


Dual Perspectives

Dual Perspectives Companion Paper: Functional Heterogeneity in the Bed Nucleus of the Stria Terminalis, by Nur Zeynep Gungor and Denis Paré

Contributions of the Central Extended Amygdala to Fear and Anxiety

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It is widely thought that phasic and sustained responses to threat reflect dissociable circuits centered on the central nucleus of the amygdala (Ce) and the bed nucleus of the stria terminalis (BST), the two major subdivisions of the central extended amygdala. Early versions of this hypothesis remain highly influential and have been incorporated into the National Institute of Mental Health Research Research Domain Criteria framework. However, new observations encourage a different perspective. Anatomical studies show that the Ce and BST form a tightly interconnected unit, where different kinds of threat-relevant information can be integrated and used to assemble states of fear and anxiety. Imaging studies in humans and monkeys show that the Ce and BST exhibit similar functional profiles. Both regions are sensitive to a range of aversive challenges, including uncertain or temporally remote threat; both covary with concurrent signs and symptoms of fear and anxiety; both show phasic responses to short-lived threat; and both show heightened activity during sustained exposure to diffusely threatening contexts. Mechanistic studies demonstrate that both regions can control the expression of fear and anxiety during sustained exposure to diffuse threat. These observations compel a reconsideration of the central extended amygdala's contributions to fear and anxiety and its role in neuropsychiatric disease.

Key words: affective neuroscience; fear and anxiety; fMRI; mood and anxiety disorders; neuroimaging; nonhuman primates

Introduction

When expressed too intensely or in maladaptive contexts, fear and anxiety can become debilitating (American Psychiatric Association, 2013). Anxiety disorders impose a staggering impact on public health and the global economy (Collins et al., 2011; Whiteford et al., 2013; DiLuca and Olesen, 2014). They are the most common family of neuropsychiatric disorders and contribute to the development of depression and comorbid substance abuse (Kessler et al., 2012). Existing treatments are inconsistently effective or associated with significant adverse effects (Bystritsky, 2006; Insel, 2012; Griebel and Holmes, 2013), underscoring the need to develop a deeper understanding of the neural circuits that control the experience and expression of fear and anxiety in humans.

Studies of rodents, monkeys, and humans demonstrate that the extended amygdala—an anatomical concept encompassing portions of the amygdala and the bed nucleus of the stria terminalis (BST) (Alheid and Heimer, 1988)—plays a crucial role in assembling states of fear and anxiety in response to a broad spectrum of learned and unlearned threats (Calhoun and Tye, 2015; Fox et al., 2015a; Janak and Tye, 2015; Tovote et al., 2015; Gungor and Paré, 2016; Oler et al., 2016a) (Fig. 1). Other work suggests that alterations in the function of this circuit contribute to the development (Fox and Kalin, 2014; McLaughlin et al., 2014; Fox et al., 2015a; Swartz et al., 2015) as well as the maintenance of anxiety and mood disorders in humans (Sheline et al., 2001; Paulus et al., 2005; Etkin and Wager, 2007; Felmingham et al., 2007; Hamilton et al., 2012; Phan et al., 2013). Although this vast literature leaves little doubt that the extended amygdala plays an important role in evaluating and responding to threat, confusion persists about the respective contributions of its major subdivisions.

In a series of thoughtful empirical studies and landmark reviews, Davis, Walker, and Grillon marshalled a wide array of mechanistic, psychophysiological, and clinical data to suggest that phasic and sustained responses to threat are mediated by different parts of the extended amygdala (Davis et al., 1997, 2010; Davis, 1998, 2006; Walker et al., 2003, 2009; Grillon, 2008; Walker and Davis, 2008). In earlier versions of the model, they emphasized a strict functional segregation (Fig. 2a),

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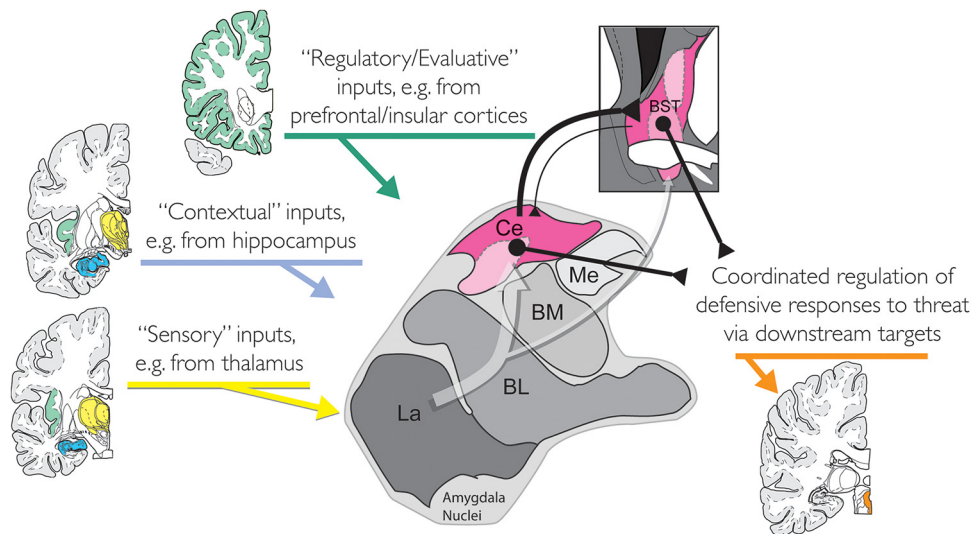


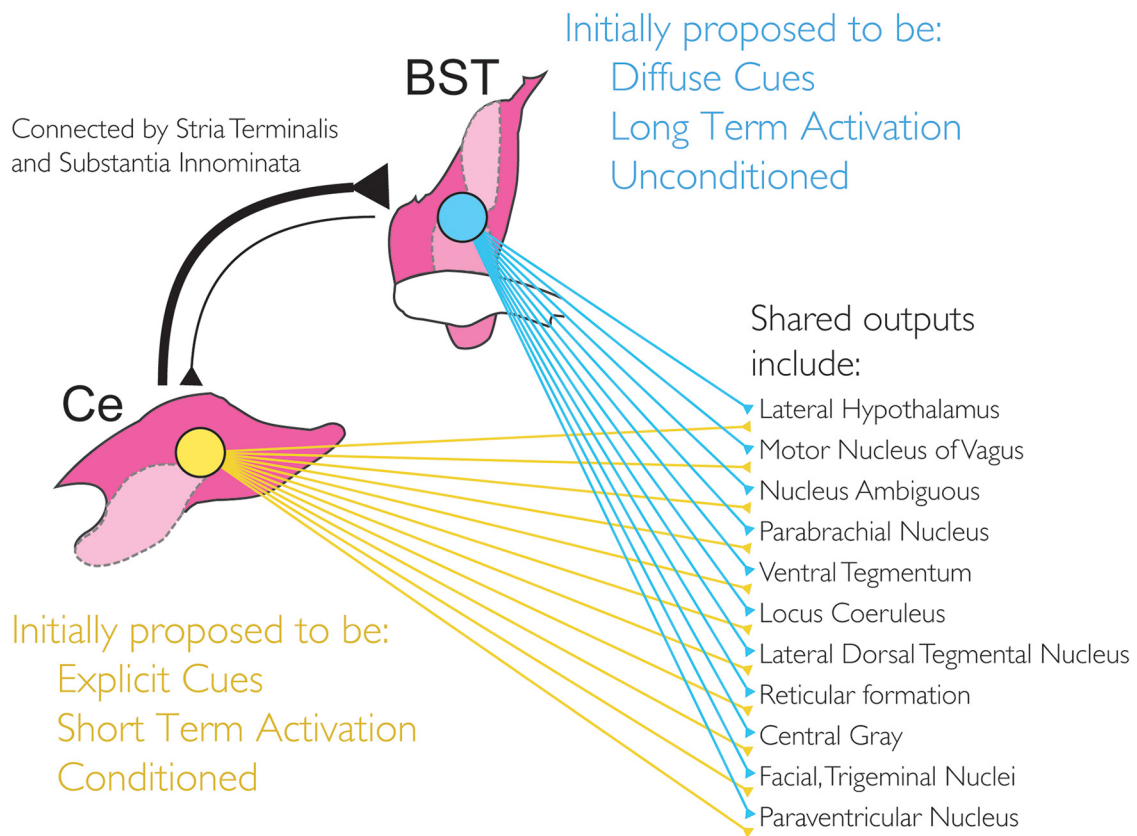
Figure 1. Primate central extended amygdala. Simplified schematic of key central extended amygdala inputs and outputs in humans and other primates. The central extended amygdala encompasses the central (Ce) nucleus of the amygdala, which lies in the dorsal amygdala, and the bed nucleus of the stria terminalis (BST), which wraps around the anterior commissure. As shown by the translucent white arrow at the center of the figure, much of the sensory (yellow), contextual (blue), and regulatory (green) inputs to the central extended amygdala are indirect (i.e., polysynaptic), 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 states of fear and anxiety via projections to downstream effector regions (orange; for additional detail, see Fig. 2). Portions of this figure were adapted with permission from the human brain atlas of Mai et al. (2007). BL, Basolateral; BM, basomedial; Ce, central; La, lateral; Me, medial nuclei of the amygdala.

arguing that the central nucleus of the amygdala (Ce) and BST represent two phenomenologically and anatomically dissociable systems (Davis, 2006). In this early model, a circuit centered on the Ce rapidly assembles short-term responses to explicit threat, such as a light or tone paired with the imminent delivery of shock. In contrast, a circuit centered on the BST comes on-line much more slowly and is responsible for orchestrating longer-lasting responses to novelty and diffuse threat, such as a context previously paired with shock or, in humans, a dark room (Baas et al., 2002). With the accumulation of new data, this hypothesis was revised to allow for a more nuanced division of labor (Davis et al., 2010). In the reformulated model, the Ce contributes to both immediate (“phasic”) and longer-lasting (“sustained”) responses to threat (compare Fig. 1). Phasic responses are mediated by projections originating in the medial division of the Ce (CeM). In contrast, responses to more persistent kinds of danger—those that are uncertain, psychologically diffuse, or remote in time in time or space—are mediated by projections from the lateral division of the Ce (CeL) to the lateral division of the BST (BSTL). In this reformulated model, the BSTL is rapidly engaged, between 4 and 60 s following the onset of threat. Somewhat later, feedback projections from the BSTL inhibit the CeM, enabling a smooth transition from phasic to sustained responses to threat.

Davis and colleagues’ general hypothesis remains highly influential. It has been adopted, wholesale or with minor modifications, by many prominent commentators (Grupe and Nitschke, 2013; LeDoux, 2015; Avery et al., 2016; Lebow and Chen, 2016) and incorporated into the National Institute of Mental Health Research Domain Criteria (RDoC) as Acute Threat (Fear) and Potential Threat (Anxiety). Unfortunately, in RDoC and elsewhere, Davis and colleagues’ hypothesis is often recast as a simple double-dissociation: “the amygdala mediates fear, the BST mediates anxiety” (see also <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>) (Kozak and Cuthbert, 2016), following their earlier model. All too often, it is this simpler axiom, with its corresponding emphasis on strict functional segregation, that serves as the lens through which neurobiological and clinical research in humans is conceptualized, interpreted, and evaluated.

Here, we review the contributions of the Ce and BST to fear and anxiety, focusing most heavily on studies in humans and nonhuman primates. This emphasis reflects the fact that anxiety disorders are defined and diagnosed on the basis of subjective symptoms and studies of humans are essential for understanding the neural mechanisms supporting the experience of fear and anxiety (Anderson and Adolphs, 2014; LeDoux, 2015). Human studies are also crucial for identifying the features of animal models that are conserved and, hence, most relevant to developing improved interventions for human suffering (Birn et al., 2014). Finally, human studies afford important opportunities for developing objective biomarkers of disease or disease risk (Borsook et al., 2006, 2014; Wise and Preston, 2010; Davis et al., 2015) and for generating novel hypotheses that can be mechanistically assessed in animal models (“reverse translation”) (Janak and Tye, 2015; Ferenczi et al., 2016). Work in monkeys, on the other hand, can be conceptualized as a bridge, one which links the elegant mechanistic and recording studies that can routinely be performed in rodents to the complex phenomenology of human feelings and human disease. Monkeys are particularly useful for modeling and understanding the neurobiology of fear and anxiety because humans and monkeys share similar genes and similar brains (Gibbs et al., 2007; Preuss, 2007; Freese and Amaral, 2009), which endow the two species with a common repertoire of complex socio-emotional responses to potential threat and enables the use of similar behavioral assays (Fox and Kalin, 2014; Kaiser and Feng, 2015; Oler et al., 2016a).

a Early working model adapted from Davis et al., 1998



b Examples that are inconsistent with the early Davis model

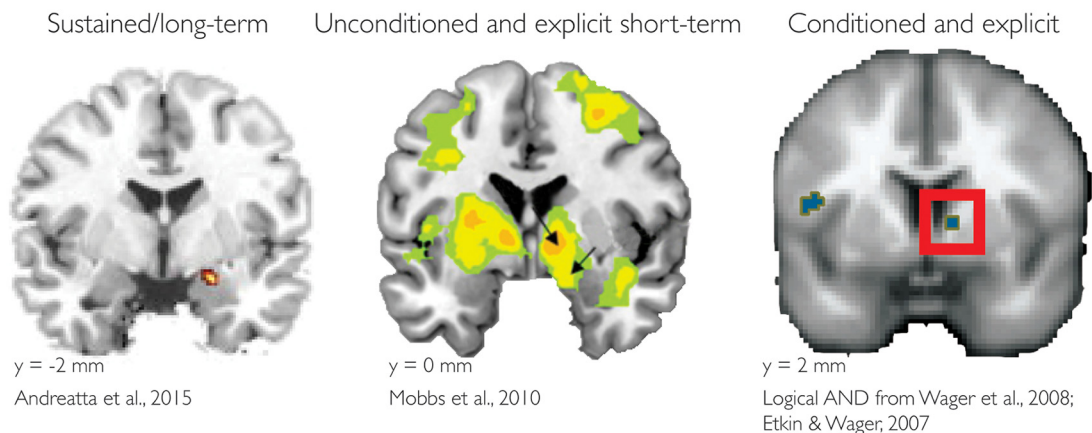


Figure 2. Early model and inconsistent human imaging evidence. **a**, Early model emphasizing strict functional segregation in the extended amygdala. In earlier work, Davis, Walker, and their colleagues noted that the Ce and BST send similar projections to the brainstem and hypothalamic regions that proximally mediate particular elements of fear and anxiety (e.g., tachycardia, startle potentiation). Based on the available evidence, they hypothesized that the Ce and BST reflected dissociable circuits that differentially processed specific types of threat. Although Davis and colleagues subsequently reformulated their hypothesis (Davis et al., 2010), the basic tenets of the 1998 model continue to permeate the literature and NIMH RDoC initiative. This figure is a redrawing of the schematic shown in Davis (1998) and incorporates elements adapted with permission from the human brain atlas of Mai et al. (2007). **b**, Examples of human imaging data inconsistent with the early model of Davis and colleagues. Left, Sustained/long-term activation in the Ce in response to a virtual reality context (30 s) paired with unpredictable electric shocks. Middle, Ce and BST both show phasic/short-term activation in response to an explicit, unconditioned threat (4 s video clips of an approaching tarantula). Right, BST activation in response to explicit, conditioned and unconditioned threats. Figure represents the minimum conjunction (logical “AND”) of thresholded maps ($p < 0.005$) derived from two imaging meta-analyses: one focused on activation associated with the experience of experimentally induced negative affect (Wager et al., 2008) and the other focused on activation elicited by aversive Pavlovian conditioned stimuli (Etkin and Wager, 2007). The two meta-analytic maps are freely available at <http://www.columbia.edu/cu/psychology/tor/MetaAnalysis.htm>. Portions of the bottom are adapted with permission from Mobbs et al., (2010), Andreatta et al. (2015), and Fox et al. (2015a).

We begin our review with a brief comment on the importance of nomenclature for conceptual understanding. We then describe new and classic anatomical evidence suggesting that the Ce and the BST form a tightly integrated circuit that is poised to organize states of fear and anxiety. Next, we highlight recent imaging studies in monkeys and humans showing that, in many regards, the Ce and the BST are more functionally alike than different (Fig. 2*b*). Finally, we review mechanistic data demonstrating that both regions can control defensive behaviors elicited by sustained exposure to diffuse threat. Together, this rapidly accumulating body of observations in humans, monkeys, and rodents refutes longstanding claims of strict phenomenological or anatomical segregation in the extended amygdala. We conclude by outlining a roadmap to the most important avenues and strategies for future research aimed at understanding the contributions of the extended amygdala to fear, anxiety, and neuropsychiatric disease.

The conceptual importance of a precise and consistent vocabulary

The words that we as scientists use to describe nature influence our ability to communicate and to understand, for better or worse (Markon, 2015; Schaafsma et al., 2015; Poldrack and Yarkoni, 2016; Zaki et al., 2016). Establishing the nature and neurobiological bases of fear and anxiety requires that researchers describe both the brain and behavior in a clear, precise, and unambiguous way.

BST versus BNST

Identifying the mechanisms that give rise to differences in the function of the central extended amygdala will ultimately require the synthesis of data acquired from different species, including optogenetic and chemogenetic manipulations in animals and imaging and postmortem gene and transcript mapping studies in humans and monkeys. Linking these disparate datasets requires a standardized vocabulary. Already, all of the major brain atlases for the rat, monkey, and human (Mai et al., 2007; Paxinos et al., 2009; Paxinos and Watson, 2014), including the more recent and comprehensive Allen Brain Atlas (<http://www.brain-map.org>), use the acronym BST to refer to the bed nucleus of the stria terminalis. Therefore, we use this nomenclature throughout our review.

Fear versus anxiety

Inspired by the work of Davis and colleagues (Davis et al., 2010), as well as psychometric analyses of psychiatric symptoms and comorbidity (Kotov et al., 2015; Lang et al., 2016), a growing number of researchers draw a sharp distinction between states of “fear” and “anxiety” (e.g., Barlow, 2000; LeDoux, 2015). Yet lay people, scholars in other areas, the American Psychiatric Association’s Diagnostic and Statistical Manual (American Psychiatric Association, 2013), and even domain experts, at least in unguarded moments, often use these terms interchangeably or inconsistently. As one psychiatrist noted almost 40 years ago, “The word ‘anxiety’ has become confused. It has so many meanings in so many languages, that...it has come to be a synonym for the generic term ‘fear’” (Gaylin, 1979, p. 18). Other commentators have emphasized the difficulty of drawing sharp operational boundaries between the terms (Perusini and Fanselow, 2015). To avoid misunderstanding, we use the undifferentiated term “fear and anxiety” throughout our review. We urge other researchers to eschew these problematic redefinitions of everyday language and instead focus on the specific parameters of the threat (e.g.,

probability) and neurobehavioral response (e.g., time course), including subjective reports of emotional experience.

The central extended amygdala is tightly interconnected and poised to assemble states of fear and anxiety

In primates, the extended amygdala encompasses a heterogeneous collection of nuclei buried beneath the medial temporal lobe. This includes the Ce, BST, intercalated masses of the amygdala, medial nucleus of the amygdala, parts of the nucleus accumbens shell, and cell columns in the substantia innominata that serve to bridge the Ce and BST (i.e., the sublentiform extended amygdala [SLEA]) (Alheid and Heimer, 1988). Like other subcortical structures involved in emotion and motivation (e.g., nucleus accumbens, periaqueductal gray), the Ce and BST are complex and can be partitioned into multiple subregions (for detailed reviews, see Fox et al., 2015a; Gungor and Paré, 2016), each containing intermingled cell types with distinct, even opposing functional roles (e.g., anxiolytic vs anxiogenic) (Janak and Tye, 2015). As a consequence, research that relies on lesions, pharmacological inactivation approaches (e.g., muscimol micro-injections), or imaging techniques necessarily reflect a mixture of cells or signals.

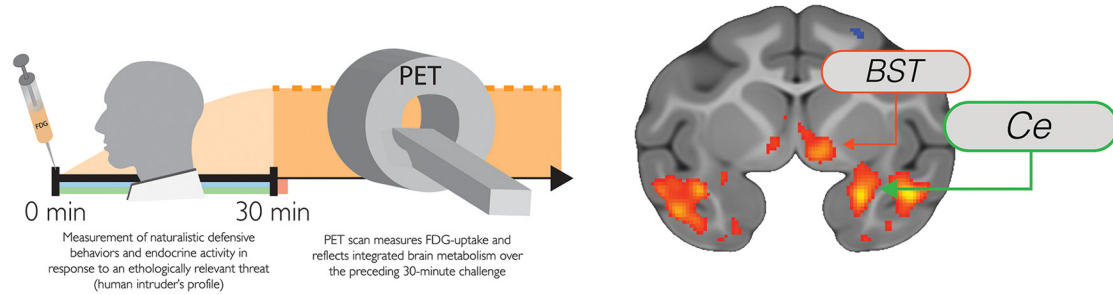
Invasive studies of anatomical connectivity first suggested that the central division of the extended amygdala (i.e., the Ce, BSTL, and portions of the SLEA) represents a tightly integrated structural and functional unit. It has long been recognized that the amygdala is connected to the BST via two major fiber bundles: the ventral amygdalofugal pathway (VA; sometimes termed the ansa peduncularis) and the stria terminalis (ST) (Nauta, 1961). Classic tracing studies showed that VA fibers project through the SLEA region of the substantial innominata, directly connecting the Ce to the BST (Novotny, 1977). In parallel, the ST exits the caudal amygdala to arch dorsally and rostrally over the thalamus, carrying with it a second set of projections from the Ce to BSTL (Klinger and Gloor, 1960; Freese and Amaral, 2009; Oler et al., 2016b). More recent tracing and diffusion imaging studies in monkeys have not only confirmed that the Ce and BSTL are structurally interconnected via these two direct pathways (primarily Ce → BSTL) but have also identified a novel indirect pathway in the SLEA (Ce ↔ SLEA ↔ BSTL) (deCampo and Fudge, 2013; Oler et al., 2016b). In both monkeys and humans, the Ce and BST also show persistently high levels of physiological coupling (Oler et al., 2012; Avery et al., 2014; Birn et al., 2014; Torrisi et al., 2015; Oler et al., 2016b), suggesting that they represent an evolutionarily conserved functional circuit.

Invasive tracing studies in monkeys and rodents demonstrate that the Ce and the BSTL are both well positioned to orchestrate key signs of fear and anxiety, including alterations in arousal, behavioral inhibition, and neuroendocrine activity, via dense monosynaptic and polysynaptic projections to brainstem and subcortical effector regions (Davis and Whalen, 2001; Freese and Amaral, 2009; Penzo et al., 2014; Fox et al., 2015a) (Figs. 1, 2). In human fMRI studies, many of these downstream regions (e.g., hypothalamus, periaqueductal gray) also show robust functional connectivity with the BST (Torrisi et al., 2015).

In sum, converging lines of anatomical and physiological evidence gleaned from *in vivo* and *ex vivo* studies of rodents, monkeys, and humans indicates that the two major subdivisions of the central extended amygdala (the Ce and the BST) form a functionally coherent circuit that is uniquely poised to integrate and evaluate potentially threat-relevant information and assemble states of fear and anxiety (Fig. 1).

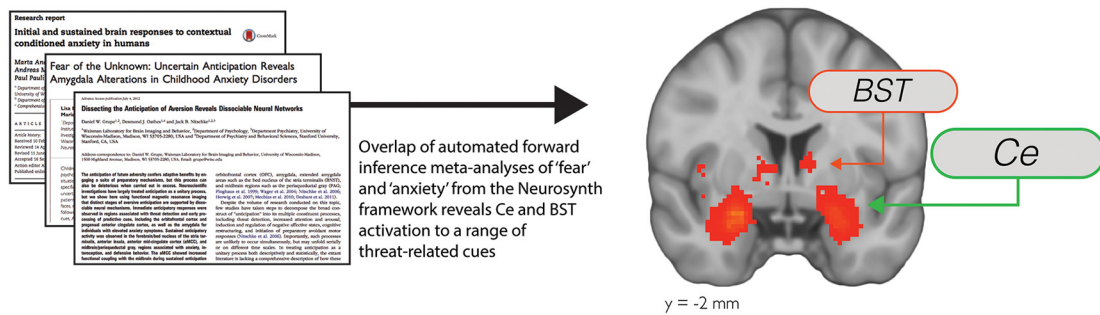
Monkey

a *BST and Ce are related to sustained threat in monkeys*



Human

b *Automated meta-analysis of 'fear' and 'anxiety' reveals BST and Ce activation*



c *Sustained BST activation during the uncertain anticipation of aversive images*



Figure 3. Assessing fear- and anxiety-relevant brain function in monkeys and humans. **a**, BST and Ce are related to sustained threat in young monkeys. In our nonhuman primate model, we simultaneously assess behavior, neuroendocrine activity, and brain metabolism. At the beginning of the session, the monkey receives an injection of a radiotracer, 18-fluorodeoxyglucose (FDG) and is placed alone in a testing cage. Paralleling behavioral paradigms (e.g., “strange situation”) used to assess fear and anxiety in children, in some experiments an unfamiliar human experimenter (“intruder”) enters the room and stands motionless outside the cage while presenting his or her profile to the subject. In contrast to other forms of stress, such as direct threats, the adaptive response in this context is to inhibit vocalizations and freeze, decreasing the likelihood of detection by the intruder. Immediately following the intruder challenge, plasma is collected for quantifying neuroendocrine activity (e.g., cortisol), and subjects are anesthetized and positioned in a stereotaxic device within the high-resolution, small-bore PET scanner. The PET scanner then measures the amount of FDG uptake during the preceding 30 min behavioral paradigm; regions that were more metabolically active during the behavioral challenge take up more radiolabeled glucose. Metabolism in the Ce and BST is associated with heightened signs of fear and anxiety (fewer vocalizations, more freezing, and elevated levels of the stress-sensitive hormone cortisol) during prolonged (30 min) exposure to the human intruder’s profile ($n = 592$) (Fox et al., 2015b). **b**, Automated meta-analysis of “fear” and “anxiety” studies in humans reveals BST and Ce activation. Figure represents the minimum conjunction (logical “AND”) of thresholded forward inference maps ($q < 0.01$) automatically generated by Neurosynth (Yarkoni et al., 2011) for studies tagged with the keyword “fear” (298 studies) or “anxiety” (312 studies). **c**, Sustained BST activation during the uncertain anticipation of aversive images. Somerville et al. (2013) presented standardized negative or neutral images (3 s) (Lang et al., 1998) in blocks (118 s) where the timing of presentations was either certain or uncertain. Analyses demonstrated that 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). Portions of this figure were adapted with permission from Somerville et al. (2013) and Fox et al. (2015b).

The central extended amygdala responds to a broad spectrum of threats
 Studies of nonhuman primates have enabled researchers to obtain concurrent measures of naturalistic defensive behaviors, neuroendocrine activity, and whole-brain metabolic activity,

something rarely attempted in humans (Fig. 3a). Well-established 18-fluorodeoxyglucose positron emission tomography (FDG-PET) procedures make it possible to examine changes in brain activity and behavior elicited by a variety of ethologically relevant threats, including diffusely threatening

contexts (i.e., a novel testing cage) and more explicit cues (i.e., an unfamiliar human intruder's profile) (Kalin and Shelton, 1989; Fox and Kalin, 2014; Fox et al., 2015a; Oler et al., 2016a). Using this approach, we have demonstrated in studies incorporating as many as 592 individuals that metabolic activity in both the amygdala and the BST is associated with heightened signs of fear and anxiety (more freezing, fewer vocalizations, and elevated levels of the stress-sensitive hormone cortisol) during sustained (30 min) exposure to either diffusely threatening contexts (Fox et al., 2005, 2008; Kalin et al., 2005) or intruder threat (Kalin et al., 2005; Jahn et al., 2010; Oler et al., 2010; Shackman et al., 2013; Fox et al., 2015b) (Fig. 3a). Importantly, we used chemoarchitectonic techniques (i.e., serotonin transporter binding, quantified *in vivo* using PET in an independent sample) to more definitively localize the functionally defined region of the amygdala to the Ce. Metabolic activity in the Ce and BST is heritable; and BST metabolism, in particular, is genetically correlated with behavioral and endocrine measures of intruder-elicited fear and anxiety (Fox et al., 2015b).

In sum, a considerable body of nonhuman primate research reveals similar functional profiles in the Ce and BST. Both regions show elevated metabolism during prolonged exposure to potentially dangerous contexts and cues, and this activity predicts concurrent variation in fear- and anxiety-relevant defensive behaviors and endocrine activity. Although imaging research in monkeys, which has relied heavily on FDG-PET techniques, lacks the temporal resolution needed to cleanly dissociate phasic from sustained neural responses (Fig. 3a), it provides an important translational framework for the kinds of mechanistic research that we describe later in the review.

A growing body of fMRI research in humans suggests that the Ce and BST are similarly engaged by a range of threat-related cues and contexts. There is ample evidence that the amygdala, including the Ce, is recruited by a variety of threat-related cues, including aversive images, Pavlovian shock-cues, and emotional faces (Costafreda et al., 2008; Sergerie et al., 2008; Fusar-Poli et al., 2009; Mechias et al., 2010; Vytal and Hamann, 2010; Sabatinelli et al., 2011; Lindquist et al., 2012, 2016). Work using high-resolution fMRI (~ 1.5 mm³) indicates that the dorsal region of the amygdala in the region of the Ce is particularly sensitive to aversive images (Hrybowski et al., 2016). Increased activation in the dorsal amygdala, in turn, is associated with elevated signs (e.g., startle potentiation, skin conductance) and symptoms (i.e., ratings) of arousal in response to acute threat (e.g., Pavlovian threat cues) (LaBar et al., 1998; Knight et al., 2005; Cheng et al., 2006, 2007; van Well et al., 2012; Wood et al., 2014; Kragel and LaBar, 2015). Furthermore, multivoxel classifier analyses suggest that the dorsal amygdala is an important component of a larger circuit that supports heightened distress and negative affect in response to aversive images (Chang et al., 2015).

The human imaging literature indicates that the BST, like the Ce, is recruited by a broad spectrum of potentially threat-relevant cues. As shown in Figure 3b, an automated meta-analysis generated using Neurosynth (Yarkoni et al., 2011) reveals that studies of "fear" (298 studies) and "anxiety" (312 studies) consistently report 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., due to omission from automated labeling tools) (Fox et al., 2015a). Paralleling the Ce, BST activation and functional connectivity covary with threat-elicited changes in cardiovascular activity, skin conductance, and self-reported fear and

anxiety (Somerville et al., 2013; McMenamin et al., 2014; Alvarez et al., 2015; Banihashemi et al., 2015). Together, this physiological evidence shows that both subdivisions of the central extended amygdala are recruited by a variety of threat-related cues and predict concurrent changes in peripheral physiology and emotional experience, converging with the results of imaging research performed in monkeys.

Recent fMRI studies have begun to more directly assess the relevance of the Davis model, which was largely derived from rodent studies, to humans. As shown in Figure 2a, Davis and colleagues originally hypothesized that the Ce and BST differ in at least two crucial ways: the kind of threat each is most sensitive to (certain/specific vs uncertain/diffuse) and the time course of their response (phasic vs sustained). Consistent with this hypothesis, several studies have demonstrated that the BST shows a persistent hemodynamic response during the uncertain anticipation of noxious reinforcers, such as shock or aversive images, whereas the dorsal amygdala shows more transient responses (Alvarez et al., 2011; Grupe et al., 2013; Somerville et al., 2013; McMenamin et al., 2014; Herrmann et al., 2016). In one of the most compelling examples, Somerville and colleagues presented either aversive or neutral images (3 s) in relatively long blocks (118 s) where the timing of image presentations was either certain or uncertain (Fig. 3c). These unique design features are important because they afford a crucial opportunity to double-dissociate phasic (to 3 s certain threat) from sustained (i.e., to 118 s uncertain threat) responses in the same individuals. Analyses revealed transient activation in the amygdala in response to the negative images, whereas the BST showed persistent activation during negative-versus-neutral blocks and during uncertain-versus-certain blocks. Furthermore, 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 limitations (e.g., perceptual confounds, failing to test the Region \times Condition interaction), these results are consistent with the idea that the central extended amygdala is functionally segregated, providing important support for the translational relevance of Davis and colleagues' original model.

On the other hand, a growing number of human imaging studies are difficult to reconcile with the early Davis model. Several studies have found heightened amygdala activation during the anticipation of uncertain threat (Andreatta et al., 2015; Williams et al., 2015). For example, Andreatta et al. (2015) observed sustained activation, verified using a finite impulse response model, in the region of the Ce during exposure to a virtual-reality context (30 s) paired with unpredictable electric shocks (Fig. 2b). Other work has revealed phasic responses in the region of the BST to punctate threats, such as a 4 s video clip of a tarantula that appears to approach the subject's foot (Mobbs et al., 2010; Choi et al., 2012; Grupe et al., 2013; Klumbers et al., 2015). Likewise, a recent large-scale imaging study ($n = 168$) reported phasic activation of the BST in response to 4 s cues that coterminated with shock delivery (Klumbers et al., 2015), consistent with evidence that a substantial proportion of BST neurons exhibit short-latency responses during exposure to both acute threat and diffusely threatening environments in rodents (Gungor and Paré, 2016).

On balance, the neuroimaging literature demonstrates that the Ce and the BST show similar functional profiles. Human studies provide compelling evidence that both subdivisions of the central extended amygdala respond to a broad spectrum of aver-

sive stimuli, including the anticipation of uncertain threat, and are correlated with concurrent changes in peripheral physiology and emotional experience. Across studies, both regions show transient responses to clear and immediate threat (<10 s) and both show sustained responses in contexts associated with uncertain, longer-lasting threat (>30 s). In studies of monkeys, both regions show increased metabolic activity during sustained exposure (30 min) to novel contexts and potential threat. The upshot of this work is that claims of strict phenomenological and anatomical segregation in the central extended amygdala (i.e., “the amygdala mediates fear [phasic responses], whereas the BST mediates anxiety [sustained responses];” Fig. 2*a*), as described in the earlier model of Davis and colleagues, are clearly unwarranted. Although the nature of their differential contributions remains unclear, both subdivisions of the central extended amygdala appear to play an important role in evaluating threat and promoting feelings of fear and anxiety.

The central extended amygdala is a crucial substrate for fear and anxiety

Converging lines of mechanistic evidence gleaned from studies of monkeys, rodents, and humans demonstrate that the Ce is a crucial substrate for fear and anxiety. In monkeys, excitotoxic Ce lesions markedly reduce the defensive behaviors and endocrine activity normally elicited by sustained exposure to the human intruder’s profile or by acute exposure to a live snake (Kalin et al., 2004). Conversely, genetic manipulations that increase Ce metabolism (i.e., via viral vector-mediated overexpression of corticotrophin-releasing hormone) potentiate defensive responses during prolonged exposure to intruder threat (Kalin et al., 2016). These experimental observations in monkeys dovetail with evidence that humans with circumscribed amygdala damage show a profound lack of fear and anxiety to diffusely threatening contexts (e.g., walking through a haunted house) as well as more acute threats (e.g., spiders, snakes, Pavlovian threat cues) (Bechara et al., 1995; Feinstein et al., 2011). Furthermore, patients report abnormally low levels of dispositional fear and anxiety on standardized psychometric measures (Feinstein et al., 2011), consistent with more informal clinician ratings of temperament (Tranel et al., 2006).

Although the causal contribution of the BST to fear and anxiety in monkeys or humans has yet to be explored, surgical lesions to the orbitofrontal cortex (OFC) in monkeys have been shown to disrupt freezing during sustained exposure to intruder threat, and this appears to be mediated by a downstream reduction in BST metabolic activity (Kalin et al., 2007; Fox et al., 2010). Humans with OFC damage also show reduced blood flow to the BST (Motzkin et al., 2015), further suggesting that these two regions work closely together to orchestrate and regulate responses to sustained threat (Fig. 1).

As described in much more detail in the accompanying review by Gungor and Paré (2016) and other recent commentaries (Calhoun and Tye, 2015; Janak and Tye, 2015; Tovote et al., 2015), mechanistic work in rodents suggests that the circuits supporting phasic and sustained responses to threat are highly overlapping. For example, inactivation of the Ce attenuates phasic responses to acute threat (Wilensky et al., 2006; Cioocchi et al., 2010; Li et al., 2013) and learning-dependent plasticity within the CeL is required for the acquisition of Pavlovian fear conditioning (Cioocchi et al., 2010). But there is also evidence that excitotoxic BST lesions can attenuate defensive responses elicited by cues as short as 20 s (Kiyokawa et al., 2015). Likewise, both regions play a critical role in regulating sustained responses to diffusely

threatening contexts (Moreira et al., 2007; Zimmerman et al., 2007; Duvarci et al., 2009; Zimmerman and Maren, 2011; Jennings et al., 2013; Kim et al., 2013). Other work demonstrates that the CeL and BST both contribute to the overgeneralization of fear and anxiety to Pavlovian safety cues (Duvarci et al., 2009; Cioocchi et al., 2010). Although researchers have sometimes interpreted null effects as indicating that the BST is “not necessary for” or “not involved in” triggering phasic responses to briefly presented (<60 s) threat cues or contexts (e.g., Hammack et al., 2015), the degree to which the experimental (e.g., lesion) and control groups are statistically equivalent (Seaman and Serlin, 1998) remains unexplored. Moreover, the translational relevance of much of this work remains unknown, making it an important avenue for future research in humans or monkeys.

Collectively, these observations demonstrate that the Ce and the BST both regulate sustained defensive responses elicited by prolonged exposure to threatening cues and contexts, contrary to earlier versions of the Davis model. This body of research also reveals a critical role for the CeL in triggering phasic responses to acute threat, and highlights a potentially important role for the BST in assembling states of fear and anxiety in response to relatively brief threat cues. Both findings are at odds with the reformulated Davis model. Finally, work in rodents indicates that both subdivisions of the central extended amygdala are mechanistically involved in the overgeneralization of fear- and anxiety-related responses to acute safety cues. The latter observation is particularly interesting because overgeneralization is known to confer elevated risk for the development of anxiety disorders (Craske et al., 2012; Lenaert et al., 2014; Barker et al., 2015) and consistently distinguishes anxiety patients from psychiatrically healthy control subjects across a range of specific diagnoses (Kheirbek et al., 2012; Lissek, 2012; Grupe and Nitschke, 2013; Duits et al., 2015).

An integrative perspective on fear and anxiety

The anatomical, physiological, and mechanistic evidence that we have reviewed shows that the central extended amygdala is a tightly interconnected functional unit, one that is poised to assemble states of fear and anxiety in response to a variety of aversive challenges. Imaging studies show that activity in the Ce and the BST covaries with signs and symptoms of fear and anxiety. Both subdivisions are engaged by uncertain, ambiguous, or temporally remote threat. Both show phasic responses to fleeting challenges and both show heightened activity during sustained exposure to novelty or threat. Mechanistic studies demonstrate that the Ce and BST both play a critical role in controlling sustained responses to diffuse or uncertain threat. These observations make it clear that claims that the central extended amygdala is strictly segregated are no longer tenable and suggest the need to reevaluate the Research Domain Criteria constructs of Acute Threat (Fear) and Potential Threat (Anxiety). This perspective is not a new theory. Indeed, much of the data and many of the ideas that we have described are already well-known among select groups of neuroscientists. It is instead a synthesis of earlier suggestions and new data into a clear working hypothesis about the contributions of the Ce and the BST to fear and anxiety. In the next section, we delineate the kinds of evidence that will be required to refine it and, ultimately, to understand the differential contributions of circuits centered on these two regions to fear, anxiety, and human disease.

A roadmap to future challenges

Research conducted in the half-decade since the publication of the reformulated Davis model has yielded a number of important and exciting new insights into the contributions of the Ce and the BST to fear and anxiety. Still, it is equally clear that our understanding remains far from complete and that considerable work remains if we are to understand the precise functional architecture and relevance of the central extended amygdala to fear and anxiety. Here, we outline some of the most crucial challenges for future research and some specific strategies and guidelines for addressing them.

Rigorous methods

Understanding the neurobiology of fear and anxiety requires that we determine how the Ce, the BST, and other brain regions represent and respond to different kinds of threat. Threats differ along several major dimensions — probability, imminence (i.e., physical distance or temporal latency), and duration (Fanselow and Lester, 1988; Blanchard et al., 1989; Fanselow, 1989, 1994; Blanchard et al., 2001; Mobbs and Kim, 2015; Mobbs et al., 2015) — and there is compelling evidence that these dimensions are psychiatrically relevant (Davis et al., 2010; Craske et al., 2012; Bradford et al., 2013; Duits et al., 2015; Shackman et al., 2016). Yet, we know remarkably little about how the brain represents and differentially responds to them. Although important strides have been made (Mobbs et al., 2010; Somerville et al., 2013), conceptual progress has been slowed by the use of paradigms and assays that confound these dimensions (e.g., if vs when threat will occur; brief cues vs prolonged contexts).

Drawing strong inferences about the neural systems supporting phasic and sustained responses to different dimensions of threat requires the use of well-matched tasks, both in humans (Luck, 2005; Shackman et al., 2006) and in animals (Hammack et al., 2015). Tasks must be equated for motor requirements and perceptual characteristics, including paired reinforcers (e.g., shocks, aversive images). Investigators should be cautious when comparing neural activity or behavior across tasks that markedly differ in duration or number of trials (i.e., in the variance of the read-out), as in paradigms where long blocks are compared with brief events. Parametric manipulations of threat probability (if threat will occur), imminence (when or where it will occur), and duration (as in Mobbs et al., 2010; Bradford et al., 2013) 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 (Bach and Dolan, 2012; de Berker et al., 2016) and for identifying circuits involved in triggering behavioral and physiological “phase transitions” (Mobbs and Kim, 2015; Mobbs et al., 2015) (e.g., from vigilance to behavioral inhibition to active defense). Putative double dissociations need to be rigorously assessed by testing the appropriate Region \times Condition interaction (as in Somerville et al., 2010). 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,” based on a null statistical test or a single assay is unwarranted (Seaman and Serlin, 1998; Button et al., 2013a; for more general statistical recommendations, see Button et al., 2013b).

Human studies also provide a crucial opportunity to establish the neural mechanisms underlying subjective symptoms of fear and anxiety, something that cannot be assessed in animal models (Anderson and Adolphs, 2014; LeDoux, 2015). To this end, it will be critical for human studies to verify the presence of target emotions separately for each task (Shackman et al., 2006) and examine relations with ongoing neural activity (Heller et al., 2014). For correlational techniques, such as fMRI, trial-by-trial relations between neural signals and emotional experience provide one of the strongest and most direct links between the brain and emotion (Lim et al., 2009; Atlas et al., 2010). Multivoxel classifier approaches, in which machine learning techniques are used to identify patterns of activation predictive of subjective states, are also likely to be fruitful (Wager et al., 2013; Woo et al., 2014; Chang et al., 2015).

Neuroanatomy

Understanding the contributions of the extended amygdala to fear and anxiety in humans requires that neuroimaging researchers begin to more fully engage with its neuroanatomical complexity. Although imaging studies reporting amygdala activation number in the thousands, far fewer studies report activations in the BST. This discrepancy partially reflects the fact that automated tools for assigning anatomical labels to activation clusters do not yet include the BST, although probabilistic anatomical masks have recently become available (Avery et al., 2014; Torrisi et al., 2015). As a consequence, investigators without a strong background in neuroanatomy may not realize that a cluster encompasses the BST or may mis-assign it to neighboring regions in the basal ganglia. Even those familiar with the BST often remain cautious in assigning this label, given the limited spatial resolution afforded by fMRI (Shmuel et al., 2007; Chaimow et al., 2011) (i.e., the upper limit of resolution at 3 Tesla, the field strength of most MRI scanners, is ~ 3.5 mm). Researchers should continue to approach cluster labeling with caution. Provisional BST clusters should always be compared with an atlas (e.g., Mai et al., 2007). Diffusion-weighted imaging approaches can be used to enhance confidence that an activation cluster includes the BST (e.g., via tracing of the amygdalofugal pathway linking the Ce to the BST). It can also be helpful to assess whether provisional BST clusters lie outside of neighboring regions incorporated in automated atlases (i.e., a Boolean NOT with nucleus accumbens, globus pallidus, and caudate) (Fox et al., 2015b). Traditional and automated atlases can also be used to assign more specific labels to clusters that encompass the amygdala. *In vivo* chemoarchitectonic techniques (i.e., serotonin transporter expression quantified using PET) can be used to more definitively localize the CeL (Oler et al., 2010; Shackman et al., 2013). Regardless of the anatomical label ultimately assigned (e.g., “basal forebrain/BST” or “dorsal amygdala in the vicinity of the Ce”), it is clear that increased attention to the functional neuroanatomy of the central extended amygdala will reveal important information relevant to understanding the neurobiology of fear and anxiety.

Coordinated cross-species research

Much of the data that we have reviewed comes from brain imaging studies. Aside from unresolved questions about the origins and significance of the measured signals (Logothetis, 2008; O’Herron et al., 2016), the most important limitation of these techniques is that they cannot address causation. A cru-

cial challenge for future studies is to develop a mechanistic understanding of the distributed neural circuits that support the expression of normal and pathological fear and anxiety. In particular, virtually nothing is known about the causal contribution of the BST to fear and anxiety in primates, including humans. Addressing these fundamental questions mandates coordinated research efforts in humans and animals. For example, mechanistic techniques (e.g., viral vector, chemogenetic, or optogenetic techniques) in animal models can be combined with the same whole-brain imaging strategies routinely applied in humans, enabling the development of integrated, bidirectional translational models of fear and anxiety (compare Borsook et al., 2006; Fox et al., 2010; Desai et al., 2011; Casey et al., 2013; Ferenczi et al., 2016). Combining targeted mechanistic interventions with whole-brain imaging is particularly valuable for determining whether changes in behavior are mediated by alterations in the function of downstream regions (e.g., BST), as occurs following OFC lesions in monkeys (Fox et al., 2010) or OFC damage in humans (Motzkin et al., 2015). The development and refinement of bidirectional translational models of fear and anxiety that incorporate optogenetic or chemogenetic techniques would also open the door to identifying the specific molecules, cells, and subregions of the central extended amygdala that mediate effects detected in imaging studies (compare to Ferenczi et al., 2016). Combining fMRI in humans with cognitive-behavioral, neurofeedback, or pharmacological interventions (e.g., anxiolytics) would provide another opportunity for understanding how regional changes in brain activity alter circuit function and, ultimately, the signs and symptoms of fear and anxiety (Paulus et al., 2005; Stoeckel et al., 2014; deBettencourt et al., 2015; Duff et al., 2015; Schnyer et al., 2015). Like other psychological processes and psychiatric disorders, fear and anxiety reflect the coordinated activity of distributed neural circuits (McMenamin et al., 2014; Janak and Tye, 2015; Shackman et al., 2015). Thus, it will also be crucial to understand how the Ce and BST functionally interact with one another (Gungor and Paré, 2016) and with other regions involved in fear and anxiety (Fox et al., 2010, 2015b; Shackman et al., 2011; Cavanagh and Shackman, 2015) to evaluate and respond to different dimensions of threat.

The real world and the clinic

Most studies of fear and anxiety rely on a limited number of well-controlled, but highly artificial, manipulations (e.g., electric shock), collected under unnatural conditions. Although these methods have afforded a number of important insights, the real-world relevance of the central extended amygdala and other brain systems that control the expression of fear and anxiety remains unclear. Recent work combining fMRI with experience-sampling techniques underscores the value of this approach for identifying the neural circuits underlying variation in naturalistic mood and behavior (Forbes et al., 2009; Berkman and Falk, 2013; Lopez et al., 2014; Heller et al., 2015), a depth of understanding that cannot be achieved in animal models or using isolated measures of brain function. Although there is emerging evidence that the BST is sensitized in patients with anxiety disorders (Straube et al., 2007; Yassa et al., 2012; Münsterkötter et al., 2015), nothing is known about the contribution of the BST to the first emergence of psychopathology. Prospective longitudinal imaging studies would provide a valuable opportunity to discover the relevance of central extended amygdala function to the development of patholog-

ical fear and anxiety (Admon et al., 2009; McLaughlin et al., 2014; Swartz et al., 2015).

In conclusion, a wide variety of evidence demonstrates that the central extended amygdala plays a crucial role in evaluating and responding to a range of threat-related cues and contexts. Across a variety of imaging paradigms, the Ce and BST have both proven sensitive to uncertain or temporally remote threat; both covary with threat-elicited changes in behavior, physiology, and emotional experience; both show phasic responses to acute threat cues; and both show heightened activity during sustained exposure to novel or diffusely threatening contexts. Work in rats and mice shows that both regions can control sustained responses to threat and that both regions are critically involved in the overgeneralization of phasic fear and anxiety to safety cues. In light of this evidence, the claim that that extended amygdala is strictly segregated into fear- and anxiety-related subdivisions is no longer tenable. Put simply, the Ce and BST are more alike than different. Developing a more detailed understanding of their common and distinct functions is important and promises to enrich our understanding of the central extended amygdala's role in emotion and temperament and accelerate the development of improved intervention strategies for pathological fear and anxiety.

Response from Dual Perspective Companion Authors—Nur Zeynep Gungor and Denis Paré

Shackman and Fox's perspective paper is an important and insightful contribution to the fear and anxiety literature. First, it reviews recent human fMRI and monkey PET studies, highlighting an emerging picture: contrary to earlier views, activation of Ce and BNST occurs in response to both short, highly probable and long, uncertain threats. Thus, the former notion that Ce and BNST are differentially involved in fear versus anxiety is impeding rather than facilitating our understanding of negative emotional states.

Second, Shackman and Fox remind the human research community that BNST, not only the amygdala, should be considered in fear and anxiety studies. They draw attention to the fact that BNST is often mislabeled or ignored in human studies due to obstacles, such as low spatial resolution or omission from automated labeling software. This is indeed unfortunate because one of the main advantages of fMRI is visualization of the whole brain *in vivo*. Although such noninvasive methods do not allow mechanistic investigations of BNST-Ce interactions, they can generate invaluable data regarding the experimental conditions that activate this network. For instance, threatening stimuli can be more easily manipulated along certainty, duration, and imminence dimensions in human studies than in rodent studies. Moreover, brain activation in response to such stimuli can be compared within the same subjects. And a vast array of experimental manipulations can be used to reveal the functional connectivity between BNST and Ce, potentially guiding the design of rodent experiments by "reverse translation." Last, human studies can relate functional assessments of BNST and Ce activity to verbal reports of subjective feelings, measures of personality, and clinical diagnoses.

Therefore, we support Shackman and Fox's call to make BNST an integral part of human fear and anxiety research and agree that integrative approaches across species are needed. Using rodents, much research has been conducted on BNST in the past decade, thanks to the influential model put forward by Walker and Davis. As we have explained in our perspective paper, BNST and Ce are comprised of many subnuclei and cell types that exert antagonistic effects on behavior. However, investigating the precise interactions between these elements is exclusively the realm of animal research, at least for the time being.

Last, Shackman and Fox remind us of the confusion surrounding the terms fear and anxiety. The demarcation between the two is not always clear, and some use these terms interchangeably. Although we believe that fear and anxiety can be distinguished based on the threat's imminence, probability, and duration, we agree that, in some circumstances, the undifferentiated term "fear and anxiety" is a better choice. At least, this approach will contribute to extinguish strict dichotomous views of negative emotional states and promote the idea of common underlying neural substrates.

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