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Nonhuman Primate Models to Explore Mechanisms Underlying Early-Life Temperamental Anxiety

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Abstract

Anxiety disorders are among the most prevalent psychiatric disorders, causing significant suffering and disability. Behavioral inhibition is a temperament that is linked to an increased risk for the later development of anxiety disorders and other stress-related psychopathology, and understanding the neural systems underlying this dispositional risk could provide insight into novel treatment targets for anxiety disorders. Nonhuman primates (NHPs) have anxiety-related temperaments that are similar to those of humans with behavioral inhibition, facilitating the design of translational models related to human psychopathology. Characterization of our NHP model of behavioral inhibition, which we term anxious temperament (AT), reveals that it is trait-like. Exploration of the neural substrates of AT in NHPs has revealed a distributed neural circuit that is linked to individual differences in AT, which includes the dorsal amygdala. AT-related metabolism in the dorsal amygdala, including the central nucleus, is stable across time and can be detected even in safe contexts, suggesting that AT has trait-like neural signatures within the brain. The use of lesioning and novel chemogenetic methods allows for mechanistic perturbation of the amygdala to determine its causal contribution to AT. Studies characterizing the molecular bases for individual differences in AT in the dorsal amygdala, which take advantage of novel methods for probing cellular and molecular systems, suggest involvement of neurotrophic systems, which point to the importance of neuroplasticity in AT. These novel methods, when used in combination with translational NHP models such as AT, promise to provide insights into the brain systems underlying the early risk for anxiety disorder development.

Anxiety disorders (ADs) are among the most common psychiatric disorders, affecting about 20% of adults and 30% of adolescents in the United States, leading to significant suffering and disability (1–3). ADs tend to emerge during childhood and adolescence (1,3–5), and many children with ADs do not receive treatment (6,7). Currently available treatments are not fully effective and many patients respond sub-optimally (8–10). Developing new, more effective treatments is critical and can be achieved by a more complete understanding of the neurobiological systems that underlie ADs and the early-life risk factors associated with AD emergence. Behavioral inhibition (BI) is an early temperament that reflects one's tendency to engage in protective behaviors when confronted with potential threats (11–13). Children with extreme BI are highly sensitive to novel and/or unfamiliar situations and have a very

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low threshold to activate defensive responses, such as inhibiting ongoing behavior and reducing vocalizations (12,14). BI emerges early in life, and, when extreme, is a prominent risk factor for the later development of ADs, depression, and other stress-related psychopathology (15,16). Many of the behavioral and physiological manifestations of BI are conserved across species (17), facilitating the design of translational animal models, which enable the testing of mechanistic hypotheses involving neural circuits and molecular systems. Nonhuman primates (NHPs) are particularly important in this regard, as they share similarities in temperament with humans (17–19), as well as in the structure and function of the neural systems proposed to underlie temperamental variation (17). Here we review progress in developing NHP models to study the early temperamental risks for later AD development, with a particular focus on our NHP model of anxious temperament (AT), which we argue models components of BI in humans. We also highlight the translational value of NHP models, where complementary behavioral, neuroimaging, and molecular techniques allow for insights into the neural bases of the early risk to develop stress-related psychopathology.

UNDERSTANDING INHIBITED TEMPERAMENT ACROSS SPECIES

BI was initially characterized based on the observation that when exposed to novelty and/or strangers in laboratory-based paradigms, a subset of young children (2–5 years of age) tend to inhibit their ongoing behavior, withdraw, and become hypervigilant (13,14,20) in a manner that is extreme relative to that of their peers. This inhibited behavior can be accompanied by increased physiological reactivity (21,22). When viewed from an evolutionary perspective, freezing and other inhibited responses decrease the likelihood of detection by predators (17) and can be adaptive when expressed at appropriate levels. However, when extreme, expressed out of context, and distressing, these responses can be thought of as antecedents of pathological anxiety. Meta-analyses suggest that when stable and extreme, BI is related to a 3- to 4-fold increase in the likelihood of developing a social AD and comorbid psychiatric disorders (15,16). Because BI is one of the strongest predictors of later social anxiety, an understanding of its neural substrates could provide insight into the brain systems underlying the early risk for developing stress-related psychopathology. This understanding can be improved by use of translational models of BI, the design of which is facilitated by the conserved freezing responses observed in response to novelty and threat across species, particularly in young rhesus monkey. Rhesus monkeys are extremely valuable models owing to their close evolutionary relatedness to humans (17), which manifests in similarities in their defensive repertoire, and the neurobiological systems underlying defensive responses.

In young rhesus monkeys, the human intruder paradigm is used to assess responses to threat across different contexts (23): attachment-related responses when separated from a conspecific (alone condition); protective inhibitory responses induced by the potential threat associated with a human intruder presenting her/his profile (no eye contact condition [NEC]); and fight or flight responses elicited by the imminent threat associated with the direct gaze of a human intruder (stare condition) (23,24). Our group has focused on young monkeys' behavioral responses to NEC as a model of human BI because the freezing behavior and inhibition of vocalizations, particularly affiliative coo calls, were similar to

inhibited children's responses when exposed to novelty and/or strangers in a laboratory setting (13). We focused on these behaviors associated with BI as they are conserved across species and can be reproducibly measured across large populations (17,25). We later incorporated NEC-induced increases in cortisol levels into the BI concept and termed this anxious temperament (AT). The addition of cortisol broadens the temperamental assessment to include threat-related physiological activation, which captures additional heterogeneity related to individual differences in responses to threat (26,27). Other approaches to modeling BI, as well as other relevant NHP temperamental constructs, are discussed in more depth in the Supplement.

Although the NEC condition of the human intruder paradigm assesses acute responses to uncertain threat, repeated testing has revealed that an individual's AT score is moderately stable (r range, .4–.7) and therefore trait-like, across both time and development (Figure 1A) (23,28,29). Context-specific responses to NEC emerge around 3 to 4 months of age in rhesus monkeys (30,31), a developmental point that corresponds to the emergence of stranger anxiety in children (32). In the context of our work, AT is typically assessed in rhesus monkeys between 1 and 3 years of age, which roughly corresponds to the childhood/preadolescent period in humans (Figure 1A). In humans, this age range encompasses both the time during which many ADs, especially the social ADs that children with high BI are predisposed to, begin to emerge (1), and the age at which BI is assessed in humans (15). Large population studies ($N = 592$) reveal that there is substantial variability in AT, with some monkeys showing inhibition that is extreme relative to their peers (25). Heritability studies in this large sample show that AT is approximately 30% heritable (25,33), which is consistent with the findings of heritability studies of BI (34) and ADs (35) in humans. Studies in a naturalistic setting on the island of Cayo Santiago, Puerto Rico, demonstrate that young rhesus monkeys with high AT tend to engage in lower levels of social interaction (19), similar to social impairments experienced by children with extreme BI. Together, these and other studies [reviewed in (13,29,30,36)] provide compelling evidence for the use of AT in NHPs as a trait-like model of childhood BI.

THE CENTRAL NUCLEUS OF THE AMYGDALA AS A CORE COMPONENT OF THE NEURAL CIRCUIT UNDERLYING ANXIOUS TEMPERAMENT

Moving beyond phenotypic characterization, several studies from our laboratory have investigated the neural circuit underlying individual differences in AT in rhesus monkeys using a variety of neuroimaging and lesioning methods, and have demonstrated involvement of a brain-wide circuit. This circuit includes prefrontal regulatory regions, such as the dorsolateral prefrontal cortex (PFC) (37) and posterior orbitofrontal cortex (38,39); limbic structures, including the anterior cingulate cortex (25,40), anterior hippocampus (25,26,29,33), and dorsal amygdala (25–27,29,41); basal forebrain structures, such as the bed nucleus of the stria terminalis (25,28,42); and striatal, thalamic, and brainstem regions (25,42). Studies in humans have revealed a largely overlapping circuit that is associated with early BI (16). The details of this circuit have been discussed in depth in several recent publications, and we refer interested readers to these articles (16,19,43). We emphasize that the brain circuit underlying AT is complex, involving structures distributed across multiple

functional networks within the brain that interact to mediate individual differences in temperament. Here we highlight the evidence linking the central nucleus (Ce) of the amygdala to individual differences in AT and, more broadly, to inhibited temperament. We focus on the Ce based on the convergence of clinical and preclinical research, which emphasizes the importance of this structure in AT. Furthermore, we use the translational work performed in the primate Ce as a way to highlight the critical translational gap bridged by NHP models, where in vivo phenotyping and imaging commonly employed in human populations can be paired with molecular techniques used in rodent models.

CROSS-SPECIES NEUROIMAGING STUDIES LINKING THE AMYGDALA TO AT

Initial studies posited that the amygdala was a critical substrate of BI, based both on observations of the increased fear, anxiety, and autonomic arousal experienced by children with extreme BI and on the known functions of the amygdala from rodent studies (11,44,45). Located in the medial temporal lobe, the amygdala is a collection of highly interconnected nuclei that play a key role in emotion, social interactions, and threat responses (46,47). Neuroimaging studies performed in individuals with a history of high BI have consistently implicated involvement of this region (15). These studies have also provided insight into the temporal dynamics of amygdala responses, with faster, more sustained amygdala responses (48–50) and less habituation of these responses (51) occurring in individuals with a history of BI. It is noteworthy that many of these neuroimaging studies assess brain function in adolescents and young adults that have a history of BI. Although these studies suggest that BI-related amygdala hyperactivity can be detected into adulthood (15,50,52,53), it is difficult to disambiguate whether this dysfunction is a cause or consequence of long-term, stable inhibited temperament. Studies that aim to assess threat-related amygdala function concomitant with the emergence of BI in children promise to provide insight into the early brain correlates of inhibited temperament.

To characterize the early neural substrates of our AT construct in young rhesus monkeys, our lab has made use of behavioral phenotyping combined with ^{18}F -fluorodeoxyglucose positron emission tomography. The radiotracer ^{18}F -fluorodeoxyglucose can be administered immediately before a 30-minute exposure to NEC, during which time it is trapped in cells that are undergoing increased metabolic demand, the distribution of which can be visualized using positron emission tomography scanning (54). Use of this method across a the previously discussed large NHP pedigree ($N=592$) revealed several brain regions in which threat-related metabolism was associated with individual differences in AT (25,33), including a cluster in the amygdala. This cluster (shown in Figure 1C) is in the dorsal amygdala region, which encompasses the Ce, dorsal portions of the basal, accessory basal, and lateral nuclei. Interestingly, the peak of this cluster fell within the Ce, which was confirmed using chemoarchitectonic localization (25). The Ce was localized based on positron emission tomography scans with a ligand with a high affinity for the serotonin transporter, which is expressed at higher levels in the lateral division of the central nucleus (55,56). This finding is largely consistent with meta-analyses of studies assessing BI in

humans, which suggest that clusters of functional activation related to differences in BI are more likely to be reported in the dorsal portions of the amygdala (57).

The anatomical inputs and outputs of the Ce make it particularly interesting in the context of AT. The Ce, which comprises a lateral (CeL) and medial (CeM) division, is primarily composed of striatal-like cells that communicate via the inhibitory neurotransmitter GABA (gamma-aminobutyric acid) (57). Local microcircuits within the Ce, as well as intra-amygdala circuits, have been shown in rodent models to be involved in mediating behavioral responses to potential threats (58,59). The CeL receives input from the ventral amygdala, which provides integrated sensory, regulatory, and contextual inputs from cortical and subcortical regions (60–63). The primary output of the amygdaloid complex is via the CeM (64), which innervates hypothalamic and brainstem nuclei that mediate threat- and stress-related behavioral and autonomic responses (63,65). Thus, individual differences in the threat-related function of the Ce, in part mediated by local microcircuits operating within it, could affect the propensity to initiate defensive behavioral and physiological responses, which have been specifically demonstrated to be enhanced in children with high BI and in monkeys with extreme AT.

When assessed with repeated exposures to the NEC, Ce metabolism is relatively stable (intercluster correlation coefficient = 0.64), suggesting that this AT-related brain signature has a trait-like nature (29). Furthermore, metabolism in the amygdala is related to AT in both stressful and nonstressful contexts (11), which is interesting as children with high BI tend to display increased anxiety in “safe” contexts (28). Metabolism in the Ce is independently associated with each of the components of AT (freezing, cooing, cortisol), suggesting that this structure is hyperactive regardless of the admixtures of its components that result in a high AT score (26). Studies performed in children (34) and in our multigenerational rhesus monkey pedigree (25) have demonstrated that both heritable and nonheritable influences are important in determining AT’s neural circuit (25). Together, these studies provide strong evidence for trait-like, context-independent, AT-related neural activity in the amygdala, particularly the Ce.

A CAUSAL ROLE FOR THE DORSAL AMYGDALA IN MEDIATING AT

Lesioning methods in animal models have been very informative in extending these correlative findings. As human studies must capitalize on disease-related or surgery-induced lesions (66,67), efforts to probe the causal contribution of the amygdala in humans are limited by a paucity of individuals with circumscribed amygdala damage. These studies are also complicated by the incomplete or unilateral nature of damage to the amygdala (68,69), the concomitant damage of several amygdala nuclei (70), and an inability to prospectively randomize lesion and comparison groups that could lead to confounds related to preexisting illnesses and behavioral dispositions (71). Furthermore, structural lesions that are indicative of those in humans often include damage to fiber tracts proximal to the amygdala (72). Neurotoxic lesioning methods performed in rhesus monkeys may induce targeted damage that is limited to neurons in the amygdala, or nuclei within it, allowing for the refinement of hypotheses about the causal contribution of the amygdala to temperament, without the confounds potentially associated with concomitant fiber damage. Complete neurotoxic

lesions of the amygdala in adult rhesus monkeys result in a variety of behavioral alterations, which influence social interactions and defensive responses (Table 1) (24,72–87). Neonatal lesions of the amygdala also result in alterations in young monkeys' tendency to display fear-related behaviors during social interactions, in both early life and adulthood (74,88). Surprisingly, although large neurotoxic lesions of the majority of the amygdaloid complex in juvenile monkeys decreased fearful responses in the presence of a snake, they did not alter AT (24). Instead, targeted neurotoxic lesions of the dorsal amygdala, which encompassed the Ce, resulted in behavioral and physiological alterations consistent with a decrease in AT (80). These results suggest that the Ce, which has been proposed to act as a “gate” (89) between incoming sensory and regulatory input and autonomic and behavioral output, is particularly important in determining the extent to which an individual inhibits behavior in the presence of a threat.

The gating function of the Ce has been linked to the activity of microcircuits comprising several cell types within the Ce, which are defined by their response properties (90), as well as expression of molecular markers (91) (Figure 2A). Furthermore, plasticity within these Ce circuits, as well as in projections to and from other amygdala nuclei and downstream targets, is also important in mediating defensive responses (92–95). Studies in rodents, facilitated by opto- and chemogenetic methods that may discretely target and activate various genetically defined subpopulations of cells in the amygdala, have been essential in refining the understanding of these circuits and their causal contributions to behavior (58,59,96). Most of these rodent studies probe the function of Ce microcircuits in the context of fear-learning paradigms (90,91), whereas most of the primate research discussed in Table 1 relies on paradigms that assess unconditioned fear- and anxiety-related behaviors [but see (97,98)]. The translation of opto- and chemogenetic technologies for use in NHPs will be important in determining the extent to which distinct cell populations are linked to the expression of AT. Although the structure and connections of the amygdala are generally well conserved between rodents and primates, there are several notable cross-species differences: in primates, the ventral amygdala is significantly expanded (approximately 30 times as large in rhesus monkeys as in rats), thought to be due increased cortical input, although the relative size of the Ce has remained fairly constant (approximately 5 times as large in rhesus monkeys as in rats) (99). Understanding how this expanded input from the ventral amygdala influences interactions with Ce microcircuits will be important. At a cellular level, studies suggest that there may be some differences between primate and rodent species in terms of the composition and distribution of relevant cell types and molecules in the Ce (27,100), though the functional significance of these species differences has not been fully explored.

Relevant to answering these questions, chemogenetic strategies are beginning to emerge as tools for manipulating neurons and understanding microcircuit function in NHPs. The use of viral vector-mediated delivery may be leveraged to induce expression of chemogenetic receptors (commonly, DREADDs [designer receptors exclusively activated by designer drugs]), which are activated by “inert” ligands that may be systemically administered, to induce reversible changes in firing in the cells where these receptors are expressed (101). Early NHP studies employing these methods have shown that manipulation of cells in the amygdala appears to alter threat-related responses elicited by the human intruder paradigm (77). Initial studies in our own laboratory, which have relied on the development of

intraoperative monitoring systems to ensure selective targeting of viral vector infusions to discrete regions (102) (Figure 2D, E), suggest that manipulations of cells in the dorsal amygdala that are based on DREADDS can alter AT. Efforts are ongoing to further advance these methods in NHPs to enable the specific activation of genetically defined cell populations (103), which will prove essential in targeting the functionally distinct amygdala cell types characterized in rodent models. Application of these methods in NHPs (104) will allow for unprecedented insights into the neural circuitry underlying AT in the primate amygdala.

Also of interest from the standpoint of manipulation of the amygdala are the many regulatory projections from the PFC (65,105), which have been linked to fear responses, anxiety, and AT (37–39,106) and primarily target the basolateral amygdala (105). Rhesus monkeys are particularly valuable in the study of prefrontal mechanisms related to psychopathology, as they share with humans many of the same chemo-architectonically and functionally defined prefrontal regions (107–109), which are connected to subcortical structures in similar ways (62,110). White matter pathways that link the prefrontal and medial temporal lobes, including the uncinate fasciculus, have similar organization in humans and rhesus monkeys (111,112), and the microstructural integrity of this tract has been linked to AT (113). Inhibition of cells in the NHP amygdala based on DREADDS results in altered frontolimbic connectivity (114), as assessed using functional magnetic resonance imaging. Dual-vector approaches (Figure 2C) have provided insight into the importance of prefrontal–amygdala interactions in rodents (115–117), but outstanding questions regarding the functional homology between rodent and human prefrontal sectors underscore the importance of performing these studies in NHPs (118–120). In addition to expanding the understanding of the regulatory relationship between the PFC and the amygdala in primates, the use of these methods could be expanded to target many other functional inputs and outputs of the Ce, such as the bed nucleus of the stria terminalis and periaqueductal gray.

IDENTIFYING AND TESTING MOLECULAR PROCESSES RELATED TO AT WITHIN THE DORSAL AMYGDALA

Although extensive work in rodents has identified molecular pathways linked to stress (121–123), there is a paucity of similar work in NHPs (124). Because of the substantial evidence linking the Ce to AT, we have focused primarily on identifying AT-related molecular alterations in this region. Characterizing molecular alterations at the level of the transcriptome allows for insight into the combined influence of structural genetic variation and epigenetic regulation on gene expression (125). To probe the molecular processes that mediate the relation between altered Ce metabolism and AT, RNA was collected from the dorsal amygdala region of 24 young rhesus monkeys that had undergone AT phenotyping and neuroimaging (29). Among other transcripts, dorsal amygdala expression levels of the transcript for the NTRK3 receptor (activated by its primary ligand, neurotrophic factor 3 [NT3]) and its downstream target RPS6KA3 were found to be inversely associated with AT, as well as with metabolism in the Ce (29,42). Neurotrophic factors, particularly BDNF (brain-derived neurotrophic factor) and FGF (fibroblast growth factor), have been

extensively linked to stress in rodent models (126–128). Neurotrophic factors bind to members of a family of tyrosine kinase receptors, such as NTRK3, to affect intracellular signaling cascades, which may result in increased plasticity either through cytoplasmic effectors or changes in gene expression (129). Ultimately, these changes result in increased spine formation and stabilization, among other structural changes (129,130). A recent study confirmed the importance of neurotrophic signaling (BDNF/NTRK2) in facilitating plasticity in CeL neurons. Deletion of presynaptic BDNF in neurons projecting from the paraventricular thalamus to the CeL or depletion of postsynaptic NTRK2 receptors on neurons in the CeL impaired fear conditioning. Furthermore, direct infusion of BDNF into the CeL facilitated fear conditioning, suggesting that the BDNF/NTRK2 pathway is important for plasticity associated with fear learning and responding in the Ce (131). Further work will be needed to determine if these principles extend to the NT3/NTRK3 system.

To causally link the NT3/NTRK3 signaling pathway to AT, we used viral vector–mediated overexpression of *NTF3* messenger RNA in young monkeys to increase NT3 protein abundance in the dorsal amygdala (41). Importantly, the effects of this manipulation resulted in decreased AT, as well as altered metabolism in both the Ce and other AT-related brain regions, suggesting that NT3/NTRK3-mediated plasticity in the dorsal amygdala affects both AT and the function of its neural circuit (Figure 3). Together, this work led us to propose a neuroplasticity hypothesis for AT that centers around the Ce (19). As the local circuits within the Ce are thought to be important for integrating information relevant to the initiation of defensive behaviors (91,132–134), decreased plasticity within these circuits could impair the ability to flexibly alter responses related to threat and safety. This impairment could lead to a persistent tendency toward activation of the Ce and its downstream targets, which could promote a tendency toward enhanced threat reactivity, including inhibition. The specific targeting of plasticity within these and other amygdala circuits (135,136), as well as prefrontal regulatory projections (105,137,138) and connections with other AT-related regions (57), could provide novel circuit-based approaches to potentially normalize the aberrant function of this region across development.

CONCLUSIONS

AT is an evolutionarily conserved, anxiety-related temperament analogous to BI in humans, which is strongly linked to the later emergence of stress-related psychopathology. Extensive characterization of AT in NHPs suggests that it has stable neural correlates, particularly in the amygdala. Using complementary *in vivo* and *ex vivo* methods, we extend these correlational findings, providing evidence for the critical importance of the primate amygdala, particularly the Ce, in AT. The use of novel methods, including chemogenetics, RNA sequencing technologies, and viral vector–mediated expression, allows for insight into the cellular and molecular bases for circuit-level dysfunction in primates, highlighting the importance of neuroplasticity systems in the dorsal amygdala in mediating individual differences in AT. Considered together with studies in humans and rodent models (11,15,16,89), these findings point to the primate Ce as a target for interventions aimed at ameliorating outcomes associated with early AT. Based on the link between neurotrophic signaling in the dorsal amygdala and AT, interventions could be designed to specifically target this system. Existing drugs such as selective serotonin reuptake inhibitors and

ketamine have been shown to interact with neurotrophic systems, though the extent to which these interventions activate neurotrophic signaling in the primate amygdala is unknown. Across medicine (139), the use of viral vector-mediated delivery has garnered increased attention in the scope of gene therapy for patients with refractory conditions, and, in relation to psychiatry, provides a basis for specifically modulating molecular systems within neural circuits. However, continued study in NHPs is necessary to determine potential efficacy, as well as safety, in the primate brain. Despite our focus on the amygdala, we emphasize that the circuit underlying AT includes many brain regions, with complex interactions between these regions (16,19,43). The studies highlighted in this review demonstrate how novel tools can be leveraged to probe the relationship between AT and amygdala function and can serve as a road map for probing molecular and circuit alterations in other regions associated with stress-related psychopathology. The ultimate aim of this work is to develop strategies, by translating from young monkeys to children, that will mitigate the likelihood of at-risk children's developing ADs and associated stress-related psychopathologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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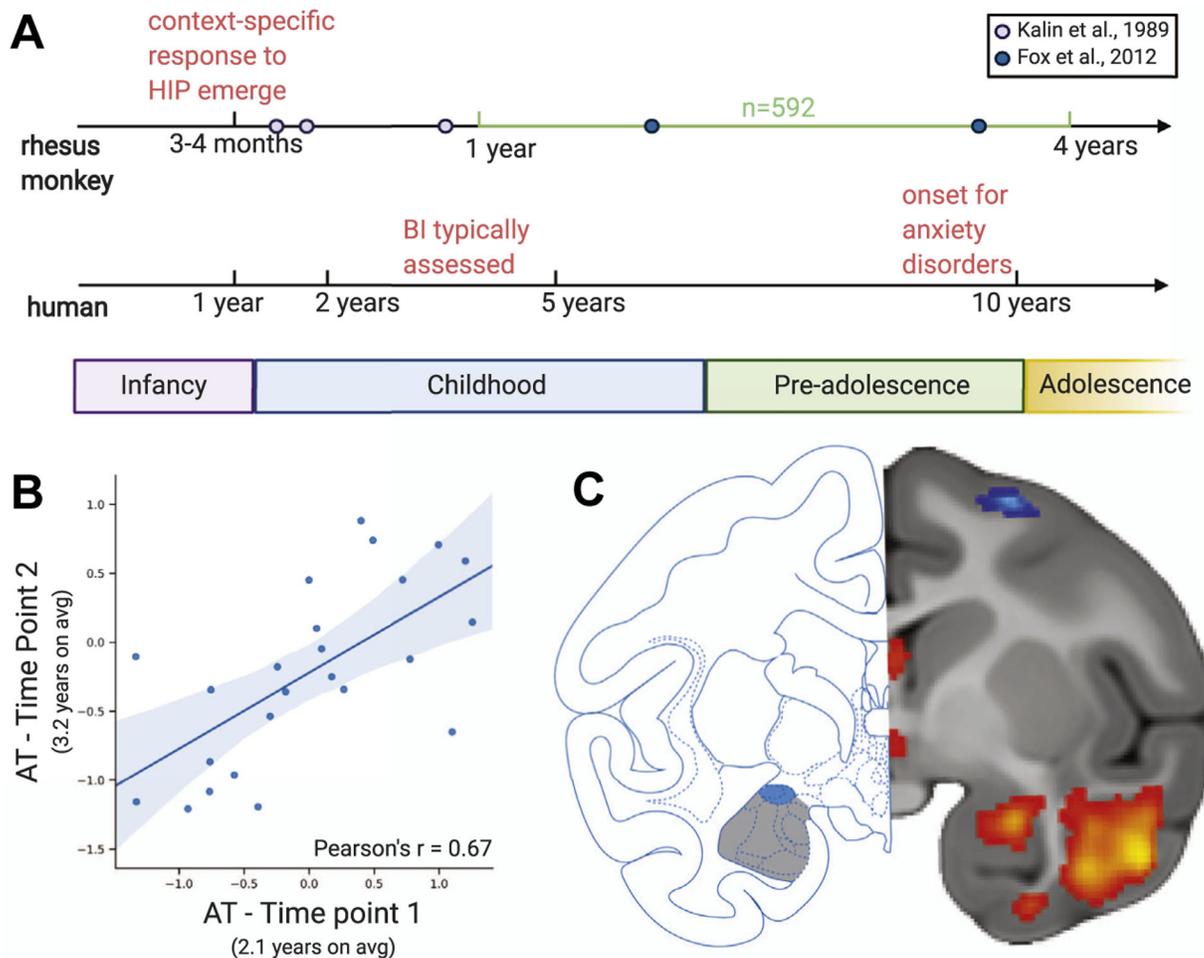


Figure 1.

(A) A schematic depicting the correspondence of developmental periods between humans and rhesus monkeys: context-specific responses to the HIP emerge around 3 to 4 months of age in rhesus monkeys (31), which corresponds to the 12- to 24-months age range in humans. In green, the age range for the large sample included in many of our analyses, which ranges from 0.87 to 3.8 years, spanning the childhood/preadolescent period. In children, social anxiety disorders that children with extreme BI are predisposed to develop begin to emerge in preadolescence (approximately 10 years of age). (Top panel) The dots on the timeline represent time points at which repeated testing for AT has been performed, with the key showing the study from which the data are drawn. In Kalin *et al.* (23), young rhesus monkeys were tested at 5, 6, and 11 months, with moderately stable AT across repeated testing (data not available). In Fox *et al.* (29), subjects were tested at 2.1 years and again at 3.2 years of age, on average. (B) The data from Fox *et al.* (29), with repeated testing from 24 rhesus monkeys. As can be seen, there is stability of AT across the childhood/preadolescent time period ($r = .67$). (C) (Left panel) This illustration shows the anatomy of the rhesus monkey amygdala. Lesions of the central nucleus, shaded in blue, result in decreased AT (80), while lesions of the entire amygdala (shaded in blue and gray), do not (24). (Right panel) Individual differences in AT are associated with individual differences in ^{18}F -

fluorodeoxyglucose uptake in the dorsal amygdala, assessed using ^{18}F -fluorodeoxyglucose positron emission tomography as discussed in the main text (25,33), as well as in the adjacent temporal cortex (141) and midline septal area. This figure also illustrates the distinction between the dorsal amygdala (used when the spatial resolution of methods employed does not allow for clear delineation of anatomical boundaries, right panel) and central nucleus (used when methods allow for an assessment of anatomical boundaries, left panel). Created with [BioRender.com](https://www.biorender.com). AT, anxious temperament; BI, behavioral inhibition; HIP, human intruder paradigm. [(C) (Left panel) Adapted with permission from Paxinos *et al.* (140).]

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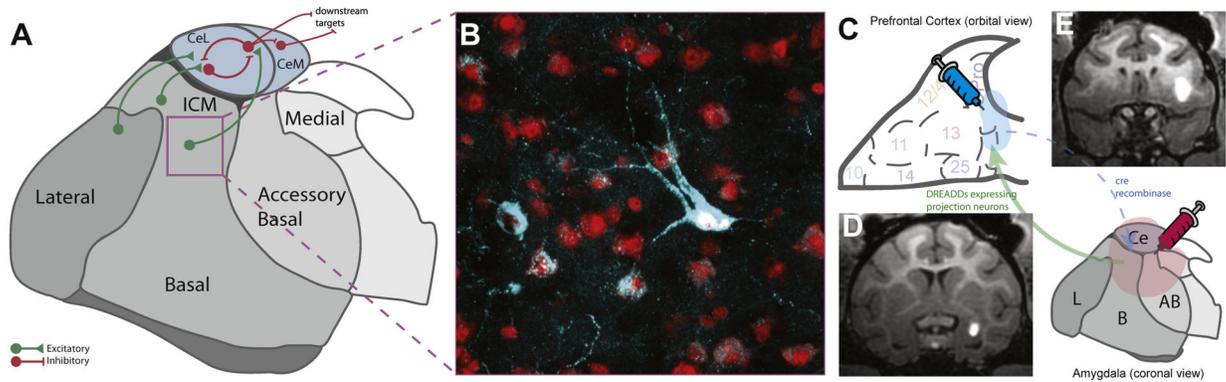


Figure 2.

(A) Anatomy of the rhesus monkey amygdala. In gray, the ventral nuclei of the amygdala receive a majority of projections from cortical, subcortical, and thalamic regions, such as the prefrontal cortex, sensory association cortices, the hippocampus, and medial dorsal thalamus (36). This region primarily contains excitatory neurons (depicted in green), which project to the inhibitory neurons of the Ce (depicted in red). Within the lateral and medial Ce, the two major divisions of the Ce, local inhibitory microcircuits interact to influence threat-related behavioral and physiological responses, which are mediated via projections to hypothalamic and brainstem nuclei (142). This simplified circuit diagram depicts general organizational principles that have been characterized in rodent models (58,90,91,96). It remains to be seen whether these circuits are conserved in primates. (B) Fluorescent images of DREADDs expressing neurons within the basal nucleus of the amygdala. Immunolabeling for the fluorescent reporter associated with the DREADDs receptor hemagglutinin is shown in blue. In red, staining for NeuN, a neuronal marker, delineates nuclei. (C) Depiction of the dual-vector approach for specific targeting of projections between the amygdala and prefrontal cortex. In pink, injection of a viral vector containing the sequence for the DREADDs receptor, under the transcriptional control of the enzyme Cre recombinase, is injected into the amygdala. In blue, a retrogradely transported virus which contains Cre recombinase, is injected into the pOFC. This virus is retrogradely transported (blue dotted arrow) along axonal projections, leading to expression of the DREADDs receptor only in cells projecting from the amygdala to the pOFC (green arrow) (117). Based on comprehensive characterization of the projections between the pOFC and amygdala (105), these projection neurons likely originate from the ventral amygdala, as projections from the Ce to pOFC are sparse. (D, E) T1-weighted intraoperative images showing co-infusions of the viral vector with a contrast agent (gadolinium, bright white) into the (D) dorsal amygdala and (E) pOFC. AB, accessory basal amygdala; B, basal amygdala; Ce, central nucleus; CeL, lateral division of the central nucleus; CeM, medial division of the central nucleus; DREADDs, designer receptors exclusively activated by designer drugs; ICM, intercalated cell mass; L, lateral amygdala; pOFC, posterior orbitofrontal cortex. [(A) Adapted with permission from Fox *et al.* (19) and Paxinos *et al.* (140).]

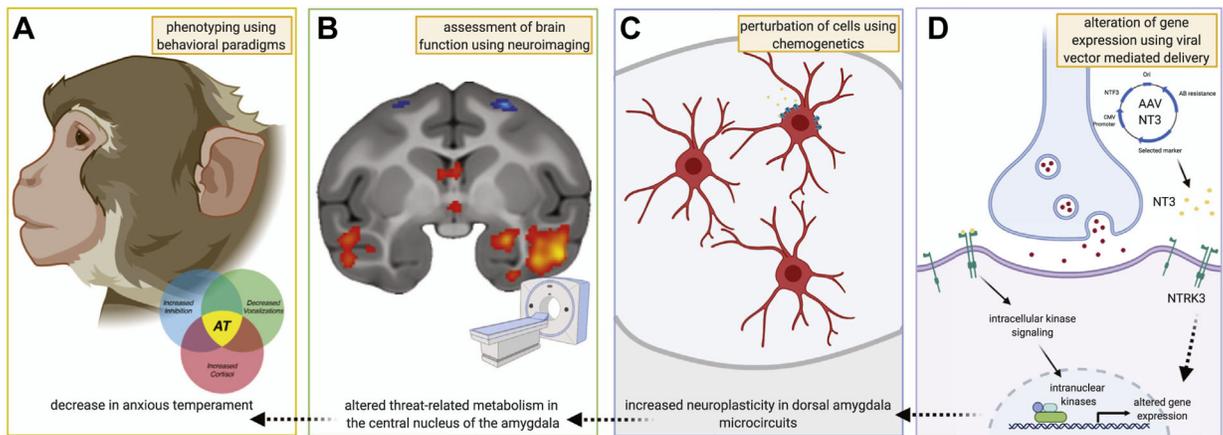


Figure 3.

(A) Translational behavioral paradigms, such as the human intruder paradigm, can be used to measure evolutionarily conserved temperaments, such as AT, that are highly relevant to understanding human psychopathology. (B) These paradigms can be administered in conjunction with ^{18}F -fluorodeoxyglucose positron emission tomography to reveal brain regions, including the central nucleus of the amygdala, where metabolic activity is related to individual differences in temperament. (C) Neurons within the dorsal amygdala form local microcircuits, which are essential for processing information relevant to threat. Perturbation of these cells can be achieved using chemogenetic technologies (blue receptors) to induce changes in firing in these cells. (D) At the level of the synapse, activation of NTRK3 by its ligand NT3 results in a variety of intracellular signaling cascades, which ultimately results in increased expression of genes that promote plasticity processes (129). Manipulation of these molecular systems can be achieved using viral vector-mediated approaches. In this case, increased expression of the messenger RNA for the NT3 protein leads to increased signaling via the NTRK3 pathway. These changes in gene expression are presumed to affect plasticity in the dorsal amygdala, resulting in altered metabolism in this AT-related region and others (42). Ultimately, this manipulation affects AT. Together, this figure shows how tools can be leveraged in rhesus monkeys to study AT at various levels—from phenotypes to circuits to cells to molecules—providing insight into the bases for the early-life risk to develop stress-related psychopathology. Created with [BioRender.com](https://www.biorender.com). AAV, adeno-associated virus; AB, antibody; AT, anxious temperament; CMV, cytomegalovirus; Ori, origin of replication.

Table 1. Summary of Findings From Selected Studies Exploring the Effects of Either Neurotoxic Lesions or Manipulations to the Amygdala on Threat- and Fear-Related Behaviors in Macaques

Reference	Developmental Stage	Type of Manipulation	Species of Macaque	No. of Amygdala Lesioned Subjects (Sex)	Control/Comparison Groups (Sex)	Age at Lesion/Manipulation	Relevant Behavioral Tests (Age or Time After Surgery)	Summary of Relevant Findings	Linked Publications
Prather <i>et al.</i> (76)	Neonatal	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	3 (2 male, 1 female)	3 unoperated controls (2 male, 1 female)	12–16 days after birth	Dyadic social interaction (6.5 mo); responsiveness testing (8.5 mo)	Amygdala-lesioned monkeys displayed normal social development and maintained species-typical interactions with peers and mothers. Lesioned subjects had heightened fear responses in social interactions and blunted fear responses to nonsocial stimuli.	
Bauman <i>et al.</i> (73)	Neonatal	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	8 (3 male, 5 female)	8 sham-operated controls (4 male, 4 female), 8 hippocampus lesion (5 male, 3 female)	12–16 days after birth	Behavioral observations (home cage, solo at 6 and 9 mo; dyads at 6, 9, and 12 mo, social group)	Despite relatively normal social development, amygdala-lesioned animals display more fear behaviors during social interaction (evident at all time points and in all social situations).	See also (74–76, 144–149)
Bliss-Moreau <i>et al.</i> (74)	Neonatal	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	8 (3 male, 5 female)	8 sham-operated controls (4 male, 4 female), 8 hippocampus lesion (5 male, 3 female)	12–16 days after birth	Object responsiveness (9 and 18 mo; temperament assessments)	Amygdala-lesioned monkeys displayed shorter latencies to interact with a variety of novel and fearful objects at 9 and 18 mo. At 18 mo, amygdala-lesioned monkeys were rated as less fearful during testing by trained observers.	See also (74–76, 144–149)
Bliss-Moreau <i>et al.</i> (75)	Neonatal	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	8 (3 male, 5 female)	8 sham-operated control (4 male, 4 female), 8 hippocampus lesion (5 male, 3 female)	12–16 days after birth	Object responsiveness (3 years)	Amygdala-lesioned animals displayed lower latencies to retrieve food rewards in the presence of emotionally salient objects and were quicker to explore objects.	See also (74–76, 144–149)
Raper <i>et al.</i> (87)	Neonatal	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	16 (9 male, 7 female)	12 sham-operated controls (6 male, 6 female), 3 behavioral	23–25 days after birth	Human intruder paradigm (2.5 and 12 mo)	While amygdala-lesioned monkeys did not show major alterations in their defensive responses, some alterations in contextual modulation of these	See also (150–154)

Reference	Developmental Stage	Type of Manipulation	Species of Macaque	No. of Amygdala Lesioned Subjects (Sex)	Control/Comparison Groups (Sex)	Age at Lesion/Manipulation	Relevant Behavioral Tests (Age or Time After Surgery)	Summary of Relevant Findings	Linked Publications
Raper <i>et al.</i> (77)	Neonatal	Chemogenetic inhibition of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	2 (1 male, 1 female)	Within-subject design	9 mo	Human intruder paradigm (14 mo); socioemotional attention task (3.5–5 mo)	Inactivation using chemogenetic technologies decreased freezing and increased attention to relevant facial features in the socioemotional attention task.	responses were reported when tested in infancy. Differences were more pronounced during the juvenile period, and amygdala-lesioned monkeys showed less freezing in the HIP. Sex differences were reported, which were more prevalent in infancy.
Kalin <i>et al.</i> (24)	Juvenile	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	17 (15 male, 2 female)	10 unoperated controls (9 male, 1 female)	22.5 mo on average	Human intruder paradigm; snake fear test (1 mo after surgery)	While no alterations were reported in freezing during the HIP, amygdala-lesioned animals displayed decreased fear in the presence of a threatening stimulus (snake).	
Kalin <i>et al.</i> (80)	Juvenile	Neurotoxic lesion of the dorsal amygdala	Rhesus (<i>Macaca mulatta</i>)	9 bilateral, 9 unilateral (all male)	16 unoperated controls (16 male)	34.6 mo on average	Human intruder paradigm; snake fear test	Dorsal amygdala-lesioned animals displayed decreased cooling, freezing and plasma corticotropin levels in the no eye contact condition of the HIP, as well as decreased latencies to reach for a food reward in the presence of a snake.	
Machado <i>et al.</i> (84)	Juvenile	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	9 (all male)	9 sham-operated controls, 9 hippocampus lesion, 6 OFC lesion (all male)	2.4–3.3 years of age	Human intruder paradigm; hormonal reactivity to conspecifics, aversive objects and physical restraint (3–12 mo after surgery);	Amygdala-lesioned subjects displayed less defensive freezing, as well as an overall decrease in ability to regulate tension related behaviors between contexts. Amygdala-lesioned subject displayed blunted cortisol following isolation from peers, but not after the HIP.	See also (155–157)

Reference	Developmental Stage	Type of Manipulation	Species of Macaque	No. of Amygdala Lesioned Subjects (Sex)	Control/Comparison Groups (Sex)	Age at Lesion/Manipulation	Behavioral Tests (Age or Time After Surgery)	Summary of Relevant Findings	Linked Publications
Wellman <i>et al.</i> (86)	Juvenile	Pharmacological activation and inactivation of the Ce/BLA	Pigtailed (<i>Macaca nemestrina</i>)	BLA: 3 (1 male, 2 female); Ce: 4 (2 male, 2 female)	Within-subject design	18 mo to 2 years of age	Dyadic interactions (18 mo to 2 years of age)	Activation of the BLA, and not the Ce suppressed social behavior, while inactivation of both structures increased social behavior within familiar social pairs.	See also (158)
Meunier <i>et al.</i> (72)	Adult	Comparison of aspiration and neurotoxic lesions of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	3 (all male) aspiration lesions; 6 (all male) neurotoxic lesions	6 controls (all male)	Adulthood	Object responsiveness (adulthood)	Though aspiration and neurotoxic lesions produce a similar set of behavioral alterations (including reduced freezing in the presence of fear-inducing objects), the alterations seen following aspiration lesions are generally more pronounced.	
Emery <i>et al.</i> (78)	Adult	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	6 (all male)	6 unoperated controls (all male)	6.15 years on average	Behavioral observations in a constrained (6.7 years), unconstrained dyad (7.1 years), and round robin dyad (7.7 years)	Across all behavioral paradigms, amygdala-lesioned subjects displayed an increased tendency towards positive social interaction with peers, as well as decreased tendency towards tension-related behaviors.	See also (82,159,160)
Stefanacci <i>et al.</i> (79)	Adult	Neurotoxic lesion of the bilateral amygdala	Cynomolgous (<i>Macaca fascicularis</i>)	5 (all male)	4 unoperated controls (4 male)	Adulthood	Object responsiveness (3 and 23 mo after surgery)	Amygdala-lesioned animals approached emotional stimuli more readily shortly after surgery (3 months), and displayed a tendency towards approaching more readily long (23 mo) after surgery.	
Izquierdo <i>et al.</i> (81)	Adult	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	4 (all male)	10 posterior OFC aspiration lesions; 10 unoperated controls (all male)	Adulthood	Human intruder paradigm; snake fear test	Amygdala-lesioned animals had lower latencies to retrieve a preferred food reward in the presence of a snake and displayed less defensive and more approach behaviors during this task. No alterations were reported in the HIP.	See also (81,85,161)

Reference	Developmental Stage	Type of Manipulation	Species of Macaque	No. of Amygdala Lesioned Subjects (Sex)	Control/Comparison Groups (Sex)	Age at Lesion/Manipulation	Relevant Behavioral Tests (Age or Time After Surgery)	Summary of Relevant Findings	Linked Publications
Mason <i>et al.</i> (82)	Adult	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	6 (all male)	6 unoperated controls (all male)	6.15 years on average	Novel object responsiveness test (5 mo after surgery); human presenting food	During initial exposure to various novel objects, amygdala lesions were more likely to interact with the novel objects than were controls. They were also food reward given by a human experimenter or placed near a complex novel object. The differences between controls and amygdala-lesioned subjects diminished with repeated testing, as controls become less inhibited.	See also (78,159,160)
Antoniadis <i>et al.</i> (83)	Adult	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	Sample 1: 6 (all male); Sample 2: 4 (all male)	Sample 1: 6 sham-operated controls, 6 hippocampus lesion (all male); Sample 2: 4 sham-operated controls (all male)	Sample 1: 11 years on average; Sample 2: 6.43 years on average	Sample 1: fear potentiated startle (4.5 years after surgery); Sample 2: lesions performed after acquisition of fear potentiated startle	Amygdala-lesioned animals failed to acquire fear-potentiated startle. However, damage to the amygdala induced after acquisition of fear-potentiated startle did not block its expression.	See also (97)
Chudasama <i>et al.</i> (85)	Adult	Neurotoxic lesion of the bilateral amygdala	Rhesus (<i>Macaca mulatta</i>)	4 (all male)	9 sham-operated controls, 8 hippocampus lesion (all male)	Adulthood	Snake fear test	Amygdala-lesioned animals had shorter latencies to retrieve a food reward in the presence of fear inducing stimuli (snake and spider) relative to neutral objects and displayed less defensive responses	See also (81,161)

A subset of selected linked publications, which probe constructs related to fear, threat, and social behavior in either the same or a linked sample of nonhuman primate subjects, are listed. A complementary table, which includes a discussion of results from aspiration lesion studies, as well as other behavioral alterations associated with amygdala lesions, can be found in (143).

BLA, basolateral amygdala; Ce, central nucleus of the amygdala; HIP, human intruder paradigm; OFC, orbitofrontal cortex.