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Human Amygdala Development in the Absence of Species-Expected Caregiving

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Abstract

In altricial species, like the human, caregiver presence is necessary for typical emotional development. Children who have been raised in institutional care early in life experience caregiver deprivation and are at significantly elevated risk for emotional difficulties. The current manuscript examines the non-human and human literatures on amygdala development following caregiver deprivation and presents an argument that in the absence of the species-expected caregiver presence, human amygdala development exhibits rapid development and perhaps premature engagement that results in some of the emotional phenotypes observed following early institutional care.

Keywords

human; amygdala; caregiver; deprivation; cortisol; HPA axis

The parent-child dyad is a very special and close relationship that is critical for normal human development. Attachment theory emphasizes the role of caregivers in providing stability, security, and safety, which greatly influences the emotional health of the offspring (Bowlby, 1963). Hofer's (1994b) work has shown that the mere physical presence of the parent may act to regulate aspects of the infant rat pup's behavior and development. That it is an external agent to the infant, yet so critical to the progression of normal development, qualifies caregiver presence as a species-expected environmental influence. Greenough et al (1987) have defined experience-expected developmental processes as those which "utilize the sort of environmental information that is ubiquitous and has been so throughout much of evolutionary history of the species" (p.540). Because of this ubiquity of the caregiving stimulus, postnatal maternal deprivation should present a serious challenge to the developing human. When these types of experiences are absent, the effects on the targeted system can be severe and long-lasting.

In the absence of this expected environmental influence, the developing system must adapt to the unexpected environment to promote survival independently. These adaptations may promote survival in the short term, but severely impair the individual's ability to optimally cope with changing environmental demands that accompany changes in age. While caregiver-deprivation is rare in human populations, children in orphanages are raised en masse by staff members in an institutional setting, which does not provide a stable caregiver. Thus, in these environments, infants experience a species-atypical care environment. There are commonly-observed behavioral consequences of such caregiver-deprivation. Orphanage environments increase a child's tendency to approach unfamiliar adults (Chisholm, 1998; Zeanah, Smyke, & Dumitrescu, 2002), decrease a child's threshold for responding to sensory stimulation (Wilbarger, Gunnar, Schneider, & Pollak, 2010), and increase a child's reactivity and sensitivity to emotional information (Fries & Pollak, 2004; Tottenham, et al., 2010). While these behaviors may be seen as examples of adaptation to orphanage life,

children raised in this situation are also at elevated risk for externalizing and internalizing problems, such as anxiety and inattention (Casey, et al., 2009; Juffer & van Ijzendoorn, 2005; Kreppner, O'Connor, & Rutter, 2001; Zeanah, et al., 2009). In these circumstances, the developmental process is severely altered to meet the demands of the highly atypical early environment. The current paper discusses human brain development in the absence of caregiving, focusing on the amygdala, a neural region that is highly susceptible to early adversity and whose activity can greatly influence emotional behaviors. The hypothesis motivating this paper is that the absence of early caregiving prematurely engages the amygdala in learning about the environment, and in the long term, these activations result in hypervigilance and altered emotional responding.

This hypothesis is not a new one and has been proposed by several researchers with regard to rodent pup development (e.g., Jones, et al., 2009; Moriceau & Sullivan, 2005; Ono, et al., 2008). The current paper will examine this hypothesis with regard to human development. We reference the rodent literature heavily to discuss potential developmental mechanisms in the human for the reason that, although the relationship is ultimately more complex in the human, both (human and rodent) species are altricial, relying on the caregiver for survival, nutrients, autonomic regulation (Hofer, 1994a; Stone, Bonnet, & Hofer, 1976) and for learning about the mother to form attachments during the infant period (Moriceau, Roth, & Sullivan, 2010). This learning forms the basis for the relationship that ensures food, protection, and physiological regulation. It is possible therefore, that the ontogeny of the neural circuitry that guide proximity seeking remained phylogenetically intact during the postnatal period. Indeed, infants, whether human or rodents, can physically survive with some nominal degree of caregiving, as was seen in Spitz's studies of children in institutional care (Spitz, 1945) and in Hofer's work examining rat pup survival with access to discrete aspects of the mother (Hofer, 1994a), but as we will discuss, the consequences of caregiver deprivation on brain development can be significant.

The Amygdala

The amygdala has been implicated in learning about the emotional significance of stimuli (Davis & Whalen, 2001). Through a process of classical conditioning, that is pairing an initially neutral stimulus with an emotionally significant stimulus until the conditioned stimulus itself elicits the emotional response, an individual learns about the relative safety or danger of environmental stimuli. In adults, this process depends on the amygdala. Neuroimaging studies have confirmed that, like in rodents and non-human primates, the human amygdala responds to both negatively and positively valenced stimuli (Breiter, et al., 1996; Hennenlotter, et al., 2005; Somerville, Kim, Johnstone, Alexander, & Whalen, 2004), suggesting it supports learning about the emotional significance of the environment in general. Studies of fear conditioning confirm the role of the human amygdala in emotional learning, where forming an association between an emotional stimulus (e.g., shock) and a shock recruits amygdala activity (Delgado, Olsson, & Phelps, 2006; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). As will be discussed, the process of emotional learning during early development may be fundamentally different.

Rodent Studies

Much of what is known about early postnatal amygdala development comes from rodent models. Studies of developing rat pups show that the amygdala is *capable* of demonstrating adult-like plasticity early in life (in that the adult, the amygdala becomes engaged during learning and thus, the animal shows avoidance learning), although under typical conditions the developing amygdala will not (Moriceau, Roth, Okotoghaide, & Sullivan, 2004). That is, early in life, the amygdala is fairly uninvolved in emotional learning (Moriceau, Wilson,

Levine, & Sullivan, 2006), despite being anatomically developed, and hence avoidance learning is not typically observed in rodent pups. More commonly, preference learning is observed, where the rat pup will show a preference for stimuli that had been paired with an aversive stimulus (e.g., shock). This drastic difference between the behavior of the adult animal and the infant pup emphasizes the developmental differences in amygdala functional phenotype across this period.

There is an intimate association between the ontogenetic engagement of the amygdala and the activity of the HPA axis. A stressor sufficiently strong will elicit a full stress response (Kemeny, 2009), which includes activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, a primary mammalian stress axis. The HPA axis response leads to peripheral (systemic) glucocorticoid (CORT) increases via hypothalamus, pituitary, and adrenal gland activity and increases in corticotropin releasing hormone in the brain (CRH, Makino, Gold, & Schulkin, 1994). Because CORT can pass the blood-brain barrier, it feeds back onto receptors in the brain including the amygdala. Numerous studies have demonstrated that stressors increase activity of the amygdala via elevations in CORT. For example, CORT administration acts to augment the CRH-enhanced startle response in rats (Lee, Schulkin, & Davis, 1994), a process dependent on the amygdala (Lang, Bradley, & Cuthbert, 1990). Cell bodies of the amygdala are rich with CORT receptors (Honkaniemi, et al., 1992), and stressors (Bonaz & Rivest, 1998), as well as direct administration of CORT, result in increased activity of the amygdala including new synthesis CRH receptors (Makino et al., 1994). The likelihood of stress impacting amygdala activity in a similar manner early in life is high given that stress hormones and CRH mRNA production in amygdala have a developmentally early onset, appearing as early as postnatal day 2 in the rat (Avishai-Eliner, Yi, & Baram, 1996; Vazquez, et al., 2006).

Despite its early structural development, rodent work suggests that under normal circumstances, that is when the mother is present, the amygdala remains functionally dormant until the pup begins to independently explore its environment. Pups stay in the nest with the mother for the first 10 days of life, and this period is characterized by very low levels of CORT production (Moriceau & Sullivan, 2006). This stress hypo-responsive period is a time in pup development when emotional learning differs dramatically from adult learning. In contrast to the adult animal, where pairing an aversive stimulus with a conditioned stimulus (CS) results in an aversion of the CS, these pairings result in a preference for the paired stimulus in the rat pup (Camp & Rudy, 1988; Moriceau, et al. 2010). The reason pups do not exhibit aversion learning lies in the fact that the amygdala plays relatively little role in mediating the association between the aversive stimulus and the CS, and in the absence of amygdala-mediated learning, other learning circuits (e.g., olfactory bulb, piriform cortex) are free to mediate the learning (which results in preference learning at this point in development) (Moriceau & Sullivan, 2004). The amygdala is not involved in learning yet because the low CORT levels keep dopamine levels at a minimum (Barr, et al., 2009), and dopamine increases are necessary for amygdala plasticity. Once the pup starts leaving the nest and the stress hypo-responsive period comes to an end, CORT production increases, learning becomes amygdala-mediated, and the animal begins to exhibit aversion learning rather than preference learning.

Critical to this whole process is the presence of the mother. The presence of the mother maintains low CORT levels (Stanton & Levine, 1988, 1990), and thus the amygdala does not participate in the emotional learning process (Moriceau & Sullivan, 2006). However, removal of the mother results in CORT increases, and amygdala-mediated aversion learning ensues. This same effect of premature amygdala involvement can be replicated by direct administration of CORT to the amygdala. It has been suggested that this pattern of emotional learning early in life facilitates caregiver identification and preference (Moriceau

& Sullivan, 2005). Indeed in expected caregiving environments, the absence of amygdala-mediated learning may serve as a means of ensuring that infant pups form preferential attachments to caregivers. However, in conditions where the mother is absent and CORT significantly increases, a neural circuitry involving the amygdala becomes engaged prematurely, switching the animal's state of one from preference learning to one where they display avoidance related behaviors towards learned stimuli.

Human studies

Structurally, the human amygdala seems to undergo rapid development early in life (Tottenham, Hare, & Casey, 2009). The basic neuroanatomical architecture of the human amygdala is present by birth (Humphrey, 1968; Ulfig, Setzer, & Bohl, 2003), and in girls, amygdala structural growth is complete by four years old (Giedd, et al., 1996). Longitudinal non-human research supports the notion that the primate amygdala is an early developing structure; based on repeated structural images, the most rapid rate of amygdala development occurs within the first 2 postnatal weeks, and this rate stabilizes early - around 8 months old (Payne, Machado, Bliwise, & Bachevalier, 2009). This rapid rate of change early in life may heighten the vulnerability of the amygdala to environmental influence early in life. It has been argued (Lupien, McEwen, Gunnar, & Heim, 2009) that the sensitive period of development for neural regions will center around its peak period of development (i.e., fastest rate of change), making periods of rapid development those times when that system is most vulnerable to environmental manipulation. For some regions, like the prefrontal cortex, this period will be quite late and extended; for the amygdala, it will be early and rapid. Given the early peak in amygdala development, we might predict that early-life is a sensitive period for the human amygdala, much in the same way that has been demonstrated in the rodent.

In humans, it is not yet known whether the mother exerts a similar buffering effect over amygdala reactivity, yet we may surmise that given the phylogenetic conservation of the amygdala and the similar need to form an attachment to a caregiver, that there exists at least a consistent process in the postnatal period of the human. Certainly, a mother's presence buffers against elevations in CORT, particularly for highly fearful children (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). Like the rat pup, a similar stress hyporesponsive period has been identified in human infants, where physical exams and inoculations do not result in CORT elevations during infancy (despite outward displays of distress and protest, Gunnar & Donzella, 2002). Basal levels are also typically low at birth and seem to reach a production low during the preschool period (Grunau, Weinberg, & Whitfield, 2004; Watamura, Donzella, Kertes, & Gunnar, 2004). Given the extended period of caregiver-dependence in the human species, we might expect this period of amygdala development to extend throughout childhood, which would afford the system several opportunities to learn about highly complex environments including caregiver preference.

Evidence from human neuroimaging with children and adolescents provides evidence of developmental change during childhood. The amygdala shows evidence of functionality in childhood that is followed by change across development (Baird, et al., 1999; Thomas, et al., 2001; Tottenham, et al., 2009), that continues until a reactivity peak in adolescence (Hare, et al., 2008). Thomas and colleagues (2001) showed that the amygdala response in children was lower for fear faces than it was for neutral faces, the opposite pattern that has been repeatedly identified in adults (reviewed in Davis & Whalen, 2001). While these data have been interpreted as indexing the ambiguity associated with neutral faces in childhood, these data might also be interpreted as indicating that the amygdala does not respond to negatively valenced faces, like fear, in the same fashion as adults. Indeed, several studies have shown that amygdala signal to emotional faces increases through childhood, reaching a peak in

adolescence (Guyer, et al., 2008; Hare, et al., 2008; Killgore & Yurgelun-Todd, 2007; Monk, McClure, et al., 2003; Somerville, Hare, & Casey, in press). Thus, throughout childhood, when less is known about the relative safety or danger of different cues, the amygdala may be playing an increasing role in assigning valence to stimuli through learning processes like those observed in fear conditioning paradigms. To date, little is known about amygdala involvement in fear conditioning during typical development. One study using an airpuff to the larynx showed adolescents recruited amygdala to cues associated with the unconditioned stimulus (Monk, Grillon, et al., 2003), suggesting that at least by adolescence, the amygdala is engaged by emotional learning in a similar fashion to adults.

Although neuroimaging based fear-conditioning studies have not yet been performed in children prior to adolescence, we may make some inferences about the normal ontogenetic engagement of the amygdala during emotional learning based on behavioral findings. In terms of general fear experience, according to self-reports, fears, nightmares, and worrying increase from the preschool period to middle childhood (Muris, Merckelbach, Gadet, & Moulart, 2000; Muris, Merckelbach, Ollendick, King, & Bogie, 2001). Some naturalistic studies examining the development of learned fears showed that trauma experienced prior to age 7 years old (needing to be rescued while swimming) was not associated with fear (of swimming) when measured at age 18 (Poulton, Menzies, Craske, Langley, & Silva, 1999), and only late (after 18 years old)-onset dental fear, but not early-onset dental fear, was associated with aversive conditioning experiences at the dentist office (Poulton, Waldie, Thomson, & Locker, 2001). While these naturalistic studies are few in number, in conjunction with the neuroimaging studies discussed above, showing a developmental increase in amygdala recruitment to emotional stimuli, the findings are consistent with the hypothesis that in typical development, the amygdala is less engaged during emotional learning than during adulthood. Consistent with findings from these naturalistic studies, laboratory-based fear conditioning studies that have measured heart rate and galvanic skin responses with young children show that fear conditioning increases from the preschool period to middle childhood (Block, Sersen, & Wortis, 1970; Gao, Raine, Venables, Dawson, & Mednick, 2010). It will be important to further examine fear conditioning processes in the preschool and childhood period, particularly in the context of neuroimaging paradigms, to determine whether the hyporesponsivity of the stress system results in preference learning in humans as it does in the rodent. While this prolonged period of amygdala development allows the individual to learn about the environment, the changes in the amygdala prior to adolescence leaves it vulnerable to adverse early experiences that can have long lasting effects. We will present findings from human imaging and animal models of amygdala development under conditions of expected rearing environments and those when the expected parental input is absent.

Adversity and the Amygdala

Adverse events do not impact the whole brain in a uniform fashion, but instead the effects are region specific, exhibiting some of the largest effects in the amygdala. In adult animals, stress or administration of stress hormones increases the growth and activity of amygdala neurons (Armony, Corbo, Clement, & Brunet, 2005; Liberzon, et al., 1999; Rauch, et al., 2000). Cells in the amygdala participate in the earliest reaction to environmental stressors, often initiating the HPA cascade. They are quickly activated by stress and express immediate-early genes (Honkaniemi, et al., 1992). Stressful events produce elevations in CRH that occur first in the amygdala and are observed only afterwards in other neural regions like the hippocampus (reviewed in Baram & Hatalski, 1998). Indeed, both psychosocial stress and administration of CORT enhance expression of CRH in the amygdala (reviewed in McEwen, 2003; Schulkin, Gold, & McEwen, 1998). Numerous

neuroimaging studies have demonstrated that the amygdala is altered structurally and functionally by psychosocial stress in humans. Studies that have examined the amygdala in individuals who experienced traumatic events (e.g., combat, physical assault) show that in adults the amygdala is both smaller (Driessen, et al., 2000; Schmahl, Vermetten, Elzinga, & Bremner, 2003) and more reactive to emotional stimuli (Armony, et al., 2005; Liberzon, et al., 1999; Rauch, et al., 2000; Shin, et al., 2004; reviewed in Shin, Rauch, & Pitman, 2006).

In the developing animal, adversity can also impact amygdala activity, perhaps even more so than adversity that occurs later in life (reviewed in Tottenham & Sheridan, 2010). This position is supported by rat models of early stress, which find that the amount of CRH required to produce amygdala-originating seizures in developing animals is 200 times smaller than required for adult animals (reviewed in Baram & Hatalski, 1998). Neonatal separation stress resulted in an increase in CRF containing neurons in the amygdala in juvenile rodents (Becker, Abraham, Kindler, Helmeke, & Braun, 2007). Adverse and/or absent caregiving is a stressor for the developing animal, and studies that have examined the timing of exposure suggest that early adverse caregiving has effects on brain development that last throughout the lifespan (Matsumoto, Yoshioka, & Togashi, 2009; McEwen, 2008). For example, poor caregiving in rodents results in increased anxiety- and aggressive-behaviors in adulthood, which is associated with accelerated amygdala development (Kikusui & Mori, 2009), early myelination (Ono, et al., 2008), more CRH-containing neurons (Becker, et al., 2007), and sensitization of the adult amygdala (Salzberg, et al., 2007). A linear dose-response association has been identified for the quality of maternal care (increased arched-back nursing and licking/grooming) and amygdala phenotype (increased benzodiazepine receptor binding)(Caldji, et al., 1998). Although the amygdala is functionally dormant in the rat neonatal period, significantly stressful events can precociously activate the amygdala (Moriceau, et al., 2004), perhaps because of the early presence of CRH mRNA (present in postnatal day 2)(Avishai-Eliner, et al., 1996; Fenoglio, Brunson, Avishai-Eliner, Chen, & Baram, 2004; Vazquez, et al., 2006), and these effects of stress on the amygdala can be observed in adulthood, as has been shown by alterations at the level of the receptor (benzodiazepine and CRH receptors), facilitated amygdala kindling, impaired emotional learning, and emotional responses to future stressors (reviewed in Caldji, Francis, Sharma, Plotsky, & Meaney, 2000; Caldji, et al., 1998; Jones, et al., 2009; Kosten, Lee, & Kim, 2006; Plotsky, et al., 2005; Sevelinges, et al., 2007; Sevelinges, Sullivan, Messaoudi, & Mouly, 2008). Non-human primate work shows that maternal deprivation stress also influences primate amygdala development, including changes in amygdala gene expression and associated emotional behaviors (Sabatini, et al., 2007) and has more devastating effects the earlier in life it occurs. This long-lasting effect may in part be related to the resistance amygdalar cells show to recovery, unlike stress-induced changes in other regions of the brain, like the hippocampus (Vyas, Pillai, & Chattarji, 2004).

Caregiving adversity and the HPA Axis

Poor or absent caregiving has similar effects on the HPA axis of developing humans. Although we have been focusing on caregiver deprivation, which might impact an experience-expected developmental processes, there may be some important insights from examining the literature on differing qualities of attachments, which may instead influence experience-dependent processes (Smyke, Zeanah, Fox, Nelson, & Guthrie, 2010). In the sustained presence of a caregiver, an attachment bond typically forms and infant behavior is organized to maintain proximity with the attachment figure (Ainsworth, 1969; Cairns, 1966), regardless of the quality of care provided by the caregiver (Kovach & Hess, 1963; Rainekei, Moriceau, & Sullivan; Reichmann-Decker, DePrince, & McIntosh, 2009). In contrast to low quality caregiving, orphanage care is institutional care, which is characterized by staff members who rotate shifts and are responsible for the survival of an

extraordinarily high number of infants. Not surprisingly, children developing in these environments may show highly atypical attachment behaviors (O'Connor, Marvin, Rutter, Olrick, & Britner, 2003; Smyke, et al., 2010) or what some have termed reactive attachments (Zeanah, Smyke, Koga, & Carlson, 2005). That a bond forms when a sustained caregiver is present may signify important differences in terms of stress physiology between children who receive poor quality caregiving and those who experience maternal deprivation. In the first case, an attachment system becomes engaged and in the second, we hypothesize that in place of the typical attachment system, a stress-mediating system, including the amygdala, becomes prematurely engaged. We present first a discussion of poor quality caregiving and the HPA axis, followed by a discussion of the role of caregiver deprivation on HPA axis function.

Low quality caregiving and the HPA axis

Relative to children in secure attachment relationships, children classified as having insecure attachment relationships (that is, those relationships typically characterized by low sensitive caregiving) in infancy are at greater risk for atypically high elevations in cortisol, especially in combination with fearful temperaments (Nachmias, et al., 1996). Maternal stress and/or maternal depression early in life has been associated with elevated cortisol production in preschool (Essex, Klein, & Kalin, 2002) as well as in 8-year old children (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002). CORT alterations may not always be evidenced as elevations, as Bruce and colleagues (2009) found that children in the United States foster care system showed significantly decreased morning cortisol production and these decreases were more likely in children who experienced caregiver neglect, as opposed to emotional maltreatment. Similarly, CORT baseline and reactivity levels were atypically low in preschool children whose mothers were clinically depressed (Fernald, Burke, & Gunnar, 2008) and in children who had experienced maltreatment from caregivers (Cicchetti, Rogosch, Gunnar, & Toth; Hart, Gunnar, & Cicchetti, 2008). While high levels of CORT production are typically associated with HPA dysfunction, lower CORT levels are not uncommon in studies of samples with histories of adversity (Gunnar & Vazquez, 2001). Because of the complex dynamics of the HPA axis, atypically low CORT levels are usually interpreted as signs of dysregulation of the axis following chronic elevations in CORT and may in part reflect the nature of the caregiving adversity (Cicchetti & Rogosch, 2001).

Caregiver deprivation and the HPA axis

Unlike children receiving low quality caregiving, children raised in institutional care experience a postnatal environment that is void of a sustained caregiver presence. Gunnar and colleagues (Gunnar, Morison, Chisholm, & Schuder, 2001; Kertes, Gunnar, Madsen, & Long, 2008) found in a sample of previously-institutionalized children adopted from Romania, that those who spent most of their first year of life in an orphanage showed cortisol production that was at levels 2 standard deviations above typically reared children. Fries and colleagues (2008) found that following interaction with the adoptive parent, previously-institutionalized children exhibited significant CORT elevations as compared to children without early caregiver deprivation.

Early alterations to HPA activity provide a cellular environment that can prematurely activate the amygdala, which is rich with stress related receptors. As animal models have shown, this early sensitivity of the amygdala to adversity makes it vulnerable to being prematurely activated under conditions when species-expected environments are not available. We have been studying a group of children who were raised in orphanages during infancy and then were subsequently removed via international adoption in order to test whether similar mechanisms operate in the developing human. Orphanage care is sparse and unstable (Gunnar, Bruce, & Grotevant, 2000) and fails to mimic the caregiving that most

children receive during infancy. Because children adopted out of orphanages are placed into stable caregiving arrangements, the timing of caregiver absence is restricted to the early postnatal period. We found that amygdala volumes were enlarged in this group of children even though measurements were taken years after the initial adversity (Figure 1, Tottenham, et al., 2010). Notably, the older children were when adopted, the larger the amygdala volumes. This dose-response association suggests that longer stays in institutional care are associated with severity of impact on amygdala development. Although these data contrast with studies of older individuals who have experienced stress, who often exhibit smaller amygdala volumes (Driessen, et al., 2000; Schmahl, et al., 2003), the developmental data have been independently replicated with a separate sample of children adopted from orphanage care (Mehta, et al., 2009), and are consistent with studies finding amygdala growth at the cellular level following chronic stress administration (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002; Vyas, et al., 2004). The contrast between the human adult data and the developmental data provides a suggestion that the timing of adverse experiences and the timing of amygdala measurement are important factors in assessing the impact of environmental exposure on amygdala phenotype (McEwen, 2003; Tottenham & Sheridan, 2010). As a group, previously-institutionalized children are more likely to exhibit behavioral phenotypes that are typically associated with increased vigilance, such as internalizing problems and heightened anxiety (Figure 2)(Casey, et al., 2009), and these anxious phenotypes are positively associated with amygdala volume (Tottenham, et al., 2010). These volumetric data are consistent with the hypothesis that the absence of early caregiving alters emotional behavior by acting on amygdala development.

Along with amygdala enlargement, previously-institutionalized children show evidence of greater sensitivity to negatively valenced faces. We tested children on the Emotional Face Go/Nogo, which assesses the degree to which emotional faces interfere with the successful execution of a behavioral response. We showed that early caregiver deprivation was associated with a greater number of errors during conditions when negative faces were present (Figure 3, left), but no effect for positively valenced faces (Tottenham, et al., 2010). These data show that the behavior of previously-institutionalized children was more affected by negative emotional information. Amygdala activity was also elevated for previously-institutionalized children for negatively valenced faces (i.e., fear) relative to the typically-reared children (Figure 3, right, Tottenham, et al., in press). The pattern of amygdala response to fearful versus neutral faces more closely resembles that of adult subjects than typically-raised children of the same age, consistent with the hypothesis that early caregiver deprivation results in premature functional developmental of the amygdala. There was no between-group difference in amygdala response to neutral faces, and similar to the findings from the Thomas et al. (2001) study, the comparison group of children showed significantly greater amygdala activation to neutral faces than for fear faces. Taken together, these behavioral and neuroimaging data support the theoretical framework that children who lack stable caregiving early in life are at risk for atypical - perhaps earlier - development of amygdala and associated function. This early engagement of the amygdala may, in part, be evidence of the system recruiting stress circuitry for short-term survival, with the system adapting to the constraints afforded by the environment. These adaptations may serve as an immediate resilience factor for the child (Masten & Obradovic, 2008), but in the long-term may have detrimental consequences for mental health (e.g., anxiety).

While elevated amygdala during childhood seems to confer hyper-reponsivity to emotional information and anxiety-like symptoms, as children typically become more independent from caregivers, it is advantageous for the amygdala to become engaged in the learning process. In the rat pup, amygdala-mediated learning coincides with the end of the stress hypo-responsive period, when rat pups begin leaving the nest and exploring independently (Moriceau & Sullivan, 2005). Although amygdala-mediating learning is advantageous for

navigating emotional environments independently (Delgado, Jou, Ledoux, & Phelps, 2009), the system must guard against overactivity of the amygdala. In mature animals, the activity of the amygdala is regulated by its connections with regions within the prefrontal cortex (PFC). Regulation of the amygdala via PFC-amygdala connections may be especially important for predicting outcome in children who experience early-adversity. Thus, individual differences in long-term resilience may in part depend on the development of regulatory processes (Masten & Obradovic, 2008) emerging from functional development of the PFC.

Regulation of the amygdala

A growing human neuroimaging literature combined with a large non-human animal literature implicates the amygdala and its connections with the ventral PFC (which includes the orbitofrontal cortex and ventral anterior cingulate cortex) as biological substrates that underlie difficulties in emotional reactivity and regulation (reviewed in Monk, 2008). This is important because a failure to effectively recruit this circuitry has been implicated in a wide range of mental illnesses (e.g., anxiety, depression, schizophrenia, bipolar disorder, sociopathy, personality disorder). The amygdala has direct structural and functional connections with the ventral PFC (Amaral & Carmichael, 1992; Ghashghaei, Hilgetag, & Barbas, 2007; Milad & Quirk, 2002). Studies of structural and functional connectivity in humans have shown that greater coupling between the two regions is associated with better regulation of emotion (Banks, Eddy, Angstadt, Nathan, & Phan, 2007). The activity of these structures represents the simplest form of emotional regulation, which is extinction learning. Extinction studies in adult animals support this position and show that inhibition of the fear response to a conditioned stimulus requires the ventral PFC (Phelps, Delgado, Nearing, & LeDoux, 2004; Quirk, Russo, Barron, & Lebron, 2000). The ventral PFC can modulate emotional responses through GABAergic inhibitory projections to the amygdala, perhaps via afferents to the intercalated cells that inhibit amygdala activity (Akirav, Raizel, & Maroun, 2006; Harris & Westbrook, 1998). Lesioning or inactivation of the ventral PFC in adults with protein synthesis blockers (e.g., anisomycin) interferes with extinction learning (reviewed in Quirk & Mueller, 2008) - the animal will continue to exhibit fear responses when they are no longer needed. Consistent with a theorized regulatory role of the ventral PFC over the amygdala (reviewed in Quirk & Beer, 2006), the human amygdala response to emotional stimuli, such as faces, habituates over the course of the fMRI scanning session (Hare, et al., 2008), presumably because the emotional face is a conditioned stimulus that, in the context of the experimental session, is not predictive of any threat. This habituation has been likened to an extinction process, whereby ventral PFC engagement results in a progressive reduction amygdala activity over the course of the session. Amygdala habituation is associated with stronger negative connectivity between amygdala and ventral PFC, and adolescents and adults who show more amygdala habituation are less anxious (Hare, et al., 2008). The habituation seems to be supported by strong connectivity between amygdala and ventral PFC (Hare, et al., 2008; Pezawas, et al., 2005).

The development of amygdala-ventral PFC connectivity is not well characterized. Animal models suggest that emotional processes involving the amygdala and ventral PFC operate differently across development (Kim, Hamlin, & Richardson, 2009). In the rodent, the PFC develops late in life, not showing its volumetric peak until the adolescent period (van Eden & Uylings, 1985). Connectivity between the PFC and the amygdala does not emerge until the adolescent period (Cunningham, Bhattacharyya, & Benes, 2002), when amygdala projections to PFC first emerge (Verwer, Van Vulpen, & Van Uum, 1996). Consistent with the notion of a late developing ventral PFC, rodent studies show that, unlike in adulthood, pre-adolescent extinction learning does not rely on the PFC, where PFC inactivation does not impair extinction learning. Consequently, pre-adolescent extinction differs in behavioral

phenotype (e.g., renewal and reinstatement) from the extinction observed in mature animals (Kim, et al., 2009; Kim & Richardson, 2009). Currently, much less is known about human amygdala-PFC connectivity.

Even less is known about the development of the PFC following early adversity, although some rodent studies show that early life adversity alters PFC development both structurally (higher synaptic densities compared to controls - Ovtsharoff & Braun, 2001) and functionally (reduced tyrosine hydroxylase-positive fiber innervation - Braun, Lange, Metzger, & Poeggel, 1999). How early adversity alters amygdala-PFC connections is unknown at this point, but we can speculate that the stress-induced changes in the amygdala may over the course of development, weaken amygdala-PFC connections and communication. Our findings with previously-institutionalized children showed group differences in ventral PFC activity in response to fearful faces during the Emotional Face Go/Nogo (Tottenham, et al., in press). Specifically, typically-reared children showed modulation of the ventral PFC in response to fearful faces by decreasing activity, whereas the fearful faces did not alter PFC activity in previously-institutionalized children. Decreased coupling between the amygdala and PFC has routinely been observed in individuals with poor emotion regulation (Hare, et al., 2008; Marsh, et al., 2008; Shin, et al., 2006). These data with previously-institutionalized children are consistent with poor communication between amygdala and PFC in the previously-institutionalized group, and diffusion tensor imaging has identified reduced white matter between amygdala and prefrontal cortex in previously-institutionalized children (Govindan, Behen, Helder, Makki, & Chugani, 2009). If this interpretation of the functional imaging data is correct, then it suggests that there may be developmental/consequential effects of early caregiver deprivation on the amygdala's emerging connectivity with those regions that regulate its activity.

Much behavioral work has demonstrated that early caregiver deprivation results in significant emotional difficulty later in life. The replicability of these findings strongly suggests that the presence of caregivers is a species-expected environment, as described by Greenough et al. (1987), whose absence can cause significant detriment to the typical developmental process. Studies that use non-human animals have provided important clues regarding the potential neurobiological mechanisms that might mediate the associations between caregiver deprivation and altered emotional behavior. In the current manuscript, we used these findings to generate hypotheses about the neurobiological development of humans following caregiver deprivation. Future studies examining the development of human prefrontal connectivity with the amygdala will provide insight into individual differences in resilience (Masten & Obradovic, 2008), particularly during adolescence - a time when we expect prefrontal cortex connections to be rapidly forming and therefore, highly amenable to environmental-resilience factors (e.g., positive family environments). While the human neural data are currently sparse, they are mounting and support the hypothesis that caregiver deprivation alters emotional behavior by taking advantage of the readiness of the developing amygdala to respond to environmental adversity.

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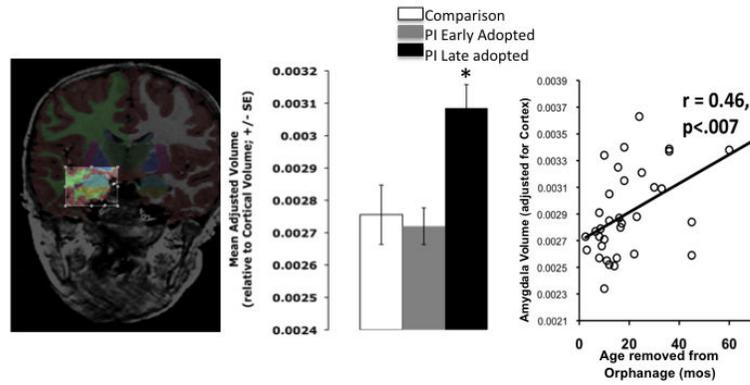


Figure 1. Amygdala Volume following Early Caregiver Deprivation. (Left) Amygdala segmentation highlighted in blue. (Middle) Post-institutionalized (PI) children who had been adopted later in life showed significant increases in amygdala volume. (Right) Longer periods of orphanage care were associated with larger amygdala volumes. PI = previously-institutionalized. Adapted from Tottenham et al., 2010.

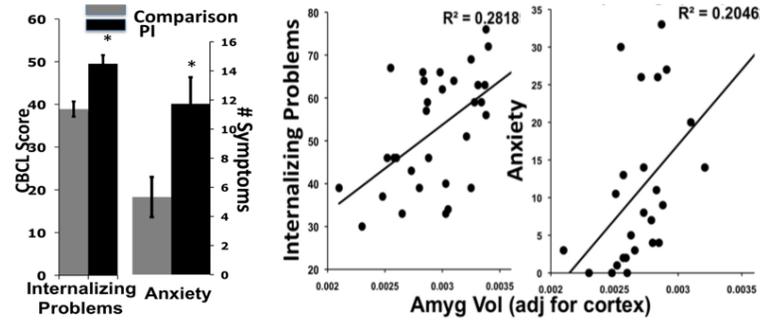


Fig 2. (left) PI children show more internalizing problems as measured by the Child Behavior Checklist (CBCL) & anxiety symptoms as measured by the SCARED-(Right 2 panels) Amygdala volume is positively associated with internalizing problems and continuous anxiety symptoms. PI = previously-institutionalized. Adapted from Casey et al., 2009 & Tottenham et al., 2010.

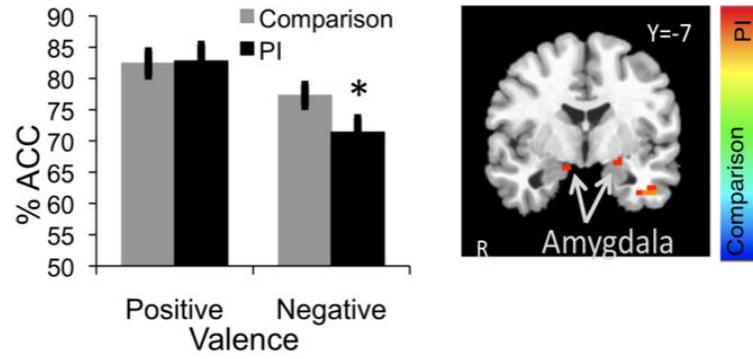


Fig 3. (Left) During performance on the Emotional Face Go/Nogo task, behavioral performance is more impaired by negatively valenced faces in the PI group. (Right) Direct group contrast show that PI children (orange) show heightened amygdala activity in response to fear faces. PI = previously-institutionalized. Adopted from Tottenham et al., 2010 and Tottenham et al., in press