL18 (CeA)</td>
</tr>
<tr>
<td>Gao et al. (2019a)</td>
<td>C57BL/6 male adult mice</td>
<td>10 days</td>
<td>Chronic</td>
<td>Long-term</td>
<td>↑CD-11b and Iba-1 cells, IL-1β, IL-6, TNF-α (HPC)</td>
</tr>
<tr>
<td>Gao et al. (2019b)</td>
<td>C57BL/6 male adult mice</td>
<td>10 days</td>
<td>Chronic</td>
<td>(3 weeks after SD) Long-term</td>
<td>↑IL-1β, TNFα (PFC) ↑mRNA expression CD11b and Iba-1 (PFC)</td>
</tr>
<tr>
<td>Kopschina Feltes et al. (2019)</td>
<td>Male outbred Wistar Unilever rats</td>
<td>5 days</td>
<td>Chronic</td>
<td>3 weeks after the last SD</td>
<td>↑IL-1β levels (Frontal Cortex) Short-term Glial brain activation</td>
</tr>
<tr>
<td>Lisboa et al. (2018)</td>
<td>C57BL/6 male adult mice</td>
<td>6 days</td>
<td>Chronic</td>
<td>Long-term</td>
<td>↑IL-1β mRNA in microglia/macrophages.</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Authors</th>
<th>Animal Description</th>
<th>Social defeat</th>
<th>Type of SD</th>
<th>Short or long term</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu et al. (2019)</td>
<td>C57BL/6 male adult mice</td>
<td>10 days</td>
<td>Chronic</td>
<td>Long-term</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>mRNA IL-1β, IL-6, CD206, Arginase1, HMGB1 (HPC)</td>
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<td>Iba-1 (HPC)</td>
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<tr>
<td>McKim et al. (2016)</td>
<td>C57BL/6 male adult mice</td>
<td>6 days</td>
<td>Chronic</td>
<td>Immediately</td>
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<td></td>
<td>Iba-1 and CD45 cells (DG)</td>
</tr>
<tr>
<td>Montagud-Romero et al.</td>
<td>C57BL/6 male adult mice</td>
<td>4 days</td>
<td>Intermittent</td>
<td>Short-term</td>
<td>IL-1β, NFkB and COX-2 (HPC and STR)</td>
</tr>
<tr>
<td>(under review)</td>
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<tr>
<td>Montagud-Romero et al. (2020)</td>
<td>C57BL/6 male adult mice</td>
<td>4 days</td>
<td>Intermittent</td>
<td>Short-term</td>
<td>CX3CL1 (STR)</td>
</tr>
<tr>
<td>Niraula et al. (2019)</td>
<td>C57BL/6 male adult mice</td>
<td>6 days</td>
<td>Chronic</td>
<td>Short-term</td>
<td>Plasma IL-6</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>(14 h after SD)</td>
<td>(CD11b+/Ly6Chi) monocytes serum</td>
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<td></td>
<td></td>
<td></td>
<td>(CD11b+/CD45hi) monocytes brain</td>
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<td></td>
<td></td>
<td>Iba 1 cells (PLC, CA3, DG)</td>
</tr>
<tr>
<td>Nozaki et al. (2020)</td>
<td>C57BL/6 male adult mice</td>
<td>10 days</td>
<td>Chronic</td>
<td>8 days after the last SD</td>
<td>TSPO in microglia (BLA, LHb, vHPC)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>TNFα (BLA, vHPC)</td>
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<td>IL-1β (BLA)</td>
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<td>IL-6 (BLA, Nacc)</td>
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<td></td>
<td>IL-12 (BLA)</td>
</tr>
<tr>
<td>Reguilón et al. (2020a, b)</td>
<td>OF1 mice</td>
<td>4 days</td>
<td>Intermittent</td>
<td>Short-term</td>
<td>CX3CL1, CXCL12 (STR)</td>
</tr>
<tr>
<td>Rodríguez-Arias et al. (2018)</td>
<td>OF1 mice</td>
<td>4 days</td>
<td>Intermittent</td>
<td>Long-term</td>
<td>Iba 1 cells (Nacc-M1-M2 monocytes).</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Iba 1 cells (PrL-M3–5 monocytes)</td>
</tr>
<tr>
<td>Sawicki et al. (2015)</td>
<td>C57BL/6 male adult mice</td>
<td>1–3–6 days</td>
<td>Chronic</td>
<td>Short-term</td>
<td>6SD: VCAM-1 and ICAM-1 mRNA (PFC, PVN)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(14 h after SD)</td>
<td>VCAM-1 and ICAM-1 mRNA (AMY, HPC)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>IL-1β, CCL2, and CXCL2 mRNA (microglia)</td>
</tr>
</tbody>
</table>
Table 1 (continued)

<table>
<thead>
<tr>
<th>Authors et al. (2020)</th>
<th>Animal</th>
<th>Social defeat</th>
<th>Type of SD</th>
<th>Short or long term</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Song et al. (2020)</td>
<td>C57BL/6 male adult mice</td>
<td>10 days</td>
<td>Chronic</td>
<td>4–5 days after the last SD</td>
<td>IL-1β, IL-18, IL-6, TNFα (HPC)</td>
</tr>
<tr>
<td>Stankiewicz et al. (2015)</td>
<td>Swiss—Webster male mice</td>
<td>8–13 days</td>
<td>Chronic</td>
<td>24 h after the last SD</td>
<td>IL-4 (Transcriptomic genes)</td>
</tr>
<tr>
<td>Tian et al. (2019)</td>
<td>C57BL/6 male adult mice</td>
<td>10 days</td>
<td>Chronic</td>
<td>6 days after the last SD</td>
<td>IL-1β, IL-6 (HPC)</td>
</tr>
<tr>
<td>Wohleb et al. (2011)</td>
<td>C57BL/6 male adult mice</td>
<td>6 days</td>
<td>Chronic</td>
<td>Short-term (14 h after SD)</td>
<td>CD11b/ CD45 high/ Ly6C high macrophages in the CNS IL-1β microglia CD14, CD86, TLR4 microglia</td>
</tr>
<tr>
<td>Wohleb et al. (2012)</td>
<td>C57BL/6 male adult mice</td>
<td>6 days</td>
<td>Chronic</td>
<td>Short-term (14 h after SD)</td>
<td>IL-6 (serum) IL-1β mRNA in enriched CD11b + cells microglia activation in the HPC, PFC, AMY, PVN 72 h after SD CCR2+ macrophages in the brain IL-1β and TNFα mRNA</td>
</tr>
<tr>
<td>Wohleb et al. (2013)</td>
<td>C57BL/6 male adult mice</td>
<td>1 day</td>
<td>Acute</td>
<td>Immediately</td>
<td>IL-1β (HPC) IL-1β, CCL2 (CTX-R); CX3CL1 (HYPO); IL1B (BG); IL1B (HPC) IL-1β, CCL2, CX3CL1 (CTX-R); IL1B, CCL2 (HYPO); IL1B, CCL2 (BG); IL1B, CCL2 (HPC)</td>
</tr>
<tr>
<td>Wohleb et al. (2014a)</td>
<td>C57BL/6 male adult mice</td>
<td>6 days</td>
<td>Chronic</td>
<td>Short-term (12 h after SD)</td>
<td>Monocytes (serum) Macrophages (brain) IL-6 (serum) CD11+ cells (serum) CD11+ cells (serum) Macrophages (brain) Microglia (PFC, AMY, HPC) deramified morphology</td>
</tr>
</tbody>
</table>

STR striatum, HPC hippocampus, PFC prefrontal cortex, AMY amygdala, HYPO hypothalamus, CTX-R rostral cortex, PVN paraventricular nuclei, LHb lateral habenula, BG basal ganglia, BLA basolateral amygdala, CeA central amygdala, DG dentat gyrus, PLC prelimbic cortex, vHCP ventral hippocampus, Nacc nucleus accumbens
3 Susceptibility or Resilience to Social Stress

Clinical and preclinical studies show that not all subjects exposed to social stress will subsequently display unhealthy behaviors (Krishnan et al. 2007). When mice undergo chronic SD, those that are susceptible to the effects of stress exhibit social avoidance, decreased sucrose preference, decreased circadian amplitude of body temperature, social hyperthermia, or weight loss (Krishnan et al. 2007; Christoffel et al. 2011). On the other hand, some animals – referred to as resilient animals – cope effectively with stressful situations, and their behavior and psychological functioning are not affected (Brockhurst et al. 2015; Pfau and Russo 2015; Dantzer et al. 2018).

A number of factors are involved in susceptibility or resilience to the deleterious consequences of stress (Dutcher and Creswell 2018; Cathomas et al. 2019). As the stress response is multidimensional and multisystem, it encompasses both behavioral responses and physiological processes (Murrough and Russo 2019). When exposed to chronic social defeat stress, phenotypes that are resilient to depression-like behavioral symptoms are observed in approximately 30% of animals, although longer exposure decreases the percentage of such phenotypes (Lu et al. 2021). Before exposure to SD, resilient mice are characterized by higher novelty-induced activity and greater exploration of social and non-social targets. On the other hand, susceptible mice perform better in the passive avoidance task (Milic et al. 2021). An interesting recent study by Zhang et al. (2021) has shown that different baseline physical activities affect susceptibility and resilience to CSDS, possibly via the dopamine system. Animals with high baseline physical activity or activation of the tyrosine hydroxylase in the ventral tegmental area tended to be resilient. The oxytocinergic system has recently emerged as a promising target to potentiate resilience to SD (Ferrer-Pérez et al. 2021). Following SD, resilient mice showed an active coping response during episodes of defeat, with fewer submission behaviors, a weaker anxiogenic response in the elevated plus maze, low levels of novelty-seeking, and high social interaction (Calpe-López et al. 2020; Ballestín et al. 2021).

Vulnerability to stress-induced depression has been linked to individual differences in the immune system (Hodes et al. 2014; Wood et al. 2015); for example, the reactivity of the inflammatory response after stress can predict if a mouse will display depressive-like consequences (such as social avoidance) or a resilient phenotype. A less reactive immune system with lower levels of proinflammatory cytokines is characteristic of resilient mice, while susceptible mice display an exaggerated proinflammatory response under chronic SD stress (Hodes et al. 2014). In line with this, active coping strategies in the resident intruder paradigm are linked with weaker inflammatory processes, while mice showing passive coping strategies tend to display proinflammatory processes (Wood et al. 2015).

In general, research shows that stress-susceptible mice exhibit an up-regulation of proinflammatory cytokines such as IL-6, IL-15, IL-7, monocyte chemoattractant protein (MCP-1), and IL-1β (Hodes et al. 2014; Stewart et al. 2015; Wood et al. 2015; Ballestín et al. 2021) in specific brain areas, such as the STR or HPC, after a
chronic SD procedure. In contrast, stress-resilient rats or mice (under chronic or repeated social defeat stress) do not display these augmented levels of proinflammatory cytokines in blood serum, but instead present an enhanced expression of anti-inflammatory IL-4 and IL-10 (Hodes et al. 2014; Stewart et al. 2015). Expression of the 18-kDa translocator protein, thought to be a biomarker of inflammation due to its expression in activated microglia in the brain, is significantly higher in the basolateral amygdala (BLA), lateral habenula (LHb), and ventral HPC (vHPC) in susceptible mice submitted to chronic SD (Nozaki et al. 2020).

To summarize, based on current reports we can affirm that chronic and intermittent social stressful experiences significantly contribute to inflammation and the alteration of immune signaling, thereby potentiating the release of several proinflammatory mechanisms. Resilience to the effects of SD seems to depend on the neuroinflammatory response and is characterized by a less reactive phenotype.

4 Role of the Immune System in the Enhanced Sensitivity to Drugs of Abuse After Social Stress Experience

4.1 The Neuroinflammatory Response to the Increase in the Rewarding Effects of Cocaine and Ethanol Induced by SD

The current literature endorses that both SD and drugs of abuse can activate the immune system and trigger the release of inflammatory markers. However, to date few studies have explored the effects of the inflammatory system on the increased rewarding effects of drug use induced by social stress. In human studies, the power of cocaine to trigger proinflammatory central immune signaling is well documented (Coller and Hutchinson 2012). Indeed, Fox et al. (2012) reported elevated levels of proinflammatory TNFα in cocaine abusers exposed to stress imagery, together with a decrease in anti-inflammatory IL-10 levels in plasma (Fox et al. 2012). In this way, cocaine-dependent individuals display an enhanced inflammatory state following exposure to stress-related cues.

Preclinical studies also support a critical role for the neuroinflammatory response in increased susceptibility to cocaine reward after social stress exposure. We have repeatedly shown that mice exposed to intermittent SD develop cocaine conditioned place preference (CPP) with a subthreshold dose of cocaine (Montagud-Romero et al. 2016a, b, 2017, 2018; Ferrer-Pérez et al. 2018), and that only these animals show increased striatal IL-6 protein levels after CPP (Ferrer-Pérez et al. 2018). Moreover, when mice were classified as susceptible (those that developed CPP) or resilient (those that behaved like controls), increases in IL-6 protein in the HPC and STR were observed only in the susceptible mice under intermittent SD (Ballestín et al. 2021). In contrast to IL-6, CX3CL1 can exert a neuroprotective or neurotoxic role depending on the type of neuroinflammatory event (Sheridan and Murphy...
In a recent study we have observed that intermittent SD decreased CX3CL1 striatal levels (Montagud-Romero et al. 2020), although cocaine exposure induced transient increases in CX3CL1 concentrations in the hippocampus (Montesinos et al. 2020), suggesting a specific response of this chemokine to social stress. Moreover, knockout mice for the CX3CL1 chemokine receptor 1 developed a similar increase in cocaine-induced CPP to that observed in defeated wild-type animals, though they also exhibited an increment of IL-1β and CX3CL1 in the hippocampus (Montagud-Romero et al. 2020). We suspect that the lack of CX3CL1/Cx3cr1 signaling under stress conditions induces further changes in protein and transcription factors, and that CX3CL1 is needed to buffer the response to SD.

Numerous studies have shown that, similarly to psychostimulants, alcohol activates the immune system, increasing inflammatory markers in both plasma and brain levels. Accordingly, while several interleukins are released due to alcohol intake, deletion of the IL-1 receptor I gene produces a modest decrease in alcohol consumption in mice, though it does not affect SD-induced chronic drinking. The combined deletion of TNF-1R and IL-1RI receptors has been shown not to affect alcohol reward, but it does prevent an increase in alcohol consumption following chronic SD stress (Karlsson et al. 2017). A rise in striatal levels of the chemokines CX3CL1 and CXCL12 has been observed in defeated mice immediately after the last defeat, and also after ethanol self-administration (Reguilón et al. 2020a, b). Finally, TLR4 seems to play a critical role in increased cocaine or alcohol intake after intermittent SD, since these increments are absent in TLR4 KO mice (Montagud-Romero et al. 2021).

4.2 How to Prevent the Neuroinflammatory Response Induced by SD

Several pharmacological or environmental interventions can influence the effects of stress on the organism, and a series of studies support the hypothesis that this positive effect is due to the immune system. Accumulating evidence supports an association between depression and inflammatory processes, a connection that seems to be bidirectional. As we have previously mentioned, chronic and intermittent SD animals (mice and rats) exhibit a depressive-like phenotype, characterized by social inhibition, anhedonia and pronounced physiological and endocrine responses, such as circadian rhythm disturbances and elevated levels of corticosterone (Meerlo et al. 2002; Fuertig et al. 2016; Montagud-Romero et al. 2016a, b; Rodríguez-Arias et al. 2017; Macedo et al. 2018). In accordance with these data, antidepressant and anxiolytic treatments administered to defeated animals have been shown to reverse anxiety and depressive-like behavior, preventing immune impairment and endocrine alteration (Beitia et al. 2005; Fuertig et al. 2016; Ramirez and Sheridan 2016; Ramirez et al. 2016). Lorazepam and clonazepam attenuate mRNA expression of corticotropin-releasing hormones in the hypothalamus and corticosterone in plasma, while they block stress-induced accumulation of macrophages.
(CD11b+/CD45high) in the CNS in mice exposed to chronic social defeat (Ramirez et al. 2016). Stress-induced increases in plasma IL-6 are prevented after administration of imipramine and anxiolytics (lorazepam and clonazepam), but only the former abolishes the development and trafficking of inflammatory myeloid progenitor cells from the bone marrow to the blood and brain (Ramirez and Sheridan 2016; Ramirez et al. 2016).

During chronic social disruption stress in mice, the sympathetic nervous system is activated, triggering the release of catecholamines (epinephrine and norepinephrine) that regulate the immune cells via the stimulation of two receptors, the alpha(α)- and the beta(β)-adrenergic receptors (Hanke et al. 2012). Furthermore, stress-dependent alterations of microglia and macrophages are prevented by propranolol (an adrenergic receptor antagonist) when administered before each confrontation with the intruder, at the same time blocking the anxiety-like behavior induced in mice by agonistic encounters (Wohleb et al. 2011; Hanke et al. 2012). Propranolol also reduces the plasma increase of IL-6, TNFα, and MCP-1 in mice experiencing chronic SD, as well as the percentage of CD11b + splenic macrophages, and the expression of TLR2, TLR4, and CD86 on the surface of these cells (Hanke et al. 2012).

On the other hand, anti-inflammatory treatments such as indomethacin administered to mice before each episode of stress (in an intermittent paradigm of SD) have been shown to prevent a rise in IL-6 levels without altering the anxiogenic consequences of social stress (Ferrer-Pérez et al. 2018). In line with this, minocycline, an anti-inflammatory agent, and thought to also be a microglia inhibitor (Henry et al. 2008), was found not to prevent the persistence of social avoidance behavior induced by chronic SD in mice, while it abolished both microglia activation and monocyte recruitment (McKim et al. 2016).

Among its multiple actions, the endocannabinoid (ECB) system regulates the neuroendocrine and inflammatory responses mediated by stress. The synthetic cannabinoid receptor agonist WIN55,212-2 reverses the anxiety-like behavior induced by daily SD in mice and reduces the accumulation of inflammatory monocytes in the circulation and brain, attenuating IL-1β mRNA expression in microglia/macrophages (Lisboa et al. 2018).

Several natural compounds with anti-inflammatory properties, such as resveratrol, were shown to block neuroinflammation in the locus coeruleus and the dorsal raphe cytokine expression produced by chronic social defeat stress (on five consecutive days) in rats (Finnell et al. 2017). Furthermore, administration of allicin – derived from garlic – down-regulates microglia activation and tempers the increment of inflammatory cytokines in the HPC in defeated mice undergoing chronic SD stress (Gao et al. 2019a). Chronic administration of Ginsenoside Rg1 (Rg1) – the major active ingredient of ginseng, with low toxicity and both neuroprotective and antidepressant-like effects – significantly decreases Iba-1 expression in hippocampal sections, reducing the microglial activation induced by chronic SD in mice (Jiang et al. 2020). Ginseng, as well as the natural product morin, reduces inflammatory mediators (TNFα, IL-6, cyclooxygenase-2 and NFkB), acetylcholinesterase activity, and NADPH oxidase expression in specific brain regions of mice affected by chronic
Other treatments, such as the probiotic *Lactobacillus rhamnosus*, which prevents deficits in social interaction, have been shown to attenuate stress-related activation of dendritic cells while increasing IL-10+ regulatory T cells in mice undergoing the chronic SD procedure (Bharwani et al. 2017). Environmental interventions, such as physical activity, have proved themselves to be a modulator of mental function. The physical exercise most commonly studied in rodents (mice and prairie voles) is voluntary wheel running (VWR), which improves learning, enhances neurogenesis and angiogenesis, modifies various signaling molecules, and prevents the behavioral dysfunctions associated with stress, such as social avoidance or anhedonia (Salam et al. 2009; Mul et al. 2018; Watanasriyakul et al. 2018; Zhang et al. 2019; Pagliusi Jr et al. 2020). Physical activity in rats induces adaptive responses to stress due to regulation of the HPA (Pietrelli et al. 2018). In a recent study in mice, Reguilón and collaborators (2020a) confirmed that VWR prevented the neuroinflammatory response induced by intermittent SD stress through a reduction in the chemokines CX3CL1 and CXCL12, while it blocked any increase in ethanol intake.

Another environmental factor that modulates the stress response, not only in mice, but also in rats, is social enrichment, a positive housing condition, which protects against or reduces the negative consequences of social stress, such as anxiety (Gasparotto et al. 2005; Nakayasu and Ishii 2008; Neisewander et al. 2012). Low-stress housing with a female, or with a male from adolescence onwards (in mice), blocks the increase in cocaine reward and release of the proinflammatory cytokine IL-6 (Ferrer-Pérez et al. 2019). A description of the pharmacological or environmental interventions that modulate the effects of SD is provided in Table 2.

5 Conclusion and Future Directions

Studies published to date leave no doubt concerning the critical role of the neuroinflammatory response in the effects of social stress. SD induces a wide range of neuroinflammatory responses that can last for months. Blockade of the immune response inhibits the development of depression and anxiety, and increases in cocaine and alcohol intake induced by stress. In this context, neuroinflammation has presented itself as a new target in the treatment of stress disorders. Among these new pharmacological treatments, the following are worthy of mention: the tetracycline antibiotic minocycline, which reduces microglia activation and neuroinflammation (Jiang et al. 2009); the nonsteroidal anti-inflammatory indomethacin, which inhibits the cyclooxygenase enzyme and reduces neuroinflammation (Ferrer-Pérez et al. 2018); and drugs that inhibit the transcription factor NFκB (Truitt et al. 2016).

In relation to biomarkers of neuroinflammation, numerous studies have highlighted increases in neuroinflammation markers in brain structures and in peripheral blood. Specific cytokines or chemokines are now considered biomarkers of ethanol binge drinking (Pascual et al. 2018) or cocaine use (Araos et al. 2015), and
**Table 2** A complete description of pharmacological or environmental interventions that could reverse the immune system activation produced by social defeat stress

<table>
<thead>
<tr>
<th>Author</th>
<th>Animal</th>
<th>Treatment</th>
<th>Dosis</th>
<th>Immune system effects</th>
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| Ben-Azu *et al.* (2020) | Swiss Albino mice | Ginseng         | 50 mg/kg | Reduction TNFa and IL-6 in STR and PFC  
Reduced the expression of COX-2 and NF-kB in STR and PFC; and of NOX-2 in PFC |
|                         |                 | Morin           | 25 mg/kg | Reduced TNFα in STR and HPC, IL-6 in STR and PFC  
Reduced the expression of COX-2 and NF-kB in STR and PFC; and of NOX-2 in PFC |
|                         |                 |                 | 50 mg/kg | Reduced TNFα in STR and HPC, IL-6 in STR  
Reduced the expression of COX-2 and NF-kB in STR and PFC; and of NOX-2 in PFC |
|                         |                 |                 | 100 mg/kg| Reduced TNFα in STR, PFC and HPC, IL-6 in STR  
Reduced the expression of COX-2 and NF-kB in STR and PFC; and of NOX-2 in PFC |
| Bharwani *et al.* (2017)| C57BL/6 mice    | Lactobacillus rhamnosus (JB-1) | 200 ul | Increasing IL-10+ regulatory T cells  
Attenuated stress-related activation of dendritic cells |
| Ferrer-Pérez *et al.* (2018)| OF1 mice | Indomethacin | 10 mg/kg | Decreased IL-6 in plasma  
Decreased IL-6 in STR |
| Ferrer-Pérez *et al.* (2019)| OF1 mice | Housing conditions | Paired with a female after SD | Decreased IL-6 in STR |
|                         |                 |                 | Paired with a male from PND42 | Decreased IL-6 in STR |
| Finnell *et al.* (2017) | Sprague Dawley rats | Reveratrol | 10 and 30 mg/kg | Decreased splenic IL-1β and TNFα levels  
Blocked LC neuroinflammation (30 mg/kg)  
Reduced DR cytokine expression |
| Gao *et al.* (2019a) | C57BL/6 mice    | Allicin         | 2, 10, 50 mg/kg | Reduced protein expression of Iba-1 and CD-11b in the HPC  
Down-regulated the levels of proinflammatory cytokines IL-6, TNFα and IL-1β in HPC |
| Hanke *et al.* (2012) | CD-1 mice       | Propanolol     | 10 mg/kg | Reduced IL-6, TNFα, and MCP-1 in plasma  
Decreased CD11b + splenic macrophages  
Decreased the expression of TLR2, TLR4, and CD86 on the surface of splenic macrophage cells |

(continued)
further studies in humans and animal models are necessary to detect similar neuroinflammatory markers of social stress. It is vital that future studies are performed in female rodents; despite the higher susceptibility of women to the effects of social stress, the number of basic science studies performed to date in female subjects is low.

<table>
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<th>Author</th>
<th>Animal</th>
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<tr>
<td>Jiang et al. (2020)</td>
<td>C57BL/6 mice</td>
<td>Ginsenoside Rg1</td>
<td>20 and 40 mg/kg</td>
<td>Attenuated microglial activation and inflammatory cytokine expression</td>
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<td>Imipramine</td>
<td>10 mg/kg</td>
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<td>Lisboa et al. (2018)</td>
<td>C57BL/6 mice</td>
<td>WIN55,212–2</td>
<td>1 mg/kg</td>
<td>Reduced the accumulation of inflammatory monocytes in circulation and brain</td>
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<td>Attenuated IL-1β mRNA expression in microglia/macrophages</td>
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<td>Ramirez and Sheridan (2016)</td>
<td>C57BL/6 mice</td>
<td>Imipramine</td>
<td>15 mg/kg</td>
<td>Attenuated IL-6 in plasma</td>
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<td>Decreased the percentage of monocytes and granulocytes in the bone marrow and circulation</td>
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<td>Prevented the accumulation of macrophages in the brain</td>
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<td>Ramirez et al. (2016)</td>
<td>C57BL/6 mice</td>
<td>Lorazepam</td>
<td>0.10 mg/kg</td>
<td>Blocked stress-induced levels of IL-6 in plasma</td>
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<td>Inhibited splenomegally and the production of proinflammatory cytokines in the spleen</td>
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<td>Blocked the accumulation of macrophages (CD11b+/CD45high) in the CNS</td>
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<td>Clonacepam</td>
<td>0.25 mg/kg</td>
<td>Blocked stress-induced levels of IL-6 in plasma</td>
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<td>Reguilón et al. (2020a, b)</td>
<td>OF1 mice</td>
<td>Voluntary wheel running</td>
<td>1 h-access</td>
<td>Reversed CX3CL1 and CXCL12 levels in STR</td>
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<td>McKim et al. (2016)</td>
<td>C57BL/6 mice</td>
<td>Minocycline</td>
<td>90 mg/kg</td>
<td>Prevented microglia activation</td>
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<td>Prevented monocyte recruitment</td>
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<td>Wohleb et al. (2011)</td>
<td>C57BL/6 mice</td>
<td>Propanolol</td>
<td>10 mg/kg</td>
<td>Reduced IL-6 plasma levels</td>
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<td>Prevented monocyte recruitment</td>
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STR striatum, HPC hippocampus, PFC prefrontal cortex
To conclude, there is clear evidence to support the role of neuroimmune signaling in many of the effects of social stress. In today’s society we are continuously exposed to stress challenges during our entire life span. Bullying during adolescence or harassment at work are only two examples of the wide range of social stresses that human beings continuously face. Social stress induces devastating consequences in the form of depression, anxiety, or substance use disorders. A better understanding of the mechanisms that underlie the effects of social stress may contribute to the development of effective therapeutic strategies to treat disorders associated with stress.

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Social Stress and Aggression in Murine Models

Aki Takahashi

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Abstract Throughout life, animals engage in a variety of social interactions ranging from the affiliative mother–offspring interaction and juvenile play to aggressive conflict. Deprivation of the appropriate social interaction during early development is stressful and disrupts the development of appropriate social behaviors and emotional responses later in life. Additionally, agonistic encounters can induce stress responses in both dominant and subordinate individuals. This review focuses on the social stress that escalates aggressive behavior of animals and discusses the known neurobiological and physiological mechanisms underlying the link between social stress and aggression. Social instigation, a brief exposure to a rival without physical contact, induces aggressive arousal in dominant animals and escalates aggressive behaviors in the following agonistic encounter. Furthermore, the experience of winning an aggressive encounter is known to be as rewarding as addictive drugs, and the experience of repeatedly winning induces addiction-like behavioral and neurobiological changes and leads to abnormal aggressive behaviors. Social isolation stress in early development from neonatal to juvenile and adolescent periods also affects aggressive behavior, but these effects largely depend on the strain, sex, and species as well as the stage of development in which isolation stress is
experienced. In conclusion, understanding neurobiological mechanisms underlying the link between social stress and aggression will provide an important insight for the development of more effective and tolerable treatments for maladaptive aggression in humans.

**Keywords**  Aggression reward · Aggressive behavior · Developmental stage · Social instigation · Social isolation · Social stress

### 1 Introduction

Social relationships are rewarding and enrich our life, but they can also be stressful and sometimes destructive to our lives. In fact, prolonged social stress can result in the development of psychological problems such as anxiety and depression as well as antisocial and violence-related behaviors. When considering stress as “a real or interpreted threat to the physiological or psychological integrity of an individual that results in physiological and/or behavioral responses” (McEwen 2016), many types of social interactions can be considered stressful including agonistic encounter in adult males as well as deprivation (neglect) or maltreatment during the juvenile to adolescence period. Thus, social stress contains a large amount of heterogeneity in terms of the type of stressors. Although Hans Selye (1936) originally proposed the stress response to be a common array of physical responses as a general alarm of an organism that is nonspecific to the type of stress (Selye 1936), it is now largely accepted that stress responses are heterogeneous depending on the type of stress (Pacák and Palkovits 2001). Social stress activates the sympathetic nervous system (fight-or-flight response), the hypothalamic-pituitary-adrenal (HPA) axis, and the immune system differently depending on the type of stressor and the status of the animal (i.e., whether they are dominant or subordinate) (Avitsur et al. 2007; Bartolomucci et al. 2003; Fokkema and Koolhaas 1985; Raab et al. 1986; Sapolsky 1982). Furthermore, the effects of stress vary between acute and chronic exposure, the predictability of the stress (predictable vs unpredictable), and whether the stress is controllable vs uncontrollable (Maier et al. 1982; Dhabhar and McEwen 1999; Sapolsky 2015). Importantly, the latter type of stress is known to cause a larger disturbance on both psychological and physiological processes. Another important aspect of social stress is individual differences in stress sensitivity which is termed as stress susceptible vs resilient (Russo et al. 2012) as well as coping style (Koolhaas et al. 2010). The mechanisms underlying coping style on social stress are covered in Prof. Sietse de Boer’s chapter in this book.

This review summarizes the types of social stress that affect aggressive behavior in rodents and discusses some recent findings regarding the underlying neurobiological and physiological mechanisms involved in each model. First, behavioral and physiological changes induced by agonistic encounters are introduced. We then discuss how social instigation and repeated winning experiences in acute and chronic stress models escalate aggressive behavior in the dominant individual.
Finally, developmental stress models including maternal separation stress and post-weaning social isolation stress are summarized. This review will not focus on repeated social defeat and social disruption stress in subordinate animals that strongly suppress aggressive behavior and lead to devastating physiological, endocrinological, immunological, and psychological effects, as these models will be discussed in several chapters by Prof. Haller, Prof. Trainor, and Prof. Miczek et al. of this book.

2 Agonistic Behavior in Rodents

In the natural environment where population density is low, male mice (Mus musculus) and rats (Rattus norvegicus) establish a territory, known as a deme, which is a small breeding unit where one male dominates a small group of females and young offspring and patrols the territory to protect it from other intruding males. If an intruder male invades his territory, the resident male shows fierce aggressive behaviors to chase it out of his territory (Crowcroft 1966; de Boer et al. 2016). As the population density increases and the resources in the niche become more abundant, rats become more socially cohesive and establish a large colony with a dominant-subordinate hierarchy among several males (de Boer et al. 2016). Although mice are considered to be a territorial species, they also become socially tolerant in high population density situations, such as a laboratory condition where males are co-housed together (Berdoay and Drickamer 2007). In most rodent species, males show stronger territorial and dominance aggression than females. However, in hamsters (Mesocricetus auratus) and California mice (Peromyscus californicus), both males and females show strong territorial aggression (Payne and Swanson 1970; Ribble and Salvioni 1990). Although female mice and rats are considered to be docile, they show intense aggressive behavior specifically when they become pregnant and are lactating (maternal aggression) to protect their offspring from intruding males (St. John and Corning 1973). In addition, even when not pregnant, female mice and rats have been known to exhibit inter-female rival aggressive behavior when they are housed with a male (Albert et al. 1991; Newman et al. 2019).

Agonistic behaviors are defined as “adaptations for situations involving physical conflict or contests between members of the same species” (Scott 1966), and it contains a whole variety of behavioral responses during an agonistic encounter. At first, animals tend to show proactive agonistic behaviors such as exploratory social sniffing and allogrooming, which then shift to aggressive behaviors including attack bites, chasing, boxing, and threat behaviors. Shortly after a dominant animal is determined during this agonistic interaction, the subordinate animals inhibit the expression of aggressive behaviors and quickly change their behavior repertoire to submissive behaviors such as submissive upright postures, fleeing, and immobility (Grant and Mackintosh 1963; Miczek and O’Donnell 1978). The dominant and subordinate determination usually occurs relatively quickly; within a few seconds to minutes of the agonistic encounter.
An agonistic encounter with a rival conspecific induces a physiological stress response in both the dominant winner and the subordinate loser in rats and mice (Brain 1980; Covington and Miczek 2005; de Boer et al. 2016). For example, an acute agonistic encounter in the resident–intruder test causes a clear increase in heart rate, blood pressure, body temperature as well as enhanced locomotor activity in both dominant and subordinate rats (Koolhaas et al. 2011). Furthermore, adrenaline, noradrenaline, and corticosterone levels in the plasma or adrenal gland are increased after an agonistic encounter in both dominants and subordinates (Brain 1980; Fokkema et al. 1988; Covington and Miczek 2005; Koolhaas et al. 2011). Therefore, an agonistic interaction activates both the sympathetic nervous system and HPA axis, two major stress response systems, in both the winner and loser animals. In addition, an agonistic encounter induces an acute activation of immune system, and not only in dominant and subordinate males but also in animals who did not engage in any aggressive behavior in the resident–intruder encounter show increased proinflammatory cytokine release in the blood (Takahashi et al. 2021; Hodes et al. 2014). Thus, an agonistic encounter itself can act as a stressor that induces several physiological and endocrinological changes in animals.

On the other hand, the winners show a faster recovery of these stress responses to baseline, whereas subordinate animals show a prolonged over-activation of the sympathetic nervous system (including a sustained rise in heart rate, blood pressure, and body core temperature) and HPA axis (increased adrenocorticotropic hormone (ACTH) and glucocorticoids) lasting hours after the agonistic encounter, and long-term hypercortisolism as well as reduction of androgen levels after continuous subordination (Ely and Henry 1978; Schuurman 1980; Raab et al. 1986; Sapolsky 1990; Tornatzky and Miczek 1993; Covington and Miczek 2005; de Boer et al. 2016). Therefore, stress responses differ between winner and loser individuals in terms of the temporal pattern and intensity after an agonistic encounter. Increasing evidence has shown that prolonged stress responses in subordinate animals after multiple defeat experiences causes an allostatic overload, which refers to the excessive cumulative results of originally adaptive allostatic stress responses, and dysregulates physiological, endocrinological, immunological, and psychological processes (McEwen 1998).

**Future Agenda**

Behavioral changes during an agonistic encounter in dominant and subordinate individuals occur even though they show a similar acute physiological stress response. Thus, there must be brain areas that monitor the dynamics of their dominance-subordinate relationship during the agonistic interaction and quickly shift their behavior repertoires to be submissive when they become subordinate. One of the brain areas that have been shown to be involved in the development of a social hierarchy is the medial prefrontal cortex (mPFC). Dominant hierarchy of male mice is often examined using dominance tube test (Lindzey et al. 1961). Dominant males in the dominance tube test have higher excitatory synaptic strength in the mPFC via AMPA receptor activation, and manipulation of the expression of AMPA receptor subunit GluR2 in the mPFC successfully changes the dominant status in
group-housed male mice (Wang et al. 2011). Furthermore, mPFC neural activity has been shown to correlate with their dominance behavior, and in vivo electrophysiological recording has shown that pyramidal neurons in the dorsomedial part of mPFC (dmPFC) are activated during dominant push and resistance behaviors in the dominance tube test, while fast spiking interneurons were activated during passive retreat behaviors in male mice (Zhou et al. 2017). Indeed, DREADD inhibition of dmPFC neurons increases retreat behavior in dominant males, and optogenetic activation of these neurons has been shown to increase dominant push behaviors in the low-rank male (Zhou et al. 2017). By contrast, optogenetic activation of the mPFC, including both the prelimbic and infralimbic cortex, in the resident–intruder test has been shown to inhibit territorial aggressive behavior in male mice (Takahashi et al. 2014), and thus the involvement of the mPFC for aggressive behavior seems to be different depending on the subregion of the mPFC or the type of aggression. One possibility is that the mPFC is involved in the calculation of the social relationship in the complex social situation that helps to promote the dominance hierarchy, while activation of the mPFC has a suppressive effect on territorial aggression, which is a simpler form of aggression in which the resident expels any type of intruder; the suppressive effect keeps aggression from exceeding the adaptive level, avoiding unnecessary energy consumption and injurious fight. Future studies using direct neural imaging during the behavioral shift from dominant to subordinate will be required to understand the neural mechanism(s) underlying this quick switch in behavior repertoire. It has been shown that activation of the mPFC has a bidirectional effect on stress responses; the infralimbic cortex activates the sympathetic nervous system while the prelimbic cortex inhibits the sympathetic nervous system (Ulrich-Lai and Herman 2009). Thus, the difference in the time course of stress responses in the dominant and subordinate animals may be regulated by the mPFC, and this will need to be addressed in future studies.

3 Social Instigation

As described above, an antagonistic confrontation itself has a stress component not only in subordinate, but also in dominant animals. It has been shown that a brief confrontation with a rival induces “aggressive arousal” or “attack readiness” in the dominant animals (Berkowitz 1993), and it increases the intensity and frequency of aggressive behaviors in a future encounter in mice, rats, and hamsters (Lagerspetz 1969; Tellegen and Horn 1972; Potegal 1991; Fish et al. 1999). Thus, the social instigation model has been used to study animal’s aggressive arousal and escalated aggressive behavior. In this model, a potential rival conspecific male (instigator) is placed into a protected cage and then presented in the home cage of the resident male, where the resident can see, smell, and hear the presence of the instigator in his/her home cage but cannot physically attack it or expel it from the territory. After exposure to this provocation for a few minutes, an intruder male is placed in the resident’s home cage allowing the resident to have direct contact with the intruder.
During this direct agonistic interaction, the resident male shows higher frequency and duration of aggressive behaviors as well as a shorter attack latency compared to his usual resident–intruder aggression without social instigation (Fig. 1). The aggression-heightening effect of social instigation lasts for hours, even after the instigator is removed from the home cage (Potegal and Popken 1985). Aggressive arousal is induced only when the resident male mouse is exposed to an adult male, and not to juvenile or female mice (Fish et al. 1999). Also, induction of aggressive arousal affects only aggressive behaviors and does not change wheel running, feeding or sexual behavior (Lagerspetz and Hautojärvi 1967; Potegal and TenBrink 1984). Importantly, there are large individual differences in the aggression-heightening effect of social instigation, with some individuals showing a strong

Fig. 1 Social instigation procedure and glutamate input in the dorsal raphe nucleus. Top: Schematics of social instigation procedure (left). A caged-instigator was introduced into the test cage of a resident male for 10 min, and then an intruder male was introduced to observe aggressive behavior. Frequency of attack bites without and with social instigation (right). The same resident males were tested for both without and with social instigation situations (n = 34). Bottom: Both social instigation and aggressive encounters increased glutamate levels in the DRN. Each ten-minute sample was collected: five samples for baseline, one sample for social instigation, one sample for during and after aggressive encounter, and five further samples after the aggressive encounter. Data are means ± SEM expressed as percentage of baseline (n = 10); *p < 0.05 compared to baseline. Adopted from Takahashi et al. (2015)
increase in aggressive behaviors after social instigation, whereas others showing suppressed or unchanged aggression after instigation (Fish et al. 1999).

Serotonin (5-HT) has been implicated in the neurobiology of aggression and emotion. Pharmacological activation of the 5-HT₁B receptor dose-dependently reduces instigation-heightened aggressive behaviors without affecting locomotor activity (Fish et al. 1999; De Almeida and Miczek 2002). The dorsal raphe nucleus (DRN) contains the largest number of 5-HT neurons that project to forebrain areas, and disruption or pharmacological manipulations of this area affect aggressive and defensive behaviors (Jacobs and Cohen 1976; Wallentschek and Raab 1982; Vergnes et al. 1986; Sijbesma et al. 1991; Mos et al. 1993; Koprowska and Romanuik 1997; van der Vegt et al. 2003; Bannai et al. 2007; Faccidomo et al. 2008; Takahashi et al. 2010a, b, 2012; Quadros et al. 2014). Previous work from our laboratory showed that excitatory input into the DRN is involved in the escalation of aggressive behaviors induced by social instigation. Furthermore, in vivo microdialysis has shown that glutamatergic input in the DRN is increased during aggressive encounters as well as during the period of social instigation, where the animal cannot physically show attack behaviors (Fig. 1). In addition, local infusion of L-glutamate into the DRN causes an increase of aggressive behaviors, suggesting that increased glutamate release in the DRN by social instigation has a pro-aggressive effect in male mice (Takahashi et al. 2015). We also observed increases in 5-HT release within the DRN and prefrontal cortex during the instigation-heightened aggression. However, the effect of 5-HT on instigation-heightened aggression has to be carefully interpreted because former studies have shown suppressive effect of 5-HT₁ agonists on escalated aggressive behaviors. Furthermore, the cell-type within the DRN that is responsible for the aggression-promoting effects of glutamatergic input into the DRN remains unclear, requiring further investigation. Social instigation has shown to activate the corticomedial amygdala in female Syrian hamsters (Potegal et al. 1996a), and high-frequency stimulation of this area induces prolonged aggressiveness (Potegal et al. 1996b). Therefore, there may be an interaction between the DRN and corticomedial amygdala in instigation-heightened aggression, and this will need to be examined.

Social instigation (or psychosocial stimulus) has been shown to increase heart rate, blood pressure, and body core temperature, as well as produce an increase in circulating corticosterone levels in the rat (Haller et al. 1995; Koolhaas et al. 2011), although these activations in the sympathetic nervous system and HPA axis were observed in dominant, subordinate, and nonaggressive individuals. In fact, acute treatment of ACTH or glucocorticoid has been shown to increase aggressive behaviors in male mice, rats, and hamsters (Brain 1980; Hayden-Hixson and Ferris 1991; Haller et al. 1997; Mikics et al. 2004). Consistent with this, pharmacological blockade of the synthesis and action of glucocorticoid has also been shown to reduce aggressive behavior in mice and rats (Haller et al. 2000a, b; Mikics et al. 2004; Fish et al. 2005). The pro-aggressive effects of glucocorticoids strongly depend on the context, and activation of the HPA axis increases aggressive behavior in dominant animals, whereas it increases submissive behavior in subordinate animals (Leshner et al. 1980; Haller et al. 1997). Thus, activation of the HPA axis increases the level of
arousal within a social environment and, in the case of dominant animals, escalates aggressive behaviors induced by stress (Kruck et al. 2004). In addition, a positive correlation between resident–intruder aggressive behavior and basal noradrenaline levels in the blood has been observed in rats (Fokkema et al. 1988). Indeed, administration of a beta-blocker has been shown to decrease aggressive outbursts in several psychiatric disorders in humans (Haspel 1995), suggesting that over-activation of the sympathetic nervous system may be related to high levels of aggressive arousal. For more on the link between glucocorticoids and aggression, see the chapter by Prof. Joseph Haller in this book.

Future Agenda
An important factor to be considered is the individual differences of the aggression-heightening effect of social instigation (Fish et al. 1999). That is, does it correlate with physiological stress responses such as glucocorticoid response or activity of the sympathetic nervous system in each individual? Although a previous study showed that both the aggressor and non-aggressor showed similar physiological changes (Haller et al. 1995), it will be important to examine the correlation between individual differences in physiological response and instigation-heightened aggression. To that end, our unpublished data show that individual differences in the sensitivity to instigation-heightened aggression does not correlate with their baseline aggression measured by the resident–intruder test, and thus different neural and physiological mechanisms are involved in aggressive arousal versus basal aggression. Furthermore, it has been shown that early life stress can affect sensitivity to social instigation. For example, early weaning from the dam in mice at 14 days of age (usual weaning is at 21 days) eliminates the aggression-heightening effect of social instigation on aggressive behaviors (Nakamura et al. 2008). Our preliminary data also show that social isolation at different stages of development causes an enhancement in sensitivity to social instigation (Takahashi in preparation). Therefore, there seem to be developmentally sensitive periods for aggressive arousal which need to be addressed in future studies.

4 Repeated Winning Experiences

In addition to the stress component of the aggressive confrontation, the experience of winning is highly rewarding (see nice reviews by Covington et al. 2019; Golden et al. 2019b). It has been shown in mice, rats, hamsters, and California mice that winners of a previous fighting bout show a higher probability to win in the next aggressive confrontation as the result of increased aggressive behaviors and testosterone (Ginsberg and Allee 1942; Van de Poll et al. 1982; Parmigiani and Brain 1983; Oyegbile and Marler 2005). Additionally, winners tend to seek the opportunity to fight, that is, the animals preferred to spend more time in the area that was associated with the presence of an intruder in the T-maze test (Tellegen et al. 1969; Kelsey and Cassidy 1976) or conditioned place preference (CPP) test (Meisel and
Furthermore, animals learn to perform operant behaviors to obtain an intruder male as a reward (Fish et al. 2002; Couppis and Kennedy 2008; Falkner et al. 2016; Golden et al. 2017b; Covington et al. 2018). In fact, about 20% of CD-1 male mice show compulsive aggression seeking in an aggression operant task; high number of lever presses even after 15 days of abstinence, high responding under the control of a progressive ratio as well as after abstinence even when aggression reward (the presence of an intruder) was combined with a punishment (foot-shock), and a preference to aggression reward over palatable food (Golden et al. 2017b). Thus, the rewarding property of aggression, or winning experience, is as strong as addictive drugs, and repeated aggressive behavior can induce addiction-like behavioral and neurobiological changes.

Social defeat stress has intensive effect on the physiology and behavior of defeated animals, such as increases of anxiety-like behavior, anhedonia, social avoidance, and sleep disturbance, especially when they receive repeated chronic defeat experiences (Buwalda et al. 2005; Kudryavtseva et al. 1991; Krishnan et al. 2007; Miczek et al. 2004). In addition, repeated fighting experiences affect behavior and physiology of dominant animals and lead to persistent and maladaptive aggressive behaviors. In the sensory contact model, two male mice are housed in a cage with a perforated divider between them, and the divider is removed once a day for 10 min (or 3 min maximum if attack behavior occurs) to allow the two males display agonistic interactions. After the physical defeat, the loser mouse is moved to the other cage that has another winner on the opposite side, and this procedure is repeated for 10 days with novel pairs every day (Kudryavtseva et al. 1991, 2014). After prolonged fighting experiences in this setting, dominant males develop abnormal aggressive behaviors such as aggression toward female mice and juveniles in addition to attack behavior toward human hands (Kudryavtseva 2000; Kudryavtseva et al. 2014). Also, the effect of repeated fighting experiences has been shown to be persistent even after days of abstinence from fighting. In fact, a longer abstinence period (18 days) and longer preceding fighting experience (30 days) caused higher attack behaviors than shorter abstinence (Kudryavtseva 2004), suggesting an incubation of aggression over time, in which aggression energy is accumulated in individuals in the absence of a discharging stimulus that leads to a lower threshold to induce aggressive acts (Lorenz 1966; Kudryavtseva 2004). Additionally, repeated fighting leads hyper locomotor activity and increased anxiety-like behavior in the elevated plus maze test (Avgustinovich et al. 1997; Kudryavtseva et al. 2002, 2004). Thus, even in dominant individuals, repeated fighting experiences results in maladaptive behavioral changes. Details and important findings from this model are discussed in the chapter by Prof. Kudryavtseva.

Although winning experience is rewarding, there are some animals that do not show any aggressive behavior (termed as non-aggressor: NON). Animals that show aggressive behavior in the resident–intruder test (termed as aggressor: AGG) usually show strong preference to intruder-paired chambers in aggression CPP test, but NON individuals avoid the intruder-paired chamber (Golden et al. 2016). The lateral habenula (LHb) has been identified as a key node for aggression reward. It has been
shown that NON animals have increased activation of LHb from an aggressive encounter compared to AGG (Golden et al. 2016; Flanigan et al. 2020). Three days of repeated aggressive encounters caused a session-by-session reduction of LHb activity in AGG males, whereas an increase in NON, indicating that winning experience causes suppression of LHb activity induced by an aggressive encounter. Thus, the LHb acts as an inhibitory switch for aggression reward, and once this switch is turned off by inhibitory inputs, animals show increased aggressive behavior by increasing its rewarding property. Indeed, the inhibitory GABAergic projections from the basal forebrain area to the LHb, as well as orexinergic neurons in the lateral hypothalamic nucleus that send discrete projection to GABAergic interneurons in the LHb have been identified to be responsible for the increase of aggression reward in AGG individuals (Golden et al. 2016; Flanigan et al. 2020).

The LHb modulates the activity of dopamine (DA) and 5-HT neurotransmission via direct and indirect projections to the VTA and substantia nigra, the DRN and median raphe nucleus, as well as the rostromedial tegmental nucleus (Proulx et al. 2014). It has been shown that an aggressive encounter induces a subsequent increase in DA release in the NAc and PFC in male rats (Van Erp and Miczek 2000). Even the expectation of an aggressive encounter increases DA release in the NAc, and male rats that were trained to fight at the same time of day for 10 days showed an increase in DA release, as well as increased heart rate, around the scheduled time on day 11 without an actual aggressive encounter (Ferrari et al. 2003). Furthermore, optogenetic activation of DA neurons in the VTA increased attack behaviors in the DATiRESCre mouse (Yu et al. 2014). Repeated chronic fighting experiences also caused increases of DA metabolites and noradrenaline in several brain areas (Kudryavtseva 2000), as well as increased innervation of dopamine neurons in the projection areas such as the bed nucleus of the stria terminals (BNST), LS, and NAc (Schwartzer et al. 2013). Activation of the DA system has been shown to be involved in aggression reward. For example, microinjection of dopamine D1- and D2-like receptor antagonists (SCH-23390 and sulpiride) into the NAc reduced operant lever-press behavior to obtain an intruder as a reward, and the effect of these drugs was higher in operant behavior than in actual aggressive behavior in male mice (Couppis and Kennedy 2008), suggesting that dopaminergic signaling in the NAc is involved in the motivation (or seeking) to fight. Consistent with this, DREADD inhibition of D1 receptor-positive medium spiny neurons (MSN) in the NAc reduced active lever-pressing behavior to obtain an intruder as well as reduced aggressive behavior itself (Golden et al. 2019a). By contrast, inhibition of D2-MSN did not affect aggression seeking nor aggressive behavior (Golden et al. 2019a), indicating that the major action of dopamine for aggression reward is mediated by D1-MSN in the NAc. Furthermore, AGG male mice that have high aggression motivation have increased expression of ΔFosB, a transcription factor which has been shown to be accumulated in the NAc by administration of drugs of abuse, specifically in D1-MSN (Aleyasin et al. 2018). Thus, it is likely that repeated winning experiences induce plastic change to the DA system. In fact, winning experiences over the course of 3 days in the home cage have been shown to increase the expression of androgen receptors in the NAc and VTA in California mice (Fuxjager et al. 2010).
By contrast, 5-HT release was decreased by the expectation of an upcoming fight in the NAc of rats (Ferrari et al. 2003), and administration of a 5-HT$_{1B}$ receptor agonist (CP-94253) suppressed aggression-seeking operant behaviors, especially when activating 5-HT$_{1B}$ receptor in the DRN of male mice (Bannai et al. 2007). Repeated chronic fighting experiences decreased 5-HT synthetic enzyme tryptophan hydroxylase (TPH) in the midbrain and striatum, but increased TPH in the hypothalamus of male mice (Kudryavtseva 2000). Consistent with this, 5-HT turnover in the frontal cortex is negatively correlated with aggressive behaviors after repeated fighting experiences in the male rat (de Boer et al. 2009). Thus, in contrast to DA, 5-HT action via 5-HT$_{1B}$ receptor has a suppressive effect on aggressive motivation.

In terms of physiological indices related to the HPA axis and autonomic system, no clear changes are observed after repeated fighting experiences compared to controls, whereas huge changes are observed in defeated animals (Kudryavtseva 2000). It has been reported that immune response to antigen is increased by repeated fighting experiences in dominant mice (Kudryavtseva 2000), which is consistent with findings that dominant animals show enhanced functional and adaptive immune responses compared to subordinate animals that show maladaptive immune responses after repeated defeat experiences (Takahashi et al. 2018). In summary, repeated fighting experiences cause maladaptive aggressive and anxiety-like behaviors accompanied with over-activation of the DA system and suppression of the 5-HT system in the brain. By contrast, its effect on physiological indices such as the activity of the autonomic system, HPA axis, and immune system are minor or rather adaptive. Only when repeated fighting experience was combined with the escalated aggression induced by social instigation, did 10 days of experiences of heightened aggression cause an increase in the corticosterone response by an aggressive encounter in winner mice (Fortes et al. 2017).

**Future Agenda**

Winning experience has been shown to be as rewarding as addictive drugs, and repeated aggressive behavior induces addiction-like behavioral changes as well as neurobiological changes in the reward system. In fact, repeated ethanol intake has shown to increase aggressive motivation measured by operant FI responding, without affecting the frequency of aggressive behavior (Covington et al. 2018). Stress has been shown to escalate drug taking behavior (Miczek et al. 2008; Yap and Miczek 2008) and thus future studies will need to clarify how other types of stress experiences change aggressive motivation and aggression reward. In addition, the developmental effect on aggression reward is another interesting topic to be considered. Adolescence has been shown to be a vulnerable period to addictive drugs due to higher sensation seeking and risk-taking behavior (Laviola et al. 2003), and stress experience during this phase strongly increases drug taking behavior (Burke and Miczek 2014). In humans, engagement of repeated violent behaviors, such as soldiers in the Democratic Republic of Congo during late puberty, particularly at the ages of 16–17, drastically increased the appetitive aggression trait in adulthood (Köbach and Elbert 2015). When this violent experience occurred either earlier or later than this time window, the development of appetitive aggression was not
observed. Thus, there seems to be a sensitive period for developing the aggressive motivation produced by repeated aggressive experiences.

5 Social Isolation Stress During Development

Social isolation increases territorial aggression in adult male mice (Pinna et al. 2003; Zelikowsky et al. 2018). Because this species changes their social structure depending on population density, social isolation in adulthood leads mice to show territorial behavioral strategies and increased aggressive behaviors toward intruding opponents (Brain 1975). Thus, social isolation in adulthood in territorial species should not be considered simply a stressful experience. By contrast, during the course of early development, mammalian species rely on care from adults to obtain milk, control body temperature, and maintain hygiene. In addition, social interaction and play with conspecifics during the juvenile to adolescent period are required for the appropriate development of social behaviors. Deprivation of social interactions during this early phase of development has been shown to cause devastating effects on emotion, cognitive, and social behaviors including aggressive behaviors (see reviews in Miczek et al. 2008; Veenema 2009; Haller et al. 2014).

5.1 Maternal Separation Stress

Rodent pups emit distress calls (ultrasonic vocalizations) when they are separated from their dam and siblings (Noirot 1972). These separation-induced calls are sensitive to anxiolytic drugs (Noirot 1972; Sales and Pye 1974; Gardner 1985; Brunelli et al. 1994; Miczek et al. 1995; Takahashi et al. 2009), and thus maternal separation is considered a stressful experience. Indeed, repeated maternal separation is known to increase plasma corticosterone levels in rat pups (Stanton et al. 1988), and maternal separation over hours leads to a reduction in heart rate and respiration rate (Hofer 1970). The consequences of maternal separation vary depending on the frequency and duration of separation from the dam. Short-term separation causes increased maternal care (licking and grooming) after the pups are returned to their dam (Liu et al. 1997) and thus those offspring show reduced HPA responses to stress. In contrast, long-term repeated maternal isolation induces a largely devastating effect on offspring’s physiology and behavior including aberrant social behavior, anxiety- and depressive-like behaviors, as well as increased stress responses (see reviews in Levine 2005; Veenema 2009; Haller et al. 2014; Wang et al. 2020).

Daily maternal separation for 3 h during the first 2 weeks of development (postnatal days 1–14) has been shown to increase territorial aggressive behaviors in male Wistar rats in adulthood (Veenema et al. 2006) as well as increased play-fighting behavior in 5-week-old juveniles (Veenema and Neumann 2009). This separation procedure has been shown to increase basal corticosterone levels in the
blood, and increased arginine vasopressin mRNA expression in the paraventricular nucleus of juvenile male rats (Veenema and Neumann 2009). Furthermore, stress responses including ACTH and corticosterone release in response to forced swim stress are increased in maternally separated rats in adulthood (Veenema et al. 2006). Therefore, it is likely that exaggerated stress reactivity, or arousal, during an agonistic encounter causes an increase of aggressive behaviors in maternally separated rats later in life.

On the other hand, the effect of maternal separation on physiology and behavior largely depends on strain, sex, and species (Wigger and Neumann 1999; Lehmann and Feldon 2000; Neumann et al. 2005; Millstein and Holmes 2007; Kember et al. 2012; Kundakovic et al. 2013; Haller et al. 2014). For example, in the mouse, the same maternal separation procedure that increased aggressive behavior in the rats reduced territorial aggressive behavior in C57BL/6 males (Veenema et al. 2007; Tsuda et al. 2011). By contrast, when lactating females were tested for maternal aggression, maternal separation experiences in juveniles caused an increase in the number of aggressive animals and reduced attack latency in adult C57BL/6 female mice (Veenema et al. 2007). However, in Long Evans rats, maternal aggression was reduced by repeated maternal separation stress (Boccia and Pedersen 2001). In addition, an important gene-by-environment (G × E) interaction has been reported on the effect of maternal separation in genetically modified mice. For example, monoamine oxidase A (MAOA), a catabolic enzyme of 5-HT, has been shown to be involved in exaggerated aggressive behavior when it is genetically deleted in the mouse (Cases et al. 1995; Scott et al. 2008) and human (Brunner et al. 1993a, b). There is an upstream variable-number tandem repeat polymorphism in the MAOA gene which is linked to an expression difference of MAOA, and individuals with the low MAOA expression (MAOA-L) polymorphism show a higher propensity for criminal arrests and violent history, adolescent conduct disorder, and higher aggressive disposition when they were reared under stressful conditions such as abuse and neglect in childhood (Caspi et al. 2002; Kim-Cohen et al. 2006; Fergusson et al. 2011; Byrd and Manuck 2014). This genetic effect disappears when MAOA-L individuals did not have childhood maltreatment experiences, indicating a G × E interaction on aggressive behavior. Similarly, in the mouse model of low MAOA enzymatic activity, MAOANeo mice do not show increased aggressive behavior without early life stress (Bortolato et al. 2011), but when MAOANeo mice are subjected to repeated maternal separation combined with physical stress (intrapitoneal saline injection) in the first week of life (postnatal day 1–7), they showed a large increase in the number and duration of attack behaviors in adulthood compared to wild-type and non-stressed MAOANeo mice (Godar et al. 2019). In wild-type mice, this early life stress causes a reduction in aggressive behaviors (Godar et al. 2019), which is consistent with other mouse studies (Veenema et al. 2007; Tsuda et al. 2011). This early life stress causes increased 5-HT2A receptor expression in the prefrontal cortex, and the administration of a 5-HT2A blocker during maternal separation stress in the first week has been shown to block this G × E interaction and reduce aggressive behaviors (Godar et al. 2019).
5.2 Post-Weaning Social Isolation Stress

From the juvenile to adolescent period, rats show a high level of social interaction and play behavior with conspecifics (Panksepp 1981), and interaction with conspecifics during this period has been shown to be rewarding using the CPP test in mice and rats (Douglas et al. 2004; Panksepp and Lahvis 2007; Trezza et al. 2009; Dölen et al. 2013). Social experiences during the juvenile to adolescent period play an important role in appropriate social and cognitive development (Vanderschuren et al. 1997; Špinka et al. 2001; Pellis and Pellis 2009).

Prolonged post-weaning social isolation (PWSI), from postnatal day 21 to adults, has been shown to increase aggressive behavior in adolescence and adulthood in male Wistar rats (Tóth et al. 2008) as well as in males and female Sprague-Dawley rats (Zhao et al. 2009; Wall et al. 2012; Fontenot et al. 2018). Rats subjected to PWSI show intensive attack behaviors (especially, hard bites) that are targeted at the vulnerable part of the intruder’s body and show attack behaviors without preceding offensive signals, or sometimes combined with defensive postures (Tóth et al. 2008, 2011, 2012). Thus, PWSI rats show both a quantitatively and qualitatively escalated form of aggressive behaviors. These PWSI rats also show an increased autonomic activation measured by heart rate, as well as stronger corticosterone release, induced by the aggressive encounter when compared to a socially housed control (Tóth et al. 2011). It has been shown that this abnormal form of aggressive behavior cannot be rescued by returning them to the social environment (resocialization) for 3 weeks in adulthood (Tóth et al. 2012). Even when PWSI was restricted to the first 2 weeks of post-weaning (from 4–5 weeks old) and following subsequent return to social housing, these rats showed inappropriate social behaviors when they were housed with or encountered an aggressive dominant male in the adulthood. These PWSI rats did not display submissive posture nor immobility when they encountered an aggressive resident rat, which elicited agitation in the opponent and resulted in increased aggression toward PWSI rats from the dominant rats (van den Berg et al. 1999; Von Frijtag et al. 2002). Therefore, it is likely that there is a time window that is critical for the development of appropriate agonistic behaviors. Interestingly, it has been shown that chronic treatment with fluoxetine for 3 weeks during resocialization in adulthood reversed the abnormal aggressive behaviors back to species-typical aggressive behaviors observed in control rats (Mikics et al. 2018). Because fluoxetine has been shown to reactivate critical period-like plasticity (Karpova et al. 2011), fluoxetine might re-open brain circuits that respond to social learning during critical periods to help reduce aggression by resocialization. This combination of fluoxetine with resocialization has been shown to restore BDNF levels in the infralimbic area of the mPFC, and pharmacological inhibition of the BDNF receptor abolishes the combined effect of fluoxetine with resocialization on abnormal aggression (Mikics et al. 2018). Therefore, plastic changes in mPFC neurons via TrkB signaling is an important target for the intervention of abnormal aggression induced by PWSI. In fact, PWSI has been shown to induce structural and functional changes in the mPFC of rats. Neural dendritic density and the number of glial cells in the mPFC have been
found to be decreased by PWSI, and PWSI rats show over-activation of both glutamatergic and GABAergic neurons in the mPFC induced by resident–intruder aggression (Biro et al. 2017). An optogenetics study demonstrated that the projection from the mPFC to LH is involved in the qualitative aspect of abnormal attack behaviors, while the projection from the mPFC to the mediobasal hypothalamus is involved in the quantitative aspects of attack bite behavior (Biro et al. 2018). Therefore, it is likely that early life stress during the juvenile period affects plastic changes in those projections from the mPFC to produce abnormal aggressive behaviors.

Similarly, in the mouse, PWSI has been shown to increase the duration of aggressive behaviors and reduce attack latency in adult male ICR and NMRI strains of mouse (Ibi et al. 2008; Shimizu et al. 2016; Amiri et al. 2017; Liu et al. 2019) as well as in adolescent Swiss-Webster, C57BL/6J, and BALB/c male mice (Dang et al. 2015; Locci et al. 2017; Yu et al. 2018) in the resident–intruder encounter in their home cage or novel arena. On the other hand, some studies have shown only a small effect (Bibancos et al. 2007; Workman et al. 2011) or no effect (Chang et al. 2015, 2020; Chang and Gean 2019) of PWSI in the territorial aggression test in adult male C57BL/6 J mice. Another study using C57BL/10 mice showed an increase in attack latency produced by PWSI experience (King 1957). Thus, the effect of PWSI depends on the strain of the mouse, possibly due to the low territorial aggressive behavior found in C57BL/6 J mice in this test. Indeed, a clear effect of PWSI has been observed in other types of aggression in C57BL/6 J mice. For example, in foot-shock induced aggression, in which animals are subjected to five 0.1 mA foot-shocks 30 min before the resident–intruder test, experience of prolonged PWSI increases aggressive behavior in C57BL/6 J males (Chang et al. 2015). These animals do not show aggressive behavior without foot-shock, so this isolation effect is specifically observed in defensive aggression or stress-provoked aggression (Chang et al. 2015). Similarly, an increase of shock-induced defensive aggression by PWSI experience has been observed in male rats (Knutson and Kane 1980; Potegal and Einon 1989).

PWSI mice that show increased shock-induced defensive aggression have increased NMDA receptor NR2B expression and enhanced activation of neurons in the ventral hippocampus (vHip) (Chang et al. 2015, 2018). Indeed, optogenetic and chemogenetic activation of glutamatergic vHip neurons enhances foot-shock induced defensive aggression in PWSI mice (Chang and Gean 2019). Chemogenetic activation of the projection from the vHip to the ventromedial hypothalamus (VMH), a locus responsible for the initiation of attack behavior (Lin et al. 2011; Hashikawa et al. 2017), increases attack behavior even without foot-shock (Chang and Gean 2019). However, chemogenetic activation of the vHip does not increase aggressive behavior in group-housed males, indicating that plastic changes in vHip neurons, possibly projecting to the VMH, by social isolation stress during early development are required to increase shock-induced defensive aggressive behavior (Chang and Gean 2019). BDNF has been shown to play an important role in this plastic change in the vHip (Chang et al. 2018). PWSI mice show reduced BDNF protein expression in the vHip due to an increase in a micro-RNA, miR-206, that suppresses BDNF expression (Chang et al. 2020). Overexpression of miR-206 in
4-week-old group-housed mice increased shock-induced defensive attack behaviors during adolescence (Chang et al. 2020). Importantly, plastic changes induced by resocialization combined with fluoxetine in PWSI-exposed rats were observed in vHip neurons that project to the mPFC (Mikics et al. 2018). Therefore, the vHip seems to be an important hub region for PWSI-induced escalation of aggression.

**Future Agenda**

Social deprivation stress during early life has a disruptive effect on wide range of physiological, neuroendocrinological, and behavioral phenotypes including aggression. At the same time, the effect of social stress on aggressive behavior differs depending on which stage of development they are exposed to isolation stress and also largely depends on the strain, sex, and species. During early postnatal development, the brain undergoes dynamic plastic changes such as neurogenesis, apoptosis, myelination, synaptogenesis, and synaptic pruning, and these changes continue until adulthood. In addition, during adolescence, there are large increases in gonadal hormone levels that induce organizational changes of the brain. How social stress differentially affects neuroplasticity of the brain and epigenetic modifications in each developmental period will need to be mapped systematically to identify sensitive periods of social isolation stress on exaggerated aggression.

**6 Concluding Remarks**

- Agonistic encounters activate stress responses including the sympathetic nervous system, HPA axis, and immune system in both dominant and subordinate individuals. Winners show faster recovery of those stress responses, while losers show prolonged over-activation of stress responses.
- Brief confrontation with rival conspecifics induces “aggressive arousal” or “attack readiness” and escalates aggressive behavior in the following agonistic encounter. Excitatory input into the DRN has been shown to be involved in this social instigation-heightened aggression. There are individual differences in the pro-aggressive effect of social instigation, and whether physiological and endocrinological stress responses are involved in these individual differences will need to be addressed.
- The experience of winning can be as rewarding as addictive drugs, and dominant animals seek to fight. Repeated winning experiences lead to addiction-like behavioral and neurobiological changes. The LHB has an inhibitory effect on aggression reward, and winning experience reduces LHB activity via orexin input from the LH. Repeated winning experiences increase DA neurotransmission in the NAc, while suppressing the 5-HT system.
- Social isolation stress during early development, including maternal separation and PWSI, induces devastating effects on emotion, cognitive, and social behaviors including aggressive behaviors. The effect of isolation stress on aggressive behavior depends on which stage of development they experience the isolation
stress. In addition, its effect largely depends on which strain, sex, and species are used for the experiment. How social isolation stress at each developmental period affects the epigenetic and neuroplastic changes that lead to escalated aggression will need to be addressed.

Box 1 Neural Circuit for Social Stress Induced Aggression (Fig. 2)

The medial prefrontal cortex (mPFC) is responsible for dominance status and possibly acts as a locus to calculate the current status of the animal (whether they are dominant or subordinate) during agonistic behaviors and changes their behavioral repertoire. It has been shown that activation of mPFC neurons that project to the lateral habenula (LHb) suppresses social interaction behavior (Beneekreddy et al. 2018). By contrast, activation of the mPFC to mediobasal hypothalamus projection increases the frequency of attack bites, whereas the mPFC to lateral hypothalamus (LH) projection is involved in abnormal aggressive behaviors (Biro et al. 2018). Therefore, it is likely that the mPFC bidirectionally regulates aggressive behaviors depending on the animal’s status and quickly changes the behavioral output.

The habenula has been shown to be involved in dominance/subordinate status. In zebrafish, subregions of the dorsal habenula (which corresponds to medial habenula in mice) has opposing effects on aggression in which the lateral subregion of the dorsal habenula facilitates winner behavior whereas the medial subregion of the dorsal habenula enhances loser behaviors (Chou et al. 2016). In mice, LHb activity is increased in nonaggressive animals (Golden et al. 2016), and suppression of LHb neurons via orexin input from the LH increases aggression reward and is responsible for the winner effect in dominant animals (Flanigan et al. 2020). The LHb modulates the activity of both dopamine (DA) and serotonin (5-HT) neurotransmission via direct and indirect projections to the ventral tegmental area (VTA) and dorsal raphe nucleus (DRN). Aggression reward has been shown to be mediated by DA transmission in the nucleus accumbens (NAc), especially DA action on D1 receptor-positive medium spiny neurons (D1-MSN) (Couppis and Kennedy 2008; Aleyasin et al. 2018; Golden et al. 2019a). By contrast, 5-HT action in the DRN has a suppressive effect on aggressive motivation (Bannai et al. 2007). On the other hand, social instigation has been shown to increase excitatory input in the DRN and increase 5-HT release in the projection sites, and thus DRN is involved in the aggressive arousal induced by social instigation.

Although social isolation stress in early life has multiscale effects on several brain areas, its effect on aggressive behavior is mainly mediated by the ventral hippocampus (vHip) and mPFC. Social isolation stress has been shown to activate the hypothalamic-pituitary-adrenal (HPA) axis and enhances glucocorticoid release via corticotropin-releasing factor (CRF) release from (continued)
the paraventricular nucleus (PVN) of the hypothalamus (Haller et al. 2014). 
Glucocorticoid binds to both mineralocorticoid (MR) and glucocorticoid 
receptors (GR), and abundant expression of these receptors is observed in 
the mPFC, hippocampus, and amygdala (Meaney et al. 1985; Reul and De 
Kloet 1986; Fuxe et al. 1987). Thus, both the vHip and mPFC are vulnerable 
areas for the effects of long-term stress and undergo plastic changes by 
glucocorticoid exposure induced by social isolation stress.

The details of the brain circuit that mediates the expression of aggressive 
behavior were not discussed in this review, but the above-mentioned brain 
areas interact with the “core aggression circuit” including the medial amygdala 
(MeA), premammillary nucleus (PMv), bed nucleus of the stria terminalis 
(BNST), and ventrolateral part of the ventromedial hypothalamus (VMHvl) 
in the mouse (see (Lischinsky and Lin 2020) and escalate aggressive behav-
iors. This “core aggression circuit” has similarity as well as differences among 
species, i.e. feline (Bandler 1982), rat (“hypothalamic attack area”; Kruk et al. 
1983), and mouse (VMHvl; Lin et al. 2011; Hashikawa et al. 2017), and which 
of these regions translates to the primate brain remains to be explored.

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Glucocorticoids and Aggression: A Tripartite Interaction

Jozsef Haller

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Abstract The effects of glucocorticoids on aggression can be conceptualized based on its mechanisms of action. These hormones can affect cell function non-genomically within minutes, primarily by affecting the cell membrane. Overall, such effects are activating and promote both metabolic preparations for the fight and aggressive behavior per se. Chronic increases in glucocorticoids activate genomic mechanisms and are depressing overall, including the inhibition of aggressive behavior. Finally, excessive stressors trigger epigenetic phenomena that have a large impact on brain programming and may also induce the reprogramming of neural functions. These induce qualitative changes in aggression that are deemed abnormal in animals, and psychopathological and criminal in humans. This review aims at deciphering the roles of glucocorticoids in aggression control by taking in view the three mechanisms of action often categorized as acute, chronic, and toxic

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stress based on the duration and the consequences of the stress response. It is argued that the tripartite way of influencing aggression can be recognized in all three animal, psychopathological, and criminal aggression and constitute a framework of mechanisms by which aggressive behavior adapts to short-term and long-term changes in the environment.

**Keywords**  Aggression · Psychopathology · Violence · Glucocorticoids · Non-genomic · Genomic · Toxic

## 1 Introduction

The MedLine database returned 5,488 hits when searched for the term (corticosterone [title/abstract] OR cortisol [title/abstract] OR glucocorticoid [title/abstract] OR stress [title/abstract]) AND (aggressi* [title/abstract] OR violen* [title/abstract]). This demonstrates that the role of stress and stress hormones in aggression control received large and increasing attention over the last five decades (Fig. 1, left-hand panel). This is not surprising as the stress response is the main mechanism by which environmental challenges are translated into neural and behavioral changes, including aggressive behavior (Fig. 1, right-hand panel).

The almost exponential increase in the number of publications may at least partly be explained by the evolution of the concepts of stress and aggression, both undergoing considerable developments in the last decades. Indirectly, this resolved many of the controversies related to their relationship. It gradually became clear that there is no general stress-aggression relationship as this depends on the type of stress and type of aggression considered. Therefore, we start this review by overviewing

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![Fig. 1](image)  *Interest in the glucocorticoid-aggression relationship over time*. The interest is partly explained by the central role of glucocorticoids in mediating between environmental influences and behavioral responses to these as shown on the right-hand panel of the figure. In addition, the relationship is often perceived as an unresolved puzzle, which likely contributes to the exponential growth of publications.
the main concepts concerning stress responses and their mechanisms as well as the various conceptualizations of aggressive behavior.

2 Contexts of the Glucocorticoid-Aggression Interaction

2.1 The Main Types of Glucocorticoid Stress Responses and Their Mechanisms

2.1.1 Acute Stress

The term “acute stress” denotes rapid and transient increases in plasma glucocorticoids that are elicited by environmental challenges and are terminated once the challenge was adequately responded. One such challenge is the aggressive encounter, which results in a strong acute stress response that vanishes once the encounter was won. This type of aggression-related stress response was documented first in mice (Bronson and Eleftheriou 1965), thereafter in various species, e.g., rats (Schuurman 1980), fish (Earley et al. 2006), lizards (Woodley et al. 2000), and birds (Ramenofsky 1985). Noteworthy, the acute nature of the stress response depends neither on the intensity of aggression nor on the status of the individual (resident or intruder) but on the act of winning (Haller et al. 1996). Similar responses were observed in people submitted to various laboratory tests of aggression, e.g., the Taylor and Point Subtraction paradigms (Böhnke et al. 2010a, b; Geniole et al. 2011), children and adults playing violent video games (Gentile et al. 2017; Aliyari et al. 2018) as well as in field studies where spontaneous aggressive behavior was accompanied by surges in glucocorticoid production (Adam 2006; Murray-Close et al. 2008).

The discovery of the genomic way of glucocorticoid action (McEwen et al. 1986; Joëls and de Kloet 1992) resulted in some controversies regarding the role of glucocorticoids in aggression, as the earliest genomic effects (which involve protein synthesis) are expressed within approximately half an hour, when the aggressive encounter is usually terminated in laboratory rodents where the interaction received the largest attention (Haller et al. 2008). Yet the non-genomic way of glucocorticoid action was recurrently demonstrated later (Haller et al. 1998a, b, c; Liu et al. 2019; Makara and Haller 2001; Panettieri et al. 2019). Briefly, glucocorticoids can alter membrane fluidity, can bind to membrane proteins (e.g., voltage-dependent calcium channels) and neurotransmitter receptors (e.g., opioid and GABA receptors), and can influence the function of intracellular signaling mechanisms. Moreover, they have their own membrane receptors besides their genomically active cytoplasmic ones. Via these mechanisms, glucocorticoids can affect behavior rapidly, well within the timeframe of aggressive encounters.
2.1.2 Chronic Stress

Chronic stress emerges when the organism was unable to adequately respond an environmental challenge. Within the context of aggression, chronic stress develops in losers if these are forced by circumstances to cohabitate with winners (Schuurman 1980). Noteworthy, the severity of chronic stress depends on the duration of winner/loser cohabitation. E.g., daily encounters of 4 h chronically increases plasma glucocorticoids without enlarging the adrenals and decreases thymus weight whereas continuous cohabitation produces all three symptoms of chronic stress (Zelena et al. 1999).

The consequences of chronic stress result from genomic alterations induced by glucocorticoids (Joëls and de Kloet 1992; McEwen et al. 1986; Nishi and Kawata 2007). The number of glucocorticoid responsive elements on the DNA is rather high; estimates range from several hundreds to several thousands. Protein synthesis may also be affected by glucocorticoid binding to certain messenger RNAs (Ing 2005).

Glucocorticoids modulate gene function constitutively, i.e., without the involvement of stress responses. Yet, chronic stress durably increases glucocorticoid receptor occupancy, and the larger number of glucocorticoid-receptor complexes may affect DNA segments that rarely bind glucocorticoids under normal conditions. This induces a qualitatively differential glucocorticoid gene modulation under stressful and non-stressful conditions. In addition, some genomic effects lead to consequences within relatively short periods (e.g., hours) whereas others develop only after prolonged periods of stress (e.g., month) involving that the effects of chronic stress change over time, and such long-term changes are brain region-specific (Datson et al. 2008; de Kloet et al. 2008; Thormann et al. 2019).

The result of gene modulation by glucocorticoids is that the protein machinery of neurons (and naturally other cells) is altered, with secondary changes in neural function and behavior. It is worth to mention, however, that the chronic effects of glucocorticoids result from increased receptor occupancy, which decreases in parallel with the decrease of plasma glucocorticoids. This involves that the genomic effects of glucocorticoids are reversible.

2.1.3 Toxic Stress

The term “toxic stress” initially was used to depict stress responses elicited by toxins (Gerwing 1958), but over the last decade its meaning changed, and is currently used to identify the persistent, often life-long consequences of stressors suffered in childhood (Garner et al. 2012), adolescence (Joos et al. 2019), young adulthood (SmithBattle 2019), adulthood (Sabri and Granger 2018), and advanced age (Andreescu et al. 2008). In contrast to chronic stress, the effects of toxic stress persist long after the hormonal response terminated and may even lead to life-long conditions. Stressors that elicit such responses are either unusually strong e.g., traumatic, or exceedingly durable.
The core mechanism of toxic stress is epigenetics, which targets the genome like chronic stress, but induces molecular alterations in the DNA. The best characterized epigenetic mechanisms are histone acetylation and the methylation of nucleobases (Holliday 1989; Turner 1998). The consequence of these are often persistent; moreover, may have life-long consequences if occur during sensitive periods of brain development (Nagy and Turecki 2012).

Glucocorticoids are among the main engines of epigenetics (Reul et al. 2014; Sasaki and Matthews 2019) and generate changes primarily in the genes that control the glucocorticoid stress response (Meaney and Szyf 2005). Although they are not the only factors of epigenetics, they constitute important modulators of brain programming and psychopathology in interaction with other factors (Klengel and Binder 2015). It was hypothesized that toxic stress and the ensuing epigenetic changes prepare the organism for harsh circumstances which are predicted by the exceptional strength of stressors suffered. Preparations may include uncommon forms of aggression (Crombach and Elbert 2014; Sommer et al. 2017; van Lange et al. 2017).

3 Concepts of Aggression

3.1 The Four Main Approaches

There are four scientific areas where aggression is of primordial importance, these being biology, psychology, psychiatry, and law enforcement. All four agree that aggression is a behavior, which, however, is differentiated from other behaviors based on unique principles. In addition, the four areas create dissimilar subdivisions within the main concept, which show virtually no overlaps. Thus, the concept of aggression is versatile, and the glucocorticoids-aggression relationship cannot be disentangled without taking this in view.

The simplest and most phenomenologically oriented approach is provided by psychologists, who consider aggression as a behavior that aims at the delivery of harm (Buss 1961). Subsequent scholars (e.g., Baron 1977; Jhangiani and Tarry 2014; Zillman 1979) changed the wording but the essence of the definition remained the same ever since. Some modern scientists (e.g., Anderson and Husemann 2003) enriched the definition with a series of details, e.g., they emphasized the importance of intention over the actual delivery of harm and specify that harm may also be psychological. Yet definitions remain behavior oriented with few exceptions. In psychology three types of aggression are differentiated, e.g., proactive, reactive, and more recently, psychotic aggression. Proactive and reactive aggression (Dodge and Coie 1987) was first differentiated by Berkowitz (1967). The terms have many synonyms, e.g., controlled-impulsive (Sneiers et al. 2007), instrumental-hostile (Atkins and Stoff 1993), predatory-emotional (Vitiello et al. 1990), premeditated-impulsive (Barratt et al. 1999). All these differentiate the deliberate use of aggression for a personal goal from uncontrolled outbursts of aggression elicited by provocation. Noteworthy, one of the main distinctive features of proactive and reactive
aggressions is the aggression-related stress response – its diminution in the former and its exacerbation in the latter. The third type, psychotic aggression involves behaviors executed under the effect of cognitive distortions (Philipp 1979).

In biology, aggression is conceptualized as a form of competition that implies the delivery of harm. The concept is often attributed to Darwin (1871) or Craig (1917). One modern formulation reads as follows “Aggressive behaviors in animals, for example, threat, attack, and defense, are commonly related to competition over resources, competition over mating opportunities, or fights for survival” (Lindenfors and Tullberg 2011). Note that the behaviors listed in the definition fulfill the criteria of the psychological approach as they involve harm or the threat of harm. Types of aggression are often differentiated based on the resource that is competed for. Aggression can be territorial, hierarchical, parental, etc. The biological approach was built into laboratory paradigms of human aggression, where participants compete for a reward (Taylor 1967; Cherek 1992).

In psychiatry, aggression is a symptom of a variety of mental disorders, without being an entity of its own right. The latter is best exemplified by the failure of the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5; American Psychiatric Association 2013) to define the term in the Glossary of Technical Terms, which defines a series of terms related to aggression, e.g., callousness, disinhibition, hostility, impulsivity, and temper tantrums, but not aggression per se. In this normative psychiatric manual, “impairments in social, occupational, or other important areas of functioning” are the primary diagnostic criteria of mental disorders (DSM-5, p. 21). Apparently, aggression per se does not fulfill these basic criteria, involving most manifestations of aggressive behavior are outside of its scope. Only those aggressions receive attention that are embedded into a wider array of mental and behavioral malfunctions. As aggression is not defined, it is not classified either. Yet, the disorders for which aggression is a symptom belong to distinct disorder groups, e.g., personality disorders, impulse control disorders as well as disorders involving cognitive dysfunctions. Roughly, these are analogous to the psychological groups called proactive, reactive, and psychotic aggression, respectively. Importantly, however, these varieties of the psychological concept become parts of psychiatric ones only if they are associated with the other, aggression-unrelated symptoms of the respective disorders.

Law enforcement also employs a restrictive approach. For instance, the Uniform Crime Reporting Program of the FBI defines violence as “…offenses which involve force or threat of force” and specifies that “violent crime is composed of four offenses: murder and nonnegligent manslaughter, forcible rape, robbery, and aggravated assault” (Violent Crime, FBI 2021, section Violent Crime subsection Definitions). As such, aggression is within the scope of the law enforcement approach only if it constitutes an offense. Minor forms of aggression, as well as self-defense, the defense of others e.g., by the police as well as sports e.g., martial arts are no subject areas of the law enforcement approach. As such, law enforcement implicitly classifies aggression into three main categories: prosocial, i.e., aggression that serves the society and is under certain conditions a social obligation, tolerated, i.e., aggression that is not a necessity from a social perspective but does not break the law, and
antisocial, i.e., violence that is socially unwanted. Naturally, there are more sophisticated systems of classification where the motive and other circumstances of violence are considered (see Douglas et al. 2013), yet all these systems classify antisocial forms of aggression only.

### 3.2 A Comparison of the Main Concepts

We suggest that the four main approaches can be united into two that may be depicted as “normal” and “abnormal” aggressions. Normal aggression, i.e., the natural manifestation of an evolutionarily shaped behavior is the study target of the biological and psychological approaches, whereas psychiatry and law enforcement study deviant behaviors.

Theoretically, the psychological approach encompasses all forms, because phenomenologically all involve the delivery of harm. In practice, however, psychological studies focus on non-criminal subjects who are mentally healthy. Both the biological and psychological approaches consider aggression as an integral part of the behavioral repertoire of the species, the total absence of which is never assumed. The link between the biological and psychological approaches is also outlined by using animal aggression as a model for human aggression. This approach – exemplified by the term comparative psychology – allows the deciphering of underlying mechanisms that cannot be studied in humans. As such, the biological approach becomes a “tool” for mechanistic studies, the conclusions of which are deemed to be valid for both animals and humans.

By contrast, psychopathologies and law infringements are typical to subgroups of the population only. The two approaches are similar not only by their focus on deviant behaviors but also by the large overlaps between their study subjects. Firstly, law infringements are often listed among the symptoms of aggression-related psychopathologies. For instance, symptom A1 of antisocial personality disorder reads as follows “Failure to conform to social norms with respect to lawful behaviors as indicated by repeatedly performing acts that are grounds for arrest” (American Psychiatric Association 2013). Similarly, DSM-5 lists the use of weapons against people, robbery, destruction of property, etc. among the symptoms of conduct disorder. In other cases, e.g., intermittent explosive disorder, the main symptom “failure to control aggressive impulses” is inherently law breaking even if law infringement is not mentioned in the description of the disorder. On the other hand, law enforcement studies quite often categorize violent offenders based on psychiatric criteria (Douglas et al. 2013; Megargee and Carbonell 1995; Sneyers et al. 2007). Finally, and most importantly, psychiatric studies often mention law breaking by study participants, whereas law enforcement studies often provide details on their psychopathologies. Sometimes it is difficult to tell the two kinds of studies apart.
4 The Glucocorticoid-Aggression Relationship: Conceptually Grouped Findings

In the followings we present the findings on the glucocorticoid-aggression relationship grouped along three statements that are used as titles of the following sections. First we address normal (species-specific) aggression, which is the study subject of the biological and psychological approach. Thereafter we address abnormal aggression from the point of view of both the psychiatric and law enforcement approach. Each section starts with human findings; we made efforts to cover all the published studies. Subsequently, we overview animal findings, which often provide mechanistic details of the phenomenon.

4.1 Acute Stress Supportively Controls Normal Aggression

4.1.1 Humans: The Psychological Approach

In healthy non-criminal populations, the role of glucocorticoids was examined so far in laboratory paradigms of aggression, as well as in nurseries, kindergartens, and summer schools, where associations were studied between behavior and cortisol that were measured several times over the day. Findings show that:

– Competitive challenges, e.g., those involved by the Taylor and Point Subtraction paradigms, Ultimatum Game and violent video games increase glucocorticoid production in both genders as well as in children and adults (Aliyari et al. 2018; Böhnke et al. 2010a, b; Geniole et al. 2011; Gentile et al. 2017; Gerra et al. 1997, 2001, 2004; Goulter et al. 2019; Probst et al. 2018). No contradictory findings were reported, but in two studies glucocorticoid increases were not observed (De Sousa Fernandes Perna et al. 2016; Gerra et al. 2007). The intensity of aggression and the blood level of the hormone correlated significantly in most cases.

– In field studies performed usually in children and adolescents, higher spontaneous aggression over the day correlated with higher plasma glucocorticoid levels (Adam 2006; Blair et al. 2005; Gunnar et al. 2010; Marsman et al. 2008; Murray-Close et al. 2008; Tout et al. 1998). We found only one study where this was not observed (Oberle et al. 2017).

– Acute stressors as well as the glucocorticoid hydrocortisone administered before the Taylor Aggression Paradigm increased aggressiveness (Böhnke et al. 2010a, b; Verona and Curtin 2006; Verona and Kilmer 2007).

In brief, aggression associates in humans with an increase in glucocorticoid production. The aggression-enhancing role of this response is indicated by disparate observations on the consequences of pre-trial stressors and in one instance of hydrocortisone. No trials to limit glucocorticoid production were performed so far,
for which the evidence remains circumstantial. Noteworthy, the glucocorticoid synthesis inhibitor metyrapone—amply employed in animal studies to investigate causality—was approved for human use both as a diagnostic tool and as a treatment (Avgerinos et al. 1996; Sigalas et al. 2012). To the best of our best knowledge, however, the compound was not studied in humans, for which one must rely on animal studies regarding mechanistic explanations.

4.1.2 Animals: The Biological Approach

As shown above, aggression rapidly increases aggression in various taxa; moreover, the mere sensing of potential opponents elicits stress responses including glucocorticoid production (Haller et al. 1995; Landys et al. 2010; Tornatzky and Miczek 1994). As actual aggression starts with a delay as compared to the sensing of opponents, this means that the behavior develops on the background of increased glucocorticoid production.

The inhibition of the social challenge-induced glucocorticoid stress response by metyrapone dramatically reduced aggression, which was restored by exogenous corticosterone treatments, an effect that was insensitive to protein blockade (Mikics et al. 2004; Ruiz-Aizpurua et al. 2013). As altered protein synthesis is a prerequisite of genomically mediated glucocorticoid actions, these findings show that glucocorticoids affect aggression rapidly by non-genomic mechanisms. An experiment involving aggression elicited by the electric stimulation of the hypothalamic attack area suggested the existence of a positive feedback loop between aggression-related brain mechanisms and glucocorticoid production (Kruk et al. 2004).

It occurs that the facilitatory role of glucocorticoids is not restricted to challenge-induced increases in glucocorticoid production as natural variations in glucocorticoid production and aggression-unrelated stressors are also relevant for aggression control. Glucocorticoid production is subject to both diurnal and ultradian variation (Lightman et al. 2002, 2008), and aggressiveness was higher during the zeniths as compared to the nadirs of oscillatory production (Haller et al. 1998a, b, c, 2000a, b). Glucocorticoid receptor blockade prevented the increase in aggression, suggesting a causal relationship. Social and non-social stressors administered before the aggressive encounter also increase aggression in a variety of taxa (Chang and Gean 2019; de Almeida and Miczek 2002; Manuel et al. 2016; Potegal et al. 1996; Takahashi et al. 2012).

4.1.3 Summary

In agreement with the fight-flight concept of Cannon (1915) and the frustration-aggression hypothesis of Miller (1941) these findings demonstrate that aggression is expressed under the supportive control of stress responses. As such, the findings briefly reviewed in this section are not surprising. Their novelty consists in attributing glucocorticoids an active role in this phenomenon. As adrenaline does not cross
the blood-brain barrier under physiological conditions (Hardebo and Owman 1980),
glucocorticoids remain the only stress hormones that acutely control aggressive
behavior. This assumption appears weakly and strongly supported by human and
animal studies, respectively.

As a second novelty, one study suggests that this effect of glucocorticoids is
exerted via non-genomic mechanisms because the effect developed rapidly (within
2 min) and was insensitive to protein synthesis inhibition (Mikics et al. 2004). We
suggested earlier that glucocorticoid effects can be assigned to non-genomic mech-
anisms if the effect develops in considerably less than 15 min and persists after either
or both the blockade of cytoplasmic receptors and protein synthesis (Haller et al.
2008). Albeit rapid effects of glucocorticoids on aggression were reported by several
studies (Brain and Haug 1992; Hayden-Hixson and Ferris 1991a, b; Poole and Brain
1974) other criteria of non-genomic mechanisms were investigated just by one. As
such, this finding awaits confirmation and clarification as it regards mechanistic
details.

4.2 Faulty Impulse Control-Linked Aggression Associates
with Glucocorticoid Overproduction

4.2.1 Humans: The Psychiatric Approach

In humans, chronic stress is inevitably associated with clinical conditions; therefore,
the role of this hormonal condition in aggression control is usually investigated from
the point of view of the psychiatric approach. For a more psychology-oriented
approach, see section “Unresolved cases.”

Typical examples of disorders where aggression is elicited by the loss of impulse
control are disruptive mood dysregulation and intermittent explosive disorders,
which are characterized by disproportional aggressive responses to provocation.
Unfortunately, these were not investigated so far regarding the relationship between
aggression and glucocorticoid production. More data are available for attention-
deficit/hyperactivity (ADHD), borderline personality (BPD), and depressive (DEP)
disorders.

In these disorders, both constitutive secretion profiles and glucocorticoid stress
responses were studied. The former covers measurements performed independently
of aggression, in ways that represent the overall propensity of the adrenals to secrete
the hormone. This includes the evaluation of the awakening glucocorticoid response
which is deemed to reflect the overall functionality of the stress system, repeated
measurements over the day, 24 h secretion profiles e.g., by measuring glucocorti-
coyds in collected urine samples, and the measurement of the hormone in hair, which
mirrors secretion profiles over long periods. Regarding glucocorticoid stress
responses we focused on studies where glucocorticoid production was evaluated
several times, e.g., before, during, and after the aggressive encounter. Point mea-
surements, i.e., single measurements performed at the end of an aggressive
encounter are rather unreliable as argued elsewhere (Haller 2014, 2020, pp. 185–186); therefore, such studies received little attention here.

Aggression in “pure” ADHD, i.e., in the absence of comorbidities, the disorder was typically associated with increased glucocorticoid stress responses (Kaneko et al. 1993; van West et al. 2009; Palma et al. 2012; Lackschewitz et al. 2008) albeit no association (Palma et al. 2012) and the inverse relationship was also reported (Maldonado et al. 2009). In BPD, aggression was associated with either or both increased stress responses (Banki and Arató 1983; Carvalho Fernando et al. 2012; Engström et al. 1997; Minzenberg, et al. 2006; Nater et al. 2010; Rinne et al. 2002; Schweitzer et al. 2001; Simeon et al. 2007; Wingenfeld et al. 2007a, b) and increased constitutive glucocorticoid production (Rausch et al. 2015; Wingenfeld et al. 2007a, b; Jogems-Kosterman et al. 2007; van Heeringen et al. 2000). Opposite findings were not reported, but no association was found in two studies (stress response: Ehrenthal et al. 2018; constitutive production: Simeon et al. 2007). Finally, aggression was associated with increased glucocorticoid secretion in depression (stress response: Van Praag 1998, 2001; López-Ibor et al. 1985; constitutive production: Van den Bergh et al. 2008; Sher et al. 2005). Note that plasma glucocorticoids are usually high in depression; we referred here to studies where aggressive and non-aggressive depression patients were compared. The number of studies using aggressiveness as an independent variable is small, but their findings are highly similar.

When ADHD was comorbid with anxiety, the acute stress response was further increased (Hastings et al. 2009), whereas comorbidity with oppositional and/or conduct disorder reversed the relationship (Hastings et al. 2009; Northover et al. 2016; Pesonen et al. 2011; Stadler et al. 2011). The latter are primarily associated with decreased glucocorticoid secretion (see next section). It occurs that their interaction with glucocorticoid production overrides that of ADHD.

4.2.2 Humans: The Law Enforcement Approach

In contrast to violent crimes of the antisocial type (see next section), those attributable to poor impulse control were rarely if ever investigated in conjunction with glucocorticoid secretion. However, all patients suffering from the above-mentioned disorders are overrepresented in violet criminal populations (Fazel et al. 2015; Látalová 2009; Mohr-Jensen and Steinhausen 2016; Sansone and Sansone 2009). In addition, studies on the glucocorticoid-aggression relationship in these psychopathologies often mention the presence of criminal violence in their study sample, which further outlines the similarity of the psychiatric and law enforcement approach.
4.2.3 Animals: Chronic Stress

As shown above, acute glucocorticoids surges promote competition by aggression ostensibly by non-genomic mechanisms. This effect, however, appears transient, as defeat – the unfavorable outcome of the aggressive encounter – decreases aggression in a variety of taxa (fish: Höglund et al. 2002; reptiles: Summers et al. 2003; birds: Carere et al. 2001; laboratory rodents: Schuurman 1980). Similarly, non-social stressors that last longer than 2 h dramatically reduce aggression (Albonetti and Farabollini 1993; Wood et al. 2003; Yohe et al. 2012). Although the stress – e.g., defeat-induced decrease in testosterone likely contributes to this effect (Solomon et al. 2009), glucocorticoids also play a role as chronic glucocorticoid treatments decrease aggressiveness and promote submission (fish: Summers et al. 2005; reptiles: DeNardo and Sinervo 1994; Tokarz 1987; birds: Meddle et al. 2002; mammals: Leshner et al. 1980). Moreover, glucocorticoid injections administered after the encounter to mimic the defeat-induced persistence of glucocorticoid secretion induced submission irrespective of the actual outcome of the fight (Timmer and Sandi 2010).

All these findings show that chronic glucocorticoid overproduction decreases aggression in animals and suggest that the human case briefly reviewed above cannot be explained by animal studies. Interestingly however, stressors repeatedly administered to laboratory rodents over prolonged periods lose their aggression-inhibiting effect; moreover, they increase aggressiveness (Yohe et al. 2012; Wood et al. 2003). This paradoxical effect of repeatedly administered homotypic (similar) stressors may be due to sensitization to heterotypic (different) stressors, which results in exacerbated stress responses to the latter (Belda et al. 2016; García et al. 2000). As a similar sensitization-like process was proposed for de development of human depression (Willner et al. 2013), and because acute stress responses promote aggression as shown above, we propose that increased sensitivity to heterotypic stressors explains anger outbursts in animals, which may model aggression in depressed patients.

4.2.4 Animals: Models of Reactive Abnormal Aggression

Beginning with the early 2000s, models of abnormal aggression became more and more popular in animal research. In such models, etiological factors of abnormal (e.g., psychopathological) human aggression are modelled in animals, and their consequences for aggressive behavior are investigated in competitive situations, e.g., the resident-intruder test which on its turn models territorial aggression (Haller et al. 2001; Haller and Kruck 2006).

In such models, aggression becomes quantitatively excessive (exceeds species-typical levels), attack patterns change qualitatively in the meaning that they potentially lead to severe injuries (e.g., attacks are aimed at the head, throat, belly, and occasionally paws and testicles of opponents), the social signaling of attacks is deficient, which makes them unpredictable for their targets, and involve direct and
indirect risks for the attacker (e.g., they may be initiated against larger individuals or may target reproductively valuable individuals, e.g., pups and females). These changes in aggressive behaviors were considered abnormal and analogous to psychopathological aggression in many respects (Haller and Kruk 2006; Miczek et al. 2013). Although never considered from this perspective so far, aggression in these models may also be comparable to law-breaking aggression as it involves the breaking of evolutionary rules that make aggression compatible with the survival of combatants and change cost/benefit relationships unfavorably for the individuals and the species.

Some of the abnormal aggression models, particularly rats selected for low anxiety as well as maternal separation, postweaning social isolation and repeated administration of cocaine or amphetamine in adolescence lead to increased stress responses and abnormal aggression in adulthood and may model aggression-related psychopathologies (Haller 2017; Haller et al. 2014). Albeit it is difficult to make direct connections between animal models and human psychopathologies, animals submitted to these models show multiple similarities with abnormal forms of human aggression that are elicited by the loss of impulse control (Haller 2017, 2018). Similarities include exacerbated responses to provocation and enhanced glucocorticoid and autonomic stress responses to aggression.

4.2.5 Summary

Enhanced constitutive glucocorticoid secretion as well as enhanced stress, including glucocorticoid stress responses is preferentially associated with disproportional aggressive responses to provocation in several psychopathologies (Fig. 2). Analogous forms of aggression can be seen in animals chronically stressed in adulthood as well as in certain models of abnormal aggression.

We suggest that the combination of homotypic and heterotypic stressors may model aggressive outbursts in depression, whereas abnormal aggression associated with enhanced stress responses may model aggression seen in the other psychopathologies discussed here.

We also suggest that the behavioral consequences of enhanced glucocorticoid secretion are mediated by two different mechanisms in the psychopathologies discussed in this section and their corresponding animal models. Depression may develop at any age, it responds positively to both psycho- and pharmacotherapy; moreover, spontaneous remission is rather frequent (Whiteford et al. 2013). Similarly, the behavioral consequences of chronic stress disappear relatively rapidly after the elimination of the stressor in laboratory animals. This is compatible with the assumption that such effects are mediated by gene modulation that is dependent on the presence of glucocorticoids. By contrast, ADHD and especially BPD develop as responses to early stressors and persist long after the termination of the triggering events. Similarly, abnormal aggression associated with excessive glucocorticoid stress responses, e.g., that triggered by postweaning social isolation are resistant to social re-integration, suggesting that the behavioral deviance persists long after the
elimination of its cause (Tulogdi et al. 2014). This is consistent with the toxic stress concept and suggests that consequences are due to epigenetic phenomena. This assumption was experimentally demonstrated for the postweaning social isolation model of abnormal aggression (Mikics et al. 2018).

4.3 Antisocial Aggression Associates with Glucocorticoid Deficits

4.3.1 Humans: The Psychiatric Approach

Here we discuss disorders which are associated with antisocial tendencies e.g., with the deliberate use of aggression for personal benefit. Albeit a poor control over emotions may also result in violent crime, the disorders discussed in the previous and this section are different regarding the motive of aggression. Typical examples of this category of psychopathologies are conduct disorder in youth and antisocial personality disorder in adults. The antisocial character of behaviors seen in these

Fig. 2 The tripartite interaction – a graphical representation of findings. An unselective view suggests that aggression can associate with both increased and decreased glucocorticoid production. However, clear trends can be observed when types of aggression are evaluated separately. Note that even when the association seems similar (left-hand and middle columns) the underlying mechanism is still different. Conclusions that can be drawn based on the majority findings are somewhat obscured by a non-negligible minority. Possible causes of such discrepancies are discussed in the section Overall summary.
disorders is amply demonstrated by their DSM-5 description. We also included oppositional-defiant disorder, where antisocial tendencies are not general but are directed against authoritative figures.

Predominantly, conduct disordered youth show decreased constitutive glucocorticoid production (Oosterlaan et al. 2005; Pajer et al. 2001, 2006; Platje et al. 2013; Stoppelbein et al. 2014; Van Bokhoven et al. 2005; von Polier et al. 2013) and/or reduced stress responses (Azar et al. 2004; Buydens-Branchey and Branchey 2004; Feilhauer et al. 2013; Fairchild et al. 2008). Increased constitutive secretion was observed in two studies (Azar et al. 2004; Fairchild et al. 2008), whereas no association was found in one (Oosterlaan et al. 2005). Note that conflicting findings were reported within the same studies. For instance, cortisol secretion did not correlate with conduct disorder symptoms when these were evaluated by parents but showed correlation when evaluated by teachers (Oosterlaan et al. 2005). In another, there were no overall associations between overall diurnal secretion patterns, but associations were seen at certain timepoints (Fairchild et al. 2008).

Relatively few studies addressed associations in oppositional defiant disorder, but all are in favor of a negative correlation between symptom severity and glucocorticoid production (Kohrt et al. 2015; Snoek et al. 2002; van Goozen et al. 1998). The quasi-psychiatric condition called disruptive behavior attracted more attention. This term covers mixed conduct and oppositional defiant disorder symptoms, which separately do not fully fulfill diagnostic criteria for either. The predominant finding was negative correlation, symptom severity being associated with low constitutive glucocorticoid responses (Loney et al. 2006; Popma et al. 2007) and/or low stress responses (Moss et al. 1995; Popma et al. 2006; Schaefer et al. 2013; Schoorl et al. 2016; van de Wiel et al. 2004; van Goozen et al. 2000). The opposite conclusion was drawn by two studies (Kobak et al. 2009; Susman et al. 2010) and no association was found in one (Sondeijker et al. 2007).

Finally, antisocial personality disorder also associates with decreased glucocorticoid secretion constitutively or during acute stress (Bergman and Brismar 1994; Fishbein et al. 1992; Holi et al. 2006; Johnson et al. 2015; Lindman et al. 1997; O’Leary et al. 2007; Sorocco et al. 2006; Virkkunen 1985). Opposite findings were reported by one study (Loomans et al. 2016) and no association was found in two (Fishbein et al. 1992; Glenn et al. 2015).

Note that in many studies, the focus was on the severity of symptoms, which included but was not restricted to aggression. However, negative correlations between glucocorticoid production and aggression were more accentuated when aggression and/or violent criminal behavior was also considered. This is also evident from the review of law enforcement studies.

4.3.2 Humans: The Law Enforcement Approach

Violent crime was invariably associated with decreased glucocorticoid production be that constitutional (Bergman and Brismar 1994; Brewer-Smyth et al. 2004; Cima et al. 2008; Dabbs et al. 1991; Gowin et al. 2013; Holi et al. 2006; Lindman et al.
1997; Platje et al. 2013; Virkkunen 1985) or phasic (Gowin et al. 2013; Moss et al. 1995; van der Meij et al. 2015). Note that part of the papers referred to here were already referred to in the previous section, because the participants of psychiatric and law enforcement studies overlap to a large extent due to the comorbidity of psychiatric diagnoses and criminal records.

The relationship between violent crime and glucocorticoid production appears to be valid for crime in general, as a similar relationship was found in incarcerated populations, which were either non-violent or included non-violent criminals. In such cases, crime was associated with either decreased constitutive glucocorticoid production (Popma et al. 2007; Gostisha et al. 2014) or decreased glucocorticoid stress responses (Couture et al. 2008, 2018; Halpern et al. 2002; Johnson et al. 2015; Popma et al. 2006). Here, however, two conflicting findings were also reported (Kobak et al. 2009; Loomans et al. 2016).

### 4.3.3 Animals: Abnormal Aggression Models

There are three models of abnormal aggression, which are associated with decreased glucocorticoid production. The subjugation model developed in hamsters consists in exposing subjects repeatedly to social defeat from large males in the peripubertal period. This results in kind of a “cyclist reaction” in adulthood (downward treading and upward bending) where aggression increases against smaller but decreases against larger opponent (Delville et al. 1998). Abnormal features of aggression include in this model the premature expression of adult forms of aggression – demonstrating that development was altered – and the readiness of treated hamsters to attack juveniles (Delville et al. 1998; Ferris et al. 2005; Wommack and Delville 2003). Glucocorticoids administered in the peripubertal period result in a similar behavioral profile in adulthood, whereas glucocorticoids receptor antagonists administered concurrently abolish the development of the behavioral abnormality (Wommack and Delville 2007; Wommack et al. 2005). This indicates causal relationships between the pubertal glucocorticoid stress response and the delayed alteration of aggressive behaviors. Peripubertal defeat increased glucocorticoid production acutely, but plasma levels normalized within two weeks, and as adults, hamsters showed reduced glucocorticoid stress responses to social challenges (Ferris et al. 2005; Wommack et al. 2004). The temporal dissociation between the stress-induced glucocorticoid production and its behavioral consequences indicates the involvement of toxic stress-like mechanisms that affect both the stress system and behavior, possibly in interaction. Consistent findings were obtained in rats (Cunningham and McGinnis 2008) but the rat variant of the model was less well characterized.

In the peripubertal stress model, rats are exposed to three non-social stressors around puberty, which dramatically increases aggressiveness in adulthood (Márquez et al. 2013). Biting attacks are poorly signaled socially and preferentially target vulnerable parts of opponents; in addition, rats submitted to the model attack anesthetized, i.e., inoffensive opponents and females (Cordero et al. 2012; Márquez
et al. 2013; Papilloud et al. 2018). The model was employed in females with similar behavioral consequences (Cordero et al. 2013). The administration of glucocorticoid receptor antagonists concurrently with the stressor in puberty abolishes behavioral consequences in adulthood (Papilloud et al. 2019) demonstrating the causal role of this hormone in the development of abnormal aggression. Basal glucocorticoid levels remain unaltered in adulthood, but rats show blunted stress responses to aggression (Márquez et al. 2013; Papilloud et al. 2018). Noteworthy, downregulated glucocorticoid production was observed already in late puberty when altered aggression was not yet apparent (Walker et al. 2018a, b; Papilloud et al. 2019), which suggests that hormonal changes precede behavioral ones.

In the glucocorticoid deficit model of abnormal aggression, glucocorticoid secretion was abolished experimentally in adult rats by bilateral adrenalectomy, but low basal levels of the hormone were ensured by subcutaneous corticosterone pellets (Haller et al. 2001). Abnormal aggression developed within a week and was characterized by a dramatic increase in attacks on vulnerable body parts of opponents and decreased social signaling of attack intentions (Halasz et al. 2008; Haller et al. 2005; Tulogdi et al. 2010). In addition, rats showed a decreased autonomic stress response when socially challenged, which is similar to decreased autonomic responses observed in antisocially aggressive humans (Haller et al. 2004). The short-term (acute) inhibition of glucocorticoid secretion decreased aggression without the induction of abnormal attack features, whereas repeated daily corticosterone injections abolished the behavioral effects of the chronic glucocorticoid deficit (Halasz et al. 2008; Haller et al. 2001), which suggest a causal relationship between the chronic decrease in plasma glucocorticoids and altered aggressive behavior.

4.3.4 Summary

The findings briefly reviewed in this section show that psychopathologies characterized by gain-motivated antisocial aggression are preferentially associated with decreased glucocorticoid secretion profiles that are manifested as decreased constitutive glucocorticoid production, decreased glucocorticoid stress responses or sometimes both (Fig. 2). It is worth to mention, however, that a non-negligible share of studies drew the opposite conclusion or found no association. We discovered no qualitative differences between these three types of studies; as such, neither seems to be attributable to experimental error. The possible explanations for conflicting findings will be addressed in the last section. Despite some contradictions, however, most findings show that antisocial aggression associates with glucocorticoid dysfunctions.

Long-term decreases in glucocorticoid production were often attributed to traumatic experiences suffered at early ages (Carpenter et al. 2009; Elzinga et al. 2008; Lovallo et al. 2012) including bullying in school (Ouellet-Morin et al. 2011) and high violence in the environment of the developing child (Busso et al. 2017). The same hormonal condition may also develop in adolescence or adulthood in response to traumas (Schindler et al. 2019; Yehuda et al. 1990), intimate partner violence.
(Pinto et al. 2016), drug addiction (Buydens-Branchey et al. 1997) chronic fatigue syndrome (Nijhof et al. 2014), or psychotic illness (Pruessner et al. 2017). Many of these glucocorticoid-downregulating conditions were proposed to underlie the development of antisociality-associated psychopathologies and violent crime.

Animal models show that adolescent stressors induce causally interrelated long-term deficits in glucocorticoid production and abnormal aggression. The glucocorticoid response to the stressor and its behavioral consequences dissociate in time. The glucocorticoid deficit model suggests that chronically low glucocorticoid levels can lead to the development of abnormal aggression in adults. Consequently, adverse behavioral effects are due to the chronic hormonal consequences of toxic stress rather than to the age when the stress was suffered.

4.4 Unresolved Cases

There are two conditions where the glucocorticoid/aggression relationship remains largely unresolved. One is related to the internalizing and externalizing psychological profiles. Without extensively reviewing the literature, we note here that both profiles were associated with decreased (Puetz et al. 2016, 2017) just as frequently as with increased glucocorticoid secretion (Cicchetti and Rogosch 2001; Marsman et al. 2008). As the two behavioral types are associated with aggressiveness, such confusing findings may undermine the assumption that glucocorticoids and aggression are related (Alink et al. 2008; Jones et al. 2020). It is worth to consider, however, that these behavioral types are defined inconsistently. Internalizing behavior is in some studies associated with mixed anxiety and depression symptoms, whereas in others it is a psychological concept that defines how emotions are dealt with. Externalizing behavior on its turn may cover mixed symptoms of attention-deficit/hyperactivity and substance use disorders, mixed conduct and oppositional-defiant disorder symptoms or simply the propensity to show emotions off. One can hypothesize that conflicting findings regarding associations with glucocorticoids derive at least partly from the versatility of these constructs.

The other unresolved case is that of post-traumatic stress disorder. Again, we do not cover the issue exhaustively here, but overall, findings on glucocorticoid changes are highly inconsistent. Part of studies performed after the initial finding by Yehuda (1990) confirmed that this disorder is associated with decreased glucocorticoid production (only as examples: Basu et al. 2013; Wessa et al. 2006). Others, however, reported that glucocorticoids increase in this disorder (Pico-Alfonso et al. 2004; Shaheen et al. 2020), whereas yet others found no association (Schindler et al. 2019; van der Hal-Van Raalte et al. 2008), not even in holocaust survivors who were investigated by Yehuda (1990). A detailed review suggests that such opposite findings were reported by an approximately equal number of studies, a minority reporting no associations (Haller 2020, pp. 196–197). To the best of our best knowledge, the relationship between glucocorticoids and aggression was not specifically investigated in this disorder so far, but the conflicting findings on the hormonal
background of the disorder *per se* forecast that the glucocorticoid-aggression relationship is also complex.

### 4.5 Overall Summary

Natural variations in glucocorticoid production appear to be among the mechanisms that control aggressiveness and understandably so. Aggressiveness requires both the fueling of this energy-demanding behavior and the activation of the brain circuitry of aggression. The former is achieved by the concerted adrenaline and glucocorticoid stress response which result in enhanced circulation and energy metabolism (Haller 1995; Haller et al. 1998a, b). Although rarely studied directly, it occurs that glucocorticoids promote aggression by effects on neurons of the aggression circuitry (Hayden-Hixson and Ferris 1991a, b), suggesting that the positive feedback between glucocorticoid production and the brain mechanisms involved in aggressive behavior (Kruk et al. 2004) is not secondary to the bodily effects of glucocorticoids.

Environmental insults can chronically alter glucocorticoid production, and the resulting hormonal state associates with psychopathological aggression. In the case of depression, the underlying mechanism appears to be the modulation of the glucocorticoid responsive elements of genes as effects vanish when glucocorticoid production returns to normal. In this case, abnormal manifestations of aggression appear to result from a faulty interplay between chronic response to homotypic and acute responses to heterotypic stressors. In the case of other disorders, the mechanism underlying the glucocorticoid-aggression relationship may be classified into the category of toxic stress as behavioral consequences emerge long after the hormonal consequences of the triggering stressor vanished. It is commonly assumed that epigenetic changes are the main mechanisms underlying toxic stressors. There is a long history of attributing such changes to a phenomenon called brain programming (Levendosky et al. 2016; Meaney et al. 1996; Walker and Sandi 2018), which involves that the toxic effects of glucocorticoids ultimately serve adaptation. For instance, it has been assumed that harsh developmental conditions prepare the organism for harsh competition in adulthood, which promotes the emergence of unusual forms of aggression (Crombach and Elbert 2014; Sommer et al. 2017; van Lange et al. 2017). Interpreting aggression as a form of competition and seeking evolutionary explanations is typical to the biological approach, which however is not shared by the other three approaches. One wonders whether aggression-related psychopathologies and violent crime can be considered adaptive in a human context. Irrespective of biological and evolutionary implications, however, certain stressors have a long-term effect on the stress system as well as on the aggression circuitry, which together lead to psychopathological/criminal violence in humans and abnormal aggression in animals.

Stressors that have toxic-like effects (e.g., traumas) can induce on the long-run either increased or decreased glucocorticoid secretion (Joos et al. 2019). Human findings suggest that the former is typical in psychopathological aggression.
characterized by the loss of impulse control, whereas the latter to antisocially motivated aggression. Both can be associated with criminal violence, as aggression is against the law irrespective to its motivational or emotional context. Animal models suggest that glucocorticoids are causally implicated in both cases. It was proposed earlier that both decreased and increased glucocorticoids may underlie abnormal aggression, rendering the dose-response curve of the glucocorticoid-aggression relationship U-shaped (Walker et al. 2018a, b). We suggest here that this proposal should be completed by considering the normal/abnormal nature of aggression (Fig. 3).

Based on the above we suggest that the glucocorticoid-aggression relationship is not as confusing as sometimes suggested. The picture is still blurred, however, by a number of conflicting findings within each type of the relationships (Fig. 2). One reason of the discrepancies might be that long-term alterations in glucocorticoid secretion have a temporal course. For instance, it was shown that an initial stress response was followed by a silent period (glucocorticoid secretion returned to normal); this was followed by a period of hypersecretion which ultimately gave way to a chronic hyposecretion (Bosch et al. 2012). As each phase lasts several years, the directionality of the glucocorticoid-aggression relationship may depend on the timing of the study respective to these phases. An additional and perhaps more important confusing factor may be the complexity of human conditions including both disorder comorbidities and variations in living conditions. Notwithstanding trends in the relationship are relatively clear.
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Neurobiological Bases of Alcohol Consumption After Social Stress

Klaus A. Miczek, Alyssa DiLeo, Emily L. Newman, Naz Akdilek, and Herbert E. Covington III

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Abstract The urge to seek and consume excessive alcohol is intensified by prior experiences with social stress, and this cascade can be modeled under systematically controlled laboratory conditions in rodents and non-human primates. Adaptive coping with intermittent episodes of social defeat stress often transitions to

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maladaptive responses to traumatic continuous stress, and alcohol consumption may become part of coping responses. At the circuit level, the neural pathways subserving stress coping intersect with those for alcohol consumption. Increasingly discrete regions and connections within the prefrontal cortex, the ventral and dorsal striatum, thalamic and hypothalamic nuclei, tegmental areas as well as brain stem structures begin to be identified as critical for reacting to and coping with social stress while seeking and consuming alcohol. Several candidate molecules that modulate signals within these neural connections have been targeted in order to reduce excessive drinking and relapse. In spite of some early clinical failures, neuropeptides such as CRF, opioids, or oxytocin continue to be examined for their role in attenuating stress-escalated drinking. Recent work has focused on neural sites of action for peptides and steroids, most likely in neuroinflammatory processes as a result of interactive effects of episodic social stress and excessive alcohol seeking and drinking.

Keywords Alcohol · Consumption · Coping · CRF · Hypothalamus · Neuroinflammation · Opioid · Oxytocin · Prefrontal cortex · Seeking · Self-administration · Striatum · Tegmentum

1 Introduction

Stress tests reveal the breaking point of materials and financial institutions. Yet, in the biomedical sciences, stress and stress disorders comprise cascades of excessive or impaired activity in neural, endocrine, immune, metabolic, and circadian systems (McEwen et al. 2015; Sapolsky 2015). An acute stressor such as an injection with a hypodermic needle, momentary exposure to heat or cold, painful or toxic stimuli results in a response that eventually restores the homeostatic equilibrium, originally attributed to the general adaptation syndrome (Selye 1946). By contrast, coping with traumatic events, including continuous food restriction, prolonged and inescapable pain, predatory pressure, or environmental toxins, involves stress responses that follow an allostatic course, thereby recovering stability through change (McEwen and Wingfield 2003).

Stressors are defined in terms of their intensity, frequency, and temporal pattern; behavioral scientists have added preparatory responses in anticipation of a predictable stressor and in recovery from an uncontrollable stressor as critical antecedents and consequences of the stress response (Koolhaas et al. 2011; Maier and Watkins 2005; Weiss 1970). Responses to stressors of low and moderate intensity activate several behavioral and endocrine metrics up to an inflection point on an inverted U-shaped curve, whereas persistent and intense stressors impair function and ultimately exert deleterious effects (Sapolsky 2015; Fig. 1). In human and veterinary medicine, the stressed individual is considered either resilient or vulnerable to the ill effects of stress (Krishnan and Nestler 2008), and these characteristics appear
relevant in the genesis of the so-called stress disorders such as depression, anxiety, PTSD, alcohol and drug use disorders (see chapter “Social Support Effects on Neural Stress and Alcohol Reward Responses”).

An important shift in our understanding of the physiology and neurobiology of stress resulted from pioneering studies of immediate early gene expression, glucocorticoid and catecholamine activation after exposure to different kinds of stressors (Mason 1968; Pacak and Palkovits 2001). Evidence for stressor-specificity rather than generality emerged, based on the unique neural pathways that were activated by either cold, insulin, restraint, or formalin challenges in laboratory model systems. For example, Pacak and Palkovits (2001) described abundant Fos-immunoreactive cells that were detected in the hypothalamic paraventricular neurons after exposure to immobilization stress, a formalin injection, or hypotensive hemorrhage, but not after an insulin injection. Similarly, prolonged immobilization and pain stimuli activated amygdaloid cells, but cold stress and blood loss did not. Examining the transcriptional outcomes of distinct stressors also revealed characteristic upregulated
Fos expression after acute stress exposure and downregulated Fos after chronic stress experience (e.g., chronic social defeat stress; Flati et al. 2020). Most such mechanistic inquiries focused on physiological stressors, yet the translational appeal of different kinds of social stress in rodent and non-human primate species derives from their significance in every developmental phase of each individual (see chapters “Methods and Challenges in Investigating Sex-Specific Consequences of Social Stressors in Adolescence in Rats: Is It the Stress or the Social or the Stage of Development?” and “Social Stress and Aggression in Murine Models”).

2 Why SOCIAL Stress? Distinctive Features of Social Stress

Social behavior and its underlying neurobiology evolved independently in several species ranging from insects, fish, birds to mammals (Wilson 1975), creating the opportunity to study the species-specific determinants of social stress under controlled laboratory conditions (Norton and Bally-Cuif 2012; Pryce and Fuchs 2017; Von Holst 1998). Here, we examine the developmental trajectory of neural and behavioral adaptations and maladaptations to social stress in mammals. In commonly studied rodent models, for example, the enduring effects of separation from maternal care, and its reversal, and from contact with littermates in early development continue to be detected in adulthood, both in terms of gene expression in hippocampal cells and in coping with stress challenges (Burke and Miczek 2014; Liu et al. 1997). Similarly, when adolescents are exposed to social isolation or to brief episodes of social stress (“bullying”) long-lasting sequelae ensue at the neural and behavioral level (Skelly et al. 2015; Wommack and Delville 2003). Studies in several species of laboratory animals such as mice, voles, rats, and hamsters have begun to map immediate early gene expression in distinctive hypothalamic and limbic pathways as a consequence of displaying aggressive, defensive-submissive and sexual behavior during adulthood (Covington et al. 2005; Joppa et al. 1995; Kollack-Walker et al. 1997; Nikulina et al. 2004). A regionally distinctive pattern of neural activation emerged with each social stressor being characterized by c-fos gene expression and increased immunoreactivity in brain stem, hypothalamic and limbic regions (vide infra).

The distinctive characteristics of social stress can be traced to the origins of social cohesion or dispersal in a particular species. When living in an impoverished niche with localized food sources, feral mice disperse, mark, patrol, and defend their territories (Berdoy and Drickamer 2007). Territorial residents pursue and attack intruders, who escape when given the opportunity. By contrast, under enriched conditions, such as in typical laboratory housing, social hierarchies may emerge. Acquiring and maintaining a dominant rank in the initial phase of a confrontation can be just as stressful as defending and submitting; however, prolonged subordination engenders pathophysiological consequences such as cardiovascular...
hypertension and disrupted circadian rhythmicity (Beitia et al. 2005; Henry et al. 1971; Schuurman 1980). A particularly striking example is the morbid course of tree shrews that were forced into submission in a confrontation with a dominant opponent, even when protected behind a screen. While initially both the dominant and the subordinate contestants sustain intense tachycardia, hypertension, and hyperthermia, only the former recovers whereas the latter does not and eventually succumbs (Von Holst 1998).

The memory of a social defeat stress is manifest by the prompt display of a species-typical defeat posture in response to an approaching non-aggressive partner, as illustrated in Mus musculus (Fig. 2) (Frischknecht et al. 1985; Miczek et al. 1982). Defeated mice assume an upright posture with limp forelimbs, head angled backward, retracted ears, emitting audible squeals upon approach by the opponent, orienting by moving the upper torso. This repertoire of defeat behavior is evident in many strains of mice, including recombinant inbred mice such as C57BL/6 (Miczek et al. 2021). In spite of their unusual social behavior and relatively modest reproductive rate, C57BL/6 mice are the most frequently studied strain in molecular genetics work, ever since their genome was mapped. This strain of mice is the current model of choice in preclinical work with alcohol on account of their...
proclivity for this beverage. The submissive supine posture and immobile crouch are the salient elements in socially stressed rats (Miczek 1979). Several laboratories have begun to implement machine learning programs in order to provide reliable, unbiased, quantifiable accounts of social defeat stress in mice and other rodent models (Goodwin et al. 2020; Kwiatkowski et al. 2021).

While responses to most acute stressors in the laboratory such as novelty, restraint, electric shock pulses or forced swim undergo habituation with repeated presentations, most animal species become sensitized when exposed to multiple episodes of social stress. Exposure to repeated brief episodes of social defeat stress or social separation in several rodent models resulted in a persistently augmented behavioral and dopaminergic response to social challenges (Holly and Miczek 2016; Kikusui et al. 2005; Tidey and Miczek 1996). By contrast, chronic inescapable exposure to an aggressive opponent induces immobility, impairing behavioral, metabolic, immune, and endocrine responses that persist and result in pathophysiological changes, summarily referred to as stress disorders. Intermittent episodes of social stress engender rapid sympathetic activation, followed by HPA mobilization and unrelenting feedback to glucocorticoid receptors (Bartolomucci et al. 2003; Sgoifo et al. 2005; Tornatzky and Miczek 1994; see chapter “More or Less: Glucocorticoid Roles in Aggression”).

Women present twice as often with major depressive disorders and PTSD, and gender has been implicated in stress-related psychopathologies that are comorbid with substance use disorders (Breslau et al. 2003; Kessler 1997; Kessler et al. 1993, 1995, 2003; Lehavot et al. 2014; Sonne et al. 2003). The gender gap between men and women is closing with regard to the rate of substance use disorders. In women, a primary stress-induced mood disorder often precedes the development of a comorbid alcohol use disorder (Lehavot et al. 2014; Sonne et al. 2003). Only very recently has a promising animal model for social stress in females been developed in C57BL/6J mice (Newman et al. 2019) that includes the development of excessive alcohol consumption (Newman et al. 2021). Social stress in female mice was induced by confronting a resident female who had engaged in aggressive behavior toward female intruders repeatedly. A critical variable for the emergence of female aggression is the cohabitation of the resident female with a male partner, suggesting that the female–female confrontations constitute a form of female rivalry dissociated from the context of maternal behavior. Increased social vigilance and defensive behavior was also evident in female California mice upon exposure to stress (Duque-Wilckens et al. 2018; chapter “Mean Girls: Social Stress Models for Female Rodents”).

3 Social Stress and Alcohol

Interactions that promote social intercourse as well as interactions that are confrontational have proven to facilitate the seeking and consumption of alcohol and relapse to alcohol drinking after periods of abstinence (Newman et al. 2018c; Pohorecky 1991). Both anti- and pro-social factors activate glucocorticoids in the HPA axis and
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<td>Dependent and social drinkers (8 adult males/group)</td>
<td>Lab: Five assertive interpersonal events</td>
<td>Lab: FR 50 (30% bourbon in water)</td>
<td>Dependent drinkers ↑FR response</td>
<td>Miller et al. (1974)</td>
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<td>Dependent drinkers after treatment (129 adult males)</td>
<td>Life: Acute and severe psychosocial events</td>
<td>Life: Self-reports of alcohol drinking and stressors</td>
<td>Relapsing participants experienced more severe stress before relapse</td>
<td>Brown et al. (1990)</td>
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<tr>
<td>Dependent drinkers after treatment (67 adult males)</td>
<td>Life: Acute and severe psychosocial events</td>
<td>Life: Self-reports of alcohol drinking and stressors</td>
<td>↑↑psychosocial Vulnerability in relapsing participants 3 Mons. After treatment</td>
<td>Brown et al. (1995)</td>
</tr>
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<td>Social drinkers (8 adult males and 36 females)</td>
<td>Lab: Public speech prompt</td>
<td>Lab: Consumption and motives questionnaires</td>
<td>↑↑in alcohol craving and seeking; “drinking to cope” ↑attentional bias to alcohol</td>
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<td>Dependent drinkers (40 adult males and 39 females)</td>
<td>Lab: Trier social stress test</td>
<td>Lab: Preferred alcohol odor (wine or beer)</td>
<td>↑↑in craving to alcohol across groups</td>
<td>Thomas et al. (2011)</td>
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<tr>
<td>Abstinent (31 males) and dependent drinkers (23 males)</td>
<td>Lab: Trier social stress test</td>
<td>NA</td>
<td>↑↑ heart rate and cortisol in dependent drinkers</td>
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<td>Non-dependent drinkers (45 adult males and 37 females)</td>
<td>Lab: Trier social stress test</td>
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<td>New Zealand adults (635 males and 630 females)</td>
<td>Life: Stressful life events</td>
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<td>Mulia and Zemore (2012)</td>
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<td>Anxious, dependent drinkers (39 adult females)</td>
<td>Lab: Trier social stress test</td>
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<td>Regular drinkers (36 adult males and 41 females)</td>
<td>Life: Interpersonal interactions (&gt;5 min for 14 days)</td>
<td>Life: Self-reports of alcohol drinking</td>
<td>Social rejection ↑day of drinking</td>
<td>Laws et al. (2017)</td>
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in extra-hypothalamic regions. The current focus is on the specific types of aversive social stress that individuals cope with in a species-normative manner.

As summarized in Table 1: Humans, exposure to both experimental social stress tests under controlled laboratory conditions and exposure to stressful social life events, documented by questionnaires or self-reports, demonstrate increased craving and seeking for alcohol as well as drinking and relapse in dependent and social drinkers. These effects are detectable in ages that range from young to older adult male and female alcohol-dependent patients as well as social drinkers. While the different methods used for subject recruitment, exposure to alcohol, stress manipulation and data collection across reports prevented a rigorous meta-analysis, the consistent key finding of social stress promoting alcohol craving and consumption is striking (Brown et al. 1990, 1995; Gilpin and Weiner 2017; Sinha 2001, 2008; Sinha et al. 2009; Stewart 1996; Uhart et al. 2006; Uhart and Wand 2009). The magnitude and persistence of social stress effects on increased seeking and drinking of alcohol are most remarkable, and these features are difficult to model in experimental laboratory procedures, either in humans or animals. Most recent experimental investigations in humans on the link between social stress and alcohol craving and consumption have employed the Trier Social Stress Test (TSST) or some variant thereof. Individuals with a family history of alcohol use disorder had higher plasma cortisol responses to the TSST, indicating maladaptive cortisol responses to stress even before individuals begin heavy drinking (Schuckit 1994). When challenged with the TSST, patients with AUD report more craving and respond more strongly to alcohol as well as increase total alcohol intake relative to healthy controls. This urge for drinking may indicate that alcohol-dependent individuals cope with negative affective states by drinking (McCaul et al. 2018; Thomas et al. 2011; Van Hedger et al. 2017). Moreover, non-abstaining, alcohol-dependent subjects respond more strongly to stress relative to abstaining patients or healthy controls (Starcke et al. 2013). In contrast to social drinkers, heavy-drinking individuals react more strongly to even mild to moderate stress which in turn may intensify alcohol craving.

The translation of the stress-alcohol link has been successfully implemented and systematically investigated in non-human primates and laboratory rodents only in

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<tr>
<td>Heavy drinkers (21 adult males and 9 females)</td>
<td>Lab: Trier social stress test</td>
<td>Lab: Alcohol-motivated response</td>
<td>↑ alcohol craving and responding</td>
<td>McCaul et al. (2018)</td>
</tr>
<tr>
<td>Binge (22 adult males and 6 females) and moderate (19 adult males and 6 females) social drinkers</td>
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<td>Lab: Alcohol taste test (two 8 oz. glasses of 4.2% beer)</td>
<td>↑↑ intake and craving in binge drinkers</td>
<td>Blaine et al. (2019)</td>
</tr>
</tbody>
</table>

_Lab_ exposures occurred in a controlled laboratory setting, _Life_ exposures occurred in the participant’s life, _FR_ fixed ratio, _AAD_ alcohol abuse/dependence; ↑, ↓: _p_ < 0.05; ↑↑, ↓↓: _p_ < 0.01

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the last decade (Becker et al. 2011; Gilpin and Weiner 2017; Helms et al. 2012; Miczek et al. 2008; Norman et al. 2015; McKenzie-Quirk and Miczek 2008). Reviews of the earlier literature pointed to many reports of the suppression of alcohol drinking in animals that were exposed to various environmental stressors (Helms et al. 2012; Pohorecky 1981, 1991). Specifically, individuals will not drink more in stressful laboratory environments, and laboratory rodents will not increase alcohol intake in response to an electric foot shock or restraint stressor, but will after the stress of forced swimming (Becker 2013; Noori et al. 2014; Table 2: Animal Models). Among the classic stress parameters, the intensity and timing of social stressors determine its effects on alcohol drinking (Table 2). Alcohol drinking, as all consummatory behavior, is suppressed during and immediately following social defeat but increases after the final episode of stress (Croft et al. 2005; Norman et al. 2015; Sillaber et al. 2002; van Erp and Miczek 2001). Social stress is not only a risk factor for drug use, but a mechanism for inducing and expressing behavioral sensitization to drugs of abuse by impacting the mesolimbic dopamine system (Anstrom et al. 2009; Covington and Miczek 2001; Tidey and Miczek 1996, 1997). Separation from maternal care during specific post-natal days of development in outbred strains of mice and rats has been found to result in behavioral sensitization and increased alcohol consumption in adulthood (Huot et al. 2001; Kikusui et al. 2005), but these long-term effects are difficult to confirm (Jaworski et al. 2005).

In adulthood, the confrontation between an aggressive resident and an intruder has emerged as a most effective social stress experience in preclinical studies of alcohol, stimulant and opioid effects (Golden et al. 2017; Kudryavtseva 1991; Miczek et al. 1982; Table 2). A hallmark feature of the social stress experience by the intruder is the lack of habituation, neither in adolescents (Watt et al. 2009) nor in adult rats or mice, neither in males nor in females (Covington and Miczek 2005), rendering this procedure a robust model to study the neuroplastic changes that result from repeated exposures to social defeat stress and to alcohol. Social defeat stress is readily studied in many strains of mice and rats, in adolescents and in adults (Newman et al. 2018a, b; Table 2). Most recently a methodological innovation was presented that implements the study of the interaction between social defeat stress and escalated alcohol consumption in female C57BL/6 mice (Newman et al. 2021).

The growing literature on social stress and alcohol in animal models begins to recognize the diverse evolutionary origins of socially cohesive and dispersive patterns, each based on distinctive gene expression in males and females. Episodes of social stress in infancy, adolescence, and adulthood are readily remembered, and upon repetition result in neural and behavioral sensitization that lead eventually to stress disorders.
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<td>Male Long Evans rats (5–10/group)</td>
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<td>Intake in non-dependent mice vs. controls</td>
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<td>Male C57BL/6J mice (exp. 1: 9–10/group; exp. 2: 7–12/group)</td>
<td>Social defeat (5 days)</td>
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<td>Male C57BL/6J mice (exp. 1: 9–10/group; exp. 2: 7–12/group)</td>
<td>Social defeat (5 days)</td>
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<td>Intake in non-dependent mice vs. controls</td>
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<td>Male C57BL/6J mice (exp. 1: 9–10/group; exp. 2: 7–12/group)</td>
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<td>Male C57BL/6J mice (exp. 1: 9–10/group; exp. 2: 7–12/group)</td>
<td>Social defeat (5 days)</td>
<td>Continuous access (20% w/v; 4 weeks)</td>
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*PND* post-natal day, *IA* intermittent access, *CIE* chronic intermittent exposure, *FR* fixed ratio, *PR* progressive ratio, *exp* experiment; − indicates an estimation from published figures; (↑), (↓): non-significant trend; ↑, ↓: $p < 0.05$; ↑↑, ↓↓: $p < 0.01$
4 Intermittent Episodes of Social Stress and Intermittent Access to Alcohol

A most significant conceptual and methodological development was the appreciation of intermittent exposure to episodes of social defeat stress and to alcohol. One of the earliest demonstrations of the power of intermittency can be traced to the behavior-analytic expose of schedules of reinforcement (Ferster and Skinner 1957). When behavior is reinforced intermittently according to a specific schedule, it occurs at higher rates and is more resistant to extinction in comparison with continuously reinforced behavior. This basic principle appears to apply also to increased alcohol consumption during intermittent access and intermittent consumption of small food pellets in outbred rats (Falk et al. 1972; Simms et al. 2008; Wise 1973) and mice (Hwa et al. 2011). In addition to alcohol, significant increases in drug seeking and consuming are evident during intermittent access to amphetamines (Kawa et al. 2016). Social stress effects are detected when intermittent access to psychomotor stimulant self-administration is repeated in episodes of <1 to 24 h “binges.” The temporal limits of intermittent access to alcohol are more narrow than those for psychomotor stimulants as illustrated by the drinking-in-the-dark model of binge drinking, when a 4-h access period on the fourth day follows 2-h access periods on the preceding 3 days in the early hours of the dark phase of the circadian cycle (Rhodes et al. 2005). When alcohol can be accessed every other day in the presence of a water alternative, outbred and C57BL/6 mice consume alcohol voluntarily, persistently and preferentially in increasing amounts (Albrechet-Souza et al. 2017; Hwa et al. 2011, 2016; Newman et al. 2015, 2018a, c). The long-term maintenance of increased alcohol consumption for several months of intermittent access is evident in C57BL/6 mice (Miczek et al. 2021) (Fig. 3). Schedule-induced or “adjunctive” alcohol consumption is detected in a significant subgroup of macaque monkeys at excessive levels for many months (Vivian et al. 2001). Intermittency is also a key variable in the timing of episodes of social defeat stress. In parametric studies with Long Evans rats, a common laboratory strain, a schedule of four brief social defeat episodes during a 10-day period induced an escalation of intravenous cocaine self-administration concurrent with increased dopamine release in the nucleus accumbens (Boyson et al. 2011, 2014; Covington and Miczek 2001; Holly et al. 2012, 2015; Shimamoto et al. 2011, 2015). The experience of repeated brief episodes of social defeat stress has also been found to be effective in increasing alcohol drinking in Swiss-derived outbred mice and in inbred C57BL/6 mice (Albrechet-Souza et al. 2017; Miczek et al. 2021; Hwa et al. 2016; Karlsson et al. 2017; Kudryavtseva 1991; Kudryavtseva et al. 2006; Nelson et al. 2018; Newman et al. 2015, 2018c).

When presented in an intermittent manner, both alcohol and social defeat stress are more effective in promoting more intense motivation for and consumption of alcohol in laboratory animals (Table 2). Moreover, both intermittent access to alcohol and intermittent social defeat stress are additive in engendering higher levels of alcohol consumption than after exposure to either alcohol or stress (Hwa et al...
It is challenging to delineate common neurobiological mechanisms for self-administered alcohol, on the one hand, and for the intensely arousing defeat experience, on the other hand. Nonetheless, it is feasible that voluntary alcohol intoxication as well as the withdrawal from such a state represents stressful experiences that prompt escalated alcohol consumption as a form of coping with stress. It

![Ethanol intake and preference for 20% W/V ethanol](image)

**Fig. 3** Ethanol intake (top) and preference for 20% W/V ethanol (bottom) in an intermittent access 2-bottle choice procedure before and after 10 days of intermittent ($n = 23$, gold triangles) or continuous ($n = 10$, pink squares) social defeat stress (red vertical bar). All data points are means ± SEM; **$p < 0.01$ and *$p < 0.05$ compared to pre-stress baseline (dashed lines); ##$p < 0.01$ and #$p < 0.05$ compared to non-stressed controls ($n = 16$, gray circles)
will be instructive to learn how the rewarding or intoxicating effects of alcohol intersect with the distinctive mechanisms of reacting to and coping with social stress. The episodic alternation of excitation and inhibition as a result of brief exposure to alcohol or social stress has been hypothesized to rely on the balance of glutamatergic and GABAergic modulation of aminergic activity in mesocortical and corticostriatal loops (vide infra).

5 Brain Circuits for Social Stress and EtOH Drinking

The continuing improvements in preclinical models of motivation for and consumption of alcohol as well as relapsing to alcohol after periods of abstinence prompt investigations into the underlying neural circuits, cellular and molecular mechanisms for different phases of alcohol action (Becker et al. 2011; Spanagel 2009). At the same time, the increasingly more precise quantification of both adaptive and maladaptive responses to social stressors from infancy and adolescence through adulthood is implemented under controlled laboratory conditions (see chapter “Social Stress and Aggression in Murine Models”). It has now become feasible to characterize the interaction between neural mechanisms for responses to social stress with those for excessive alcohol consumption.

Abundant evidence demonstrates the importance of impulse flow in neural circuits involving the medial, basal, lateral, and central amygdala, the bed nucleus of the stria terminalis, lateral septum, preoptic area, anterior hypothalamic area, paraventricular nucleus of the hypothalamus, ventral portion of the premammillary nucleus, periaqueductal, and central gray (Fig. 4). Neural activity in the mesolimbic circuitry connecting these brain regions contributes to the display of social stress responses. A new research frontier defines the role of cortical mechanisms that support active and passive coping strategies in response to social, environmental, and pharmacological stressors.

5.1 Cortical Control

Decisions about offensive and defensive response patterns in socially stressful confrontations depend on activity in the orbital frontal cortex. This brain region integrates details of the prevailing context, the proximal and distal past and triggering sensory cues; “affective defense,” as originally characterized by W.R. Hess in cats, requires an intact orbital frontal cortex when receiving impulses of limbic origin, especially from the amygdaloid complex. When neural activity in the anterior medial prefrontal cortex is suppressed by alcohol, impulsive decision making is more likely, especially in psychopathic individuals. Clinical studies point to increased amygdaloid activity that is inversely related to anger-induced inhibition of orbital frontal cortical activity; damage to the OFC is often implicated in the
development of sociopathic behavior (Blair 2016). Neuroimaging studies have confirmed the frontal lobes as critical in regulation of emotion, particularly the prefrontal cortex for impulsive actions (Bufkin and Luttrell 2005; George and Koob 2010). Clinical and preclinical studies point to the critical role of the mPFC in the transition to ethanol dependence (George et al. 2012; Holmes et al. 2012; Kroener et al. 2012) and ethanol-heightened aggression (Quadros et al. 2014).

Distinct cellular ensembles in subregions of the prefrontal cortex contain molecular determinants of offensive actions and defensive reactions (Holly et al. 2015, 2016; Hwa et al. 2015). In comparison with limbic and hypothalamic structures (vide infra), the role of cortical regions in coping with social stress remains to be investigated in more detail (Czeh et al. 2007; Nikulina et al. 2008). The mPFC receives projections from the ventral tegmental area that release increased amounts of dopamine in socially stressed rats (Holly et al. 2015; Tidey and Miczek 1996). This rise in extracellular dopamine was rapid, as soon as the intruder rat was threatened, and much more potent than the response to the mild stress of novelty. The dopamine rise in response to social stress appears to precede the sympathetic stress response (Anstrom et al. 2009; Tornatzky and Miczek 1993). A rise in cortical and accumbal dopamine as well as in accumbal CRF has been detected preceding
imminent attacks and threats by an aggressive opponent rat. It appears feasible that these increases in signaling enable increases in drug consumption (Leonard and Miczek, unpublished data).

During the early hours of withdrawal from several weeks of voluntary alcohol consumption, glutamine and glutamate are increased in the mPFC, pointing to neuronal and glial sources in socially stressed mice (Hwa et al. 2016). The precise origin of the glutamate projections to the mPFC during EtOH withdrawal needs to be determined. Anatomical studies have identified pyramidal neurons in the mPFC that project to the nucleus accumbens and ventral tegmental area where they release glutamate (Sesack and Pickel 1992).

5.2 Hypothalamic Control

Among the subcortical regions that enable social stress coping responses, medial hypothalamic structures emerged as the most critical. Early evidence stems from pulsatile electrical stimulation with microelectrodes that evoked feline affective defense reactions (Egger and Flynn 1962; Hess and Bragger 1943). Afferent and efferent connections of the hypothalamic sites established a network of limbic and mesencephalic structures that mediated defensive responses (Siegel et al. 1999). The early electrical stimulation studies were complemented by electrophysiological recordings of single cell activity in the central gray area during hypothalamically evoked affective defense (Adams 1968).

Starting with an influential report by Lin et al. (2011), chemo- and optogenetic methods permitted more precise characterization of the hypothalamic cells that differentially activate offensive, defensive, indiscriminate biting, mating, or other, pro-social behaviors. So far, a specific hypothalamic neuronal ensemble for coping with social defeat stress is only beginning to be identified in C57BL/6 mice (Wang et al. 2019). The excitability of ventromedial hypothalamic cells adapts with repeated social defeat stress experiences. Daily episodes of social defeat stress fine-tune ensembles of neurons expressing steroid receptors, suggesting that specific cell populations are active during the execution of a fight and become increasingly more specialized with a winning or losing experience (Remedios et al. 2017). Particularly, the anterodorsal preoptic area, the ventrolateral part of the ventromedial hypothalamus, and the dorsal part of the premammillary nucleus have been found to be activated in several species of laboratory animals such as mice, voles, rats, and hamsters; immediate early gene expression in distinctive neural pathways has been mapped in animals displaying species-specific defensive reactions to aggressive threats (Covington et al. 2005; Kollack-Walker et al. 1997; Motta et al. 2009; Newman et al. 2019; Nikulina et al. 2004, 2008). In addition to the canonical amines and acids such as catecholamines, indolamines, GABA, and glutamate, several candidate molecules have been investigated such as BDNF, CRF, opioid peptides, oxytocin, vasopressin, and orexin (Berton et al. 2006; Dayas et al. 2008; Gianoulakis et al. 1996; Jurek and Neumann 2018), but all of them exert critical roles in the...
control of various survival functions such as ingestive, reproductive, thermoregulatory, or circadian domains. Chemo- and optogenetic tools as well as novel sensors for specific molecules promise to identify critical hypothalamic mechanisms for active and passive coping with different kinds of social stress.

5.3 Midbrain and Brainstem Control

The hierarchical neural organization of species-typical aggression and defense becomes evident from studies focused on midbrain structures, including the central gray at the level of the superior colliculi. Single-cell recordings from the dorsal periaqueductal gray reveal its functional activation during fights and electrical stimulation of projections to the medial hypothalamus rapidly evokes intense affective defense (Adams 1968). Tracing studies have identified descending second-order glutamatergic periaqueductal gray projections to the pontine nucleus, raphe magnus, and locus coeruleus that modulate arousal, sympathetic tone, and motor aspects of the well-characterized feline “affective defense.” Ascending attack-promoting tracts densely target areas of the VMH (vide supra) also coordinates elements of defensive responses through reciprocal periaqueductal gray innervations. The early appreciation of a “fight or flight” syndrome and its relationship to the hypothalamus (Bard 1928) was followed by systematic investigations of midbrain and brainstem structures, most prominently the raphe nuclei, locus coeruleus, and ventral tegmentum for their role in aggressive and defensive behavior.

6 Interaction between Neural Mechanisms of Social Stress and Alcohol

Either acute or chronic exposure to alcohol by forced ingestion, vapor, or experimenter-delivered injections is intensely stressful. While voluntary alcohol self-administration in animal models translates more readily to the human condition, the activation of CRF signaling and other stress hormones are indicative of the arousal in anticipation of self-administered alcohol and other drugs (Miczek et al. 2021; Vena et al. 2020).

6.1 Catecholamines

Several decades of research have demonstrated that voluntarily self-administered alcohol and other drugs of abuse are associated with increased release of accumbal dopamine (Siciliano et al. 2018; Vena et al. 2020). Once the animals have been
conditioned to consume alcohol as reward, the increased dopamine response is evident not only in the nucleus accumbens, but also in the dorsolateral striatum and medial PFC (Doherty et al. 2016; Shnitko and Robinson 2015; Weiss et al. 1993). These and other pharmacological effects provide evidence for the important role of mesolimbic and mesocortical dopamine in the rewarding effects of alcohol.

Concurrent with the dopamine-drug reward hypothesis (Spanagel and Weiss 1999), but in contrast are accumulating data that identify intensely painful, aversive, and stressful stimuli as prompting accumbal and cortical dopamine signaling (Cabib and Puglisi-Allegra 1996; Lammel et al. 2012; Marinelli and McCutcheon 2014; Tidey and Miczek 1996; Ungless et al. 2010) (Leonard and Miczek, in press). One resolution to these contrasting sets of data is the proposed heterogeneity of the mesolimbic and mesocortical dopamine pathways. A significant number of dopamine cells in the VTA actually increase their rate of firing when experimental animals are exposed to brief noxious, aversive, stressful stimuli (Ungless et al. 2010). At present, the topographical organization of functionally distinctive DA neurons remains to be verified; either projections from the posterior vs. anterior portions of the VTA, alternatively from the medial vs. lateral or from the dorsal vs. ventral VTA are critical for mediating aversive vs. rewarding effects. Future electrophysiologic studies are required to differentiate phasic vs. tonic responses to aversive, stressful stimuli, including coping with episodes of social stress.

DA cells in the VTA receive projections from the noradrenergic cells in the locus coeruleus and from the serotonergic cells in the dorsal raphe nucleus (Mejias-Aponte 2016; Phillipson 1979). Some evidence suggests a dose-dependent alcohol effect in the mPFC, with low doses increasing and higher doses suppressing noradrenergic responses (Vena et al. 2020). Alcohol-seeking and consumption as well as the rewarding effects of alcohol are inhibited by drugs that block alpha-1 adrenoceptors and activate alpha-2 adrenoceptors such as prazosin and clonidine, possibly by action in the VTA (Rommelfanger et al. 2009; Shelkar et al. 2017). Prazosin has already begun to be studied as a potential treatment for AUD, with some moderate effects on reducing heavy drinking (Simpson et al. 2018). The precise sites of the adrenoceptors that are critical for the modulation of alcohol seeking and consuming need to be delineated in order to ascertain the potential interaction between alcohol- and social stress-responsive adrenoceptor populations in the VTA and in the mPFC.

6.2 Glutamate

Low to moderate doses of alcohol, whether administered by the experimenter or self-administered, are associated with heightened glutamate level in the mesolimbic DA pathways of rats (Das et al. 2015; Ding et al. 2013; Quertemont et al. 2002), although these effects depend on the way in which alcohol becomes available (Vena et al. 2020). Beyond these correlational assay data, behavioral augmentation as a result of repeated exposure to alcohol requires activation of ionophoric glutamate receptors
such as N-methyl-d-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Broadbent et al. 2003; Phillips and Shen 1996). Antagonism of NMDA or AMPA receptors with ketamine, dizocilpine, or NBX reduced alcohol-escalated aggressive behavior, but not aggressive arousal (Covington et al. 2018). The inhibition of NMDA receptors in the mPFC, particularly in the prelimbic subregion of the mPFC, appears to be a site and mechanism by which alcohol and ketamine increase aggressive behavior, at least in a significant subset of individual mice (Newman et al. 2018a). NMDA and AMPA receptors in the prelimbic region of the mPFC represent promising candidate mechanisms for the interaction between alcohol and social stress. Considerably less is known about the role of glutamate acting on metabotropic receptors, particularly at extrasynaptic sites and at glia in socially stressed targets of aggression.

6.3 GABA

Electrophysiological evidence documents the potentiation of the inhibitory action of GABA by alcohol in several brain regions, among them the VTA, nucleus accumbens, and central amygdala (Roberto et al. 2004; Theile et al. 2008). It will be informative to ascertain whether the increased accumbal levels of GABA after repeated experimenter-injected ethanol doses can be detected during self-administered ethanol (Pavon et al. 2019).

Positive allosteric modulation of GABAA receptors is an important mechanism of action for low to moderate doses of alcohol. The precise synaptic and extrasynaptic localization of alcohol action on the GABAA receptor at doses that promote social drinking remains to be determined (Krystal et al. 2006). However, significant attention has been awarded to extrasynaptic delta-containing GABAA receptors where low levels of alcohol (3–30 mM) increase tonic inhibition in recombinant receptor systems and in vivo (Glykys et al. 2007; Santhakumar et al. 2007; Wallner et al. 2003). Mice lacking the delta subunit, whether through embryonic gene deletion or cre-mediated viral approaches, have reduced alcohol consumption and preference and exhibit lower withdrawal hyperexcitability and anxiety-like behaviors (Darnieder et al. 2019; Melón et al. 2019; Mihalek et al. 2001). Interestingly, delta-GABAA receptors are also sensitive to positive allosteric modulation from neurosteroids which can alleviate anxiety-like behavior, depressive behavior, alcohol consumption and effects of withdrawal (Mihalek et al. 1999; Stell et al. 2003; Sundstrom-Poromaa et al. 2002; for review, see Morrow et al. 2020).

Considerable evidence identifies a link between alcohol use disorder (AUD) and single nucleotide polymorphisms in GABRA2, the gene encoding the GABAA receptor alpha2 subunit protein (Covault et al. 2004). Preclinical and clinical data reveal a correlation between dependence on alcohol and fewer GABAA receptor sites (Laukkanen et al. 2013). Future studies will have to ascertain how the GABAA receptor gene expression and sensitivity to modulators is regulated during development, possibly involving early life stresses. It has been hypothesized that shifts in the
expression levels of GABAA receptor subunits may contribute to alcohol tolerance, dependence, and withdrawal symptoms (Krystal et al. 2006), and social stress experiences may accentuate these shifts. Antagonists or point mutations of GABAA receptors containing alpha2 subunits prevent benzodiazepine- and alcohol-escalated aggressive behavior in outbred and C57BL/6 mice (Newman et al. 2016), possibly related to a role in impulse control for this receptor.

Several investigations explore further targets in GABA signaling that appear promising for reducing excessive alcohol consumption. Furthermore, baclofen and small molecule allosteric modulators of GABAB receptors have been found to effectively reduce alcohol consumption in several rodent models of excessive intake (Colombo et al. 2003; Hwa et al. 2013; Maccioni and Colombo 2009; Maccioni et al. 2007, 2008, 2010, 2012; Walker and Koob 2007) and in initial clinical trials (Addolorato and Leggio 2010).

6.4 Neuropeptides

Extrahypothalamic CRF and its receptors have emerged as a most significant signaling system that is associated with increased alcohol seeking and consumption as well as with the rewarding effects of alcohol, extending from acute action to dependent, abstinence, and relapsing phases mostly in laboratory rat models (Baldwin et al. 1991; Chu et al. 2007; Funk et al. 2006, 2007; George et al. 2012; Gilpin et al. 2008; Lê et al. 2000; Marinelli et al. 2007; Merlo Pich et al. 1995; Rassnick et al. 1993; Richter et al. 2000; Valdez et al. 2002; Zorrilla et al. 2001, 2014). The sites of action for CRF in these initial studies were the central amygdala and mPFC, regions that are also activated by social stress (vide supra). The action of CRF on receptor 1 (CRF R1) has been the focus of most studies exploring the possible utility of this target for pharmacological intervention in excessive drinking.

The earlier work with CRF R1 antagonists has been extended from outbred rats and selectively bred alcohol-preferring rats to outbred and recombinant inbred mice, using systemic and cell-specific administration, and to non-human primates (Albrechet-Souza et al. 2017; Hwa et al. 2013, 2016; Newman et al. 2018b; Ostroumov et al. 2016). By now, the CRF system and alcohol have been studied in animal species which have evolved distinctive social behavior such as exclusive territorial or, alternatively, group-living adaptations. Stressful species-specific confrontations and pro-social interactions in voles, hamsters, and mice reveal to be effective in escalating alcohol consumption (Anacker and Ryabinin 2010; Lowery et al. 2008).

The translational significance of preclinical studies demonstrating the consistent, selective reduction of alcohol intake in preference to alternative non-alcoholic fluids by CRF R1 antagonists has become a matter of debate (Schwandt et al. 2016; Shaham and de Wit 2016; Spierling and Zorrilla 2017; Vena et al. 2020; Zorrilla et al. 2013). Spierling and Zorrilla (2017) discuss important issues that may contribute to the failure of relatively small clinical studies; one key issue pertains to the role
of CRF R1 in adapting to episodic or “dynamic” social stress. Clinical studies need to determine whether or not CRF R1 antagonists with longer receptor occupancy are effective in reducing alcohol craving and consumption that is escalated by episodes of social stress.

6.5 Neuroimmune Signaling

In response to a stressor, the peripheral nervous system releases immune cells that travel to the brain to initiate a neuroimmune response via toll-like receptors (TLRs) expressed on microglia, which subsequently release chemokines and cytokines, measurable signatures of a neuroimmune response (Kettenmann et al. 2011; Montesinos et al. 2016; Prinz and Priller 2017). Transcriptome analysis in humans with AUDs and rodent models of drinking show overlap in the upregulation of neuroimmune mechanisms in the brain (Alfonso-Loeches et al. 2010; Crews et al. 2013; Ferguson et al. 2019; He and Crews 2008; Liu et al. 1997; Saba et al. 2015). Social defeat stress increases the expression of some cytokines and chemokines released by alcohol, which have been found to persist in specific brain regions up to a month after cessation of social stress (Ramirez et al. 2015; Wohleb et al. 2013, 2014). Additionally, microglia activation has been identified in adolescent and adult animal models of drinking and is also thought to contribute to the development of anxiety-like behavior after social defeat stress (Lehmann et al. 2019; McClain et al. 2011; Peng et al. 2017; Walter et al. 2017).

Importantly, individual differences in the immune system activation may underlie the emergence of vulnerability or resilience to social defeat stress. Lower immune reactivity has been associated with resilient mice, while vulnerable mice mount an exaggerated inflammatory response to social defeat (Hodes et al. 2014). Social defeat also induces glucocorticoid insensitivity, disabling a mechanism to suppress inflammation, which may prime the neuroimmune system for dysfunction during future stress experiences (Reader et al. 2015; Tornatzky and Miczek 1994). Deletions of neuroimmune-associated targets in preclinical models have proven effective at reducing alcohol intake, preference, and social defeat-induced increases in alcohol intake (Alfonso-Loeches et al. 2010; Blednov et al. 2011; June et al. 2015; Montesinos et al. 2016; Truitt et al. 2016) (chapters “Social Stress-Induced Neuroinflammation and Mitochondrial Dysfunction as a Link to Depression and Cardiovascular Disease Comorbidity” and “Unravelling the Neuroinflammatory Mechanisms Underlying the Effects of Social Defeat Stress on Use of Drugs of Abuse”). These findings invite the possibility of using neuroimmune modulators as therapeutic treatments for AUDs and stress disorders. Indeed, the reduction of neuroinflammation and neuroimmune signaling reduces voluntary alcohol consumption in rodent models (Agrawal et al. 2011; Pascual et al. 2007; Stopponi et al. 2011; Truitt et al. 2016).
7 Future Directions

The intersection of the neurocircuits for excessive alcohol seeking and drinking with those for adapting and coping with social stress represents a most intriguing frontier. The emerging tools of chemo- and optogenetics allow molecularly specific interventions with more adequate regional and temporal resolution.

Cycling through repeated episodes of social stress followed by periods of recovery engender neuroadaptations that lead to alcoholic-like drinking. It will be informative to gain a mechanistic understanding of the molecular changes that are responsible for excessive drinking resulting from intermittent episodes of social stress.

Evidence from animal models of social stress in infancy, adolescence, and adulthood highlights how readily and persistently traumatic episodes are remembered. Moreover, repetitive experiences with social confrontations result in neural and behavioral sensitization that lead eventually to stress disorders. At a neural circuit level, glutamatergic and GABAergic modulation of mesolimbic dopamine projections are critical for stress-sensitized behavior. Future anatomical studies are bound to delineate the precise cascade of interactions between glucocorticoid, glutamatergic, GABAergic actions on aminergic cells that drive stress-escalated drinking. Accruing evidence points to discrete ensembles of dopamine projection neurons that subserve separate functions ranging from coping with aversive stress to intensely pleasurable experiences such as stimulant “highs.”

Adequate quantification of the salient behavioral elements that constitute the evolving responses to social stress is required for the study of social stress in animal models. Machine learning promises to be a most useful approach to standardize and quantify social stress in the most frequently used laboratory rodent species. The salient acts and postures of the defeated mouse are consecutively recruited in the transition from active defense to passive coping.

The emerging focus on sex differences in coping with social stress is gradually revealing sex-specific neuroendocrine mechanisms that contribute to excessive alcohol seeking and drinking (Newman et al. 2021; Yohn et al. 2019).

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Epigenetics of Aggression

Florian Duclot and Mohamed Kabbaj

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Abstract Aggression is a complex behavioral trait modulated by both genetic and environmental influences on gene expression. By controlling gene expression in a reversible yet potentially lasting manner in response to environmental stimulation, epigenetic mechanisms represent prime candidates in explaining both individual differences in aggression and the development of elevated aggressive behaviors following life adversity. In this manuscript, we review the evidence for an epigenetic basis in the development and expression of aggression in both humans and related preclinical animal models. In particular, we discuss reports linking DNA methylation, histone post-translational modifications, as well as non-coding RNA, to the regulation of a variety of genes implicated in the neurobiology of aggression including neuropeptides, the serotoninergic and dopaminergic systems, and stress response related systems. While clinical reports do reveal interesting patterns of DNA methylation underlying individual differences and experience-induced aggressive behaviors, they do, in general, face the challenge of linking peripheral

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observations to central nervous system regulations. Preclinical studies, on the other hand, provide detailed mechanistic insights into the epigenetic reprogramming of gene expression following life adversities. Although the functional link to aggression remains unclear in most, these studies together do highlight the involvement of epigenetic events driven by DNA methylation, histone modifications, and non-coding RNA in the neuroadaptations underlying the development and expression of aggression.

Keywords Aggression · DNA methylation · Early-life adversity · Epigenetics · Individual differences · Monoamines

1 Introduction

Aggression is a complex set of behaviors critical to one’s adaptation to its environment. In both humans and animal models, however, maladaptive levels of aggressive behaviors can lead to escalated aggression (Miczek et al. 2013; Covington et al. 2019) and are thus a common feature in severe neuropsychiatric disorders such as schizophrenia, psychosis, antisocial personality disorder, depression, or autism spectrum disorder (Raine et al. 2002; Comai et al. 2012; Takahashi and Miczek 2014; Matson and Jang 2014; Manchia and Fanos 2017; Takahashi et al. 2018; Cupaioli et al. 2020). Depending on the subtype of aggressive behavior considered, methodology, or dataset, estimates of the average heritability of human aggression range between 50 and 60% (Burt 2009; Tuvblad and Baker 2011; Porsch et al. 2016; Waltes et al. 2016; Luningham et al. 2020), indicating that both genetic and non-genetic factors are at play. A wide range of factors promoting such elevated levels of aggression have been identified, some of environmental nature – such as early-life experiences, stress, or substance abuse – and others of genetic nature. In this context, it appears particularly important to better understand how such external factors can interact with and influence the neurobiological underpinnings of aggression.

By controlling gene expression in a dynamic yet potentially long-lasting manner, epigenetic processes provide a powerful interface between external factors and gene expression (Box 1), and thus represent particularly promising candidates in mediating gene–environment interactions (Meaney 2017), including those relevant to aggression (Manchia and Fanos 2017; Palumbo et al. 2018; Chistiakov and Chekhonin 2019). In light of their dynamic and inducible nature, epigenetic processes have been well described as consequences of exposure to aggression. Histone post-translational modifications, in particular, are critical factors controlling the development of a depressive-like phenotype following social defeat stress in mice and rats (Hollis et al. 2011; Covington et al. 2011a, b; Duclot and Kabbaj 2013; Sun et al. 2016; Kim et al. 2016; Hamilton et al. 2018). Notably, the repeated exposure to physical aggression, such as occurring during the social defeat stress paradigm, can
lead to neuroadaptations associated with escalated aggression in animal models (Takahashi et al. 2018), thereby suggesting that the molecular consequences of exposure to aggression could themselves contribute to the neuroetiology of escalated aggression. In this chapter, however, we will focus on the epigenetic mechanisms underlying the gene expression programs driving aggressive behaviors, over those resulting from physical aggression. Overall, the combination of genetic, functional, and anatomical studies uncovered a complex interaction of the serotonergic system, dopaminergic system, as well as neuroendocrine regulations in the neurobiology of aggression (Takahashi and Miczek 2014; Rosell and Siever 2015; Miczek et al. 2015; Asherson and Cormand 2016; Fanning et al. 2017; Covington et al. 2019; Cupaioli et al. 2020), which will thus be the main focus of this chapter. Furthermore, as the current evidence for an epigenetic contribution to the neurobiology of aggression in humans bears a clinical focus, this chapter will primarily relate to aggressive traits over transient aggressive states. First, we will summarize evidence for an epigenetic basis in inter-individual differences in aggression levels in humans, before describing similar observations in preclinical models. Finally, we will describe in more detail the epigenetic processes associated with experience-induced aggression in rodents.

Box 1
In this manuscript, we will refer to epigenetics as the layer of transcriptional regulations mediating a sustained homeostasis in the absence of the original perturbation (Greally 2018), thus reflecting the set of molecular alterations induced by external factors leading to a modulation of gene expression underlying differences in the aggression phenotype. In particular, we will focus on the main types of post-translational modifications targeting histones – acetylation and methylation – as well as DNA methylation and long non-coding RNA. Acetylation and methylation of histone N-terminal tails are among the best described histone post-translational modifications and promote gene transcription or repression in a site- and modification-specific manner through recruitment of transcriptional co-factors as well as chromatin remodeling (Jaenisch and Bird 2003). Notably, levels of histone acetylation and methylation are dynamically regulated by “writers” (lysine acetyltransferases, or lysine methyltransferases, respectively), or “erasers” (histone deacetylases, or lysine demethylases, respectively), themselves controlled by intracellular signaling pathways. In contrast to histone post-translational modifications, DNA methylation occurs on the nucleotide sequence itself, but similarly, can promote or repress gene transcription in a site-specific manner through recruitment of transcriptional co-factors (Jaenisch and Bird 2003; Jones 2012; Schübeler 2015). DNA methylation is also dynamic, mediated by DNA methyltransferases (DNMT) and removed by Ten-eleven translocation methylcytosine dioxygenase (TET) enzymes. Accordingly, histone post-translational modifications and DNA methylation are key mediators of neuronal plasticity in a variety of high-level processes such as learning and memory, stress response, or addiction (Gräff and Mansuy 2008; Gräff et al. 2011; Gräff and Tsai 2013; Nestler 2014; Fortress and Frick 2014; Liyanage et al. 2014; Mews et al. 2019; Browne et al. 2020). Finally, long
non-coding RNA are a subclass of regulatory RNA that control transcription in a highly tissue-specific manner through a variety of sequence-specific genomic processes (Quinn and Chang 2016; Wei et al. 2017). As a result, long non-coding RNA have emerged in recent years as important regulators of central nervous system plasticity in physiological and pathological states (Tielking et al. 2019; Liu et al. 2020b; Pascale et al. 2020; Acharya et al. 2020; Saba et al. 2020).

Dynamic regulation of post-translational modifications of histones (top) and DNA (bottom). Histone acetylation and methylation are “written” to the N-terminal tails of histones by histone acetyltransferases (KAT) and histone methyltransferases (KMT), respectively, and “erased” by histone deacetylases (HDAC) and histone demethylases (KDM), respectively. Similarly, DNA methylation is performed by DNA methyltransferases (DNMT) and removed by Ten-eleven translocation methylcytosine dioxygenase (TET) enzymes. Transcription start sites are denoted by the “+1” nucleotide, and the first exon depicted as a gray rectangle.

2 Human Evidence

Multiple efforts to evaluate the genetic component of human aggression based on large-cohorts twin studies indicate a heritability averaging around 50–60% with variations depending on the cohort, sex, or raters (Burt 2009; Porsch et al. 2016; Waltes et al. 2016; Luningham et al. 2020). These observations led to efforts in identifying the non-genetic or environmental factors contributing to the etiology of human aggression and brought valuable information when considering their interaction with genetic risk factors following a gene–environment model (Laucht et al. 2014). One such early study revealed, for instance, an interaction between the effects of childhood maltreatment on the children’s development of antisocial behavior and individual differences at a functional polymorphism in the promoter of the monoamine oxidase A (MAOA) gene known to affect its expression (Caspi et al. 2002).
Individuals with high MAOA activity thus presented with significantly weaker effect of childhood maltreatment on the development of a composite scoring of antisocial behavior. In particular, maltreated males with low MAOA activity were more likely to be convicted of a violent crime than nonmaltreated males, whereas maltreatment did not confer such risk in males with high MAOA activity (Caspi et al. 2002). Many subsequent reports revealed similar involvement of other genes associated with the neurobiology of aggression, including the serotonergic transmission (SLC6A4, MAOA), or dopaminergic transmission (DRD2, DAT, COMT) (Laucht et al. 2014). Although particular care must be taken on the generalization of such gene–environment observations (Byrd and Manuck 2014; Duncan et al. 2014), these studies do highlight how external factors can influence the development of aggressive behaviors through interaction with gene expression and as a result, warrant the consideration of an epigenetic component in individual differences in aggression in humans.

2.1 Evidence from Targeted Studies

In line with the pioneering identification of the MAOA gene as an important center of gene–environment interaction in the development of human aggression following childhood maltreatment described above (Caspi et al. 2002; Byrd and Manuck 2014; Nilsson et al. 2018), levels of DNA methylation at the MAOA gene promoter have been associated with the interaction between MAOA genotype and the development of antisocial personality disorder (ASPD) following childhood abuse in women. Philibert et al. (2011) identified a second variable nucleotide repeat (VNTR) region upstream to the one previously reported by Caspi et al. (2002) and found that the low-activity allele at this novel region was associated with greater vulnerability to the development of ASPD symptoms following childhood maltreatment in females, but not males (Philibert et al. 2011). This novel VNTR is rich in CpG and exhibits differential methylation levels depending on the genotype. Indeed, females carrying the low-activity allele at this novel VNTR, but not males, present with higher methylation levels than non-carriers (Fig. 1a), thereby suggesting an epigenetic basis for the interaction between childhood maltreatment in this cohort and the development of ASPD.

It is important to note, however, that such link between MAOA methylation levels and ASPD symptoms – including aggression –is likely population and locus-specific. Indeed, in a separate cohort of male incarcerated participants with ASPD, the MAOA VNTR region was found hypermethylated when compared to healthy controls (Fig. 1a) (Checknita et al. 2015). It is important to note, however, that although childhood maltreatment is an important factor in determining ASPD development (Hill and Nathan 2008), this study could not evaluate the contribution of such adverse early-life events to the differences in MAOA VNTR methylation observed. Recently, however, a detailed analysis of MAOA promoter methylation provided valuable insights into the nature and extent of the interplay between DNA methylation, MAOA genotype, sex, childhood adversity, and aggressive behaviors.
Checknita et al. thus found that in male participants carrying the MAOA short allele, aggressive behaviors were elevated only among those with hypermethylation of the CpGs 7–12 located on MAOA first exonic region (Fig. 1a) (Checknita et al. 2020). In females, however, no interaction between childhood maltreatment, MAOA genotype, and DNA methylation with aggressive behaviors was found. Altogether, these findings indicate that DNA methylation at the MAOA locus can represent a key modulator of the interaction between early life adversity and the development of aggressive behaviors.

Such effects of early life adversity are not restricted to MAOA, however, but expand to additional important modulators of the serotonergic neurotransmission. The methylation of the serotonin transporter gene SLC6A4, for instance, can be altered by early life adversity. In a study of monozygotic twins discordant for bullying victimizations, SLC6A4 was found hypermethylated in bullied twins when compared to non-bullied twins (Fig. 1b) (Ouellet-Morin et al. 2013). As

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**Fig. 1** Genomic location of epigenetic modifications with direct evidence for an association with aggression in humans and rodents. Each gene of interest is schematically represented from 5' to 3' (left to right) with transcription start sites denoted with an arrow at the “+1” nucleotide, and the first exon depicted as a gray rectangle. The genomic locations for each epigenetic modification were estimated from each referenced study to the latest reference assemblies: GRCh38 for human, GRCm38 for mouse, and Rnor_6.0 for rat. MAOA: Monoamine oxidase A, SLC6A4: Solute carrier family 6 member 4

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Checknita et al. thus found that in male participants carrying the MAOA short allele, aggressive behaviors were elevated only among those with hypermethylation of the CpGs 7–12 located on MAOA first exonic region (Fig. 1a) (Checknita et al. 2020). In females, however, no interaction between childhood maltreatment, MAOA genotype, and DNA methylation with aggressive behaviors was found. Altogether, these findings indicate that DNA methylation at the MAOA locus can represent a key modulator of the interaction between early life adversity and the development of aggressive behaviors.

Such effects of early life adversity are not restricted to MAOA, however, but expand to additional important modulators of the serotonergic neurotransmission. The methylation of the serotonin transporter gene SLC6A4, for instance, can be altered by early life adversity. In a study of monozygotic twins discordant for bullying victimizations, SLC6A4 was found hypermethylated in bullied twins when compared to non-bullied twins (Fig. 1b) (Ouellet-Morin et al. 2013). As
such differences cannot be attributed to genetic or shared environmental factors due to the study design, this illustrates SLC6A4 methylation’s responsiveness to childhood adversity. Similarly, the SLC6A4 gene promoter is hypermethylated in patients-derived lymphoblasts of women victims of sexual abuse during childhood, and the degree of methylation was associated with ASPD symptoms (Beach et al. 2011). Moreover, the authors provide preliminary evidence for a potentiation of SLC6A4 genotype effects to predict ASPD symptoms by DNA methylation, with the latter tending to be more strongly associated with ASPD symptoms in individuals carrying the short SLC6A4 allele (Beach et al. 2011). Notably, such association might likely be phenotype and tissue specific as no association between SLC6A4 genotype and its methylation was found in peripheral blood cells (T-cells and monocytes) of healthy adult males (Wang et al. 2012). SLC6A4 methylation was higher, however, at specific CpG sites (Fig. 1b) of participants with a history of high childhood-limited aggression (C-LHPA) than individuals with low aggression in childhood (LPA). Higher methylation of CpG sites associated with childhood aggression in T-cells – and to a lower extent in monocytes – was associated with lower in vivo serotonin synthesis in the lateral left and right orbitofrontal cortex (Wang et al. 2012). Combined with the observation that DNA methylation of these CpG sites reduced SLC6A4 promoter transcription in vitro in a luciferase reporter assay, this not only provides further support for a modulation of the serotoninergic system by early life adversity through DNA methylation, it illustrates the potential for DNA methylation in readily-available tissue such as peripheral blood cells to proxy regulations of the serotoninergic transmission in specific brain areas.

2.2 Evidence from Genome-Wide Studies

While the studies described above targeted known modulators of aggression, the democratization of high-throughput methods to measure DNA methylation across the genome has further expanded our knowledge of the epigenetic component of aggression. The immune system, for instance, has been increasingly associated with the long-term behavioral consequences of early life adversity in humans (Provençal et al. 2015; Takahashi et al. 2018). Boys on a chronic physical aggression (CPA) trajectory, for instance, exhibit lower plasma levels of the pro-inflammatory cytokines IL-1α and IL-6, as well as anti-inflammatory cytokines IL-10 and IL-8 than the control group on the same background (Provençal et al. 2013b). Through methyl-DNA immunoprecipitation followed by targeted hybridization on microarrays, Provençal et al. revealed differential methylation profiles in males on a CPA trajectory at genomic loci encompassing these cytokines as well as their regulatory transcription factors. Forty-eight differentially methylated regions associated with CPA were thus identified (20 in T-cells, 28 in monocytes), 7 of which correlated with cytokine expression levels in the plasma (Provençal et al. 2013a). Moreover, a total of 66 differentially methylated regions were identified in the loci encompassing the cytokine-regulating transcription factors NFκB1, NFAT5, and STAT6 (Provençal
et al. 2013a), thereby revealing a coordinated DNA methylation response regulating cytokine expression levels in men on a CPA trajectory when compared to controls. A similar but non-targeted approach revealed a total of 448 differentially methylated regions in T-cells between males on a CPA trajectory and males with the same background on a normal physical aggression trajectory (277 and 171 were hypo- and hyper-methylated in the CPA group, respectively) (Provençal et al. 2014). Supporting previous observations, the related genes are associated with inflammatory response and cytokine signaling, but revealed additional processes of interest such as metabolism, gene expression, or hyperactivity (Provençal et al. 2014). Notably, five of these genes (AVPRIA, HTR1D, GRM5, DRD1, and SLC6A3) were previously associated with aggressive behaviors in humans, which thus further supports the importance of DNA methylation in regulating aggression-related genes and expands the breadth of candidates.

It is of particular interest to note that a similar investigation of DNA methylation underlying CPA in women yielded very similar results, with a total of 430 differentially methylated regions (353 and 77 were hypo- and hyper-methylated in the CPA group, respectively) (Guillemin et al. 2014), 3 of which with known link to aggression (TPH2, CRHBP, and NR3C1). The set of genes related to these differentially methylated regions in females and males significantly overlap and encompass similar biological pathways than in males such as inflammatory response, cell signaling, and behavior (Guillemin et al. 2014). Most of the individual CpG sites included in the differentially methylated regions commonly linked to CPA in both males and females shared directionality of regulation, which thus suggests that the epigenetic mechanisms driving such DNA methylation following early life adversity overlap at least partially between men and women.

Recently, the genome-wide analysis of DNA methylation in peripheral blood cells of subjects with a diagnosis of intermittent explosive disorder (IED) – characterized by recurrent, problematic, and impulsive aggression (American Psychiatric Association 2013) – revealed a total of 27 CpG sites with differential DNA methylation between individuals with and without IED (Montalvo-Ortiz et al. 2018). Although none of these reached the threshold for genome-wide significance, the biological pathways related to the genes nearing these 27 CpG sites were enriched in terms related to inflammatory response and cytokine processes, neuronal differentiation, and the hormonal system (Montalvo-Ortiz et al. 2018), thereby strengthening the association between DNA methylation of immune system-related genes, and the development of aggression.

2.3 Epigenome-Wide Association Studies

Beyond such case–control genome-wide studies, a few investigations sought to identify genomic loci of DNA methylation associated with aggression behavior in epigenome-wide association studies (EWAS). In a cohort of 2,029 subjects from the Netherlands Twin Register (NTR) and NTR biobank project, DNA methylation in
whole blood was measured by microarray and compared to self-evaluation of aggression. Based on the entire cohort, two CpG sites of interest emerged – located near the zinc-finger transcription factor tri-chorhinophalangeal syndrome I (TRPS1), the non-coding RNA ARD6G antisenseRNA 1 (PARD6G-AS1), and the activity-dependent neuroprotective protein 2 gene (ADNP2) – with a positive relationship between DNA methylation and aggressive behavior, but none reached the genome-wide statistical threshold (van Dongen et al. 2015). Nevertheless, functional enrichment analyses revealed a strong enrichment of central nervous system-related processes and pathways, with some overlap with the pathways associated with CPA in females such as “behavior” (Guillemin et al. 2014). Thanks to the nature of the cohort, the authors were also able to test DNA methylation levels for association with aggression behavior between twins discordant for aggression levels. While substantial differences in DNA methylation were observed between discordant twins with, on average, 30% or more difference in methylation on 24 CpG sites, these differences were generally twin pair-specific and thus no CpG site reached genome-wide statistical threshold, similar to the entire NTR cohort (van Dongen et al. 2015). It is nevertheless interesting to note that the top three CpG sites are located near three genes relevant to aggression. The top two genes are indeed involved in inflammatory and immune response, with RAS oncogene family 39 gene (RAB39A) regulating interleukin-1beta secretion (Becker et al. 2009), and sialic acid-binding Ig-like lectin 10 gene (SIGLEC10) repressing immune response induced by tissue damage (Chen et al. 2009). The third gene, prolyl endopeptidase (PREP) modulates the biosynthesis of peptide hormones such as vasopressin and oxytocin (García-Horsman et al. 2007), both critical components of the neurobiology of aggression (Veenema 2009, 2012). Moreover, PREP is associated with aggressive behavior and inversely correlated with the immunoglobulin IgG2 in autistic patients (Frenssen et al. 2015). Despite statistical shortcomings, these observations are supported by their overlap in biological pathways with case–control studies conducted in CPA (Guillemin et al. 2014; Provençal et al. 2014) and thus suggest an important role for DNA methylation in modulating gene expression underlying aggression levels at the population level.

Recently, a similar approach aimed at characterizing genome-wide DNA methylation patterns associated with physical aggression provided additional evidence. In a cohort of 119 participants with self-report engagement in physical fights, an integrated analysis of DNA methylation in buccal swabs, an epigenome-wide association study revealed four differentially methylated probes meeting the threshold of genome-wide significance (Cecil et al. 2018). Functionally, the biological pathways enriched in genes annotated to all differentially methylated probes shared a surprising overlap with the other pathways of interest described above, including inflammatory and immune response, central nervous system, and behavior (Cecil et al. 2018), thus further reinforcing the link between DNA methylation regulation of these pathways and aggression. Notably, by using a region-based approach – combining closely-related CpG sites to increase power (Robinson et al. 2014) – the authors identified one region of DNA methylation in the body of the dopamine receptor D4 (DRD4) gene with a strong negative association with engagement in
physical fights (Cecil et al. 2018). This association remained significant even after correcting for shared variance such as drug use, indicating that the association between DRD4 methylation and physical aggression was not entirely explained by co-occurring symptomatology such as drug use. In particular, lower DNA methylation at the underlying CpG sites was not associated with childhood maltreatment. Given this association between lower DRD4 methylation with higher physical aggression was replicated in a distinct blood T-cells, and the separate observation that the DNA methylation of the underlying CpG sites in peripheral tissue reflect those in several areas of the brain, the authors propose DRD4 DNA methylation as a biomarker for physical aggression (Cecil et al. 2018).

Altogether, these studies do support an epigenetic component in the neurobiology of aggression. Despite diversity in cohorts and design, these studies surprisingly reveal the association of common pathways under epigenetic control with aggression represented mainly by inflammatory and immune response, but also serotonergic and dopaminergic transmission (Table 1). It is important to note, however, that despite some evidence of correlation between DNA methylation changes in peripheral tissues with those occurring in the brain, their functional relevance in the central nervous system as mediators of aggression behaviors remains unclear. Similarly, the causal nature of such associations remains difficult to establish for most.

3 Epigenetics of Individual Differences in Aggression in Animal Models

Animal models have been extensively used to decipher the neurobiology underlying aggressive behaviors as well as uncovering evidence to better our understanding of the etiology of aggression-related disorders in humans. Although more limited, these studies yielded valuable evidence for an epigenetic basis in inter-individual differences in aggression (Table 2). In an effort to provide a comprehensive view of the genetics of aggression, Zhang-James et al. compared genome-wide and transcriptome-wide studies of regulations associated with aggression conducted in humans and rodents, and found varying degree of overlap at the gene level, but substantial similarities at the biological pathway level (Zhang-James et al. 2019). Notably, this integrated analysis allowed for the identification of 40 genes associated with aggression across species and revealed the involvement of a few key epigenetic factors such as the histone deacetylase 4 (HDAC4), the euchromatic histone lysine methyltransferase 1 (EHMT1), and the RNA splicing factor RNA binding fox-1 homolog 1 (RBFOX1). While these associations are correlative and their functional roles in mediating aggression remain to be clarified (Fernàndez-Castillo et al. 2020), they do warrant further investigations of specific epigenetic events driving gene expression relevant to aggressive behaviors.

One of the advantages of animal models lies in the ability to investigate spatial and gene-specific epigenetic regulations throughout the animal’s lifetime, allowing
Table 1  Evidence for an epigenetic component in gene regulations associated with aggression in humans

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Tissue</th>
<th>Condition</th>
<th>Epigenetic observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAOA</strong></td>
<td>Patient-derived lymphoblasts</td>
<td>ASPD</td>
<td>Greater vulnerability to childhood maltreatment-induced development of ASPD symptoms in women carrying low activity allele Higher DNA methylation in low-activity allele carriers than non-carriers</td>
<td>(Philibert et al. 2011)</td>
</tr>
<tr>
<td><strong>MAOA</strong></td>
<td>Blood</td>
<td>ASPD</td>
<td>Higher DNA methylation at 31 of 71 CpG sites analyzed (~5% increase overall) in incarcerated males with ASPD than healthy controls</td>
<td>(Checknita et al. 2015)</td>
</tr>
<tr>
<td><strong>MAOA</strong></td>
<td>Saliva</td>
<td>N/A</td>
<td>Elevated aggressive behaviors only among those with hypermethylation of CpGs 7-12 (at least one standard deviation above the mean)</td>
<td>(Checknita et al. 2020)</td>
</tr>
<tr>
<td><strong>SLC6A4</strong></td>
<td>Buccal swabs</td>
<td>MZ twins</td>
<td>~90% higher DNA methylation at CpG 8 in bullied vs. non-bullied twin</td>
<td>(Ouellet-Morin et al. 2013)</td>
</tr>
<tr>
<td><strong>SLC6A4</strong></td>
<td>Patient-derived lymphoblasts</td>
<td>Sexual abuse/ASPD</td>
<td>Higher DNA methylation in women victims of sexual abuse during childhood. DNA methylation level associated with ASPD symptoms ($r_{25} = 0.573$, $p = 0.001$, for those homozygous for the s allele)</td>
<td>(Beach et al. 2011)</td>
</tr>
<tr>
<td><strong>SLC6A4</strong></td>
<td>Blood T-cells and monocytes</td>
<td>C-LHPA</td>
<td>Higher DNA methylation (72–157% increase at select CpG sites) in males with a history of high childhood-limited aggression (C-LHPA) than in males with low aggression (LPA)</td>
<td>(Wang et al. 2012)</td>
</tr>
<tr>
<td><strong>DRD4</strong></td>
<td>Buccal swabs</td>
<td>N/A</td>
<td>Low DNA methylation associated with high engagement in physical fights (bivariate: $r = −0.39$, $p = 1.10^{−05}$, partial: $r = −0.28$, $p = 0.01$)</td>
<td>(Cecil et al. 2018)</td>
</tr>
<tr>
<td><strong>MAALIN</strong></td>
<td>HPC</td>
<td>N/A</td>
<td>Low DNA methylation (~78% of controls) and H3K27me3 (~30% of controls), but not H3K4me3 in suicide completers with high aggression-impulsivity</td>
<td>(Labonté et al. 2020)</td>
</tr>
<tr>
<td><strong>5-HTR2c</strong></td>
<td>dlPFC</td>
<td>Suicide completers</td>
<td>RNA-editing imbalance (14% increase in ratio) leading to higher levels of 5-HTR2c-Tr than in controls</td>
<td>(Dracheva et al. 2008b)</td>
</tr>
</tbody>
</table>

5-HTR2c 5-hydroxytryptamine receptor 2C, ASPD antisocial personality disorder, dlPFC dorsolateral prefrontal cortex, DRD4 dopamine receptor D4, HPC hippocampus, MAALIN MAOA-associated IncRNA, MAOA monoamine oxidase A, MZ monozygote, SLC6A4 solute carrier family 6 member 4
for the identification of specific epigenetic regulation of gene transcription in relevant brain areas and their association with individual differences in aggressive behaviors. For instance, in rats, the locomotor response to the mild stress of a novel environment, akin to the “sensation-seeking” behavioral trait, predicts subsequent propensity to take drugs (Piazza et al. 1989). Notably, rats selectively bred for high (bHR) or low (bLR) response to novelty exhibit robust behavioral differences reflecting distinct emotional responses, including impulsivity and aggression (Kerman et al. 2011), alongside widespread and structure-specific differences in gene expression (Birt et al. 2020). These distinct transcriptomic patterns are accompanied by structure and gene-specific alterations in the repressive histone post-translational mark H3K9me3. Indeed, the more aggressive bHR rats show lower global H3K9me3 but not H4K20me3 in the hippocampus, amygdala, and nucleus accumbens than bLR rats, but higher H3K9me3 at the glucocorticoids receptor.

Table 2: Evidence for an epigenetic component in gene regulations associated with aggression in rodents

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Tissue</th>
<th>Model</th>
<th>Aggression phenotype</th>
<th>Epigenetic observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAOA</td>
<td>PFC</td>
<td>Peripubertal stress</td>
<td>Escalated aggression in adulthood, mediated by MAOA up-regulation in the PFC</td>
<td>Hyperacetylation of histone H3 but not H4 in stressed rats</td>
<td>(Márquez et al. 2013)</td>
</tr>
<tr>
<td>MAOA</td>
<td>PFC</td>
<td>Peripubertal stress</td>
<td>Escalated aggression in adult males but not females, associated with increased MAOA expression and activity in the PFC</td>
<td>DNA hypomethylation in PFC, DNA hypermethylation in hypothalamus of stressed male mice Increased SIRT1 binding in PFC, reduced in hypothalamus of stressed male mice</td>
<td>(Konar et al. 2019)</td>
</tr>
<tr>
<td>NR3C1</td>
<td>HPC, Amy, NAc</td>
<td>bHR/bLR</td>
<td>Higher aggression in bHR than bLR rats</td>
<td>Higher H3K9me3 in bHR than bLR rats</td>
<td>(Chaudhury et al. 2014)</td>
</tr>
<tr>
<td>FGF2</td>
<td>NAc</td>
<td>bHR/bLR</td>
<td>Higher aggression in bHR than bLR rats</td>
<td>Lower H3K9me3 in bHR than bLR rats</td>
<td>(Flagel et al. 2016)</td>
</tr>
<tr>
<td>DRD2</td>
<td>NAc</td>
<td>bHR/bLR</td>
<td>Higher aggression in bHR than bLR rats</td>
<td>Higher H3K9me3 in bHR than bLR rats</td>
<td>(Flagel et al. 2016)</td>
</tr>
<tr>
<td>OXTR</td>
<td>HPC</td>
<td>TET1 mutant mice</td>
<td>Sustained aggression towards an intruder and pups</td>
<td>DNA hypermethylation in TET1 mutant mice</td>
<td>(Towers et al. 2018)</td>
</tr>
</tbody>
</table>

Amy Amygdala, bHR/bLR bred High Responder/bred Low Responder, DRD2 Dopamine receptor D2, FGF2 Fibroblast growth factor 2, HPC Hippocampus, MAOA Monoamine oxidase A, NAc Nucleus accumbens, NR3C1 Nuclear receptor subfamily 3 group C member 1, OXTR Oxytocin receptor, PFC Prefrontal cortex, SIRT1 Sirtuin 1, TET1 Tet methylcytosine dioxygenase 1
(NR3C1) gene promoter in these brain areas (Chaudhury et al. 2014). Similarly, in the nucleus accumbens, H3K9me\(^3\) is lower at the promoter of the fibroblast growth factor (FGF2) gene promoter, but higher at the dopamine receptor D2 (DRD2) gene promoter in bHR rats than in bLR rats (Flagel et al. 2016). In line with the transcriptionally repressive nature of H3K9me\(^3\), FGF2 and DRD2 mRNA levels are higher and lower in the nucleus accumbens of bHR than bLR rats, respectively. Early-life exposure of bLR rats to FGF2, which affects their “emotional” phenotype to resemble the profile of bHR rats, affects H3K9me\(^3\) at the NR3C1 promoter in the hippocampus, and FGF2 promoter in the hippocampus and nucleus accumbens (Chaudhury et al. 2014). Altogether, these evidence highlight a gene- and structure-specific pattern of histone methylation between bHR and bLR rats that is susceptible to an external insult in early life, and associated with a distinct “emotional” phenotype related to aggression. In addition to distinct histone methylation patterns, it is interesting to note that bHR and bLR rats also exhibit different expression of the DNA methyltransferase 1 (DNMT1) mRNA in the hippocampus and amygdala at postnatal day 7 but not later in development (P14 or P21), suggesting that DNA methylation could also be associated with differences in aggression observed between these two rat strains (Simmons et al. 2012).

While the above associations with aggression remain correliative, a recent translational investigation brought functional evidence for an epigenetic mediation of aggressive behaviors. In the dentate gyrus of suicide patients diagnosed with high levels of impulsive-aggressive behaviors, Labonte et al. found lower DNA methylation in an intergenic region located between the MAOA and MAOB genes when compared to healthy controls (Labonté et al. 2020). This locus of DNA hypomethylation in patients with high levels of aggression revealed to also present with lower H3K27me\(^3\) but not H3K4me\(^3\) levels, controlling the expression of a novel gene coding for a long-coding RNA (lncRNA) named MAOA-associated lncRNA (MAALIN) (Labonté et al. 2020). In vitro, MAALIN overexpression or knockout induced a robust down- or up-regulation of MAOA, respectively, whereas in vivo MAALIN overexpression in the mouse hippocampus increased aggressive behaviors and down-regulated MAOA expression (Labonté et al. 2020). Combined with the higher MAALIN expression in the hippocampus of suicide patients with high levels of aggression, these observations establish a functional link between a coordinated epigenetic regulation involving DNA methylation, histone post-translational marks, as well as lncRNA, and aggressive behaviors through regulation of MAOA expression in the hippocampus. This hypomethylation was reproduced in blood samples of ASPD patients as well (Labonté et al. 2020), highlighting a robust link between DNA methylation at this locus and aggressive behaviors and providing preliminary evidence for its use as a biomarker.
4 Epigenetics of Experience-Induced Aggression

While a few studies (as mentioned above) describe an epigenetic component in individual differences in aggressive behaviors, substantial evidence exists describing the involvement of epigenetic mechanisms in the development of aggression as a consequence of stress (Table 2). Adversity early in life, for instance, is a major factor contributing to the development of elevated or excessive aggression in rodents (Veennema 2009), as in humans (Barnow and Freyberger 2003; Ethier et al. 2004; Fonagy 2004). Chronic social isolation represents another stress paradigm leading to heightened aggression in rodents and proves valuable to investigate the underlying neurobiology in adulthood (Matsumoto et al. 2005; Araki et al. 2016; An et al. 2017; Zelikowsky et al. 2018). In the following section of this manuscript, we will thus summarize the recent evidence of an epigenetic basis in the development of aggressive behavior following these two stress paradigms in rodents. Notably, we will focus on the main systems associated with the neurobiology of aggression: serotonergic transmission, glucocorticoids signaling, and neuropeptides.

4.1 Focus on Serotonergic Transmission

In line with the known involvement of the serotonergic transmission in response to stress and the development of aggression, it is not surprising to find several reports of an epigenetic regulation of key serotonergic-related genes underlying stress-induced aggression. In male and female rats, the repeated and unpredictable exposure to fear-induction procedures – predator odor and elevated platform – between postnatal days 28 and 42 (peripubertal stress) leads to the development of sustained aggression at adulthood (3 months of age) in a resident-intruder paradigm (Márquez et al. 2013; Cordero et al. 2013). Notably, this aggressive behavior is observed even against unthreatening intruders such as smaller or anesthetized animals, indicative of an escalated aggression phenotype. In line with its known association with aggression, MAOA expression was up-regulated in the prefrontal cortex but not in the amygdala of stressed male rats when compared to unstressed controls (Márquez et al. 2013). A treatment with the MAOA inhibitor clorgyline during adulthood reversed stress-induced escalated aggression, thereby indicating that MAOA up-regulation mediates the behavioral consequences of peripubertal stress. In this study, this group reported a hyperacetylation of histone H3, but not H4, at MAOA gene promoter in the prefrontal cortex of stressed rats (Fig. 1c). Such epigenetic control of MAOA transcription was recently further detailed in Balb/c mice, in which peripubertal stress also leads to enhanced aggressive behaviors in adulthood. Similar to rats, this stress-induced hyper-aggression phenotype in mice is associated with increased neuronal activation as well as MAOA expression and activity in the prefrontal cortex when compared to unstressed mice (Konar et al. 2019). Contrary to rats, however, the effects of peripubertal stress on adulthood aggression were sex-specific and
observed only in males (Konar et al. 2019). MAOA expression was negatively correlated with DNA methylation at its promoter (Fig. 1d), being hypomethylated in the prefrontal cortex, but hypermethylated in the hypothalamus. Moreover, binding of the histone deacetylase Sirtuin 1 (SIRT1) at the MAOA promoter was increased in the prefrontal cortex and decreased in the hypothalamus of stressed male mice when compared to unstressed controls (Konar et al. 2019). Altogether, these studies depict a model in which peripubertal stress triggers a coordinated epigenetic regulation of the MAOA gene involving local histone acetylation and DNA methylation events that facilitate a sustained up-regulation of MAOA in the prefrontal cortex leading to escalated aggression in adulthood.

In addition to controlling gene transcription through DNA methylation and histone post-translational modifications, the epigenetic control of serotonin-related genes extends to the post-transcriptional via RNA editing and splicing processes. The pre-mRNA for the serotonin receptor 5-HTR2c, in particular, can undergo editing by Adenosine Deaminase Acting on RNA 1 (ADAR1) or ADAR2 (Werry et al. 2008), which in turn affects its subsequent splicing, leading to variation in the generation of a full-length (5-HTR2c-Fl) or truncated (5-HTR2c-Tr) form of the receptor. Importantly, both isoforms are endogenously expressed and interact with each other in the endoplasmic reticulum, ultimately modulating levels of the full-length, physiologically active, form of the receptor at the cytoplasmic membrane (Martin et al. 2013). In this context, variations in 5-HTR2c editing can lead to an imbalance in 5-HTR2c isoforms, affecting 5-HT turnover, and ultimately modulating 5-HT dependent behaviors such as anxiety or aggression (Martin et al. 2013). Accordingly, variations in 5-HTR2c editing as well as the ratio between its two splice variants in the human dorsolateral prefrontal cortex are associated with suicide but not with comorbid neuropsychiatric diagnoses (schizophrenia or bipolar disorder) (Dracheva et al. 2008a, b), thereby pointing at a more specific link with aggression. Accordingly, mice carrying the fully edited isoform of 5-HTR2c (VGV mice) present with high levels of aggression with congeners when compared to their wild-type controls (Martin et al. 2013).

In this context, it is particularly interesting to note that 5-HTR2c editing can be modulated by external factors promoting aggression such as chronic social isolation. Balb/c mice isolated for 2 weeks post-weaning thus present with higher aggression towards a same-sex intruder in the resident-intruder paradigm, associated with a reduction in the RNA editing enzyme ADAR1 in the amygdala when compared to group-housed controls (Yu et al. 2018). No change in 5-HTR2c editing, however, could be detected. Nonetheless, isolated mice treated with the 5-HTR2c antagonist SDD243213 or 5-HTR2c inverse agonist SB206553 showed ADAR1 expression and aggression levels similar to controls, as well as reduced 5-HTR2c RNA editing (Yu et al. 2018), thereby providing evidence for a link between stress-induced aggression and epigenetic control of 5-HTR2c expression.
4.2 Focus on Glucocorticoids Signaling

As discussed above (see Sect. 2), the glucocorticoid receptor gene \((NR3C1)\) is among the loci of DNA methylation associated with CPA in women with a history of childhood abuse (Guillemin et al. 2014). This association is not specific to CPA, however, and has been reported in other conditions as well (Palma-Gudiel et al. 2015; Turecki and Meaney 2016). For instance, methylation of the \(NR3C1\) exon 1F is lower in young adults with a lifetime diagnosis of an externalizing disorder than in healthy controls or patients with lifetime depressive disorder (Heinrich et al. 2015). In line with this observation, the \(NR3C1\) methylation is higher in patients with internalizing disorders (Efstratopoulos et al. 2018). Similarly, multiple individual CpG sites in the \(NR3C1\) gene have been significantly associated with aggressive behavior in an adult males cohort (Liu et al. 2020a).

In this context, it is particularly interesting to mention the pioneer work by Weaver et al. describing how DNA methylation of \(NR3C1\) in rats mediates the effects of early life experience on stress response in adulthood (Weaver et al. 2004). Through cross-fostering experiments, Weaver et al. indeed showed that variations in the amount of maternal care received by the offspring of mothers with either high or low pup licking/grooming behavior (high/low LG, respectively) guide \(NR3C1\) expression levels in the hippocampus and stress response in adulthood (see (Zhang et al. 2013; Meaney 2017)). Notably, \(NR3C1\) expression in the hippocampus following maternal care (licking and grooming) is under control of coordinated epigenetic events involving DNA methylation alongside histone acetylation and methylation. In particular, maternal stimulation by licking and grooming triggers a cascade of signaling events in the offspring hippocampus leading to the binding of the transcription factor and immediate early gene Early Growth Response 1 (EGR1) together with the transcription co-factor and histone acetyltransferase CREB binding protein (CREBBP) to \(NR3C1\) promoter exon 1\(_7\), which will in turn activate \(NR3C1\) transcription (Weaver et al. 2007; Hellstrom et al. 2012). Accordingly, pups receiving high levels of maternal care (high-LG) present with more EGR1 binding to \(NR3C1\) exon 1\(_7\) promoter and higher \(NR3C1\) expression levels in the hippocampus than low-LG pups (Weaver et al. 2004). In line with the acetyltransferase activity carried by CREBBP and the ability of EGR1 to recruit the DNA demethylase Ten-Eleven Translocation protein 1 (TET1) (Sun et al. 2019), EGR1 overexpression increases H3K9 acetylation levels and reduces local DNA methylation levels at the \(NR3C1\) exon 1\(_7\) promoter (Weaver et al. 2007). As a result, high-LG pups show higher H3K9 acetylation and H3K4 methylation, alongside lower DNA methylation at the \(NR3C1\) exon 1\(_7\) promoter in the hippocampus than low-LG pups, leading to higher \(NR3C1\) expression (Weaver et al. 2004, 2007; Zhang et al. 2013). Altogether, the \(NR3C1\) locus of high-LG individuals exhibits an epigenetic profile favorable to the sustained \(NR3C1\) expression found in the hippocampus at adulthood when compared to low-LG counterparts. Furthermore, it is interesting to note that serotonin has been reported to reduce \(NR3C1\) DNA methylation and increase \(NR3C1\) expression in hippocampal neurons cultures via an PKA and EGR1-dependent
mechanism, likely downstream of the 5-HTR7 receptor (Weaver et al. 2007; Hellstrom et al. 2012).

These findings thus establish a mechanism through which experience (maternal stimulation of the pup by licking and grooming) triggers a signaling cascade leading to a coordinated recruitment of epigenetic factors and processes promoting a sustained alteration of gene expression responsible for individual differences in stress response. Beyond stress response, the effects of natural variations in maternal care expand to other behavioral domains, including aggression. In male juvenile rats, for instance, the offspring of low-LG mothers present with higher levels of aggressive behaviors than high-LG offspring during play fighting, including pinning, pouncing, and aggressive grooming (Parent and Meaney 2008). Similarly, low-LG male rat offspring exhibits higher levels of aggression-related behaviors in the resident-intruder at adulthood than high-LG offspring (Menard and Hakvoort 2007). In this context, it is tempting to hypothesize that the epigenetic control of NR3C1 in the hippocampus following maternal care participates to explain differences in aggression later in life. Moreover, the observation that such epigenetic reprogramming can be under serotoninergic control provides an interesting bridge between the widespread involvement of serotonin and glucocorticoids in the neurobiology of aggression (Quadros et al. 2020; Cupaioli et al. 2020). The contribution of epigenetic processes to such modulation of stress response in adulthood by early life experiences could also represent valuable therapeutic targets due to their dynamic and reversible nature. Indeed, treatment in adulthood with either the histone deacetylase inhibitor Trichostatin A or methyl supplementation can reverse the effects of maternal care on the epigenetic modulation of NR3C1, its expression, and thus subsequent stress response (Weaver et al. 2004, 2005).

4.3 Focus on Neuropeptides

In addition to the serotoninergic and dopaminergic systems, growing evidence in both clinical and preclinical studies point towards an epigenetic basis in the role of the neuropeptides oxytocin (OXT) and vasopressin (AVP) in the development of aggressive behaviors following stress exposure, in line with their known involvement in the expression of aggression itself (Veenema 2009, 2012; Maud et al. 2018). While OXT levels in the cerebrospinal fluid, for instance, are inversely correlated with life history of aggression in humans (Lee et al. 2009; Jokinen et al. 2012), differences in DNA methylation at the OXT promoter have been reported in the blood cells of a major depressive disorder cohort (Sanwald et al. 2020). In particular, mean methylation levels as well as the methylation of two specific CpG sites in the OXT promoter were negatively associated with the history of stressful life events. Notably, no association was found between DNA methylation at the OXT promoter and depression symptoms severity, however, thus suggesting that control of OXT promoter was related to early life stress (Sanwald et al. 2020). The receptor for oxytocin (OXTR) shows similar epigenetic regulation by early life experiences, with
greater DNA methylation of the \textit{OXTR} gene in the blood cells of adults with history of low maternal care in childhood (Unternaehrer et al. 2015). Furthermore, in a cohort of youth with established conduct problems, \textit{OXTR} DNA methylation was predictive of resilience in the conduct domain, but in the emotional or hyperactivity domains following exposure to early life adversity (Milaniak et al. 2017). Altogether, these observations do support an underlying regulation of the oxytocicnergic transmission through epigenetic mechanisms – and DNA methylation in particular – following early life experiences, that is sustained in adulthood.

Some evidence linking DNA methylation at the \textit{OXTR} gene locus and aggressive behaviors also exist in rodents. Mutant mice lacking the DNA demethylase Tet1, for instance, exhibit a down-regulation of \textit{Oxtr} mRNA levels in the hippocampus, along with a variety of behavioral impairments including higher levels of threatening behaviors towards an intruder in the resident-intruder paradigm, as well as higher aggression directed towards pups (Towers et al. 2018). Notably, a select locus of the \textit{Oxtr} gene was hypermethylated in \textit{Tet1} mutant mice when compared to controls, which in light of the known association of \textit{OXTR} in the development and expression of aggressive behaviors (LoParo et al. 2016; Caldwell et al. 2017) would tend to suggest that the aggression-related behavioral impairments observed in \textit{Tet1} mutant mice might relate to \textit{Oxtr} reduced transcription by DNA methylation (Towers et al. 2018). It is important to note, however, that other genes showing altered DNA methylation in the brain of \textit{Tet1} mutant mice might also contribute to their heightened aggression (Towers et al. 2018).

Differential DNA methylation of the \textit{OXTR} gene in adulthood between individuals with a history of low or high maternal care was also observed in rats, albeit with different results than in humans. Indeed, while \textit{OXTR} methylation was greater in the blood cells of humans with a history of low maternal care, \textit{OXTR} methylation in peripheral blood mononuclear cells (PBMC) was greater in male rats with a history of high maternal care (Beery et al. 2016). Notably, this difference was not found in the brain, however, as DNA methylation at the \textit{OXTR} gene was similar between high or low maternal care rats. To estimate whether such discrepancy would be the result of a tissue-specific effect, the authors conducted a detailed comparison of \textit{OXTR} methylation levels between PBMC and three brain structures including the hypothalamus, the hippocampus, and the striatum. \textit{OXTR} DNA methylation was highly concordant at the group level between brain structures and between PBMC and the brain to a lower extent, whereas DNA methylation was highly discordant across these tissues when compared at the individual level (Beery et al. 2016). As a result, while \textit{OXTR} methylation in the blood can reflect DNA methylation profiles in the brain, it is not likely to represent a reliable indicator of individual variations in DNA methylation at specific CpG sites.

Similar to the OXT system, the AVP system is a target of sustained epigenetic regulation through DNA methylation following early life adversity in the brain. Male mouse pups exposed to maternal separation stress for 3 h per day for their first 10 postnatal days develop an altered stress response in adulthood characterized by hypersecretion of corticosterone accompanied by a sustained up-regulation of AVP expression in the hypothalamic paraventricular nucleus neurons (Murgatroyd et al. 2018).
This enduring transcriptional alteration is mediated by reduced DNA methylation at a key regulatory element of the AVP gene, an enhancer containing binding sites for the transcription co-factor methyl CpG-binding protein 2 (MECP2). As MECP2 binding at this enhancer was reduced as well in adult mice with a history of early life stress when compared to controls, this suggests that the sustained AVP expression in early life stressed mice reflects a transcriptional de-repression resulting from reduced MECP2 occupancy due to DNA hypomethylation at this promoter (Murgatroyd et al. 2009). The mechanisms leading to the sustained AVP DNA hypomethylation at this specific locus responsive to early life stress were later elucidated in a cellular model of hypothalamic-like differentiation. In this in vitro model, the AVP enhancer switches from a repressive state in undifferentiated cells to a transcriptionally active state following differentiation (Murgatroyd and Spengler 2014). These two states are associated with opposite chromatin contexts. In its undifferentiated, repressed state, the AVP enhancer is bound by the histone methyltransferase SUZ12 member of the polycomb complex and exhibits high levels of the transcriptionally repressive H3K27 trimethylation (H3K27me³). Following differentiation, however, a time dependent and dynamic regulatory process occurs, involving eviction and recruitment of a variety of transcriptional co-factors such as the DNA methyltransferase 3a (DNMT3a), HDAC1, HDAC2, and MECP2. Notably, in hypothalamic tissues, MECP2 co-associates with the transcriptionally repressive co-factors HDAC1 and HDAC2, as well as DNMT3a and 3b (DNMT3b), suggesting a coordinated transcriptional repression of AVP (Murgatroyd and Spengler 2014). In light of the known dissociation of MECP2 from the AVP enhancer following its phosphorylation by neuronal activation signaling pathways (Murgatroyd et al. 2009), these findings thus paint a model in which neuronal activation in the paraventricular nucleus of the hypothalamus following exposure to maternal stress in mice would trigger MECP2 eviction from the AVP enhancer resulting in a coordinated epigenetic de-repression of AVP transcription involving DNA methylation, as well as histone acetylation and methylation (Murgatroyd et al. 2009; Murgatroyd and Spengler 2014).

The V1aR vasopressin receptor expression (encoded by the AVPR1A gene), in addition to its ligand, can also be epigenetically modulated by life experiences. In male mice, Bodden et al. thus investigated whether the epigenetic regulation of the AVPR1A by adverse experiences was dependent on sensitive life phases (Bodden et al. 2017). Male mice were thus exposed to adverse experience early in life and/or in adulthood, which affected anxiety-like behaviors, as well as the expression of a variety of aggression-related targets in the hippocampus such as the serotonin receptor 1a (HTR1A), MAOA, NR3C1, or AVPR1A. AVPR1A mRNA levels were lower in mice exposed to adversity in adulthood than those exposed to a beneficial experience at adulthood, regardless of the nature of their early life experience – adverse or beneficial. Moreover, AVPR1A was hypermethylated in mice exposed to late adversity when compared to those exposed to late beneficial experience, thereby suggesting an epigenetic regulation of AVPR1A specific to late adversity but not early life adversity in the hippocampus (Bodden et al. 2017).
5 Conclusions and Perspectives

Throughout this manuscript, we summarized some of the most relevant work highlighting an involvement of epigenetic mechanisms in the neuroadaptations underlying the development of aggressive behaviors in both humans and rodents. In direct relation to the role of epigenetic processes as mediators of gene–environment interactions, it is important to note that the vast majority of studies providing evidence for an epigenetic basis in the development of aggressive behaviors revolves around the interference of past life experiences. In this context, and guided by current knowledge on the neurobiology of aggression, a growing body of evidence suggests the involvement of epigenetic mechanisms and DNA methylation in particular, in the modulation of gene expression related to the serotonergic and dopaminergic neurotransmission underlying the development of aggressive behaviors following early life adversity (Table 1). It is therefore not surprising to find, in preclinical studies, a particular focus on the effects of early life experiences on the expression of important factors regulating the serotonergic and dopaminergic neurotransmissions, as well as stress response (Table 2). A variety of paradigms centered on the exposure to negative experiences either prenatally or early in postnatal development have thus been developed, and they have provided extensive information on the neuroadaptations, including those epigenetically-based, relevant to aggression. It is important to note, however, that their direct, causal relationship to the development and expression of aggressive behaviors remains in general uncertain and thus cannot provide a clear answer as to the exact involvement of epigenetic processes in the mediation of elevated aggression following early life adversity.

Nevertheless, both human and rodent studies share a substantial overlap in the genes and biological pathways involved, supporting the notion that aggression is evolutionary conserved across species (Lischinsky and Lin 2020). The directionality of association between specific genes and experience-induced epigenetic regulations, however, can differ between clinical and preclinical contexts. For instance, while aggressive behaviors were generally associated with MAOA hypermethylation in blood samples in humans (Table 1), the development of escalated aggression following peripubertal stress in mice was linked to MAOA hypomethylation in the prefrontal cortex (Table 2). Although these observations could very well illustrate species-specific regulations of the MAOA gene, it remains critical to consider the discrepancies in the tissues used for DNA methylation analysis. A major unknown in clinical studies lies in the extent to which readily-available tissues such as blood or buccal samples reflect neuroadaptations occurring in areas of the central nervous system relevant to the phenotype of interest. With regard to DNA methylation – the main epigenetic modification studied in human samples – few studies have provided evidence addressing this issue in the context of aggressive behaviors. Cecil et al. nevertheless conducted an extensive comparison across tissues of the methylation status of the DRD4 gene locus linked to engagement in physical fights in humans (Cecil et al. 2018). Not only was DRD4 methylation pattern and its association with aggressive behaviors consistent within peripheral tissues (saliva vs. blood samples),
a good concordance in its methylation pattern was found between the periphery and several brain areas including the prefrontal cortex, entorhinal cortex, superior temporal gyrus, or Brodmann area 10 (Cecil et al. 2018). As a result, these findings do support the use of DNA methylation measurements in readily-available peripheral tissues as indicators of biologically relevant neuroadaptations in the central nervous system. It is important not to unilaterally generalize these findings, however, as further comparisons in rodents revealed a more complex relationship between peripheral and central patterns of DNA methylation in the vicinity of the OXTR gene. Indeed, while measuring OXTR methylation in adult rats who received either high or low levels of maternal care, Beery et al. found that methylation of each individual CpG of interest within each individual was highly discordant between PBMC and the hippocampus, despite a significant positive correlation between these two tissues at the group level (Beery et al. 2016). Therefore, individual variation in OXTR DNA methylation pattern in PBMC is not predictive of its methylation in the hippocampus. Altogether, these reports highlight the need to consider the biological relevance of peripheral measurements of DNA methylation patterns with those occurring in brains areas relevant to aggressive behaviors for each gene of interest.

Despite these limitations, the main systems mentioned in this chapter showing evidence for an epigenetic role in the development of aggression in humans and rodents have also been implicated in a wide range of species. In Drosophila, reducing methyl-CpG binding domain proteins controls reproductive strategies and reduces aggression in males (Gupta et al. 2017). In dogs, DNA methylation of the MAOA promoter strongly associates with MAOA expression levels in the brain and varies between breeds with different behavioral characteristics such as aggression (Eo et al. 2016). Similarly, DNA methylation profiles in the brain differ between highly aggressive and gentle honey bees subspecies (Cingolani et al. 2013). In addition, threat-induced aggression alters DNA methylation profiles in the honey bee brain around genes related to neural plasticity, chromatin remodeling, and hormone signaling (Herb et al. 2018). Experience-dependent epigenetic regulations can also be expanded to other species. In chicken, in ovo exposure to high dose corticosterone leads to increased aggressive behaviors when compared to controls, and altered expression of several genes related to the serotoninergic system and stress response in the hypothalamus, including NR3C1, 5-HTRIA, and MAOA, alongside increased DNA methylation at the NR3C1 and corticotropin releasing hormone (CRH) promoters (Ahmed et al. 2014). Finally, in the small carpenter bee, removal of the mother during larval development leads to increased aggression and avoidance in adulthood, as well as genome-wide changes in gene expression and DNA methylation (Arsenault et al. 2018). Although this overlap in genes and systems across species likely results from the targeted nature of these investigations, it nonetheless suggests that like the neurobiological underpinnings of aggression (Lischinsky and Lin 2020), the epigenetic component in the development of aggressive behaviors are, to some extent, evolutionary conserved.

Although the current manuscript focused on some of the main neurobiological systems underlying aggression, such as the serotoninergic, dopaminergic systems, other factors known to modulate aggression have also shown evidence for an
epigenetic component. In rats, social isolation from weaning to adulthood leads to social deficits including elevated levels of aggression, associated with reduction in brain-derived neurotrophic factor (BDNF) in the infralimbic cortex (Mikics et al. 2018). Re-socialization combined with fluoxetine treatment, but not either alone, attenuated the stress-induced escalated aggression and reduced DNA methylation at the BDNF P4 promoter, suggesting that fluoxetine induced a transcriptionally permissive state through local DNA hypomethylation thereby potentiating the effects of re-socialization on BDNF expression (Mikics et al. 2018). Like in rats, social isolation-rearing in mice leads to escalated levels of aggression. These behavioral impairments are mediated by an up-regulation of the GABA\(_B\) receptor subunit 1a in the dorsal raphe nucleus, with local hypomethylation and histone H3 hyperacetylation around its gene transcription start site (Araki et al. 2016). In this context, the range of potential targets of an epigenetic regulation underlying the development of aggressive behaviors – either induced by experience or not – thus appears to extend far beyond the commonly studied members of the serotonergic, dopaminergic, or neuropeptidic systems. This was particularly well illustrated by the analysis of genome-wide DNA methylation patterns related to chronic physical aggression in men and women revealing the extent of the epigenetic regulation targeting the immune system (Guillemin et al. 2014; Provençal et al. 2014). Furthermore, detailed investigation of the epigenetic signatures associated with aggression can also reveal novel regulatory mechanisms, as evidenced by the discovery of the novel lncRNA MAALIN (Labonté et al. 2020). As the range and accessibility of high-throughput techniques allowing the unbiased exploration of genome-wide epigenetic modifications keep increasing, we can thus expect to further our understanding of the epigenetic basis in the development of aggression.

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Part II
Human Research
Early Life Stress and Neurodevelopment in Adolescence: Implications for Risk and Adaptation

Jonas G. Miller, Rajpreet Chahal, and Ian H. Gotlib

Abstract An alarming high proportion of youth experience at least one kind of stressor in childhood and/or adolescence. Exposure to early life stress is associated with increased risk for psychopathology, accelerated biological aging, and poor physical health; however, it is important to recognize that not all youth who experience such stress go on to develop difficulties. In fact, resilience, or positive adaptation in the face of adversity, is relatively common. Individual differences in vulnerability or resilience to the effects of early stress may be represented in the brain as specific patterns, profiles, or signatures of neural activation, structure, and connectivity (i.e., neurophenotypes). Whereas neurophenotypes of risk that reflect the deleterious effects of early stress on the developing brain are likely to exacerbate negative outcomes in youth, neurophenotypes of resilience may reduce the risk of experiencing these negative outcomes and instead promote positive functioning. In this chapter we describe our perspective concerning the neurobiological mechanisms and moderators of risk and resilience in adolescence following early life stress and integrate our own work into this framework. We present findings suggesting that exposure to stress in childhood and adolescence is associated with functional and structural alterations in neurobiological systems that are important for social-affective processing and for cognitive control. While some of these neurobiological

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alterations increase risk for psychopathology, they may also help to limit adolescents’ sensitivity to subsequent negative experiences. We also discuss person-centered strategies that we believe can advance our understanding of risk and resilience to early stress in adolescents. Finally, we describe ways in which the field can broaden its focus to include a consideration of other types of environmental factors, such as environmental pollutants, in affecting both risk and resilience to stress-related health difficulties in youth.

**Keywords**  Adaptation · Adolescence · Early life stress · MRI · Resilience · Risk

### 1 Introduction

More than half of youth in the United States are exposed to at least one kind of adverse, stressful experience, such as family violence, poverty, and emotional abuse (McLaughlin et al. 2012). In addition to being highly prevalent, these types of early life stressors carry considerable burden for children and adolescents; indeed, they have been linked to increased risk for psychopathology (Green et al. 2010; LeMoult et al. 2020), accelerated biological aging (Colich et al. 2020; Sosnowski et al. 2020), and poor physical health (Flaherty et al. 2006; Oh et al. 2018). Despite these well-documented adverse effects of early life stress (ELS), not all youth who are exposed to ELS go on to develop difficulties. In fact, resilience, or positive adaptation and decreased vulnerability in the face of adversity (Luthar et al. 2000; Rutter 2006), appears to be quite common (Bonanno and Diminich 2013; Masten 2001). Thus, there are significant individual differences in the psychosocial and neurobiological factors that are involved in vulnerability, and resilience, to the adverse effects of ELS.

Some of these individual differences could be related to variation in the severity, type, duration, and timing of specific events that represent forms of ELS (Cohodes et al. 2020; Malhi et al. 2019), as well as to the presence or absence of both individual- and environment-level protective factors (Holz et al. 2020). Collectively, these various factors may be represented in the brain as specific patterns, profiles, or signatures of neural activation, structure, and connectivity (i.e., neurophenotypes) that confer risk versus resilience. Neurophenotypes of risk that reflect the persistent and deleterious effects of ELS on the developing brain are likely to underpin or exacerbate negative outcomes in children and adolescents. Conversely, neurophenotypes of resilience may reduce the risk of negative outcomes and preserve or promote positive emotional, cognitive, and behavioral functioning following ELS.

There are two main approaches to studying the interplay of ELS, brain development, and risk versus resilience. The first, and most prevalent, approach focuses on the effects of ELS on the brain and examines implications of these relations for child and adolescent well-being (i.e., a mechanism- or mediation-based approach)
In other words, this approach considers how the effects of ELS on well-being are explained by or linked to the effects of ELS on brain function and structure. The second, less common, approach focuses on the interactive effects between ELS and neurobiology on well-being (i.e., a moderation-based approach) (Guyer 2020). This approach considers how the specific effects of ELS on well-being (i.e., strength of effects, positive or negative direction) change depending on individual differences in brain function or structure. Although it may seem paradoxical that neurobiological systems can serve both as mechanisms that link ELS to outcomes and as moderators of the effects of ELS, there are at least three reasons to posit that these processes work in concert.

First, although ELS can affect neurobiological functioning and structure, it is important to recognize that neurodevelopment is not shaped solely by early experience. In fact, research suggests that some aspects of neurobiology are highly heritable, and that genetic variation contributes to neurobiological systems that underlie risk for stress-related psychopathology (Fox et al. 2015; Teeuw et al. 2019). This inherent variability may influence the moderation of the effects of ELS on well-being.

Second, there are sensitive periods during which neurobiology is particularly responsive to input from the environment (Dunn et al. 2019; Feldman 2015; Gee 2020). ELS may have more lasting, powerful effects on neurobiological systems when they are undergoing rapid development, as is the case during childhood and adolescence. After the sensitive periods for these systems have passed, additional stressful experiences may have a weaker effect. Importantly, however, these neurobiological systems continue to regulate how children and adolescents perceive and respond to subsequent stressors, potentially moderating their impact on well-being.

Third, it is possible that neurobiological systems that are sensitive to ELS play a role in filtering and encoding information from the environment. Some researchers have posited that reactivity in stress-response systems (e.g., HPA axis, parasympathetic nervous system, and sympathetic nervous system) may help children and adolescents extract and store information about the social environment (Del Giudice et al. 2011). The degree of reactivity in stress-response systems may help to calibrate their long-term functioning (Del Giudice et al. 2011). Importantly, these stress-response systems are regulated by brain regions that are sensitive to ELS (Ulrich-Lai and Herman 2009). Thus, forms of ELS that lead to increased neurobiological reactivity in brain regions important for child and adolescent well-being may also underlie openness and responsiveness to environmental input.

In this chapter we review the current literature in presenting our perspective on the neurobiological mechanisms and moderators of risk and resilience in adolescence following ELS, with particular attention to integrating our own work into this framework. We are conducting an ongoing longitudinal study with a sample of over 200 adolescents to examine the relations among ELS (largely social stress), neurobiology, and adolescent development and well-being. Adolescents in our study completed interviews to assess exposure to 30 different types of stressful events as well as the age at onset of each event. Table 1 shows the types of ELS events and rates of exposure to ELS in our sample. A panel of trained raters coded each
### Table 1  Measurement of early life stress

<table>
<thead>
<tr>
<th>Type of ELS</th>
<th>Examples</th>
<th>Percentage endorsed</th>
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<tr>
<td></td>
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<td>Total sample (%)</td>
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<td>Female (%)</td>
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<td>Male (%)</td>
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<td>1. Witnessed illness/injury</td>
<td>Caregiver cancer, relative heart attack</td>
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<tr>
<td>2. Moved/family moved in and out</td>
<td>Moving many times, eviction</td>
<td>48</td>
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<td>45</td>
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<td>53</td>
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<td>3. Parental verbal fighting</td>
<td>Nonphysical yelling</td>
<td>39</td>
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<td>41</td>
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<td>36</td>
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<td>4. Death of someone close</td>
<td>Relative to cancer</td>
<td>34</td>
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<td>5. Bullying</td>
<td>Peer name-calling, hitting</td>
<td>33</td>
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<td>27</td>
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<td>6. Divorce</td>
<td>Caregivers separated (legally or non-)</td>
<td>32</td>
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<td>32</td>
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<td>7. Experienced illness/injury</td>
<td>Hospitalized for asthma</td>
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<td>8. Witnessed accident</td>
<td>Sibling hit by car</td>
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<td>9. Experienced accident</td>
<td>Car crash</td>
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<tr>
<td>10. Family mental illness/substance abuse</td>
<td>Caregiver depression, caregiver alcoholism</td>
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<td>22</td>
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<tr>
<td>11. Separation from family – rehab/foster care/detention</td>
<td>Caregiver in rehab, caregiver in jail</td>
<td>13</td>
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<td>12. Family financial problems</td>
<td>Food/housing insecurity</td>
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<tr>
<td>13. Family legal problems/ imprisonment</td>
<td>Sibling DUI arrests, caregiver in prison</td>
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<td>9</td>
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<td>15</td>
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<tr>
<td>14. Separation from family – work/travel</td>
<td>Separation due to work, child going to camp</td>
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<td>15. Witnessed community conflict – physical</td>
<td>Robbery, gunshots</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>16. Domestic violence – physical</td>
<td>Family hitting, throwing objects</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>17. Attacked by animal</td>
<td>Dog bite</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>18. Suicide/self-harm of someone close</td>
<td>Classmate suicide, caregiver attempts</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>19. Neglect</td>
<td>Lack of food/supervision</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>20. Disaster</td>
<td>Hurricane, tornado, forest fire</td>
<td>5</td>
</tr>
<tr>
<td></td>
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<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>21. Witnessed community conflict – verbal</td>
<td>Neighbors yelling/threatening people</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>22. Physical assault/abuse</td>
<td>Hitting by adult family</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>23. Emotional abuse</td>
<td>Yelling, threatening by adult family</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>24. Mugging</td>
<td>Robbery</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>25. Witnessed live war/terrorism on TV</td>
<td>Live news coverage</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>26. Sexual assault/abuse</td>
<td>Rape, sexual touching</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>27. Domestic violence – threats</td>
<td>Family threats of injury</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
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</tbody>
</table>

(continued)
interview response for stress severity. Here, we discuss ELS as any adversity occurring before or concurrent to the assessment of our participants as adolescents. Recent findings from our study indicate that ELS is associated with anomalous patterns of functional and structural brain outcomes (Chahal et al. 2020b; Colich et al. 2017; Kircanski et al. 2019), and that adolescents who have been exposed to ELS are at greater risk for poor psychosocial outcomes (Humphreys et al. 2019b). Importantly, we are also finding evidence that ELS-related alterations in the brain may confer resilience against adverse outcomes, at least in the short-term (Chahal et al. 2020b; Miller et al. 2020b).

We begin by presenting findings from functional neuroimaging studies suggesting that exposure to ELS is associated with alterations in neurobiological systems that are important for social-affective processing (e.g., amygdala, ventral striatum, orbitofrontal cortex, and medial prefrontal cortex [PFC]) and for cognitive control (e.g., dorsolateral and ventrolateral PFCs, parietal cortices, and the anterior cingulate cortex [ACC]). Next, we review evidence for ELS-related structural alterations in similar regions, particularly the PFC and hippocampus. We then discuss research examining the effects of ELS on functional connections within and between neural circuits (e.g., frontoamygdala circuitry) and on structural properties of white matter tracts (e.g., uncinate fasciculus). Within each section, we focus on findings that point to neurobiological alterations following ELS as potential mechanisms of risk and resilience, but also consider findings that highlight brain-based markers of individual differences in vulnerability, sensitivity, or resilience to the adverse effects of ELS.

After examining the patterns of structural and functional brain development that are implicated in risk and resilience, both in the broader literature and in our own study, we discuss potential ways that researchers can more effectively characterize risk- and resilience-related neurobiological factors following ELS that may serve as mechanisms underlying the association between ELS and the subsequent development of psychopathology. We also discuss strategies that can be used to advance our understanding of which adolescents are more likely to be vulnerable, and which

<table>
<thead>
<tr>
<th>Type of ELS</th>
<th>Examples</th>
<th>Percentage endorsed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total sample (%)</td>
</tr>
<tr>
<td>28. Physical assault/abuse –</td>
<td>Threat of injury by adult family</td>
<td>2</td>
</tr>
<tr>
<td>threats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. Sexual assault/abuse of</td>
<td>Rape, sexual touching</td>
<td>1</td>
</tr>
<tr>
<td>someone close</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. Kidnapping</td>
<td>Attempted kidnapping, relative kidnapped</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>Friend moving away, bomb threat at school</td>
<td>7</td>
</tr>
</tbody>
</table>

Type of ELS presented in order of prevalence of exposure
adolescents are more likely to be resilient, to the adverse effects of ELS. In this context, we focus on two specific avenues for future research: (1) integrating neuroimaging with advances in methodology and data science to identify neural circuit-defined biotypes and biomarkers of risk for psychopathology; and (2) using multilevel frameworks that consider neurobiology and ELS embedded in the context of other individual- and environment-level variables.

2 Brain Functional Activation

Amygdala Activation as a Mechanism of the Effects of ELS  A growing body of research is demonstrating that ELS is associated with altered functioning of brain regions important for affective and cognitive control processes, such as the PFC, amygdala, and ventral striatum. These alterations appear to underpin both risk and resilience following ELS. One of the most consistent neural abnormalities associated with ELS is hyperactive amygdala reactivity to threatening or salient stimuli, particularly emotional faces (Hein and Monk 2017; Tottenham et al. 2011). Different types of ELS, characterized by both deprivation and threat, have been linked to heightened amygdala activation. For example, compared to family-reared youth, previously-institutionalized youth with a history of psychosocial deprivation exhibit greater amygdala reactivity to emotional faces (Gee et al. 2013); in turn, greater amygdala reactivity to fearful faces was associated with lower levels of social competence (Tottenham et al. 2011). Young adolescents exposed to maltreatment and family violence also demonstrate heightened amygdala reactivity to emotional faces (McCorry et al. 2011). Studies considering specific subdivisions of the amygdala have found that greater exposure to ELS is related to heightened centromedial amygdala reactivity to emotional faces (Suzuki et al. 2014). Similarly, in our own work we found that a cumulative measure of ELS severity was positively associated with right centromedial amygdala reactivity to emotional faces in a community sample of adolescents (Miller et al. 2020b). The centromedial amygdala mediates autonomic, endocrine, and behavioral responses supporting attention to salient stimuli (LeDoux 2007). These aspects of emotional processing may be particularly sensitive to ELS, potentially serving adaptive purposes in stressful contexts (Frankenhuis and de Weerth 2013).

Heightened amygdala activation supports hypervigilance to threat that is important for staying safe in chaotic and dangerous environments, but this neurophenotype could also constitute emotional processes, such as learned fear responses, that increase long-term risk for difficulties (Maren et al. 2013). Indeed, many studies have implicated heightened amygdala reactivity during socioemotional processing in risk for psychopathology in adolescence (Beesdo et al. 2009; Kerestes et al. 2014). Our recent work provided novel evidence that amygdala hyperreactivity also marks risk for accelerated biological aging; specifically, we found that heightened left centromedial amygdala reactivity to emotional faces was associated with more rapid cellular aging, assessed by telomere shortening over 2 years in adolescence.
One interpretation of this finding is that centromedial amygdala hyperreactivity may regulate psychological and biological processes involved in accelerated cellular aging, such as negative affect and hypothalamic pituitary adrenal (HPA) axis activation. Although hyper-vigilant amygdala activity may be adaptive in the context of adversity and impeding threat, this neurophenotype may come at the cost of accelerated aging via allostatic load and serve as a risk factor for psychopathology outside of stressful contexts, in which amygdala hyperreactivity is no longer adaptive.

**Reward- and Cognitive Control-Related Regions**

ELS has been also been linked to blunted reactivity in other brain regions that are important for evaluating reward-related stimuli and regions involved in cognitive control. Experiences of emotional neglect have been linked to attenuated ventral striatum reactivity during a monetary reward task which, in turn, predicted and partially mediated subsequent depressive symptoms (Hanson et al. 2015a). Similarly, previously-institutionalized youth have been found to demonstrate lower ventral striatum reactivity to happy faces, which was related to higher depression scores (Goff et al. 2013). The ventral striatum plays a crucial role in attributing value to reward-related stimuli (Berridge 2007); diminished reactivity in this region following ELS may underlie specific types of mental health difficulties, such as reduced anticipation of, and motivation to pursue, reward (Pechtel and Pizzagalli 2011) – a central component of anhedonia commonly observed in depressed individuals (Sherdell et al. 2012; Wu et al. 2017). ELS may also disrupt regions involved in cognitive control of emotion (McLaughlin et al. 2020). Adolescents exposed to violence have decreased dorsal ACC reactivity to fearful faces and reduced dorsomedial PFC and superior frontal gyrus reactivity to neutral faces (Weissman et al. 2020a). Further, decreased dorsal ACC reactivity was found to partially mediate the association between violence exposure and symptoms of psychopathology. This lower activity in cognitive control regions may contribute to the deficits in executive functioning that have been documented in adolescents who have experienced ELS (Pechtel and Pizzagalli 2011), and in individuals with a range of mental health disorders (McTeague et al. 2016).

It is important to note that neurophenotypes that may be implicated in risk for psychopathology have also been documented in asymptomatic individuals following ELS (e.g., van Harmelen et al. 2013), suggesting that other neurobiological factors play a compensatory role in promoting resilience (Teicher et al. 2016). Adaptive functioning in prefrontal regions involved in emotion regulation is an example of a trait-level factor that may contribute to resilience. Consistent with this perspective, dynamic and flexible responses in the ventromedial PFC (vmPFC) during stress induction, marked by initial deactivation followed by increased activation, have been found to be correlated with positive coping responses (Sinha et al. 2016). This flexible neural activity may help to offset sustained activation in other brain regions, including the amygdala, hypothalamus, and insula, that were observed during the stress induction (Sinha et al. 2016). Measures of peripheral nervous system flexibility that are linked to vmPFC functioning, such as high-frequency heart rate variability (Thayer et al. 2012), have also been found to be related to positive
social-emotional competencies in children (Miller et al. 2013; Miller 2018). Although the convergence of these results is intriguing, research examining brain-based functional activation markers of resilience is still in its early stages compared to investigations of the relation between functional brain activation and risk for psychopathology.

**Amygdala Activation as a Moderator of the Effects of ELS** In addition to being a **mechanism** by which ELS contributes to risk, neural activation in the brain regions described above may also **moderate** the impact of ELS (Schriber and Guyer 2016). For example, amygdala reactivity supports increased engagement with challenging and salient events that, in the context of environmental stressors, often include threat-related cues. Thus, amygdala hyperreactivity supports processes that may increase sensitivity to stressful events, potentially increasing vulnerability to their adverse effects; in contrast, dampened amygdala response supporting decreased sensitivity may contribute to resilience. This pattern of activation would be consistent with the formulation that amygdala functioning is a diathesis for the adverse effects of ELS. Alternatively, there is growing evidence for **differential susceptibility** models, which posit that specific patterns of neurobiological activity indicate increased openness to supportive and adverse environments, both for better and for worse (Boyce 2016). Amygdala hyperreactivity is linked to putative markers of differential susceptibility to environmental influence, such as negative emotionality and increased reactivity in peripheral stress-response systems (Blackford et al. 2013; Ulrich-Lai and Herman 2009).

Consistent with the differential susceptibility model, Gard et al. (2018) demonstrated that increased amygdala reactivity to emotional faces marked differential susceptibility to the presence versus absence of socioeconomic resources, which can be conceptualized as a proxy for exposure to stressors. Specifically, young adult men who exhibited higher amygdala reactivity to emotional faces self-reported the most and the least antisocial behaviors in the context of low and high socioeconomic resources, respectively; conversely, men who had lower amygdala reactivity appeared to be buffered from the association between socioeconomic resources and antisocial behavior. Interestingly, Weissman et al. (2018) found the opposite – that low amygdala reactivity during an emotion introspection task represented increased risk for externalizing problems in the context of increased exposure to community violence. Recent research on brain-based moderators of risk for internalizing symptoms related to ELS has yielded more consistent findings. For example, in a study of previously-institutionalized youth, those who demonstrated decreased amygdala reactivity to parent cues showed reductions in anxiety across 3 years; anxiety symptoms were high and stable over time in those youth who had increased amygdala reactivity (Callaghan et al. 2019). Similarly, in a sample of adolescents exposed to maltreatment, those who decreased amygdala activation during an emotion regulation task showed improvements in depressive symptoms over time (Rodman et al. 2019). These studies demonstrate that amygdala reactivity during affective processing may render some adolescents more or less sensitive to the consequences of ELS and related risk factors; it is still unclear, however, whether
this neurophenotype is a brain-based moderator of environment that conforms more to a diathesis-stress or differential susceptibility model. Further research in this area would help us gain a more comprehensive understanding of why some adolescents do better or more poorly following ELS, and how these individual differences are rooted in the functioning of different brain regions (Guyer 2020).

Table 2 presents a summary of the findings and implications of studies focused on ELS and brain activation in adolescents.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Functional activation alteration following ELS</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>Hyperractivity to salient/threatening stimuli</td>
<td>Hypervigilance that may heighten the adverse effects of ELS on psychopathology, particularly internalizing symptoms</td>
</tr>
<tr>
<td>Ventral striatum</td>
<td>Blunted reactivity to monetary and social rewards</td>
<td>Altered reward-related processing</td>
</tr>
<tr>
<td>Anterior cingulate cortex and dorsomedial prefrontal cortex</td>
<td>Blunted reactivity to emotional faces</td>
<td>Altered cognitive control of emotion</td>
</tr>
<tr>
<td>Ventromedial prefrontal cortex</td>
<td>Unclear</td>
<td>Positive coping that could be important for resilience following ELS</td>
</tr>
</tbody>
</table>

Table 3 Brain Structure

Prefrontal and Limbic Structural Alterations as Mechanisms of ELS Another brain-based variable implicated as a mechanism and/or moderator of the association between ELS and risk/resilience for psychopathology is brain structure. Human neuroimaging studies have consistently found that ELS is associated with structural alterations of PFC and limbic regions that are important for multiple forms of emotional processing and are implicated in the development of mental health problems. PFC regions that undergo protracted development may be particularly vulnerable to the effects of ELS. For example, the orbitofrontal cortex (OFC), which plays an important role in emotion, motivation, and psychopathology (Rolls 2019), is one of the last brain regions to mature in humans (Toga et al. 2006), and research suggests that the development of this region is affected by exposure to ELS. Adolescents who experience physical abuse and child maltreatment have been found to have smaller OFC volumes than do typically-developing adolescents (De Brito et al. 2013; Hanson et al. 2010). Research with adults has also found that the cumulative number of stressful life events experienced is associated with smaller volume in the OFC, as well as in regions that are implicated in salience processing, including the insula and ACC (Ansell et al. 2012). Compared to a control
group of adolescents who were reared by their biological families, post-
institutionalized youth with a history of psychosocial deprivation were found to
demonstrate broad, global reductions in gray matter volume and more specific
reductions in PFC and hippocampal volume (Hodel et al. 2015). In turn,
ELS-related reductions in prefrontal and hippocampus volume in adolescents have
been associated with social difficulties and behavioral problems (Hanson et al. 2010,
2015b). ELS has also been linked to cortical development in community samples
exposed to a range of more normative ELS events. For example, in a study of
adolescent girls, recent common life stress was associated with thinner parietal
cortices which, in turn, predicted the future development of depressive symptoms
(Bartlett et al. 2019).

Although ELS has been linked to volumetric reductions across a number of
cortical regions and the hippocampus, some studies suggest that ELS is related to
larger amygdala volume. Previously-institutionalized youth have been found to
have larger amygdala volume than a comparison group of adolescents without a
history of institutionalization (Mehta et al. 2009); prolonged institutional rearing in
eyear life (i.e., later adoption) has also been found to be associated with larger
amygdala volume (Tottenham et al. 2010). It is worth noting, however, that other
studies have failed to find an effect of institutionalization on amygdala volume in
youth (Sheridan et al. 2012), and that other studies have linked violence exposure
and a cumulative measure of ELS exposure with smaller, not larger, amygdala
volume (Hanson et al. 2015b; Weissman et al. 2020b). These inconsistencies in
the literature on amygdala volume are somewhat surprising given the relatively
consistent effects of ELS on amygdala functional activation (Teicher et al. 2016).

Our group posited that some of these discrepancies in findings across studies may
be due, in part, to sex differences. In our community sample of adolescents, we
found that self-reported experiences of childhood neglect were associated with larger
right amygdala volume in boys but not in girls (Roth et al. 2018); further, larger right
amygdala volume mediated the association between greater childhood neglect and
more severe anxiety symptoms in boys. These sex differences mirror findings of
recent research that neglect has stronger associations with hippocampal volume in
males, whereas threat-related experiences are more strongly correlated with hippo-
campal volume in females (Teicher et al. 2018). Sex-specific effects of ELS on brain
development in adolescence may be mediated by sex hormones. More specifically,
the amygdala is rich in glucocorticoid and androgen receptors (LeDoux 2007;
Martini and Melcangi 1991), and amygdala volume in adolescent boys has been
positively associated with circulating levels of testosterone (Neufang et al. 2009).
ELS may lead to atypical coupling of stress and sex hormones in adolescents,
although research on the nature of this effect has yielded inconsistent findings
(Dismukes et al. 2015; King et al. 2020; Ruttle et al. 2015). Nevertheless, it is
possible that neuroendocrine functioning mediates sexual dimorphic effects of ELS
on amygdala volume as well as on other brain structures, but further research is
clearly needed.

Discrepancies in the literature concerning amygdala volume could also be related
to differences in the timing of assessments or in the ages of participants across
studies. Weems et al. (2015) found that, compared to same-age healthy control adolescents, younger participants exposed to trauma had smaller right amygdala volume, but older participants exposed to trauma had larger right amygdala volume. Interestingly, associations between higher ELS and smaller amygdala volume have been found in samples of participants who were, on average, in early adolescence (Hanson et al. 2015b; Weissman et al. 2020a; but see Tottenham et al. 2010 for an exception), whereas Mehta et al. (2009) found that ELS was associated with larger amygdala volume in middle adolescence. In general, the amygdala increases in volume during adolescence (Scherf et al. 2013); longitudinal studies are necessary to determine whether this developmental trajectory of the amygdala is altered following exposure to ELS. ELS may be associated with a trajectory characterized by relatively smaller amygdala volume in early adolescence but relatively larger amygdala volume later in adolescence, and it is possible that this trajectory is related to pubertal development more strongly than to age. Pubertal stage and levels of circulating testosterone have been found to be associated with larger amygdala volume (Neufang et al. 2009); further, we have observed pubertal shifts in the effects of ELS on other neurobiological outcomes, such as the cortisol awakening response (King et al. 2017).

Brain Structure in Relation to Types and Timing of ELS
Beyond degree of ELS severity, number of ELS events, or comparing individuals with and without a history of ELS, researchers are increasingly considering the effects of different forms of ELS on alterations in brain structure (Everaerd et al. 2016). For example, we have used a person-centered approach (i.e., latent class analysis) to identify unique subgroups of adolescents exposed to particular combinations of ELS experiences, and examined whether this approach provided novel information about ELS-related effects on hippocampal volume (King et al. 2019). The hippocampus is rich in glucocorticoid receptors and plays a role in the regulation of stress responding (Jacobson and Sapolsky 1991). Exposure to glucocorticoids as a result of stress can disrupt synaptogenesis and neurogenesis, thereby leading to alterations in hippocampal volume (Andersen and Teicher 2008). In our community sample of adolescents, taking a person-centered approach to measuring ELS yielded three different subgroups that were distinguished by experiences of family instability (e.g., parental divorce, separation from family) and victimization (e.g., maltreatment). The largest subgroup was composed of adolescents who experienced less exposure to both experiences of family instability and victimization; adolescents in the second largest subgroup experienced high levels of family instability, and the smallest subgroup was characterized by direct victimization experiences. These subgroupings were similar to those identified in prior work with a high-risk sample of young children (Hagan et al. 2016). In our community sample, adolescents in the subgroup characterized by direct victimization had smaller bilateral hippocampal volume than did adolescents in the subgroup characterized by low exposure to family instability and victimization. These findings are consistent with findings from cumulative severity and extreme-group models of ELS suggesting that hippocampal volume is specifically sensitive to threatening experiences (Sheridan and
Importantly, however, King et al. (2019) found that using a person-centered approach to modeling ELS explained more variation in hippocampal volume than did using a cumulative measure of ELS, highlighting the need for research explicitly considering the advantages and disadvantages in using different approaches to modeling ELS.

In addition to examining combinations of different types of ELS, it will also be important to consider the timing of ELS exposure, given that there may be sensitive periods of development during which neurobiology is particularly malleable and shaped by experiences of ELS (Dunn et al. 2019; Feldman 2015; Gee 2020). Specifically, we found that more severe stressful experiences in early childhood (through 5 years of age), but not in late childhood (age 6 years and older), were associated with smaller hippocampal volume in early adolescence (Humphreys et al. 2019a). Importantly, stress in early childhood outperformed a cumulative measure of stress severity in predicting hippocampal volume. These findings add to a growing body of research underscoring the importance of ELS experiences in childhood for brain development, and particularly the hippocampus region (Luby et al. 2016).

**Brain Structure as a Moderator of the Effects of ELS** Compared to research examining the relation between ELS and structural alterations in the brain, there are fewer studies that have assessed structural markers of individual differences in susceptibility, vulnerability, and resilience to ELS in adolescents (i.e., treating brain structure as a moderator). In a study of Mexican-origin adolescents, Schriber et al. (2017) found that larger hippocampal volume was a marker of differential susceptibility. Specifically, in adolescents with larger hippocampal volume, community violence and family connectedness were associated with more and less severe depressive symptoms, respectively; in contrast, these factors were not associated with depressive symptoms in adolescents with smaller hippocampal volume.

Deane et al. (2020) focused on sensitivity to the adverse effects of maternal aggression, which could be a proxy for common family-related stressors. Assessing individual differences in cortical thinning at three timepoints across adolescence, Deane et al. (2020) found that adolescents with less cortical thinning were more susceptible to both the adverse and the positive effects of higher and low maternal aggression, respectively, on their well-being. Although more research is needed, these early findings suggest that larger hippocampal volume and reduced cortical thinning mark heightened sensitivity to both negative and positive experiences during adolescence.

Table 3 presents a summary of the findings and implications of studies focused on ELS and brain structure in adolescents.

**4 Brain Circuits**

Functional connections between the amygdala and regions of the PFC constitute a critical circuit for socioemotional processing (Banks et al. 2007; Phelps et al. 2004). Several studies have reported altered amygdala PREFRONTAL functional connectivity
following ELS (see review by VanTieghem and Tottenham 2018). Children and adolescents who had been exposed to trauma (e.g., physical abuse, neglect, domestic violence, sexual abuse) have been shown to exhibit weaker negative connectivity between the pregenual ACC and amygdala than do non-trauma-exposed youth when viewing and labeling facial emotions in the presence of emotional distractor words; further, lower connectivity was associated with poorer performance on this emotional conflict task (Marusak et al. 2015). These findings suggest that heightened emotional reactivity following ELS (as shown via activation studies of amygdala) is not properly modulated by prefrontal regions, leading to difficulties in emotion regulation.

Researchers have provided evidence of a mediating role of amygdala-prefrontal circuitry in the association between ELS and psychopathology in adolescence. Adolescents who experienced childhood maltreatment (e.g., physical and sexual abuse, physical and emotional neglect) have been shown to have weaker resting-state connectivity between the amygdala and subgenual ACC; importantly, this amygdala-prefrontal alteration mediated the association between ELS and internalizing symptoms at age 18, such that weaker connectivity contributed to higher levels of symptoms (Herringa et al. 2013). Weaker resting-state amygdala-ACC connectivity in ELS-exposed children and adolescents has also been shown to predict higher anxiety symptoms 1 year later (Pagliaccio et al. 2015). The long-term impact of ELS on amygdala-PFC connectivity and stress response has also been documented in adults. Young adult males who reported a history of ELS, specifically childhood abuse, showed weaker resting-state functional connectivity between the pregenual ACC and amygdala; these adults also exhibited higher levels of state anxiety following a psychosocial stress task (Fang et al. 2012). Similarly, young adults with history of childhood maltreatment have been found to exhibit atypical connectivity between the amygdala and inferior frontal gyrus when processing threat-related emotional stimuli (Jedd et al. 2015).

Importantly, the Stress Acceleration Hypothesis posits that fronto-limbic circuitry develops at an accelerated rate following ELS as a temporary adaptation to adversity

<table>
<thead>
<tr>
<th>Regions</th>
<th>Structural alteration following ELS</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal and parietal cortices</td>
<td>Reduced volume, increased cortical thinning</td>
<td>Increased risk for psychopathology, but may also indicate reduced plasticity and sensitivity to negative and positive experiences in adolescence</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Reduced volume</td>
<td>Increased risk for psychopathology, but may also indicate reduced plasticity and sensitivity to negative and positive experiences in adolescence</td>
</tr>
<tr>
<td>Amygdala</td>
<td>Unclear; may be sex-, age-, or pubertal development-specific</td>
<td>Altered emotion processing implicated in risk for psychopathology</td>
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</tbody>
</table>
(Callaghan and Tottenham 2016; Herzberg and Gunnar 2020). For example, adolescents who were exposed to maternal deprivation were found to have more mature (i.e., negative) amygdala-prefrontal functional connectivity; further, this neural pattern was related to fewer symptoms of anxiety and, thus, may be characterized as being adaptive (Gee et al. 2013). In addition, previously-institutionalized youth have been shown to have stronger functional connectivity between prefrontal and limbic regions during aversive learning than do typically-developing youth (Silvers et al. 2016); stronger connectivity also predicted improvement in anxiety symptoms. Finally, negative prefrontal-amygdala connectivity has been found to be stronger in adolescents with ELS, a pattern that was also associated with lower internalizing symptoms. Recently, we similarly demonstrated that adolescents who were exposed to more severe ELS exhibited negative prefrontal-amygdala connectivity during socioemotional processing (Colich et al. 2017; Miller et al. 2020b); further, this neurophenotype predicted slower biological aging over 2 years, assessed by telomere shortening and pubertal tempo (Miller et al. 2020b). The protective associations of negative prefrontal-amygdala connectivity were strongest in adolescents who had been exposed to more severe ELS, suggesting that this neurophenotype confers some positive adaptation following ELS. We also recently found that adolescents with more exposure to ELS exhibited greater age-related changes in white matter pathways connecting the frontal lobes to limbic, temporal, and parietal regions; further, higher fiber density and cross-section of the uncinate fasciculus, which connects frontal and limbic regions, was associated with lower levels of internalizing problems in mid-adolescence (Chahal et al. 2020b). We should note, however, that in these studies we conducted cross-sectional analyses and found that adolescents exposed to ELS have more “mature”-appearing neurophenotypes that are often accompanied by fewer symptoms of psychopathology. No study has yet examined longitudinally the development of brain functional or structural connections in the context of ELS to investigate whether neural alterations remain throughout development, or alternatively, whether lower-ELS youth eventually “catch up” with their more ELS-exposed peers.

Although less common than research examining fronto-limbic connectivity, some studies also suggest that reward circuitry is altered in ELS. Lower functional connectivity between the ventral tegmental area and hippocampus, as well as between the substantia nigra and hippocampus, has been reported in adolescents who were exposed to threat-related forms of ELS (Marusak et al. 2017). Another study found that post-institutionalized youth exhibited higher functional connectivity between the ventral striatum and anterior medial PFC, a neural pattern that was also related to greater social problems (Fareri et al. 2017). Finally, Herzberg and Gunnar (2020) posited that accelerated fronto-limbic circuit development may come at the cost of delayed reward circuit development, although this formulation has not yet been tested longitudinally.

ELS has also been linked to alterations in the executive control network (ECN). The ECN includes frontoparietal brain regions and supports executive functioning skills (Cole and Schneider 2007). Stress experienced in the first year of life has been associated with greater resting intra-regional synchronization of activity in the left
prefrontal cortex in young children, and this pattern of brain activity was associated with poorer cognitive control ability (Demir-Lira et al. 2016). In a study that compared adolescents with a history of severe child abuse with a healthy control group, participants exposed to child abuse demonstrated reduced functional connectivity in the ECN network when engaging in a sustained attention task, a neural pattern related to poorer performance on the task (Hart et al. 2017). Taken together, ELS may contribute to abnormal functional connectivity within the ECN and between the ECN and other regions, potentially impeding the development of executive functions that help buffer against the development of psychopathology.

In addition to possibly being altered following ELS, recent research on moderator effects indicates that functional connectivity within the ECN may be a source of resilience. Higher resting-state ECN connectivity appears to buffer adolescents against cardiometabolic risks associated with exposure to violence in the community (Miller et al. 2018). Similarly, we are finding that higher ECN connectivity buffers against risk for internalizing problems during the COVID-19 pandemic in adolescents who previously reported being in more advanced stages of puberty relative to their same-age peers (Chahal et al. 2021); further, ELS was associated with more advanced puberty in females, a finding consistent with results of past research with adolescents exposed to maltreatment (Mendle et al. 2011). Taken together, it appears that neural circuits that support executive functioning contribute to resilience against physical and mental health problems in adolescents who may otherwise be at risk (e.g., those exposed to ELS).

Table 4 presents a summary of the findings and implications of studies focused on ELS and brain circuits in adolescents.
5 Common Themes Across ELS Research on Function, Structure, and Circuits

The literature shows that across various samples, across measures of ELS, and across neuroimaging methods, exposure to ELS is associated with altered brain development in adolescence. ELS may have selective strengthening and downregulatory effects on different neural systems. ELS is linked not only to neurophenotypes that underlie increased engagement with and processing of emotionally salient stimuli (Hein and Monk 2017; Humphreys et al. 2019a; Teicher et al. 2018), but also to blunted reward-processing (Hanson et al. 2015a) and to decreased activation and connectivity in regions important for executive functioning (Chahal et al. 2020c; Weissman et al. 2020a). The combination of these neural alterations following ELS may support psychological processes that serve as core mechanisms that link ELS to multiple forms of psychopathology.

Many of the observed associations both in the broader literature and in our own study are consistent with the stress acceleration hypothesis – that ELS can lead to faster maturation of neural regions and circuits implicated in affective processing (Callaghan and Tottenham 2016). For example, ELS has been linked to amygdala hyperreactivity to salient and threatening stimuli (Hein and Monk 2017; Suzuki et al. 2014; Tottenham et al. 2011), to negative prefrontal-amygdala functional connectivity (Colich et al. 2017; Gee et al. 2013; Miller et al. 2020b), to increased integrity in fronto-limbic white matter tracts (Chahal et al. 2020b; Kircanski et al. 2019), and to cortical thinning and reduced gray matter (Bartlett et al. 2019; Hanson et al. 2010). Although these outcomes may reflect more mature neurophenotypes in adolescence, they may also vary in whether they confer risk or resilience (short-term) following ELS (Aghajani et al. 2014; Callaghan and Tottenham 2016; Gee et al. 2013). More mature functional and structural connections between frontal and limbic regions may confer resilience to adverse outcomes following ELS (Chahal et al. 2020b; Gee et al. 2013; Miller et al. 2020b), whereas amygdala hyperreactivity and accelerated cortical maturation may contribute to elevated risk for ELS-related difficulties (Bartlett et al. 2019; Hanson et al. 2010; Tottenham et al. 2011). Further research is necessary to clarify when and for whom ELS leads to neurophenotypes that reflect accelerated maturation that supports resilience versus vulnerability.

One potential consequence of ELS-related acceleration in neurodevelopment is an earlier reduction in plasticity (Callaghan and Tottenham 2016). Interestingly, some of the brain-based moderators of sensitivity to environmental influence are affected by ELS. For example, smaller hippocampal volume and greater cortical thinning following ELS may indicate more mature development in these systems, with implications for increased risk for psychopathology (Bartlett et al. 2019; Hanson et al. 2015b), but may also indicate reduced sensitivity to environmental influence (Deane et al. 2020; Schriber et al. 2017). This relative insensitivity to the environment may represent a developmental adaptation following ELS that helps to reduce vulnerability to subsequent adverse experiences during adolescence (i.e., plasticity) that may exacerbate difficulties. Indeed, research with nonhuman primates
suggests that ELS places a limit on reactivity to subsequent stressors (Parker et al. 2006). Conversely, reduced sensitivity/plasticity may also render some adolescents less open to the benefits that can be derived from subsequent positive, supportive experiences. It is important to note, however, that this interpretation is speculative given that few studies have focused on brain-based moderators of ELS and related experiences. Indeed, research on brain-based resilience in general is a relatively recent undertaking compared to research on ELS-related mechanisms of risk. More studies are urgently needed to increase our understanding of whether putative neurophenotypes of resilience serve as compensatory factors that help to offset ELS-related risk, or whether they act as protective moderators that buffer against the adverse effects of ELS.

6 Future Directions

One intriguing direction for the field is to apply more person-centered methodologies to define brain-based biotypes. Brain-based biotyping is a relatively novel concept that involves examining patterns of resting-state (or task-based) functional connectivity (within and between networks) to determine whether subgroups of individuals can be identified at the neural level. Figure 1 presents a visual summary of the brain-based biotyping approach and how it might reveal otherwise undetected heterogeneity in a sample of adolescents exposed to ELS (e.g., who is more or less likely to develop symptoms of psychopathology). Biotyping can be accomplished by selecting regions of interest in candidate networks (e.g., default mode, salience, cognitive control networks) and testing whether individuals with different subtypes of disorders differ in their neural signatures (Williams 2017). A second approach to biotyping is utilizing data-driven techniques to parse neurophysiological patterns, rather than examining differences in a priori groups; this approach allows researchers to distinguish neural patterns that may otherwise be masked by group-averaged approaches (e.g., Chahal et al. 2020d; Price et al. 2017). For example, subtypes of adult depression have been identified based on heterogeneous connectivity-based profiles using the Subgroup Group Iterative Multiple Model Estimation (S-GIMME; Gates et al. 2017). Further, a community sample of adolescents have been parsed using S-GIMME into two ventral affective network biotypes that differ in the past, current, and future internalizing symptoms (Chahal et al. 2020d). Importantly, S-GIMME is an unsupervised approach that examines directed functional connections between regions of interest and uses Walktrap (Pons and Latapy 2006), a community detection algorithm, to identify subgroups of individuals with shared patterns of directed connectivity. Because no symptom information is required beforehand, this approach relies entirely on brain-derived information to search for connectivity-based subgroups that can later be compared on external variables. Therefore, biotyping has great potential for use in research examining ELS risk and resilience, as it can reveal brain-based differences that may be related to different
types or duration of ELS, and to differences in susceptibility to adversity with regard to psychopathology, without any prior model information.

To date, however, only two studies have investigated biotypes in the context of ELS. One study found that adolescents with high violence exposure were more likely to belong to a connectivity-based subgroup (i.e., biotype) that showed few shared connections and low network density of the salience and default mode networks, compared to adolescents who were not exposed to violence (Goetschius et al. 2020). In the second study, we examined the utility of biotyping in understanding resilience to psychopathology following ELS (Chahal et al. revise and resubmit). We first identified regions of the executive control network, given its involvement in adolescent psychopathology (Chahal et al. 2020a). We then tested whether we could identify connectivity subgroups, based on similarities in directed functional connections within the ECN. We found three separable ECN subgroups, and then tested whether these subgroups differed in the association between ELS severity and depression, in order to examine whether there are susceptible or resilient brain-based groups. Interestingly, we found that one ECN connectivity subgroup, consisting of 25% of the sample and characterized by more directed connections between frontoparietal brain regions, did not show an association between ELS and
general psychopathology (based on a latent factor score of six psychopathology measures). This ECN subgroup could be characterized as resilient, given that those participants were protected from ELS effects. In contrast, in the other two subgroups there were strong positive associations between ELS and psychopathology (i.e., risk groups). Importantly, we found that differences in risk and resilience could not be uncovered by raw correlation values that are typically used in connectivity studies. These findings highlight the importance of examining biotypes to understand individual differences in the sequelae of ELS. The community detection approach is blind to information about symptoms and ELS exposure; thus, the resulting subgroups represent meaningful partitions of psychological processes.

Finally, we believe it is important for researchers to adopt theoretical perspectives and use methodological approaches that conceptualize ELS as being embedded in the context of other types of environmental factors. For example, ELS often co-occurs with different physical environmental factors, such as environmental pollutants, that are also implicated in the development of neurobiological systems important for risk and resilience (Olvera Alvarez et al. 2018). Few studies of ELS and brain development have considered pollutants, despite recent calls for more research both on the effects of pollutants on psychological development (Trentacosta et al. 2016) and on the joint, synergistic effects of ELS and pollutants on neurobiology and health (Olvera Alvarez et al. 2018). Interestingly, many of the adverse effects of ELS and environmental pollutants appear to be mediated through similar neurobiological pathways, such as chronic activation of stress-response systems (Thomson 2019). Our own work with adolescents suggests that living in areas with higher concentrations of air pollution is associated with increased biological reactivity to social stress (i.e., increased autonomic and HPA axis reactivity to lab-based stress test), a link that we found to be magnified in adolescents who were experiencing high levels of psychosocial stress in the form of internalizing difficulties (Miller et al. 2019, 2020a). In addition, we recently found that for adolescents living in psychologically controlling families, higher levels of drinking water contaminants were associated with increases in depressive symptoms over 2 years (Manczak et al. 2020). Taken together, these findings highlight the interplay of psychosocial and physical environmental factors in influencing risk and resilience to psychopathology. More research on adolescent brain development is needed that considers exposure to physical environmental and psychosocial factors that are more closely related to ELS, such as experiences of deprivation and threat.

7 Conclusions

The studies we discuss in this chapter converge to suggest that exposure to ELS organizes and consolidates brain development in a manner that places adolescents at risk for experiencing psychopathology and other kinds of negative health outcomes. The field is making considerable progress in identifying specific brain-based pathways that link ELS to subsequent problems in mental and physical health.
Nevertheless, not all adolescents are equally sensitive or vulnerable to the adverse effects of ELS on well-being. The complex coordination of ELS and neurobiology that underlies individual differences in risk and resilience is only beginning to be considered by researchers in the area of developmental neuroscience. Methodological advances in our modeling of ELS and neurobiology, as well as a consideration of ELS in the context of other risk- and resilience-promoting factors, will advance our understanding of mechanisms, of early detection of high-risk adolescents, and of efforts to develop targeted preventions and interventions aimed at fostering resilience.

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Effects of Parenting Environment on Child and Adolescent Social-Emotional Brain Function

Tara M. Chaplin, Kelsey L. Mauro, and Claire E. Niehaus

Abstract The caregiving environment that children and adolescents experience is critically important for their social-emotional development. Parenting may affect child social-emotional outcomes through its effects in shaping the child’s developing brain. Research has begun to investigate effects of parenting on child and adolescent brain function in humans using functional magnetic resonance imaging (fMRI). Here we review these initial studies. These studies find associations between parenting...
behavior and child and adolescent functional activation in neural networks involved in emotional arousal, emotion regulation (ER), reward processing, cognitive control, and social-emotional information processing. Findings from these studies suggest that higher negative parenting and lower positive parenting are generally associated with heightened activation in emotional arousal networks in response to negative emotional stimuli in youth. Further, findings indicate that lower positive parenting is associated with higher response in reward processing networks to monetary reward in youth. Finally, findings show that lower positive parenting predicts lower activation in cognitive control networks during cognitive control tasks and less adaptive neural responses to parent-specific stimuli. Several studies found these associations to be moderated by child sex or psychopathology risk status and we discuss these moderating factors and discuss implications of findings for children’s social-emotional development.

**Keywords** Adolescence · Emotion · Emotion regulation · fMRI · Neural function · Parenting · Reward

Parenting as a topic of inquiry has attracted the attention of scientists for decades due to the powerful relationship between the parenting environment and child outcomes. Indeed, across theories of parenting, positive parenting, characterized by high levels of parent warmth, involvement, and sensitivity (e.g., Baumrind 1967) may act as a protective factor and may promote adaptive child social, emotional, and cognitive development. Positive parenting longitudinally predicts positive child social-emotional functioning, including children’s development of understanding of social interaction and prosocial behaviors, as well as positive cognitive, academic, and physical health outcomes across the developmental span (Amato and Fowler 2002; Beckwith et al. 1992; Malonda et al. 2019; Morris et al. 2017). In contrast, negative parenting, characterized by high levels of harsh or inconsistent discipline, may be considered a social stressor to children and, similar to other social stressors (e.g., neighborhood stress, peer victimization), negative parenting may lead to problems in children’s social-emotional development (e.g., development of prosocial behaviors and empathy, social reward learning) and in their cognitive and physical development (Amato and Fowler 2002; Malonda et al. 2019; Morris et al. 2017). In addition to a large number of longitudinal studies, experimental studies that randomly assign parents to parenting interventions find that changing parenting behavior experimentally can lead to changes in child social-emotional behavior (e.g., Webster-Stratton and Herman 2008). This work suggests that parenting is not merely correlated with child behavior, but plays a causal role in shaping child social-emotional development. In sum, parenting, which is itself a social context (and can be a social stressor), has been shown to play a critical role in shaping children’s social-emotional development. Further, parenting affects youth both in childhood and in adolescence. Although parent-child relationships change as youth enter adolescence, research still finds that parenting behavior during adolescence predicts adolescent outcomes (Steinberg 2001).
Despite our growing understanding of the nature of relationships between parenting and youth outcomes, the mechanisms by which parenting affects child outcomes are not yet fully understood. There are several theories of how parenting exerts its influence on children. Parenting may exert its influence by genetic transmission of psychological and other functioning, through modeling behaviors for the child that they imitate, or, as has recently gained attention, by environmental shaping of the child brain. The current review will focus on effects of the parenting environment as one important social environment that can shape child and adolescent brain development. We aim to inform future directions and clinical implications for this exciting field with important implications for prevention of adverse child outcomes. We will also describe potential moderators of effects of parenting on child brain function, since parenting may operate differently depending on child gender, child temperament, and/or socio-cultural context (Rodriguez et al. 2009).

1 Parenting and Child and Adolescent Brain Function

As described above, a large literature has documented that the parenting environment affects children and adolescents’ social, emotional, and cognitive development and their risk for negative social-emotional and health outcomes. Recently, researchers have begun to explore whether parenting affects child development through its effects on the developing brain. Understanding neural systems affected by parenting is important because it can inform our understanding of typical and atypical brain development and can lead to neurobiological targets for intervention, including parenting interventions (Tan et al. 2020). Theory, animal research (Rilling and Young, 2014), and recent research in humans suggest that parenting may influence child and adolescent brain development across several neural networks, including emotional arousal and emotion regulation (ER), reward processing, social-emotional information processing, and cognitive control-related networks.

The parenting social environment can affect child brain development in several ways. Negative parenting may act as a social stressor on the child and can lead to alterations in child brain development similar to other social environmental stressors such as poverty (Lupien et al. 2009). In contrast, positive parenting can act as a social environmental condition that promotes feelings of safety and adaptive brain development. Negative parenting may also reflect parents’ own stress reactivity or shared stressors in the family environment that may affect child brain development. Indeed, studies have found that mothers’ higher negative emotion and stress levels are associated with their adolescents’ heightened neural responses to negative emotional stimuli in emotion processing and ER regions including amygdala, anterior cingulate cortex (ACC), and medial prefrontal cortex (PFC) (Turpyn et al. 2018; Niehaus et al. 2019). And studies find that parental social responses may be a critical pathway for shaping social reward learning in youth. For example, parental depression is associated with altered striatal response to reward in children and adolescents (e.g., Morgan et al. 2019).
Below we review findings from the emerging human studies of associations between negative and positive parenting behavior and child neural functional activation to negative emotional stimuli, rewarding stimuli, personalized parent-specific stimuli, and cognitive control tasks. We limited our review to studies of normative variations in parenting, as there are other reviews of child maltreatment and brain development (e.g., Teicher and Samson 2016). We also limited our review to fMRI studies of neural function since we are particularly interested in effects of parenting on patterns of neural activation. Thus, we did not review studies on parenting effects on brain structure (e.g., Whittle et al. 2014). And we did not review studies on peripheral physiology or EEG measures, although these studies find effects of parenting on youth physiological responses (e.g., Chaplin et al. 2012). For another review of parenting behaviors related to emotion socialization and brain function and structure, see Tan et al. (2020).

In addition to the above, our inclusion criteria for the present review were that studies (1) were an original empirical article; (2) included a measure of parenting collected before the child was 18; (3) examined associations between the parenting measure and youth fMRI activation or functional connectivity in a task. To find published work, we conducted a literature search using the following key terms in PubMed: (fMRI>Title/Abstract) AND (parent*[Title/Abstract] OR caregiv*[Title/Abstract] OR mother[Title/Abstract] OR father[Title/Abstract] OR paternal[Title/Abstract] OR maternal[Title/Abstract]) AND (child OR youth OR adolescent) AND filtered for Journal Articles. This search yielded 332 results, which were scanned for relevant titles and abstracts. Of those 332, 25 studies met our inclusion criteria. Findings from those studies are reviewed below, organized by task type: negative emotion tasks, reward tasks (including monetary and social reward tasks), parenting-specific tasks, and cognitive control tasks.

2 Empirical Studies: Negative Emotion Tasks

Several studies have found associations between parenting and youth’s neural activation while processing negative emotional stimuli in neural networks involved in emotional arousal and emotion regulation (ER). Study information can be found in Table 1. Emotional arousal-related networks involve interconnected regions that support detecting and appraising salient stimuli and include amygdala (and other limbic regions), anterior insula, and subgenual (sg) and perigenual (pg) ACC (Lindquist et al. 2012; Menon 2011). ER-related networks include prefrontal regions such as dorsolateral and ventrolateral PFC (dLPFC, vLPFC), ventromedial PFC (vmPFC)/orbitofrontal cortex (OFC), and dorsal ACC (dACC), which support explicit ER (intentional down-regulation of emotion, e.g., re-appraisal) and/or implicit ER (automatic regulation of emotion, e.g., unintentionally shifting attention away from emotional stimuli) (Niendam et al. 2012; Phillips et al. 2008).
<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Age Par. Meas.</th>
<th>Age at MRI</th>
<th>Sample (Youth) Char.</th>
<th>Parent sex*</th>
<th>Par. construct</th>
<th>Par. Meas.</th>
<th>MRI task</th>
<th>MRI contrasts</th>
<th>Main finding</th>
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<tr>
<td><em>Negative emotion studies</em></td>
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<tr>
<td>Romund et al. (2016)</td>
<td>83</td>
<td>14–16</td>
<td>13–16</td>
<td>Community</td>
<td>1</td>
<td>Pos Par</td>
<td>Child report</td>
<td>Emo. Face matching</td>
<td>Fear &gt; neutral faces</td>
<td>Lower Pos parenting associated with higher L amygdala response (ROI)</td>
</tr>
<tr>
<td>Guyer et al. (2015)</td>
<td>39</td>
<td>7</td>
<td>M = 17.89</td>
<td>High and low behavioral inhibition (BI)</td>
<td>1</td>
<td>Neg &amp; Pos Par</td>
<td>Parent report</td>
<td>Peer rejection</td>
<td>Peer rejection &gt; acceptance</td>
<td>For all youth, low pos parenting associated with higher R caudate (ROI)</td>
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<td>For youth with high BI, neg parenting associated with lower R vlPFC (ROI)</td>
</tr>
<tr>
<td>Butterfield et al. (2019)</td>
<td>120</td>
<td>9–14</td>
<td>9–14</td>
<td>Half with anxiety disorder</td>
<td>1</td>
<td>Pos coping socialization</td>
<td>Observed (P-C anxiety discussion)</td>
<td>View threat words</td>
<td>Threat words &gt; baseline period</td>
<td>In healthy youth, lower pos parenting is associated with higher L/R anterior insula &amp; pACC. In anxious youth, lower pos parenting associated with lower L/R anterior insula &amp; ACC (ROI)</td>
</tr>
<tr>
<td>Farber et al. (2019)</td>
<td>232</td>
<td>M = 13</td>
<td>M = 13</td>
<td>Half with relative with MDD</td>
<td>2</td>
<td>Warm family envir.</td>
<td>Child report</td>
<td>Emo. Face matching, shape matching</td>
<td>Angry face &gt; shapes</td>
<td>Lower pos parenting associated with lower L amygdala response (ROI)</td>
</tr>
<tr>
<td>Pozzi et al. (2020)</td>
<td>86</td>
<td>8–9</td>
<td>9–11</td>
<td>Low-income</td>
<td>1</td>
<td>Neg affect</td>
<td>Observed (P-C discussion)</td>
<td>Emo. Face matching, shape matching</td>
<td>Fear &amp; angry faces &gt; shapes</td>
<td>Maternal neg affect associated with increased R amygdala response (ROI)</td>
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<td></td>
<td>For girls only, maternal neg affect associated with decreased lingual gyrus response (WB)</td>
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<tr>
<td>Study</td>
<td>N</td>
<td>Age Par. Meas.</td>
<td>Age at MRI</td>
<td>Sample (Youth) Char.</td>
<td>Parent sexᵃ</td>
<td>Par. construct</td>
<td>Par. Meas.</td>
<td>MRI task</td>
<td>MRI contrasts</td>
<td>Main finding</td>
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<tr>
<td>La Buissonnierre-Ariza et al. (2019)</td>
<td>84</td>
<td>2–9</td>
<td>13–16</td>
<td>High and low anxiety</td>
<td>1</td>
<td>Neg Par</td>
<td>Parent report</td>
<td>Face paired with fear stimulus (CS+), and unpaired fear (CS-)</td>
<td>CS+ &gt; CS-</td>
<td>– Lower neg parenting associated with deactivation in CS+ &gt; CS- for L amygdala and L anterior hippocampus (ROI)</td>
</tr>
<tr>
<td>Gard et al. (2017)</td>
<td>167</td>
<td>2</td>
<td>20</td>
<td>Low-income, all boys</td>
<td>NS</td>
<td>Neg Par</td>
<td>Parent report, observed (at home)</td>
<td>Emo. Face matching, shape matching</td>
<td>Fear face &gt; shapes; angry face &gt; shapes</td>
<td>– Neg parenting associated with lower R amygdala response (ROI)</td>
</tr>
<tr>
<td>Chaplin et al. (2019)</td>
<td>66</td>
<td>12–14</td>
<td>12–14</td>
<td>Community</td>
<td>1</td>
<td>Neg Par</td>
<td>Observed (P-C discussion)</td>
<td>Neg and neutral emotional images</td>
<td>Neg &gt; neutral image</td>
<td>– For girls, neg parenting associated with higher R sgACC &amp; R pACC (ROI) -For boys neg parenting associated with lower L/R anterior insula, L pACC, &amp; L dACC (ROI)</td>
</tr>
<tr>
<td>Kopala-Sibley et al. (2020)ᵇ</td>
<td>65</td>
<td>3</td>
<td>10–11</td>
<td>Community, oversampled for high neg emo and high BI</td>
<td>1</td>
<td>Neg Par</td>
<td>Observed (P-C interaction) &amp; mother report</td>
<td>Emotional faces</td>
<td>Sad face&gt; neutral face</td>
<td>– Neg parenting associated with more neg connectivity between amygdala and insula, operculum, and mPFC</td>
</tr>
</tbody>
</table>

**Reward studies**

| Holz et al. (2018)        | 172 | 3 months      | 25         | Community            | 1           | Pos Par        | Observed (P-C interaction) | Monetary reward (MID) | Monetary > verbal anticipation; win > no win | – For children with familial risk, low pos parenting associated with lower caudate (ROI), SMA, cingulum, and MFG (WB) to reward anticipation. For children with no risk, low pos parenting associated with |
higher caudate (ROI), SMA, cingulum, and MFG (WB) to reward anticipation – For children with familial risk, low pos parenting associated with higher caudate to reward receipt (ROI), and higher caudate mediated relationship between pos parenting and youth ADHD diagnoses.

- Increases in pos parenting associated with decreased VS and dLPC over time (WB)
- Increases in pos parenting associated with decreased risk taking through decreased VS over time (WB)

Qu et al. (2015) b 24 15–17 (T1) & 16–18 (T2) Community 2 Child report BART T2 cash out > T1 cash out

- Low pos parenting associated with higher activation in the bilateral NAcc (ROI) in boys only, which predicted increased substance use 1 year later

Morgan et al. (2014) 120 18/24 months and 10/11 years Community, low-income, all boys 1 Pos Par Observed (P-C interaction) Reward anticipation > baseline; reward outcome > baseline; loss anticipation > baseline; loss outcome > baseline

Chaplin et al. (2021) 71 12–14 Community 1 Pos Par Observed (P-C interaction) Monetary reward (card guessing) Win > neutral

- Lower pos parenting at 18–24 months associated with greater mPFC in reward anticipation, and lower pos parenting at 10–11 years associated with greater mPFC to reward outcome (ROI)
<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Age</th>
<th>Par. Meas.</th>
<th>Sample (Youth) Char.</th>
<th>Parent sex&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Par. construct</th>
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<th>MRI task</th>
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<tr>
<td>Casement et al. (2014)</td>
<td>120</td>
<td>11–12</td>
<td>16</td>
<td>Community, all girls, oversampled for MDD</td>
<td>1</td>
<td>Pos Par</td>
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<td>Monetary reward (card guessing)</td>
<td>Reward anticipation &gt; baseline</td>
<td>For boys exposed to maternal depression only, lower pos parenting at 18–24 months associated with higher mPFC to reward anticipation and higher striatum to reward win and anticipation, and lower pos parenting at 10–11 associated with lower caudate to reward anticipation (ROI)</td>
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<tr>
<td>Morgan et al. (2017)</td>
<td>122</td>
<td>10, 11, &amp; 12</td>
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<td>Community, low-income, all boys</td>
<td>1</td>
<td>Neg Par</td>
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<tr>
<td>Tan et al. (2014)</td>
<td>40</td>
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<td>Miller et al. (2020)</td>
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<tr>
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<tr>
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<td>53</td>
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<td>Community ethnically diverse (63% minority)</td>
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**Cognitive control studies**

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<th>Study</th>
<th>N</th>
<th>Age Par. Meas.</th>
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<td>Trait words rating for self and mother, and control word sorting</td>
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<td>Neg Par</td>
<td>Observed (P-C discussion)</td>
<td>Own and other parent emotion video clips</td>
<td>Own &amp; other Pos &gt; Neu; own &amp; other Neg &gt; Neu; [own Neg-Neu] &gt; [other Neg-Neu]; [own Pos-Neu] &gt; [other Pos-Neu]</td>
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<td>Pos Par</td>
<td>Child report</td>
<td>Pictures of own and other mother in positive or neutral expressions</td>
<td>Own mother Pos &amp; neu &gt; other mother Pos &amp; neu</td>
<td>Higher pos parenting associated with negative amygdala to PFC connectivity (PPI)</td>
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<td>Marusak et al. (2018)</td>
<td>27</td>
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<td>9–16</td>
<td>Community low-income</td>
<td>2</td>
<td>Neg &amp; Pos Par</td>
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<td>Emotional conflict: match happy or fear faces w diff words (incongruent) or same word (congruent)</td>
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ᵃPar. sex: M = male, F = female.
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<tr>
<th>Study</th>
<th>Sample</th>
<th>Age Range (T1)</th>
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<th>Environment</th>
<th>Parenting</th>
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<td>Lauharatanahirun et al. (2018)</td>
<td>167</td>
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<td>167</td>
<td>13–14</td>
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<td>Interference &gt; neutral</td>
</tr>
</tbody>
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Ages reported in years unless otherwise specified


*1* = mothers only sample or 90% or greater mothers, *2* = both mothers and fathers

*The same study presented under the two constructs the study explored*
2.1 Positive Parenting

A few studies have examined positive parenting, including parental warmth, involvement, and sensitivity, and youth neural responses to negative emotional stimuli. Romund et al. (2016), in healthy 13–16-year-olds, examined adolescent-reported maternal positive parenting (warmth). They found that lower maternal positive parenting correlated with adolescents’ higher L amygdala activation while matching fear faces (versus matching neutral faces). In addition, Guyer et al. (2015) found that lower reported positive parenting at age 7 predicted increased R caudate response to peer rejection at age 17 in a sample of youth high and low in behavioral inhibition. R caudate is a striatal region involved in positive and also negative emotional arousal. Taken together, these two studies suggest that lower positive parenting is associated with higher responses in emotional arousal-related regions to negative stimuli. Butterfield et al. (2019), in healthy and clinically anxious 9–14-year-olds, examined observed positive parenting (parents’ coping socialization statements) during parent-adolescent anxiety discussions, with mostly mothers. In the healthy youth, consistent with Romund et al. and Guyer et al., lower positive parenting was associated with higher L and R anterior insula and pgACC response to threat words (versus a baseline period).

However, Butterfield and colleagues found that lower positive parenting was associated with lower activation in these emotion arousal regions in the anxious youth. Notably, in the anxious adolescents, higher activation was adaptive; it was associated with less disengaged coping in their daily lives. Similar to this, Farber et al. (2019) found that lower positive parenting (warmth in the family environment) was associated with lower L amygdala response to matching angry faces in 13-year-olds, half with a relative with depression. Thus, lower positive parenting may be associated with heightened responses to negative emotional stimuli in typically developing youth, but with lower responses in youth with (or at risk for) internalizing disorders. Perhaps youth with internalizing disorder risk have a tendency to respond to negative stimuli with high arousal in general, even when they experience positive parenting. Or, parents of youth with internalizing disorders that may appear positive and warm are actually over-controlling, leading high positive parenting scores to correlate with high negative emotional arousal in youth with internalizing symptoms (Borelli et al. 2015).

2.2 Negative Parenting

A number of studies have examined negative parenting (including harsh parenting, criticism, and high negative affect during parenting) and child neural responses to negative emotional stimuli. In one study, Pozzi et al. (2020), in a low-income sample, examined observed maternal negative affect during parent-child conflict and positive discussions. They found that higher maternal negative affect at age 8–9
predicted, 18 months later, children’s increased right (R) amygdala activation in an emotion matching task while matching angry and fear faces (versus matching shapes). La Buissonniere-Ariza et al. (2019), in youth who were high and low in anxiety symptoms, examined mothers’ self-reported negative parenting from ages 2 to 9 years predicting children’s neural response to a fear conditioning task at age 13–16. They examined responses to a neutral face that was paired with fear stimuli (CS+) versus an unpaired neutral face (CS-). They found that lower negative parenting predicted deactivation to CS+ versus CS- in left (L) amygdala and L anterior hippocampus. Taken together, these two studies both suggest an association between more negative parenting and higher response to negative emotional stimuli in amygdala and also in hippocampus (a limbic structure that is involved in processing negative emotion along with other cognitive processes). In contrast to these studies, Gard et al. (2017), in a sample of low-income boys, examined negative (harsh) parenting via home observation and parent interview at age 2 predicting youth MRI responses at age 20. This study of young adult males’ neural responses found, in contrast with the above studies with male and female adolescents, that higher negative parenting at age 2 predicted lower R amygdala activation while matching fear and angry faces (versus shapes) in the young adult males. This divergent finding may be due to the older age or the sex of the sample. In terms of age, children may initially respond to negative parenting environments with heightened reactivity to negative emotion, but eventually over repeated exposure to negative parenting, this reactivity may wear down, leading to a more blunted response. This is consistent with some literature on HPA axis functioning and social environmental stress (Koss and Gunnar 2018).

Consistent with a sex differences hypothesis, Chaplin et al. (2019) found that adolescent sex moderated the association between negative parenting and adolescent neural response to negative stimuli. In a community sample of 12–14 year olds, Chaplin and colleagues examined associations between observed maternal negative parenting during parent-adolescent challenging discussions and adolescents’ neural responses to negative emotional images (versus neutral images). They found that higher negative parenting was associated with higher R sgACC and pgACC responses to negative emotional images for girls, but lower L and R anterior insula, pgACC and dACC and R amygdala responses for boys. This is similar to the Gard and colleagues’ study that found that negative parenting predicted blunted amygdala responses in a male-only sample. Taken together, these findings may suggest that negative parenting predicts heightened responses to negative emotional stimuli in emotional arousal-related regions for girls, but is associated with blunted responses in these regions for boys. One other study of parenting and child neural responses also found sex differences. Pozzi et al. (2020) study, described above, found that maternal negative affect predicted decreased lingual gyrus (a region involved in early perceptual processing of faces) responses to anger and fear face matching for girls, but not boys. This may suggest that negative parenting leads to heightened arousal for girls in networks involved in processing of the salience of emotion (e.g., ACC), perhaps at the cost of perceptual processing of facial features.
In addition to moderation by sex, one study (Guyer et al. 2015) found moderation by behavioral inhibition. They found that higher mother-reported harsh/punitive parenting when youth were toddlers predicted decreased R vIPFC response in adolescence for youth with high behavioral inhibition (Guyer et al. 2015), suggesting that negative parenting may lead to lower emotion regulatory system responses to negative social-emotional stimuli, particularly for those at risk for anxiety.

2.3 Functional Connectivity

One other consideration for how parenting relates to child brain responses to negative emotional stimuli is how neural responses in these networks are interconnected. Three studies examined functional connectivity of the amygdala to other regions during negative emotional stimuli processing. Two studies found that higher negative parenting was associated with more negative connectivity between amygdala and emotion processing and ER regions in childhood and early adolescence (Kopala-Sibley et al. 2020; La Buissonniere-Ariza et al. 2019). Given that, in typical development, amygdala connectivity to PFC becomes more negative over time into adulthood, this indicates that youth with high negative parenting showed a more “mature” pattern of connectivity, which is consistent with studies of children who have experienced other forms of social stress, such as maltreatment (Tottenham 2012). However, one other study (Pozzi et al. 2020) found negative parenting to predict increased amygdala to parietal lobule positive connectivity for 10-year-old boys versus girls. This may suggest that the pattern of negative parenting predicting greater negative connectivity may be more common for girls than boys. Future studies should further examine sex differences in parenting effects on connectivity.

2.4 Summary and Implications

In sum, most studies found that lower positive parenting and higher negative parenting correlated with (and, in some studies, longitudinally predicted) higher reactivity to negative emotional stimuli (and possibly more negative connectivity) in neural networks involved in emotional arousal in children and adolescents. This is consistent with negative parenting (and a lack of positive parenting) being a social environmental stressor and leading to heightened arousal in response to negative stimuli. However, higher negative parenting correlated with blunted neural responses to negative stimuli for older youth (Gard et al. 2017), for boys (Chaplin et al. 2019; Gard et al. 2017), and for youth with anxiety or at risk for depression (Butterfield et al. 2019; Farber et al. 2019). In terms of age differences, as noted above, children may initially show a high neural reactivity to the stress of negative
parenting, but over time, these responses may wear down and become blunted by later adolescence.

**Clinical Implications** In terms of sex differences, our review suggests that girls may respond to social environmental stressors (including maladaptive parenting) by showing heightened emotional reactivity, whereas boys may show a more blunted emotional response (Chaplin and Aldao 2013; Hankin et al. 2007). One clinical implication of this is that girls’ heightened neural responses to negative stimuli may contribute to their greater risk for internalizing disorders. Indeed, the Chaplin et al. (2019) study found that higher anterior insula, pgACC, and dACC responses to negative emotional stimuli were associated with higher depressive symptoms and substance use for girls. The two studies with adolescents with or at risk for internalizing problems (Butterfield et al. 2019; Farber et al. 2019) found that these youth showed a pattern of high reactivity to negative emotional stimuli (although they surprisingly found that to be the case even when they experienced high positive parenting), further supporting heightened neural reactivity as a marker of risk for internalizing problems. Whereas girls’ heightened reactivity to negative emotional stimuli may lead to internalizing symptoms, boys’ under-reactivity to negative emotional stimuli may contribute to their greater risk for externalizing symptoms, given that blunted amygdala responses to negative stimuli have been shown to predict externalizing behaviors (Jones et al. 2009). Consistent with this, the Gard et al. (2017) study found that lower amygdala responses predicted higher antisocial behavior in their sample of male young adults.

3 **Empirical Studies: Reward Tasks**

A number of studies have examined associations between parenting and youth’s functional brain response to reward receipt or the anticipation of reward. While the majority of this research has used fMRI paradigms using monetary reward feedback, two studies used peer acceptance or feedback, which were included here as a measure of social reward. This research has broadly identified parenting predicting responses to reward in networks involved in reward processing (striatum, medial PFC (mPFC)), emotional arousal (insula, ventral ACC (vACC includes sgACC and pgACC), amygdala), and prefrontal regions involved in regulation of reward responses and decision making (dLPFC).

3.1 **Positive Parenting**

Several studies have found associations between lower positive parenting and heightened response in brain regions associated with reward processing to reward. Holtz et al. (2018), in a sample of youth who were currently healthy, assessed
observed maternal stimulation (trying to gain the infant’s attention or make contact with their infant), which is one aspect of positive parenting, during a parent–infant interaction task at age 3 months. They found that lower positive parenting (stimulation) predicted higher response in reward processing regions (caudate head), as well as the supplementary motor area, cingulate cortex (ACC and posterior cingulate cortex (PCC)), and middle frontal gyrus during reward anticipation when youth were 25. Consistent with this, using a longitudinal design, Qu et al. (2015), in a community sample, assessed the relationship between youth report of positive parenting (parental support and youth’s disclosure to parents) and youth’s brain response to reward during a risk-taking task at Time 1 (age 15–17) and again 1 year later at Time 2 (age 16–18). The authors found that increases in positive parenting from Time 1 to Time 2 were associated with decreases in R ventral striatum from Time 1 to Time 2 during receipt of reward. Further, these neural decreases mediated the relationship between increases in positive parenting and decreases in adolescent task-based risk taking. Qu et al. (2015) also found that increases in positive parenting from Time 1 to Time 2 were related to decreases in the R dlPFC response from Time 1 to Time 2 to reward receipt. There is some evidence that the dlPFC is involved in modulating striatal activation during reward processing (Staudinger et al. 2011), so this finding is perhaps consistent with the lack of striatal response. Taken together, these studies found that lower positive parenting is related to higher activation in reward processing regions during monetary reward receipt and anticipation.

Three other studies also found that lower positive parenting predicted higher neural response to reward. Chaplin et al. (2021), in a community sample, measured maternal involvement (one aspect of positive parenting) during a parent-adolescent discussion task with 12–14 year olds and found lower positive parenting correlated with higher activation in the bilateral Nucleus Accumbens (NAcc), a striatal region involved in reward processing, to monetary reward receipt. Follow-up analyses indicated that this finding was significant for boys, but not for girls. Further, for boys, this higher NAcc activation to reward receipt predicted increased substance use 1 year later. Similar to this, Morgan et al. (2014), in a low-income sample of all boys, examined maternal positive parenting (observed warmth during a parent–child interaction task) during early childhood and adolescence predicting MRI responses to reward at age 20. They found that less positive parenting (maternal warmth) during childhood and adolescence predicted greater response in the mPFC during anticipation and reward receipt, respectively, in this sample of boys. Taken together, these studies may suggest that associations between a lack of positive parenting and higher neural response to reward are stronger for boys than girls. However, counter to this, Casement et al. (2014), in a sample of all girls oversampled for girls with depression, found that lower positive parenting (mother report of maternal warmth) at age 11–12 predicted higher response in reward processing and emotional arousal regions, including the ventral striatum, dorsal and rostral mPFC, and amygdala during monetary reward anticipation when girls were 16 years old. They also found that greater mPFC and ventral striatum activation mediated the relationship between low positive parenting and youth depressive symptoms. Further research should examine moderation by sex to determine if there are reliable sex differences
in effects of positive parenting on youth’s development of reward processing neurobiology.

Two studies found that associations between low positive parenting and youth brain response to reward were moderated by parental psychopathology. First, Morgan et al. (2014) found that, in a sample of low-income boys, for those who were exposed to maternal depression, while low positive parenting (maternal warmth) during childhood (age 18 and 24 months) predicted higher activation in mPFC during reward anticipation and striatum during reward anticipation and receipt at age 20, low positive parenting during adolescence (age 10 and 11) actually predicted lower activation in caudate to reward anticipation. A second study, Hotz et al. (2018) (reviewed above), in a sample of youth with maternal psychiatric diagnoses before age 11 who are currently healthy, also found that, among youth exposed to parental psychiatric diagnosis, lower maternal positive parenting at age 3 months was associated with both lower and higher activation to reward at age 25. They found that, in youth with a parental psychiatric diagnosis prior to youth age 11, low positive parenting was associated with lower activation in a reward processing region (caudate head), as well as in the supplementary motor area, cingulum, and middle frontal gyrus during reward anticipation and greater reward response during reward anticipation and during reward receipt. Further, they found that greater reward response during receipt mediated the relationship between low positive parenting and greater youth ADHD severity. Taken together, this suggests that the findings above on lower positive parenting predicting higher neural responses to reward may be reversed for some youth who have been exposed to parental psychopathology. It may be that, for some youth, low positive parenting leads to heightened reward system response, which may lead to risk for risk taking and externalizing behaviors, but for those at risk for depression, a lack of positive parenting may lead to a blunted reward system response, which may then lead to risk for depression (Forbes et al. 2006).

### 3.2 Negative Parenting

Two studies have found associations between higher negative parenting and lower neural response to reward in emotional arousal and reward processing regions. Morgan et al. (2017), in a sample of low-income boys, found a significant interaction between maternal negative parenting (observed disengagement) when youth were 10–12 and maternal rumination in predicting youth response to monetary reward, such that a combination of negative parenting and rumination predicted lower activation in a region involved in emotional arousal (vACC) to reward receipt in boys aged 20. This might suggest that youth experiencing negative parenting and maternal rumination are responding less to positive events (i.e., winning a reward). Tan et al. (2014), in a sample of 11–17 year olds who were oversampled for depression, found that higher observed maternal negative affect during a parent–youth interaction (which is one form of negative parenting) was associated with...
lower response in regions related to reward processing and emotional arousal (L NAcc, sgACC, amygdala, L anterior insula) during a social reward (peer acceptance). Considering that negative parental affect provides youth negative social feedback, this pattern might prevent youth from recognizing and responding to social reward. Notably, lower neural response to reward has been associated with risk for depression (Forbes et al. 2006); therefore, youth who experience high negative parenting, possibly in combination with parental modeling of maladaptive ruminative coping strategies (or in combination with their own higher depression – Tan et al. 2014) may be at risk for lower neural responsivity and subsequent increases in depression.

Finally, it is notable that we found one study that failed to find a significant association between negative parenting and neural response to reward. Miller et al. (2020), in a community sample of 8–12 year olds, did not find an association between youth-reported parent-youth poor attachment quality (which measured attachment anxiety and avoidance), and youth brain response to positive social feedback. The Miller et al. study was different from others in that it measured attachment, which does not solely reflect negative parenting but also characteristics of the child.

### 3.3 Functional Connectivity

One study examined parenting and functional connectivity during reward processing. Kopala-Sibley et al. (2020), in a community sample oversampled for high negative emotion and behavioral inhibition, assessed for observed maternal negative parenting (maternal hostility) and mother-reported positive parenting (appropriate rules and restrictions) during childhood (age 3) and youth’s functional connectivity during monetary reward processing at age 10–11. They found that higher negative parenting predicted more negative connectivity between reward regions (L ventral striatum) and the R posterior orbital frontal cortex and R inferior frontal gyrus, while greater positive parenting predicted more positive connectivity between the ACC and the bilateral insula/operculum and the R anterior striatum/pallidum. Negative parenting may lead to a lower capacity of prefrontal regions to effectively modulate striatal response to reward.

### 3.4 Summary and Implications

In summary, research shows that low positive parenting is generally associated with youth’s higher responses in reward processing regions to monetary reward anticipation and receipt among early to late adolescents (Casement et al. 2014; Chaplin et al. 2021; Holtz et al. 2018; Morgan et al. 2014; Qu et al. 2015), although it can predict blunted responses to reward in some at-risk youth with parental psychopathology
This suggests that, generally, a lack of warmth and engagement from parents may lead to an over-sensitive reward system, perhaps especially for monetary reward. For negative parenting, two studies found that higher negative parenting was associated with lower responses in reward processing and emotional arousal regions to monetary and social reward (Morgan et al. 2017; Tan et al. 2014). Thus, a lack of positive parenting may lead to higher sensitivity to monetary reward for most youth, whereas harsh negative parenting may lead to blunted responses to monetary or social reward. So, interestingly, these two forms of parenting may lead to different outcomes.

**Clinical Implications** Parenting effects on reward system function may have important implications for youth’s risk for psychopathology. Three studies found that lower positive parenting predicted higher response to reward in reward processing regions and that, in turn, this higher responsivity predicted greater substance use (for boys- Chaplin et al. 2021), greater risk-taking behavior (Qu et al. 2015), and greater ADHD severity among boys with a history of parental psychiatric diagnosis (Holtz et al. 2018). This is consistent with other research finding that heightened response to reward in adolescence is associated with risk for externalizing symptoms and risk behaviors (Bjork et al. 2010). In terms of internalizing disorders, one study found that a combination of negative parenting and maternal rumination (which is associated with depressive symptoms) predicted blunted response to monetary reward (Morgan et al. 2017) and two studies found that lower positive parenting predicted blunted responses to monetary reward for some youth who had a parent with depression or other psychiatric diagnosis (Hotz et al. 2018; Morgan et al. 2014). This may indicate that, for youth at risk for depression, maladaptive parenting may lead to more blunted neural response to reward, which then may lead youth to develop depressive symptoms themselves, given that blunted response to reward has been shown to predict depression in youth (Forbes et al. 2006). One study (Casement et al. 2014) of all girls, oversampled for girls with depression, was counter to this, finding that lower positive parenting predicted higher mPFC and ventral striatum responses to reward, and this higher reward response then led to greater depressive symptoms. This counter finding may be due to the inclusion of girls (who are at higher risk for depression) or the oversampling of youth with depression. Future research should examine interactions between neural response to reward and sex predicting depression and other psychopathology outcomes.

### 4 Empirical Studies: Parenting-Specific Tasks

A few initial studies have begun to examine associations between parenting behavior and child neural responses to parent-related stimuli, including personalized stimuli of their own parents expressing negative and positive emotion. These studies find associations between parenting and child and adolescent neural responses and
connectivity while processing parent stimuli in emotional arousal (e.g., amygdala, insula, sgACC), social cognitive (e.g., Temporal Parietal Junction (TPJ)/Inferior Parietal Lobule (IPL), mPFC, PCC, precuneus), emotion regulation (e.g., vlPFC, and reward processing (e.g., putamen, lentiform nucleus, mPFC) networks (Blakemore 2008).

4.1 Positive Parenting

Two studies examined associations between positive parenting and child neural responses to audio clips of personalized parent criticism statements toward the child. First, a longitudinal study of adolescents with an anxiety disorder examined adolescent-reported positive parenting (warmth) at age 9–14 predicting adolescent neural responses at age 11–16 to audio clips of their parents (97% mothers) making critical or neutral statements about them (Butterfield et al. 2020). Lower positive parenting predicted higher adolescent neural response in regions involved in emotional arousal and emotion regulation (L amygdala, bilateral insula, sgACC, ACC, vlPFC) to parent critical versus neutral statements (Butterfield et al. 2020). Further, lower positive parenting predicted higher anxiety and depressive symptoms 2 years later through higher sgACC response to parent criticism, suggesting the important effect of low positive parenting on internalizing symptoms through increased emotional arousal region response. Second, in a non-clinical sample of 9–17-year-olds, lower youth-reported positive parenting (warmth) was associated with higher social cognitive network response (TPJ/IPL, PCC, precuneus) to mother critical versus neutral statements (Lee et al. 2015). Taken together, these studies indicate that lower positive parenting predicts heightened activation of emotional arousal and regulation related regions and of social processing regions to hearing parent critical statements. Possibly youth who have experienced low positive parenting have a greater negative emotional response and also require greater regulation of that response. They also overly engage social processing of negative comments, which may be maladaptive. Notably, however, while the two studies above found an association between positive parenting and youth’s neural responses to parental criticism, one study of 11–17 year olds failed to find a relationship between youth-reported positive parenting (maternal acceptance) and youth neural responses to parental praise (Sequeira et al. 2019). Thus, the association between parenting and parental statements may be stronger for criticism than praise.

One study examined associations between positive parenting and a parent versus self trait word sorting task (Van der Cruijsen et al. 2019). In this study, 11–21-year-olds completed a trait word sorting task in the scanner, where they sorted if positive and negative trait words described themselves or their mother, and researchers examined response to positive and negative mother trait words versus self trait words. Lower observed maternal positive parenting (maternal emotional support) was associated with lower youth putamen (a reward processing region) response to mother trait words. Further, lower youth-reported positive feelings about their
mother were related to lower vPFC activation to mother trait words, which may indicate lower engagement of emotion regulation regions.

4.2 Negative Parenting

One study examined negative parenting predicting youth response to own and other parent video clips from a parent–adolescent interaction task and did not find significant associations (Whittle et al. 2012). In a sample of adolescents with a range of depressive symptoms, observed maternal negative parenting (aggressive behavior) in a parent–adolescent interaction task in early adolescence (mean age 12.85) did not significantly predict adolescent neural response to their own and another mothers’ negative or positive emotional video clips (versus neutral emotional video clips) 4.5 years later. This null finding may be because the authors examined negative parenting (and the findings above were for positive parenting) or because they used naturalistic videos rather than more standardized maternal criticism audio clips or maternal trait word sorting.

4.3 Functional Connectivity

It may also be important to consider associations between parenting and functional connectivity of neural networks during processing of parent stimuli. One study examined this in an ethnically diverse sample with a wide range of child ages (4–17 years) (Gee et al. 2014). This study found that higher youth-reported positive parenting (attachment security) was related to negative connectivity between amygdala (an emotional arousal region) and mPFC (which is involved in reward, decision making, and social cognition) during the processing of own mother posed positive and neutral emotional pictures compared to a fixation cross. The authors describe that negative connectivity between the amygdala and the mPFC is characteristic of mature connectivity seen in adolescence and adulthood (compared to positive connectivity in childhood) and may be adaptive particularly for the older adolescents in this sample.

4.4 Summary and Implications

Overall, lower positive parenting is related to higher child and adolescent neural responses in networks involved in emotional arousal, emotion regulation, and social cognition, but lower neural responses in reward processing regions to negative parental stimuli. For one study that included responses to both positive and negative maternal stimuli, lower positive parenting was related to lower reward and emotion
regulation region responses. Finally, one study found that higher positive parenting was associated with greater amygdala to mPFC connectivity while processing mother positive and neutral pictures. Taken together, a lack of positive parenting may have an effect on youth’s emotion, social cognition, and reward-related networks responses to parent-specific stimuli. Also, parents who use less positive parenting may have higher emotional reactivity themselves, which could be passed down genetically or modeled for the child, resulting in higher child emotional arousal. Lastly, lower positive parenting may lead to a lowered reward network response to one’s parent, which may indicate that the parent is not a rewarding stimulus, and the dyad may not have a positive relationship.

**Clinical Implications** Higher child social-cognitive and self-referential responses to parental stimuli, especially to parent criticism, may lead children to identify with the negative remarks of their parents and “check out” from trying to perspective take with the parent in order to protect themselves, which may lead to negative social-emotional outcomes. Having higher emotional arousal and social cognitive and lower reward region reactivity to parent stimuli may contribute to higher levels of psychopathology such as depression and anxiety (Forbes et al. 2010; Hall et al. 2014), which was found in the Whittle and colleagues’ study above.

5 Empirical Studies: Cognitive Control Tasks

Only a small number of studies have looked at the relationship between parenting and youth’s functional neural response during tasks that involve cognitive control. These studies find associations with regions involved in cognitive control, including the superior frontal gyrus and corpus callosum and in a region involved in detecting salient information and triggering cognitive control (the insula) (Hinkley et al. 2012; Niendam et al. 2012).

5.1 Positive Parenting

Marusak et al. (2018), in a low-income urban sample of 9–16 year olds, examined correlations between youth-reported positive parenting (maternal and paternal care) and youths’ MRI responses to emotional conflict (identifying an emotional [fear or happy] face while the opposite emotion word is on the screen) versus emotional non-conflict (identifying an emotional face while the same emotion word is on the screen). The authors found that lower positive parenting was associated with lower activation in the superior frontal gyrus (SFG), a region involved in inhibition and other cognitive functions (Niendam et al. 2012).

Two studies used risk-taking paradigms in measuring parenting and youth brain response. Lauharatanahirun et al. (2018), in a low-income sample of 14- and
15-year-old adolescents, found that during the decision phase of risk taking, lower youth-reported positive parenting (parental monitoring) was associated with lower adolescent emotional arousal network response (insula) only among households with low chaos. Of note, this fMRI analysis did not involve a contrast. The insula’s role in processing salient information and triggering cognitive control suggests that this activation of this network might be more adaptive for youth during risk taking. Qu et al. (2015), in a community sample, longitudinally studied changes in youth-reported parent-child relationship quality, including positive parenting (parental support), and adolescent functional response during the decision period of a risk-taking task from ages 15–17 to ages 16–18. They found that longitudinal increases in the positive measures of parent-child relationships predicted longitudinal increases in activation from age 15–17 to age 16–18 in the corpus callosum and occipital lobe during the decision period. The corpus callosum is critical in communication between the brain’s two hemispheres, and its structure has previously been implicated in cognition (Hinkley et al. 2012).

Finally, one study (Kim-Spoon et al. 2017) found positive parenting to be associated with neural response during cognitive control. They, in a community sample of 13–14 year olds, measured cognitive control based on neural response during a multi-source interference task (determining which of three numbers differed from the other two when target number was placed in both congruent and incongruent locations) in several regions related to cognition, emotion regulation, sensorimotor function, and emotional arousal (L posterior-medial frontal cortex, bilateral inferior frontal gyrus, bilateral IPL, R insula, R SFG, and L middle frontal gyrus). They found that lower adolescent-reported positive parenting (monitoring) at age 13–14 was related to higher activation in these regions during interference trials for youth in households with low household chaos (i.e., home disorganization and confusion). This finding of lower positive parenting associated with higher activation in these regions is inconsistent with the three other studies finding that positive parenting was associated with lower activation in these regions. This might reflect differences in the task, as perhaps lower activation is maladaptive when judging emotional conflict (e.g., Marusak et al.) or risk taking (e.g., Lauharatanahirun et al.), whereas higher activation is maladaptive in interference tasks (e.g., Kim-Spoon et al.) Marusak et al. and Lauharatanahirun et al. also used low-income community samples, whereas Kim-Spoon et al. did not, suggesting that perhaps higher activation in these regions is particularly adaptive in low-income samples.

5.2 Negative Parenting

Marusak et al. (2018), mentioned above, was the only study to report associations between negative parenting and youth’s responses in cognitive control tasks. They found that higher youth-reported maternal and paternal negative parenting (psychological control) was associated with lower response in L insula during emotional conflict versus emotional non-conflict in 9–16 year olds, which may suggest lack of
engagement of a region involved in recognizing salient stimuli and signaling to initiate cognitive control (Menon and Uddin 2010).

5.3 Summary and Implications

While more research is needed on the relationships between parenting and neural response during cognitive control, most of the existing studies suggest that lower positive parenting (and, in one study, higher negative parenting) is associated with (and longitudinally predicts) decreased activation in emotional arousal and cognitive control regions including insula, corpus callosum, and occipital lobe during emotion related conflict (Marusak et al. 2018) and risk taking (Lauharatanahirun et al. 2018; Qu et al. 2015). However, counter to this, one study found that lower positive parenting was associated with increased activation in emotion arousal and cognitive control regions during a cognitive type of interference task (Kim-Spoon et al. 2017).

Clinical Implications Overall, decreased engagement of emotional arousal and cognitive control regions during cognitive control and decision-making tasks may be maladaptive, leading youth to lack ability to attend to salient information and initiate cognitive control mechanisms. This may lead them to poor impulse control and increased risk-taking behaviors in adolescence. Importantly, two studies’ findings only held true in low chaos households, suggesting that higher risk family contexts might involve distinct effects of parenting on youth’s brain response to cognitive control tasks.

6 Summary

Overall, the studies reviewed above suggest that the parenting environment is a key social environment that is associated with (and, in some studies, longitudinally predicts) children and adolescents’ neural response (and functional connectivity) in networks involved in emotional arousal, ER, reward processing (including of monetary and social reward), cognitive control, and social-emotional information processing (as shown in Fig. 1). High negative parenting and low positive parenting may act as a social environmental stressor and were associated with higher reactivity in emotion arousal networks to negative emotional stimuli, although this was moderated in some studies by child sex, age, and psychopathology risk. Lower positive parenting was also associated with heightened response in reward processing networks to monetary reward stimuli, again with some studies finding that this was moderated by psychopathology risk or child sex. A few initial studies found that lower positive parenting (and, for one study, higher negative parenting) generally predicted lower engagement of networks that support cognitive control during cognitive control tasks. And, finally, a few studies found that lower positive
Fig. 1 Summary of findings on parenting and youth neural responses. "Psych Risk indicates presence of risk for psychological symptoms, including parenting psychopathology or temperament. Emo Emotion, Amy Amygdala, sgACC subgenual Anterior Cingulate Cortex, pACC perigenual Anterior Cingulate Cortex, Reg Regulation, vlPFC ventrolateral Prefrontal Cortex, esp especially, NAc Nucleus Accumbens, mPFC medial Prefrontal Cortex, dlPFC dorsolateral Prefrontal Cortex, hi high, TPJ Temporoparietal Junction, IPL Inferior Parietal Lobule, PCC Posterior Cingulate Cortex, SFG Superior Frontal Gyrus"
parenting was associated with lower reward system response and higher emotional arousal and social cognition network response when processing parent-specific social-emotional stimuli.

Interestingly, both negative parenting and positive parenting predicted neural activation in response to negative emotional stimuli, suggesting that both high negative parenting and a lack of protective positive parenting act as a social stressor and affect the development of stress and negative emotion processing circuitry. In contrast, most of the findings for parenting impacting response to reward, parent-specific stimuli, and cognitive control tasks were for positive parenting. Positive parenting may more specifically impact the development of response to more positive and rewarding stimuli, to positive feelings toward parents, the development of social reward learning, and toward adaptive social-emotional and cognitive development. Thus, interventions should take care to not just reduce negative parenting, but also work to increase positive parenting in order to promote adaptive development in these important domains.

These altered neural activation patterns may be key mechanisms by which parenting contributes to child and adolescent social-emotional development and development of psychopathology. Negative parenting was associated with heightened neural responses to negative emotional stimuli, possibly particularly for girls. These responses may lead to high negative emotional arousal and rumination on negative emotion, which may interact with environmental stressors to lead to the development of depression or anxiety symptoms, possibly particularly for girls. Consistent with this, Chaplin et al. (2019) found that higher ACC response to negative emotional stimuli predicted depressive symptoms for girls. Also, lower positive parenting was linked to heightened neural response to monetary reward and this high reward system sensitivity may lead to risk for substance use or other risk behaviors (Cope et al. 2019). Notably, for some youth at risk for psychopathology (due to parental psychopathology), lower positive parenting predicted blunted striatal response to reward. Blunted striatal response to reward may be a mechanism by which parent depression leads to risk for depression in youth. Lower positive parenting was also linked to lower engagement of networks that support cognitive control, which may lead youth to risk for impulsivity and possibly for disorders involving poor impulse control. Finally, higher positive parenting predicted children’s higher reward system response and lower emotional arousal system response to their own parent, which may lead to closer parent-adolescent relationships, which may provide a key social environment that may protect youth from development of psychopathology. Indeed, one study found that higher sgACC response to own parent critical statements predicted higher internalizing symptoms in youth (Butterfield et al. 2020). More studies are needed to continue to examine psychopathology outcomes.
7 Strengths, Limitations, and Future Agenda

One limit of the studies reviewed above is that they mostly examined parenting behavior in mothers. Given that both mothers and fathers (and/or other caregivers) contribute to children’s social-emotional development, future research should assess effects of fathers’ parenting and should explore parent sex differences. For example, given theory suggesting that mothers attend more to emotional aspects of parenting and fathers more to caregiving through play (Cabrera et al. 2018), mothers’ parenting may be more strongly related to child neural responses to negative emotional stimuli and fathers’ parenting may be more linked to reward or positive emotion processing.

In addition, many of the studies above (but not all) either focused on middle-income predominantly White families or studied low-income youth but did not fully examine contextual processes related to income and race that may affect connections between parenting and child brain. For example, stern/strict parenting behavior (that may appear as “negative” parenting) may be maladaptive in some contexts but adaptive in others, such as when it may be useful to prepare youth to face racism, economic disadvantage, and challenging neighborhood characteristics (e.g., Nelson et al. 2013). One of the studies above (Gard et al. 2017), in a low-income urban sample, found that harsh parenting and also neighborhood deprivation predicted blunted amygdala response to negative emotional stimuli. Thus, parenting may affect brain development in conjunction with, or possibly in interaction with, contextual factors, and these will be important to examine in future research.

There are several methodological advances in the studies reviewed above and also potential future directions methodologically. First, some of the studies prospectively examined parenting behavior in early childhood predicting outcomes in adolescence or adulthood (e.g., Gard et al. 2017). This design can start to untangle directionality of effects between parenting and youth brain function. However, to better understand direction of effects, future studies should conduct MRI at repeated longitudinal time-points and examine whether parenting behavior predicts changes in MRI responses over time. Only one study reviewed above conducted repeated MRI (Qu et al. 2015). Furthermore, to test whether parenting behavior has a causal role in child neurobiology, experimental studies are needed. Such work could randomly assign parents with high negative parenting to receive (or not receive) a parenting intervention and examine effects of intervention-related changes in parenting on changes in youth brain function.

Second, the studies showed innovation in measurement of parenting. Several examined observed parenting behaviors in laboratory parent–child interactions that modeled real-life parenting situations, such as discussing a conflict topic, which is innovative and adds to the literature on self-reported parenting behavior. One future direction would be to utilize ecological momentary assessment (EMA) to examine daily parenting behaviors and youth reactivity. This information could be combined with observed behavior in the laboratory to fully characterize the parenting environment in and out of the lab.
Third, the studies showed innovation in the fMRI tasks. Many of the studies used standardized, well-validated tasks, which is useful to show effects of parenting on core emotional and cognitive processes. However, some studies used personalized recordings of adolescents’ own parents’ voices or behaviors (e.g., Butterfield et al. 2020). This novel work gets closer to tapping youth’s neural responses to actual parent–youth interactions. It would be interesting to see if youth’s neural responses to their parents’ negative emotions in the MRI correlate with youth’s peripheral physiological responses in laboratory-based parent–youth interactions, and future research could explore this.

One additional future direction is to probe the neuroscience of parenting by conducting MRI scans of both parent and child. A handful of studies have conducted parent-child scanning and have found, for example, that similarity in neural responses between parent and child was associated with higher family connectedness (Lee et al. 2018). More work could be done in this area.

In sum, emerging findings suggest that the quality of the parenting social environment that children and adolescents experience is associated with, and likely shapes, their neural activation in emotional arousal, ER, reward processing, social-emotional, and cognitive control networks. These findings help us better understand the roots of typical child social-emotional development and can inform our knowledge of how parenting affects the development of psychopathology. These findings also point to potential targets for interventions aimed at improving parenting, normalizing child brain development, and improving social-emotional outcomes.

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Conflicts of Interest: The authors have no conflicts of interest to declare.

References


The Stressed Brain: Neural Underpinnings of Social Stress Processing in Humans


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Abstract As humans, we face a variety of social stressors on a regular basis. Given the established role of social stress in influencing physical and psychological functioning, researchers have focused immense efforts on understanding the psychological and physiological changes induced by exposure to acute social stressors. With the advancement of functional magnetic resonance imaging (fMRI), more recent work has sought to identify the neural correlates of processing acute social stress. In this review, we provide an overview of research on the neural underpinnings of social stress processing to date. Specifically, we summarize research that has examined the neural underpinnings of three types of social stressors commonly studied in the literature: social rejection, social evaluation, and racism-related stress. Within our discussion of each type of social stressor, we describe the methods used to induce stress, the brain regions commonly activated among studies investigating that type of stress, and recommendations for future work. This review of the current literature identifies activity in midline regions in both prefrontal and parietal cortices, as well as lateral prefrontal regions, as being associated with processing social rejection. Activity in the insula, thalamus, and inferior frontal gyrus is often found in studies using social evaluation tasks. Finally, racism-related stress is associated with activity in the ventrolateral prefrontal cortex and rostral anterior cingulate.
cortex. We conclude by taking a “30,000-foot view” of this area of research to provide suggestions for the future of research on the neuroscience of social stress.

**Keywords** fMRI · Neuroimaging · Racism-related stress · Social rejection · Social stress

Think about the last time you had a really stressful day. What was the source of the stress? Perhaps you had an argument with a family member, had to give a presentation at work or school in front of an intimidating audience, got rejected by a date you really liked, or maybe someone treated you unfairly due to factors beyond your control, like your race, gender, or sexuality. For many humans, facing these social stressors is associated with both short- and longer-term changes in affect and physiology (Dickerson and Kemeny 2004; Sin et al. 2015). Over time, these psychological and physiological reactions to stressors can contribute to physical health and emotional well-being, including the development of chronic disease and psychopathology (Gianaros and Jennings 2018; Slavich 2020). Given the importance of social stressors in contributing to physical and psychological functioning, researchers in psychology and neuroscience across a variety of subdisciplines (e.g., social psychology, clinical psychology, psychoneuroimmunology, affective science, psychoneuroendocrinology) have focused their efforts on understanding the psychological and physiological changes induced by exposure to acute social stressors, both in the laboratory and “in the wild.” More recently, research in this area has begun to focus on how the brain responds to social stressors, which will be the focus of the present chapter.

Advancements in neuroimaging technology and the widespread use of functional magnetic resonance imaging (fMRI) over the past two decades have provided stress researchers with the exciting opportunity to understand the neural correlates of social stress processing in vivo in humans. Using fMRI to study the neural underpinnings of social stress provides a unique opportunity to answer a number of questions of interest in stress research. These include shedding light on how the brain initially orients to and ultimately regulates responses to social stressors, providing insight into the neurocognitive “ingredients” that comprise the experience of social stress. Further, fMRI research on stress facilitates our understanding of the neural signals that predict the extent to which individuals show physiological changes in response to a social stressor (Ginty et al. 2017; Muscatell and Eisenberger 2012; Thayer et al. 2012). Of course, utilizing brain imaging to understand the neural underpinnings of social stress in humans also presents some unique challenges. These include difficulties in inducing meaningful experiences of social stress within the confines of the MRI scanner environment, computational challenges related to adequately correcting for the multiple comparison issues inherent in fMRI research and the multivariate nature of neuroimaging data, and issues related to data interpretation, including making causal claims from correlational studies and utilizing reverse inference to infer psychological processes from neural data. Despite these challenges, dozens of studies investigating the neural underpinnings of social stress have been conducted in recent years, leading to exciting insights about the nature of social stress.
Given the ever-growing literature on the neural underpinnings of social stress, the present review is intended to provide an overarching summary of research in this area to date, while also looking to the future and offering suggestions for next steps in this area of research. Specifically, we provide an overview of research that has examined the neural underpinnings of three types of social stressors commonly investigated in the literature: social rejection, social evaluation, and racism-related stress. Within our discussion of each type of social stressor, we provide details regarding the methods used to induce stress (see also Noack et al. 2019), the brain regions commonly activated among studies investigating that type of stress, and recommendations for future work (see Table 1 for an overarching summary of tasks used and commonly activated brain regions). We conclude by taking a “30,000-foot view” of this area of research to provide suggestions for next steps. This review is not intended to be comprehensive nor summative (see Dedovic et al. 2009a; Muscatell and Eisenberger 2012), but rather to provide an overview of the literature on the neural correlates of social stress to orient readers to the methods and results of past work, and new opportunities for moving the field forward.

1 Neural Correlates of Social Rejection

Overview Social rejection (sometimes called ostracism and/or social exclusion) is one of the most commonly studied social stressors in fMRI research. This is not by accident – indeed, social rejection experiences are commonly experienced in everyday life (Murphy et al. 2015) and are also among the most potent predictors of the development of psychopathology (Slavich 2020; Slavich et al. 2009), making them important to study empirically. Further, the relative ease with which social rejection experiences can be created despite the constraints of the MRI scanner makes this a popular social stressor to study. More details about specific methods for inducing social rejection in fMRI research are outlined below.

Methodological Approaches Cyberball. The most popular task used to study the neural correlates of social rejection is Cyberball (Eisenberger et al. 2003; Williams et al. 2000). In this task, participants are told they are playing a virtual game of catch with two other players, in which they will press a button to “throw” the (virtual) ball to each of the other players. Studies vary in the extent to which participants are actually introduced to two other players in-person; in these cases, the “other players” are typically members of the research team posing as study participants. In other studies, participants are just shown pictures or avatars representing the other players and are told they will be playing over the Internet. In the first round of Cyberball (the “inclusion” condition), players pass the (virtual) ball around equally, with each player, including the participant, receiving a roughly equal number of passes. In the next round of the game, the participant initially receives a few passes from the other players, but is subsequently completely excluded from the game (the “exclusion” condition) as the other players pass the ball back-and-forth to one another but
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<td>Participants complete difficult time-limited mental arithmetic while being evaluated on their performance. Feedback on performance is provided via computer screen</td>
<td>Social and time pressure to perform mental arithmetic; negative emotions associated with negative performance feedback</td>
<td>Insula, thalamus, claustrum, inferior frontal gyrus, precentral gyrus, inferior/middle temporal gyrus</td>
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<td><strong>Social evaluation</strong></td>
<td>ScanSTRESS (Streit et al. 2014)</td>
<td>Participants completed difficult visuo-spatial and mental arithmetic tasks while being evaluated on their performance. Feedback on performance is provided by a panel of evaluators, which participants can view and hear via live video streaming</td>
<td>Social and time pressure to perform mental rotation and arithmetic; negative emotions associated with negative performance feedback</td>
<td>Insula, thalamus, inferior frontal gyrus, precentral gyrus, inferior/middle temporal gyrus</td>
<td>Berretz et al. (2021)</td>
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<td><strong>Social rejection</strong></td>
<td>Cyberball (Williams et al. 2000)</td>
<td>Participants play a computerized ball-tossing game with two confederates. After initially being included in the game, the confederates only pass the ball to each other, excluding the actual participant from the game</td>
<td>Negative emotions and cognitive processes associated with being socially excluded/rejected</td>
<td>Anterior cingulate cortex, posterior cingulate cortex, medial/lateral prefrontal cortex, ventrolateral prefrontal cortex, insula, thalamus, claustrum, inferior frontal gyrus</td>
<td>Berretz et al. (2021), Vijayakumar et al. (2017), Wang et al. (2017)</td>
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(continued)
never to the participant. This experience of being excluded from the game leads to negative emotions and self-reported feelings of being socially rejected (K. D. Williams et al. 2000; Zöller et al. 2010), as well as changes in neural activity, which are further detailed below.

**Other Social Rejection Tasks.** While Cyberball has certainly been the dominant task used to study the neural correlates of social rejection to date, a number of other MRI-compatible social rejection tasks exist and have also been utilized in research in this area. For example, an early task that attempted to disentangle the neural correlates of expectancy violation from social feedback (which are confounded in the Cyberball task) had college students submit a photograph of themselves prior to scanning, which they were told would be rated by individuals at other universities (Somerville et al. 2006). During the scan, participants received “feedback” about how others rated them, such that on each trial, they were told that either the rater thought they would like the participant (positive feedback) or that the rater thought they would not like the participant (negative, rejecting feedback). A similar task (sometimes called the “Chatroom task”) wherein participants receive negative, rejecting feedback from peers based on their photograph has been primarily used in studies of the neural correlates of social rejection in adolescents (Guyer et al.

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<td>Racism-related stress</td>
<td>Adaptation of Cyberball (Masten et al. 2011)</td>
<td>Racial/ethnic minoritized participants play a computerized ball-tossing game with two white confederates. After initially being included in the game, the white confederates only pass the ball between each other, excluding the actual participant and leading some participants to believe their exclusion is due to race</td>
<td>Negative emotions associated with socially excluded/rejected based on one’s race (i.e., racial discrimination)</td>
<td>Ventrolateral prefrontal cortex, rostral anterior cingulate cortex</td>
<td>Masten et al. (2011)</td>
</tr>
</tbody>
</table>
Another, arguably more ecologically valid, social rejection task asked participants to view photographs of a person who had recently “dumped” them (i.e., an ex-romantic partner) and recall their feelings of rejection upon being dumped, and contrasted neural activity to these images/memories to viewing images of a friend while recalling a recent positive experience with that friend (Kross et al. 2011). Thus, while many studies of the neural correlates of social rejection utilize Cyberball to create a rejecting experience in the scanner, a number of other social rejection tasks also exist and have been used in the literature, albeit less frequently.

Summary of Findings What neural regions are commonly activated in response to social rejection tasks, as outlined above? Given widespread interest in this question in recent years, a number of quantitative and narrative reviews have already been published on this question. For example, a scoping review (Wang et al. 2017) identified 28 fMRI studies published prior to 2016 that had investigated the neural correlates of social rejection induced by the Cyberball task. Results of the scoping review showed that the anterior cingulate cortex (ACC), including ventral, dorsal, and subgenual sections, was significantly activated in 24 of the 28 published studies, with six studies also reporting significant activity in the posterior cingulate cortex (PCC). Other regions commonly activated in response to social exclusion during Cyberball identified in the scoping review included medial and lateral areas of the prefrontal cortex (PFC; observed in 18/28 studies) and the insula (observed in 17 of 28 studies), including both anterior and posterior subsections. Finally, in addition to examining activation of individual brain regions, a few studies in this area of work also examined functional connectivity, or assessments of interactions between regions. Among those studies, five of seven that examined connectivity also observed significant functional connectivity between the ACC and PFC in response to social exclusion in Cyberball; typically, this connectivity was negative, suggesting an inverse relationship between ACC and PFC during social exclusion. Taken together, this review highlights that the ACC (and, to a lesser extent, PCC), PFC, and insula are likely to engage in response to social rejection using the Cyberball task, demonstrating the critical role of regions of the salience network and default mode network in responding to social rejection.

In contrast to this qualitative approach to summarizing the Cyberball literature taken by Wang et al. (2017), a quantitative, coordinate-based meta-analysis of the same fMRI literature (Vijayakumar et al. 2017) suggested a different, largely non-overlapping, set of regions that activated in response to the Cyberball task. Specifically, the medial prefrontal cortex (MPFC; including VMPFC and medial orbitofrontal cortex and extending into perigenual and subgenual ACC), posterior cingulate cortex, and left ventrolateral prefrontal cortex (VLPFC) were observed to be reliably activated during social exclusion (vs. inclusion) in Cyberball in this meta-analysis. These results suggest that, rather than engaging neural regions thought to be involved in processing salience/pain (e.g., dACC, anterior insula), social exclusion is instead associated with greater activation in regions of the default mode network (i.e., MPFC, PCC), as well as regions of the fronto-parietal control network (i.e., VLPFC). While speculative, this pattern of results suggests that the
brain responds to social rejection with greater activity in regions that are associated with social cognitive processing and mentalizing (i.e., MPFC, PCC), as well as negative affect regulation (i.e., VLPFC).

What are we to make of these seemingly opposing findings revealed in recent reviews regarding the neural underpinnings of processing social rejection? One likely contributor to the discrepancy in findings is the intentional omission of papers that solely utilized region-of-interest (ROI) analyses from the meta-analysis (Vijayakumar et al. 2017). Indeed, many papers in this literature report results from ROI analyses focused on the ACC (and, to a lesser extent, the anterior insula), which may be why ACC appears so prominently in a scoping review of the literature but not a quantitative meta-analysis focused on whole-brain activation patterns (Vijayakumar et al. 2017). While a-priori ROI analyses have the advantage of being hypothesis-driven rather than exploratory, such analyses are also often performed at a lower statistical threshold in which BOLD signal from many adjacent voxels is averaged together. As such, ROI analyses may be biased toward confirmatory findings (Buhle et al. 2013).

Opportunities for Future Research As outlined above, the neural correlates of social rejection are quite widely studied in the literature, particularly using the Cyberball task. While informative, such studies are plagued by a number of methodological concerns, which provide opportunities for future work in this area. First, whenever an area of research is over-reliant on a single task, this creates a situation in which we do not have a comprehensive understanding of the neural correlates of a broad social experience (i.e., social rejection) but rather a more limited knowledge of the neural correlates of a specific task (i.e., Cyberball) (Poldrack and Yarkoni 2016; Yarkoni et al. 2010). As such, we currently lack breadth in our understanding of how the brain responds to social rejection, including what neural activity might be common to many different social rejection experiences, and what neural activity might be specific to the particular task conditions of any individual social rejection task. While there is utility in using the same task in multiple studies and harmonizing data collection across projects (particularly when one is interested in moderators of neural activity previously established using that task), such an approach severely limits our knowledge of the “neural reference space” for social rejection writ large. Indeed, undersampling the psychological construct of interest is likely to bias the field to converge on oversimplified understanding of how these processes are represented in the brain (Jolly and Chang 2019). As such, it will be important for future research on the neural correlates of social rejection to move beyond Cyberball and utilize other approaches, making use of other social rejection tasks that already exist (including those mentioned previously) or developing new methods for studying social rejection in the MRI scanner.

Second, it is worth noting that some prior research suggests that laboratory-based social rejection paradigms such as Cyberball do not elicit strong physiological responses. For example, some work suggests that Cyberball does not lead to changes in the neuroendocrine hormone cortisol (Zöller et al. 2010) that is widely studied in social stress research (Dickerson and Kemeny 2004). This could be in part due to the
fact that most MRI-compatible social rejection paradigms are not “motivated performance tasks” and thus do not require metabolic activation and physiological arousal to complete them (Blascovich and Tomaka 1996). As such, they are relatively passive compared to other types of social stressors studied in the literature (see Social Evaluation section below) and thus less likely to engender the physiological reactivity that is characteristic of other acute stress social paradigms that involve motivated performance. Future research efforts might thus focus on creating an MRI-compatible social rejection task that requires more metabolic and cognitive effort and is associated with physiological activation.

In sum, social rejection experiences are critical predictors of psychopathology and physiological functioning (Slavich 2020) and thus are important social stressors to study. Widespread use of the Cyberball paradigm to induce social rejection in neuroimaging research to date has led to a substantial literature on the neural correlates of Cyberball, which has reliably associated midline regions in both prefrontal and parietal cortices, as well as lateral prefrontal regions, with processing social rejection. Some work also suggests that dACC and anterior insula are commonly activated during social rejection, though such results are tenuous given overreliance on ROI analyses of these regions. Future research should explore new tasks beyond Cyberball to further our understanding of the broad neural reference space for processing social rejection.

2 Neural Correlates of Social Evaluation

Overview A second type of stressor commonly studied in research on the neural underpinnings of social stress is social evaluation. The focus on social evaluation is undoubtedly due to the ubiquity of the Trier Social Stress Test (TSST; Kirschbaum et al. 1993), which is by far the most commonly used acute social stress paradigm used in stress research in humans. Though the specific parameters of the TSST vary somewhat from study to study, the overall approach is similar: A participant is asked to complete an impromptu performance-based task (e.g., giving a speech, performing mental arithmetic aloud, or both) in front of a panel of evaluators who are trained not to provide positive feedback to the participant about their performance and instead to remain neutral and stoic (or, in some cases, provide negative nonverbals, such as eye rolling and doodling). As such, the TSST encompasses features of uncontrollability and social evaluation, two characteristics of acute laboratory stressors that are associated with the largest cortisol responses (Dickerson and Kemeny 2004).

Given that the TSST reliably elicits psychological and physiological reactions and is widely used in the literature, it makes sense that neuroimaging researchers thought to adapt features of this paradigm for use in the MRI scanner to study the neural correlates of social stress. Of course, the constraints of the MRI environment make certain features of the TSST more amendable for use than others, and researchers made additional methodological decisions that deviate from the standard
TSST approach. More details regarding the specific social evaluation tasks used in the neuroimaging literature to date are outlined below.

**Methodological Approaches** *Montreal Imaging Stress Test (MIST).* The most widely used social evaluative stressor task used in fMRI research to date is the MIST (Dedovic et al. 2005, 2009b). At the core of the MIST is a mental arithmetic task, in which participants complete mental math while undergoing MRI scanning. Critically, during stressor trials (or blocks), there is time pressure for completing the math problems, which is calibrated to each individual’s average reaction time such that participants fail to answer correctly within the given time limit 55–80% of the time. Further, to create a feeling of social evaluation, the participant’s overall task performance is displayed on the screen, as well as the supposed average performance of other participants, though in reality the average performance listed is rigged such that participants are always performing worse than others, with the intention of creating feelings of failure/inferiority. Finally, in between runs of the task the experimenter reminds the participant of their poor performance relative to others, and states that the participant must reach a minimum performance level in order for their data to be used. This feedback from the experimenter is intended to further enhance the social evaluation present in the MIST procedures. To contrast with neural responses to these stressful trials (or blocks), the MIST also includes control trials in which participants complete mental arithmetic at a similar difficulty level and rate to the stressful trials, but with no performance feedback or time pressure. Performance on control trials is much higher, with participants failing to provide a correct answer only 10% of the time (on average). Thus, contrasts comparing neural responses to the stressful vs. control trials control for neural activity involved in solving mental arithmetic problems, and what (theoretically) remains is neural activity associated with the stress of time pressure, social evaluation, negative performance feedback, and lower task performance.

*ScanSTRESS.* A second social evaluation task that has been developed for use in the MRI scanner is the ScanSTRESS paradigm (Lederbogen et al. 2011; Streit et al. 2014). ScanSTRESS is similar to the MIST in many ways, incorporating elements of time pressure, performance pressure, forced failure, social evaluative threat, uncontrollability, and unpredictability. During scanning, participants complete two challenging cognitive tasks: a mental rotation task and a mental arithmetic task. Task speed and difficulty are adapted to the participant’s performance to ensure frequent failure. Further, participants are also shown a live video transmission of a panel of evaluators seated in the scanner control room, who are monitoring the participant’s behavior and task performance and providing negative visual and auditory feedback when the participant makes a mistake. Activity during these stress blocks is contrasted to activity in control blocks, during which no time pressure is provided, performance is much higher, and no social evaluation is present.

*Other Social Evaluation Tasks.* It is worth mentioning two other social evaluation tasks that have been used in the literature and are distinct from the MIST and ScanSTRESS in that they attempt to isolate distinct components of these social stressors. First is a task that largely mimics the “speech preparation” phase of the
TSST (Wager et al. 2009a, b). In this task, participants are told they are going to have to give a speech to a panel of experts, and that they have 2 min to prepare for the speech in their heads, while they are being scanned. This creates a feeling of anticipatory stress in participants as they must quickly prepare, without the use of writing or research material, to give a challenging, evaluative speech.

The second task (Eisenberger et al. 2011; Muscatell et al. 2015) removes cognitive performance pressure entirely and isolates the social evaluative feedback component of the other stressor tasks mentioned above. In this task, prior to being scanned, participants complete an interview that is either video or audio recorded. During the scan, they are given feedback from a “fellow participant” (actually a member of the research team) about how they come across during their interview. Critically, participants receive positive, neutral, and negative evaluative feedback (in the form of different adjectives being selected from a grid) over the course of the scan. This task thus isolates the evaluative feedback component of social stress, as the cognitive effort from the interview is completed prior to scanning.

**Summary of Findings** A recent meta-analysis of research on the neural correlates of social stress processing attempted to identify BOLD signal changes that were common to many social stressor tasks (e.g., Cyberball, MIST, ScanSTRESS; Berretz et al. 2021). Analyses identified convergent activity in the bilateral insula, bilateral claustrum, thalamus, and the inferior frontal gyrus across all social stress induction paradigms. Meanwhile, there was convergent deactivation in the parahippocampal gyrus, extending into right amygdala, across all stressor tasks. When coordinates derived from papers that used Cyberball as a stressor were omitted from analyses, two additional clusters of activation were identified; one in precentral gyrus extending into the insula, and a second in the inferior/middle temporal gyrus extending into the middle occipital gyrus. Two additional clusters of reliable deactivation were also identified in the analysis that omitted Cyberball; one in precuneus/posterior cingulate cortex, and a second in superior temporal gyrus.

The reliable activation of the insula, thalamus, and inferior frontal gyrus during social evaluative stress processing suggests that regions that play a critical role in coordinating information transfer across different regions of the brain (e.g., insula, thalamus) and in regulating psychological and physiological responses (i.e., inferior frontal gyrus) are especially likely to activate in response to a stressor. This makes sense because during stress, the brain must be especially nimble at integrating visual input from the environment, sending signals to subcortical and brainstem regions that start cascades of physiological responding, and also to receiving afferent signals from the body about its metabolic state. These integrated processes occur in an effort to coordinate adaptive behavioral responses to a stressor. As such, activation in coordination/relay centers like the thalamus and insula, as well as regions that facilitate attention and cognitive control like the inferior frontal gyrus, are critical to orchestrating this complex stress response.

It is interesting to note that, contrary to what some may have predicted, this meta-analysis actually found consistent deactivation in the parahippocampus/amygdala in response to social stressors. It should be noted that deactivation results from fMRI
meta-analyses should be interpreted with caution, as there is inconsistent reporting of deactivation across studies in the literature and thus meta-analytic estimates of deactivation patterns may be less reliable than those for activation. Nevertheless, Berretz et al. (2021) suggest that, as a region critical for processing salience, the amygdala is active during the first few moments of a stressor task, facilitating the identification of relevant salient information in the environment. Over time, however, the amygdala may habituate to the repeated task demands (given that many social evaluation tasks used repeated stimuli/cognitive processes) and thus appear as deactivation when averaged over the course of a long scan. Alternatively, it may be the case that the amygdala is particularly involved in signaling salience/uncertainty of visual and auditory stimuli, but is not responsive to all forms of threat, especially not those that are more abstract or self-generated, such as the social stress tasks outlined here. Consistent with this idea, other meta-analytic work finds that amygdala responses are associated with affectively evocative visual stimuli (as in the perception of fearful facial behaviors or disgusting images) but not experiences of fear or affective states induced by imagery or recall (Lindquist et al. 2012). Thus, while it may seem surprising that studies associated with threat such as social evaluative stressors report consistent deactivation of the amygdala, it is likely a misconception that the amygdala consistently underlies all instances of threat responding. Rather, amygdala-prefrontal functional connectivity – as well as the broader swath of areas involved in visceromotor control and representation of affective states such as the insula, thalamus, and inferior frontal gyrus – may be what is critical during social stress processing.

**Opportunities for Future Research**

What are critical next steps for research addressing the neural correlates of social evaluation? There are a number of interesting opportunities for future work in this area. First, work exploring the neural correlates of physiological responses to social evaluation is needed. While some work has been done in this area (for a review, see Ginty et al. 2017; Kraynak et al. 2018; Muscatell and Eisenberger 2012; Thayer et al. 2012), more research that examines the neural activity patterns that are common to many physiological responses vs. unique to a specific physiological channel (e.g., autonomic responses; immune responses) is needed (Eisenbarth et al. 2016). Unlike Cyberball, which, as mentioned above, does not elicit reliable physiological reactions in participants, many of the social evaluation tasks described above do lead to changes in physiological parameters of interest to stress researchers, including heart rate and skin conductance (Wager et al. 2009a, b), cortisol (Dedovic et al. 2009b), and inflammation (Muscatell et al. 2015). As such, future work that continues to explore the neural predictors of physiological responses to social evaluative stressors is needed.

A second future direction for this area of work is to “break down” the complex social evaluation tasks that include elements of performance stress, uncertainty, time pressure, cognitive effort, and social feedback into their component parts to isolate the neural underpinnings of each individual process. Currently, many tasks include all of these various elements which combine together to create a stressor, but it is also important for translational efforts to determine which brain activity is specific to each
component part. As mentioned above, one recent task isolated the social evaluative feedback component that is a feature of the other tasks in this area to examine the neural correlates of that component specifically (Eisenberger et al. 2011; Muscatell et al. 2015). Future work could examine how the brain responds to time pressure, performance evaluation, and/or cognitive effort, independently of the other factors. This would facilitate our understanding of the neural “ingredients” that combine to construct an overall experience of stress. As we discuss next, another avenue of future work is to build on work of racism-related stress to identify the specific aspects of social stress that are particularly pernicious for members of individuals from marginalized communities.

To conclude, a number of different tasks exist that can be used to elucidate the neural underpinnings of social evaluative stress. A recent meta-analysis of this area of work identified that the insula, thalamus, and inferior frontal gyrus are reliably active across these different tasks (Berretz et al. 2021), highlighting the important role that coordination/integration regions as well as cognitive control regions play in responding to social evaluative stress. Future research that builds on a small but critical literature investigating the neural correlates of physiological responses to social evaluative stress, as well as work that isolates the specific neural correlates of the various components of these stressor tasks, will move the field forward.

3 Neural Correlates of Racism-Related Stress

Overview Within the social stress literature, a small but emerging body of work has begun to examine how racism-related stress (e.g., racial discrimination) is represented in the brain. Like general social stress, racism-related stress has been clearly associated with negative physical and mental health outcomes, particularly among Black Americans (D. R. Williams and Mohammed 2013). Moreover, research suggests that exposure to racism-related stress can alter reactivity to other general social stressors (Akdeniz et al. 2014; Brondolo et al. 2009; Wright et al. 2020), thus creating compounding effects of stress among individuals from marginalized racial/ethnic backgrounds.

Despite these well-documented associations between racism-related stress and negative outcomes, much is still unknown about the mechanisms through which this type of stress impacts the brain and body. As such, it is important to investigate the neural correlates of racism-related stress processing. Currently, there are a limited number of studies in this area and very little work to date has examined how the brain responds to a racism-related stressor. However, one existing study by Masten et al. (2011) offers promising insights for future work investigating the neural correlates of racism-related social stress. Here we review the methods in this study, summarize results, and discuss future directions for this line of research.

Methodological Approaches Masten et al. (2011) utilized an adapted version of Cyberball in order to examine neural responses during an experimentally-
manipulated experience of racial discrimination among Black Americans (Masten et al. 2011). In this paradigm, a real, interactive experience of interpersonal discrimination is simulated, as Black participants engaged in a game of Cyberball with two White individuals (confederates). Similar to the Cyberball protocol described earlier, participants were excluded by the White “players,” allowing the possibility that Black participants would attribute the exclusion to being about their race, thus inducing the stress of racial discrimination. To increase the likelihood that discrimination attributions would be made by the Black participants, race was made salient by having participants meet the White confederates prior to the scanning procedure. While in the scanner, participants were also able to see images representing the other players. Besides the visual cueing of race, race was not brought up at any point during the experimental procedures.

Following scanning, participants completed self-report measures of distress and discriminatory attributions. Participants also completed a videotaped interview, describing their thoughts and feelings about being excluded during the game. These interviews were then rated by trained observers to index participants’ non-verbal negative affect. Taken together, these post-scan measures provided a way to assess whether participants attributed their social exclusion during the Cyberball task as being due to their race and measure the immediate behavioral and affective consequences of such racism-related stress. Moreover, the inclusion of the questions about discrimination allowed the researchers to investigate differences in neural activity based on whether the participants experienced the exclusion episode as an instance of racial discrimination, thus, creating a paradigm to examine the direct effects of racism-related stress on neural activity.

Summary of Findings  Results from the Masten et al. (2011) study offer novel findings about the neural correlates of perceiving racial discrimination in a social context. Specifically, findings demonstrated that Black individuals who attributed the experience of social rejection to racial discrimination displayed decreased activity in the dACC, which has been established as a key region in the salience network (Menon and Uddin 2010; Perini et al. 2018; Uddin 2015). Furthermore, findings of Perini et al. (2018) demonstrate that the dACC is involved in monitoring salient, self-relevant social information, and thus deactivation in this region may indicate disengagement or a lack of monitoring/attention. Though speculative, this suggests the possibility that individuals who experienced their rejection as racial discrimination may have been reducing attention to or disengaging from such rejection, perhaps in effort to cope with the negative experience of racism-related stress. This pattern of neural activity aligns with behavioral findings that show adopting a self-distanced (vs. self-immersed) approach to processing stressful experiences can result in reduced feelings of distress and negative affect, thus reflecting an effective coping strategy (Kross et al. 2014; Kross and Ayduk 2011; Mischkowski et al. 2012). This possibility could be addressed in future research on the neural underpinnings of coping processes engaged in response to racism-related social stress. Finally, discrimination attributions were also linked to increased activity in VLPFC and rostral ACC, which have been implicated in emotion
regulation processes (Buhle et al. 2013; Eisenberger et al. 2003; Masten et al. 2011), further suggesting that individuals who attributed rejection to racial discrimination may have engaged to a greater degree in emotion regulating coping strategies.

Overall, results of this study provide seemingly counterintuitive findings regarding the neural underpinnings of racism-related stress. Indeed, given the well-established literature on the adverse effects of experiencing racial discrimination for health and well-being among minority individuals, it would seem that such experiences would trigger neural activity consistent with experiencing adversity. Yet, Masten et al. (2011) found neural activity associated with potentially “buffering” effects of perceiving negative treatment as being due to racial discrimination. Given these findings and the general lack of similar studies, further research is certainly needed to disentangle the nuances of neural activity associated with experiencing racism-related stress.

Opportunities for Future Research Given that there is just this one known study investigating the neural underpinnings of race-related social stress, more research is needed in this area, and opportunities for future studies abound. Research exploring how the brain responds to racism-related stress would offer important insights into the ways in which the brain may give rise to downstream physiological changes in response to such stress, and subsequently contribute to racial inequities in health and well-being. However, before successful research in this area can progress, a few challenges must be addressed.

First, studies investigating the neural correlates of racism-related stress have remained difficult due in part to the challenges of simulating “real-world” experiences of racism-related stress within the scanner. As reviewed previously, there currently exist a number of “standard” tasks used to elucidate the neural mechanisms of experiencing general social stress (e.g., Cyberball, ScanSTRESS, MIST). However, no such “standard” tasks exist for inducing racism-related stress in the scanner. One potential solution to this methodological issue may lie in the development of a standardized, scanner-based racism-related stress task that would allow for the replicable and generalizable study of the neural correlates of racism-related stress. One approach to this task development may be to adapt elements of existing general social stress tasks, such as what was done in Masten et al. (2011) for the Cyberball task. Similarly, such a task could capitalize on the stressful nature of social evaluation and involve monitoring neural activity of historically oppressed racial/ethnic groups while they are being evaluated by oppressors (i.e., in the U.S., White confederates).

Second, racism is increasingly appreciated as a systemic and structural problem in addition to an interpersonal issue (Bailey et al. 2017; Jones 2000; Neblett 2019). As such, it will be important for future research to examine if and how exposure to structural racism shapes neural functioning. Along these lines, the recent “bias of crowds” model (Payne et al. 2017) suggests that, rather than racial bias being something that occupies the minds of specific individuals, it is rather reflective of the history and current day practices of particular contexts and places (Payne and Hannay 2021). As such, Black individuals (and likely those from other oppressed
groups) may experience greater racism-related stress as a function of where they live (Payne et al. 2019). Given this, it would be interesting for future work to utilize a multi-cite collaborative model wherein researchers in different areas of the country that vary in their history of racial oppression and current levels of systemic racism could each scan a set of participants, and examine if there are regional differences in neural responses as a function of both historical (e.g., history of slavery) and present day (e.g., prevalence of red-lining) structural racism.

Finally, it will be important to conduct future research in large samples such that additional risk and resilience factors that influence neural reactivity to racism-related stress can be examined as moderators. For example, past experiences of perceived discrimination and high identification with one’s race (i.e., racial centrality) have been found to increase physiological reactivity to experimentally manipulated experiences of racism-related stress (Neblett 2019). As such, future work may seek to investigate how race-related individual differences may moderate neural activity while experiencing racism-related stress. The inclusion of such relevant moderators may then further highlight the nuances of racial/ethnic minorities experiences with racism-related stress. fMRI research investigating individual differences as predictor of neural responses to tasks is notoriously challenging, given that it requires large sample sizes to produce reliable, replicable effects. Thus, such work will need to be conducted in larger-than-typical samples and will require significant funding; we encourage funding agencies to take note of this and to allocate funds for well-powered fMRI studies to investigate predictors of neural responses to racism-related stress.

In sum, despite a few challenges, many opportunities exist for social neuroscientists to uniquely contribute to existing knowledge about the neural pathways through which racism can impact health and well-being.

4 Overall Next Steps for Research Investigating the Neural Underpinnings of Social Stress

The past 20 years of neuroimaging research on stress and the brain have shed significant light on the neural correlates of social stress processing. As outlined above, we now have robust literatures on the neural correlates of social rejection and social evaluation, and we also have the foundation for a new literature on the neural correlates of racism-related stress. While a number of future research directions are included in the separate sections above, we end by “zooming out” and recommending two critical, overall next steps for the literature on the neuroscience of social stress moving forward.

A first overall next step is to move beyond merely studying the neural correlates of social stress and to instead work to build a literature that examines how neural responses to social stress are predictive of subsequent cognitions, emotions, behaviors, physiology, and ultimately, downstream consequences on health. For example,
behavioral research shows that exposure to a stressor is associated with subsequent changes in reward processing (Boyle et al. 2020), emotion regulation success (Raio et al. 2013), and cognitive control strategies (Steinhauser et al. 2007). However, only a few known neuroimaging studies have investigated the neural contributors to these effects, and particularly, how neural activity during a stressor might be predictive of neural activity and behavioral performance during a subsequent cognitive task, or at rest (Shermohammed et al. 2017; Zhang et al. 2020). Thus, we currently have only a cursory knowledge of the neural predictors of alterations to behavior following stress, as well as how stress impacts neural responses during subsequent tasks. This is an important next step for research in this area, as it is likely that many of the negative health effects of social stress are driven not solely by responses during a stressor, but also how one behaves (and how one’s brain functions) after encountering a stressor. Similarly, as we note above, little research examines how neural responses to social stress are associated with physiological shifts in the periphery of the body (e.g., autonomic nervous system responding, HPA-axis responding, inflammatory responding), responses themselves that likely have long-term consequences for health and well-being.

A second overall next step for work in this area is to utilize advanced quantitative techniques to create more reliable, replicable “neural signatures” of social stress reactivity. For example, machine learning techniques characterizing multivariate patterns of neural activity to distinguish between different psychological and/or physiological states have become more widely utilized in neuroimaging research over the past few years, but are only starting to be used in stress research. One seminal recent paper, for example, used this technique to identify both common and unique neural patterns that distinguish heart rate from skin conductance reactivity to a social evaluative threat task (Eisenbarth et al. 2016). We encourage future research in this area to test whether similar (or different) neural patterns in response to social evaluation predict other types of physiological and psychological reactivity. Another example of a quantitative technique that should be applied to future neuroimaging research on social stress is network analysis (Bassett and Sporns 2017; Bassett et al. 2018). Network analytic tools can facilitate the quantification of the degree to which different brain networks are relatively more integrated or segregated in response to changing task demands (i.e., dynamic functional connectivity). The relative level of network segregation/integration has emerged as an important predictor of behavioral task performance in recent work (Cohen 2018; Cohen and D’Esposito 2016; Kucyi et al. 2018), and yet, this approach is only just beginning to apply to understand how brain networks reconfigure in the face of a social stressor (Wheelock et al. 2018). Thus, future research should also use graph theoretic techniques to further our understanding of how brain networks connect (or disconnect) in response to social stress. We look forward to these future approaches that will further weigh in on how social stressors impact the brain, body, and ultimately, human health and wellness.
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Social Acts and Anticipation of Social Feedback

Irene Perini, Sara Kroll, Leah M. Mayo, and Markus Heilig

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Abstract Socialization happens so regularly in humans that it can be perceived as an effortless activity. However, it reflects a sophisticated behavior, pervaded by anticipation and emotion. The fast-paced social interplay, strongly mediated by facial expressions, can be considered one of the most frequent high-order motor acts within the human behavioral repertoire. The ability to adequately process social feedback is critical for appropriate socialization and affects well-being. The social difficulties often observed in psychiatric patients highlight the link between mental health and successful socialization and the importance of characterizing the behavioral and neural mechanisms of social interaction. This chapter will present some cross-species evidence on the cortical regions engaged during social interactions including facial expressions, and the impact of induced or perceived social stress on the experience of social interactions.

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1 Introduction

Social stress can trigger negative feelings that can occur in the presence of an objective social environmental stressor, for example when receiving a negative social judgment. Still, social stress can also be triggered or enhanced by a biased subjective evaluation of the social interaction (Perini et al. 2019), with the latter being a particularly relevant aspect in psychiatry.

While models of sustained social stress and its behavioral consequences are feasible in animals, the investigation of social stress and more generally social interactions in humans faces more ethical and methodological obstacles. Social neuroscientists are particularly confronted with the challenging trade-off between ecological validity and experimental control. To ensure real-world validity in the artificial magnetic resonance imaging (MRI) setting while keeping experimental control, we designed a task that simulates the social media environment, a widely used contemporary channel for socialization. While this task, that simulates an “online game,” was limited in its ecological validity by the constraints of the MRI environment and analysis, it specifically included key components of the complex experience of socialization, namely fast-paced positively- or negatively-valenced feedback toward self and others. Using the online game task, we investigated the neural substrates of social interaction in a group of healthy adolescents (Perini et al. 2018), in healthy adults after stress induction (Kroll et al. 2021), and in adolescents that engage in nonsuicidal self-injury (NSSI), a behavior often triggered by perceived social rejection (Nock et al. 2009).

In the first half of this chapter, we will present evidence on the neural mechanisms behind the salient experience of being judged by others, using the online game task. Based on human and monkey brain evidence, we will also highlight how the high-order motor functions localized in to the anterior midcingulate cortex (aMCC) and adjacent perigenual anterior cingulate cortex (pACC) might support socialization. In the second half of the chapter, we will present the psychophysiological effects of stress on self-referential processing in healthy adults. Finally, we will present the effects of perceived social stress in triggering a negative social bias in a group of adolescents with NSSI.
2 The Salience of Self During Social Feedback

Social interactions are essential for well-being across the lifespan, and particularly so during adolescence. Socialization into peer groups during adolescence increases the importance of social interactions and their outcomes. Adolescence is also a period characterized by development of neural circuits that mature along different time-scales and under the control of partially distinct biological processes (Steinberg 2005).

In our MRI studies in adolescents, we investigated the interaction of social judgment, balanced across negative and positive feedback, with whether the social judgment was directed toward the participant or other players (Perini et al. 2018, 2019). Participants were instructed to decide on a trial-by-trial level whether they liked or disliked pictures of other putative players. They also received positive or negative feedback from others. The task also included an anticipatory phase, in which the participants waited for the collective feedback which was either toward another player or toward self. By introducing a “self” versus “other” dimension we identified anterior insula (AI) and aMCC/pACC as regions involved in processing self-referential social evaluation. While our findings are consistent with previous studies, where the self-relevant condition coincided with the salient experience of feeling rejected by others, they also expand on them and challenge the idea of AI and aMCC as rejection-specific areas [for meta-analyses, see Cacioppo et al. (2013); Rotge et al. (2015); Vijayakumar et al. (2017)].

Instead, in accordance with more recent findings (Dalgleish et al. 2017), our data in healthy adolescents show that when the salience of the positive and negative feedback is balanced in a trial-by-trial manner across the experimental run, then these regions are equally responsive using univariate statistics (Perini et al. 2018). Our findings resonate well with the theorization of AI and aMCC as domain-general structures and key hubs of the salience network, a network that integrates internal and external feedback to guide action selection (Seeley 2019; Seeley et al. 2007; Uddin 2015). In addition, and relevant in the context of social judgment as a potential probe of social stress, we show that the engagement of AI and MCC is present already during anticipation of social feedback from others, suggesting that these regions might be involved in monitoring and forecasting the expected judgment from others.

The fact that this task engaged the salience network during the processing of self-relevant judgment might be challenged by two alternative interpretations. First, the salience network is usually active when participants are engaged in nearly any task (Menon and Uddin 2010; Seeley 2019). Our findings in AI and aMCC might therefore just be interpreted as a simple task-related activity. However, the different task requirements across the two dimensions suggest otherwise. Only in the other-condition are participants instructed to indicate their decision, based on their preference. Therefore, only then, participants must be engaged and exert an overt behavioral choice to successfully address task demands. This is not the case during self-trials, in which participants can only anticipate and receive the feedback. This
difference between the two perspectives suggests that if the AI and aMCC activations were simply due to task demands then their engagement would be higher during the most active condition, i.e. the other-condition. Instead, these regions were more active during the self-condition, where no action was required. Therefore, we interpret this finding as an indication of an experiential difference between the two conditions, where monitoring and possibly forecasting the potential valence of the self-directed feedback was more salient to the participant than expressing and witnessing judgment toward other players.

Secondly, one might find that salience network activity does not resonate well with typical findings for introspection, which classically tend to activate the default-mode network (DMN) (Spreng et al. 2009). However, while being more passive in terms of behavioral output, the self-condition does not reflect disengagement. On the contrary, it demands more attention, as reflected by increased activity in the salience network. Therefore, we propose that the identified activations in AI and aMCC reflect a “reactive” versus an elaborative mode of self-relevant information processing (Perini et al. 2018), in which anticipated and received mutual feedback are integrated at present (Fig. 1).

In the following section we will present some evidence of aMCC and pACC, often reported in social neuroimaging studies, as relevant moderators of the complexities of social behavior, including facial expressions.
3 Social Interactions Require Prompt Motor Action

Brodmann’s anterior cingulate has been often referred to as a region subserving “affective” and “cognitive” processes, whether in the context of pain or social interactions. In this section we will try to break down these psychological terms into more tangible cognitive functions within the anterior cingulate, in an attempt to provide a parsimonious explanation of its role during social processing.

Throughout the chapter, instead of “dACC,” we use the term MCC, from the “four-region model” proposed by Vogt and colleagues [for an extensive summary, see Vogt (2009)], and the term “anterior cingulate” when referring to Brodmann’s dual-subdivision. The subdivision of the human cingulate cortex into four main regions has been shown to better capture the heterogeneity of this structure compared to Brodmann’s dual model, in which the cingulate was divided into an anterior and posterior portion (Brodmann 1909). In the four-region model, the anterior cingulate is divided into two portions: the anterior cingulate cortex (ACC) and the midcingulate cortex (MCC) (Palomero-Gallagher et al. 2008). Although the term “midcingulate” has seen an exponential increase in the literature (Vogt 2016), the field of social neuroscience still seems to favor the less precise term “dACC.” However, the term “dACC” reflects general localization and it implies a homogeneity within the anterior cingulate which is not confirmed by microanatomical, neurochemical, and functional evidence (Margulies and Uddin 2019; Palomero-Gallagher et al. 2008).

Crucial support for the ACC-MCC division in humans comes from a large presurgical electrical stimulation study, showing affective responses, such as laughter, interoceptive sensations and autonomic responses, more located in ACC and goal-directed behavior in MCC (Caruana et al. 2018). Given the functional and structural differences between MCC and ACC, it becomes relevant for the field of social neuroscience to leverage this evidence, to better understand the contribution of these regions during social processing. Here, we present some nonhuman and human primate findings that highlight the role of aMCC and adjacent pACC in exerting complex motor behavior, including monitoring and execution of facial expressions, supporting the hypothesis that activity in this region during socialization is related to integrative high-order behavior.

3.1 High-Order Motor Control in aMCC

Lesion studies in macaques have shown the role of the anterior cingulate in social behavior and attention to salient social stimuli (Hadland et al. 2003; Rudebeck et al. 2006). After removal of the anterior cingulate, macaques engage less in social interactions and instead, favor manipulation of inanimate toy objects (Hadland et al. 2003). Rudebeck et al. corroborated this finding and showed that the anterior cingulate modulates behavior by allocating attention between basic rewards and
salient social stimuli (Rudebeck et al. 2006). Anterior cingulate lesions reduced the
typical interference in task-related reactivity when presented with socially salient
stimuli, such as videos depicting a dominant staring male and female genitalia
(Rudebeck et al. 2006). This effect was driven by gyral lesions (including area
24 and 32) which also induced decreased affiliative and aggressive behaviors. These
results present some evidence on the role of the anterior cingulate in social interplay
and highlight potential complementary contributions of sulcal and gyral regions,
with the first reflecting emotion-driven motor control and the latter evaluation of
salient social stimuli.

The notion that the midcingulate is involved in skeletomotor control goes decades
back (Talairach et al. 1973) and is supported by extensive work in monkeys (Dum
Vogt et al. 1987) and by brain imaging findings in humans (Amiez and Petrides
2014; Picard and Strick 1996). For their premotor properties, cingulate sulcal sub-
fields in areas 24 and 23 have been named “cingulate motor areas” in monkeys, as
they project to the primary motor cortex and to the spinal cord in a topographic
manner (Dum and Strick 1991; He et al. 1995; Muakkassa and Strick 1979) [for a
review, see Dum and Strick (2002)]. The motor actions mediated by cingulate motor
areas follow a caudo-rostral gradient. Caudal portions are thought to regulate
visuo-spatial monitoring and specific body orientation, as evidenced by extensive
connectivity to parietal regions (Morecraft et al. 2004; Vogt et al. 1987) and by their
short-latency anticipatory activity to simple movements (Shima et al. 1991). In
addition, they contain large pyramidal neurons, typical of “primitive” motor
processing and absent in other parts of the anterior cingulate (Braak 1976; Matelli
et al. 1991). Conversely, single-cell recording indicated that rostral premotor regions
are preferentially responsive to self-paced voluntary reward-dependent behaviors

In humans, imaging studies identified a correspondence between cingulate motor
areas of the monkey and “cingulate motor zones” in humans, located in MCC (Misra
and Coombes 2015; Picard and Strick 1996). While often activated during pain,
MCC is not selectively engaged by pain (Wager et al. 2016). Using functional
magnetic resonance imaging (fMRI), we showed that MCC is activated during
task-contingent action independently of co-occurring thermal noxious or innocuous
stimulation (Perini et al. 2013, 2020). In her model of pain perception, Morrison
highlights the role of MCC as a site of adaptive behavior within a prediction coding
system framework and suggests a “proactive” more than a reactive role of this region
(Morrison et al. 2013). This is consistent with the view of aMCC as an integrative
region serving “adaptive control” (Shackman et al. 2011).

Meta-analytic evidence from positron emission tomography (PET) and intracra-
nial electrical stimulation findings support the caudo-rostral gradient observed in
monkeys (Caruana et al. 2018; Picard and Strick 1996) [for a review, see Margulies
and Uddin (2019)]. Basic organization of movement is represented more caudally,
near the vertical line (Vca), in the posterior portion of MCC (pMCC). Conversely,
self-generated actions, conditional movements, and movements reflecting goal-
directed behaviors are represented more anteriorly in aMCC (Caruana et al. 2018;
(Petersen et al. 1988; Picard and Strick 1996) [in humans aMCC includes cytoarchitectonic regions a24’ and 32’ (Fig. 2), the latter is lacking in monkeys (Vogt 2016)]. Accordingly, receptor architecture evidence supports this motor complexity-based gradient within MCC and shows that aMCC contains most of dopamine D1 receptors, whereas the posterior portion presents higher GABA_B binding (Palomero-Gallagher et al. 2009), with the latter typical of lateral motor and premotor areas (Zilles and Palomero-Gallagher 2001).

3.2 Facial Expressions and the aMCC/pACC Border

While evidence in the macaque brain suggests that the rostral sulcus in area 24 regulates self-generated, cognitively demanding actions, its most anterior portion participates in the modulation of facial muscles during high-order actions and social behavior. Anterograde tracing shows that facial representation fields in the cingulate
and in premotor and motor regions are interconnected (Luppino et al. 2003; Morecraft et al. 1996; Muakkassa and Strick 1979). The face representation territory in rostral cingulate sulcal area 24c (area M3 by Morecraft) projects bilaterally to the facial nucleus in a topographic manner (Morecraft et al. 1996, 2001). More specifically, it projects bilaterally to the dorsal and intermediate subnuclei which innervate the orbicularis oculi and frontalis-corrugator muscles, located around the eye region and important at conveying facial expressions (Morecraft et al. 2001). While area 24c triggers saccades (Mitz and Goodschalk 1989) it is unlikely to be involved in the primary control of eye moments, but rather in high-order oculomotor control (Luppino et al. 2003; Schall et al. 2002). Single-unit recordings show that activity in this region in concert with the supplementary eye field is involved in action performance monitoring (Schall et al. 2002). Consistently, area 24c is connected to the supplementary eye field (Luppino et al. 2003) and less so to the frontal eye field (Huerta and Kaas 1990), with the latter more implicated in primary control of movements.

Importantly, amygdalo-cingulate motor projections are preferentially targeting the face representation territory, suggesting that the projections of area 24c to the facial muscles and primary motor regions might be modulated by emotions (Morecraft et al. 2007; Morecraft and Van Hoesen 1998). Accordingly, using microanatomical evidence and intracortical stimulation, Jezzini et al. show that area 24c is connected to mid-ventral portions of the insula that trigger lip-smacking in macaques, a social behavior that signals cooperation (Caruana et al. 2011; Fedurek et al. 2015; Jezzini et al. 2012; Jezzini et al. 2015). While the presented evidence suggests that this cingulate premotor region is involved in modulating socially intended facial muscles, crucial evidence comes from a more naturalistic paradigm, consisting of an actual face-to-face interaction in monkeys (Livneh et al. 2012). Intracortical recordings in the dorsal anterior cingulate (likely including subregions of a24’ and extending to 24c) and amygdala revealed that these regions are rapidly and synchronously engaged in monitoring peer-facial expression, and crucially, in modulating self-executed facial expressions (Livneh et al. 2012). This important convergence at single-neuron level between the monitoring and the execution of facial expressions is consistent with the idea of socialization as a rapid integration of incoming and outgoing signals (Livneh et al. 2012). Altogether the presented evidence suggests that area 24c contributes to the articulation of facial communicative acts while integrating emotional and internal states.

The evidence from the monkey brain presented above resonates well with Damasio’s classic report on the effects of anterior cingulate versus primary motor damage in humans (Damasio 1994). Patients with lesions to the anterior cingulate cortex, including area 24 presented with blunted affect, importantly including blunted emotion-related expressions, while the ability to voluntarily contract facial muscles was preserved. Conversely, patients with left motor cortex damage were impaired in moving contralateral facial muscles, while surprisingly, spontaneous facial expressions such as laughter were unaffected. This observation highlights the compensatory role across face representation fields in the cortex and led to the hypothesis that the anterior cingulate might be involved in modulating facial
expressions associated with social communication and emotions (Damasio 1994; Morecraft et al. 2007). The face representation territory identified by Morecraft in monkey area 24c was located on the lower bank of the cingulate sulcus, with the genu of the corpus callosum as vertical anterior limit. In humans, this sulcal region lies in pACC adjacent to rostral aMCC, and for its pregenual location it has been renamed to p24c (Palomero-Gallagher et al. 2008; Vogt 2016) (Fig. 2). Importantly, area 24c is much wider in humans, and that, together with its ventro-dorsal cytoarchitectonic subdivision, might reflect a wider spectrum of emotions supported by facial expressions in humans (Palomero-Gallagher et al. 2008).

Broadly speaking, since MCC is involved in motor control and ACC in autonomic processing and emotions, the border between these regions is unlikely to reflect a clear-cut segregation during social behavior, but rather, on the contrary, a necessary functional integration. Functional gradients are common elsewhere in the cortex. For example, functional gradients in motor and premotor cortex led to the re-interpretation of the somatotopy within the motor cortex, classically assumed to reflect simple body parts segregation, when in fact it reflects ethologically relevant action maps (Graziano 2016). Anatomical connectivity models within the human insular cortex using probabilistic tractography suggest a smooth transition in connectivity patterns in the insula consistent with its cytoarchitectonic gradient (Cerliani et al. 2012). The insula is a highly integrative, heterogenous region, within which its anterior portion is considered to be a site of complete integration of salient bodily, visceral, and contextual information (Craig 2009).

Evidence in monkeys has shown that the anterior portion of the insula is mainly connected to anterior sulcal cingulate motor regions (Mesulam and Mufson 1982; Mufson and Mesulam 1982; Vogt et al. 1987). In humans, resting state connectivity shows anterior insula to aMCC/pACC connectivity (Taylor et al. 2009), supporting the hypothesis that current bodily information and contextual demands are integrated into adaptive motor actions. This is consistent with intracortical stimulation in humans, where pACC triggered facial affective responses (i.e., laughter) and interoceptive sensations (Caruana et al. 2018). While some facial expressions might reflect basic hard-wired emotions, facial acts during socialization most likely reflect subtle “indicators” supported by emotional, autonomic, and contextual information. More studies in humans should characterize potential different and/or complementary contributions of aMCC and pACC in the execution and processing of facial expressions during socialization. To identify their potential contributions has implications for understanding stress-related mechanisms during socialization.

In a recent investigation of cortical top-down influence on sympathetic responses in monkeys (Cebus apella) and rats, Dum et al. showed important across-species differences in descending cortical multisynaptic input to the adrenal medulla, a crucial effector site of stress responses (Dum et al. 2019). In monkeys, descending cortical influence was identified in three independent cortical networks related to movement, cognition, and affect. Rostral cingulate motor areas corresponded to the cognitive network, reflecting high-level motor control and more anterior cingulate regions including 24c to the affective network. Cingulate influence was absent in the rat brain, where the major cortical input to the adrenal medulla was from primary
motor and sensory cortices. The evidence of a more extensive cortical modulation of stress responses in monkeys emphasizes the higher complexity of the behavioral repertoire in primates. It also suggests that in humans, aMCC and pACC might serve a complementary influence in modulating stress responses during high-order behavior, including socialization.

Socialization is a fast-paced, anticipation-filled, emotion-modulated interaction, in which the promptness to act, whether to form facial expressions, or carry out approach and avoidance behaviors is a necessary component. The observed functional rostrocaudal gradient within MCC demonstrates that aMCC is a region fundamentally involved in high-order actions, and together with pACC, in the monitoring and execution of facial expressions. The connectivity to insular and amygdala regions suggests that the degree of complexity of actions/facial expressions triggered by aMCC/pACC might be the result of an integrative process, where interoceptive information and emotions are weighted in to calibrate adaptive behavior.

In conclusion, activation in aMCC and adjacent pACC is often reported in social neuroimaging studies, and it has been suggested that it is specifically related to social rejection. In our view, however, the presented evidence does not provide support for a role of this cortical territory to be valence specific. Instead, it favors the view that this region is an integrative site during socialization, well-equipped to support social behavior across its qualitative spectrum.

In this last section, we discussed the contribution of the aMCC/pACC in high-order facial expressions, key players of socialization. In the following section, we will show in healthy adults that stress affects facial muscles during the processing of self-pictures while anticipating social judgment from others.

4 The Effects of Acute Stress on Self-Referential Processing During Social Feedback

Socialization is a high-order, complex behavior, that is continuously modulated by environmental and internal states. The need to belong is a fundamental motivation in humans and relies on social interactions (Baumeister and Leary 1995). Social evaluation of self and others is an important element of these interactions, which can be impaired by socially threatening situations, such as receiving negative feedback from others (Dickerson et al. 2008). Systematic negative biases in social evaluation are found in several psychiatric disorders, such as depression and anxiety, suggesting a clinical relevance of this process (Bradley et al. 2016; Frye 2018; Gara et al. 1993; Hedrick and Berlin 2012; Nordahl et al. 2017). Therefore, characterizing the psychophysiological correlates of stress on social interaction in healthy individuals might have important implications for psychiatry.

Recently, we addressed the effects of stress on social interaction processing in a combined behavioral and psychophysiological study, where participants engaged in
the online game task while their facial muscle reactivity was collected (Kroll et al. 2021). The aim of this approach was to explore the effects of stress on social behavior and to objectively quantify affective responses to social judgment using facial electromyography (facial EMG). Several studies have investigated the neural and psychological effects of social stress in the form of simulated rejection using the classic “Cyberball” [for meta-analyses, see Cacioppo et al. (2013); Rotge et al. (2015); Vijayakumar et al. (2017)]. While the Cyberball induces potent self-reported negative psychological effects, as measured by the “need-threat” questionnaire, it doesn’t necessarily target the effects of stress in the context of social interactions. In addition, despite an extensive literature on the effects of stress on physiology and non-social behavior (Godoy et al. 2018; Skoluda et al. 2015), little is known about the effects of physical and social stress on the processing of social interactions.

Facial expressions are key “moderators” of the continuous exchange of feedback that occurs during socialization (Darwin 1872). Based on the facial expression theory by Lang et al. (1993), facial muscle activity is tightly linked to the internal affective state of an individual. In this view, activation of the zygomaticus major, that mediates smiling, reflects enhanced positive affect, whereas the corrugator supercilii, that mediates frowning, signals increased negative affect. Consistently, findings by Larsen et al. (2003) show an association between facial muscle activity and self-reported affective state induced by affective pictures and sounds (Larsen et al. 2003). In their study, negative affect was strongly correlated with corrugator activity, whereas positive affect was associated with zygomatic activity. An alternative but complementary theory views facial expressions as communicative tools during socialization and highlights the role of facial muscle activity as an important mediator of behavioral intentions such as approach and avoidance (Fridlund 1994). The data presented in the previous section suggest that these two facial expression theories are complementary. The role of facial expression during socialization is undeniable and so is the modulation of facial muscles by emotions and internal states.

We used a within-subject design to investigate behavioral stress responses and facial muscle reactivity on 35 healthy subjects during the online game task (Fig. 3a) (Perini et al. 2018, 2019) following stress exposure or a non-stressful control condition. Facial muscle activity was assessed using facial EMG, a psychophysiological measure of affective response and social behavior. Stress was induced by the Maastricht Acute Stress Test (MAST), that combines a cold pressor test with a social stress component and uses a physiologically and psychologically neutral control condition (Smeets et al. 2012). After stress, we found increased corrugator and decreased zygomatic reactivity when participants viewed their own picture while anticipating feedback from others. This indicates that, in healthy individuals, acute stress triggers a negative self-referential processing during anticipation of social judgment from others. In addition, participants’ subjective feeling of stress predicted corrugator activity, suggesting that the stress-induced negative state affected psychophysiological self-evaluation. Crucially, corrugator responses predicted the percentage of dislikes given to other participants only in the stress condition, suggesting a link between facial expressions and social behavior (Fig. 3b). Overall, our findings
Fig. 3  The effects of acute and chronic stress on social processing. (a) Online game task. Chronological sequence of self- and other-trials. Total feedback for self-trials was fixed to 50% positive and 50% negative. (b) Acute stress manipulation in healthy adults ($N = 35$). Facial muscle reactivity during anticipation of social feedback and its relationship to social behavior (Kroll et al. 2021). Top. Increased corrugator reactivity after acute stress exposure compared to non-stress control condition. Bottom. Corrugator reactivity predicted the amount of negative feedback toward others only after acute stress ($p = 0.021$) but not in the
non-stress condition \((p = 0.4)\). (c) Negative bias in female adolescents with nonsuicidal self-injury (NSSI) \((N = 27)\) (Perini et al. 2019). Top. NSSI participants were more sensitive to being rejected and disliked more to see their own face during the game. In the NSSI group, rejection sensitivity scores correlated positively to multivariate group classification scores based on functional brain activity during anticipation of social feedback. Bottom. Compared to controls, adolescents with NSSI perceived being disliked more often than controls, and at trend level, disliked others more. Cutting frequency was positively correlated to perceived rejection. Error bars reflect standard error of the mean. \# = 0.08, *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\)
are consistent with both leading facial expression theories, that of facial expressions as a reflection of affective state, (Lang et al. 1993) and that of facial expressions communicating behavioral intentions (Fridlund 1994).

Given that maladaptive stress responses as well as inadequate social evaluation and interaction are key features in several psychiatric disorders such as depression, anxiety disorder, and addiction, our findings support the notion that facial expressions may have utility as biomarkers of psychiatric diseases (Mayo and Heilig 2019). In addition, our findings in healthy adults support the importance of self-evaluation as treatment target in treatment of individuals with stress-related psychiatric disorders.

In the following section, we will present data on altered self-evaluation in a group of female adolescents that engage in nonsuicidal self-injury, a behavior often triggered by perceived social rejection and stress.

5 Social Stress and Negative Bias: Evidence from Nonsuicidal Self-Injury

As previously discussed, social interactions are essential for well-being across the lifespan, but particularly so during adolescence, a period of important neurodevelopmental changes. External factors likely play a key role in neurodevelopment, as the fine-tuning of these circuits occurs in an experience-dependent manner (Giedd 2004; Giedd et al. 1999). The increasing importance of social interactions at a time when neural processes governing these behaviors are in flux contributes to a heightened vulnerability in developing maladaptive social and emotional behaviors.

Chronic stress, including interpersonal stress and perceived criticism, can contribute to the development of nonsuicidal self-injury (NSSI). NSSI consists of behaviors performed intentionally to harm oneself without suicidal intent, such as cutting, burning, or hitting oneself (Nock 2010). It has been suggested that NSSI, just like other formally different risky behaviors, such as hazardous drinking, might serve the function of “experiential avoidance” and reflect an attempt to control or modify an internal or external state (Hayes et al. 1996; Kingston et al. 2010). The risk of engaging in NSSI is particularly high during adolescence, with prevalence rates around 17% in community samples (Muehlenkamp et al. 2012) and between 40 and 80% in clinical samples of psychiatric patients (Klonsky and Muehlenkamp 2007). NSSI is more common in females, particularly in clinical samples (Bresin and Schoenleber 2015). NSSI can occur together with or independent of other psychiatric diagnoses, including anxiety and depression (Kiekens et al. 2018). It is currently considered among the defining symptoms of borderline personality disorder (BPD), but is far more common in adolescents than is BPD, suggesting that NSSI can exist independently of or co-occur with BPD (Kiekens et al. 2018). Recently, NSSI was suggested as a diagnostic entity of its own and was included in the third section of
the Diagnostic and Statistical Manual of Mental Disorders, Fifth edition (APA 2013) as a condition requiring further study, highlighting the importance of research in this area.

The age of onset for NSSI is around 12–14 years and peaks during adolescence, with rates declining in adulthood (Klonsky and Muehlenkamp 2007; Moran et al. 2012). However, even after cessation of NSSI behaviors, lasting effects on anxiety and depression are evident in adults (Daukantaite et al. 2020). High emotional distress, sensitivity to interpersonal stress, and chronic romantic stress are suggested to play a role in the development and maintenance of NSSI (Miller et al. 2018). NSSI is often related to distress, with the function of NSSI behaviors attributed to the regulation of negative affective and social experiences. This is supported by ecological momentary assessment (EMA) studies, which have identified an association between NSSI and interpersonal instability (Santangelo et al. 2018). NSSI-related thoughts are often triggered while socializing with others, and the likelihood of NSSI is greater as a function of increased perceived rejection in these social contexts (Nock et al. 2009). Longitudinal data suggest that frequency of lifetime and past year NSSI predicts the occurrence of stressful interpersonal life events and increases in depressive symptoms 6 months later (Burke et al. 2015). Thus, NSSI appears to be both an antecedent and consequence of interpersonal stress, highlighting the need for understanding the mechanisms facilitating this association.

Accumulating evidence from longitudinal and EMA studies supports the association between interpersonal stress and NSSI, but experimental studies are only beginning to provide mechanistic insights. Compared both to controls and to those with a suicide attempt, adolescents with NSSI endorse greater interpersonal sensitivity and report more perceived stress during an interpersonal conflict task (Kim et al. 2015). Pilot neuroimaging studies suggest potential brain regions important for feelings of social rejection, but these studies are preliminary in nature due to their small sample sizes (Brown et al. 2017). To address this dearth of knowledge, we have recently begun to explore the neurobiological mechanisms contributing to interpersonal stress sensitivity and NSSI using our online game task (Perini et al. 2018, 2019).

As described previously, the simulated online game task aims to identify neural regions involved in the processing of self-relevant information during social interaction (Perini et al. 2018). Like in a social media platform, participants indicate whether they like or dislike pictures of other adolescents. Similarly, participants anticipate and view their own picture being judged by other simulated players. After the game, participants are asked about their perceived amount of total negative feedback, how much they liked seeing their own face, how bad it felt to be disliked, and how good it felt to be liked. Importantly, the task is fixed such that 50% of the self-trials result in acceptance, while the other 50% end in rejection.

We used the online game task in 27 adolescent females with NSSI and 27 age-matched controls to explore potential mechanisms contributing to the observed interpersonal difficulties in NSSI (Perini et al. 2019). In accordance with clinical observations, we found a striking negative bias in NSSI patients (Fig. 3c). Patients perceived being rejected more than controls, with some NSSI patients even
reporting 100% rejection rates, even though all rejection rates were in fact fixed at 50% across all participants. NSSI patients also displayed greater sensitivity to rejection, rating the feeling of being rejected worse as compared to control participants. Finally, NSSI patients also liked seeing their face much less than controls. Interestingly, this bias was specific for the negative domain, as there was no group difference in the sensitivity to acceptance, with both groups reporting similar ratings on how good it felt to be accepted. Thus, NSSI patients perceive social evaluative experiences with a profound negative bias when compared to matched controls.

Critically, the behavioral findings reported above appear to have a significant relationship to the clinical characteristics of NSSI patients. The average cutting frequency, which reflects the number of cuts in the past 12 months, was positively correlated with greater perceived rejection, suggesting that those with more frequent cutting episodes perceived being rejected more often. Cutting frequency was also negatively correlated with how much participants liked seeing their own face. In addition, there was a negative relationship between the recency of the latest NSSI episode and perceived rejection, such that the more recent the NSSI episode, the more rejected the patient reported feeling. Together, the behavioral data suggest that more severe clinical characteristics are associated with a greater negative perception of social interaction.

In our previous study in healthy adolescents (Perini et al. 2018), we had found that right AI and aMCC are key regions for processing self-relevant information. We replicated these findings in our NSSI patients and matched controls, with clusters in bilateral AI and aMCC/pACC and activating more in response to the “self” versus “other” contrast (Perini et al. 2019). For the right AI, there was a perspective-by-outcome interaction with activation in the self-perspective greater during the rejection condition than acceptance, possibly reflecting increased salience processing to negative-social feedback in this group of female adolescents. For the anticipation interval, activation of the right AI, aMCC/pACC, and bilateral supplementary motor area showed greater activation in the “self” versus “other” contrast. However, somewhat unexpectedly given the marked group differences in behavioral responses, using a univariate approach, none of these analyses detected differences in activations between NSSI patients and healthy controls.

Assuming that robust behavioral differences must have a basis in differential brain activity, the likely implication is not that group differences between NSSI patients and healthy controls do not exist; but rather that conventional, univariate analyses fail to detect them, possibly because the responses involve spatially distributed patterns of activity that do not lend themselves well to cluster-based analyses. We therefore applied a multivariate machine-learning approach to determine whether brain activity outside the salience network during the online game task could be used to classify participants’ group membership (NSSI patients versus controls). This also allowed us to identify brain regions outside the salience network that most significantly contribute to multivariate discrimination of groups. Applying a support vector machine to voxel-wise indices of task-activation from the anticipation epoch produced statistically significant classification of participants, with 68% accuracy in classifying patients versus controls. This allowed us to identify unique
brain-behavior associations that were specific to the NSSI patients. In particular, classification scores derived from the multivariate analysis significantly correlated with the behavioral measure of rejection sensitivity in NSSI patients, while this relationship was absent in control participants. This association between classification scores and rejection sensitivity remained significant even after controlling for several variables, including borderline personality traits, depression traits, and medication use.

Using this multivariate approach, we were able to identify brain regions outside of the typical salience network nodes that significantly contributed to the discrimination between NSSI patients and matched controls. These regions included the dorsomedial prefrontal cortex (dmPFC), posterior cingulate cortex (PCC), and importantly, subgenual anterior cingulate cortex (sgACC). The dmPFC and PCC are involved with self-referential processing and autobiographical memory (Spreng et al. 2009), while the sgACC is involved in the generation of affective states and is reported to be functionally and anatomically altered in mood disorders (Drevets et al. 2008). This pattern of activation during anticipation of social judgment might contribute to the negative interpretation bias evidenced in the patient group (Perini et al. 2019) (Fig. 3b).

This negative bias appears to be specific to social evaluation, and not representative of a general deficit in emotion processing or perception (Perini et al. 2019). In the same study, participants also completed an emotion identification task in which neutral faces morphed into six standard emotions. Participants had to indicate how quickly they could identify the emotion, providing an index in sensitivity to emotion detection, in addition to identifying the emotion. We also used facial electromyography (EMG) of the corrugator (“frown”) and zygomatic (“smile”) muscles to assess affective reactions to the emotional faces. This provided us the opportunity to determine if the NSSI patients had a similar negative bias in their perception of emotions, which could influence their experience in the social interaction task. However, we found no differences between NSSI patients and matched controls in either the sensitivity or accuracy in identifying emotions or emotional reactions to the faces. Thus, the negative bias NSSI patients experience seems to be specific to a socio-evaluative context and not representative of broader difficulties in the perception of emotions.

More recently, we have reported that we do find dysregulation of more general, e.g. non-social, emotion processing in NSSI (Mayo et al. 2020). In particular, affective images elicit greater emotional reactivity, again indexed by EMG of the corrugator and zygomatic muscles, in response to both positive and negative stimuli. While we found no group differences in neural response to affective images, we did find that greater EMG activity correlated with neural response in the anterior insula in NSSI patients but not controls. Critically, this association held regardless of valence, that is, greater positive affect (e.g., zygomatic response to positive images) and greater negative affect (corrugator response to negative images) individually and additively correlated with anterior insula responding. This further supports the specificity of the negative bias in interpersonal contexts, as we failed to find a similar negative bias in the processing of affective images.
6 Conclusion and Future Agenda

Socialization hinges on cortical regions exerting high-order motor control. This chapter discusses the role of portions of the cingulate in the implementation of social acts, and the behavioral and physiological effects of acute and chronic social stress. Using a simulated social online environment, we show that aMCC and pACC are activated when receiving, and importantly, when anticipating judgment from others.

This finding, together with existing observations on the role of these regions in high-order motor control and facial expressions, highlights the link between socialization and promptness to act. In a combined behavioral and psychophysiological study in healthy adults, we found that acute stress in healthy individuals increased frowning during anticipation of social feedback, which predicted negative feedback toward others. We also identified a negative bias in interpreting social feedback in a psychiatric condition characterized by chronic interpersonal stress. The observed negative bias is likely supported by regions involved in introspection during anticipation of social feedback. Altogether, these findings show that social acts are highly integrative and bidirectional processes, where interoceptive information, physiological responses, and emotions are weighted in and integrated with current social feedback to generate the upcoming social behavior. The aMCC and pACC might represent potentially meaningful and complementary sites in supporting the demands of socialization and in the modulation of stress-related responses. We believe that future social neuroscience studies in humans should consider the MCC/ACC distinction discussed in this chapter.

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Clinical Outcomes of Severe Forms of Early Social Stress

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Abstract Early social stress, particularly severe but nevertheless frequent forms such as abuse and neglect, are among the major risk factors for the development of mental disorders. However, we only have very limited knowledge of the psychobiological disease mechanisms underlying the influence of early life stress and stress-related disorders during this vulnerable phase of life. Early stress can have long-lasting adverse effects on the brain and other somatic systems, e.g. through influences on brain development. In adulthood, the prior experience of abuse or neglect can result in complex clinical profiles. Besides conditions such as mood and anxiety

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disorders as well as posttraumatic stress disorder, substance use disorders (SUD) are among the most prevalent sequelae of early social stress. Current social stress further influences the development and maintenance of these disorders, e.g., by increasing the risk of relapses. In this chapter, we will first give an overview of currently used methods to assess the phenomenology and pathophysiology of stress-related disorders and then focus on the phenomenological and neurobiological background of the interaction between early social stress and SUD. We will give an overview of important insights from neuroimaging studies and will also highlight recent findings from studies using digital tools such as ecological momentary assessment or virtual reality to capture the influence of early social stress as well as current social stress in everyday life of persons with SUD.

Keywords Abuse · Adverse childhood experience · Ecological momentary assessment · Neglect · Neuroimaging · Social stress · Substance use disorders

1 Introduction

More than 450 million people worldwide fulfill the criteria of a mental disorder, accounting for 13% of the Disability Adjusted Life Years of all diseases (Vigo et al. 2016). Adverse childhood experiences (ACE), i.e., abuse and neglect, are severe forms of early social stress and currently considered to be among the major risk factors for the development of mental disorders (Gilbert et al. 2009). This is particularly relevant as community surveys from Europe and worldwide show high prevalence rates of sexual (9.6%), physical (22.9%), and emotional abuse (29.1%), as well as physical (16.3%) and emotional neglect (18.4%) (Sethi et al. 2013). However, we only have very limited knowledge of the psychobiological disease mechanisms underlying the influence of ACE – particularly early life stress – and stress-related disorders during this vulnerable life phase (Fava et al. 2014).

ACE can have long-lasting adverse effects on the brain and other somatic systems, specifically through epigenetic mechanisms and influences on brain development (Heim and Binder 2012; Raabe and Spengler 2013). Although an ACE in general lead to increased risk for brain-related disorders (Teicher and Samson 2013), the role of type and timing of ACE is of particular interest in terms of prevention and treatment of ACE-related psychiatric and somatic conditions (Herzog and Schmahl 2018). It has been postulated that during certain vulnerable developmental phases, the risk for subsequent ACE-related disorders is increased (Kaplow and Widom 2007; Heim and Binder 2012; Baker et al. 2013; Teicher and Samson 2013). Preliminary evidence points to sensitive periods and specificity of ACE-subtypes in the development of neurobiological alterations, e.g., volumetric or functional changes in the amygdala and hippocampus (Andersen et al. 2008; Pechtel et al. 2014; Riem et al. 2015; Zhu et al. 2019). Extending morphological findings to brain function, a recent study reported that amygdala reactivity to emotional faces was actually decreased in individuals who experienced physical maltreatment in a
prepubertal phase between the age of 3 and 6, but increased for peer emotional bullying in a postpubertal phase between the age of 13 and 15 (Zhu et al. 2019). These findings could partially be corroborated with ACE during both a prepubertal (ages 3 & 4) and a postpubertal (ages 16 & 17) period emerging as particularly predictive for amygdala function, while total ACE severity did not contribute to the prediction (Sicorello et al., in preparation).

Also, evidence suggests that certain types of ACE predispose individuals for different disorders: The experience of physical violence may induce aggression-related conditions (Fanning et al. 2014), whereas the experience of neglect may be related to the development of emotional difficulties (Colvert et al. 2008). Therefore, it is not surprising that prior experience of these severe forms of early social stress can result in complex clinical profiles with several co-occurring mental and somatic disorders. ACE are a risk factor for many psychiatric conditions (Teicher and Samson 2013) and the risk for developing a mental disorder after ACE is highest for major depressive disorder, posttraumatic stress disorder, borderline personality disorder, and substance use disorder (SUD) (Maercker et al. 2004; Danese et al. 2009; Cutajar et al. 2010; Hägele et al. 2014; Lindert et al. 2014). Furthermore, psychological and psychosocial mechanisms known to contribute to mental disorders are affected after ACE. Impairments in cognitive and affective processing (Dannlowski et al. 2012), including social emotions such as heightened experience of loneliness (Boyda et al. 2015), as well as social cognitive functioning (Pechtel and Pizzagalli 2011) and social interactions (Tost et al. 2015; Halevi et al. 2016), including aggressive behavior (Ferguson et al. 2008) have been observed. Furthermore, emerging evidence suggests that ACE promote the development of obesity, diabetes, and other non-communicable as well as inflammatory diseases (Felitti and Anda 2010; Alastalo et al. 2013; Danese and Tan 2014; Fuller-Thomson et al. 2015).

Somatic symptom disorder, including abnormal pain perception with and without corresponding somatic pathology (e.g., chronic pain vs. pain during childbirth) has also shown to be enhanced after ACE (Leeners et al. 2016; Tesarz et al. 2016).

Mental disorders in individuals with ACE are likely to develop earlier with more severe symptomatology (Hovens et al. 2012), increased risk of comorbidity (Harkness and Wildes 2002), and are less likely to respond to standard treatment (Agnew-Blais and Danese 2016). It has therefore been suggested that individuals with ACE within a diagnostic category represent specific ecophenotypes (Teicher and Samson 2013). In a meta-analysis, Norman et al. (2012) reported a higher odds ratio for depression when exposed to emotional abuse, compared to physical abuse, and a higher odds ratio for drug abuse when exposed to physical abuse, compared to emotional abuse.

In this chapter, we focus on the impact of early social stress in the form of abuse and neglect in psychiatric conditions, particularly in SUD. The next sections will describe important methods for psychometric and neurobiological assessment of the influence of ACE in these conditions. We focus particularly on two important methods, the assessment of psychopathology in everyday life via ecological momentary assessment (EMA) and the measurement of brain activity via functional magnetic resonance imaging (fMRI). The last section will give an overview of clinical
and neurobiological findings on the intersection of early and current social stress with psychopathology of SUD. An outlook on the future research agenda, particularly on the combination of EMA and fMRI, will round up the chapter.

2 Methodological Challenges

Social stress is experienced on multiple levels – physiological, psychological, and social. It involves individual and environmental factors, histories of stress exposure (early life stress), current chronic stressors, protective factors, and multiple individuals interacting in context. Efforts to measure early social stress in humans face some methodological challenges. First, due to the difficulties in obtaining data from young children on traumatic experiences or assessing the same individual over years (prospective studies), most evidence comes from cross-sectional studies on adults who retrospectively report ACE. This yields a limited causality and generalizability of associations between measured stress exposure and adult psychopathology (Newbury et al. 2018). Second, although neuroimaging techniques enable us to study neural correlates underlying psychopathology in great detail, spatial and physical restrictions of the MRI scanners do not allow for investigating social interactions in naturalistic and ecologically valid setups. Furthermore, behavior under a controlled laboratory setting with experimental paradigms emulating social contact has limited ecological validity (Ebner-Priemer and Trull 2009; Tost et al. 2015). Third, exposure of people to real-life social stress is ethically difficult in most types of experimental research (Caspi and Moffitt 2006; Tost et al. 2015). In the following, we review selected established and more novel approaches and challenges of their application for studying the effect of social stress exposure in real life. We also provide exemplary studies highlighting the promising potential of using the novel integrated methods.

3 Ecological Momentary Assessment: Alternative to Retrospective Self-Reported Measures

The standard approach in trauma research and its impact on mental health relies heavily on retrospective assessment of trauma (Hardt and Rutter 2004) which is affected by recall bias, an individual’s subjective interpretation of the event(s), as well as current mental health and, therefore, is lacking ecological validity (Cohen and Java 1995; Shiffman et al. 2008).

An innovative approach, EMA (or ambulatory assessment), can partially help to address this challenge. EMA, using smartphones, enables a more naturalistic multimodal daily assessments of behavior, affective states, physiology, subjective experience, location information, and environmental context in real-time and real-life
settings (Fahrenberg et al. 2007; Shiffman et al. 2008; Ebner-Priemer and Trull 2009; Ebner-Priemer and Kubiak 2010; Loeffer and Peper 2014). Applying EMA as an alternative or addition to questionnaires, apart from instantaneous assessment of what is happening at the moment, also helps to avoid recollection bias and enables to assess context-dependent relationships (Ebner-Priemer and Trull 2009). Importantly, it also allows capturing the fluctuation of emotions and cognitions over a time period.

EMA is reliable, valid, and ideally suited to study the effect of early life social stress on moment-to-moment emotions and social experiences (stress perception, stress reactivity) later in daily life (Glaser et al. 2006; Reininghaus et al. 2016; Baker et al. 2019), and the link between early social stress, daily life stress reactivity, and psychopathology (depressive, anxiety, and psychosis symptoms) (Cristóbal-Narváez et al. 2016; van Nierop et al. 2018). EMA is also very helpful when studying participants at risk for mental disorders (e.g., a depressive episode, alcohol craving and consumption, or suicidal thinking) (Stange et al. 2019; Griffin and Trull 2021). Since substance use is related to momentary emotional state and psychosocial stress experienced at particular places and times, EMA offers a unique possibility to study complex interaction between contextual influences and psychopathological symptoms by recording context-specific data (e.g., substance-related cues - pubs, bars, restaurants, wine shops), immediate internal experience (e.g., mood, craving, or withdrawal state), and social interactions (e.g., social pressures to use) (Shiffman 2009; Epstein et al. 2014; Gustafson et al. 2014; Kwan et al. 2019). Furthermore, smartphone-based digital phenotyping, which combines many streams of participants social and behavioral footprints (like emotional state, GPS location, social behavior, and sleep quality) with machine learning algorithms, has the potential to improve relapse detection, prediction and intervention, and overdose intervention (Onnela and Rauch 2016; Ferreri et al. 2018; Hsu et al. 2020).

4 Neuroimaging as a Method of Choice

Advances in neuroimaging over the past decades have turned it into a central method for studying the biologic foundations of mental illness. In addition, neuroimaging also enables one to study the effects of specific biological, environmental, or psychological factors, such as genes or personality (Meyer-Lindenberg and Tost 2012). Due to low risk, high spatial resolution, and its non-invasive nature, MRI is the most widely used tool to examine the neural correlates of the effects of early life trauma. Furthermore, it permits the investigation of both the structure and the function of the human brain. MRI studies that examine the neural mechanisms underlying the development of psychopathology have employed several different approaches. The structural characteristics of stress-related brain alterations can be assessed using structural MRI (sMRI) (Symms et al. 2004). Structural volumetric studies, examining the association between exposure to traumatic stress and regional volumes, are most prevailing in trauma sMRI research (Kribakaran et al. 2020). A
growing number of research also uses cortical thickness measurements and morphological imaging techniques such as voxel-based morphometry and the (MRI-based) diffusion weighted imaging method (Teicher et al. 2003; Symms et al. 2004; O’doherty et al. 2015; Kribakaran et al. 2020).

fMRI studies assess brain activity of regions during specific emotional or cognitive tasks, as well as connectivity between regions of interest, either task-dependent (psychophysiological interaction) or task-independent (resting state functional connectivity) (Helpman et al. 2017). Trauma-related studies often employ tasks with emotional stimuli associated with previous stressful events (Kleim et al. 2012; Sartory et al. 2013; Herzog et al. 2019), or stimuli unrelated to the specific trauma to study general emotion processing (Koch et al. 2016; Van Rooij et al. 2016). Another approach is to explore the neural correlates of regulation of emotional responses using different emotion regulation tasks (Phan et al. 2005; New et al. 2009; Marusak et al. 2015). Yet, other studies apply psychological and social stress induction paradigms in the MRI environment, such as the Montreal Imaging Stress Task with mental arithmetic challenges under time pressure (Dedovic et al. 2005), or the ScanSTRESS task, which includes cognitively demanding tasks, pressure to perform, time pressure, and social-evaluative stress (Streit et al. 2014). Another example is the Sing-a-song Stress test, where participants are asked to sing a song aloud (Brouwer and Hogervorst 2014). Resting-state fMRI is particularly useful to study adaptive emotion regulation in the period following stress, because brain activity is not disturbed by task demands (Veer et al. 2011).

The complex nature of social stress calls for generating ecologically valid situations similar to what we usually encounter in our daily life. The combination of neuroimaging with EMA could take neuroscience outside the controlled laboratory environment, and thus shed new light onto the neural substrates of real-life social-environmental risk factors (Tost et al. 2015). Heller et al. using this combined innovative approach, showed with fMRI that prolonged daily positive affect is associated with a sustained engagement of the ventral striatum to rewards (Heller et al. 2015). Further, Tost and colleagues found that when participants are surrounded by green space, higher affective valence ratings are associated with lower prefrontal cortex activation during negative-emotion processing (Tost et al. 2019). Another study using positron emission tomography in combination with EMA found that daily life stress has a blunting effect on reward-induced dopamine release in the ventral striatum in individuals at risk for psychosis (Kasanova et al. 2018).

5 Virtual Reality

Daily social activity, environments, and events are very individually specific, which can be considered as a shortcoming of the EMA method. Virtual reality (VR refers to computer-generated simulations (Biocca and Levy 1995)) can function as a useful option for more controlled experiments with high ecological validity: It simulates
fictitious, safe, interactive, and controllable scenarios with high emotional engagement, similar to those in a physical environment (Bohil et al. 2011; Veling et al. 2014; Holz and Meyer-Lindenberg 2019). Due to cost reduction and increased quality, modern VR devices can produce a high level of immersion (Segawa et al. 2019).

A promising application of VR has also been found in stress studies, e.g. in the association between early life stress, psychosis liability in response to VR social stress (Veling et al. 2016), inducing social stress using the Trier Social Stress Test (Zimmer et al. 2019), in therapeutic applications for fear conditioning, anxiety disorder, or in the treatment of phobias and posttraumatic stress disorder (Bohil et al. 2011). Importantly, VR is compatible with neuroimaging technologies (Bohil et al. 2011). Such an integrated approach can, for example, provide important information about the neural effect of treatments for SUD (Lee et al. 2009).

6 Hyperscanning as a Tool to Assess Social Dynamics

As an alternative to traditional neuroimaging studies that record neural activity of one participant responding to the stimuli emulating social contact (like recorded videos, human-avatar, or human–computer interactions), hyperscanning is a new, promising method to overcome some of these shortcomings in order to increase the natural component of social interactions in experimental settings. Hyperscanning refers to measuring the brain activity of multiple subjects simultaneously, i.e., to examine freely forming social interactions. This technique allows for real-time reciprocal social contact in a truly interactive manner, and thus investigation of the real-time dynamics between two or more interacting brains (Hari and Kujala 2009; Hari et al. 2013).

Hyperscanning has been successfully applied to measure emotions and affect in social contexts (Bilek et al. 2015; Czeszumski et al. 2020), as well as social impairments in patients with borderline personality disorder and a history of childhood trauma (Bilek et al. 2017). Also, neural activations in schizophrenia, major depressive disorder or bipolar disorder patients during live face-to-face conversation were investigated (Takei et al. 2013, 2014). Specifically, near-infrared spectroscopic studies during face-to-face conversation found decreased frontal activation in both MDD and BD, and decreased activation in the temporal lobe and inferior frontal gyrus in patients with schizophrenia and could be related to functional deficits and might reflect the pathophysiological character of disease (Takei et al. 2013, 2014).
Detrimental consequences of SUD affect the within-person level but also our entire society: Worldwide, around 35 million individuals suffer from SUD. In 2016, 283 million individuals over the age of 15 years (5.1% of adults) were diagnosed with alcohol use disorder (AUD). A harmful consumption of alcohol is associated with approximately three million deaths per year (United Nations Office on Drugs and Crime 2019). Further underlining this extensive social and economic damage, the US was faced with an alcohol-related loss of 249 billion USD in 2010 (Sacks et al. 2015). From a clinical perspective, loss of control over substance consumption, substance craving, and interpersonal problems are key criteria of SUD. Further, SUD is marked by a chronic progression and instances of relapse (Koob 2008; Heilig et al. 2010; Koob and Volkow 2010; Sinha 2011; Hasin et al. 2013; Koob 2013; Witteman et al. 2015). By their nature, SUDs are complex, manifold and of a multifactorial etiology. Therefore, we should approach the investigation of mechanisms underlying this disorder on different levels. Cognitive control, motivation, mood states, substance cue exposure, stress vulnerability, and social interactions play major roles in the development and maintenance of the disorder (Shalev et al. 2002; Heinz et al. 2003; Sinha 2008; Shiffman 2009; Sinha et al. 2009; Simons et al. 2010; Koob 2013; Dvorak et al. 2014b, Hägele et al. 2014; LaBrie et al. 2014; Serre et al. 2015; Cabrera et al. 2016; Everitt and Robbins 2016; Blaine and Sinha 2017; Ghiță and Gutiérrez-Maldonado 2018; Riva et al. 2018). Using psychometric assessments, as well as EMA, VR, and neuroimaging, the dynamic interaction of individual and situational or contextual factors can be revealed (Heinz et al. 2020).

In the following, we will outline the intersection of SUD as one psychiatric outcome influenced by early social stress, and ACE from a neurobiological perspective. For this, we will mostly focus on AUD as one form of SUD, since this substance type is the most prevalent one (Peacock et al. 2018). Neuroplasticity is pivotal throughout the course of AUD – and a concomitant shift from positive to negative reinforcement of the alcohol consumption behavior is observed. This may also be modulated and moderated by predisposing factors, such as personality traits or (epi-) genetic processes. Over the course of the development and maintenance of AUD, and considering the multifactorial etiology of AUD, adaptation takes place in neural circuits related to (1) reward and motivation, (2) memory, conditioning, and habituation, (3) executive functioning and inhibitory control, (4) interoception and self-awareness, and (5) stress (Koob and Volkow 2010). These domains are functionally related to the ventral (incentive learning) and dorsal striatum (habit learning and action initiation), thalamus (regulation of arousal and attention), hippocampus (memory and contextual conditioning), insula (interoception, craving), amygdala (conditioned learning, emotion processing), dorsolateral prefrontal cortex (executive functioning, inhibitory control), orbitofrontal cortex (salience attribution), ventromedial prefrontal cortex (regulation of autonomic stress response, reward and...
emotion regulation), and cingulate gyrus (inhibitory control and conflict monitoring) (Koob and Volkow 2010; Volkow et al. 2012).

During an early, binge, and intoxication stage, fast changes of the dopaminergic system in the ventral striatum are perceived as being rewarding by the individual. Context and expectation regarding the effect of alcohol further influence neural reactivity (Wrase et al. 2007; Koob and Volkow 2010; Nees et al. 2012; Vollstädt-Klein et al. 2019; Zhornitsky et al. 2019; Vollstädt-Klein et al. 2020a). In addition, alterations in thalamic activity may lead to an impaired regulation of arousal and attention modulation (Koob and Volkow 2010; Pitel et al. 2015). A later withdrawal and negative affect stage is marked by a reduced reactivity of the dopaminergic system to reinforcing stimuli with a simultaneous sensitivity to conditioned, alcohol-related stimuli. Additionally, disruptions in frontal regions (dorsolateral prefrontal cortex, cingulate gyrus, orbitofrontal cortex) lead to impairments in inhibitory control and in the brain stress system further maintaining the AUD, e.g., through recurrent relapses (Koob and Volkow 2010; Blaine and Sinha 2017; Zahr et al. 2017). A final preoccupation and anticipation stage is marked by further reorganization of the reward and memory circuits. Conditioned cues (alcohol-, stress-, and emotion-related) trigger alcohol craving and lead to a continued consumption of alcohol (Sinha et al. 2009; Koob and Volkow 2010). Underlining the transition from impulsive to compulsive, and therefore, more habit-related alcohol consumption, a shift from ventral to dorsal striatal reactivity to alcohol-related stimuli was observed when comparing light social drinkers to heavy drinkers (Vollstadt-Klein et al. 2010; Everitt and Robbins 2013).

8 The Role of Early Social Stress

One major maintaining factor in AUD are changes in stress-sensitivity and stress-associated brain regions following chronic alcohol consumption (Blaine and Sinha 2017). Deactivation in the posterior cingulate cortex and ventromedial prefrontal cortex in response to threatening stimuli was observed in AUD (Wilcox et al. 2020). In addition, Sinha et al. (2011) were able to predict relapse rates via the relation of brain activity in the ventral and medial prefrontal cortex and anterior cingulate cortex, and the stress markers cortisol and adrenocorticotropic hormone (Sinha et al. 2011). Both, AUD and stress lead to a loss of inhibitory control over striatal reward and behavior related networks. Changes in neurobiological processes due to alcohol and stress are further associated with emotion regulation, alcohol craving, and an increase in impaired control over impulsive behavior, alcohol intake, and eventually relapse (Koob 2008; Sinha et al. 2009, 2011; Seo et al. 2013, 2016; Wade et al. 2017). Additional factors are deficits in emotion regulation per se (Oscar-Berman and Bowirrat 2005; Volkow et al. 2012). Therefore, some individuals with AUD use the substance as a means of affect regulation, which further promotes relapse (Kopera et al. 2015). Then again, from a neurobiological perspective, reduced efficiency of the mesocorticolimbic network in individuals with AUD
leads to a reduced processing of, e.g., emotional faces, which may also hamper an integration of memory in current, emotional states (Dvorak et al. 2014b).

SUD are not only a cause (e.g., children of parents affected by SUD) but also a major psychiatric outcome of ACE, as mentioned above (Gilbert et al. 2009; Cutajar et al. 2010; Hägele et al. 2014; Choi et al. 2016; Schäfer et al. 2016). Parents with SUD might be a source of ACE, due to inadequate parenting style, such as aggression toward the child (Eiden and Leonard 2000), or neurobiological changes, both leading to a dysfunctional interaction with the child (Rutherford and Mayes 2017). Therefore, and besides biological factors, a parent–child transmission of SUD is possible due to adverse environmental factors – namely, ACE resulting from a parent with SUD. 51.7% of individuals with SUD reported at least one adverse event during their childhood (Choi et al. 2016). Early experiences of adversities are further related to early substance use and can be seen as a moderator, contributing significantly to the risk of developing, e.g., an AUD (Koss et al. 2003; Dube et al. 2006; Pilowsky et al. 2009; Whitesell et al. 2009; Afifi et al. 2012; Hägele et al. 2014). This association may be due to deficits and alterations in cognitive performance, memory, processing of social and affective stimuli, processing and regulating emotions, and stress-sensitivity (Hien et al. 2005; Lloyd and Turner 2008; Gilbert et al. 2009; Pechtel and Pizzagalli 2011; Ansell et al. 2012; Dannlowski et al. 2012; Vilhena-Churchill and Goldstein 2014; Zorrilla et al. 2014; Choi et al. 2016). In addition, a higher vulnerability for drinking as a coping mechanism within the framework of a self-medication hypothesis, e.g., as a response to stressful events, has been indicated (Schmid et al. 2010; Temmen and Crockett 2020). Animal studies further support these findings. In rodents, an increasing risk for AUD due to alterations in the medial prefonsal cortex and amygdala signalling following traumatic stress has been observed (Edwards et al. 2013). Epigenetic processes on glucocorticoid receptors after ACE may be responsible for a following, enhanced vulnerability for alcohol use in stressful situations (Spanagel et al. 2014).

A cumulative exposure to ACE is associated with smaller gray matter volume in the medial prefrontal cortex, insula, and anterior cingulate cortex (Ansell et al. 2012; Dannlowski et al. 2012). Further alterations following early social stress are an enlarged right amygdala and a hyper-responsiveness to threatening stimuli (Dannlowski et al. 2012; Pechtel et al. 2014), as well as smaller hippocampal volume (Andersen et al. 2008; Dannlowski et al. 2012; Riem et al. 2015). Due to early social stress leading to alterations in brain regions that are involved in memory, stress, emotion and reward regulation (Holz et al. 2020), an increase in the vulnerability for AUD seems plausible. It is also known that early and chronic stress affect mesolimbic dopamine pathways which lead to alterations in the responsiveness of the hypothalamic-pituitary-adrenal axis (Sinha 2008). A dysregulation of the HPA-axis, in turn, leads to greater stress- and cue-induced alcohol craving (Sinha et al. 2011) – possibly resulting in alcohol intake increasing the cortisol secretion (Junghanns et al. 2005) to a quasi-normal level.

Contribution to the possible parent–child transmission of SUD, neurobiological and functional changes in the reward and stress systems of a parent with SUD are also related to a decreased salience of infant cues and, in consequence, passive or
disengaged behavior toward the child. Additionally, a parent with SUD might perceive the act of taking care for a child as less rewarding and more stressful (Rutherford and Mayes 2017), due to the abovementioned neural alterations. Both aspects might lead to ACE such as neglect or abuse. Starting the vicious circle in their childhood, individuals exposed to early social stress are likely to develop maladaptive emotion regulation and coping abilities due to neurodevelopmental alterations. They may later rely on alcohol to regulate emotional or stressful states (Hien et al. 2005; Dube et al. 2006; Rothman et al. 2008; Sinha 2008; Afifi et al. 2012). At the same time, alcohol-related alterations affect brain regions that are relevant for emotional functioning (e.g., amygdala, hippocampus, prefrontal cortex) (Oscar-Berman and Bowirrat 2005; Dvorak et al. 2014b, Kopera et al. 2015), similar to ACE, and thus strengthening the adverse effect. Additionally, chronic alcohol consumption further sensitizes the impaired stress response system (e.g., anterior cingulate cortex, prefrontal cortex, ventral striatum, amygdala) inducing a heightened affective reactivity to daily stress (Koob 2008; Blaine and Sinha 2017). This boosts the vicious circle and possibly contributes to a further parent–child transmission of SUD (Fig. 1).

9 Use of Modern Technology in Early Stress-Related SUD

Recent progress in digital technology such as EMA and VR (as described above) enables real-life monitoring of affective states and response to daily events. In the case of the multifactorial etiology of SUD, use of this technology provides a fine-grained and close-to-life examination of the influence of early and current social stress on this disorder. Indeed, EMA findings support the relation between daily stress, stress reactivity or negative affect and substance craving (Simons et al. 2010; Neupert et al. 2017; Waters et al. 2020) as well as the use of alcohol to decrease social anxiety (Battista et al. 2015). Negative affect mediates the relationship between posttraumatic stress symptoms and same-day alcohol consumption and can be interpreted within the framework of a self-medication hypothesis (Cohn et al. 2014; Alexander and Ward 2018). It was also noted that these observations are similar to laboratory findings (Serre et al. 2015) which further contributes to a comprehensive explanatory model of SUD.

A few recent reviews have revealed evidence supporting the use of VR in the study and treatment of SUD (Ghiță and Gutiérrez-Maldonado 2018; Trahan et al. 2019; Segawa et al. 2020; Vollstäd-t-Klein et al. 2020b). Since VR allows to modulate the environment and relevant (substance-related) stimuli, hypotheses regarding craving, stress, and interpersonal relations can be assessed (Lee et al. 2008; Simon et al. 2020). Some studies have successfully used a virtual exposure to induce alcohol craving under social pressure situations in individuals with SUD (Lee et al. 2008), as well as in SUD therapy, e.g., aversive therapy (Ghiță and Gutiérrez-Maldonado 2018). Additionally, this novel method has been applied to several other psychiatric mental disorders, such as phobia and posttraumatic stress disorder (Park...
Fig. 1 Schematic display of the interaction between early social stress and substance use disorders. Inadequate parenting, e.g., aggression toward or dysfunctional interaction with the child, of parents with substance use disorders can lead to early social stress and ultimately results in a vicious circle. Both, early social stress and substance use disorder can mutually influence each other. This interaction is mediated by structural and functional alterations in the brain. Additionally, environmental factors and (epi)-genetic processes contribute to this interaction.
Therefore, it appears to be useful across specific psychiatric diagnosis and stress-related conditions also regarding social stress and emotion regulation.

## 10 Conclusions and Outlook

Early social stress, as described here in the form of the adverse childhood experiences (abuse and neglect), is a central factor in the emergence of mental disorders such as SUD. On the neural level, ACE can lead to impairments in brain circuitries related to stress, emotion, reward, and inhibitory control. If present, these alterations serve as a major driving factor for the development of SUD later in life. Due to overlapping pathways and mechanisms following exposure to stress and chronic substance consumption, SUD can further enhance these deficits, and thus maintain a vicious circle. When examining consequences of and alterations due to ACE or current social stress in inhibitory control, emotion, and stress networks, a variety of validated or newly developed fMRI paradigms can be applied. Using a combination of established and advanced methods, including psychometric assessment, EMA, neuroimaging, and VR, the dynamic interaction of individual, situational, or contextual factors can be revealed.

Emerging EMA-based technologies offer promising opportunities and can improve our understanding of psychopathologies, substance use behaviors, assessment, and treatment. Given first empirical evidence, we can assume that new technical tools in the near future might help in prediction of daily alcohol consumption (Dvorak et al. 2014a), in identification of at-risk individuals (Simons et al. 2010), and in individually tailored solutions (Hsu et al. 2020). As the field rapidly progresses, there are a few concerns that need to be addressed. Privacy and security questions are critically important, since EMA captures highly sensitive personal data and it is vulnerable to security threats. Another is intellectual challenge – large volumes of data to process on a (cloud) server and subsequent data analysis (Onnela and Rauch 2016; Hsu et al. 2020).

A combination of brain imaging and behavior related methods such as EMA and VR (Gustafson et al. 2014; Riva et al. 2018) yields great potential not only for studying, but also treating mental disorders such as SUD. Due to methodological challenges and relatively high costs, studies using novel technologies such as virtual reality, hyperscanning, and/or a combination of neuroimaging and EMA are scarce. However, they hold a promising potential for application in psychiatric conditions related to early social stress. Incorporating such innovative approaches can provide striking new insights and advance our understanding of the complex interplay of neurobiology, psychology, and social environment that shape healthy and stress-influenced brain development and function.

In terms of consequences for interventions, parents with SUD and own ACE should be an important target group since intervening here might help to reduce the risk for further ACE as well as the development of SUD in the offspring. Such interventions should take into focus not only substance consumption, e.g.,
SUD-related therapy for the parents. Further, stress and emotion regulation of parents with SUD as well as the child–parent interaction (including parents and children) should be addressed. Additionally, (cognitive-behavioral) therapy to develop skills for stress and social regulation should be provided for children of parents with SUD to break the above described vicious circle.

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Clinical Outcomes of Severe Forms of Early Social Stress


Childhood Violence Exposure, Inflammation, and Cardiometabolic Health

Eric D. Finegood and Gregory E. Miller

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Abstract Exposure to interpersonal violence during childhood, a severe and often traumatic form of social stress, is an enduring problem that an emerging body of work suggests may be relevant to cardiometabolic health and the progression of cardiovascular disease (CVD) across the life course. Less is known about this association causally, and consequently, the biological mechanisms that may confer risk for, and resilience to, poor health outcomes in the aftermath of violence are not well understood. Drawing on recent theoretical insights and empirical research in both humans and non-human animal models, the current paper articulates a hypothesis for one way that childhood violence could get “under the skin” to influence CVD. Based on this emerging literature, one plausible way that childhood violence exposure could increase susceptibility to CVD in later life is by sensitizing stress-response neurobiology and immune processes that regulate and promote inflammation, which is a key pathogenic mechanism in CVD. This is inherently a developmental process that begins in early life and that unfolds across the life course, although less is known about the specific mechanisms through which this occurs.

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The goal of this paper is to articulate some of these plausible mechanisms and to suggest areas for future research that aims to reduce the burden of disease among individuals who are exposed to violence.

**Keywords** Cardiovascular disease · Child development · Childhood violence · Inflammation · Monocytes

### 1 Introduction

Exposure to interpersonal violence is a severe and often traumatic form of social stress that afflicts a large number of youth in the United States, and, relative to other age groups, children are disproportionately at risk of exposure (Margolin and Gordis 2004). The World Health Organization defines interpersonal violence as the “intentional use of physical force or power, threatened or actual, by a person or small group of people against another person or small group that either results in or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment or deprivation” (World Health Organization 2014, p. 82). Violence against youth takes many forms and it can be experienced directly (e.g., experiences of maltreatment or abuse by parents, being victimized by violent crime in the community, or being bullied at school) and indirectly (e.g., witnessing interparental violence or violent acts in the community; Margolin and Gordis 2004). Data on rates of exposure indicate that violence against children is highly prevalent. According to the National Survey of Children’s Exposure to Violence, an estimated 51% of youth (children below the age of 17) in the US report having been physically assaulted in their lifetime and nearly 1 in 4 have experienced maltreatment (e.g., been physically, emotionally, or sexually abused, or neglected; Finkelhor et al. 2015). Nearly 1 in 3 youth has witnessed an assault in their community and nearly 1 in 12 has been exposed to a shooting (Finkelhor et al. 2015).

The psychological and socioemotional consequences of violence for youth are many and have been documented across decades of research (Cicchetti and Toth 1995; Foster and Brooks-Gunn 2009; Fowler et al. 2009; Margolin and Gordis 2000). At a psychological level, exposure to violence can undermine a child’s sense of safety in their environment (e.g., their home, their school, their neighborhood), it can diminish their feelings of self-worth, and it can disrupt their attachment relationships and social relationships broadly (Cicchetti and Toth 1995; Margolin and Gordis 2000, 2004). Extensive literature in child development and epidemiology indicates that childhood violence exposure is associated with emotion regulation problems and maladjustment (e.g., Kim and Cicchetti 2010), difficulties in school (Perkins and Graham-Bermann 2012), and with increased risk of developing psychopathology including anxiety disorders, depression, and posttraumatic stress disorder (PTSD) (Cicchetti and Toth 1995; Foster and Brooks-Gunn 2009; Margolin and Gordis 2000, 2004; McLaughlin et al. 2013; Slopen et al. 2012). Of course, none
of these adverse outcomes is inevitable; some youth maintain good health even after severe violence exposure (Yule et al. 2019). But on average, violence increases children’s vulnerability to difficulties in multiple life domains across the life course.

2 Violence and Cardiovascular Health

In addition to these psychological and mental health consequences, an emerging body of literature suggests that childhood violence may also have consequences for cardiovascular health – and it is this specific association that is the primary focus of the current paper. Recent work suggests, for example, that interpersonal violence in home and community contexts may increase children’s risk of developing cardiovascular risk factors including obesity (Midei and Matthews 2011), and higher daytime (Gooding et al. 2016; Murali and Chen 2005) and nighttime (Wilson et al. 2002) blood pressure (also see Wright et al. 2017 for review of health correlates of community violence specifically). Over the long run, childhood violence exposure forecasts higher rates of adult morbidity from clinical manifestations of cardiovascular disease (CVD), including hypertension, diabetes, stroke, and myocardial infarction (Basu et al. 2017; Dong et al. 2004; Felitti et al. 1998; Midei et al. 2013; Riley et al. 2010; Suglia et al. 2015).

Despite the critical importance of these epidemiological observations linking childhood violence and adult CVD morbidity, it is also important to acknowledge two significant limitations that characterize many of these studies: the use of cross-sectional designs and retrospective self-reports of childhood violence (Basu et al. 2017; Suglia et al. 2015). These approaches have well-known limitations that complicate interpretation of the findings, including the possibility of response and recall biases (Baldwin et al. 2019; Hardt and Rutter 2004) as well as the more common threats to causal inference associated with observational and cross-sectional research (e.g., selection bias and residual confounding) (Shadish et al. 2002). With this in mind, a recent study has been particularly informative. In a large UK sample, Chandan et al. (2020) utilized electronic health record data to identify cases of documented and suspected maltreatment before the age of 18 and estimated their association with the development of CVD in adulthood. The authors found that childhood maltreatment was associated with increased risk of CVD events, type 2 diabetes mellitus, hypertension, and all-cause mortality. Relative to earlier studies, these findings allow for stronger inferences about long-term health consequences of violence because they draw on a large sample size (over 80,000 exposed to maltreatment and over 160,000 unexposed) and, importantly, because they make use of data prospectively collected by health care providers.

Of course, this was still an observational study and the associations could reflect the influence of underlying confounders that predispose the patients to both childhood violence and subsequent disease. These concerns are somewhat alleviated by another recent study that utilized a time-series fixed-effects design, linking spikes in community violence (local homicides and assaults) in California to hospitalizations
for stress-related diseases (Ahern et al. 2018). The authors found that when spikes in community violence occurred, rates of acute myocardial infarction, as well as asthma, substance use, and anxiety disorders rose in tandem. Because of this study’s within-community design – comparing neighborhoods to themselves during periods of higher versus lower community violence – many of the usual concerns about underlying confounds are minimized.

Studies of non-human animals are also highly informative from a causal inference perspective because they allow for experimental manipulation of stress exposure, eliminating much of the concern about potential underlying confounds. Furthermore, animal models can elucidate biological mechanisms underlying risk for health problems that are not directly measurable in humans. A valuable animal model for understanding causal effects of violence on health is the rodent model of repeated social defeat (RSD) – a social stress paradigm involving chronic (i.e., repeated) bouts of fighting between dominant “aggressor” and submissive “resident” mice, in which resident mice are eventually defeated (reviewed in Weber et al. 2017). Studies show that after RSD, defeated mice evidence anxiety-like behaviors (Wohleb et al. 2013), and in terms of physical health consequences, defeated mice show evidence of increased atherosclerotic plaque formation (Bernberg et al. 2012). Others have shown that social defeat increases abdominal fat deposits when paired with a fat- and sugar-rich diet (Kuo et al. 2007). These experimental data from animals, along with the extensive and large-scale observational findings in humans, suggest a scenario in which exposure to interpersonal violence causally increases risk for later cardiovascular health problems.

3 Inflammation as a Biological Mechanism Linking Childhood Violence to CVD

If there is empirical support for a causal association between childhood violence and CVD vulnerability, then it is important to understand mechanism(s) that could explain these associations. To be sure, any causal pathway from violence to disease is likely to be complex and to involve multiple systems and processes across development. For example, recent perspectives suggest that broad and interacting mechanisms involving mental health, lifestyle, and peripheral biology are likely involved (Suglia et al. 2018). That said, there is mounting evidence from both animal and human studies that excessive inflammation may represent a final common biological pathway onto which these processes converge (Elwenspoek et al. 2017; Fagundes et al. 2013; Miller et al. 2011a; Miller and Chen 2013; Nusslock and Miller 2016; Slavich and Irwin 2014; Wright 2011). As we discuss below, violence exposure appears to reliably upregulate inflammatory activity, particularly in innate immune cells known as monocytes. These cells play a key role in the precursor conditions that often set the stage for CVD, like obesity, insulin resistance, diabetes, hypertension, and metabolic syndrome (reviewed in Hotamisligil 2006). Monocytes
also trigger and maintain much of the vascular inflammation that drives growth of atherosclerotic plaque (Nahrendorf 2018). More generally, inflammation is now recognized as a key pathogenic mechanism in all stages of the atherosclerotic process, from the early formation of plaque, through its expansion and dissemination, and the eventual rupture (Libby et al. 2018; Teague et al. 2017) that precipitates acute coronary events like myocardial infarction and unstable angina.

3.1 Violence and Inflammation in Humans

What evidence is there for the hypothesis that childhood violence exposure is associated with inflammatory activity? Prior studies have reported associations between childhood abuse and biomarkers of low-grade inflammation in adulthood, including higher circulating levels of the cytokine interleukin-6 (IL-6; Bertone-Johnson et al. 2012; Gouin et al. 2012). Associations between childhood abuse and another commonly measured biomarker, the acute phase protein C-reactive protein (CRP) have also been observed in studies of adults (Bertone-Johnson et al. 2012; Matthews et al. 2014), although findings with CRP measured in adulthood have been mixed. For example, a recent meta-analysis (Baumeister et al. 2016) found no overall association between childhood sexual abuse or physical abuse and CRP levels in adulthood. They did, however, find modest significant associations between abuse and levels of IL-6, as well as tumor necrosis factor-α, another commonly measured inflammatory biomarker. Several recent large-scale epidemiologic studies have also examined associations between adverse childhood experiences (ACEs), which often include violence exposures (e.g., physical or sexual abuse) but also other types of adversities that can co-occur with violence (e.g., parent substance abuse, parent separation, parent death). In general, these studies have found that the accumulation of ACEs is associated with high circulating CRP levels in adulthood (e.g., Iob et al. 2020; Lin et al. 2016). In long-term epidemiologic studies of apparently healthy individuals, higher circulating levels of these inflammatory biomarkers forecast the development of diabetes, metabolic syndrome, coronary heart disease, and stroke (Ridker 2007, 2016). However, it is important to keep in mind that these biomarkers are probably not direct causal actors in disease, but instead proxies for a chronic low-grade inflammatory response occurring within arterial plaque, skeletal muscle, and visceral fat.

Again, there is ambiguity about the meaning and direction of many of these findings because most relied on single assessments of inflammation in adulthood and a simultaneous retrospective report of childhood adversity. In this regard, studies of children and adolescents are informative because they reduce the potential for error due to recall bias. For example, a recent study observed that sexual abuse before age 16 was associated with higher CRP levels at age 16, although there was some indication that this association was explained by body mass and health behaviors (i.e., nicotine or alcohol use; Jonker et al. 2017). Another recent study found that maltreatment was positively associated with a composite of circulating inflammatory
cytokines, but only in girls (and not boys) whose earliest experience of maltreatment was before the age of 5 years (Ehrlich et al. 2020). This finding is particularly informative from a policy perspective because it suggests a window of time early in development when the systems that regulate inflammation may be especially sensitive to violence-related stress.

Others have focused on community-level exposures and shown that children residing in high-crime or high-poverty neighborhoods have over twice the likelihood of elevated CRP than children residing higher-income or lower-crime neighborhoods (Broyles et al. 2012). And, noting the limitations of traditional inflammatory biomarkers such as IL-6 and CRP, a recent study of adolescents (Finegood et al. 2020) examined the relationship between community violence and pro-inflammatory classical monocytes, which, as noted above, are key cells involved in driving the early stages of atherosclerosis (Nahrendorf 2018). Finegood and colleagues observed a positive association between the level of neighborhood violence (as reflected in homicide rates) and counts of classical monocytes in circulation. Further, they observed that this association was moderated by youths’ own personal exposure to violence. Specifically, the positive association between neighborhood violence and monocyte count was only evident among youth who reported having been directly victimized by violence, witnesses of violence, or who had friends or family who had been victimized in the past year. These patterns suggest the possibility that violence at the neighborhood level may be an especially potent stressor for youth who have been victimized or witnesses to it in the past.

Studies with prospective longitudinal designs have also been conducted, and they provide more clarity on temporal precedence. For example, in one study of 972 individuals, those who had experienced maltreatment before age 10 were at increased risk of clinically high levels of CRP at age 32 (Danese et al. 2007). Another prospective study showed that severe childhood adversity and violence before age 12 was associated at age 18 with higher levels of soluble urokinase plasminogen activator receptor (suPAR) – an inflammatory biomarker that may be particularly sensitive to early life experience, and that has only recently begun to be measured in studies of childhood adversity (Rasmussen et al. 2020). Lastly, a twin analysis with a prospective design provided additional evidence to support a causal interpretation. It found that childhood victimization was associated with higher CRP in females but not males at age 18, independent of genetic influences on inflammation. Because of this study’s genetically informative design, it is possible to rule out that this association was confounded by genetic and/or family-level factors that could cause spurious associations (Baldwin et al. 2018).

3.2 Violence and Inflammation in Animals

Even the most rigorous human studies of violence exposure have observational designs, leaving open the possibility of confounding, selection, and other threats to causal inference. Fortunately, there have been numerous experimental studies in
animals, where causal effects of violence and biological mechanisms can be examined more closely. As discussed previously, a common paradigm involves repeated social defeat (RSD), where a dominant and aggressive intruder mouse is repeatedly placed into the cage of “resident” mice, which results in repeated violent interactions between intruder and residents – and the eventual defeat of residents (reviewed in Weber et al. 2017).

In an elegant series of studies, Sheridan and colleagues have shown that social defeat induces myelopoiesis, where classical monocytes are mobilized from the bone marrow into peripheral circulation. Once in circulation, these monocytes migrate to the spleen and brain, and other tissues where injuries or infections have occurred. In tissue these monocytes mature into macrophages and dendritic cells, assuming an aggressive pro-inflammatory phenotype marked by exaggerated cytokine responses to stimuli and relative insensitivity to inhibitory signals from glucocorticoids, which normally regulate the extent and duration of their activity (Bailey et al. 2007; Sheridan et al. 2006; Wohleb et al. 2012; Weber et al. 2017). As reviewed in Weber et al. (2017), this activity circles back to the brain, with inflammatory cytokines, particularly IL-1, modulating neural circuitry involved in threat and fear responding. The sympathetic nervous system is a key mediator of this chain-of-events. For example, these studies show that when beta-adrenergic receptor antagonists are given to animals before RSD, the stressor’s inflammatory consequences are prevented – indicating that stress-induced myelopoiesis is mediated by up-regulated sympathetic signaling after RSD (Wohleb et al. 2011).

Other studies have used a vicarious social defeat paradigm, where a “witness” mouse looks on while other mice engage in RSD (reviewed in Warren et al. 2020). The witness, despite not having actually engaged physically in the altercations, goes on to show increased expression of inflammatory cytokines (Finnell et al. 2018; Hodes et al. 2014). Taken together, the results from animal models converge with human findings and suggest that a causal effect of violence (e.g., direct victimization and even witnessing violence) on inflammation is plausible. Furthermore, this work suggests a bidirectional signaling pathway between the brain and monocytes in the periphery may be involved – a biological mechanism for how the perception of chronic threat in the environment, induced by violence, can promote a pro-inflammatory state in the periphery and activate fear circuitry in the brain.

### 3.3 Implications for CVD Risk?

Although these studies indicate that violence triggers inflammation, they focus on otherwise healthy animals and humans. Thus, it remains unclear whether this violence-induced inflammatory response is sufficient to hasten the progression of CVD. One recent study of adults addressed this question indirectly, using full-body PET/CT scanning to simultaneously quantify metabolic activity in patients’ amygdala (reflecting threat sensitivity), bone marrow (reflecting myelopoiesis), and aorta (reflecting arterial inflammation) (Tawakol et al. 2019). After observing that adverse
neighborhood conditions (including poverty and violence) significantly forecasted CVD morbidity and mortality over the subsequent 4 years, it incorporated PET/CT data into path analyses to identify underlying mechanisms. The results were consistent with a scenario wherein adverse neighborhood conditions, acting through higher amygdala activity, triggered myelopoiesis, arterial inflammation and contributed to the development of CVD (Tawakol et al. 2019). Although this study elegantly defines possible mechanistic pathways linking violence to the clinical manifestations of CVD in later life, there are important scientific and practical reasons to consider the life course developmental context in which this scenario unfolds. First, it is well known that atherosclerosis begins in the early decades of life (Berenson et al. 2005; Juonala et al. 2010; Strong et al. 1999). Second, as reviewed earlier, there is accumulating evidence to suggest that childhood violence exposures accelerate this process (Suglia et al. 2015). Finally, from a translational and policy perspective, childhood is likely to offer a window of opportunity for intervention, when the body has maximal plasticity to accommodate change, and multiple decades over which any benefits would compound.

4 Neural Adaptation to Violence During Childhood and Adolescence

Social experience in early life shapes brain development in ways that maximize survival within the environment (Cameron et al. 2005; Del Giudice et al. 2011; Gunnar and Quevedo 2007; Hanson and Gluckman 2014; Hofer 1987). During childhood, exposure to violence over prolonged periods can skew brain development toward phenotypes that support heightened vigilance and threat reactivity (Pollak 2008) and does so, in part, by sensitizing or enhancing activity in the neurocircuitry involved in emotion processing and threat detection (e.g., subcortical brain areas including the amygdala and limbic system; reviewed in McLaughlin et al. (2014)). For example, functional magnetic resonance imaging (fMRI) studies of adolescents suggest that youth previously exposed to family violence and maltreatment show increased activity in the amygdala and related brain areas while observing angry faces and other negatively-valenced stimuli (McCrory et al. 2011; McLaughlin et al. 2015). Community violence may also be associated with the development of amygdala and hippocampal volumes in adolescence (e.g., Saxbe et al. 2018). Furthermore, rodent models show that rats who as pups had been exposed to abuse-like maternal behaviors later show increased amygdala activity to subsequent stressors during adolescence (Raineki et al. 2012). The amygdala and other subcortical structures regulate sympathetic outflow from the brain (e.g., catecholamines) during acute psychosocial stress (Gunnar and Quevedo 2007; Sapolsky et al. 2000). Consistent with the idea that this neural circuitry and output can be calibrated by violence, earlier observational work in humans has shown that sexually abused girls tend to display higher levels of catecholamine excretion (De Bellis et al. 1994). Similarly,
Wilson et al. (2002) observed that boys who had heard about violence in their community had higher daytime catecholamine excretion than those who had not. Collectively, these insights from studies of children and adolescents suggest a scenario consistent with the animal work, whereby violence in early life tunes the brain toward a phenotype that supports heightened vigilance and reactivity via upregulation of the neural circuitry involved in threat processing (McLaughlin et al. 2014).

The developmental consequences of these early neurobiological adaptations to violence are not yet fully understood. However, studies of adults who experienced childhood violence suggest that such effects may be long lasting. For example, Dannlowski et al. (2012) found in a sample of adults retrospectively reporting on childhood maltreatment that those who had experienced maltreatment showed increased amygdala activity in response to angry faces in fMRI. Others found in a sample of adults who had been studied longitudinally since childhood that maltreatment was associated prospectively with altered functional connectivity between the amygdala and other brain areas including regions of the prefrontal cortex and the hippocampus (Jedd et al. 2015). If early neurobiological adaptations to childhood violence involving the amygdala are indeed sustained into adulthood, a viable hypothesis is that they contribute across the life course, via upregulation of pro-inflammatory processes, to the gradual progression of CVD.

5 Psychosocial Stress and Neuro-Immune Interaction

As we noted above, communication between the brain and monocytes in the periphery is bidirectional. That is, just as neural signals can regulate peripheral inflammation, so too can peripheral inflammation regulate neural activity in certain brain circuits and regions (Dantzer et al. 2008; Glaser and Kiecolt-Glaser 2005; Irwin and Cole 2011; Miller and Raison 2016; Nusslock and Miller 2016). Human evidence indicating peripheral-to-brain signaling comes from experimental laboratory studies of healthy volunteers administered a low-dose endotoxin (a component of the cell wall of gram-negative bacteria), which triggers a mild inflammatory response in the periphery. Compared to a group receiving placebo, those who received the endotoxin showed increased amygdala responses to threatening stimuli during fMRI (Inagaki et al. 2012; Muscatell et al. 2016). Furthermore, in response to monetary rewards, they showed decreased activity in the ventral striatum, a key brain area involved in reward processing (Eisenberger et al. 2010; Moieni et al. 2019). Given their experimental designs, these studies indicate that peripheral inflammation can modulate the way the brain processes threat and even reward.

Experimental work in animals substantiates these findings and further suggests that psychosocial stressors like violence may initiate this process, with implications for behavioral sensitivity to threat. Again, the evidence from models of social defeat is valuable. For example, studies show that RSD-induced anxiety-like behaviors are no longer present 24 days after the end of RSD. However, a single bout of acute
social defeat after 24 days is sufficient to re-establish anxiety-like behavior in previously-defeated mice – a pattern indicating a sensitization of the neurocircuitry regulating fear responses (Mckim et al. 2016; Wohleb et al. 2014). Importantly, this work shows that this reestablishment of anxiety-like behavior is dependent on monocytes in the periphery initiating a cascade of events culminating in inflammatory cytokines modulating brain areas involved in threat processing (e.g., the amygdala, hippocampus, and prefrontal cortex (Mckim et al. 2016; Wohleb et al. 2014).

Recent observational human research provides further evidence that chronic psychosocial stress could enhance the bi-directional signaling or “cross-talk” between peripheral inflammatory activity and these brain circuits (Nusslock and Miller 2016). For example, Miller and colleagues (2021b) observed in a sample of adolescents that higher levels of circulating inflammatory biomarkers were associated with increased amygdala reactivity to threatening stimuli during fMRI, but, importantly, this was only evident among youth who came from lower-income homes, where exposure to psychosocial stressors tends to be greater. Low-grade inflammation was also associated with increased activity in the ventral striatum, an area involved in reward and motivation, during a monetary reward task. Again, this pattern was only evident among adolescents from lower-income homes, suggesting that the chronic psychosocial stress associated with low socioeconomic status might potentiate this neuro-immune signaling (Nusslock and Miller 2016). The involvement of neurocircuitry mediating threat processing (i.e., the amygdala) but also motivation and reward (i.e., ventral striatum) implicates this neuro-immune signaling in other health processes known to both be associated with childhood adversity and to sustain inflammation (e.g., poor health behaviors and substance abuse, as well as psychiatric conditions including major depression; Miller and Raison 2016; Nusslock and Miller 2016). Severe psychosocial stress-induced upregulation of this neuro-immune network in the context of violence, if sustained across the course of development, would be expected to promote an atherogenic pro-inflammatory state in the periphery and the gradual development of cardiovascular disease. Figure 1 provides a visual description of this possible developmental process.

6 Risk and Resilience Processes

Even if violence does activate CVD-relevant neurobiological and inflammatory processes, disease is more likely but not certain. That is, many youths will not develop health problems after violence. Thus, a critical scientific question is whether there are factors (e.g., psychosocial, contextual, or person characteristics) that contribute to resilience along this developmental pathway (Luthar et al. 2000).

Work in developmental science indicates that biological stress response systems are, in part, socially-regulated, and that positive interpersonal relationships that young people have with others such as with parents and peers can be protective against stress-related health risks associated with adversity (reviewed in Hostinar
Fig. 1 Depiction of how exposure to violence during childhood could translate into a pro-inflammatory state that increases disease risk.
et al. 2014). Studies of adults have been consistent with this, in providing some indication that the family environment, and maternal warmth specifically, during childhood may be capable of buffering individuals against some of the immunologic and cardiometabolic health problems associated with early life adversity (Carroll et al. 2013; Chen et al. 2011; Miller et al. 2011b). Although again, insights in this area remain somewhat limited given that much of what is known has been derived from studies of adults that were not truly longitudinal with repeated measures of biology and health over time. Furthermore, because many of these studies rely heavily on adults’ retrospective reports of early life adverse experiences. Having said this, recently applied work helps to alleviate some of these concerns. For example, Miller et al. (2014) showed that a family- and parenting-based intervention designed to strengthen relationships among family members lowered inflammation in youth from low-income homes.

Person-level factors such as young peoples’ capacity for self-regulation may also be health-protective in the context of violence and adversity broadly. Self-regulation is broad construct reflecting an individual’s “…primarily, but not necessarily, volitional management of attention and arousal, including stress physiology and emotional arousal, for the purposes of goal-directed action” (Blair and Ursache 2011, p. 305). Exposure to acute and chronic stressors associated with early life adversity is hypothesized to shape the development of self-regulation (Blair and Raver 2012; Finegood and Blair 2017; Raver et al. 2013; Sharkey et al. 2012) and may do so in ways that undermine physical health (Hostinar et al. 2015). Recent findings suggest that functional connectivity in a network of brain areas supporting self-regulation, the central executive network (CEN), may be health-protective for youth exposed to community violence (Miller et al. 2018). For example, in a recent analysis of adolescents living in Chicago, Miller et al. (2018) found that youth living in neighborhoods with high homicide rates displayed worse cardiometabolic health – as reflected in obesity, insulin sensitivity, serum leptin, and a count score of metabolic risk – but only if they also evidenced lower functional connectivity in this brain network thought to support self-regulation. Consistent with this finding, in a follow-up analysis, Miller and colleagues (2021a) observed that youth living in high-violence neighborhoods and who evidenced low functional connectivity in the CEN brain network also had monocytes that expressed a pro-inflammatory phenotype. This phenotype was characterized by larger in vitro inflammatory cytokine responses to multiple stimuli, and lower sensitivity to agents that normally inhibit these responses. There was no such association between neighborhood violence and leukocyte activity for those with higher connectivity in this network. Collectively, these findings suggest a possible protective role of this brain network (and possibly of self-regulation or self-control more broadly) for youth living in high-violence contexts. More work is needed to understand the longer-term implications of this buffering for cardiovascular risks in the context of violence, and to understand whether the possible buffering effects of this brain network indeed extend to other components of self-regulation that have been studied in the developmental literature (e.g., executive functions, emotion regulation, and attention control).
7 Future Agenda, Unresolved Issues, and Conclusions

Looking ahead, there are an abundance of questions related to these hypotheses that are not yet resolved. For example, basic developmental questions concerning the importance of timing of exposure, chronicity, severity, and even type of violence exposure are not well understood in terms of their immune and CVD relevance and will require further theoretical and empirical elaboration. A focus on developmental and change processes within studies of children and adolescents will be especially important for addressing the many mechanistic questions that remain about how exactly childhood violence could come to accelerate inflammation and CVD progression. Aligned with this, efforts to boost causal inference in this area of inquiry will be important, given for example that most of the prior work has been conducted in large-scale cross-sectional studies of adults self-reporting on childhood violence, and also self-reporting on health. More emphasis on longitudinal study designs with repeated measures of violence exposure and biological mechanisms and health across development, which would allow for more rigorous testing of causal relationships, is needed. Because these designs can clarify the temporal patterning of risk exposure and biological processes, and thus allow for the exploration of change over time, they can facilitate answers to policy-relevant questions about risk and resilience – to identify factors exacerbating or reducing risk of developing non-resolving inflammation and accelerating CVD progression after violence.

Another consideration is that for many children and adolescents, violence exposure is only one of several co-occurring risk factors that they may confront in their daily lives. For example, low income and poverty are risk factors for violence exposure in the home and community, and are also risk factors for a range of other environmental and psychosocial stressors relevant to children’s development and physical health (e.g., chaotic home environments, harsh caregiving and parental separation, exposure to toxins/pollutants in the neighborhood; Evans 2004; McLoyd 1998). How to measure and understand co-occurring risk factors that can accumulate and interact with one another to affect developmental outcomes and health processes is a current topic of much research (Evans et al. 2013; McLaughlin et al. 2014; Raver et al. 2015). Applying developmental frameworks that acknowledge the multiple and changing contexts in which young people develop will be needed to better assess the health impact of childhood violence across development.

In conclusion, deeper understanding of the association between childhood violence exposure and cardiometabolic health across human development is needed. Inflammation is increasingly recognized as a possible biological mechanism in this association, and its association may be causal, although more human work along the lines we have outlined is needed to substantiate this claim. We also note the paucity of human research, especially in child and adolescent samples, concerning the specific stress-related neuro-immune mechanisms that could link childhood violence to inflammation and CVD. In this paper, we have thus drawn on a number of recent and relevant findings as well as the theoretical insights of others (Cicchetti and Toth 1995; Irwin and Cole 2011; Margolin and Gordis 2000; McLaughlin et al. 2014;
Miller and Raison 2016; Nahrendorf 2018; Nusslock and Miller 2016; Pollak 2008; Sheridan et al. 2006; Suglia et al. 2015; Tawakol et al. 2019; Weber et al. 2017) to suggest a developmental mechanistic hypothesis for one way that childhood violence exposure could get “under the skin” to increase CVD vulnerability. To further elaborate this possible mechanism, closer attention should be paid to developmental processes as well as to contexts that could support resilience along this developmental pathway. This understanding will be needed to inform future research and practice aiming to reduce disease burden among those who are exposed to violence in early life.

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Social Support Effects on Neural Stress and Alcohol Reward Responses

Nia Fogelman, Seungju Hwang, Rajita Sinha, and Dongju Seo

Abstract  Social support (SS), or having people to depend on during times of stress, may offer an emotional and neurological buffer to problem drinking. Specifically, SS may modulate reward and stress-related brain responses to mitigate perceptions of alcohol reward and stress. There is limited evidence, however, on this topic and specifically on brain networks that may modulate SS effects on stress and alcohol reward. Here we present a review of the current literature on this topic as well as data from a large community sample of 115 social drinkers. Findings from a novel fMRI task viewing alcohol cue, stress, and neutral images, in separate blocks, while providing ratings on subjective feelings of alcohol craving, stress, and arousal are included. Lower SS significantly predicted greater alcohol craving during alcohol...
cue and stress conditions, higher baseline levels of stress, and greater arousal in the alcohol cue, relative to neutral condition. Remarkably, individuals with low SS showed greater reward activation (ventral medial prefrontal cortex (VmPFC) and ventral striatum) during alcohol cue exposure, while those with high SS showed no such activation ($p < 0.001$, family wise error corrected at 0.05). Furthermore, individuals with lower SS showed greater stress circuit (VmPFC, dorsal striatum, and periaqueductal gray) activation not observed in the high SS groups. Both groups showed increased amygdala activation under stress condition. The findings support the notion that SS is a powerful modulator of stress response and reward motivation. High SS buffers neural and subjective stress responses, while low SS potentiates greater reward seeking with higher alcohol craving and greater brain activation during alcohol cue exposure. Previous research and current results suggest the need to further explore the role of SS in those at risk of developing alcohol use disorder and assess novel prevention strategies to boost SS in at-risk drinkers.

**Keywords** Alcohol use · fMRI · Neural response · Social support · Stress

### 1 Introduction

Social Support (SS) is the presence of a network of individuals that one can turn to in a time of need or stress, whether for emotional discussion, practical help, validation of self worth, or sense of group membership (Cohen and Wills 1985; Cohen and Hoberman 1983a). SS is a well-known and critically important, healthy method of coping with stress (Schwarzer and Knoll 2007). Receiving SS has been subjectively and neurologically identified as a rewarding experience (Bhanji and Delgado 2014). Given that these properties overlap with alcohol use and its related circuits (Sinha 2001; Volkow et al. 2011), SS may represent one pathway for mitigating the desire to drink and subsequent development of Alcohol Use Disorder (AUD). Finding a mitigating pathway is paramount, as AUD is a pervasive illness (Substance Abuse and Mental Health Services Administration 2019), linked to the development of physical health problems (Fuster and Samet 2018; Roerecke and Rehm 2014; Hillbom et al. 2011), mental health problems (Burns and Teesson 2002; Grant et al. 2004), and greater overall mortality (Stockwell et al. 2016). In this chapter, we review the literature connecting SS to drinking, as well as explore how SS and drinking may offer competing coping mechanisms via reward and stress pathways. We then present some of our own work supporting this theory. We conclude with future questions and directions for the field of neuroscience in relation to SS, problematic drinking, and alcohol addiction.
1.1 The Behavioral Connection Between Social Support and Drinking

Prior work has found that greater SS is associated with a lower risk of drinking across populations. For instance, greater SS lessened the positive relationship between stress and drinking in US military personnel (Kelley et al. 2017). SS has also been associated with less drinking in college students (Pauley and Hesse 2009), among those who are socioeconomically disadvantaged (Budescu et al. 2011), and in older adults (Villalonga-Olives et al. 2020). Indeed, a recent Cochrane review highlighted the benefits of Alcoholics Anonymous and similar 12-step therapies rooted in SS as beneficial to long-term abstinence in those with AUD (Kelly et al. 2020).

There are a few theories about why SS would have an inverse relationship to alcohol consumption. The Main or Direct Effect Theory suggests that increasing SS leads to a generally positive attitude, thereby promoting healthy behaviors overall and diminishing one’s desire to behave in an unhealthy manner, including problematic alcohol consumption (Cohen and Wills 1985; Uchino 2004; Moon et al. 2019). This means that someone would benefit from SS regardless of initial SS levels. The Optimal Matching Model, alternatively, suggests the benefits of SS are variable, where different types of SS are more or less beneficial in preventing drinking behaviors depending on stressors and other factors in peoples’ lives (Moon et al. 2019; Beattie and Longabaugh 1997; Cutrona 1990). The Stress Buffering Model represents a hybrid, suggesting that SS is most useful in decreasing drinking and increasing help seeking in the presence of stress or strain, but that it is not dependent on the stressor type (Cohen and Wills 1985; Dobkin et al. 2002; Peirce et al. 1996). Taken together, these theories provide a useful framework for assessing the role of SS on alcohol intake and risk of problem drinking, both in the context of stress and in the direct potential benefit of SS. Critically, when people feel stress, strain, or craving, they may be more prompted to cope with these negative emotions by drinking (Sinha 2001, 2007) and those with high SS may be less likely to drink even in the absence of a stressor.

1.2 The Neuroscience of Social Support and Alcohol Intake

Although there is evidence of behavioral effects of SS on alcohol intake, the neurobiological basis for this relationship has yet to be fully understood. One proposed common and overlapping area is that both SS and alcohol stimulate reward pathways in the brain. SS is a positive social experience and has been subjectively and neurologically reported as rewarding (Bhanji and Delgado 2014). Consistent with this, receiving SS has been linked to greater ventromedial prefrontal cortex (vmPFC) activity, a region that modulates striatum activity, particularly ventral striatum activity, a reward center of our brain (Bhanji and Delgado 2014;
Alcohol is also rewarding, eliciting euphoria as well as inducing the desire to drink in order to cope with a multitude of emotions (Koob 2011). Brain imaging research has shown that alcohol increases activity in the brain reward regions of the ventral striatum, midbrain (ventral tegmental area), prefrontal cortex (PFC; including anterior cingulate cortex (ACC), and lateral orbital frontal cortex (latOFC)), as well as emotional limbic regions (amygdala and hippocampus, insula) (Volkow et al. 2011; Koob et al. 1994). With heavy and consistent alcohol use, tolerance, withdrawal and sensitization processes drive neuroadaptations in these same circuits that promote greater craving, higher stress, and increased risk of continuing alcohol intake (Volkow et al. 2011; Koob 2011; Sinha 2012; Robbins and Everitt 2002). Critically, questions remain of whether adaptations in these overlapping stress and reward regions also render the neurobiology susceptible to not only greater stress but also to increased reward seeking and whether these neural responses are modulated by SS.

An important aspect of SS is its ability to mitigate threat and stress brain networks (e.g., dorsal ACC (dACC), anterior insula (AI), periaqueductal gray (PAG), amygdala, and vmPFC (Eisenberger 2013)), which overlaps with alcohol reward circuitry (Blaine and Sinha 2017). Past research suggests that alcohol and acute stress cues are both associated with vmPFC and ACC activity (Seo et al. 2013). Dysregulation of this circuitry may be highly involved in alcohol craving and compulsive alcohol intake lapse in problematic drinking (Sinha 2012). If SS acts along these same pathways, increased SS may modulate not only reduction in neural stress responses, but also a reduction in reward seeking, buffering both the ill neural effects of stress and excessive alcohol reward. Therefore, SS may have an impact on both reward and stress-related circuitry.

### 1.3 Social Support Mitigating Social Stress

Although there is strong theoretical evidence that SS would affect alcohol and stress-related neural pathways, few studies have tested this directly and, to the best of our knowledge, never in the context of alcohol stimuli (for an overview see Table 1). A number of studies have examined SS in the context of social stress and pain. Some research indicates that SS lowers dACC, AI, and PFC activity, amongst other regions when facing a social stressor (Coan et al. 2006; Eisenberger et al. 2007; Onoda et al. 2009; Masten et al. 2012; Morese et al. 2019). However, others have also seen no relationship between receiving SS and brain activity during social stress (Hyde et al. 2011; Inagaki et al. 2016) and suggest providing rather than receiving SS may be a buffering pathway (Inagaki et al. 2016). Of note, studies with null findings employed a priori region of interest (ROI) analysis, whereas the studies that did have significant findings conducted whole brain (corrected) analyses; whole brain analysis may enable the identification of additional significant regions involved in SS. While current SS and stress-related studies provide insights into neural stress regulation by SS, one limitation is that many of these studies focus on only one
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Sample</th>
<th>Average alcohol consumption</th>
<th>Social support measurement</th>
<th>fMRI stress paradigm</th>
<th>Findings</th>
<th>Social support and neural activation relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coan et al.</td>
<td>2006</td>
<td>16 Women (31 years old)</td>
<td>NP</td>
<td>3 Social support conditions: holding husband’s hand, holding male stranger’s hand, or control (no support)</td>
<td>Shocks to the ankle while viewing a fixation cross</td>
<td>Greater marriage quality + husband hand holding = less activity in left SFG, left AI, and hypothalamus</td>
<td>—</td>
</tr>
<tr>
<td>Eisenberger et al.</td>
<td>2007</td>
<td>32 Adults (20.6 years old)</td>
<td>NP</td>
<td>Ecological momentary assessment data on “supportiveness of their most recent social interaction partner”</td>
<td>Cyberball task</td>
<td>Social support negatively correlated with dACC, BA8, thalamus, caudate, BA20, and PAG</td>
<td>—</td>
</tr>
<tr>
<td>Onoda et al.</td>
<td>2009</td>
<td>26 Adults (21.7 years old)</td>
<td>NP</td>
<td>Emotional support text messages during a social exclusion task</td>
<td>Modified cyberball task (3 conditions: social inclusion, social exclusion, emotional support)</td>
<td>Emotional support relative to social exclusion: greater activation in vACC, right SPL, left VC and lower activation of the left dIPFC, left STS, and right caudate</td>
<td>—/+</td>
</tr>
<tr>
<td>Karremans et al.</td>
<td>2011</td>
<td>15 Adults (22 years old)</td>
<td>NP</td>
<td>Choosing an “attachment” and “non-attachment” figure and reporting how securely attached to each they were</td>
<td>Modified cyberball task (3 conditions: social inclusion, social exclusion, and control run twice imagining the)</td>
<td>Greater attachment during social exclusion to the attachment figure was associated with decreased activation of the left MFG</td>
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<tr>
<th>Authors</th>
<th>Year</th>
<th>Sample</th>
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<th>fMRI Stress paradigm</th>
<th>Findings</th>
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</thead>
<tbody>
<tr>
<td>Hyde et al.</td>
<td>2011</td>
<td>103 Adults (44.5 years old)</td>
<td>Interpersonal support evaluation list (ISEL)</td>
<td>NP</td>
<td>Social support did not predict amygdala activity</td>
</tr>
<tr>
<td>Masten et al.</td>
<td>2012</td>
<td>21 Adolescents (17.8 years old)</td>
<td>Daily diary hours spent with friends</td>
<td>NP</td>
<td>No relationship with social support giving and receiving social support negatively related to amygdala, dACC, AI and right amygdala</td>
</tr>
<tr>
<td>Inagaki et al.</td>
<td>2016</td>
<td>36 Adults (22.4 years old)</td>
<td>2-Way social support scale</td>
<td>NP</td>
<td>No relationship with receiving social support giving negatively correlated with dACC, AI and right amygdala</td>
</tr>
<tr>
<td>Morose et al.</td>
<td>2019</td>
<td>81 Women (21.7 years old)</td>
<td>Multidimensional scale of perceived social support (MSPSS)</td>
<td>NP</td>
<td>No emotional support or informational support activity in the amygdala was associated with lower SS</td>
</tr>
<tr>
<td>Sato et al.</td>
<td>2020</td>
<td>51 Adults (22.5 years old)</td>
<td>Multidimensional scale of perceived social support (MSPSS)</td>
<td>NP</td>
<td>No emotional support or informational support activity in the amygdala was associated with lower SS</td>
</tr>
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</table>

Table 1 (continued)
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Group Description</th>
<th>AUD: 14 day abstinence</th>
<th>Control: 14 day abstinence</th>
<th>Cyberball task (4 conditions: explained social exclusion, social inclusion, social exclusion, re-inclusion)</th>
<th>Social exclusion &gt; explained social exclusion: AUD: right dACC, insula, precentral gyrus, IFG, left PCC AUD &gt; control: greater right insula, less left VPFC and less MFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maurage et al.</td>
<td>2012</td>
<td>22 Males with AUD (47.2 years old) 22 control males (45.1 years old)</td>
<td>19.6 (10.59) drinks per day</td>
<td>1.54 (1.15) drinks per day</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Groefsema et al.</td>
<td>2019</td>
<td>153 Males (AUD and SD) (22.8 years old)</td>
<td>Total average weekly drinks: 18.09 (13.26)</td>
<td>None</td>
<td>4 Image conditions: social alcohol, non-social alcohol, social soda, non-social soda</td>
<td>[(Social alcohol &gt; non-social alcohol) − (social soda &gt; non-social soda)] greater STS and left inferior parietal lobe shared by alcohol &gt; soda and social &gt; non-social: greater left SFG and vmPFC</td>
</tr>
</tbody>
</table>

NP not provided, AUD those with alcohol use disorder, SD social drinkers, SFG/IFG/MFG Superior frontal gyrus/Inferior frontal gyrus/middle frontal gyrus, dACC or vACC dorsal or ventral anterior cingulate cortex, PAG periaqueductal gray, BA8 Brodmann’s Area 8, part of frontal cortex, BA20 Brodmann’s Area 20, ventral temporal cortex, AI anterior insula, TPJ temporal parietal junction, vmPFC and dlPFC ventromedial or dorsolateral prefrontal cortex, SPL superior parietal lobule, VC visual cortex, STS superior temporal sulcus, STG superior temporal gyrus, PCC posterior cingulate cortex
social stress paradigm (Cyberball; (Eisenberger et al. 2007; Onoda et al. 2009; Masten et al. 2012; Morese et al. 2019; Karremans et al. 2011)). Such a paradigm has been considered a proxy for social exclusion and social pain (Williams and Jarvis 2006; Eisenberger 2012), which does not necessarily generalize to other types of stressors (e.g., acute stress). It remains unclear whether the experience of SS alters bio-behavioral responses to acute stress.

1.4 Social Support Mitigating Alcohol-Related Effects

Evidence in human neuroscience assessing the effects of SS in the context of alcohol-related cues is limited. One study examined neural activity during social stress provocation in those with AUD compared to those without (Maurage et al. 2012), finding greater right insula, left VPFC, and left middle frontal gyrus (MFG) activity in those with AUD. Another study presented alcohol-related and non-alcohol (soda) related visual stimuli in social and non-social contexts amongst those with a range of drinking habits (Groefsema et al. 2020). They found that alcohol stimuli in a social context was uniquely related to increased superior temporal sulcus (STS) and left inferior parietal lobe activity, but that both the alcohol and social stimuli were associated with heightened left superior frontal gyrus and VmPFC activity. Neither of these studies examined SS directly.

1.5 Questions and Hypotheses

Overall, the literature suggests that SS may buffer against neural stress and reward responses by modulating key regions involved in such responses, namely the striatum (ventral and dorsal), amygdala, and their modulation by the VmPFC and the anterior cingulate cortices. However, whether SS mitigates subjective and brain response to alcohol cues and non-social acute stress in social drinkers, ranging from light/moderate to binge heavy drinkers, is not known. Therefore, we recruited a group of healthy social drinkers to address these questions. On the basis of the previous literature review, we hypothesized that greater SS would be associated with diminished reward responses (i.e., lower VmPFC and striatal response) to alcohol cues, and reduced amygdala response to stress stimuli, indicative of buffering of both stress and reward responses. We therefore also expected reduced subjective experience of stress and alcohol craving in the stress and alcohol cue conditions, respectively.
2 Methods

2.1 Participants

One hundred and fifteen healthy social drinkers (ages 18–57; 53\% female) were recruited from the greater New Haven, CT area. Participants were excluded from participation if they had a history of psychotic disorders, were on medications for current medical, psychiatric, or substance use disorders (not including nicotine), and pregnancy for female participants. Additionally, since this study involved fMRI, participants were excluded for having metal in their bodies, reporting claustrophobia, or having an incident of loss of consciousness for longer than 30 min. The Yale University Institutional Review Board approved all study procedures.

2.2 Procedures

After an initial phone screening, participants came into the laboratory for two to three intake sessions to ensure eligibility and complete psychological assessments. The Structured Clinical Interview for DSM-5 (SCID-I) (First et al. 2015) was used to rule out current moderate to severe AUD and other current psychiatric diagnoses. Baseline measures and questionnaires, including SS, alcohol usage, and demographics questions, were also captured during intake. All subjects then underwent an fMRI scan.

2.3 Measures

2.3.1 Demographics and Drinking Levels

Participants provided demographic information via interview, including their age and sex. The sample included 59 light to moderate (never binging) individuals and 56 who were binge and heavy drinkers, and none who met DSM-5 criteria for current moderate to severe AUD.

A binge episode was defined as four or more drinks for women and five or more drinks for men during a 2 h period (Abuse, N.I.o.A. and Alcoholism 2004). Anyone who was meeting these criteria more than 4 times a year (AUDIT, drinking severity score of eight or higher for borderline cases (Saunders et al. 1993)) was considered a binge/heavy drinker. Everyone else was classified as a light drinker.
2.3.2 Interpersonal Support Evaluation List (ISEL)

The ISEL is a 40-item questionnaire assessing social support (Cohen and Hoberman 1983b). Items like “Most people think highly of me” and “I don’t often get invited to do things with others” (reverse coded) are rated as true or false. Items are summed, with higher numbers indicating greater SS. This questionnaire had excellent reliability within our sample (Cronbach’s $\alpha = 0.91$).

2.3.3 Alcohol Use Disorder Identification Test (AUDIT)

The AUDIT is a 10-item questionnaire, where higher scores are associated with greater drinking severity and problematic drinking levels (Saunders et al. 1993). Questions ask about typical numbers of drinks and the how often severe consequences from drinking have occurred, measured on a 5-point scale. Reliability was good within our sample (Cronbach’s $\alpha = 0.76$).

2.3.4 fMRI Acquisition

fMRI acquisition followed the procedures described previously (Blaine et al. 2020). A 3T multiband Siemens Trio or Prisma MRI scanner with a standard quadrature head was employed. Functional scans were acquired with a T2*-sensitive gradient-recalled single echo planar pulse sequence. Blood oxygenation level dependent (BOLD) signal was measured with a 64-channel head coil with multiband-accelerated, echo planar imaging sequence. Across the whole brain, 75 axial slices parallel to the anterior commissure-posterior commissure (AC-PC) line were captured with TR = 1,000 ms, TE = 30 ms, bandwidth = 1,894 Hz/pixel, flip angle = 55°, field of view = 220 × 220 mm, slice thickness = 2 mm and no gap.

2.3.5 fMRI Provocation

Using a well-validated sustained emotion provocation task (adopted from (Blaine et al. 2020; Sinha et al. 2016)), participants were presented with three blocks of images, counterbalanced across participants: (1) alcohol cue related (i.e., pictures of alcoholic drinks, and individuals consuming alcohol), (2) stress (i.e., highly aversive, disgusting, and disturbing images), and (3) neutral/relaxing (i.e., calming scenery). Each block was comprised of nine runs. This included three baseline runs (gray screen with fixation cross) and six 1-min provocation runs with condition-specific visual images. Eleven images per run, with each image lasting for 5s plus a 1s interstimulus interval were included for the six condition-based provocation runs. At the end of each of the nine runs per condition, participants rated their craving for alcohol, and subjective experiences of stress and arousal on a
Likert-type scale ranging from 1 (not at all) to 9 (the most possible). For more details on the provocation task, see (Blaine et al. 2020).

2.4 Data Analyses

All behavioral data analyses were conducted in R v. 3.6.1 (Team RC 2019). T-tests and Fisher’s exact tests were used to examine demographic differences by SS. We employed linear mixed effects (LME) models with a random intercept using the lmerTest package (Kuznetsova et al. 2017) to test differences in alcohol craving, stress, and arousal. Within subject effects included comparing conditions (alcohol cue, stress, and neutral) as well as comparing runs (3 baseline and 6 condition based). SS was included as a continuous predictor in these models. Models were covaried for age and gender.

All fMRI data were analyzed using AFNI (https://afni.nimh.nih.gov) and whole brain voxel-based analyses. Preprocessing and data analyses steps follow those previously described (see (Blaine et al. 2020) for details), including motion correction for three translational and three rotational directions. First level individual data were processed with BioImage Suite (www.bioimagesuite.org) using a General Linear Model (GLM) to process whole brain data by run and condition. For each condition, functional images were spatially smoothed with a 6 mm Gaussian kernel, and then individually normalized beta maps were generated (3.44 mm × 3.44 mm × 4 mm). For second-level group analysis, age and gender covaried whole brain voxel-based analyses with random intercept LME models were conducted using 3dLME in AFNI. Whole brain significance threshold was set at $p = 0.001$ and a cluster correction of $\alpha = 0.05$ was employed to correct for multiple comparisons. SS was assessed as a categorical predictor via median split of the ISEL score in order to aid in contrast interpretability. High and low SS groups were equivalent on drinking intensity.

3 Results

3.1 Sample Characteristics

The sample of 115 individuals was slightly more female (53%), predominantly Caucasian (59.1%), and approximately 28 years old. SS was generally high in the sample, with an average score of 33.4 (SD = 6.6). Descriptive information on the sample overall and by SS (median split: ISEL score of 35) are presented in Table 2. The low and high SS group were not significantly different in demographic variables, binge drinking status (split of low/moderate versus binge/binge heavy drinkers), drinking severity or average number of daily drinks.
3.2 Subjective Responses to Alcohol Cue, Stress, and Neutral Provocation

LME models testing SS effects on condition by run interactions predicting alcohol craving, stress, and arousal were conducted. Significant effects of SS were present on all three subjective ratings outcomes. While no three-way interaction was present, SS significantly interacted with the condition (F(2, 2,925) = 20.94, p < 0.001), and with the run (F(8, 2,925) = 2.04, p < 0.039) to predict alcohol craving. Across runs, lower SS predicted more craving in the alcohol and stress conditions (p’s < 0.006), but not in the neutral condition (p = 0.16). Across conditions, lower SS was associated with more alcohol craving in the provocation runs (p’s < 0.019), while no association was seen in the baseline runs (p’s > 0.063). See Fig. 1a, b.

SS significantly interacted with runs to predict stress (F(8, 2,925) = 2.22, p < 0.024). Lower SS significantly predicted higher stress ratings in the baseline runs across conditions (p’s < 0.045), but not during the task-related runs (p’s > 0.21). See Fig. 1c.

SS × Condition effects emerged in predicting subjective arousal (F (2, 2,925) = 4.21, p < 0.015). Averaging across all runs, lower SS predicted more arousal in the alcohol cue relative to neutral condition (p < 0.005) and a trending difference for alcohol cue relative to stress (p < 0.052). However, there was no significant relationship between SS and arousal across baseline and provocation runs within each condition (p’s > 0.28). See Fig. 1d.

<table>
<thead>
<tr>
<th>Table 2 Demographics by social support (SS)</th>
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<tbody>
<tr>
<td>Total (N = 115)</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Race and ethnicity</td>
</tr>
<tr>
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<tr>
<td>Asian</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Agea</td>
</tr>
<tr>
<td>Educationa</td>
</tr>
<tr>
<td>Binge heavy drinkersb</td>
</tr>
<tr>
<td>AUDIT scorea,b</td>
</tr>
<tr>
<td>Average daily drinksc</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>*Mean (Standard Deviation) are provided for continuous variables, with group differences assessed by t-tests. Categorical variables are represented by number and proportion (N[%]) by column, with group differences assessed with Fisher’s exact test. Groups were not significantly different on any of these variables.</td>
</tr>
<tr>
<td>aIndicates the variable was continuous</td>
</tr>
<tr>
<td>bThe Low SS has data on 58 participants</td>
</tr>
<tr>
<td>cTotal drinking data available for 92 participants, 50 low SS and 42 high SS</td>
</tr>
</tbody>
</table>
Fig. 1 Subjective ratings to alcohol cue, stress, and neutral stimuli. 

Panel (a) A Condition × Social Support interaction predicted craving during the imaging task ($F(2, 2,925) = 20.94, p < 0.001$), with significantly greater average craving across all runs in the alcohol and stress, but not neutral, conditions (Alcohol: $-0.295, t(120) = 3.6, p < 0.001$; Stress: $-0.236, t(120) = 2.68, p < 0.006$; Neutral: $-0.118, t(120) = 1.42 p < 0.16$). Panel (b) Run × Social Support interaction predicted greater craving across conditions, where lower social support was associated with greater craving across conditions in runs 1–6 (task-related runs; $p$’s < 0.019), but no baseline differences were observed (3 baseline runs $p$’s > 0.063). Panel (c) A Run × Social Support effect significantly predicted stress ratings during the imaging task ($F(8, 2,925) = 2.22, p < 0.024$) such that lower social support was associated with greater stress during the baseline runs ($p$’s < 0.045) but not during the task-related runs ($p$’s > 0.21). Panel (d) A Condition × Social support effect emerged in predicting arousal ratings during the imaging task ($F(2, 2,925) = 4.21, p < 0.015$). Further exploration of the effect suggested that social
3.3 **Functional Neural Responses to Alcohol Cue and Stress Relative to Neutral Provocation**

As SS significantly predicted subjective stress, arousal, and craving ratings, fMRI analyses were conducted dividing the sample into those with high and low SS and comparing the fMRI neural responses to stress and alcohol cues. Results showed different patterns of neural activity between high SS and low SS during stress and alcohol cues relative to the neutral condition ($p < 0.001$, FWE corrected at 0.05). Exposure to the stress relative to neutral condition led to greater left amygdala activity in both high and low SS groups. In addition, the low SS group showed significantly greater activity in the right amygdala, VmPFC, right ventral and dorsal striatum, and midbrain (including PAG) and decreased activity in the cerebellum during stress relative to neutral contrast, which was not seen in the high SS group. Furthermore, in the alcohol cue relative to neutral condition, those with low SS exhibited greater VmPFC, ventral striatum, and right dorsal striatal activity and decreased activity in posterior brain regions including the cerebellum and fusiform gyrus, not observed in the high SS group. On the other hand, greater left amygdala but lower right AI activity was seen in the high SS group (Fig. 2).

4 **Discussion**

SS is known to buffer stress and pain (Eisenberger et al. 2007; Morese et al. 2019; Hyde et al. 2011) but its examination of impact on reward has been limited. Social reinforcement models have framed SS in positive reinforcement terms (Ditzen and Heinrichs 2014). Emerging evidence also suggested the potential, beneficial role of SS in reducing alcohol consumption (Pauley and Hesse 2009; Groh et al. 2007), but its effects on modulating acute stress and alcohol reward have been limited. Thus, here we sought to explain how SS influences alcohol coping by presenting primary findings on the effects of SS on behavioral and neural responses to stress and alcohol reward cues in social drinkers.

Even after controlling for drinking levels of the sample, lower SS was associated with greater alcohol craving and arousal in processing alcohol stimuli and greater overall subjective stress. This is congruent with previous research showing that high SS lessens the relationship between daily stressors increasing the probability of craving in an AUD recovery population (Ames and Roitzsch 2000). It also matches findings of a significant inverse relationship between SS and basal stress in other

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**Fig. 1** (continued) support did not predict arousal significantly within any condition ($p$’s > 0.28), but that there was a significantly stronger inverse relationship between social support and arousal in the alcohol relative to neutral condition ($p < 0.005$) and a trending difference between alcohol and stress ($p < 0.052$)
populations, including healthy adult women (Stein and Smith 2015) and college students (Chao 2012). The significant effects of SS on alcohol craving and subjective arousal and stress led to evaluating SS categorically in the assessment of neural stress and reward responses. We saw evidence that low SS was associated with a hyperactive response to stress exposure in ventromedial prefrontal as well as limbic (bilateral amygdala, hippocampus, insula) and striatal (ventral and dorsal) regions, suggesting sensitized responses in stress processing networks (Sinha et al. 2016). In the stress condition relative to neutral, activation of the left amygdala was present regardless of level of social support. However, those with low social support also displayed greater activity in the VmPFC, dorsal striatum, and PAG. Panel (b) In the alcohol condition relative to neutral, those with high social support displayed greater amygdala activity. Low social support was associated with increased activity in the vmPFC and in the dorsal and ventral striatum (related to reward processing). Results represent significance at voxel $p < 0.001$ and cluster corrected to $p = 0.05$.

**Fig. 2** SS group effects on brain response to alcohol and stress cues relative to neutral. Red/Yellow color = Stress > Neutral, Alcohol cue > Neutral; Blue/Purple color = Stress < Neutral, Alcohol cue < Neutral; PAG periaqueductal gray matter, VmPFC ventromedial prefrontal cortex, ACC anterior cingulate cortex. Panel (a) The stress condition elicited amygdala activity, regardless of level of social support. However, those with low social support also displayed greater activity in the VmPFC, dorsal striatum, and PAG. Panel (b) In the alcohol condition relative to neutral, those with high social support displayed greater amygdala activity. Low social support was associated with increased activity in the vmPFC and in the dorsal and ventral striatum (related to reward processing). Results represent significance at voxel $p < 0.001$ and cluster corrected to $p = 0.05$. 

Social Support Effects on Neural Stress and Alcohol Reward Responses 475
is integral to habit learning (Knowlton et al. 1996), stress processing (Seo et al. 2011), and anticipating errors in prediction (Balleine et al. 2007). Activation of these regions overall in the low SS group (but not high SS) supports the stress sensitization effects of those with low stress buffering and also supports a role for SS in mitigating instrumental emotion-action and habit based responses during acute stress.

Brain responses to alcohol cues relative to the neutral cues were also modulated by SS. Those with low SS showed greater activity in the ventral and dorsal striatum during alcohol cue exposure, concurrent with greater subjective alcohol craving, an effect not seen in those with high SS. This suggested that lower SS was associated with greater neural and subjective reward seeking including activation in brain regions involved in reward processing, motivation, craving and instrumental and habit learning (Sinha et al. 2005; Wrase et al. 2007; Vollstädt-Klein et al. 2010). The VmPFC, a region involved in modulating reward and emotional salience (Hiser and Koenigs 2018), also displayed greater activity. This is congruent with prior findings. For example, in an odor/visual imaging task, an alcohol odor elicited greater VmPFC when compared to both a neutral appetitive odor and a control odor (Cyders et al. 2014). The simultaneous hyperactivity of the VmPFC, dorsal, and ventral striatum in those with low SS suggests that these individuals may have difficulties regulating alcohol-related urges in response to alcohol cues. Together, these results point to the beneficial effects of higher SS on regulating behavioral and neural responses to alcohol cues and conversely, increased risk of higher alcohol seeking and greater reward processing in those with lower SS.

To summarize, the neurobehavioral results presented here suggest that low SS is associated with increased response to the ventromedial-limbic-striatal circuit during stress and alcohol cues (for a schematic overview, Fig. 3). During stress, those with low SS may have a sensitized response in the stress pathways involving the VmPFC,
right amygdala, striatum, and PAG, as well as during alcohol cues, showing hyper-active responses in the reward pathways including the VmPFC and striatum. This sensitized neural activity in response to emotionally challenging cues may lead to difficulties regulating emotional distress or rewarding cues resulting in greater alcohol craving and increased risk of using alcohol, perhaps even as substitute for natural social reward in low SS individuals. An inability to reduce or modulate these subcortical regions of emotion may lead to greater perceived levels of social distress and difficulty resisting alcohol urges. This is consistent with a recent neuroimaging study showing an inverse relationship between SS and resting state amygdala activity (Sato et al. 2020). Since SS represents an important coping mechanism, lack of SS may increase the likelihood of turning to drinking during stressful and high alcohol reward situations (Catanzaro and Laurent 2004). When drinking alcohol is implemented as a means of coping, this greatly increases the likelihood of developing AUD (Cooper et al. 1988). Supporting this, those who experience social isolation and loneliness engage in hazardous drinking and smoking behaviors more frequently (Shankar et al. 2011; Niño et al. 2016). Therefore, those with high SS may show greater resilience in stress-related coping and lower vulnerability to alcohol misuse. To illustrate the neural link between social stress and alcohol use, a schematic diagram (Fig. 4) representing an overview of brain regions involved in social stress and alcohol reward related circuitry as well as supporting regions overlapping both circuits. Altered activity in the VmPFC, dACC (stress and reward regulation), and dorsal striatum (habitual processing) may play a role in promoting stress and alcohol cue related habitual and disinhibited behaviors, leading to
maladaptive coping (e.g., alcohol craving and use). Other supporting regions include the ventral striatum (reward processing), amygdala, and periaqueductal gray matter (stress processing). However, given a substantial lack of literature in this field, future studies are needed to further validate this overlapping circuitry of social stress and alcohol reward.

Across both brain and subjective response findings, there was evidence in support of both the Direct Effect and Buffering hypotheses. The Direct Effect hypothesis postulates that SS will be beneficial regardless of initial stress levels while the Buffering hypothesis suggests SS will be most impactful under stress-related circumstances (Cohen and Wills 1985). Since we saw that basal subjective stress levels were heightened in those with low SS, independent of stress and alcohol contexts, SS may exert a direct health benefit by lowering subjective stress overall. The remainder of our results support a Buffering hypothesis. SS impacted subjective alcohol craving and arousal scores, as well as reward and stress-related brain responses, in the context of alcohol and stress relative to neutral cues.

Our work fills an important gap in the literature, both in the literature review of SS and alcohol reward and in directly examining how SS may act along neural pathways to potentiate alcohol risk in a healthy drinking sample. Our paradigm allowed us to assess the impacts of SS in different contexts, specifically in alcohol cue, stress, and neutral conditions. However, there are some limitations to our findings. We did not consider if there were interactive effects between SS and different types of at-risk drinkers (e.g., heavy versus light/moderate drinkers) since there was an even split of drinking intensity in our SS groups and our goal was to understand the main effects of SS, if any. Future work would benefit from exploring these nuances, as there is evidence that striatal activity may differ in heavy vs. light drinkers (Vollstädt-Klein et al. 2010).

There are additional lingering questions on the role of SS on drinking behaviors and health. For instance, in a population based study, receiving SS lowered stroke and cardiovascular disease risk in light/moderate but not heavy drinking men (Ikehara et al. 2009). This suggests the efficacy of SS as an intervention may vary based on drinking context as well as for whom and how the SS is implemented. Further adding complexity to this issue is the role of pro-social experiences, which at times can be sources for SS that reduce drinking while other times acting as sources of social stress, and thus increasing drinking or interfering with recovery. For example, SS provided by light drinking friends is likely to encourage less drinking, compared to friends that also drink heavily (Buckman et al. 2008). Evidence suggests that having a supportive marital partner is significantly beneficial in initiating and maintaining recovery from AUD (Klostermann and O’Farrell 2013; O’Farrell and Clements 2012). However, estrangement from family and family interactions is often a source of great social stress, which could interfere with recovery. Similarly, the success of Alcoholics Anonymous has been attributed at least in part to building a new alcohol free SS network rather than simply having a network in general (Kelly et al. 2009). College students engaged in pro-social behaviors, such as being members of clubs and teams, had fewer heavy drinking episodes whereas stronger friendships were associated with more heavy drinking.
further highlighting nuances to the way social influences affect drinking behavior (Fenzel 2005). Overall, the type and context of SS (for instance, pro-social versus social stress) need to be better studied to predict drinking behavior more accurately.

Taken together, this review and findings presented herein suggest important implications in addressing risk of hazardous drinking. To prevent future alcoholism in social drinkers, assessing stress and perceived levels of SS could be essential. Furthermore, incorporating knowledge of neural susceptibility towards reward and reward seeking may also help develop adaptive stress coping strategies in socially stressful situations to promote healthy social interactions amongst those most at risk for hazardous drinking patterns.

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# Neural Underpinnings of Social Stress in Substance Use Disorders

Vyoma Sahani, Yasmin L. Hurd, and Keren Bachi

## Abstract

**Background:** Drug addiction is a complex brain disorder that is characterized by craving, withdrawal, and relapse, which can be perpetuated by social stress. Stemming from an acute life event, chronic stress, or trauma in a social context, social stress has a major role in the initiation and trajectory of substance use. Preclinical literature shows that early life stress exposure and social isolation facilitate and enhance drug self-administration. Epidemiological evidence links childhood adversity to increased risk for drug use and demonstrates that cumulative stress experiences are predictive of substance use severity in a dose-dependent manner. Stress and drug use induce overlapping brain alterations leading to downregulation or deficits in brain reward circuitry, thereby resulting in greater...
sensitization to the rewarding properties of drugs. Though stress in the context of addiction has been studied at the neural level, a gap in our understanding of the neural underpinnings of social stress in humans remains.

Methods: We conducted a systematic review of in vivo structural and functional neuroimaging studies to evaluate the neural processes associated with social stress in individuals with substance use disorder. Results were considered in relation to participants’ history of social stress and with regard to the effects of social stress induced during the neuroimaging paradigm.

Results: An exhaustive search yielded 21 studies that matched inclusion criteria. Social stress induces broad structural and functional neural effects in individuals with substance use disorder throughout their lifespan and across drug classes. A few patterns emerged across studies: (1) many of the brain regions altered in individuals who were exposed to chronic social stress and during acute stress induction have been implicated in addiction networks (including the prefrontal cortex, insula, hippocampus, and amygdala); (2) individuals with childhood maltreatment and substance use history had decreased gray matter or activation in regions of executive functioning (including the medial prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex), the hippocampal complex, and the supplementary motor area; and (3) during stress-induction paradigms, activation in the anterior cingulate cortex, caudate, and amygdala was most commonly observed.

Conclusions/Implications: A distinct overlap is shown between social stress-related circuitry and addiction circuitry, particularly in brain regions implicated in drug-seeking, craving, and relapse. Given the few studies that have thoroughly investigated social stress, the evidence accumulated to date needs to be replicated and extended, particularly using research designs and methods that disentangle the effects of substance use from social stress. Future clinical studies can leverage this information to evaluate the impact of exposure to trauma or adverse life events within substance use research. Expanding knowledge in this emerging field could help clarify neural mechanisms underlying addiction risk and progression to guide causal-experimental inquiry and novel prevention and treatment strategies.

Keywords Early and lifetime adversity · Human drug addiction · Neuroimaging · Social stress · Substance use disorder

1 Introduction

Substance use has profound individual, societal, and economic impacts (Gomes et al. 2018a; Substance Abuse and Mental Health Services Administration 2017; Volkow 2017; Hedegaard et al. 2017), yet effective evidence-based treatments to circumvent this public health emergency are limited (Volkow 2017). Individuals with substance use disorders (SUD) frequently use drugs to relieve physical and/or emotional pain, a pattern that can lead to increased morbidity and mortality (Blum et al. 2013; Gomes
et al. 2018b). A major source of emotional pain experienced by individuals with SUD is linked to chronic social stress (rejection/isolation; childhood maltreatment; loss of a caregiver) (Lawson et al. 2013; Stein et al. 2007), which may shape neural and physiological responses to drugs and exacerbate illness risk (Quinn et al. 2016; Conroy et al. 2009). Indeed, negative social emotions inclusive of social stress have been associated with activation of the opioid system which is intricately related to systems mediating physical pain (Stein et al. 2007; Eisenberger et al. 2003; Dewall et al. 2010).

Clinical and epidemiological evidence has long shown the involvement of stress induced by social factors as a critical contributor to the initiation and progression of addiction in humans (Sinha 2001; Sandi and Haller 2015; Jordan and Andersen 2017). Neurobiological evidence that links social stress and drug use stems primarily from animal models that have provided mechanistic insights about the impact of the neural stress system on reward circuits that mediate drug-seeking and taking behaviors through negative reinforcement (e.g., self-medication, withdrawal avoidance) (Koob 2008). While this knowledge provides a foundation for the neural underpinnings of how social stress can potentially trigger drug use and relapse, direct neurobiological insights about the human brain are of significant importance. The development of in vivo neuroimaging tools in recent decades has begun to examine social stress in addiction. This review evaluated such neuroimaging studies in an attempt to provide possible neural-mechanistic clarity into the impact of social stress in SUD. Knowledge gleaned may carry important translational utility to guide causal-experimental inquiry and possible intervention development.

1.1 Stress: Definition and Overview of Neurobiology

Stress may be defined as a real or interpreted threat to the physiological or psychological integrity of an individual that impacts behavior, subjective experience, and cognitive function (McEwen 2017a; Levine 2005; de Kloet et al. 2005). The term “stress” refers to a disruption in equilibrium resulting in a cascade of physiological and behavioral responses to reinstate allostasis (e.g., achieving stability through change) (McEwen 2017a; Koob and Schulkin 2019). This complex multidimensional concept involves perception (of stress stimuli), appraisal (processing of stress), response (to challenging or threatening events or stimuli), and eventually adaptation (Lazarus and Folkman 1984; Sinha 2008). While it has been recognized that stress could have positive impacts (“good” and “tolerable” stress promote resilience/growth experiences via allostasis), “toxic” stress (hereafter referred to as “stress”) is likely to result in pathophysiology due to cumulative burden with higher levels of allostatic load/overload (overuse and dysregulation that exacerbate disease processes) (McEwen 2017a).

The stress response is mainly mediated by stress hormones including corticotropin-releasing factor (CRF) and cortisol that is released by the hypothalamic–pituitary–adrenal (HPA) axis and adrenal cortex; and catecholamines,
and norepinephrine (or noradrenaline) released by the adrenal medulla and sympathetic nerves (McEwen 2013; McEwen and Gianaros 2011). Peripheral stress hormones provide feedback to the brain, regulating the activity of the HPA axis. This negative feedback loop depends on the activation of two types of glucocorticoid receptors in the brain: high-affinity mineralocorticoid receptors, activated by lower concentrations of cortisol, preventing further release of CRF; and low-affinity glucocorticoid receptors, activated by higher concentrations of cortisol, and resulting in the opposite effect, an increase in the release of CRF (McEwen 2013; McEwen and Gianaros 2011). Stress-related imbalance involves additional systemic physiological responses via neuroendocrine, autonomic, cardiovascular/immune, and metabolic mediators. Inter-individual variation in vulnerability or resilience to stress are complex but partially relate to genetic background through stress-related gene expression (Agorastos et al. 2019) and epigenetic modifications (e.g., DNA methylation associated with early life stress (Meaney and Szyf 2005)).

The experience of stress and dysregulation of stress hormones affect structural and functional neuroplasticity (e.g., dendritic remodeling and synapse turnover) (McEwen 2013; McEwen and Gianaros 2011). However, the stress experience, whether acute or chronic, induces different effects that have been characterized in brain regions such as the hippocampus, amygdala, and prefrontal cortex (PFC) that mediate fear-related memories, working memory, and self-regulatory behaviors. Traumatic stressors induce region-specific neuroplasticity effects that may lead to profound behavioral impact. In the PFC, chronic stress causes medial PFC (mPFC) neurons to debranch and shrink dendrites, a process associated with cognitive rigidity, whereas orbitofrontal cortical neurons expand dendrites that may be related to increased vigilance (Radley et al. 2004; Liston et al. 2006; McEwen 2017b). Additional acute and chronic stress-related neural alterations (Vyas et al. 2002; Bennur et al. 2007; Lau et al. 2017) are implicated in increased anxiety, posttraumatic stress disorder (PTSD)-like behaviors, and social avoidance (Bennur et al. 2007; Lau et al. 2017), conditions that are closely tied to drug-seeking and drug-using behaviors.

1.2 Social Stress and Substance Use: Preclinical, Clinical, and Neurobiological Context

Stress is a well-known contributor to drug use and relapse vulnerability. On the behavioral level, drug use was proposed as a coping strategy to alleviate acute and chronic stress (Wills and Shiffman 1986; Conger 1956; Khantzian 1985). Initially used to modulate tension, with repeated administration, drug use may become a frequent response for mood enhancement. On the neurobiological level, stress and drug use involve overlapping brain changes (Ruisoto and Contador 2019) where drug addiction has been viewed as an allostatic disorder (Koob and Schulkin 2019). Stress and drug use lead to downregulation or deficits in the brain reward circuitry.
resulting in greater sensitization to the rewarding properties of drugs (Koob and Schulkin 2019; Koob and Le Moal 1997). The effects of stress on the reward system enhance the reward and craving induced by drugs or drug cues and increase the motivation to use drugs compulsively (Piazza and Le Moal 1998). Stress-induced hyperactivity of the HPA axis and upregulation of the amygdala is involved in negative emotionality during withdrawal and drives drug-seeking and taking behaviors through negative reinforcement (Belujon and Grace 2011). The hippocampus is also down-regulated by both stress and drug use leading to further impairments of memory and emotion regulation (Belujon and Grace 2011). Importantly, stress leads to surges of dopamine and norepinephrine in the PFC, resulting in impairments of the executive function network, thereby disrupting decision making and the ability to inhibit relapse, the hallmark of drug addiction (Mather and Lighthall 2012). Collectively, these models provide explanations for the crucial roles of stress in the transition from casual substance use to SUD.

Converging lines of evidence indicate that the stressors correlated with increased risk of substance use were primarily social in nature (versus other forms of stress, such as economic or natural disasters) (Thoits 2010). In animal models, individual differences in susceptibility to cocaine use within a population, measured by the availability of dopamine D2 receptors, may be mediated by social dominance rank (Morgan et al. 2002). Moreover, early life stress, induced through neonatal or maternal separation, facilitates drug self-administration, while social defeat stress or social isolation escalates drug use (Sinha 2001). Conversely, operant access to social reward in addicted rats prevented compulsive drug self-administration and incubation of craving and relapse (Venniro et al. 2018).

Studies in humans extend these findings. Epidemiological evidence links childhood adversity to increased risk for cannabis, cocaine, and prescription drug use among adolescents (Carliner et al. 2016; Khoury et al. 2010). In adults, alcohol and drug use can contribute to unstable housing or homelessness; likewise, increased duration of homelessness was shown to be associated with increased risk of substance use (Kipke et al. 1997). Furthermore, the cumulative number of stress events an individual experienced was predictive of alcohol and drug use severity in a dose-dependent manner (Sinha 2008; Turner and Lloyd 2003; Lloyd and Turner 2008). Collectively, these findings indicate that chronic exposure to social stress, trauma, and/or an adverse life event enhances an individual’s vulnerability to substance use (Sinha 2008).

Animal models of stress have been a useful tool to investigate mechanisms of substance use through which stress alters neuroplasticity and animal behavior in a social context (Mumtaz et al. 2018). However, in vivo neuroimaging models are limited in clinical studies, leading to a gap in the understanding of the brain effects of social stress in SUD. This chapter presents the current state of knowledge of the neural underpinnings of social stress in human drug addiction. This review considers the multi-faceted roles of social stress in SUD. It seeks to understand how the prevalence of chronic and/or acute social stress may impact the neural underpinnings of SUD vulnerability and its trajectory. Studies are contextualized within the scope of preclinical, neurobiological, and clinical findings. Future directions are discussed
to inform priorities for enhancing the knowledge base of this emerging field, which could progress prevention targets and opportunities for novel treatments.

1.3 Search Method

We searched Pubmed and the Neurosynth database (www.neurosynth.org) (Yarkoni et al. 2011), for relevant studies published between January 1, 2000, and July 1, 2021. A combination of the following keywords was used: social stress, trauma, childhood trauma, childhood maltreatment, social isolation, rejection, drug addiction, human drug addiction, substance use, substance use disorder, cocaine, heroin, opioid, nicotine, alcohol, cannabis, marijuana, imaging, neuroimaging, structural integrity, functional magnetic resonance imaging (fMRI), functional connectivity, resting-state functional connectivity, paradigm. Studies were included if they met the following criteria: (1) conducted neuroimaging (functional connectivity, structural or functional magnetic resonance imaging); (2) studied a substance using population with a history of social stress and/or employed a stress-inducing paradigm in a substance using population; and (3) used standardized methods to assess for SUD and/or its severity.

Twenty-one studies were identified and summarized in Table 1. Studies are grouped by the social stress characteristics of the sample first, and the type of stress-induction paradigm used second.

2 Early and Lifetime Adversity

Early and lifetime adversity encompass a wide range of stressors with varying magnitudes of severity. Individuals may be affected by a single traumatic event, a series of stressful events, chronic stress, live in a socially dysfunctional environment, and more. The developmental timing of the stress exposure and the amount and duration of stress exposure can affect an individual’s vulnerability to SUD and their disorder trajectory (Teicher et al. 2016). This section describes studies in individuals with SUD who have experienced chronic stress or trauma within a social context in the early periods of their life (before the age of 18; see Fig. 1, top panel) or throughout their life (see Fig. 1, bottom panel).

2.1 Social Stress in Early Life

Studies that examined early life stress typically focused on the effects of childhood maltreatment in individuals with SUD, which includes physically, emotionally, and sexually abusive events and incidences of physical and emotional neglect (Van Dam
### Table 1  Summary of neuroimaging studies of social stress in human drug addiction

<table>
<thead>
<tr>
<th>Reference</th>
<th>Social stress type (measure)</th>
<th>Substance use/disorder</th>
<th>Sample</th>
<th>Paradigm (social stress or other)</th>
<th>Imaging tool/ procedure</th>
<th>Analysis type</th>
<th>Prime brain regions</th>
<th>Brain-behavior associations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Past social stress/trauma</strong></td>
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</tr>
<tr>
<td>(Van Dam et al. 2014)</td>
<td>Childhood maltreatment (CTQ)</td>
<td>Alcohol, cocaine, and/or cannabis</td>
<td>79 in-treatment SUD; 98 CON</td>
<td>N/A</td>
<td>3T MRI VBM</td>
<td>Whole-brain; ROI</td>
<td>CM (controlling for SUD): -L hippocampus, -parahippocampus, -anterior fusiform gyrus</td>
<td>L CM-related gray matter volume, %severity of substance use after initial relapse</td>
</tr>
<tr>
<td>(Bachi et al. 2018)</td>
<td>Childhood maltreatment (CTQ)</td>
<td>Cocaine</td>
<td>24 CUD + CM; 23 CUD-CM; 29 CON</td>
<td>N/A</td>
<td>3T MRI VBM</td>
<td>Whole-brain</td>
<td>CUD + CM &gt; CON</td>
<td>R lateral OFC, %depression and %constraint</td>
</tr>
<tr>
<td>(Leong and Yuan 2018)</td>
<td>Childhood maltreatment (Adverse Childhood Experiences; (Felitti et al. 1998))</td>
<td>Heroin and/or nicotine</td>
<td>7 heroin (abstinent &gt;3 months)-nicotine dependence +CM; 7 nicotine</td>
<td>Reading the mind in the eyes</td>
<td>Continuous wave; functional near-infrared spectroscopy; Resting-state FC</td>
<td>ROI</td>
<td>Heroin-nicotine dependence &gt; nicotine dependence: ↑OFC, ↑mPFC, ↑dorsolateral PFC</td>
<td>N/A</td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Social stress type (measure)</th>
<th>Substance use/disorder</th>
<th>Sample</th>
<th>Paradigm (social stress or other)</th>
<th>Imaging tool/procedure</th>
<th>Analysis type</th>
<th>Prime brain regions</th>
<th>Brain-behavior associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Casement et al. 2015)</td>
<td>Stressful life events in late adolescence (Life Event Questionnaire for Adolescents (Masten et al. 1994) and Interpersonal Problem Situations Inventory for Urban Adolescents; (Farrell et al. 1998))</td>
<td>Alcohol (problematic use)</td>
<td>152 males</td>
<td>Reward-guess task</td>
<td>3T fMRI BOLD</td>
<td>ROI</td>
<td>Stress scores; severity of alcohol use, association with reward; Anticipation of reward and reward outcome =</td>
<td>mPFC, cumulative life stress events and severity of alcohol use</td>
</tr>
<tr>
<td>(Poppa et al. 2019)</td>
<td>PTSD (lifetime sexual trauma; Life Stressors Checklist-Revised; (Wolfe and Kimerling 1997))</td>
<td>Methamphetamine and/or cocaine</td>
<td>14 female</td>
<td>Interoceptive-exteroceptive attention</td>
<td>3T fMRI FC of task-modulated networks</td>
<td>Whole-brain</td>
<td>SUD-PTSD &gt; SUD: OFC network located in the mid-posterior insula, angular gyrus, precuneus, and lateral prefrontal cortex areas</td>
<td></td>
</tr>
<tr>
<td>(Gawrysiak et al. 2017)</td>
<td>Trauma history (lifetime; Addiction Severity Index; (McLellan et al. 1992))</td>
<td>Cocaine</td>
<td>18 CUD-trauma; 16 CUD</td>
<td>N/A</td>
<td>3T fMRI Resting-stateFC</td>
<td>Seed-based (amygdala)</td>
<td>Trauma: amygdala and limbic-striatal regions connectivity</td>
<td>N/A</td>
</tr>
<tr>
<td>Study Information</td>
<td>Social Stress Induction</td>
<td>Material</td>
<td>Gender</td>
<td>Methodology</td>
<td>Imaging</td>
<td>Region of Interest</td>
<td>Findings</td>
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<tr>
<td>Seo et al. (2011)</td>
<td>Alcohol (social drinking)</td>
<td>Guided imagery induction</td>
<td>20 males; 23 females</td>
<td>Whole-brain</td>
<td>3T fMRI BOLD</td>
<td>Stress script (both): ↑L dorsal striatum, Stress script (male &gt; female): ↑mPFC, ↑rostral ACC, ↑rostral ACC, ↑posterior insula, ↑putamen, ↑amygdala, ↑hippocampus, ↑parahippocampal gyrus</td>
<td>↑dorsal striatum, ↑craving in males</td>
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<tr>
<td>Li et al. (2005)</td>
<td>Cocaine</td>
<td>Guided imagery induction, Post-task: Anxiety, craving</td>
<td>17 male CUD; 10 female CUD</td>
<td>Whole-brain; ROI</td>
<td>1.5T fMRI BOLD</td>
<td>Stress script (both): ↑mid temporal gyrus, Stress script (female &gt; male): ↑middle, medial, inferior frontal cortical areas, ↑anterior cingulate, ↑insula, ↑middle and inferior frontal cortices, ↑R posterior cingulate cortex</td>
<td>↑L anterior cingulate and ↑R posterior cingulate cortices, ↑craving rating during stress imagery in female CUDs</td>
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<tr>
<td>Li et al. (2006)</td>
<td>Cocaine</td>
<td>Guided imagery induction</td>
<td>17 male CUD; 10 female CUD</td>
<td>ROI</td>
<td>1.5T fMRI BOLD</td>
<td>Stress script (both): ↑R inferior frontal cortex, ↑mPFC, ↑R anterior cingulate</td>
<td>↑mPFC and ↑L posterior cingulate cortex, ↓socialization in female CUDs</td>
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<td>Sinha et al. (2005)</td>
<td>Cocaine</td>
<td>Guided imagery induction</td>
<td>20 CUD; 8 CON</td>
<td>Whole-brain</td>
<td>1.5T fMRI BOLD</td>
<td>Stress script (CUD): ↑medial orbitofrontal/ anterior cingulate region, ↑R postcentral gyrus, ↑R fusiform gyrus, ↑L precentral</td>
<td>↑caudate and ↑dorsal striatum, ↑stress-induced cocaine craving ratings</td>
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<th>Sample</th>
<th>Paradigm (social stress or other)</th>
<th>Imaging tool/ procedure</th>
<th>Analysis type</th>
<th>Prime brain regions</th>
<th>Brain-behavior associations</th>
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<td>Social stress paradigm</td>
<td>Nicotine</td>
<td>11 smokers in mindfulness training; 12 smokers in freedom from smoking</td>
<td>Guided imagery induction</td>
<td>1.5T fMRI BOLD</td>
<td>Whole-brain</td>
<td>Stress script (both): 1 cluster in amygdala, anterior insula, mid insula, hippocampus, parahippocampal gyrus, thalamus, middle occipital gyrus, midbrain, and R posterior cingulate cortex; 1 cluster across cuneus/posterior perimesial cortex</td>
<td>Neural activity during stress, post-treatment cigarette reduction. Post-treatment effect in similar brain regions at 3 months follow-up</td>
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<td>Social stress paradigm</td>
<td>Alcohol</td>
<td>22 AUD; 22 CON</td>
<td>Cyberball</td>
<td>3T MRI BOLD</td>
<td>Whole-brain</td>
<td>AUD &gt; CON (social exclusion): R insula, R ventral PFC; L ACC, parahippocampal gyrus</td>
<td>R insula, R ventral PFC, L medial FG</td>
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<td>Social stress paradigm</td>
<td>Cocaine</td>
<td>18 CUD; 25 CON</td>
<td>Cyberball</td>
<td>3T MRI BOLD</td>
<td>Whole-brain</td>
<td>CUD &gt; CON (social exclusion &gt; inclusion): L R medial FG; D regional cingulate</td>
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<th>Study (Year)</th>
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<tr>
<td>(Bach et al. 2019a, b)</td>
<td>Opioid</td>
<td>19 OMT; 20 CON</td>
<td>Cyberball</td>
<td>3T fMRI VBM</td>
<td>ROI</td>
<td>Whole-brain</td>
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<td>L ventral FG, R caudate</td>
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<td>(Bach et al. 2019a, b)</td>
<td>Opioid</td>
<td>17 OMT; 21 CON</td>
<td>Cyberball</td>
<td>3 T fMRI BOLD</td>
<td>ROI</td>
<td>Whole-brain</td>
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<tr>
<td>(Beard et al. 2021)</td>
<td>Alcohol and/or cannabis use</td>
<td>181 adolescents</td>
<td>Cyberball</td>
<td>3T fMRI BOLD</td>
<td>ROI</td>
<td>Whole-brain: ROI</td>
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<td>dorsal ACC and ↑ anxiety, ↑ substance use later in adolescence</td>
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<td>↓ dorsal ACC and ↑ distress (game-related), ↑ substance use later in adolescence</td>
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<td>↑ anterior insula,</td>
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<th>Reference</th>
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<th>Imaging tool/ procedure</th>
<th>Analysis type</th>
<th>Prime brain regions</th>
<th>Brain-behavior associations</th>
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<tr>
<td>(Charlet et al. 2014)</td>
<td>N/A</td>
<td>Alcohol</td>
<td>33 AUD; 33 CON</td>
<td>Facial emotion recognition (aversive faces)</td>
<td>3T fMRI BOLD &amp; VBM</td>
<td>ROI</td>
<td>AUD &gt; CON (BOLD): ↓ FG, ↑L rostral ACC, ↑L anterior cingulate cortex/ medial FG, ↑R precuneus AUD &gt; CON (VBM): ↓ FG, ↓ rostral ACC, ↓ frontal regions (ACC, insula, temporal, occipital gyri)</td>
<td>↑ substance use in females ↑L rostral ACC, ↑ days of abstinence and ↑ days of binge drinking ↑FG activation, ↑ lifetime drinking</td>
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<td>(Bedi et al. 2009)</td>
<td>N/A</td>
<td>MDMA (administration)</td>
<td>9 individuals with past MDMA use</td>
<td>Facial emotion recognition (aversive faces)</td>
<td>3T fMRI BOLD</td>
<td>Whole-brain; ROI</td>
<td>MDMA 1.5 mg/kg &gt; MDMA 0.75 mg/kg Placebo (angry &gt; neutral faces): ↑L amygdala MDMA 0.75 mg/kg &gt; MDMA 1.5 mg/kg Placebo (happy &gt; neutral): ↑R ventral striatum</td>
<td>↑R ventral striatum, no effect on self-reported sociability in MDMA (0.75 mg/kg)</td>
</tr>
<tr>
<td>(Flanagan et al. 2019)</td>
<td>N/A</td>
<td>Alcohol, cannabis and/or cocaine</td>
<td>10 couples (n = 20) w/ one partner w/ hazardous drinking or SUD</td>
<td>Relationship conflict</td>
<td>3T fMRI BOLD</td>
<td>ROI</td>
<td>Female &gt; male (conflict &gt; neutral): ↑R amygdala-L inferior FG and mid-cingulate connectivity, ↑L OFC-R mid temporal gyrus connectivity</td>
<td>↑R amygdala and L prefrontal cortex FC during the conflict or neutral cues, ↑ intimate partner violence</td>
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Notes: Childhood Trauma Questionnaire (CTQ; Bernstein et al. 2003)) Substance use disorder (SUD); Control (CON); Magnetic resonance imaging (MRI); Voxel-based morphometry (VBM); Childhood maltreatment (CM); Left (L); Cocaine use disorder (CUD); Right (R); Orbitofrontal cortex (OFC); Functional magnetic resonance imaging (fMRI); Blood-oxygen-level-dependent (BOLD); Region-of-interest (ROI); Anterior cingulate cortex (ACC); Functional connectivity (FC); Prefrontal cortex (PFC); Medial PFC (mPFC); Post-traumatic stress disorder (PTSD); Frontal gyrus (FG); Alcohol use disorder (AUD); Opioid methadone treatment (OMT); 3,4-Methylenedioxymethamphetamine (MDMA)
Fig. 1  Putative neural underpinnings of childhood and lifetime trauma in substance use disorder. Images depicting the potential effects of exposure to childhood maltreatment on gray matter volume or concentration and functional activity, and the effects of exposure to trauma during adulthood on brain connectivity. **Top left and right:** Childhood maltreatment was associated with decreased gray matter volume in the left hippocampus, parahippocampus, and anterior fusiform gyrus in individuals with substance use (alcohol, cocaine, and/or cannabis) (Van Dam et al. 2014) as well as decreased gray matter concentration in the right lateral orbitofrontal cortex and middle temporal gyrus in individuals with cocaine use disorder (CUD) (Bachi et al. 2018). Social stress (induction) was associated with decreased activation in the left supplementary motor area, rostral anterior cingulate cortex, and left dorsal-anterior precuneus, in individuals with past childhood maltreatment and CUD (Elton et al. 2015). Life stress during adolescence and severity of alcohol use was associated with decreased activation in the medial prefrontal cortex during reward processing in males (Casement et al. 2015). **Bottom left:** In adulthood, females with posttraumatic stress disorder, primarily from sexual trauma, and substance use (methamphetamine and/or cocaine) had reduced orbitofrontal cortex task-modulated functional connectivity in the lateral prefrontal cortex, mid-posterior insula, angular gyrus, and precuneus, during the interoceptive-exteroceptive attention task (Poppa et al. 2019). **Bottom right:** In individuals with CUD and trauma history, enhanced amygdala resting-state functional connectivity with limbic-striatal regions was observed (Gawrysiak et al. 2017).
et al. 2014; Bachi et al. 2018; Elton et al. 2015; Ieong and Yuan 2018). Extending this line of inquiry beyond the specific definition of childhood trauma, one study assessed cumulative stressful life experiences during adolescence (ages 15–18) with stressors ranging from poor performance in school to arguments with family members (Casement et al. 2015).

Several studies report structural brain alterations in individuals with SUD and early life trauma, which in turn may contribute to behaviors underlying SUD (see Fig. 1, top panel). Decreased hippocampal, parahippocampal, and anterior fusiform gyrus gray matter volume in a sample with alcohol, cannabis, and/or cocaine use was associated with childhood maltreatment while controlling for SUD status (Van Dam et al. 2014). Altered structural integrity in regions of the hippocampal complex overlaps with findings from preclinical studies on early life stress (Lupien et al. 2009; McEwen 2010), thus providing an important translational link. Altered reward-based learning, anxiety, depression, and/or addiction may be mediated by deficits in the hippocampal complex (Van Dam et al. 2014; Hyman and Malenka 2001). Moreover, reduced gray matter concentration in the right lateral orbitofrontal cortex (OFC) observed in individuals with cocaine use disorder (CUD) with a history of high childhood trauma was correlated with increased depression and reduced constraint (Bachi et al. 2018). The OFC (and other prefrontal regions) are highly linked to drug-seeking motivation (however, decreased motivation for other goals), awareness and interoception, decision making, learning and memory, and salience attribution (Goldstein and Volkow 2011). Deficits in the right OFC/superior temporal gyrus were observed in individuals with childhood maltreatment history and without addiction in an extensive meta-analysis (Lim et al. 2014). Evidence of decreased gray matter in the OFC in addiction literature is abundant too, however, these brain morphology effects, previously attributed to substance use, could be shaped in a premorbid stage by early exposure to trauma or an additive effect of both characteristics (Bachi et al. 2018).

Functional outcomes provide further clarity on neural pathways connecting early life stress contributions to drug-seeking behaviors. Individuals with CUD and high trauma showed decreased activation in the left dorsal-anterior precuneus and left supplementary motor area during the stress portion of a stress-induction paradigm (as compared to the baseline neutral script) (Elton et al. 2015). Diminished responses in the dorsal-anterior precuneus and supplementary motor area in individuals with childhood maltreatment history suggest a functional compromise in the ability to engage parietal-motor networks and controlled versus automated action selection in response to stressful experiences (Elton et al. 2015; Nachev et al. 2007). Similarly, stress-induction led to a loss in volitional control of behavior, indicating it could be an intermediate mechanism in a nonlinear pathway, beginning in childhood adversity and leading to SUD (Hosking and Winstanley 2011). An interaction between maltreatment severity and drug craving indicated reduced activity in the rostral anterior cingulate cortex (Elton et al. 2015), a region important for emotional conflict resolution (Etkin et al. 2006). This deactivation suggests that childhood maltreatment may attenuate a key mechanism of conflict resolution vital for adaptive stress responses (Elton et al. 2015). Hence, the anterior cingulate cortex, a region identified
as a central “hub” for addiction-related neural networks (Zhao et al. 2020), may also mediate the relationship between social stress and craving. These findings are broadened in a longitudinal fMRI study of male participants, which found that higher cumulative stressful life events during adolescence were associated with problematic alcohol use and decreased mPFC activation during a reward task (Casement et al. 2015). Previous neuroimaging research found that acute psychosocial stressors resulted in a significant decrease in reward-related responses in the mPFC (Ossewaarde et al. 2011), however, this study is the first to show a link between cumulative life stress and blunted mPFC reward response (Casement et al. 2015). As the mPFC plays an important role in reward processing and drug reinstatement (Perry et al. 2011), it is plausible that chronic stress during adolescence blunts neural mechanisms that help regulate alcohol-motivated behavior; thus, stress exposure diminishes mPFC response to naturally rewarding events and heightens the perceived benefits of alcohol use (Koob and Le Moal 1997; Casement et al. 2015).

Beyond enhancing drug-seeking behaviors, early exposure to trauma may increase the risk of relapse in individuals with SUD. Childhood maltreatment predicted a shorter time to drug relapse and gray matter volume reductions in the hippocampal complex were correlated to greater severity of substance use after initial relapse in individuals with SUD (Van Dam et al. 2014). Activation of the occipital cortex in response to drug cues was attributed to modulation of visual attention to the conditional motivational properties of drug cues in individuals with CUD (Hogarth et al. 2009). This heightened response leads to enhanced drug craving responses, and likely increases the potential to relapse (Elton et al. 2015). The processes of drug-seeking and drug use behaviors are proposed to be automatically engaged during stress (Pierce and Vanderschuren 2010), thereby supporting the observation that acute social stress and/or chronic exposure to childhood adversity is linked to risk of relapse (Elton et al. 2015).

Taken together, these in vivo neuroimaging findings suggest that early life stress has widespread effects on individuals suffering from addiction at the brain, behavioral, and clinical levels, that lead to long-lasting marks. Examination of structural integrity in gray matter and task-based brain activation revealed that individuals with childhood maltreatment and substance use disorder have decreased volume or activation in key regions of executive function and the hippocampal complex (see Fig. 1, top panel). Interestingly, these results are consistent with studies examining structural and functional alterations in individuals with childhood trauma and without substance use history (Teicher et al. 2012, 2016; Hanson et al. 2015; Andersen et al. 2008; Carrion et al. 2007; Teicher and Samson 2016). As such, it is difficult to disentangle the neural effects of early life stress from the neural effects of SUDs. The observed differences in brain structure and function have been associated with SUD severity, risk of relapse, depression, and other important clinical symptomatology, indicating that individuals with SUD and a history of early life stress may represent a clinically and biologically distinct phenotype (Bachi et al. 2018; Dannlowski et al. 2012). There is likely overlap between brain regions implicated in early life stress and substance use, however, the severity and pathology may be expected to be worse in individuals with childhood trauma and SUD given their clinical differences.
Within this subset of studies that explored the neural underpinnings of childhood maltreatment in SUD, the mesocorticolimbic pathways which are central to SUD are impacted by a history of early life trauma. These neural alterations may contribute to an individual’s pattern of SUD, drug-related behaviors, and clinical symptomatology that might inform models of trauma-informed treatment.

2.2 Social Stress in Adulthood/Lifetime

While significant attention in the field has focused on the impact of stress during childhood, the neurobiological correlates of trauma exposure in adult SUD or cumulative lifetime chronic stress are understudied. We provide an overview of cross-sectional studies that contrast brain connectivity in adult individuals with SUD and healthy controls.

Sexual trauma in adulthood is a significant stressor that has long-lasting consequences. In females with polysubstance use (primarily methamphetamine and cocaine), those with a PTSD diagnosis showed diminished functional connectivity of the OFC network in the precuneus, mid-posterior insula, lateral prefrontal cortex, and angular gyrus during an interoception task (Poppa et al. 2019) (see Fig. 1, bottom left). These observations are consistent with prior studies of posttraumatic stress in individuals without substance use history, which found reduced orbitofrontal or ventromedial prefrontal cortex activity during trauma-related tasks (Daigre et al. 2015; Moser et al. 2015). Moreover, across the sample of females with SUD, OFC network strength was inversely associated with sexual violence exposure, which accounted for an additional variance beyond the effects of PTSD. This indicates that not only is diminished OFC network strength predictive of PTSD, the OFC is functionally sensitive to the cumulative effects of sexual trauma in females with SUD (Poppa et al. 2019). Individuals with CUD and a history of trauma showed enhanced amygdala resting-state functional connectivity with limbic-striatal regions (Gawrysiak et al. 2017) (see Fig. 1, bottom right). Hyperactivity in the amygdala, a region implicated in fear processing and learning in response to threat, has been linked to traumatic stress (Brown et al. 2014; Lanius et al. 2006); increased amygdala response to evocative cues has been observed in individuals with PTSD and anxiety disorders (Patel et al. 2012; Shin and Liberzon 2010). Additionally, amygdala resting-state functional connectivity has been sensitive enough to distinguish between CUD patients (Gu et al. 2010) and heroin-dependent patients (Ma et al. 2010) from healthy controls, therefore these results may reveal further within-group heterogeneity. The impact of prior adversity on resting-state functional connectivity emphasizes the relevance of these brain regions as potential biomarkers for clinical vulnerability (Gawrysiak et al. 2017). These findings of functional connectivity, both task-modulated and at rest, indicate that individuals who experienced trauma in adulthood and are battling SUD may have attenuated connectivity in brain regions involved in executive function and fear processing (Poppa et al. 2019; Gawrysiak et al. 2017).
2.3 Social Stress in Early Life and Adulthood: Cumulative Interacting Dysregulations

While the majority of social stress studies in SUD populations have focused on a particular lifetime period (e.g., childhood/adulthood), notably exposure to stress in early life is associated with heightened vulnerability to social stress in adulthood (Miller et al. 2011). Early life stress during critical periods of neurodevelopment could have broad effects on networks related to the stress response and thus may lead to an evolving phenotype with altered allostatic processes and reduced adaptability to stress later in life (Agorastos et al. 2019; Taylor 2010). A chronically impaired stress response involves enduring hyper- or hypo-activation of the stress system and altered glucocorticoid signaling, alterations in emotional and autonomic reactivity, circadian rhythm disruption, functional and structural changes in the brain, immune and metabolic dysregulation, and epigenetic modifications [see review: 25]. The extent of subsequent vulnerability to later-life stress involves factors such as the timing, duration, intensity/severity, and type of early life stress as well as other later-life challenges, such as type of additional stressors, coping strategies, support systems, lifestyle, and aging (Agorastos et al. 2019; Teicher et al. 2016; Teicher and Samson 2016). Although most findings point to a causal relationship between early life stress and psychobiological maladaptation in later life, the precise developmental trajectories and their temporal coincidence remain unclear (Agorastos et al. 2019).

3 Stress-Induction Paradigms

In addition to studying the long-term consequences of stress, the neural responsivity during an acute stress condition is also of importance in understanding neural systems particularly sensitive to stress. Such knowledge is accomplished by studies with simultaneous in vivo neuroimaging and stress-inducing tasks performed in a social context. Such tasks include guided imagery induction, social ball tossing, facial emotion recognition, psychosocial stress, and relationship conflict. Neural responses elicited during these tasks have been used to predict clinical outcomes in SUD as summarized in Table 1 and Fig. 2.

3.1 Guided Imagery Induction: Stress Script

The guided imagery induction uses a scene development interview to calibrate the response to a stressful scenario (Miller et al. 1987; Sinha et al. 1992). Stress scripts are based on a recent personal event and are followed by a neutral script that is developed based on the participant’s description of a personal neutral situation.
Participants listen to an audio recording of these personalized stress, neutral, and in some situations, drug use, scenarios while in the MRI scanner. Studies contrast brain activation during the stress or drug use scripts to activation during the neutral stimuli.
Results from such studies report increased frontostriatal and frontolimbic activity during subjective social stress as important predictors of craving. Increased activation in the dorsal striatum (Seo et al. 2011) and caudate (Sinha et al. 2005) were associated with greater post-stress craving scores in individuals with CUD and social drinkers. As the dorsal striatum has been associated with learned habits and procedural learning (Berke and Hyman 2000), stress-induced craving related to dorsal striatum/caudate activity highlights the habitual nature of substance use and suggests that chronic stress may alter habit-based decision making (Dias-Ferreira et al. 2009).

An inverse relationship was found between anterior cingulate and posterior cingulate cortices activity and craving in females with CUD (Li et al. 2005; Li et al. 2006), suggesting the distinct role of the cingulate cortices in modulating stress-induced craving (Goldstein and Volkow 2002) and that females may employ more verbal coping strategies than males when experiencing stress (McCrae and Costa 1986). Furthermore, decreased posterior cingulate cortex and increased mPFC activity during stress were associated with low socialization scores in females with CUD (Li et al. 2006). This finding may reflect that the posterior cingulate cortex was engaged during emotional distress and the mPFC is involved with the suppression of emotional distress, thereby suggesting that reduced socialization impacts the processing of negative emotional stimuli (Li et al. 2006; Phan et al. 2005). In addition to cocaine and alcohol use, nicotine addiction has been studied in relation to acute stress using the guided imagery induction. Activity in the amygdala, insula, and hippocampal regions in nicotine smokers undergoing mindfulness or cognitive behavioral treatment was negatively correlated with post-treatment reduction in smoking (Kober et al. 2017). As the amygdala and insula have been implicated in drug craving (Chase et al. 2011; Garavan 2010; Jasinska et al. 2014; Mihov and Hurlemann 2012), this suggests that smoking reduction treatments may reduce feelings of craving that could lead to positive clinical outcomes (Kober et al. 2017).

Due to its subjective nature, the effect of performing the guided imagery task can elicit an experience of enhanced memories and emotional activation related to past traumas. Structural and functional alternations across early and lifetime adversity involved similar brain regions as those engaged by this paradigm, indicating that these brain regions may have a predisposed vulnerability to the effects of social stress (see Table 1, and Figs. 1 and 2).

### 3.2 Social Ball Tossing: Cyberball

The social ball-tossing paradigm, called “Cyberball,” is an online task where participants are informed that they are playing with actual people nearby to enhance the authenticity of the social context. The paradigm induces states of social inclusion, exclusion, and re-inclusion (after social exclusion) (Williams and Jarvis 2006). During social exclusion, the task has been shown to engage heightened insula activity in individuals with alcohol use disorder (AUD), which suggests an association between AUD and higher emotional reactions to ostracism (Maurage et al.
2012). Functional activation and structural deficits of the insula have demonstrated the pertinence of this region in aspects underlying SUD, particularly in indexing a higher emotional reaction to social rejection (Eisenberger et al. 2003; Moor et al. 2012). A similar outcome was found in individuals with OUD (Bach et al. 2019a). Decreased gray matter volume in the anterior insula in opioid methadone treatment patients was associated with stronger feelings of exclusion during the exclusion trial and reduced feelings of inclusion during the inclusion trial. Moreover, insula gray matter volume was negatively correlated with social interaction anxiety symptoms, thereby highlighting the role of this brain region in emotion- and anxiety-processing (Bach et al. 2019a).

Select frontal regions, as well as limbic-related regions (e.g., parahippocampal gyrus), have also been implicated in the processing of feelings of social ostracism (Thoits 2010; Maurage et al. 2012; Bach et al. 2019a; Hanlon et al. 2018). The frontal gyri, including the inferior frontal gyrus in individuals with OUD (Bach et al. 2019a) and medial frontal gyrus in individuals with AUD and CUD (Maurage et al. 2012; Hanlon et al. 2018), were shown to be involved in the processing of negative affect during social ostracism. Intriguingly, opposite activations were observed in individuals with AUD versus CUD in this region: individuals with AUD had reduced response in the medial frontal gyrus (Maurage et al. 2012), whereas individuals with CUD had a greater response (Hanlon et al. 2018), indicating possible drug-specific neural effects. AUD was also associated with increased activation in the dorsal anterior cingulate and parahippocampal gyrus during re-inclusion after exclusion (Maurage et al. 2012). As the frontal gyrus, anterior cingulate, and parahippocampal gyrus have previously been identified as potential treatment targets (Konova et al. 2013), these findings extend the translational relevance of these brain regions.

Recent literature suggests that neural sensitivity in the anterior cingulate induced by the Cyberball paradigm was predictive of increased substance use in adolescents (Beard et al. 2021). In particular, blunted dorsal anterior cingulate cortex response to exclusion was associated with an increased risk for substance use in adolescents with high anxiety (Beard et al. 2021). This highlights that neural response to social stress, especially the anterior cingulate cortex, may be a vulnerability marker for the propensity of substance use.

3.3 Facial Emotion Recognition

Facial emotion recognition tasks are used to assess the recognition of basic facial expressions. In some tasks, brain activation during emotional expressions is contrasted with neutral expressions (Ekman and Friesen 1976; Bedi et al. 2009); in others, brain activation while matching faces (selecting the face which matches the target face) is counterbalanced with neutral shapes (Hariri et al. 2002; Charlet et al. 2014). Through these paradigms, the neural response to viewing socially threatening faces (fearful, angry, disgusted) was analyzed in individuals with SUD.
Acute substance use dysregulated the processing of social signals (Bedi et al. 2009), whereas chronic substance use in treatment-seekers affected neural response in regions implicated in childhood maltreatment (Charlet et al. 2014) (see Fig. 1, top panel, and Fig. 2) and predicted positive treatment outcomes. Healthy individuals administered a high dose of ±3.4 Methylenedioxymethamphetamine (MDMA) responded to angry faces with decreased activation in the left amygdala, while those treated with low dose MDMA responded to happy faces with enhanced ventral striatum activation (Bedi et al. 2009), a region predictive of reward signals (Knutson and Cooper 2005). Collectively, these results indicate that MDMA increased sociability, even in situations of social threat (Bedi et al. 2009) which is in line with enhanced empathy and prosocial behavior induced by MDMA. Abstinent individuals with AUD displayed strong left rostral anterior cingulate cortex, left mPFC, and right precuneus response to aversive facial stimuli versus neutral shapes (Charlet et al. 2014), regions implicated in childhood and adult trauma effects (Elton et al. 2015; Casement et al. 2015; Poppa et al. 2019) (see Figs. 1 and 2). The anterior cingulate cortex, a brain region implicated in emotion regulation (Phelps and LeDoux 2005; Kienast et al. 2008), was positively associated with days of abstinence and negatively associated with binge drinking, suggesting that it may represent a resilience factor that protects against relapse in patients recovering from AUD (Charlet et al. 2014).

### 3.4 Relationship Conflict: In the Context of Intimate Partner Violence

A relationship conflict task explores sex differences to social stress and conflict resolution in individuals in a romantic relationship by requiring the couple to work toward a resolution together (Flanagan et al. 2019). Similar to the guided imagery induction, the script for this task is personalized to the participant; a topic of relationship difficulty is identified by each partner, and a recording of the couple discussing the topic is played in the MRI scanner. Brain response during conflict resolution with a partner is contrasted with brain response during the neutral script (recording of participant discussing their morning routine) in couples with one partner engaging in hazardous drinking or meeting DSM-IV criteria for SUD (primarily cocaine or cannabis) (Flanagan et al. 2019). Results from the neuroimaging study show that for both sexes the amygdala is functionally engaged during the relationship conflict (Flanagan et al. 2019). However, females show greater functional connectivity between the right amygdala-left inferior frontal cortex, whereas males have stronger functional connectivity between the OFC-right amygdala/hippocampus (Flanagan et al. 2019). Intimate partner violence, highly prevalent and salient in SUD populations (Afifi et al. 2009; Chermack et al. 2008; Leonard and Homish 2008), is associated with increased functional connectivity between the right amygdala-left prefrontal cortex. These findings suggest that the amygdala circuitry
plays a crucial role in both males and females with substance use during processing relationship conflict (Flanagan et al. 2019).

### 3.5 Psychosocial Stress: Montreal Imaging Stress Task

Psychosocial stress is induced by the Montreal Imaging Stress Task (Dedovic et al. 2005), based on the Trier Mental Challenge Task (Kirschbaum et al. 1993), by having participants solve difficult mental arithmetic problems on a computer with external pressure. This includes adjusting the time limit to ensure a >50% failure rate, having direct negative feedback from one of the investigators, and showcasing performance progress on the monitor. The combination of these external pressures creates an anxiety-producing social environment during an already challenging task. Neural activation during the Montreal Imaging Stress Task was contrasted with a non-stress control version of the task in nicotine smokers (Dagher et al. 2009). Widespread deactivations in limbic and paralimbic systems, notably in the hippocampus, amygdala, and nucleus accumbens were observed (Dagher et al. 2009). The deactivations predicted smoking cue-activity and craving in brain areas controlling attention and motivation, thereby again suggesting mesocorticolimbic circuits underlie stress in addiction and relapse (Dagher et al. 2009).

In summary, task-related fMRI activation during a range of social contexts has provided insights into neural circuits affecting acute stress response in relation to SUD. The different stress paradigms – guided imagery induction, social ball tossing, facial emotion recognition, relationship conflict, and psychosocial stress – revealed activations in distinct regions and overlapping regions predictive of addiction trajectory (Fig. 2). A few patterns emerged among these tasks: (1) there was an overlap between regions implicated in SUD or behaviors related to drug use, and the processing of social stress, (e.g., anterior insula, amygdala, hippocampus, and medial prefrontal cortex); (2) the anterior cingulate cortex, caudate, and amygdala activations were most commonly observed among stress paradigms; and (3) greater activation to drug cues and higher reporting of craving emerged post-stress. Paradigm-specific trends emerged as well. During the stress script paradigm, hypoactivation was observed in regions involved in emotion regulation, while hyperactivation was observed in regions involved in drug craving and substance use-related behaviors (Elton et al. 2015; Seo et al. 2011; Sinha et al. 2005; Li et al. 2006; Kober et al. 2017). Feelings of social exclusion elicited by Cyberball produced increased neural responses in frontal regions involved in processing negative affect, while decreased responses in frontal regions were associated with regulating feelings of social ostracism (Maurage et al. 2012; Bach et al. 2019a; Hanlon et al. 2018; Bach et al. 2019b). The aversive face paradigm induced hyperactivation in brain regions implicated in control, decision making, and higher-order executive functioning (Bedi et al. 2009; Charlet et al. 2014).
4 Future Directions

Main results from 21 neuroimaging studies were reviewed, corroborated, and ultimately deemed instrumental in identifying key brain regions that are dysregulated in individuals with early and lifetime social stress with SUD, and those involved in the processing of social stress stimuli in individuals with substance use. These findings should, however, be considered in light of methodological limitations in this emerging research area. The fact that the body of knowledge is based on only 21 studies to explore the intersection of substance use/disorder and social stress should question the generalizability of these findings.

One of the main challenges of investigating social stress in SUD is disentangling the effects of social stress from the effects of substance use. Most studies utilized a cross-sectional design comparing individuals with substance use history and trauma history to healthy controls. Only a few included a sample with substance use history and no trauma history or healthy individuals with trauma history. Moreover, none of the studies did all of the above. The absence of a healthy control group with trauma and an SUD group without trauma means that it is impossible to confirm the unique neural effects of social stress in individuals with SUD. Many of the structural and functional alterations identified in the early and lifetime studies corroborated findings from previous research on stress or trauma in samples without substance use history, indicating that these effects may be trauma-specific. Studies of social stress in adulthood largely did not account for histories of childhood maltreatment in their sample, diminishing the possibility to identify effects of cumulative stress dysregulation. The neuroimaging studies exploring the acute response to stress in SUD also failed to account for past trauma history, except for one study (Elton et al. 2015). Without acknowledging that prior stress may impact current stress response, a complete and accurate depiction of the neural underpinnings of stress in SUD cannot be determined. To disentangle the brain effects due to addiction versus social stress, future studies must include a control group with high trauma and no substance use history.

In the same vein, the effects of non-social stress must be disentangled from social stress. Capturing social stress in a natural setting is a difficult undertaking. It requires longitudinal studies and in vivo neuroimaging tasks that include naturalistic stimuli related to an individual’s social context. However, rather than developing novel in vivo neuroimaging paradigms, extending subjective neuroimaging paradigms such as the Guided Imagery Induction, and supplementing them with ecological momentary assessments (EMA) is a more pragmatic approach (Stone and Shiffman 1994). EMA enables data collection during the daily life of a person and has the potential to capture important information (feelings, thoughts, emotions, cravings, and more) related to social stress. Such methodological advances could guide further research.

The neurobiological sex differences in response to social stress in SUD populations is another area that requires further exploration. As sex-specific risk factors for mental illness disproportionately affect females, particularly sexual
violence (Flanagan et al. 2019; Oram et al. 2017), further analysis into sex-specific effects is warranted. While currently limited, some studies are suggesting important sex differences. For example, in healthy participants, fMRI studies revealed different neurobiological alterations associated with psychological stress and during processing social stress in females as compared to males (Wang et al. 2007; Goldfarb et al. 2019). A few of the studies summarized in this chapter also suggest unique neural effects of social stress in females versus males with alcohol or cocaine use associated with craving and reduced social functioning (Seo et al. 2011; Li et al. 2005; Li et al. 2006). However, the sex differences in neurobiological signatures associated with social stress have not been fully characterized in SUD. The clear roles that sex plays in various aspects of stress sensitivity and mental illness emphasize the importance of expanding knowledge of the female brain that could also lead to enhanced sex-specific interventions.

In addition to the issues raised above, several questions remain. How do brain alterations in SUD with a history of chronic stress or induced (acute) stress differ depending on drug class? What are the unique neural pathways of social stress in SUD as compared with other types of stress (e.g., stress associated with withdrawal)? What targeted treatments may be suitable to address neural and behavioral impairments resulting from social stress in SUD? Systematic research on these questions through rigorous clinical studies in diverse addictions samples accounting for past trauma as well as measuring stress response could contribute to a greater understanding of the multi-faceted role of social stress in SUD.

5 Conclusion

Neuroimaging studies provide remarkable insight into the effects of social stress on brain structure, function, and connectivity in SUD. As summarized in this chapter, social stress history and induction alter brain regions implicated in drug-seeking, taking, and craving pathways. These results lead to a few hypotheses: (1) chronic social stress leads to neural alterations underlying a propensity for drug use; (2) brain effects previously attributed to drug use may be, at least in part, impacted also by stress exposure; (3) brain regions impacted by chronic substance use affects stress response thereby modulating social function; and (4) synergistic effects of social stress and SUD may result in further increased SUD severity and social dysfunction. The burgeoning interest in the “social factors of addiction” will hopefully accelerate our understanding of how an individuals’ exposure to adverse life events, trauma, or chronic stress affects their substance use trajectory. Solidifying the knowledge of neural pathways of social stress in SUD is crucial to inform causal research, prevention, and targeted addiction treatments.

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