Developmental Contributors to Trauma Response: The Importance of Sensitive Periods, Early Environment, and Sex Differences

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Abstract This review considers early factors that interact with development to contribute to later trauma responses, including developmental sensitive periods, the effects of early environment, and the emergence of sex differences. We also describe development of neural substrates that have been associated with posttraumatic stress disorder and specifically focus on fear behavior and circuitry. Emerging evidence suggests that there may be developmental shifts around age 10 in these underlying circuits that may contribute to vulnerability. We also discuss age-related changes in the importance of caregiver availability as positive buffering factors. Hormonal changes later in development with onset during puberty appear to further shape development trajectories toward risk or resilience. We highlight these recent findings as well as the great need for further longitudinal research from middle childhood through early adulthood.

Keywords Amygdala • Child development • Early environment • Fear conditioning • Sensitive periods • Sex differences • Social buffering • Trauma

Contents

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Many of the most potent risk factors for posttraumatic stress symptoms and other negative responses to trauma begin early in life, as the early environment interacts with the developing brain. Childhood adversity is one of the most potent and well-known risk factors for the development of posttraumatic stress disorder (PTSD). It is well established that early deprivation, for example, institutional rearing, but also childhood abuse, or exposure to other traumatic events, such as witnessing violence, can have a long-lasting effect on the individual. However, other factors such as risk genotype, brain development, and emergence of pubertal sex differences also influence the later risk for developing PTSD. A great deal of research has established retrospective links between these early factors and psychiatric and medical outcomes in adulthood. Further research is now needed to address why early life is such an important period and to outline the specific windows of development that interact with environmental factors that increase risk for negative outcomes.

Here, we highlight the importance of developmental timing as a key factor influencing the impacts of early experiences on risk for trauma-related pathology. We focus on the development of fear learning systems, as deficits in fear inhibition and extinction have been demonstrated to be critical mediators of the trauma response in adults [1, 2]. We begin by outlining sensitive periods in development for trauma exposure and emergence of psychopathology, and early development in the neurobiology of fear, as well as windows of divergence in brain development: toward risk-related or healthy outcomes. We then highlight protective aspects of the early rearing environment, which may buffer against the negative effects of early-life stress. Finally, we end with the developmental timing of sex differences in risk for trauma-related pathology. While there is an emerging literature on genes that may contribute to vulnerability for trauma-related mental illness, here we focus primarily on environmental factors during development.

1 Sensitive Periods

1.1 Sensitive Periods for Trauma Exposure and Related Pathology

The prevalence of anxiety disorders has been shown to increase during late childhood and early adolescence, suggesting that this period may be developmentally critical in identifying individuals at risk for adult psychopathology [3, 4]. In a

replication of the National Comorbidity Survey, anxiety diagnoses were found to be highly prevalent at 28.8% with the earliest disorder emerging at a median age of 11 [5]. Separation anxiety and specific phobias emerge at the earliest age, followed by social phobia in adolescence and generalized anxiety, which can emerge in adulthood [3, 4]. Several longitudinal studies of children and adolescents found no sex differences in childhood (prior to age 12), but a highly significant increase in anxiety disorders in girls relative to boys in adolescence [6]. PTSD can be difficult to diagnose in children using adult criteria; therefore several modifications have been made to account for developmental stage, such as using trauma reenactment during play and frightening dreams as symptom presentations ([7]). The most common PTSD-eliciting event in children is injury or motor vehicle accident, with prevalence rates of about 25%. Physical or sexual abuse can result in rates as high as 58% ([7]). Because onset of PTSD is dependent on the timing of the traumatic event, it is difficult to determine developmentally sensitive periods for the emergence of symptoms in childhood; however some studies have diagnosed children as early as preschool age [8]. Longitudinal studies of PTSD in children have found it to be a stable diagnosis over 2 years, indicating the need for treatment intervention with children [8].

Exposure to trauma during childhood has long been recognized as a significant predictor of PTSD in adulthood in addition to other mental disorders, such as depression [9]. Adversity includes several different negative experiences, such as child maltreatment and poverty. Child maltreatment is a pervasive public health problem as more than three million children received intervention from Child Protective Services in 2012 [10]. Poverty is even more prevalent; currently over half of all students in US public schools come from low-income families according to the National Center for Education Statistics [11]. The long-lasting impact of adversity during early life on the brain has been well established over the last several decades in animal research [12] and in human imaging studies (see [13] for recent review).

Although there is clear evidence of the impact of childhood trauma on brain development, specific age-related sensitive periods have not been well defined. Yet, the developmental timing of trauma exposure can have a significant impact on risk for altered brain development and psychopathology [14]. While large cohort studies of child abuse using the Adverse Childhood Experiences (ACEs) in over 80,000 individuals underscore the tremendous negative impact of childhood trauma on adult health [15], most do not examine timing of trauma exposure very specifically, but rather report ACEs prior to age 18. While there are a limited number of prospective studies that have examined the effects of trauma exposure in childhood, a recent study using retrospective recall identified age 10 as the period when trauma severity recall was greatest [16]. In addition, a survey of US families recently reported that cumulative stressors prior to age 13 significantly increase the odds of psychological distress in adulthood [17]. Early adolescence is marked with increased independence resulting in higher likelihood of exposure to trauma. It has been recognized for decades that middle school children from low-income urban neighborhoods experience high levels of community violence [18]. Further,

PTSD symptoms in children aged 8–13 are highly correlated with trauma exposure within the last year [19]. However, longitudinal developmental studies defining age-related sensitive windows of trauma exposure and PTSD symptoms are still lacking in the literature.

1.2 Sensitive Periods for Development of Fear Neurobiology

Adult neurobiological models of risk for negative outcomes following trauma focus primarily on hyper-reactivity of the amygdala [20], a part of the limbic system located in the medial temporal lobe of the brain and an integral component of mammalian fear circuitry [21–23]. Studies of humans with brain damage have also found that the amygdala modulates the fear response: temporal lobectomy in patients results in loss of fear-conditioned startle [24]. Amygdala hyper-reactivity is observed in patients with chronic PTSD [25–28], and, more importantly, amygdala reactivity prior to trauma exposure may predict later PTSD severity [29, 30]. Amygdala reactivity is moderated by inhibitory connections from the ventromedial prefrontal cortex (vmPFC), which appear to be abnormal in PTSD [27, 28]. Pediatric PTSD is marked by the same underlying neurobiology, i.e., children and youth with PTSD show heightened amygdala reactivity and impaired amygdala-prefrontal connectivity [31, 32]. The development of fear circuitry may thus contribute to risk for later PTSD and other trauma-related pathology.

Animal models using fear conditioning have suggested very robust age effects on fear-related learning. For example, early postnatal development is associated with different neural circuitry underlying fear regulation compared to the juvenile period of development, such that the prelimbic cortex is involved in expression of learned fear in juvenile but not infant rats [33]. In humans, early studies using structural magnetic resonance imaging (MRI) outline a pattern of similarly prolonged development in the brain regions supporting fear learning, from early childhood through early adulthood. For example, amygdala volume increased in males from ages 4 to 18, while hippocampal volume increased in females in the same age range [34]. On the other hand, cerebral gray matter development follows an inverted U-shape, showing early increases in volume and thickness that peak in late childhood, followed by decreased volume and density after adolescence [35–38] with the medial prefrontal cortex (mPFC) showing the longest developmental trajectories [39]. White matter integrity in the uncinate and cingulum, the primary tracts connecting the amygdala and hippocampus with the PFC, show steep linear increases from childhood through early adulthood, not peaking until after age 35 [40]. Such prolonged patterns of development for the amygdala and prefrontal cortex, and connections between these regions, indicate that there is a large window spanning childhood and adolescence during which experience, particularly traumatic experience, can shape trajectories of brain development.

In addition to the aforementioned studies suggesting that gray matter volumes for the prefrontal and temporal lobes peak from ages 12 to 16 [35, 36]; a recent study found a developmental shift in functional connectivity between the amygdala and the mPFC during the viewing of fearful faces [41]. The cross-sectional study included children from 4 years of age to adults and found that these areas were positively connected prior to age 10 years and negatively connected after age 10 years [41]. The observed negative functional connectivity continued to increase from adolescence to adulthood. Earlier studies using similar methods found that adolescents showed greater amygdala reactivity to fearful faces than adults [42]. Fear conditioning studies comparing adolescents to adults have found that adolescents show greater fear-conditioned responses compared to adults [43], suggesting blunting of fear responses with adulthood. Together, these structural and functional data point to developmental decreases in activation in limbic subcortical structures in response to fear-related cues from childhood to adulthood.

Similar developmental effects on inhibition have been observed using acoustic startle responses in children and adolescents. One study examined habituation of startle responses in 7–9-year-olds compared to 10–12-year-olds in children with anxiety disorders and controls [44]. This study found that startle increased with age only in the at-risk group and was already higher in the younger children with anxiety disorders. While this study suggested that risk phenotypes emerge between 9 and 10 years of age, it was based on a relatively small sample size. A more recent study of 40 healthy children between 8 and 13 years old used fear conditioning methods and found that adult-like patterns in fear-potentiated startle emerged around 10 years of age [45]. One of the key findings of this study was the inability of younger children to inhibit fear to safety signals, suggesting that this ability develops in healthy children around 10 years of age. Data from our studies at the Grady Trauma Project (GTP) support a developmental shift in safety signal processing around age 10, in that participants who were older than 10 years of age showed higher fear-potentiated startle and significant discrimination between the CS+ (danger signal) and the CS- (safety signal) [46]. In contrast, children up to 9 years of age showed deficits in fear inhibition – this trait has been associated with PTSD in adults [47]. Of note, developmental studies that have used skin conductance response (SCR) as a physiological measure have had mixed results. A recent study that compared age groups between 5- and 10-year-old healthy children did not find age-related differences in fear conditioning or extinction [48]. In our studies in children from the Grady Trauma Project, we have found that SCR to the CS+ was greatest in children under 10 with high anxiety [46]. We also found that SCR to the CS+ was associated with fear-related symptoms of PTSD in children [49].

The abovementioned sensitive periods in brain development and physiology also point to the importance of middle childhood as a sensitive period in which environmental insults such as early adverse experiences can have long-lasting neurobiological impacts. Studies by Tottenham and colleagues investigated fear circuitry in children adopted from institutional care with high rates of neglect. These studies found larger amygdala volumes in children with prolonged maternal deprivation

early in life [50]. In an MRI study of orphaned children, those that were adopted prior to 15 months of age had similar amygdala volumes to controls, whereas children adopted after 15 months of age showed increased amygdala volumes later in childhood (tested around 10 years of age). Although this early trauma may increase risk for anxiety disorders in children, the MRI results in the study were not directly related to anxiety, since the relationship remained significant even after exclusion of children with anxiety [50]. However, the study did find that amygdala volume was positively correlated with internalizing and anxiety symptoms in the children. In addition to increased amygdala volume, amygdala reactivity to fearful faces and functional connectivity between the prefrontal cortex and the amygdala are altered in children and adolescents with early-life stress. Moreover, normal developmental changes appear to be disrupted [14, 41]. A recent study using retrospective data from adults recalling childhood trauma exposure found a dose response between trauma severity and amygdala volume, with a significant increase in volume with trauma recalled between ages 10 and 11 [16]. Finally, studies of pediatric PTSD found that PTSD was associated with a smaller PFC and hippocampus and that symptom severity correlated with the decrease in these areas [31, 51]. On the other hand, pediatric PTSD was also associated with increased dorsal cingulate reactivity to threatening cues in children ages 8–18, with age 10 and 11 reflecting highest activity [32]. Taken together, these studies indicate that timing of trauma exposure during development has significant consequences on PTSD symptoms as well as its neural underpinnings and that middle childhood around age 10 may be a sensitive period for these effects.

2 Early Environmental Factors Influencing Risk for PTSD

2.1 Rearing Environment

Growing up in an adverse environment is a known risk factor for the emergence of mental disorders; however, a positive rearing environment may exert protective or beneficial effects on the development of psychiatric disorders. The effect of rearing condition is often investigated in nonhuman primates by comparing monkeys reared by their mother with monkeys reared by age-matched peers or nursery-reared monkeys. Peer- or nursery-reared monkeys show more behavioral fear [52], lower baseline cortisol levels [53], and larger stress-sensitive brain regions [54] compared to mother-reared monkeys, indicating that rearing condition may have long-term effects on stress responses and related neurobiology. The availability of the mother may have some specific effects by means of maternal buffering, which are discussed in a separate section below.

Environmental enrichment (EE) methods have been used in order to investigate the effect of a positive rearing environment. EE is thought to improve cognitive, motor, and sensory functions compared to standard housing [55]. In a typical EE

study, laboratory rodents are placed in single-sex housing in groups with unlimited access to food and water. EE cages, however, also consist of toys, nesting materials, tunnels, running wheels, and ladders, which are regularly changed to bring novelty and complexity to the rodents' environment [56]. In the first study, the protective effects of EE against stress were investigated. Rats were tested in an EE 2 weeks prior to an inescapable foot shock procedure, which was used to induce PTSD-like anxious behavior. However, the study found that EE did not protect against the effect of this foot shock procedure [57]. Second, in a follow-up study, the rats that underwent the foot shock procedure were placed in an EE after the shock, which reversed the PTSD-like anxiety behavior and increased cell proliferation in the hippocampus [57]. Third, the effects of EE as treatment for prenatal chronic stress and early-life stress were tested. Prenatal chronic stress was induced by means of unpredictable foot shocks [58–60], and early-life stress was induced by housing newborn rats (postnatal day (PND) 2–21) in cages with limited nesting/bedding materials [61]. Both resulted in depressive-like behavior, impaired spatial learning and memory, and impaired hippocampal long-term potentiation in young adulthood (PND 53–57). EE treatment during childhood and adolescence (PND 22–52) nullified the negative effects of prenatal and early-life stress [58–61]. Furthermore, EE during the peri-pubertal period reversed the negative effects of postnatal maternal separation on endocrine and behavioral stress responses [62]. It can be concluded that EE does not protect against the immediate effects of stress, but EE treatment can reverse the negative effects of prenatal and early-life stress.

Investigating the effects of rearing environment in a human sample is far more challenging than in a laboratory setting. However, studies investigating children who were raised in institutional care and were adopted early in life have provided some insights in effects of rearing conditions. In a groundbreaking randomized trial of foster care, the Bucharest Early Intervention Project (BEIP) assigned children who were raised in Romanian orphanages to either a care-as-usual or foster care intervention [63]. A recent follow-up of the study found that foster care attenuated many of the symptoms of mental illness that developed in the institutionalized children. Several other studies from the same project showed improved cognitive development [64], emotional responses, attachment, a better psychiatric outcome [65], and improved neural activity [66, 67] in children who were adopted compared to children who remained in orphanages.

Another line of research on internationally adopted children from orphanages outside the USA has also yielded data on the effects of early environment. In most cases, institutionalized children were adopted by high-functioning and high-socioeconomic-status (SES) parents, who could provide an enriched environment for their children. Although the effects of parental care cannot be separated from the effect of EE, this situation is the best opportunity to study EE as an intervention in humans. When children were compared to never-institutionalized children, early deprivation was found to have long-lasting effects on brain and behavior. Previously institutionalized children showed difficulties in emotion regulation, increased amygdala volumes [50], and reactivity [68], as well as early maturation of amygdala-prefrontal connectivity [69]. In sum, even though adoption improves

many aspects of cognitive and emotional development, EE does not completely counteract the negative effects of the early environment and therefore does not seem to have the same effects as observed in rats.

2.2 *Maternal Buffering*

Parental availability has a particularly large stress-reducing effect on offspring [70]. In species where the primary caregiver is the mother, this phenomenon is called maternal buffering [71]. Nonhuman primate studies have shown that maternal separation induces significant stress in the infants, indicated by increased behavioral stress and cortisol responses. Reunion with the mother, or having access to visual or auditory stimuli associated with the mother, significantly decreases cortisol levels of the offspring [72–74]. In rat pups, pairing a shock with a neutral odor typically results in avoidance of the odor in the future; however, fear learning does not occur when the mother is present during odor conditioning [75, 76]. It is thought that this mechanism prevents the pups from learning to fear their mother, even when she (accidently) induces pain by, for example, stepping on the pups. This promotes attachment to the mother and thus survival in these pups that are dependent on her for care [77]. With increasing age, the effects of maternal buffering on cortical activity in the rat decrease [78].

A dampened fear response in the presence of the mother has also been observed in human studies. The cortisol response to a social stressor in children, but not in adolescents, is eliminated by maternal support [79]. In an fMRI study, children showed dampened amygdala activation to pictures of their mother compared to pictures of a stranger, whereas there was no difference in adolescents [80]. In another study that used fear conditioning of startle responses, children showed an attenuated fear response to a safety signal when the mother was available during conditioning [81]. However, when the mother was not in the same room, children were not able to discriminate danger and safety signals. Importantly, the effect of maternal buffering was only observed in children and not in young adolescents [81]. This is most likely explained by the functional emergence of the prefrontal cortex, which is important for learning to discriminate fear and danger and inhibiting the fear response when appropriate [82]. This postulation is supported by an fMRI study in which more adult-like connectivity between the prefrontal cortex and the amygdala was observed when viewing maternal versus stranger pictures [80]. Again, the effects of maternal buffering were only observed in children and not in adolescents [80].

In contrast to childhood where the individual is dependent on the parent, adolescence is a time of increasing independence. The reduced effects of maternal buffering in this phase might reflect this transition. Although adolescents or adults do not directly benefit from maternal presence, a study with mother- versus nursery-reared rhesus macaques showed that maternal presence during childhood may have

long-term effects on the ability of adult monkeys to benefit from social support later in life [83].

2.3 Social Support

Social support is thought to influence responses to stress and trauma during development or later in life. The importance of social support as a protective factor against psychiatric disorders has been demonstrated in several preclinical and human studies. In one rodent study, mice that were isolated from conspecifics showed higher anxiety-like responses and lower basal plasma corticosterone, but a larger corticosterone increase to novel or stressful situations compared to mice exposed to social or enriched conditions [84]. Similarly, socially isolated rats show increased freezing behavior after fear conditioning, whereas socially partnered rats enhance their grooming behavior [85]. These findings underscore the importance of social interaction for a healthy behavioral and neuronal development in response to stress and trauma.

In a human study, social support was found to protect against depression in a genetically at-risk group of maltreated children [86]. In an adult sample, baseline social support was found to protect patients with chronic illness from the development of PTSD [87]. Likewise, low levels of post-deployment social support were associated with increased PTSD and depression symptoms in a military cohort [88, 89]. The positive effects of social support on trauma survivors may explain the therapeutic effects of some innovative drugs that promote social affiliation, such as oxytocin [90] and methylenedioxymethamphetamine [91].

The studies reviewed here suggest that the environmental factors of rearing environment, maternal availability, and social support influence the response to stress and trauma and may protect against the development of mental disorders. Being able to successfully control mild-to-moderate stressors can result in the development of an adaptive stress response, which may protect the individual from negative effects of future uncontrollable stressors. Enriched environments and maternal or other social support during development promote adaptive stress responses. A recent finding that maternally reported warmth did not impact the effects of maternal availability on fear conditioning in children with trauma exposure [81] suggests that the quality of the mother-child relationship may not be as important as maternal availability. It is clear that childhood is a particularly sensitive period for environmental influences. As was observed in most of the maternal buffering studies, maternal presence did not impact adolescents older than 10 years of age, only children. Considering the steep increase in psychiatric disorders during adolescence, it is important to advance our understanding of environmental influences on the more plastic period before age 10.

3 Developmental Emergence of Sex Differences in Risk for PTSD

From a public health perspective, sex differences in the response to trauma are a critical factor for further investigation, given that women are at greater risk than men for developing trauma-related mental health disorders Women are nearly twice as likely as men to be diagnosed with PTSD and mood disorders [5, 92–95]. Interestingly, women do not experience a greater number of traumas than men; in fact, it has been estimated that a woman is only 77% as likely as a man to experience trauma [96]. However, women and men show differences in specific types of traumas they are most likely to experience. For example, women are more likely than men to have experienced forms of trauma that are particularly severe, such as sexual assault or abuse, starting in childhood [96]. More importantly for informing prevention and early intervention strategies, there may also be sex-dependent vulnerabilities that predate trauma. Studies of child development have the potential to reveal mechanisms that produce the striking sex differences in trauma-related psychopathology in adults.

3.1 Sex-Dependent Risk Factors for Trauma-Related Pathology

Although it is clear that women are at greater risk for PTSD than men, research on preexisting vulnerability in women is only in very early phases, with promising recent findings pointing to risk factors in brain circuits, peripheral autonomic physiology, and genetics. Exaggerated reactivity of the amygdala [25, 30] and an impairment in its connections with the rostral and subgenual anterior cingulate cortex (ACC) [28, 97] appear to be key risk factors for trauma-related psychopathology, particularly hyperarousal and other anxiety symptoms [28]. Notably, both the amygdala and prefrontal cortex are sensitive to the effects of gonadal steroid hormones [98–100]. Meta-analysis of the neuroimaging literature indicates that women show greater amygdala reactivity to negative emotional stimuli than men [101], which may increase their risk for trauma-related pathology.

Evidence suggests that estrogen levels in women may influence physiological and neural reactivity to threat, possibly providing one mechanism for women's increased PTSD risk. In an fMRI study of fear conditioning and extinction, naturally cycling women during a high-estrogen phase showed greater activation of fear-processing regions than men or women with low estrogen levels [102]. Similarly, pregnancy may be a particularly vulnerable time for women, as circulating estrogens and the stress hormone cortisol increase over the course of pregnancy. A recent study showed that pregnant women reported greater levels of hyperarousal symptoms than nonpregnant women and showed greater fear-potentiated startle to a safety signal, reflecting impaired fear inhibition [103]. Interestingly, low estrogen

levels may also increase risk: trauma-exposed women with low estrogen levels (both naturally cycling and postmenopausal) showed impaired fear inhibition [104] and impaired fear extinction [105] in a fear-potentiated startle paradigm. Women with low (versus high) estrogen also showed greater connectivity between the amygdala and dorsal ACC [106], a region associated with increased fear expression and arousal. Further studies are needed in order to investigate the possibility of an inverted U-shaped dose-response relationship between estrogen and risk for PTSD and to outline whether there are specific circumstances (e.g., pregnancy, menopause) in which high versus low levels of estrogens may promote risk.

Rodent work has demonstrated a possible mechanism by which estrogen levels may interact with stressors to increase fear reactivity in females. Ovariectomized female rats who received exogenous estrogen replacement during stress exposure showed an increase in dendrite length and density in an inhibitory pathway between the prefrontal cortex and amygdala, whereas those without estrogen replacement showed no such increase [107]. In contrast, male rats exposed to stress showed no dendritic remodeling in the same pathway [108]. A human analog of this pathway has been shown to be impaired in women with PTSD; relative to trauma-exposed women without PTSD, traumatized women with PTSD showed less connectivity between the amygdala and a prefrontal region that regulates amygdala activity, the subgenual anterior cingulate cortex [28].

Genetic variation may also contribute to PTSD risk in women. For example, a data-driven analysis of genetics associated with fear in both humans and mice identified a sex-specific risk factor for PTSD [109]. Genetic polymorphisms associated with PTSD in humans were examined for overlap with genes whose expression in the mouse amygdala showed large changes before and after a fear-conditioning experiment. In this study, 17 overlapping genes were identified, with the strongest effects for genes coding for pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor PAC1R. High peripheral levels of PACAP and a genetic polymorphism in *PAC1R* gene were associated with PTSD in women but not men [109]. In women, the *PAC1R* polymorphism was also associated with greater dark-enhanced startle, a psychophysiological correlate of anxiety, and less discrimination between danger and safety signals with fear-potentiated startle [109]. Further, the same risk allele was associated with greater amygdala reactivity to threat and less functional coupling of the amygdala with regulatory regions including the hippocampus and rostral ACC [110, 111].

3.2 Emergence of Sex-Dependent Risk Factors over Development

In order to understand how sex differences influence fear neurocircuitry and interact with trauma response, it is critical to study the emergence of sex differences over development. Although most sexually dimorphic brain changes occur with the

increase in gonadal hormone activity during puberty, it is possible that even before puberty environmental influences and maturational factors differentially impact girls and boys. An emerging literature in humans points to sex differences in emotional neurophysiology before puberty. Psychophysiological measures of emotional arousal such as heart rate and skin conductance response indicate that reactivity to negative stimuli is greater in prepubertal girls than boys [112]. In a recent study of prepubertal sex differences in fear conditioning, girls showed less discrimination between danger and safety signals compared to boys, a phenotype that has been associated with PTSD [49]. Further, the skin conductance response to the conditioned danger signal was correlated with different PTSD symptoms in girls and boys, suggesting sex-specific patterns in physiological underpinnings of trauma-related pathology [49]. Finally, in girls, but not boys, early-life stress was associated with increased basal cortisol, and this predicted later impaired resting state connectivity between the amygdala and mPFC [113].

In order to examine whether the *PAC1* genotype has sex-specific effects on anxiety prior to puberty, we examined dark-enhanced startle in 28 boys and 22 girls aged 10 ± 0.2. Dark-enhanced startle was greater in children with the risk (CC) genotype when girls and boys were considered together, with no effect of sex [114]. This indicated that the sex-dependent effects of this genotype on anxiety are not observed prior to the activational effects of estrogens postpuberty. However, it is notable that PAC1 genotype was associated with heightened startle in boys [114], but not in adult men [109], pointing to an intriguing possibility that postpubertal hormonal changes may confer a protective effect upon men with the risk genotype. This idea is supported by preclinical research showing protective effects of testosterone in males. For example, in a study of fear conditioning in which female mice showed greater freezing behavior than males after conditioning to a tone, estrogen levels and even ovariectomy in females had no effect on their freezing behaviors. Instead, males showed an *increase* in freezing after orchidectomy, which returned again to a lower level after testosterone administration [115]. Additional experiments that manipulate hormone levels in males are needed to replicate these findings and to examine links with *PAC1* genotype.

Important sex differences appear to occur with the increase in gonadal hormones during puberty. For example, it appears that sex differences in amygdala reactivity may not emerge until after puberty. A small study comparing adolescents aged 9–17 and adults aged 25–36 showed greater amygdala reactivity in female versus male adults, but not adolescents [116]. These findings were replicated in larger studies showing no sex differences in the amygdala response to emotional stimuli in children aged 7–13 [42, 117]. In addition, a study of regional volumes in the amygdala and hippocampal complex in a large sample of children aged 4–18 showed no sex differences before puberty, but with increasing pubertal maturity, the volume of hippocampal complex structures decreased in boys and increased in girls [118]. Girls and boys did not differ in patterns of amygdala volume development in this study. Further research specifically targeting late adolescence and early adulthood is needed to determine the specific developmental period during which sex differences in amygdala function emerge.

By adolescence, children exposed to stress already show similar patterns of brain activation as trauma-exposed women and men: in adolescent girls, but not boys, childhood maltreatment was associated with reduced functional connectivity between the amygdala and subgenual ACC [119]. Recently, a large study was conducted to examine how early stress exposure might interact with genetic polymorphisms in genes regulating HPA axis function, in girls and boys aged 7–12 [120]. Pagliaccio and colleagues found that the experience of stressful life events was associated with greater amygdala reactivity to negative emotional stimuli, consistent with previous studies. However, genetic factors showed an interaction with sex and pubertal status: polymorphisms in HPA axis-related genes predicted greater amygdala reactivity to fearful stimuli in pubertal girls and greater amygdala reactivity to *neutral* stimuli in pubertal boys. Interestingly, amygdala reactivity may be a more stable trait across development in boys than in girls: a study following 4-month-old infants into adulthood showed that infant boys with a "high-reactive" pattern of behavior (vigorous motor activity, crying to unfamiliar stimuli) also showed greater amygdala reactivity to novel face stimuli as adults, relative to men who were "low-reactive" as infants [121], and this was not the case in women. It is possible that amygdala function in women is more responsive to environmental and hormonal effects than in men.

4 Summary and Conclusions

Several important developmental factors contribute to risk for PTSD, including childhood adversity and hormonal activation. The neuroplasticity of the developing neural circuitry of fear responses leads to putative sensitive periods when trauma exposure may be particularly detrimental. It is important to fully understand the timing of such sensitive periods in order to apply optimal intervention and prevention strategies for PTSD. Figure 1 shows the putative timing for the primary factors that increase risk for PTSD during development such as early deprivation, child abuse and trauma exposure, and activation of gonadal hormones. In contrast, factors that decrease risk for PTSD include maternal buffering and social support. In the aftermath of early deprivation, enriched environments have been shown to potentially attenuate negative risk.

Fear learning behavior continues to change and develop over a long window of development, with the ability to differentiate threatening and safe cues strengthening in middle childhood around age 10 (e.g., [46]). Similarly, the amygdala and its connections with the vmPFC continue to develop into young adulthood. During this long window of developmental change, trauma can shift the trajectory of development toward outcomes associated with risk, such as the greater amygdala reactivity and volume observed in previously institutionalized children [50, 68]. Interestingly, retrospective studies of adults reporting their childhood experiences suggest that the impacts of trauma on amygdala structure are greatest in middle to late childhood [16]. However, the specific boundaries of sensitive periods during which trauma has

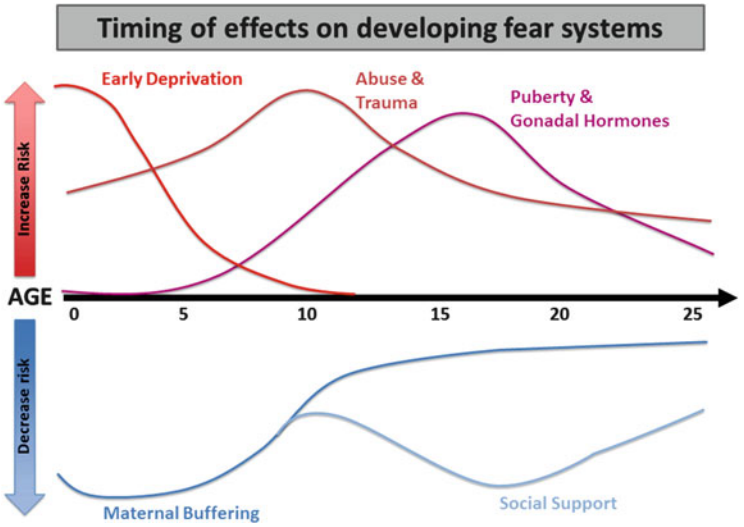

Fig. 1 The figure depicts the factors that increase and decrease risk for PTSD and the putative timing during development when these factors exert their greatest influence on underlying brain circuitry

its greatest impacts have not yet been defined. Longitudinal studies following participants through childhood and adolescence are needed to address this question.

During this same window of early childhood through middle childhood, the brain also appears to be sensitive to positive effects of the early environment. Aspects of the rearing environment can buffer against the negative effects of early-life stress, including trauma [65]. In addition, social buffering provides a powerful protective influence against stress and changes with development such that maternal presence provides greatest buffering influence through middle childhood and less so during adolescence [81]. Importantly, the potential to benefit from social support in adulthood is thought to depend on maternal availability during childhood [83].

Finally, the increase in gonadal steroid hormones during the pubertal period in adolescence appears to be a later window that shapes the brain toward risk or resilience. Developmental studies of amygdala reactivity suggest that sex differences in amygdala reactivity emerge only after puberty, with the increase in levels of steroid hormones [115, 116], and additional studies targeting late adolescence are needed in order to define a specific developmental window. However, it is notable that there are also intriguing findings pointing to prepubertal sex differences in physiological measures of arousal and HPA axis responses to stressors [49, 112, 113].

In summary, development is a highly plastic period that is influenced by environmental factors. Neural development points to several sensitive periods during development, including an early childhood period prior to age 3, a late childhood period around age 10, and an adolescent period associated with puberty.

Figure 1 depicts the putative periods when environmental factors can exert their influences. Future studies should carefully define these and other sensitive periods in order to provide critical windows of opportunity for intervention and even prevention of trauma-related pathology such as PTSD.

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