Review article

The central extended amygdala in fear and anxiety: Closing the gap between mechanistic and neuroimaging research

Andrew S. Fox[a, b, **, 1]

dfox@ucdavis.edu

Alexander J. Shackman[c, d, e, *, 1]

shackman@umd.edu

[a]Department of Psychology and University of California, Davis, CA 95616, United States

[b]California National Primate Research Center, University of California, Davis, CA 95616, United States

[c]Department of Psychology, University of Maryland, College Park, MD 20742, United States

[d]Neuroscience and Cognitive Science Program, University of Maryland, College Park, MD 20742, United States

[e]Maryland Neuroimaging Center, University of Maryland,College Park, MD 20742, United States

*Corresponding author~~s~~ at: Department of Psychology, University of Maryland, College Park, MD 20742, United States.

**Corresponding author~~s~~ at: Department of Psychology ~~and~~ , University of California, Davis, CA 95616, United States.

[1]These Authors contributed equally to this work; the order of authors was determined by a coin flip.

Abstract

Anxiety disorders impose a staggering burden on public health, underscoring the need to develop a deeper understanding of the distributed neural circuits underlying extreme fear and anxiety. Recent work highlights the importance of the central extended amygdala, including the central nucleus of the amygdala (Ce) and neighboring bed nucleus of the stria terminalis (BST). Anatomical data indicate that the Ce and BST form a tightly interconnected unit, where different kinds of threat-relevant information can be integrated to assemble states of fear and anxiety. Neuroimaging studies show that the Ce and BST are engaged by a broad spectrum of potentially threat-relevant cues. ~~Likewise, m~~Mechanistic work demonstrates that the Ce and BST are critically involved in organizing defensive responses to a wide range of threats. Studies in rodents have begun to reveal the specific molecules, cells, and microcircuits within the central extended amygdala that underlie signs of fear and anxiety, but the relevance of these tantalizing discoveries to human experience and disease remains unclear. Using a combination of focal perturbations and whole-brain imaging, a new generation of nonhuman primate studies is beginning to close this gap. This work opens the door to discovering the mechanisms underlying neuroimaging measures linked to pathological fear and anxiety, to understanding how the Ce and BST interact with one another and with distal brain regions to govern defensive responses to threat, and to developing improved intervention strategies.

Keywords: Affective neuroscience; Anxiety disorders; Bst/bnstBST/BNST (These are acronyms: It should be 'BST/BNST'); Emotion; Individual differences; Neuroimaging; Nonhuman primate

When extreme, fear and anxiety can become debilitating [1]~~.~~[2]~~.~~ Anxiety disorders impose a staggering burden on public health; they are common, costly, and contribute to the etiology of depression and substance abuse [2–4]. Existing treatments are inconsistently effective or associated with significant adverse effects [5,6], underscoring the need to develop a deeper understanding of the distributed neural circuits that control the expression of fear and anxiety. Converging lines of physiological and mechanistic evidence indicate that the central extended amygdala—including the central nucleus of the amygdala (Ce) and bed nucleus of the stria terminalis (BST)—is a key hub in this circuitry and motivates the hypothesis that local alterations in the central extended amygdala can drive changes in remote regions of the brain in ways that promote the development and maintenance of anxiety, mood, and substance use disorders [7–13] (Fig. 1).

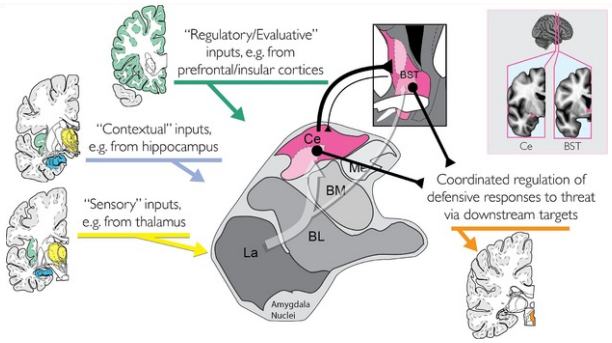

Fig. 1 The central extended amygdala helps organize defensive responses to threat. Simplified schematic of key inputs and outputs to the central extended amygdala (*magenta*) in humans and other primates. The central extended amygdala encompasses th central nucleus of the amygdala (Ce), which lies in the dorsal amygdala, and the bed nucleus of the stria terminalis (BST), which wraps around the anterior commissure. As shown by the translucent white arrow at the center of the figure, much of the sensor (*yellow*), contextual (*blue*), and regulatory (*green*) inputs to the central extended amygdala are indirect (i.e., poly-synaptic), and often first pass through adjacent amygdala nuclei before arriving at the Ce or BST. In primates, projections linking the Ce with the BST are predominantly from the Ce to the BST. The Ce and BST are both poised to orchestrate or trigger momentary negative affect via projections to downstream target regions (orange), such as the periaqueductal grey (PAG). ***Inset:*** Coronal slices depicting the relative locations of the Ce and the BST (*magenta*). Portions of this figure were adapted with permission from [14]. The Ce and BST regions depicted in the inset ~~is~~are described in [] and ([47] R.M. Tillman, M.D. Stockbridge, B.M. Nacewicz, S. Torrisi, A.S. Fox, J.F. Smith and A.J. Shackman, Intrinsic functional connectivity of the central extended amygdala, bioRxiv 2017.)[15]~~.~~, respectively The Ce region depicted in the inset ~~was kindly provided by Dr. Brendon Nacewicz~~. **Abbreviations:** Basolateral (BL), Basomedial (BM), Central (Ce), Lateral (La), and Medial (Me) nuclei of the amygdala; Bed nucleus of the stria terminalis (BST).

alt-text: Fig. 1

Here, we provide an updated ~~mini-~~review of the contributions of the Ce and the BST to fear and anxiety, focusing most heavily on studies ~~in~~of humans and nonhuman primates (for other recent reviews, see [16,17,10,18,19]. This emphasis reflects the fact that anxiety disorders are defined and diagnosed on the basis of subjective symptoms and human studies are essential for understanding the neural mechanisms supporting the experience of fear and anxiety (Please add the following 2 references: Pine, D. S., & LeDoux, J. E. (2017). Elevating the role of subjective experience in the clinic: Response to Fanselow and Pennington. *American Journal of Psychiatry, 174*, 1121-1122. || Zoellner, L. A., & Foa, E. B. (2016). Applying Research Domain Criteria (RDoC) to the study of fear and anxiety: A critical comment. *Psychophysiology, 53*, 332-335.). Human studies are also crucial for identifying the features of animal models that are conserved and, hence, most relevant to understanding human disease and to developing improved interventions for human suffering ('forward translation;' [20,21]. Finally, human studies afford important opportunities for developing objective biomarkers of disease or disease risk [22]—accelerating the development of new diagnostic and treatment strategies [23–25]—and for generating novel hypotheses that can be mechanistically assessed in animal models ('reverse translation;' [26]. Work in monkeys can be conceptualized as a bridge, one that links the precise mechanistic studies that can most readily be performed in rodents to the complexities of human feelings and human disease. The brains of monkeys and humans are genetically, anatomically, and functionally similar, reflecting the two species relatively recent evolutionary divergence (25 million years ago for monkeys vs. 70 million years ago for rodents; [27–30]. Homologous biological substrates, including a well-developed prefrontal cortex (PFC), endow monkeys and humans with a shared repertoire of complex socio-emotional responses to potential threat [7,31], increasing the likelihood of successful translation to human disease [32,33].

1 Anatomy of the ~~Central Extended A~~central extended amygdala

The extended amygdala encompasses a heterogeneous collection of subcortical nuclei along the borders of the amygdala and the ventral striatum. Classical studies of structural connectivity first suggested that the central division of the extended amygdala—including the Ce, lateral BST (BSTL), and portions of the sublenticular extended amygdala (SLEA; a neuronal bridge nestled within the substantia innominata)—represents an integrative unit [34]. Indeed, it has long been recognized that the amygdala is connected to the BST via two major fiber bundles: the ventral amygdalofugal pathway (VA; sometimes termed the *ansa peduncularis*) and the stria terminalis (ST) [35] (Fig. 2a). More recent studies in monkeys have confirmed that the Ce and BSTL are structurally interconnected via these two direct pathways (primarily Ce → BSTL) and have identified a novel indirect pathway centered on the SLEA (Ce ↔ SLEA ↔ BSTL) [40,41] (Please add: Fudge, J. L., Kelly, E. A., Pal, R., Bedont, J. L., Park, L., & Ho, B. (2017). Beyond the classic VTA: Extended amygdala projections to DA-striatal paths in the primate. *Neuropsychopharmacology, 42*, 1563-1576.). In parallel, magnetic resonance imaging (MRI) studies have revealed evidence of both structural [42–44] and functional connectivity between the Ce and BST [42,20]; Gorka et al., in press; [46,41,47,48], reinforcing the hypothesis that they represent a functionally meaningful circuit [34,8].

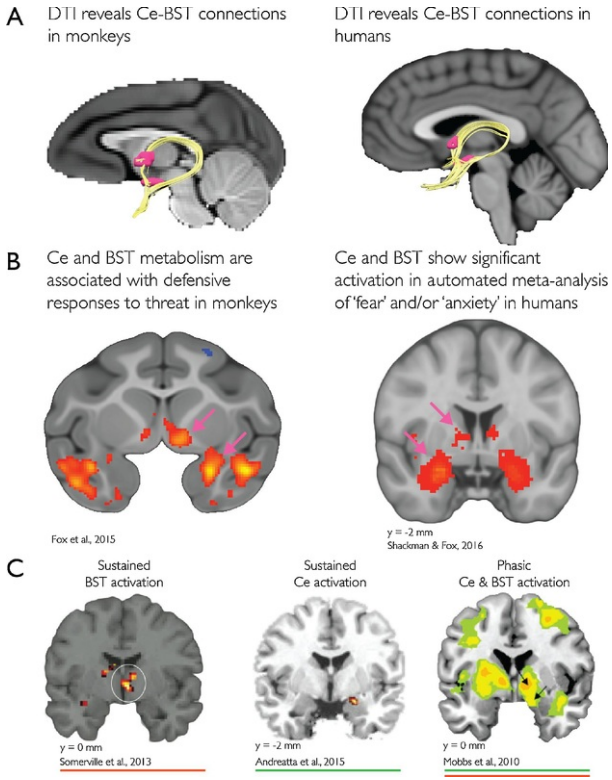

Fig. 2 Imaging the central extended amydala in nonhuman primates and humans. (A) *Diffusion tensor imaging (DTI).* Deterministic DTI reveals the evolutionarily conserved white matter pathways (*yellow*) that link the Ce (*magenta*) to the BST (*magenta*) in monkeys (*left*) and humans (*right*). Images kindly provided by Do Tromp. (B) *Functional neuroimaging of fear and anxiety.* In monkeys (*left*), metabolic activity in the Ce and the BST (*magenta arrows*) is associated with heightened behavioral and neuroendocrine responses during prolonged (30 min) exposure to a potentially threatening human intruder's profile (n = 592). Likewise, an automated ~~*meta*~~*meta*-analysis of human imaging studies (*right*) reveals activation in the BST and the Ce during studies of 'fear' and/or 'anxiety.' Figure depicts the minimum conjunction (logical 'AND') of thresholded forward-inference maps ($q < 0.01$) automatically generated using Neurosynth [36] for studies tagged with the keywords 'fear' (298 studies) and/or 'anxiety' (312 studies). Given the strengths and limitations of automated ~~*meta*~~*meta*-an (meta should not be italicized)alysis; (please insert the following 3 references just prior to the semi-colon: Muller, V. I., Cieslik, E. C., Laird, A. R., Fox, P. T., Radua, J., Mataix-Cols, D., . . . Eickhoff, S. B. (in press). Ten simple rules for neuroimaging meta-analysis. Neuroscience and Biobehavioral Reviews. ||| Salimi-Khorshidi, G., Smith, S. M., Keltner, J. R., Wager, T. D., & Nichols, T. E. (2009). Meta-analysis of neuroimaging data: a comparison of image-based and coordinate-based pooling of studies. Neuroimage, 45, 810-823. ||| Wager, T. D., Atlas, L. Y., Botvinick, M. M., Chang, L. J., Coghill, R. C., Davis, K. D., . . . Yarkoni, T. (2016). Pain in the ACC? Proceedings of the National Academy of Sciences of the United States of America, 113, E2474-2475.) this finding simply indicates that these regions are routinely recruited by a variety of potentially threat-relevant cues. It does not indicate that these regions are consistently co-activated in particular studies and it does not imply that these regions are similarly responsive to particular kinds of threat (e.g.; uncertain or diffuse danger). (C) *The Ce and the BST show phasic and sustained responses to potentially threat-related cues in human imaging studies. Left:* The BST and neighboring regions (*white ring*) show sustained (< 118-s) activation during the uncertain anticipation of aversive images. *Middle:* The Ce shows sustained activation (30-s) during exposure to a virtual reality context paired with unpredictable electric shock. *Right:* The Ce and the BST (*black arrows*) both show phasic responses to video clips of an approaching tarantula (4-s). Portions of this figure were adapted with permission from [37,8,9,38,12,13,39].

alt-text: Fig. 2

From an anatomical perspective, the central extended amygdala is poised to integrate potentially threat-relevant information and assemble states of fear and anxiety. Invasive tracing studies in rodents and monkeys show that the Ce and the BST receive direct and indirect projections from brain regions that encode sensory, contextual, and regulatory information [49] (Fig. 1). Both regions are poised to trigger somatomotor and neuroendocrine signs of fear and anxiety via dense mono- and poly-synaptic projections to brainstem and subcortical effector regions [9,49] (please add: Fudge, J. L., Kelly, E. A., Pal, R., Bedont, J. L., Park, L., & Ho, B. (2017). Beyond the classic VTA: Extended amygdala projections to DA-striatal paths in the primate. *Neuropsychopharmacology, 42*, 1563-1576.
) (Fig. 1). Leveraging the increased anatomical resolution afforded by ultra-high field strength functional MRI (7 T~~esla~~), human studies indicate that many of these downstream regions show robust functional connectivity with the Ce and the BST Gorka et al., in press; [48]. Other work shows that the Ce and BST contain cells with similar architectonic and neurochemical features and that the two regions show similar patterns of gene expression (for a detailed

review, see [8](there should be a parenthesis after the reference: [8])). Collectively, these anatomical observations suggest that the Ce and the BST represent an evolutionarily conserved, functionally coherent circuit that is poised to use information about threat, context, and internal states to initiate a range of defensive responses.

2 Physiology of the ~~Central Extended A~~central extended amygdala

Studies of nonhuman primates afford an important opportunity to obtain concurrent measures of naturalistic defensive behaviors, neuroendocrine activity, and brain metabolism in response to a range of ethologically relevant threats, including explicit cues (i.e., an unfamiliar human intruder's profile) and more diffuse contexts (i.e., a novel testing cage) [7,10,31]. Using a combination of 18-fluorodeoxyglucose-positron emission tomography (FDG-PET) and well-established behavioral assays, we have demonstrated that the Ce and BST are exquisitely sensitive to potential danger. In studies including between 238 and 592 monkeys, elevated levels of metabolic activity in the Ce and BST are associated with heightened signs of fear and anxiety (e.g., freezing, cortisol) during sustained (30-min) exposure to intruder threat [9,50] (Fig. 2b). Heightened metabolic activity in the Ce and BST is also associated with elevated defensive responses during sustained exposure to an unfamiliar testing cage (i.e., in the absence of intruder threat; [51,52].

Consistent with work in monkeys, imaging research in humans suggests that the Ce and BST are both engaged by a broad range of threat-related cues and contexts. The amygdala responds to a variety of threat-related stimuli [53–57][3] and work using high-resolution fMRI indicates that the dorsal region of the amygdala in the ~~vicinity~~region of the Ce is particularly sensitive to aversive visual stimuli [58]. Increased activation in the dorsal amygdala is, in turn, associated with elevated signs and symptoms of arousal in response to acute threat (e.g., Pavlovian threat cues; [59–65]. Likewise, multi-voxel classifier analyses suggest that the dorsal amygdala is an important component of a larger circuit that underlies negative affect elicited by aversive images [66].

Like the Ce, the BST is recruited by a variety of potentially threat-relevant cues in humans, including emotional faces Sladky et al., in press). In fact, as shown in Fig. 2b and described in more detail in the accompanying caption, an automated ~~*meta*~~*meta* *('meta' should NOT be capitalized)*-analysis generated using Neurosynth [36] reveals that studies tagged with the keywords 'fear' and/or 'anxiety' consistently reveal activation in the vicinity of the Ce and the BST, although the latter region is rarely labeled as such for a variety of reasons (e.g., omission from automated anatomical labeling tools; [8,9,19](There is an extra reference inserted here. The 2015 PNAS paper should NOT be included).[4]~~.~~ Like the Ce, BST activation and functional connectivity co-vary with threat-elicited changes in peripheral physiology and self-reported fear and anxiety [68–70,39].

Among researchers focused on humans, it is widely believed that the Ce and BST are functionally dissociable (for critical reviews, see [19,13]. Inspired by an earlier generation of lesion and inactivation studies in rodents [71], this hypothesis suggests that the Ce, or the amygdala more generally, rapidly assembles phasic responses to clear-and-immediate threats (e.g., a cue associated with the imminent delivery of shock), whereas the BST comes on-line more slowly and is responsible for orchestrating sustained responses to dangers that are diffuse, uncertain, or remote. This hypothesis has been adopted with minor modifications by many investigators and commentators and has even been incorporated into the National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) as Acute Threat ('Fear') and Potential Threat ('Anxiety') (https://www.nimh.nih.gov/research-priorities/rdoc/constructs/acute-threat-fear.shtml;https://www.nimh.nih.gov/research-priorities/rdoc/constructs/potential-threat-anxiety.shtml;https://www.nimh.nih.gov/research-priorities/rdoc/negative-valence-systems-workshop-proceedings.shtml).(spaces should be inserted after each of the semi-colons)

Consistent with the 'double-dissociation' or 'strict-segregation' model, several human imaging studies have demonstrated that the BST shows persistent hemodynamic responses during the uncertain anticipation of noxious stimuli, such as shock or aversive images, whereas the dorsal amygdala shows transient responses that are time-locked to the onset of the threat-anticipation period or the actual delivery of the noxious stimulus7 [72–75]; Klumpers et al., in press(Klumpers, F., Kroes, M. C. W., Baas, J., & Fernandez, G. (2017). How human amygdala and bed nucleus of the stria terminalis may drive distinct defensive responses. *Journal of Neuroscience, 37*, 9645-9656.); [70,39]. In one of the more compelling examples, Somerville and colleagues presented either aversive or neutral images (3~~-sec~~ s) in relatively long blocks (118~~-sec~~ s) where the timing of image presentations was either certain or uncertain (Fig. 2c). These unique design features are important because they afford a crucial opportunity to double-dissociate *phasic* (to 3~~-sec~~ s certain threat) from *sustained* (i.e., to 118~~-sec~~ s uncertain threat) responses in the same individuals. Analyses revealed transient activation in the amygdala in response to the negative images. In contrast, the BST showed persistent activation for negative-vs.-neutral blocks and for uncertain-vs.-certain blocks. Moreover, the level of sustained activation in the BST closely tracked mean differences in self-reported fear and anxiety across the four blocked conditions (i.e., uncertain-negative > certain-negative > uncertain-neutral > certain-neutral). Despite some important limitations (e.g., perceptual confounds, failing to test the Region × Condition interaction), these results are often taken as strong support for the 'strict-segregation' model.

On the other hand, a growing number of imaging studies are difficult to reconcile with the hypothesis of strict functional segregation based on threat uncertainty or duration (Fig. 2c). Several studies have reported increased amygdala activation during the anticipation of uncertain threat, both in children [77] and in adults [37,78]. For example, Andreatta and colleagues observed sustained activation—confirmed using a finite impulse response approach—in the region of the right Ce during exposure to a virtual-reality context (30~~-sec~~ s) paired with unpredictable electric shocks. Leveraging a game-like 'virtual predator' paradigm, Mobbs and colleagues observed significantly greater activation in the dorsal amygdala when the predator was first encountered and presented no immediate danger, relative to a subsequent period when shock delivery was imminent and signs and symptoms of fear and anxiety were maximal [79], which runs counter to the idea that t~~at th~~e amygdala is primarily responsible for organizing transient responses to ~~immediate~~acute danger. Herry and colleagues observedpersistently elevated amygdala activity in humans (60-s) and mice (120-s) exposedto a temporally unpredictable, anxiogenic train of auditory stimuli []. (Herry, C., Bach, D. R., Esposito, F., Di Salle, F., Perrig, W. J., Scheffler, K., . . . Seifritz, E. (2007). Processing of temporal unpredictability

in human and animal amygdala. *Journal of Neuroscience, 27*, 5958-5966.
)Other (please insert a new paragraph break here, just before the word 'Other')work has revealed phasic responses in the region of the BST to brief threats, such as a 4-sec s video clip of an approaching tarantula [80,74,38,81]. Likewise, Brinkmann and colleagues very recently demonstrated that the CeBST and the BSTCe show statistically indistinguishable responses to briefly presented (800 ms) aversive images Brinkmann et al., under review/personal communication 7/20/2017(Brinkmann, L., Buff, C., Feldker, K., Neumeister, P., Heitmann, C. Y., Hofmann, D., . . . Straube, T. (2018). Inter-individual differences in trait anxiety shape the functional connectivity between the bed nucleus of the stria terminalis and the amygdala during brief threat processing. *Neuroimage, 166*, 110-116.)). The latter result is particularly compelling given the relatively large sample (n = 93) and formal test of the Region × Condition interaction. It implies that the magnitude of regional differences (i.e., Ce vs. BST) is much smaller than implied by the strict segregation hypothesis, conditional on unknown moderators, or is simply non-existent, at least for briefly presented aversive images. Another, relatively large imaging study (n = 168) reported phasic activation of the BST in response to 4-sec s shock-predictive cues [83], indicating that the BST is sensitive to certain threat. Other recent work suggests that the BST is maximally engaged when shock-threat is psychologically imminent [84]. These imaging observations are broadly consistent with evidence from recording and loss-of-function studies in rodents indicating that a substantial proportion of BST neurons exhibit short-latency responses during exposure to both acute threat (e.g., foot- or tail-shock) and diffusely threatening environments [85,17,86].

On balance, the brain imaging literature suggests that the Ce and BST, while certainly not interchangeable, are more alike than different. In addition to the anatomical similarities described in the previous section (e.g., connectivity, gene expression), both regions respond to a broad spectrum of threat-related cues and contexts and both are correlated with concurrent changes in physiology and subjective experience. Variation in the strength of functional connectivity between the two regions has been associated with individual differences in dispositional anxiety in humans [] and monkeys [Fox, Oler, Birn, Shackman, Alexander & Kalin, *unpublished observations*]. (the 1st reference [] is:
Brinkmann, L., Buff, C., Feldker, K., Neumeister, P., Heitmann, C. Y., Hofmann, D., . . . Straube, T. (2018). Inter-individual differences in trait anxiety shape the functional connectivity between the bed nucleus of the stria terminalis and the amygdala during brief threat processing. *Neuroimage, 166*, 110-116.)In humans, the Ce and the BST both show transient responses to clear-and-immediate threat and sustained activation in contexts associated with uncertain, longer-lasting threat. Both regions show heightened activation in patients with anxiety disorders and individuals at risk for developing such disorders Brinkmann et al., in press;(Brinkmann, L., Buff, C., Feldker, K., Tupak, S. V., Becker, M. P. I., Herrmann, M. J., & Straube, T. (2017). Distinct phasic and sustained brain responses and connectivity of amygdala and bed nucleus of the stria terminalis during threat anticipation in panic disorder. *Psychological Medicine, 47*, 2675-2688.) [73]; Buff et al., in press (Buff, C., Brinkmann, L., Bruchmann, M., Becker, M. P. I., Tupaka, S., Herrmann, M. J., & Straube, T. (2017). Activity alterations in the bed nucleus of the stria terminalis and amygdala during threat anticipation in Generalized Anxiety Disorder. *Social Cognitive and Affective Neuroscience, 12*, 1766-1774.); [89,90,12,91–93], although the studies to date have been small in size, have frequently relied on data acquisition and processing techniques that are less than optimal for resolving subtle differences in regional function (e.g., linear spatial normalization algorithms, large smoothing kernels), and prospective longitudinal data are mostly lacking. In monkeys, individuals expressing more intense signs of fear and anxiety show increased FDG metabolism in the Ce and BST during sustained exposure to novel contexts and potential threat. Although FDG-PET lacks the temporal resolution needed to cleanly dissociate phasic from sustained neural responses, work in monkeys hints at some potential differences between the two regions—activity in the BST is associated with heritable individual differences in fear and anxiety? [8,9] and the BST appears to be involved in organizing persistently elevated signs of fear and anxiety following threat exposure (i.e., mood 'spillover;' [94]. Nevertheless, the critical tests of regional differences have yet to be performed in monkeys (e.g., Region × Condition; Fox et al., in press (Fox, A. S., Lapate, R. C., Davidson, R. J., & Shackman, A. J. (2018). The nature of emotion: A research agenda for the 21st century. In A. S. Fox, R. C. Lapate, A. J. Shackman, & R. J. Davidson (Eds.), *The nature of emotion. Fundamental questions* (2nd ed.). New York: Oxford University Press.); [19]. The upshot of this work is that the available imaging literature provides, at best, mixed evidence for claims—including those embodied in RDoC—of strict functional segregation between the Ce and the BST on the basis of threat uncertainty or duration (i.e., '*the Ce mediates phasic responses to clear-and-imminent danger; the BST mediates sustained responses to uncertain threat*')—a conclusion that echoes that reached by Gungor and Paré on the basis of mechanistic work in rodents [86].

Understanding the neurobiology of human fear and anxiety is important, both conceptually and clinically. As reviewed elsewhere Fox et al., in press (Fox, A. S., Lapate, R. C., Davidson, R. J., & Shackman, A. J. (2018). The nature of emotion: A research agenda for the 21st century. In A. S. Fox, R. C. Lapate, A. J. Shackman, & R. J. Davidson (Eds.), *The nature of emotion. Fundamental questions* (2nd ed.). New York: Oxford University Press.); [8,9,19], drawing strong inference about the neural circuits supporting phasic and sustained responses to different dimensions of threat requires the use of well-matched tasks. Parametric manipulations of threat probability (*if* threat will occur), imminence (*when* or *where* it will occur), and duration (as in [96,84,79,97,38] would be particularly useful. The use of dynamic parametric tasks (e.g., where threat imminence or probability is smoothly and continuously varied) would also afford powerful new opportunities for understanding the kinds of uncertainty most relevant to fear and anxiety and for identifying circuits involved in triggering behavioral and physiological 'phase transitions' (e.g., from vigilance to behavioral inhibition to active defense; [98]; [99]. Putative double dissociations need to be rigorously assessed using the appropriate Region × Condition interaction (as in [73,100], preferably in adequately powered samples [101–103]. Absent that, claims of anatomical dissociation are unwarranted. Likewise, concluding that a particular brain region is 'not involved' in a complex, multidimensional psychological function, like 'fear' or 'anxiety,' based on a null statistical test or a single assay is unwarranted. Given mounting evidence that fear and anxiety, like other emotions, reflect widely distributed neural circuits (e.g(The other 'uncited' reference is supposed to be included in this block of references: Shackman, A. J., Fox, A. S., & Seminowicz, D. A. (2015). The cognitive-emotional brain: Opportunities and challenges for understanding neuropsychiatric disorders. Behavioral and Brain Sciences, 38, e86.)., [104]; Nummenmaa and Saarimaki in press; [106]; Shackman and Fox, in press (Shackman, A. J., & Fox, A. S. (2018). How are emotions organized in the brain? In A. S. Fox, R. C. Lapate, A. J. Shackman, & R. J. Davidson (Eds.), *The nature of emotion. Fundamental questions* (2nd ed.). New York, NY: Oxford University Press.); [108], one of the most important challenges for future research will be to extend models of fear and anxiety to encompass interactions between the central extended amygdala and distal regions of the brain, a point that we discuss in more detail in the final section.

3 Mechanistic ~~Studies of the Central Extended A~~studies of the central extended amygdala

There is ample evidence ~~that~~ that the Ce and the BST are critical for assembling states of fear and anxiety. Summarizing the data available nearly a decade ago, just prior to the widespread adoption of high-precision optogenetic and chemogenetic techniques, Davis and colleagues outlined a 'partial-dissociation' model, hypothesizing that the Ce plays a critical role in organizing both immediate and longer-lasting responses to threat [85]. This model suggests that phasic responses are mediated by circuits coursing from the basolateral amygdala (BL) to the medial division of the Ce (CeM) and from there to downstream effector regions. In contrast, responses to more persistent kinds of danger—those that are uncertain, psychologically diffuse, or remote in time in time or space—were thought to be mediated by circuits passing from the lateral division of the Ce (CeL) to the lateral division of the BST (BSTL) and, ultimately, to effector regions.

More recent work in rodent models has refined our understanding of the brain bases of fear and anxiety (e.g.,[109,110], while highlighting the anatomical and functional complexity of the central extended amygdala (e.g., [111,86,112,113]. It has become abundantly clear that the Ce and the BST, like many other brain regions, harbor a variety of distinct cell 'types'—groups of neurons that can be distinguished based on protein expression, firing characteristics, connectivity, and other features—and that different cell types within the same region of the central extended amygdala (e.g., Ce) are functionally dissociable (e.g., [112,113]. Some of these neurons are response-specific, while others are threat-specific. For example, PAG-projecting cells in the CeM trigger freezing, whereas an independent, but anatomically intermingled, set of medulla-projecting neurons trigger changes in cardiovascular activity [113]. These response-specific neurons can be activated by different threat-specific neurons. For example, serotonin receptor 2a-expressing neurons in the CeL play a key role in regulating the competition between innate and learned defensive responses: *amplifying* freezing elicited by sustained exposure (10-min) to innate threat (i.e. an odor derived from fox feces) and *attenuating* freezing to learned threat (i.e., a neutral odor associated with foot-shock) [114]. These kinds of observations underscore the conceptual importance of understanding how different cell types in the central extended amygdala contribute to fearful and anxi~~ety~~ous states and traits.

Despite this complexity, mechanistic work in rodents reinforces the general conclusion that the microcircuits responsible for assembling phasic and sustained responses to threat overlap in the central extended amygdala~~3~~ [115,86,116]. For example, the Ce and the BST ~~have~~are both ~~been shown to be~~ important for assembling sustained responses to diffuse threat~~10~~ (e.g., [117–121,112]; Mazzone et al., in press; [123–125]. Projections from the BL to the Ce exert bi-directional control over defensive responses to the elevated-plus maze (EPM) and open-field test, which can be considered diffusely threatening contexts [126]; chemical inactivation of the Ce reduces defensive responses to the elevated-plus maze [127]; and CRF-expressing neurons in the Ce modulate conditioned freezing to threatening contexts and longer-lasting (30- s) threat cues Asok et al., in press; [129]. With regard to the BST, serotonergic projections from the dorsal raphe to the BST modulate the recall of conditioned defensive responses to both contextual and cued threats [130]. ~~Moreover, w~~Work using temporally unpredictable shock paradigms demonstrates that cannabinoid projections from the BL and the Ce to the BST are necessary for sustained defensive responses in response to uncertain danger [131]. This observation, which harnesses a task adapted from that ~~of~~developed by Davis, Walker, and colleagues [132–134], provides important evidence that the BL, Ce, and BST all play a role in responding to uncertain or diffuse threat, consistent with other recent work (e.g., [135–137]. While our understanding remains far from complete, taken together, these observations show that specific microcircuits within and between the Ce and the BST are important for orchestrating defensive responses to a variety of threats.

Although the causal contribution of the BST to fear and anxiety has yet to be explored in humans or other primates, monkeys with fiber-sparing excitotoxic lesions of the Ce show a marked reduction in defensive behaviors and endocrine activity during sustained exposure to human intruder threat and during acute exposure to a live snake [10,138,31]. Likewise, humans with amygdala damage exhibit a profound lack of fear and anxiety in response to both diffusely threatening contexts (e.g., walking through a haunted house, foraging in the presence of uncertain threat) and acute threat (e.g., spiders, snakes, conditioned threat cues) [139–141](A reference is missing and should be added. This is one of the 'uncited references' identified by the copy editor. Feinstein, J. S., Adolphs, R., & Tranel, D. (2016). A tale of survival from the world of Patient S.M. In D. G. Amaral & R. Adolphs (Eds.), *Living without an amygdala*. New York: Guilford.). A major caveat is that such deficits may reflect damage to axonal fibers passing through the Ce *en route* to other regions, such as the BST, or more subtle kinds of long-range functional disconnection [142], a point that we take up more fully in the next section.

4 Closing the ~~Gap between Mechanistic and Imaging R~~gap between mechanistic and imaging research

The Ce and the BST are anatomically complex and can be partitioned into multiple sub-regions (Fig. 1), each containing a variety of intermingled cell types [8,86]. Although unfamiliar to many brain imagers, recently developed opto- and chemogenetic tools provide numerous opportunities for deciphering this complexity and identifying the specific circuit components that control defensive responses to threat [143–147]. Developing a basic understanding of these methods is a key step to dissolving artificial academic 'silos' and developing more thoughtful hypotheses about the role of the central extended amygdala in human emotion and emotional disorders. Typically, a DNA vector encoding a target ligand or receptor is engineered into a virus. The virus is injected into the brain, inducing expression of the target protein in the infected region (e.g., BST). Regional and cell-type specificity is achieved using recombinase-dependent viruses or cell type-specific promoter viruses. For example, a virus containing a Cre-dependent vector can be injected into the Ce of transgenic mice engineered to express Cre recombinase in somatostatin-expressing neurons, resulting in selective expression of the targeted receptor protein in somatostatin-expressing neurons in the Ce. More sophisticated approaches enable the inclusion (Boolean AND) or exclusion (Boolean NOT) of cells with specific efferent or afferent projections, specific behavioral profiles (e.g., activated by reward vs. punishment), or combinations of these criteria. By overexpressing receptors that respond to light ('optogenetics') or designer drugs with ~~minimal~~limited off-target effects ('chemogenetics'), it is possible to reversibly activate or silence specific cell populations on demand. The application of these approaches to rodent models of fear and anxiety has revealed a

level of architectural intricacy in the central extended amygdala far beyond what can be resolved using existing neuroimaging techniques, including mutually inhibitory circuits within the Ce that control freezing and fleeing [111,114] and neuronal populations within the BST that can promote or dampen signs of fear and anxiety Garcia-Garcia et al. in press; [112].

These exciting observations raise two very important questions. First, are these mechanisms conserved in humans and other primates? If so, then they are likely to be relevant to our understanding of anxiety disorders and could guide the development of improved treatments [21]. Second, what role do these extended amygdala mechanisms play in the kinds of large-scale brain circuits that have been linked to maladaptive fear and anxiety in humans and monkeys? Which molecules and micro-circuits underlie heightened activation in the central extended amygdala and how do they influence the function (i.e., activity, functional connectivity) of distal regions of the brain implicated in pathological fear and anxiety? Reconciling these two levels of analysis—one global, the other local—is mandatory, if we are to develop a complete and clinically useful understanding of fear and anxiety.

Nonhuman primate research provides a powerful opportunity to combine focal perturbation techniques with whole-brain surveys of brain function and has begun to address some of these fundamental questions. For example, imaging studies in monkeys demonstrate that metabolic activity in the posterior orbitofrontal cortex (OFC)/anterior insula is associated with heightened passive avoidance of threat (i.e., freezing; [8]. Although surgical lesions of the OFC markedly reduce threat-elicited freezing, suggesting a causal role [149,150], neuroimaging measures collected before and after surgery suggest that this anxiolytic effect is proximally mediated by 'downstream' alterations in BST metabolism [151] (Fig. 3a). Reduced BST activity has also been observed in humans with OFC damage [152], suggesting that this circuit is conserved across primate species (Fig. 3b). In more recent work, Kalin, Fox, and colleagues have extended this strategy to gain-of-function studies [153]. Harnessing a viral vector approach, they demonstrated that overexpression of corticotropin-releasing hormone (CRH) in the dorsal amygdala increases defensive behaviors during sustained exposure to potential threat, consistent with work in rodents highlighting the importance of the Ce CRH system for responding to diffusely threatening contexts, such as the elevated plus-maze [154]. These behavioral changes were associated with increased metabolism in the dorsal amygdala and posterior OFC as well as enhanced functional connectivity between the two regions, highlighting the importance of a distributed brain network underlying fear and anxiety (Fig. 4a).

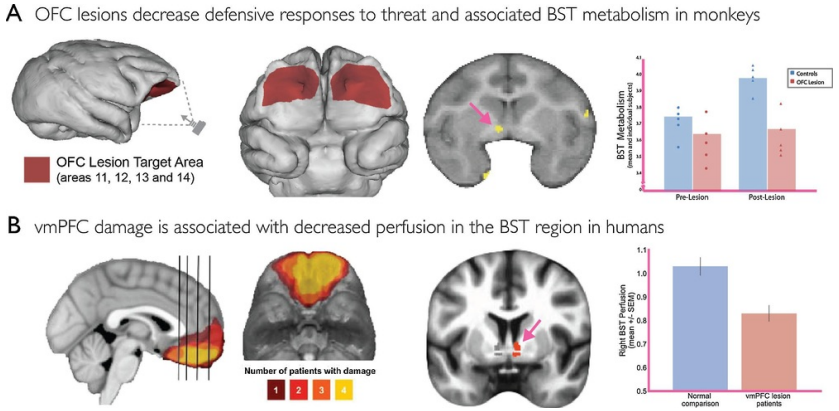

Fig. 3 Focal damage to the ventral PFC is associated with distal changes in BST function. (A) *Monkeys.* Experimental lesions of the OFC reduce threat-elicited freezing (not depicted) and BST metabolism (*magenta arrow*). The orbitofrontal regions targeted by the surgery (*maroon*) can be seen from the lateral (*far left*) and basal views (*middle*). Bar-plot depicts the significant Group × Time interaction for BST metabolism. **(B)** ***Humans.*** Damage to the ventromedial PFC (vmPFC) is associated with reduced perfusion in the BST (*magenta arrow*). The ventromedial regions showing damage can be seen from the mid-sagittal (*far left*) and basal views (*middle*). Bar-plot depicts the significant reduction in right BST perfusion in the patient group. Portions of this figure were adapted with permission from [151,152].

alt-text: Fig. 3

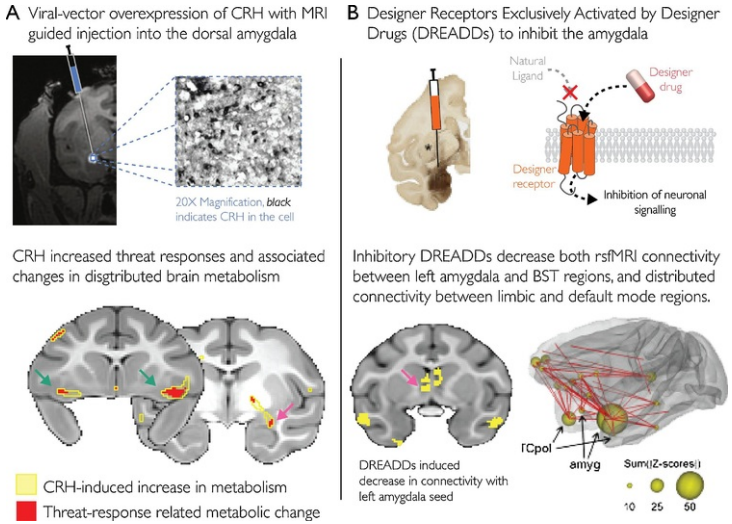

Fig. 4 Nonhuman primate research provides a ~~powerful~~ n opportunity to combine focal manipulations of the amygdala with whole-brain surveys of brain function. (A). *Molecular activation*. Using MRI-guided injections of a viral vector (*upper left*), Kalin, Fox and colleagues overexpressed corticotropin-releasing hormone (CRH) in the dorsal amygdala. MRI image depicts the gadolinium flume (*white*) in the target region. Photomicrograph shows CRH-expressing cells in the same region (*upper right*). CRH overexpression in the amygdala enhanced threat-elicited defensive responses (not shown) and increased metabolism (*yellow clusters*) in the dorsal amygdala (*magenta arrow*) and the posterior OFC (*green arrows*). CRH-induced increases in defensive responses and metabolism were correlated in both regions (*red clusters*). (B) *Chemogenetic inhibition*. Leveraging a chemogenetic approach, Grayson and colleagues reversibly inhibited the amygdala while using fMRI to assess intrinsic functional connectivity across the brain. A viral vector encoding an inhibitory designer receptor exclusively activated by a designer drug with minimal off-target effects (DREADD) was injected into the amygdala (*upper left*). Systemic administration of the designer drug reversibly inactivated the amygdala (*upper right*). DREADDs-mediated inhibition of the amygdala was associated with decreased amygdala-BST connectivity (*magenta arrow*), decreased amygdala-OFC connectivity, and increased corticocortical coupling (*lower panels*). In the coronal section, clusters (*yellow*) depict the minimum conjunction (logical 'AND') of regions significant for four designer-drug vs. vehicle contrasts that were made available by Grayson and colleagues on the publisher's website. Portions of this figure were adapted with permission from [155,153]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) (should this last comment be added to the other color images???)

alt-text: Fig. 4

Other recent work demonstrates the feasibility of using opto- and chemogenetic approaches in nonhuman primates (e.g., [156–161]—including cell-type specific perturbations in wild-type (i.e., genetically unmodified) monkeys [162]—and highlights the value of combining mechanistic interventions and cellular recordings with whole-brain imaging techniques Mazzone et al., in press; [163]; Park et al., in press (Park, S. H., Russ, B. E., McMahon, D. B. T., Koyano, K. W., Berman, R. A., & Leopold, D. A. (2017). Functional subpopulations of neurons in a macaque face patch revealed by single-unit fMRI mapping. *Neuron, 95*, 971-981.
); Shiba et al., in press (Shiba, Y., Oikonomidis, L., Sawiak, S., Fryer, T., Hong, Y. T., Cockcroft, G., . . . Roberts, A. C. (2017). Converging prefronto-insula-amygdala pathways in negative emotion regulation in marmoset monkeys. *Biological Psychiatry, 82*, 895-903.
)). In a landmark study, Grayson and colleagues showed that transient chemogenetic inactivation of the amygdala produces widespread alterations in intrinsic functional connectivity, including decreased amygdala-BST connectivity, decreased amygdala-OFC connectivity, and increases in corticocortical coupling [155] (Fig. 4b). This finding is consistent with work in rodents [26,166] and neurological patients [167–169] demonstrating that the behavioral consequences of focal brain damage can emerge from physiological alterations in distal brain regions (for a related perspective, see [106]. (a parenthesis should be added [106]).) These findings highlight the importance of a distributed circuit centered on, but not limited to, the central extended amygdala and they underscore the added value of combining the focal perturbation strategies that are widely used in rodent studies with the whole-brain imaging techniques that are routinely used in basic and clinical research in humans.

5 Conclusions

The central extended amygdala plays a crucial role in evaluating and responding to a broad spectrum of threat-related cues and contexts. While they are certainly not interchangeable, the Ce and the BST show similar patterns of connectivity, cellular composition, neurochemistry, and gene expression. Both are sensitive to uncertain or temporally remote threat; both co-vary with threat-elicited changes in behavior, physiology, and experience; both show phasic responses to acute threat; and both show heightened activity during sustained exposure to diffusely threatening contexts. Work in rodents indicates that both regions play a critical role in organizing sustained defensive responses to a range of potentially threatening cues and contexts. More generally, studies leveraging opto- and chemogenetic techniques have begun to reveal the specific molecules, cells, and microcircuits within the central extended

amygdala that support signs of fear and anxiety in rats and mice. A major challenge is to understand the relevance of these discoveries to human experience and human disease. Recent work in nonhuman primates provides a bridge to addressing this fundamental issue. Using a combination of focal perturbations and whole-brain imaging, this new generation of nonhuman primate research sets the stage for discovering the mechanisms within the central extended amygdala that underlie neuroimaging metrics linked to extreme fear and anxiety in humans; for understanding how the Ce and BST functionally interact with one another and with remote regions of the brain, such as the OFC; and ultimately for accelerating the development of improved strategies for diagnosing, treating, and preventing pathological fear and anxiety.

Conflict of interest

Authors declare no conflicts of interest.

Uncited references

[170] and [171].

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Footnotes

2It has become increasingly common to draw a distinction between ‘fear’ and ‘anxiety’ (e.g., [172]. Yet lay people, scholars in other areas, the American Psychiatric Association’s *Diagnostic and Statistical Manual* [173], and even domair experts often use these terms in interchangeable, inconsistent, or overly inclusive ways Kagan, in press; [12,13,175]. To avoid misunderstanding, we have adopted the undifferentiated term ‘fear and anxiety’ (for a more detailed discussion of nomenclature, see Fox et al., in press;(Fox, A. S., Lapate, R. C., Davidson, R. J., & Shackman, A. J. (2018). The nature of emotion: A research agenda for the 21st century. In A. S. Fox, R. C. Lapate, A. J. Shackman, & R. J. Davidson (Eds.), *The nature of emotion. Fundamental questions* (2nd ed.). New York: Oxford University Press.) [19]. Understanding the neurobiology of fear and anxiety is both theoretically and clinically important and requires that we determine how the Ce, the BST, and other brain regions work together to evaluate and respond to different kinds of threat. We urge other researchers to eschew potentially problematic redefinitions of everyday language and, instead, focus on the specific parameters of the threat, the context in which it occurs (e.g., prospects for escape), and the neurobehavioral response (e.g., time course), including subjective experience.

3Interestingly, the amygdala is not consistently recruited by conditioned threat cues in human fMRI studies [176,177], contrary to electrophysiological and mechanistic work in rodents, monkeys, and humans [178,139,116]. In addition,

several groups have reported ‘de-activation’ of the amygdala in a variety of aversive paradigms [80,179,180,70,84,79,181–183]. The mechanisms underlying these effects remain enigmatic.

4Although automated anatomical labeling tools do not yet include the BST, probabilistic masks are now available for the supracapsular portion [15,48], as shown in Fig. 1. It can also be helpful to assess whether provisional BST clusters lie *outside* of neighboring regions incorporated in probabilistic atlases (a Boolean NOT with nucleus accumbens, pallidum, caudate, putamen, thalamus, and ventricles) (Klumpers, Kroes, Baas, & Fernandez, *in press*; [19].

Highlights

- Central extended amygdala (EAc) encompasses Ce and BST.
- EAc is engaged by a range of threat-relevant cues.
- Optogenetic/chemogenetic studies reveal key molecules and microcircuits.
- Next-generation nonhuman primate studies…
- …bridge the gap between mechanistic work in rodents and imaging studies in humans.

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