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Permalink

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Journal

American Journal of Psychiatry, 171(11)

ISSN

0002-953X

Authors

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Publication Date

2014-11-01

DOI

10.1176/appi.ajp.2014.14040449

Peer reviewed



Published in final edited form as:

Am J Psychiatry. 2014 November 1; 171(11): 1162–1173. doi:10.1176/appi.ajp.2014.14040449.

A Translational Neuroscience Approach to Understanding the Development of Social Anxiety Disorder and its Pathophysiology

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Abstract

This review brings together recent research from molecular, neural circuit, animal model, and human studies to understand the neurodevelopmental mechanisms underlying Social Anxiety Disorder (SAD). SAD is common, debilitating, and often leads to further psychopathology. Numerous studies demonstrate that extremely behaviorally inhibited and temperamentally anxious young children are at marked risk to develop SAD. Recent work in human and nonhuman primates has identified a distributed brain network that underlies early-life anxiety including: central nucleus of the amygdala, anterior hippocampus and orbitofrontal cortex. Moreover, studies in nonhuman primates demonstrate that alterations in this circuit are trait-like in that they are stable over time and across contexts. Importantly, the components of this circuit are differentially influenced by heritable and environmental factors and specific lesion studies demonstrate a causal role for multiple components of the circuit. Molecular studies in rodents and primates are pointing to disrupted neurodevelopmental and neuroplastic processes within critical components of the early-life dispositional anxiety neural circuit. The possibility of identifying an early-life at-risk phenotype, along with an understanding of its neurobiology, provides an unusual opportunity to conceptualize novel preventive intervention strategies aimed at reducing the suffering of anxious children and preventing them from developing further psychopathology.

Keywords

Stress - AJP0097; Other Disorders - AJP0042; Molecular Biology - AJP0072; Anxiety Disorder-Generalized - AJP0004; Child Psychiatry - AJP0102

Social anxiety disorder (SAD) is highly prevalent and debilitating (1). SAD is characterized by marked fearfulness and anxiety in social and/or performance situations frequently resulting in avoidance, and significant disability. In addition to suffering with SAD, afflicted individuals often develop co-morbid depressive and substance abuse disorders (1). It is estimated that the prevalence of SAD is approximately 18% (2; 3). Data suggests SAD is approximately 20%–40% heritable with environmental factors accounting for the remaining variability (4). Although SAD is commonly diagnosed during adolescence, a period during

which teenagers attempt to adjust to social change, SAD can onset prior to adolescence and its antecedents often manifest very early in life (5; 6). Accumulating evidence suggests that a behaviorally inhibited (BI) or temperamentally anxious disposition during childhood can lead to the development of SAD (5; 7). This at-risk phenotype is characterized by heightened, but non-pathological, levels of anxiety and may constitute a prodromal phenotype for SAD. Importantly, although this phenotype is moderately stable over development, it does not consistently predict the development of SAD nor is it invariant. This suggests that early-life interventions targeting highly anxious children have the potential to prevent the development of more full-blown SAD and its common comorbidities.

Focused on developing novel early interventions, we take a cross-species approach to examine the behavior, neural circuits, and molecular systems that underlie the risk to develop SAD. We will discuss the biological basis of temperamental anxiety using data from humans and rodents, but will focus on insights gleaned from our studies of non-human primates. Rhesus monkeys are ideal for studying mechanisms underlying human development because of their relatively recent divergence from humans (i.e. 25 million years ago, as opposed to 70 million years ago when rodents diverged). Because of this recent divergence, the organization and function of the neural systems relevant to human anxiety, including the amygdala and prefrontal cortex, are conserved in rhesus monkeys (8; 9). Critically, rhesus monkeys and humans also share similar complex social environments that rely on parent-child bonding and peer-relationships. These early relationships can both encourage and discourage the adaptive social and emotional learning that helps regulate anxiety and promotes survival. Thus, we aim to understand how inborn and environmental influences converge on the specific biological systems that underlie extreme temperamental anxiety and the risks it confers. It is our hope that understanding the mechanisms modulating these biological substrates will guide the development of novel early-life behavioral and pharmacological interventions that will provide effective treatment for the numerous children at risk to suffer from SAD and related disorders.

The earliest manifestations of adaptive anxiety-related responses to potential threat occur during infancy and childhood and are characterized during infancy by increased excitability, and later as behavioral inhibition. Behavioral inhibition occurs in response to novelty and potential threat and is associated with autonomic and pituitary-adrenal activation. Childhood behavioral inhibition is thought to manifest during the second year of life, around the time that a child emerges from the normative stage of stranger anxiety and is developing the ability to behaviorally cope with threat (10; 11). Extreme behavioral inhibition has received considerable attention from the pioneering work of Jerome Kagan and often manifests as excessive shyness and extremely reserved and avoidant behavior in social situations (12).

Several prospective longitudinal studies have found that extreme behavioral inhibition is associated with increased odds of developing anxiety and depressive disorders (13–18). A recent meta-analysis suggests that early-life behavioral inhibition is the single highest predictor for the later development of SAD, as nearly 50% of highly behaviorally inhibited children go on to develop SAD (Fig. 1a) (7). Although behavioral inhibition fluctuates throughout childhood, studies suggest that individuals with stable levels of high behavioral

inhibition have the greatest risk to develop SAD (15; 19–21). A number of prospective and retrospective self-report studies have implicated childhood behavioral inhibition as a risk-factor for depressive disorders (22–24) and children of mothers with anxiety and/or depressive disorders tend to display increased levels of behavioral inhibition (25–27). Estimates of the heritability of behavioral inhibition are consistent with the ~20–40% estimates of heritability of anxiety disorders and anxiety related neuroticism (4; 28; 29). Many anxiety disorders, including those likely to later develop in highly behaviorally inhibited children, demonstrate partial shared heritability with each other (4; 30; 31). These studies suggest that extreme behavioral inhibition is an early phenotype that confers risk for the development of a broad range of stress-related psychopathology (32–35) (Fig 1b).

To further understand the biology of extreme early anxiety, our group has extensively validated a developmental rhesus monkey model. To assess behavioral inhibition, our initial studies developed the no-eye-contact (NEC) condition of the human intruder paradigm in which the duration of freezing behavior, analogous to behavioral inhibition, was assessed in response to the uncertain threat of a human intruder looking away and presenting only her profile, being careful to make no eye contact with the monkey (Fig. 2) (36). The NEC context can last for 30 minutes, during which the human intruder remains motionless, continuously presenting her profile throughout the entire test-period. Our extensive studies examining various fear- and anxiety-related contexts demonstrated that the NEC-context specifically and reliably elicits freezing. In contrast, direct eye contact by the human intruder often elicits an overt aggressive response from the monkey.” To assess behavioral inhibition, our initial studies developed the no-eye-contact (NEC) condition of the human intruder paradigm in which the duration of freezing behavior, analogous to behavioral inhibition, was assessed in response to the uncertain threat of a human intruder looking away and presenting her profile, assuring to make no eye contact with the monkey (Fig. 2) (36). Our extensive studies examining various fear- and anxiety-related contexts demonstrated that the NEC-context specifically and reliably elicits freezing. Freezing responses are evolutionarily conserved across diverse species functioning to help organisms remain undetected in the presence of a potential predator (37). Freezing is often accompanied by reduced coo vocalizations. Although, rhesus monkey coo vocalizations are affiliative and can be used to recruit support from conspecifics, they can also attract predators. Thus, the reduction in coo calling occurring during the NEC context is adaptive, as in the presence of a potential predator, the value of remaining undetected is a survival imperative. The NEC context also induces physiological changes, such as increased cortisol levels and right-frontal EEG asymmetry (38; 39). Moreover, similar to symptoms of anxiety in humans, behavioral inhibition in monkeys can be decreased with administration of the GABA-enhancing anxiolytic agent, diazepam (36).

To extend the assessment of behavioral inhibition, we developed the concept of anxious temperament (AT) to more completely reflect an individual’s dispositional physiological and behavioral responses to potential threat. In the monkey model, this composite AT measure is an average of standardized levels of NEC-context induced freezing, coo vocalization reductions, and NEC-induced cortisol levels. More broadly, in relation to humans, we use the term AT to define the temperamental predisposition to display behavioral inhibition (increased freezing and decreased vocalizations) along with increased

physiological reactivity (increased levels of the stress hormone cortisol) when exposed to novelty, unfamiliar individuals, or other potentially-threatening situations. Compared to behavioral inhibition alone, we believe that the composite AT measure better estimates the at-risk human phenotype (40).

Although there are no diagnostic criteria for mental disorders in nonhuman primates, data suggest that monkeys with high AT are functionally impaired. Anecdotally, the veterinary records from one extremely high AT monkey in our colony revealed significant stress-related symptomatology, including: hair loss, chronic diarrhea, and extreme fearfulness (e.g., refusal to take treats, retreating to the back of the cage, and excessive ‘crying’). Moreover, we characterized AT during the NEC-context in a large free-ranging colony of monkeys on the island of Cayo Santiago. Naturalistic observations of these animals revealed that high AT, tested in a field-improvised laboratory, was associated with elevated social inhibition (unpublished data). Specifically, increased AT predicted fewer conspecific approaches ($\rho = -.44, .007$) when animals were free ranging, and high-AT animals maintained larger distances between themselves and their peers ($\rho = -.31, p = .03$). At the most extreme, some of the highest AT animals were never observed to approach their peers, while in the same period of time their low-AT counterparts approached their peers upwards of 50 times. These data add to the relevance of the extreme monkey AT model as it relates to the dysfunction experienced by extremely anxious children. Further supporting the homology between human and monkey AT is our demonstration that individual differences in AT are relatively stable across development and that AT is ~20–40% heritable (41; 42). Like human children, some high-AT monkeys display a reduction in their levels of AT as they mature, providing a unique opportunity for future studies to prospectively characterize brain mechanisms underlying recovery and resilience.

Brain regions associated with Anxious Temperament

In an initial functional magnetic resonance imaging (fMRI) study, Schwartz, Kagan and colleagues examined young adults previously characterized as inhibited or uninhibited during their second year of life (43). Strikingly, their results demonstrated that inhibited individuals had increased amygdala activation in response to novel neutral faces approximately 20 years after original assessment. More recently, researchers demonstrated increased novelty-related amygdala activation in inhibited males approximately 18 years after being characterized as highly-reactive at 4-months of age (44). Similarly, young adolescents (~12.5 years old) who were inhibited during childhood had increased amygdala activation when instructed to rate their emotional responses to fear faces (45). Extending these studies in prospectively characterized children, Blackford and colleagues studied young adults who self-reported current and past BI. These studies provide additional evidence that amygdala activation is associated with an inhibited temperament, and extend previous findings by implicating specific processes within the amygdala. Specifically, Blackford et al. demonstrated that highly inhibited young adults show faster amygdala responding (46), prolonged and exaggerated amygdala reactivity (47; 48), decreased amygdala habituation (49), and increased amygdala volume (50). In addition to elucidating the role of the amygdala, Blackford and colleagues have begun to extend the set of regions associated with behavioral inhibition to include the hippocampus (49), lateral and medial

orbitofrontal cortex (47; 48), insular cortex (48), as well as altered BI-related connectivity between these regions and the amygdala (51).

In the neuroimaging studies of behavioral inhibition, the amygdala regions associated with behavioral inhibition are in the dorsal amygdala (Fig. 3a). The dorsal amygdala is anatomically distinct from the more ventrally located quasi-cortical basal and lateral amygdalar nuclei. The central nucleus of the amygdala (Ce), which is located within the dorsal amygdala region, is primarily comprised of striatal-like GABAergic neurons, and is considered to be the primary output structure of the amygdala-complex (52). In imaging studies, the precise localization of dorsal amygdala activations should be considered tentative, in light of the relatively large spatial confidence intervals associated with fMRI, particularly in studies examining relatively small groups of subjects (i.e. less than 50). Nevertheless, these studies suggest that the Ce, may be important for instantiating the increased emotional reactivity characteristic of high-AT individuals.

fMRI studies in normal individuals experiencing fear and anxiety have consistently identified an underlying neural circuit that includes the amygdala, hippocampus, prefrontal cortex, and insula. Moreover, adults with anxiety disorders (e.g. social and specific phobia) show increased activation in these same regions (53). Interestingly, novel social stimuli, such as emotional faces and eye-whites, are sufficient to activate the amygdala (54). Consistent with the continuity between dispositional anxiety and anxiety disorders, children with generalized anxiety disorder also show increased amygdala activation (55). In addition to activation in the amygdala, children with generalized anxiety disorder demonstrate increased activation in prefrontal cortical area 47 near the anterior limb of the insula, and increased amygdala-mid-insula functional coupling (55).

To further examine the temperamental nature of anxiety-related brain metabolism, we examined young rhesus monkeys, (i.e. 1–4 years old in monkeys, approximately corresponding to 3–12 years of age in humans) by phenotyping them for AT and performing ¹⁸fluoro-deoxyglucose positron emission tomography imaging (FDG-PET). Because the FDG-PET human intruder paradigm allows for the simultaneous assessment of brain metabolism and AT, this model can provide insights into the neural substrates underlying AT. As described before, AT was assessed by combining the NEC-context induced decreased spontaneous ‘coo’ vocalizations (though to reflect “calls for help”), increased freezing (or, behavioral inhibition) and increased stress-induced cortisol (36; 40). Our initial studies demonstrated that the components of AT are associated with metabolism in the extended amygdala, including the Ce and bed nucleus of stria terminalis (Fig. 3b), as well as hippocampus, anterior temporal lobe, and brain stem periaqueductal grey (56; 57). The extended amygdala is comprised of the Ce, bed nucleus of stria terminalis, as well as other forebrain structures that play an important role in the initiation of fear and maintenance of anxiety. Moreover, we found that our composite AT measure predicted significantly more variance in amygdala metabolism than any of the components that comprise AT (40; 58). Later studies examining FDG-PET in relation to AT during the NEC context in over 200 young rhesus monkeys revealed that metabolism in anterior temporal lobe structures including Ce, anterior hippocampus and anterior temporal pole robustly predicted AT (41). These findings are consistent with human research in highly behaviorally

inhibited individuals and in patients with anxiety disorders by providing evidence for the involvement of anterior temporal systems in the at-risk phenotype.

The fMRI and FDG-PET studies discussed above, have been limited to studying brain activity in potentially stressful contexts, i.e. the MRI scanner (59) and the NEC context. To further elucidate the temperamental nature of brain metabolism we extended these studies to examine brain activity during non-stressful conditions. Specifically, we performed FDG-PET scans on animals that were each exposed to 2 different *stressful conditions* (i.e. NEC and separation from cage-mate into a test-cage), and 2 different *non-stressful conditions* (alone in home-cage without cage-mate and life as usual in home-cage with cage-mate). Trait-like positive correlations between individual differences in AT and metabolism in the amygdala, hippocampus, anterior temporal pole, and periaqueductal grey were found in each condition regardless of the level of stress (40). Additionally, we examined the stability of AT's neural substrates across time by assessing AT and FDG-PET in 24 animals that were exposed to the NEC context 3-times over 6–18 months. Results demonstrated inter-individual stability over time in brain metabolism within AT-related regions (60). Additionally, the mean metabolism across the 3 observations predicted an individual's mean AT (60). These data indicate that context-independent and temporally stable neural substrates underlie the trait-like nature of AT. These findings provide insight into AT, as they suggest that the neural substrates of AT are present even when no behavioral manifestations are apparent.

While our definition of AT is fairly circumscribed, there remains substantial variability in how AT presents. This variability is similar to the symptom heterogeneity observed within anxiety and affective diagnostic categories. For example, some monkeys display substantial freezing behavior, while maintaining average levels of cortisol and emitting a normative number of coo calls. In contrast, other monkeys display high levels of cortisol relative to their behavioral responses. Studies in rodents by the Blanchards and others examining threat responses have suggested that while the activation of different physiological and behavioral responses can adaptively work together, they may also have different adaptive functions (37). This raises the intriguing possibility that animals expressing different anxiety response-profiles have tendencies to activate common neural circuits that via their effects on specific neural substrates can bias physiology and behavior toward different adaptive responses. In examining the neural substrates underlying AT's components, we found both *common* and *specific* brain regions that underlie the phenotype's heterogeneity (58). The common brain regions included Ce and anterior hippocampal regions, in which metabolism was independently associated with variation in freezing, cooing and cortisol. This finding suggests that regardless of their "symptomatic" presentation, individuals with high levels of AT have increased metabolism in these brain regions. We also identified regional metabolism that was specific to each component of AT. For example, metabolism in the mid-hippocampus was uniquely associated with cortisol levels, as compared to freezing or coo vocalizations. Together, these findings demonstrate that AT has both common and presentation-specific neural substrates and highlight the opportunity for understanding neural substrates that cut-across phenotypic heterogeneity.

To understand how different brain systems relate to the heritability of AT, we used our large sample of brain imaging data from a multi-generational family pedigree to perform whole-brain heritability analyses. This was the first study to examine the heritability of brain metabolism across the entire brain. We were surprised to find differential heritability within AT's neural substrates. Our results demonstrated significant heritability of anterior hippocampal metabolism, but no significant heritability of Ce metabolism (41). These findings call into question the view that it is solely amygdala altering genes that are responsible for the inter-generational transmission of anxiety. Rather, our findings suggest that AT-related genes are more likely to exert their influence by altering function in other components of the AT-related circuit. Moreover, the lack of heritability within Ce implies that this region may be more likely to mediate the environmental influences known to modulate AT, such as parenting, behavior modeling, and exposure to stress.

Causal brain regions and Anxious Temperament

Studies have been performed in patients with varying degrees of amygdala damage (61–63). Consistent with functional imaging findings, a rare human patient with bilateral damage to her entire amygdala was shown not to experience psychological discomfort in response to invasions of her personal-space (64), did not have normative distrust of strangers (65; 66), nor did she report normal fearfulness (67) – all of these features are associated with decreased AT. This work supports the role of an amygdala-centered network in adaptive fear and anxiety as well as in anxiety disorders.

Targeted lesion studies in non-human primates reveal a causal role for dorsal amygdala regions in AT. Specific amygdala lesions decrease one's reticence to act in potentially threatening situations and alter stress-induced cortisol release (68–70). Amygdala lesions also decreased anxiety in novel social situations, consistent with its role in social anxiety (71; 72). Our studies employing specific neurotoxic Ce lesions, demonstrated decreased freezing behavior and increased spontaneous coo-vocalizations, two-core components of AT (73). Although the Ce lesions did not directly affect cortisol, they reduced plasma concentrations of adrenocorticotrophic releasing hormone (ACTH) and cerebrospinal fluid concentrations of corticotrophin releasing hormone (CRH), the two key upstream mediators of cortisol release.

Targeted lesion studies in primates have also assessed the causal influences of hippocampus and orbitofrontal cortex on components of AT. Both of these regions have direct connections to the amygdala and are thought to play regulatory roles and provide contextual/regulatory information to the amygdala (Fig. 4). Of particular interest is the finding that orbitofrontal cortex aspiration lesions decrease freezing behavior and cortisol (69; 70; 74). By combining the lesion strategy with FDG-PET imaging, we found that the effects of orbitofrontal cortex lesions on AT could be explained by orbitofrontal cortex-induced changes in the extended amygdala (i.e. bed nucleus of stria terminalis), a region we previously found to be associated with AT (75). Because orbitofrontal cortex aspiration lesions can also disrupt axons passing through this region, it is possible that the effects of these lesions are not due to orbitofrontal cortex damage *per se*, but rather result from damage to fibers originating in other PFC regions. Consistent with this possibility, our

recent fMRI study found that increased Ce metabolism and AT are associated with decreased Ce-dorsolateral prefrontal cortical intrinsic connectivity (76). In contrast to the effects of orbitofrontal cortex and Ce lesions, the evidence for hippocampal lesions affecting primate AT is mixed (68; 69). Together, these data suggest that Ce, orbitofrontal cortex and possibly hippocampus might each causally influence AT, emphasizing the contribution of multiple regions to dispositional anxiety.

Lesion studies in rodents, though not assessing AT specifically, have demonstrated a causal role for many AT-related regions in unconditioned anxiety behaviors. In particular, rodent studies of unconditioned anxiety have causally implicated the amygdala (77), ventral hippocampus (similar to anterior hippocampus in primates)(78; 79), and the extended amygdala, including both the Ce and the bed nucleus of stria terminalis (77; 80). In both rodents and primates, the Ce and bed nucleus of stria terminalis project to the downstream structures necessary for initiating specific behavioral and physiological aspects of the fear-response (Fig. 4). Elegant rodent studies demonstrate dissociable roles for the Ce and bed nucleus of stria terminalis, such that the Ce is required for processing immediate and imminent threats, whereas the bed nucleus of stria terminalis is required for responding to prolonged and more distant threats (81). More recently, targeted optogenetic functional manipulations of specific projections and detailed anatomical studies have begun to elucidate projection- and cell-specific function within AT-related circuits. For example, some basolateral amygdala neurons provide excitatory input to the hippocampus and Ce, which can initiate unconditioned fear- and anxiety-related behaviors (82; 83). Moreover, specific subregions and cell-types within the Ce and bed nucleus of stria terminalis have been demonstrated to mediate specific phenotypic expressions of anxiety (84; 85). This suggests that selective alterations in the extended amygdala could give rise to the phenotypic heterogeneity observed in high-AT primates and humans with anxiety disorders. The rodent studies complement the human and non-human primate studies, strengthening support for the Ce in unconditioned anxiety and drawing attention to other components of the extended amygdala.

Molecular processes underlying Anxious Temperament

To develop ideal interventions aimed at preventing the long-term negative consequences of early-life AT, it is important to understand the molecular alterations occurring within the AT neural circuit. Genetic studies indicate that either extremely rare critical polymorphisms or many polymorphisms with small additive effects influence anxiety. Moreover, the influence of parents can have effects on anxiety via alterations in DNA methylation and other epigenetic phenomena that, in the case of methylation, can be passed down as alterations in parental methylation profiles and modified by parental behavior (86–88). Because mRNA levels reflect the confluence of genetic and environmental effects, we believe that examining gene expression within the neural substrates of AT can provide important clues. Because genetic methylation and expression profiles vary by tissue, region, and time, animal models are critical for developing a better understanding of the molecular alterations that underlie the altered brain function occurring in early-life AT.

Because the strongest evidence linking brain alterations to AT points to the Ce, we examined individual differences in Ce gene expression in relation to AT (60; 89). We performed prospective longitudinal brain imaging with behavioral and physiological assessments on 24 rhesus monkeys, prior to measuring Ce gene expression. Altered gene expression occurred in some prominent anxiety-related neurochemical systems, i.e. neuropeptide Y and serotonin systems, such that individuals with high levels of Ce *NPY1R* or *5HT2C* gene expression demonstrated lower levels of AT. AT was also negatively associated with alterations in neurodevelopmental systems within neurotrophic and cellular adhesion pathways (Fig. 5). In particular, we observed a negative correlation between AT and the expression of neurotrophic receptor kinase 3 (*NTRK3*, also known as *trkc*) as well as its downstream partners, insulin receptor substrate 2 (*IRS2*) and ribosomal protein S6 kinase, 90kDa, polypeptide 3 (*RPS6KA3*, also known as *RSK2*). These genes are involved in growth factor membrane signaling (*NTRK3*), intracellular signaling (*IRS2*) and nuclear activation (*RPS6KA3*) (Fig. 5b), all of which contribute to synaptic plasticity and development (Fig. 5c). Importantly, individual differences in expression levels of Ce *NTRK3* predicted trait-like Ce metabolism. *NTRK3* is a growth-factor receptor that when activated can initiate wide spread changes in cell-growth and plasticity, similar to those seen after injection of *BDNF* which binds to *NTRK2* (also known as *TrkB*). In relation to potential epigenetic mechanisms associated with AT, we observed that high AT individuals had decreased Ce levels of Growth arrest and DNA-damage-inducible, beta (*GADD45B*). *GADD45B* is known to be involved in plasticity and neurogenesis via activity dependent methylation of growth factors and thus may be relevant to the observed decreases in levels of *NTRK3* and its downstream partners (90). Other non-human primate studies of early-life stress have also implicated neurodevelopmental pathways (91; 92). Interestingly, recent research in squirrel monkeys demonstrated that coping in response to mild stress increased hippocampal neurogenesis, and identified neurogenesis-related hippocampal gene expression in growth-related pathways that included *NTRK3* (91).

Akil and colleagues have found similar results in relation to anxiety and depression (93). Studies of gene expression in the frontal cortex of patients with major depressive disorder identified decreased expression of *fibroblast growth factor 2* (*FGF2*) (94). Much like activation of *NTRK3*, activation of *FGF2* can increase neurogenesis and synaptic plasticity. *FGF2* was also down regulated in rodents bred to be behaviorally inhibited (95). Excitingly, a single injection of *FGF2* into the behaviorally inhibited rodents during the first days of life, prior to formation of the blood-brain-barrier, was sufficient to decrease behavioral inhibition (96). Follow-up study of these rodents revealed increased hippocampal neurogenesis and increased expression of neuroplasticity-related genes, including *NTRK3*, in relevant brain regions (96). These data implicate the *FGF* family as important for the development of anxiety during early life, and further support plasticity-related interventions aimed at decreasing AT (93).

Researchers investigating the serotonin system in rodents have suggested that similar neuroplasticity-related mechanisms underlie anxiety and the efficacy of selective serotonin reuptake inhibitors (SSRIs). SSRIs are effective in treating anxiety disorders but often take weeks to fully work, suggesting an indirect mechanism. Stress impairs, whereas SSRIs

increase hippocampal neurogenesis (97; 98), and preventing hippocampal neurogenesis via irradiation blocks the effects of SSRIs (98; 99). Recent studies suggest that immature hippocampal neurons, in part, mediate the effects of SSRIs by enhancing the ability to discriminate complex threat relevant information (100; 101). Interestingly, the effects of SSRIs on neurogenesis seem to be mediated by the BDNF receptor, NTRK2 (i.e. TrkB). These data further support the role of tyrosine kinase pathways, and neuroplasticity, in relation to anxiety.

Research and Treatment Implications

SAD is common and debilitating. Lifelong SAD often leads to further psychopathology, including mood and substance abuse disorders. Because it is possible to identify children at risk for SAD early in life, the field has an unusual opportunity to conceptualize novel preventative intervention strategies. Treatments for SAD are not completely effective and no treatments exist for children with extreme AT, the forerunner of SAD. While some children with extreme AT overcome their anxiety, early interventions promise to increase the number of children that grow-up to be psychopathology free (19; 21).

Evolutionarily conserved anxiety-related phenotypes have facilitated cross-species translational research. Studies of the neural circuits of SAD and non-human primate AT implicate dorsal amygdala, anterior hippocampus, brainstem regions, and orbitofrontal cortex. The homology between rhesus AT, childhood dispositional anxiety, and SAD provide a framework for the valid use of non-human primates in new treatment development.

Alterations in brain function associated with AT are stable over time and context-independent. In contrast, the symptoms associated with AT and SAD are elicited by specific cues and contexts associated with potential threat. These findings provide a conceptual basis for new treatments directed at changing the stable altered neural tendencies of individuals affected by AT and SAD. Attempting to modify one's trait-like brain function has the potential advantage of targeting mechanisms that may result in relapse, and failure to respond. Lesions to different components of AT's neural circuit diminish, but fail to completely normalize AT. Thus, treatments targeting multiple AT-related brain regions are likely to be most successful in treating SAD and preventing its development. Our research provides insights into which brain regions should be targeted. We identified anxiety-general regions that underlie anxiety regardless of how it is expressed, and phenotype-specific regions that are uniquely involved in a particular expression of anxiety, such as freezing. Therefore, fully effective treatments will need to target anxiety-general regions as well as response-specific regions as they relate to the diverse presentations of individuals with AT and SAD. The data demonstrate that the inter-generational transmission of anxiety is mediated by a widely distributed set of brain regions with large variation in the extent to which altered metabolism in these regions is heritable. This raises the possibility that optimal neural treatment targets could vary depending on one's family history of anxiety. As treatments become more neuroscientifically focused it is likely that neural measures reflecting treatment-related changes in AT's neural circuits will be useful predictors of longterm treatment outcomes.

Characterizing the molecular alterations in brain regions associated with anxiety and AT has begun to identify novel treatment targets. Numerous rodent studies have focused on the hippocampus, where plasticity and neurogenesis have been associated with decreased anxiety. Our group initially focused on the Ce for molecular analyses because the Ce is a core and causal component of AT's stable neural substrate. Finding a reduction in neuroplasticity-related genes that are associated with increased Ce metabolism and AT led us to speculate that extreme early-life AT may result from a diminished ability to modify intra-Ce circuits. Studies of amygdala development in non-human primates demonstrate that the Ce undergoes protracted development (102), which seems to parallel the developmental time course for children's increased tendency to react by freezing to uncertainty and novelty (i.e. normative stranger anxiety). Thus, plasticity within this network is likely to be critical for the capacity to emerge from this period of heightened childhood fearfulness. Further, we hypothesize that the maturational ability to overcome or 'un-learn' normative childhood fears relies on neuroplasticity mechanisms within AT's neural substrates. Although the work above specifically implicates *NTRK3*, *FGF2*, and other plasticity-related targets in relation to anxiety, we believe that these results reflect involvement of broader neuroplasticity-related systems. Based on these results, it is likely that treatments specifically increasing neuroplasticity within the Ce, and other components of AT's neural substrates, will be most effective for modulating early-life AT and preventing the development of SAD. Neuroplasticity mechanisms in the hippocampus have been well studied and linked to antidepressant effects (98; 99). Because of the central role of the Ce in AT, studies elucidating Ce specific neuroplasticity molecular pathways will be important in conceptualizing novel treatments.

We have made the case for developing early interventions that are aimed at preventing high-AT children from converting to anxiety disorders. Because AT emerges during a period when the brain is rapidly changing, treatments aimed at altering mechanisms underlying aberrant brain development are likely to have the potential for long-term changes in anxiety trajectories. Animal studies provide evidence for many ways to influence neuroplasticity that are relevant to treatment, such as exercise, SSRIs, and brain electrical stimulation. The building of synapses, neurons, and the resulting refinement of brain networks is a complex process involving many diverse molecules. Future work should focus on behavioral, pharmacological, and neuromodulatory strategies aimed at modulating diverse neuroplasticity-related molecules within specific components of AT's neural substrates, such as the Ce and anterior hippocampus.

Research relevant to AT and the development of SAD has revealed a number of important insights that can be helpful in formulating neuroscientifically-based early-life interventions: 1) children with extreme AT are at great risk to develop further psychopathology, especially SAD; 2) the neural circuits that underlie SAD and human temperamental anxiety are similar to those implicated in monkeys with extreme AT; 3) the neural circuits that underlie AT are trait-like; 4) heterogeneous presentations of AT are associated with activity in both shared and phenotype-specific neural substrates; 5) environment and heritability differentially influence components of AT's neural circuit; and 6) preliminary evidence points to altered neuroplasticity-related gene expression in the genesis of AT. It is our hope that this review

will focus research efforts on early interventions that are designed to not only reduce the suffering of anxious children but also to prevent them from developing further psychopathology.

Acknowledgments

The authors would like to thank Jonathan A. Oler, Alexander J. Shackman, Do P.M. Tromp, Richard J. Davidson, Brad Postle, Wen Li, and Rick Jenison for their comments on early versions of this manuscript. We would like to thank Steven E. Shelton, Helen VanValkenberg, Marissa Riedel, and the staff at: the Harlow Center for Biological Psychology, HealthEmotions Research Institute, Waisman Center, Waisman Laboratory for Brain Imaging and Behavior, and Wisconsin National Primate Center. The authors of this work were supported by the National Institutes of Health (NIH; Intramural Research Program and extramural grants R21MH91550, R01MH81884, R01MH46729, P50MH84051, MH100031, R21MH092581), the HealthEmotions Research Institute, and Meriter Hospital.

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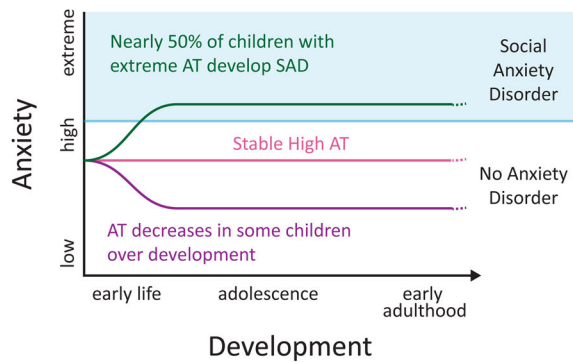
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**a) Pathological Anxiety:
Trajectories for Children with High AT**



**b) Extreme AT children are at risk for
psychopathology across the lifespan**

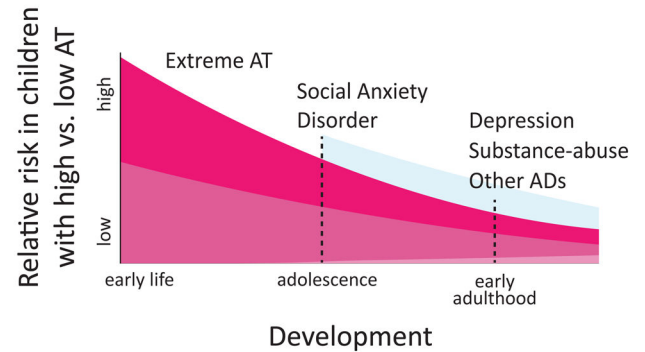


Figure 1. Early-life Anxious Temperament (AT) is a risk factor for the later development of anxiety and depressive disorders

(a) Extreme AT exists on a continuum with social anxiety disorder (SAD), and many children with high levels of AT go on to experience disabling anxiety. Nearly 50% of children with extreme AT eventually develop social anxiety disorder (green), while in other children levels of AT remain stable (pink) or diminish with experience and maturation (purple) (7). (b) During childhood and adolescence children with extreme AT are most likely to develop SAD, but throughout life individuals with extreme AT remain at a higher risk to develop other disorders that typically onset later in life, i.e. major depressive disorder and substance abuse (1; 13; 22).

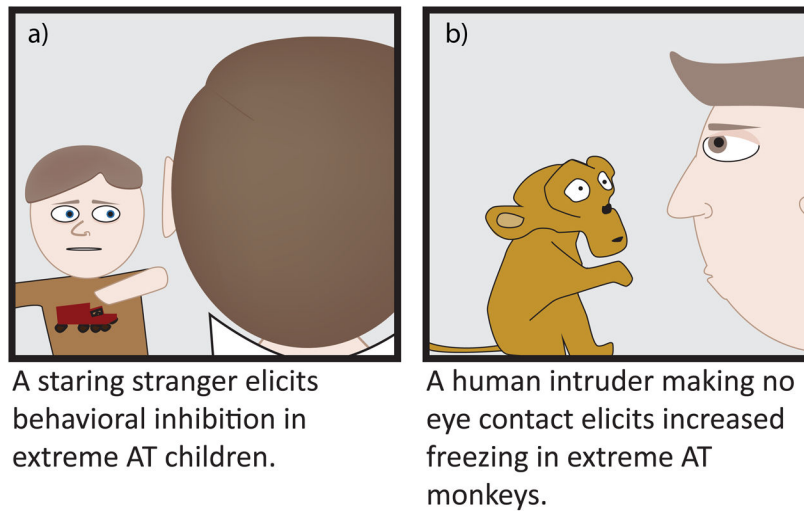


Figure 2. Anxious Temperament (AT) can be measured in (a) human children and (b) young monkeys by exposing them to potentially threatening contexts

During the NEC context, an unfamiliar human intruder stands approximately 2.5 meters from the monkey and remains still while looking away and presenting her profile to the monkey, making sure to make no eye-contact with the monkey (NEC). This potentially threatening NEC context elicits robust behavioral inhibition. In contrast, other contexts more robustly elicit “fight or flight” responses, such as when a human intruder stares at the monkey (36). Depending on the experiment, the NEC context can last for 10–30 minutes, during which time the human intruder remains motionless, continuing to present her profile throughout the entire test-period.

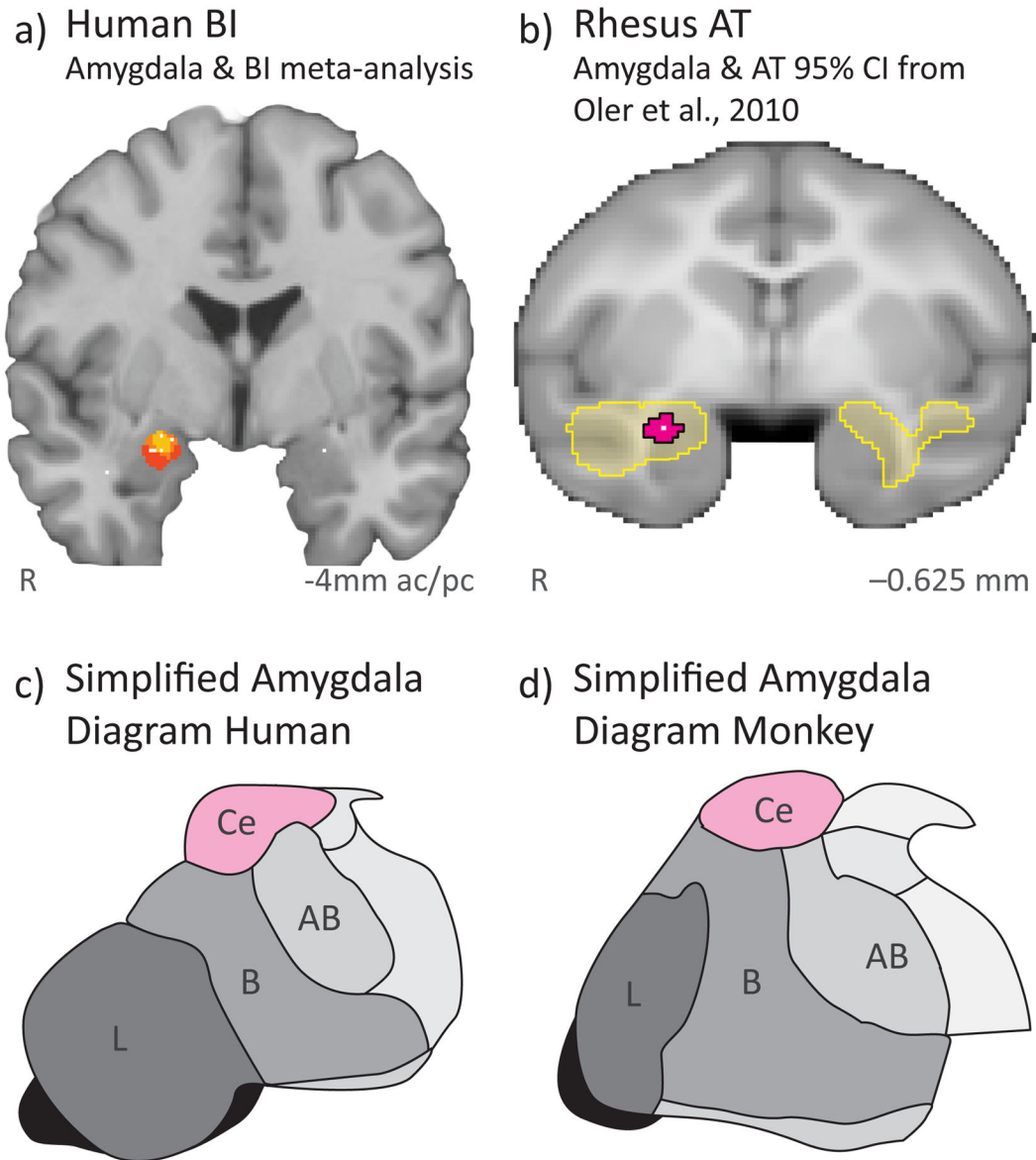


Figure 3. Dorsal amygdala activation predicts variation in Anxious Temperament (AT) in (a) humans and (b) monkeys

(a) From 7 published reports examining the role of the amygdala in individuals with a history of childhood BI, we performed a two-dimensional activation likelihood meta-analysis of the location of activation peaks in the dorsal/ventral and medial/lateral dimensions. After dilating each peak with a 4 mm² sphere, we found that 6 of the 8 amygdala peaks overlapped (yellow) in the dorsal amygdala region (4/8 peaks extended into the region shown in red). (b) FDG-PET imaging of 238 rhesus monkeys revealed that metabolism within the anterior temporal lobe predicted AT (yellow). Similar to the human studies, the peak of this region was located in the dorsal amygdala (peak in white and 95% spatial confidence interval in Red). In both humans (c) and monkeys (d) the peak activations correspond to the location of the Ce (103).

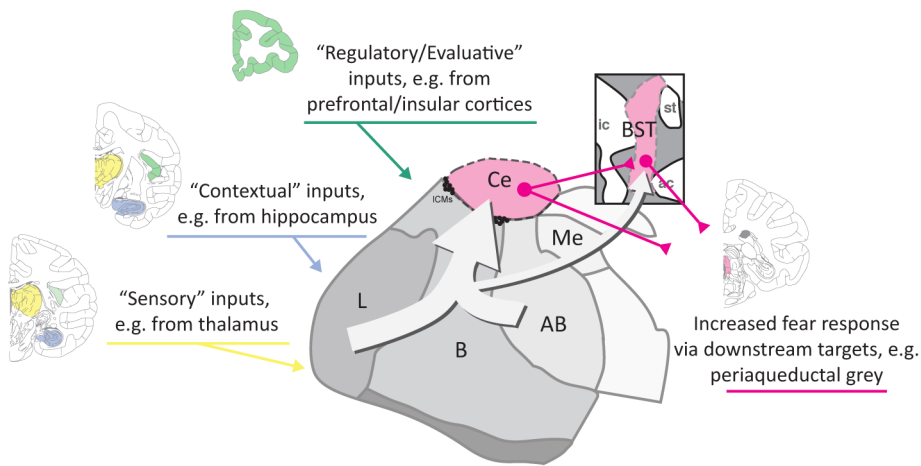
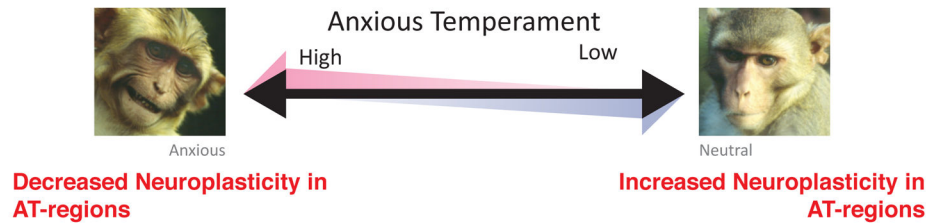


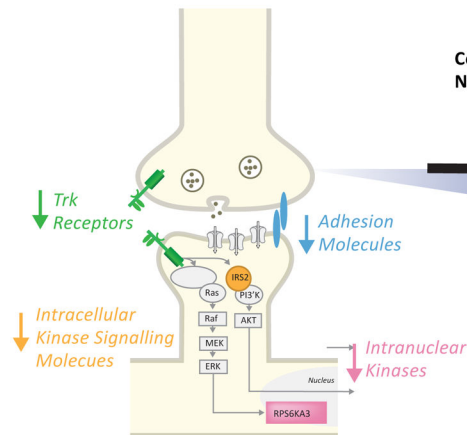
Figure 4. Simplified amygdala-centric model of the brain systems that contribute to monkey Anxious Temperament (AT)

Although the full extent of AT’s neural substrates remain unknown, neuroimaging work is beginning to identify regions that are more active in individuals with extreme AT, and lesion work suggests at least some of these regions are causally involved in the genesis of AT. The most compelling evidence exists for the amygdala, which is a critical component of AT’s neural substrates, and the focus of extensive research which implicates it in fear- and anxiety-related processing. Here, we present a simplified diagram of the monkey amygdala, and how it fits into the larger set of brain systems that influence AT. The amygdala receives input from AT-related regulatory/evaluative (green), contextual (blue), and sensory (yellow) neural systems, each of which is distributed throughout the brain. In general, amygdala information flows from the more ventral basal regions toward the Ce and bed nucleus of stria terminalis, which, via their projections to brainstem and hypothalamic structures (pink), initiate fear- and anxiety- related physiological and behavioral responses. All images are shown on slices adapted from ref: 103.

- a) Disrupted gene expression pathways in AT lead to disrupted neuroplastic processes



- b) Mechanisms of decreased neuroplasticity



- c) Function of increased neuroplasticity

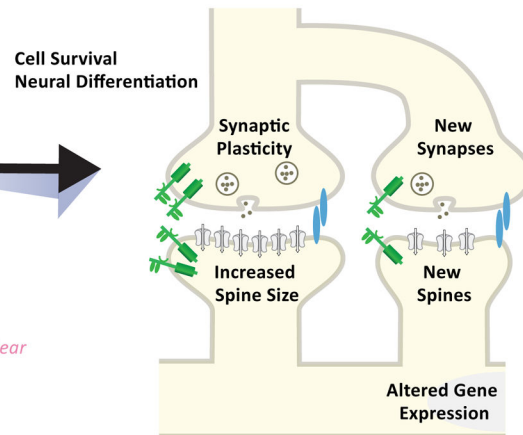


Figure 5. Mechanisms of decreased neuroplasticity mechanisms in the maintenance of early-life Anxious Temperament (AT)

(a) Research suggests that anxious individuals have decreased neurodevelopmental- and neuroplasticity-related gene activation in brain regions underlying AT, such as the Ce and hippocampus. (b) Within these regions, genes that encode adhesion molecules (e.g. EPHA4), trk receptors (e.g. NTRK3), intracellular kinase signaling molecules (e.g. IRS2), and intranuclear kinases (e.g. RPS6KA3) are inversely associated with individual differences in AT. (c) These specific genes function to increase neuroplasticity via their influences on: synaptic plasticity, increasing spine size, creating new synapses, new spines and new neurons.