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.

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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 (Calhoon 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. 2*a*),

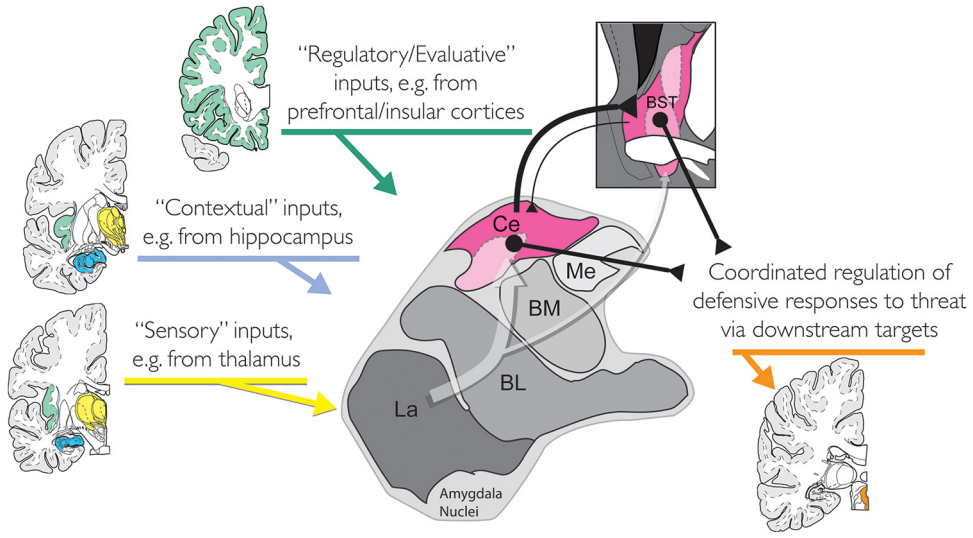

Figure 1. Primate central extended amygdala. Simplified schematic of key central extended amygdala inputs and outputs in humans and other primates. The central extended amygdala encompasses the central (Ce) nucleus of the amygdala, which lies in the dorsal amygdala, and the bed nucleus of the stria terminalis (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).

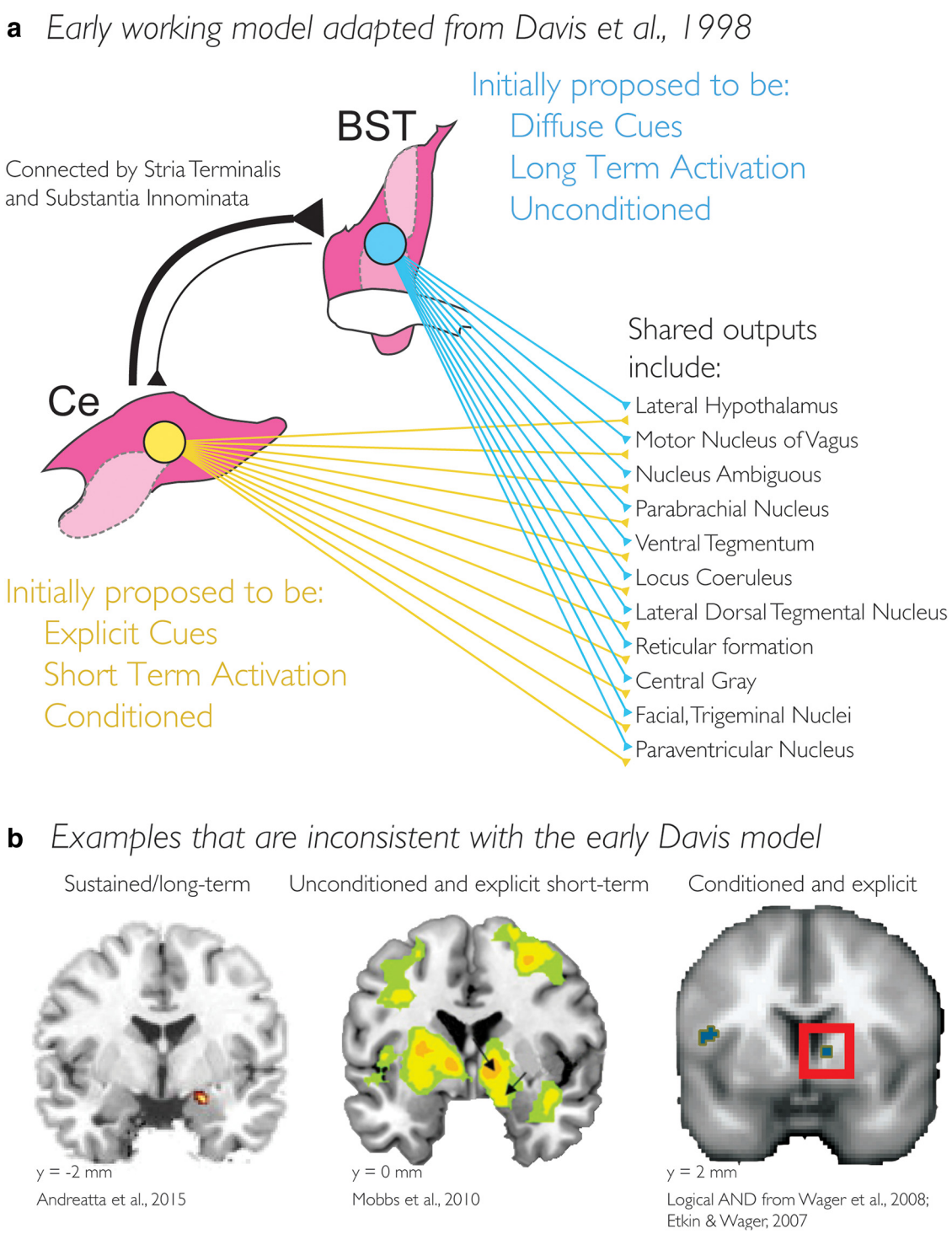

Figure 2. Early model and inconsistent human imaging evidence. ***a***, Early model emphasizing strict functional segregation in the extended amygdala. In earlier work, Davis, Walker, and their colleagues noted that the Ce and BST send similar projections to the brainstem and hypothalamic regions that 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 sublenticular 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 microinjections), 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 (Klingler 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).

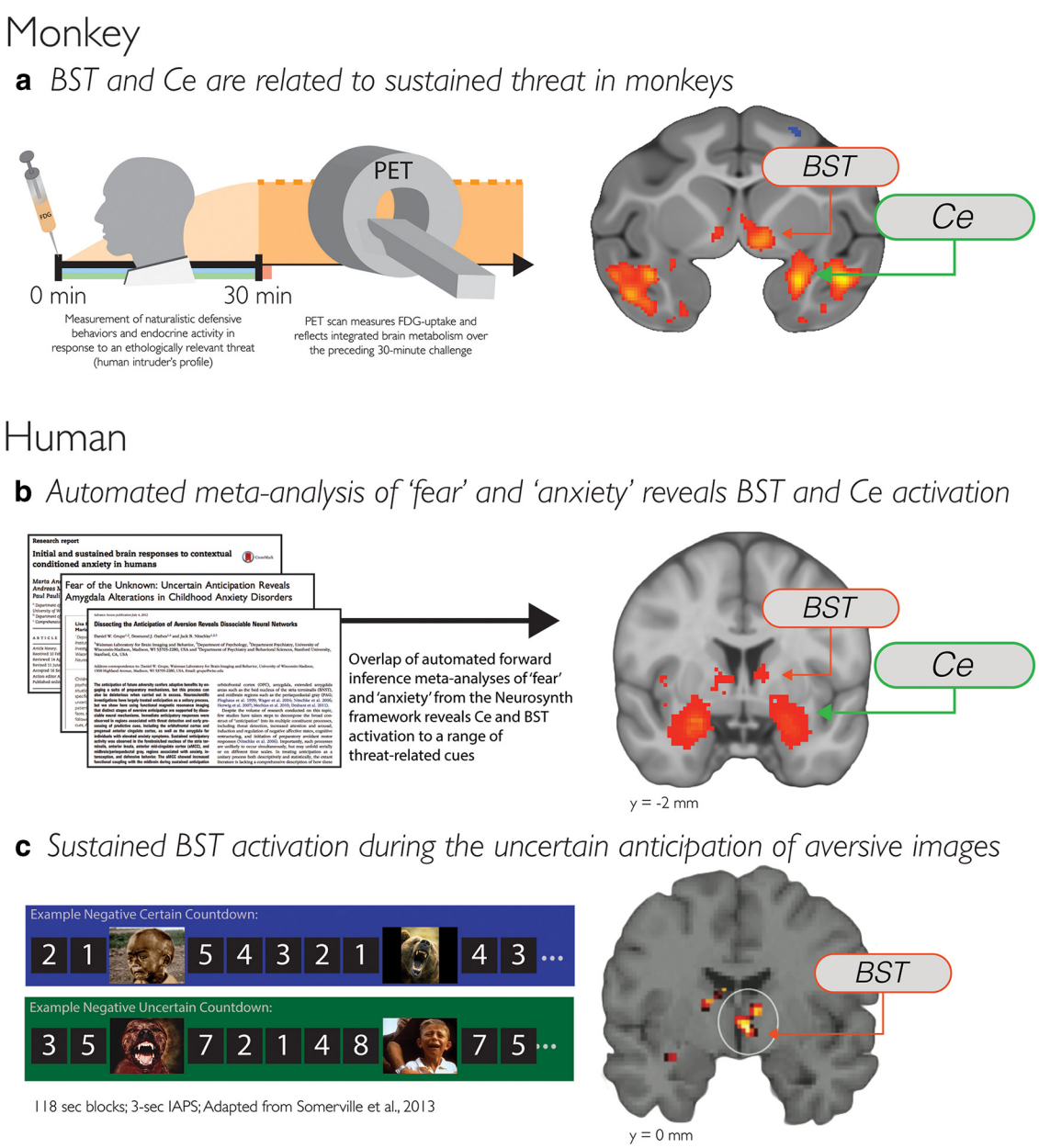

Figure 3. Assessing fear- and anxiety-relevant brain function in monkeys and humans. ***a***, BST and Ce are related to sustained threat in young monkeys. In our nonhuman primate model, we simultaneously assess behavior, neuroendocrine activity, and brain metabolism. At the beginning of the session, 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 stereotactic 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. 3*a*). 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. 3*a*). 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. 3*a*), 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 ($\sim$1.5 mm^3) indicates that the dorsal region of the amygdala in the region of the Ce is particularly sensitive to aversive images (Hrybouski 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 3*b*, 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 2*a*, 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. 3*c*). 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 × 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. 2*b*). 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; Klumpers 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 (Klumpers 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 (Calhoon 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; Ciocchi et al., 2010; Li et al., 2013) and learning-dependent plasticity within the CeL is required for the acquisition of Pavlovian fear conditioning (Ciocchi 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 for 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; Ciocchi 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 × 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 is 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 pathological 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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Editor Column

Dual Perspectives

Discussion of new, sometimes controversial, ideas is an essential and exciting component of scientific progress. Neuroscientists often have strong views on how a given question should be approached, how results should be interpreted, and how important new findings are for re-evaluating existing data. These discussions frequently occur at small meetings or in casual conversations among experts in a particular area of neuroscience and are not always readily apparent to interested scientists outside that field or to junior scientists just entering a field. To remedy this situation and, we hope, enliven these discussions, the *Journal of Neuroscience* has begun a new feature called Dual Perspectives, which appears for the first time in this issue.

Dual Perspectives are pairs of articles that present opposing or complementary views about an important issue in neuroscience research. The editors invite two sets of authors to write separate articles discussing their point of view, after which each author is invited to write a brief comment on the companion article. Our first pair of articles discusses different perspectives on the role of the extended amygdala in fear and anxiety, based on human and animal studies. Next month will bring a pair debating the role of TMC channels in hair cell mechanotransduction. We plan to publish a new pair every month or so.

These discussions are meant to be provocative, so neither piece is solicited to be fully balanced across the various viewpoints in the field. We hope that by allowing scientists to take a strong point of view, we will make clear where the points of agreement and disagreement are most profound, and perhaps where future research might clarify the issue. We invite readers of *JNeurosci* to weigh in on the discussion online at the *JNeurosci* website by clicking the "Submit a Response" link in the sidebar of the articles and adding your comments and ideas about the issues raised in these Dual Perspectives.

With this new feature, we hope not only to keep *JNeurosci* at the forefront of discussions of new, interesting, multi-level and controversial issues in neuroscience, but also to highlight voices that are not always heard, including those of younger PIs.

Dual Perspectives join TechSights, short reviews about advantages and pitfalls of novel techniques that are broadly useful to neuroscientists, and ViewPoints, more general mini reviews highlighting current topics in neuroscience, as Featured Articles meant to promote discussion of issues in neuroscience today.

If you have ideas about current controversies in neuroscience that you think should be highlighted in a Dual Perspective article, please contact us at JN_EIC@sfn.org or JN_Features@sfn.org.

Marina Picciotto, EiC, *JNeurosci*
Teresa Esch, Features Editor *JNeurosci*
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Dual Perspectives

Dual Perspectives Companion Paper: Contributions of the Central Extended Amygdala to Fear and Anxiety, by Alexander J. Shackman and Andrew S. Fox

Functional Heterogeneity in the Bed Nucleus of the Stria Terminalis

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Early work stressed the differing involvement of the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST) in the genesis of fear versus anxiety, respectively. In 2009, Walker, Miles, and Davis proposed a model of amygdala-BNST interactions to explain these functional differences. This model became extremely influential and now guides a new wave of studies on the role of BNST in humans. Here, we consider evidence for and against this model, in the process highlighting central principles of BNST organization. This analysis leads us to conclude that BNST's influence is not limited to the generation of anxiety-like responses to diffuse threats, but that it also shapes the impact of discrete threatening stimuli. It is likely that BNST-CeA interactions are involved in modulating responses to such threats. In addition, whereas current views emphasize the contributions of the anterolateral BNST region in anxiety, accumulating data indicate that the anteromedial and anteroventral regions also play a critical role. The presence of multiple functional subregions within the small volume of BNST raises significant technical obstacles for functional imaging studies in humans.

Key words: **amygdala; anxiety; BNST; fear**

Introduction

In 2009, Walker et al. (2009a) proposed a parsimonious explanation for earlier observations suggesting that the amygdala and bed nucleus of the stria terminalis (BST) play different roles. This model galvanized interest for the field and still stimulates much research. Here, we review the functional and anatomical organization of BNST and then consider empirical findings for and against this model.

It was first observed that BNST lesions do not affect conditioned fear responses elicited by discrete conditioned sensory cues (CSs) (LeDoux et al., 1988; Hitchcock and Davis, 1991; Gewirtz et al., 1998) (Table 1), unless they were very long (≥8 min) (Waddell et al., 2006; Walker et al., 2009a). In contrast, BNST lesions impaired the acquisition and recall of contextual fear responses (Sullivan et al., 2004; Duvarci et al., 2009; Poulos et al., 2010), an effect that might depend on the diffuse nature of contextual cues (Hammack et al., 2015).

Other work indicated that BNST's involvement in the genesis of anxiety-like responses is not limited to learned associations but that it extends to unconditioned threats, such as bright lights (Walker and Davis, 1997), predator odors (Fendt et al., 2003; Xu et al., 2012), and alarm pheromones (Breitfeld et al., 2015). Consistent with this, exploratory behavior in the elevated plus maze (EPM), which assesses the fear of open spaces rodents naturally display, was also found to be dependent on BNST activity (Waddell et al., 2006; Duvarci et al., 2009; Kim et al., 2013).

Overall, these findings led to the theory that BNST mediates sustained anxiety-like responses to diffuse environmental threats (Walker et al., 2009a), as opposed to the central amygdala (CeA), which generates defensive behaviors in response to imminent threats. This parsimonious explanation is well accepted in the BNST literature and guides not only animal (Daniel and Rainnie, 2016), but also human research (Avery et al., 2016). Indeed, despite their psychological and physiological similarities, anxiety and fear are triggered by distinct stimuli. Fear-eliciting cues signal imminent threats with a high probability of occurrence. On the other hand, anxiety arises in the anticipation of uncertain perils (Grupe and Nitschke, 2013). Although most of the studies implicating BNST in aversive responses used such distal and unpredictable threats, other data suggest that BNST also modulates responses to discrete cues. However, before addressing this question, we will briefly summarize major principles of BNST organization.

Anatomical and physiological substrates of BNST functions

Nuclear systematization. BNST's structure is complex and, compared with the amygdala, still poorly understood. BNST is in fact a collection of nuclei, with much disagreement regarding their number and location (e.g., compare Moga et al., 1989 and Ju and Swanson, 1989). Posteriorly located BNST nuclei are involved in

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Table 1. Glossary

Term	Definition
Unconditioned stimulus (US)	Any stimulus that can trigger a response without prior learning experience
Conditioned stimulus (CS)	An initially neutral stimulus that gains the ability to evoke responses after being paired with an unconditioned stimulus
Cue fear conditioning	The process by which a discrete and salient sensory stimulus, such as a neutral tone, is paired with a noxious US, usually a mild foot shock. As a result, the CS acquires the ability to elicit the responses originally associated with the US
Contextual fear conditioning	The process by which a context where the animal received a noxious US acquires the ability to elicit fear responses
Elevated plus maze	An apparatus that consists of open and enclosed arms, used commonly for probing anxiety in rodents
Fear	Various types of short-lived defensive responses elicited by imminent threats. Whereas fear is accompanied by feelings of fright in humans, it is unclear whether animals also experienced such states
Anxiety	Various types of enduring responses elicited by diffuse and uncertain treats. Whereas anxiety is accompanied by feelings of dread in humans, it is unclear whether animals also experienced such states
Anxiogenic	Stimulus or process that promotes anxiety
Anxiolytic	Stimulus or process that reduces anxiety
Fear-potentiated startle	A paradigm where fear is assessed by measuring startle responses elicited by loud noise bursts. After CS-footshock pairings, noise bursts presented during the CS elicit higher startle responses
Cue-induced reinstatement	In drug dependence experiments, animals are often trained to lever press for drug self-administration when cued with the presentation of a light and tone compound stimulus. Following self-administration, animals undergo an extinction period where lever responses decrease but do not completely disappear. Next, in cue-induced reinforcement, reintroduction of the light-tone CS results in an increase of lever presses, even though no drug is delivered in this part of the experiment

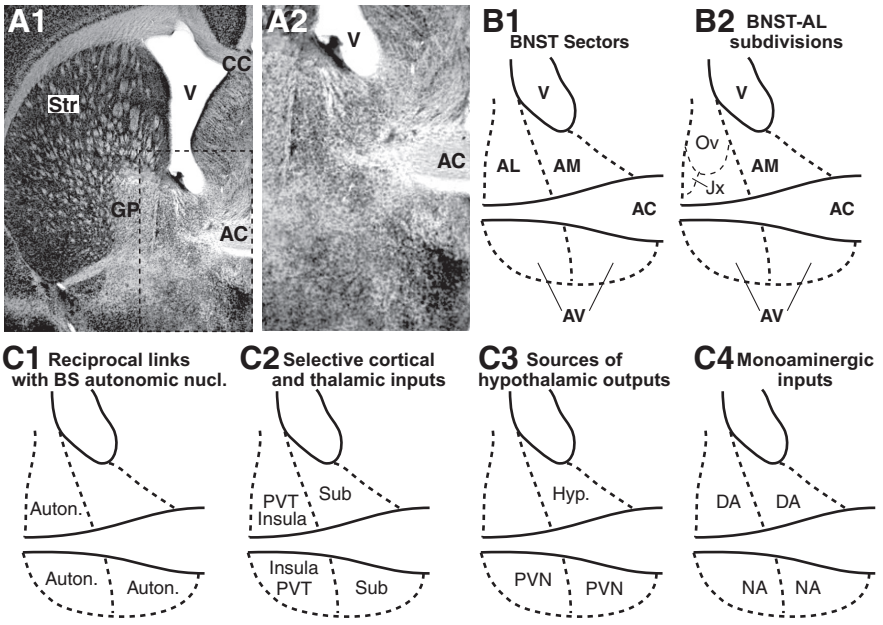

Figure 1. Structure and main connections of BNST. ***A***, Anterior BNST at low (1) and high (2) magnification. Coronal sections processed to reveal NeuN immunoreactivity. ***B***, Nomenclature. ***C***, Connections. Two major fiber bundles, the intra-BNST segment of the stria terminalis (ST) and the anterior commissure (AC), naturally divide the anterior part of BNST in three sectors: Dorsal to the AC are the AL and AM sectors, located lateral and medial to the ST, respectively. Ventral to the AC is the AV region. In contrast with BNST-AL, BNST-AM receives little or no CeA inputs (see references in main text), (1) it does not project to brainstem autonomic centers (***C1***) (Norgren, 1976; Ricardo and Koh, 1978; Saper and Loewy, 1980; Schwaber et al., 1982; Sofroniew, 1983; Gray and Magnuson, 1987, 1992; Shin et al., 2008; Panguluri et al., 2009; Bienkowski and Rinaman, 2013); (2) it is innervated by largely distinct cortical areas and thalamic nuclei (***C2***) (Cullinan et al., 1993; McDonald et al., 1999; Reynolds et al., 2005; Li and Kirouac, 2008; Shin et al., 2008; Bienkowski and Rinaman, 2013); and (3) moreover, its hypothalamic projections are comparably massive (***C3***) (Conrad and Pfaff, 1976a, b; Saper et al., 1976; Swanson, 1976; Swanson and Cowan, 1979; Kita and Oomura, 1982a; b; Dong and Swanson, 2003, 2004, 2006a, b, c; Dong et al., 2000, 2001b). Although the connectivity of the lateral and medial portions of BNST-AV is similar to that of BNST-AL and AM, respectively, it must be considered separately because of its heavy noradrenergic innervation, among the densest in the brain (***C4***) (Fallon and Moore, 1978; Forray et al., 2000), as well as its strong projections to the VTA (Dong et al., 2001b; Georges and Aston-Jones, 2002) and PVN of the hypothalamus (Sawchenko and Swanson, 1983; Moga and Saper, 1994). AC, Anterior commissure; Auton, autonomic centers; BS, brainstem; CC, corpus callosum; DA, dopamine; GP, globus pallidus; Hyp, hypothalamus; Jx, juxtacapsular; NA, noradrenaline; Ov, oval; PVT, paraventricular nucleus of thalamus; Sub, subiculum; Str, striatum; V, ventricle.

reproductive behavior (Simerly, 2002) and have received little attention from fear/anxiety researchers. Instead, their experiments initially focused on the anterior BNST region (LeDoux et al., 1988) because it is the main termination zone of CeA axons (Krettek and Price, 1978a). However, anterior BNST nuclei are small, often smaller than the dendritic arbor of the neurons they contain (McDonald, 1983; Larriva-Sahd, 2006), precluding their selective targeting *in vivo*. Moreover, with few exceptions, differences in connectivity between adjacent nuclei are minor. Thus, it seems more productive to use a grouping of anterior BNST nuclei based on regional differences in connectivity. According to this criterion, BNST should be divided in three sectors: anterolateral (AL), anteromedial (AM), and anteroventral (AV). Figures 1 and 2*A* summarize how different BNST regions receive distinct inputs and contribute contrasting projections.

BNST receives few exteroceptive sensory afferents via the thalamus and cortex. Thus, the massive glutamatergic projections it gets from the basolateral complex of the amygdala (BLA; Fig. 2*B*) probably play a critical role in determining how organisms respond to environmental contingencies. The three BLA nuclei contribute differentially to this pathway, with the lateral amygdala having no projections, and the basal nuclei contributing prominently (Krettek and Price, 1978a; Weller and Smith, 1982; Dong et al., 2001a). Although both basal nuclei project to BNST's three anterior sectors, their projections are complementary (Fig. 2*B*). The basomedial (BM) nucleus preferentially targets BNST-AM, whereas the basolateral nucleus (BL) preferentially projects to BNST-AL (Krettek and Price, 1978a; Dong et al., 2001a). Of note, the oval portion of BNST-AL is reportedly devoid of BLA inputs (Dong et al., 2001a).

Physiological cell types and the transmitters they use. So far, five physiological classes of BNST neurons have been described (Hammack et al., 2007; Francesconi et al., 2009; Szucs et al., 2010;

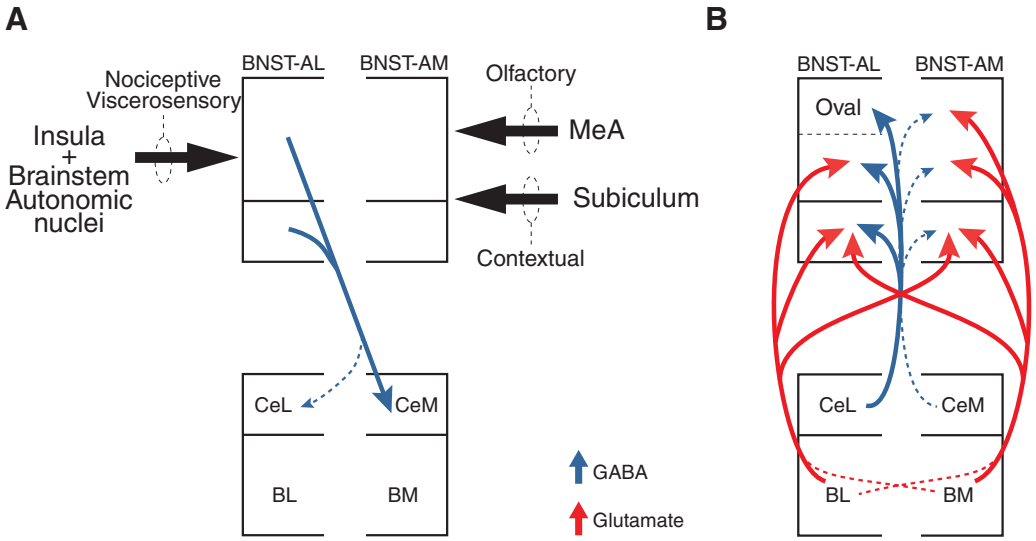

Figure 2. Reciprocal connections between the amygdala and the anterior part of BNST. ***A***, BNST projections to the amygdala. Black arrows indicate dominant sensory inputs. MeA, Medial nucleus of the amygdala. ***B***, Amygdala projections to BNST.

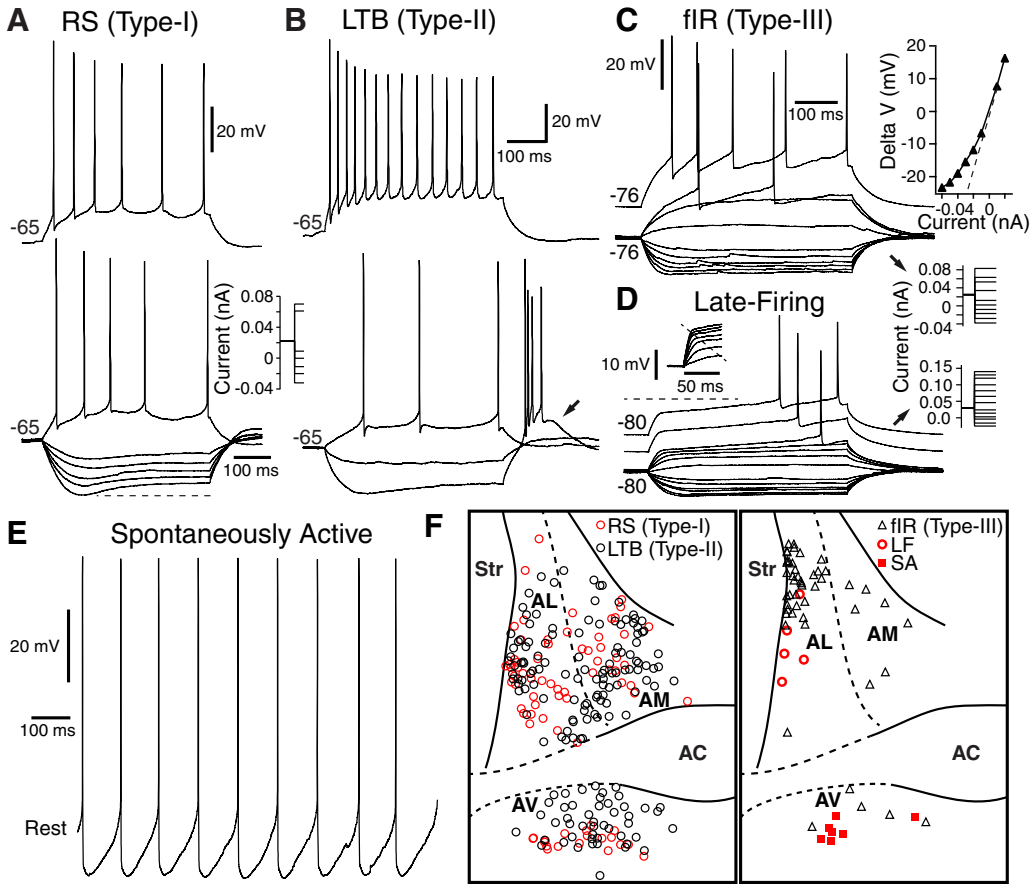

Figure 3. Physiological properties of BNST neurons. Five types have been described (***A–E***). In decreasing order of incidence, they are low-threshold bursting (LTB; Type II; ***B***), regular spiking (RS, Type I; ***A***), with a fast inward rectifying K^+ conductance (fIR; Type III; ***C***), late-firing (***D***), and spontaneously active (***E***) neurons. The relative incidence of Type I and II cells is similar in the three BNST regions (***F***, left), but the other three cell types are mostly found in one of the three regions (***F***, right). Type III cells are concentrated in the oval nucleus, spontaneously active cells in BNST-AV, and late-firing cells in BNST-AL. ***C***, Inset, Amplitude of voltage response to current pulses (*y*-axis) as a function of current (*x*-axis). ***D***, Inset, Expanded view of initial voltage response to current injection. Modified from Rodriguez-Sierra et al. (2013).

Rodriguez-Sierra et al., 2013) (Fig. 3). Importantly, in BNST-AL, the most common three cell types (Fig. 3*A–C*) were accurately clustered by their mRNA expression for different ion channel subunits (Hazra et al., 2011). Most BNST-A neurons, including projection cells, are GABAergic neurons (Cullinan et al., 1993; Sun and Cassell, 1993; Polston et al., 2004; Poulin et al., 2009) that can express a variety of peptides in multiple combinations (Gray and Magnuson, 1987; Ju et al., 1989; Moga et al., 1989). This is the

case of the corticotropin releasing factor (CRF) cells located in the oval nucleus (Sakanaka et al., 1987; Phelix and Paull, 1990), which also express a fast inwardly rectifying K^+ conductance (known as Type III cells; Fig. 3*C*) (Dabrowska et al., 2013a; but see Silberman et al., 2013). It should be noted that the number of CRF-positive BNST neurons is higher in rats than in mice (Wang et al., 2011). In addition to the prevalent GABAergic neurons, BNST-AM and AV also contain a low proportion of glutamatergic cells (Poulin et al., 2009), some of which are projection neurons (Kudo et al., 2012).

Role of the oval nucleus and CRF. Much evidence suggests that CRF exerts anxiogenic effects through its actions in BNST. For instance, intra-BNST (Sahuque et al., 2006) or intracerebroventricular injections of CRF (Lee and Davis, 1997) cause anxiogenic effects, and the latter are blocked by intra-BNST infusions of antagonists for CRF Type 1 receptors (CRF-R1) (Lee and Davis, 1997). Less definitive but also suggestive, oral administration of a CRF-R1 antagonist blocks light-enhanced startle but not conditioned fear to discrete cues (Walker et al., 2009b). Moreover, stressors, such as footshocks, cause an increase in the expression of CRF mRNA in BNST-AL and AV, indicating that CRF cells are activated during stress (for review, see Daniel and Rainnie, 2016). Consistent with this, chemogenetic inhibition of CRF cells (Pleil et al., 2015) or optogenetically inhibiting BNST-AL cells expressing D1-receptors (Kim et al., 2013), thought to be selectively expressed by CRF cells (Daniel and Rainnie, 2016), decrease anxiety in the EPM and open field.

Despite the strong link between CRF and anxiety in BNST, there is still uncertainty regarding the underlying mechanisms. First, given the lack of BLA inputs to the oval nucleus, which structures "inform" CRF cells of environmental contingencies? The oval nucleus is devoid of inputs from the subiculum (Cullinan et al., 1993; McDonald et al., 1999) and medial amygdala (Dong et al., 2001a), sites thought to convey contextual or olfactory information required for responses to threatening contexts and predator odors, respectively. However, it receives viscerosensory afferents from the insula (McDonald et al., 1999; Reynolds et al., 2005) and brainstem autonomic nuclei (Saper and Loewy, 1980; Schwaber et al., 1982) as well as mixed dopaminergic-glutamatergic inputs from the periaqueductal gray (Li et al., 2016). Whether these structures provide the critical anxiogenic signals remains to be tested.

Second, in contrast with CRF cells of the paraventricular hypothalamic nucleus (PVN), those found in BNST-AL do not control the release of stress hormones via projections to the pituitary. Thus, their anxiogenic influence likely depends on a modulation of synaptic transmission within BNST itself or at their projection sites. Indeed, CRF cells of the oval nucleus project to various brainstem autonomic nuclei thought to mediate defensive behaviors (Gray and Magnuson, 1987, 1992). Third, these neurons are not the only CRF-expressing elements in BNST. Indeed, BNST-AL receives strong CRF inputs from the lateral sector of CeA (CeL) (Sakanaka et al., 1986). Fourth, while the somatic expression of CRF-R1 mRNA is low to moderate in BNST (Potter et al., 1994; van Pett et al., 2000; Dabrowska et al., 2013a), BNST-AL is heavily innervated by axons expressing this receptor (Justice et al., 2008; Jaferi and Pickel, 2009; Jaferi et al., 2009).

Consistent with this, multiple CRF effects, so far all CRF-R1-dependent, have been described. In BNST-AL, CRF presynaptically potentiates glutamatergic transmission (Kash et al., 2008; Nobis et al., 2011; Silberman et al., 2013). Postsynaptically, CRF was reported to depolarize low-threshold bursting (Type II) cells (Ide et al., 2013), an effect that might explain why CRF increases spike-dependent inhibitory inputs to Type III neurons in the oval nucleus (Nagano et al., 2015). Last, in BNST-AV, CRF postsynaptically increases GABA-A IPSC amplitudes but does not alter EPSCs (Kash and Winder, 2006). Given these multiple and in some cases opposite effects, it remains unclear how CRF contributes to anxiety through its actions in BNST.

BNST-AL. Interestingly, other lines of evidence support the possibility that BNST-AL exerts anxiolytic influences. For instance, BNST-AL stimulation reduces corticosterone levels (Dunn, 1987), whereas BNST-AL lesions increase gastric erosions after stress exposure (Henke, 1984). Moreover, intra-BNST infusions of calcitonin gene-related peptide, a peptide that inhibits non-Type III neurons in BNST-AL (Gungor and Paré, 2014), actually increases acoustic startle and fos expression in targets of BNST-AL (Sink et al., 2011).

In addition, in a variety of stress paradigms, the efficacy of glutamatergic inputs to BNST-AL is reduced. For example, chronic restraint stress causes a depression of glutamatergic inputs to BNST-AL neurons via α-1 adrenoreceptors (McElligott et al., 2010). Similarly, chronic cortisol administration and social isolation interfere with the induction of long-term potentiation (Conrad et al., 2011), and withdrawal from various drugs of abuse reduces the intrinsic excitability of BNST-AL neurons (Francesconi et al., 2009). The only exception to this trend was obtained in Type III neurons, in which chronic restraint stress causes a potentiation of glutamatergic inputs (Dabrowska et al., 2013b).

The opposite results obtained in Type III (CRF-expressing) cells suggest that anxiety involves the differential recruitment of different types of BNST-AL neurons. Supporting the notion that functionally distinct cell subpopulations exist in BNST-AL, it was reported that different subsets of BNST-AL cells show lower (~25%) or higher (~10%) firing rates during high than low fear states (Fig. 4*B*,*D*) (Haufler et al., 2013). Interestingly, BNST-AM cells show the opposite trend (Fig. 4*A*,*D*). Below, we propose a mechanism for how BNST-AM activity might promote high fear states.

BNST-AM. An analysis of BNST-A's connections (Figs. 1, 2) indicates that BNST-AM is well positioned to mediate BNST's anxiogenic influence. Indeed, BNST-AM is the main recipient of the amygdalar (particularly BM), subicular, and olfactory (medial amygdala) signals that are needed for anxiety-like responses to threatening contexts and odors (Cullinan et al., 1993; McDonald et al., 1999; Dong et al., 2001a). On the output side, BNST-AM projects massively to the hypothalamus. Particularly intriguing in this respect are the complementary projections of BM and BNST-AM to the ventromedial hypothalamic nucleus (VMH), a node implicated in the genesis of defensive and aggressive behaviors (Gross and Canteras, 2012; Silva et al., 2013; Lee et al., 2014; Wang et al., 2015). Indeed, whereas BM sends glutamatergic projections to the core of VMH (VMH-C) (Petrovich et al., 1996), where the nucleus' glutamatergic output neurons are located, BNST-AM projects to its shell (VMH-S) (Dong and Swanson, 2006a), which contains GABAergic neurons that inhibit core neurons (Fu and van den Pol, 2008). This arrangement suggests that BM might increase its impact on VMH-C by recruiting GABAergic BNST-AM cells, which would then inhibit VMH-S cells, disinhibiting VMH-C neurons. Thus, the synergistic actions of BM and BNST-AM on the VMH are expected to enhance defensive and aggressive behaviors.

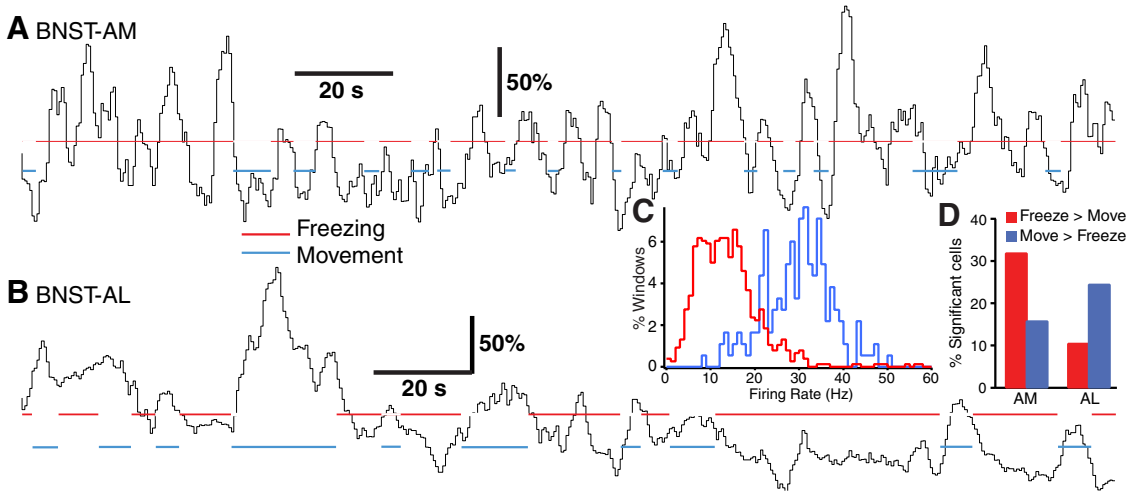

Figure 4. Inverse fluctuations in the firing rate of BNST-AM and BNST-AL neurons in relation to contextual freezing. Rats were subjected to a classical auditory fear conditioning protocol. The next day, while recording BNST neurons, rats were exposed to the conditioning context in the absence of CS. Rats froze 40%–50% of the time during exposure to the aversive context. ***A***, ***B***, Black traces represent average firing rates of 5 BNST-AM (***A***) and 3 BNST-AL (***B***) cells during epochs of contextual freezing (red lines) or movement (blue lines). ***C***, For all available cells, multiple epochs of freezing (red) or movement (blue) were segmented and distribution of firing rates compared. ***D***, Percentage cells with significantly different firing rates during freezing versus movement in BNST-AM versus AL. Red and blue bars represent cells with higher versus lower firing rates during freezing than movement. Modified from Haufler et al. (2013).

Opposite to this conclusion, however, two recent *Nature* studies from the same laboratory reported that BLA inputs to BNST-AM (Kim et al., 2013) and BM (Adhikari et al., 2015) exert anxiolytic influences, the latter being "necessary and sufficient" for anxiolysis. This conclusion is puzzling given that their common target, VMH, mediates aversive behaviors, such as avoidance, freezing (Wang et al., 2015) and attack (Lee et al., 2014). Not to mention that both BL and BM also send glutamatergic projections to the medial sector of the central amygdala (CeM) (Krettek and Price, 1978b), thought to be the amygdala's main output station for conditioned fear. A possible explanation for these contradictions is that these two *Nature* reports depended heavily on behavioral observations in the EPM and open field, where predatory or active avoidance behaviors might have been mistakenly interpreted as decreased levels of anxiety.

BNST-AV. BNST-AV is also well positioned to contribute to anxiety-like responses. Indeed, BNST projections to PVN mostly originate from BNST-AV (Sawchenko and Swanson, 1983; Moga and Saper, 1994) and they regulate the HPA axis (Herman et al., 2005). Although some glutamatergic (Csáki et al., 2000) and CRF-expressing (Moga and Saper, 1994) cells project to PVN, most are GABAergic (Radley et al., 2009; Radley and Sawchenko, 2011). These inhibitory neurons receive excitatory inputs from the mPFC and subiculum (Radley et al., 2009; Radley and Sawchenko, 2011) but few from CeA (Prewitt and Herman, 1998). In keeping with this, mPFC (Radley et al., 2009) and hippocampal lesions (Radley and Sawchenko, 2011) decrease the number of fos-positive GABAergic cells in BNST-AV while increasing fos expression in PVN. Although these findings indicate that GABAergic BNST-AV neurons inhibit PVN, other results indicate that the overall influence of BNST-AV over PVN is excitatory. Indeed, global BNST-AV lesions interfere with the recruitment of PVN by various stressors (Crane et al., 2003; Spencer et al., 2005; Choi et al., 2007), whereas selective ablation of GABAergic cells in BNST-AV increases adrenocorticotropic hormone and corticosterone levels after restraint stress (Radley et al., 2009). Overall, these findings suggest that GABAergic cells of BNST-AV inhibit PVN, whereas its glutamatergic cells do the opposite. Surprisingly, although they account for a minority of BNST-AV cells, the influence of glutamatergic neurons appears to dominate. As a result, BNST-AV as a whole exerts an excitatory influence on PVN.

Interestingly, a similar situation may prevail in BNST-AV's projections to the ventral tegmental area (VTA). Indeed, VTA-projecting glutamatergic cells of BNST-AV increase their firing rate during both aversive unconditioned and conditioned stimuli. In contrast, GABAergic cells are inhibited by both. Optogenetically activating glutamatergic cells produces place aversion and anxiogenic effects, whereas activation of the GABAergic cells produces place preference and anxiolytic effects (Jennings et al., 2013).

Intrinsic BNST connectivity. The data reviewed above emphasizes that BNST is comprised of several functionally important sectors. This situation raises the possibility that anxiety involves inter-regional coordination of activity. Consistent with this idea, tracing (Dong and Swanson, 2003, 2004, 2006a, b, c) and glutamate uncaging (Turesson et al., 2013) studies have revealed that BNST neurons form connections with other cells located in the same or different BNST sectors (Fig. 5). While inhibitory intraregional connections prevail overall, in BNST-AV and the ventral part of BNST-AM, the incidence of glutamatergic and GABAergic connections is similar (Turesson et al., 2013). Although this is surprising given that glutamatergic cells account for minority of the cells (Poulin et al., 2009), this finding is consistent with evidence that glutamatergic BNST-AV cells exert an outsized influence over PVN and VTA neurons (Choi et al., 2007; Radley et al., 2009; Radley and Sawchenko, 2011; Jennings et al., 2013). Importantly, inter-regional connections can be asymmetric or reciprocal, purely inhibitory, or dependent on a mixture of glutamatergic and GABAergic connections (Turesson et al., 2013). For instance, BNST-AL to AM and AV projections are purely GABAergic and markedly stronger than return connections (Fig. 5). Although it is currently unknown whether CRF cells in the oval nucleus contribute to these connections, suppression of firing in GABAergic BNST-AL neurons during high fear states (Haufler et al., 2013) might cause a disinhibition of BNST-AM neurons, contributing to their higher activity levels during contextual freezing (Haufler et al.,

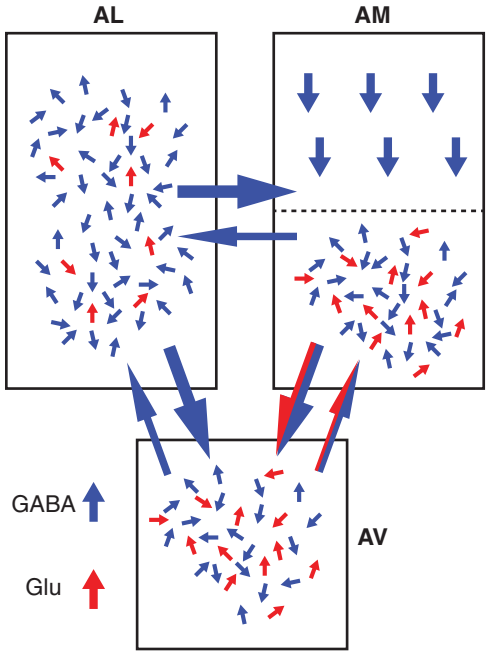

Figure 5. Intrinsic BNST connections. Pattern of intrinsic connections in the anterior BNST, as revealed with glutamate uncaging. Neurons were recorded with the whole-cell method in slices *in vitro*. Glutamate was uncaged by applying brief flashes of ultra-violet light to a circumscribed region (250 μm in diameter) of BNST. The light stimulus was moved to systematically scan the slice in search of BNST sites containing neurons projecting to the recorded cell. For intraregional connections, the number of blue (GABAergic) and red (glutamatergic) arrows approximates the relative frequency of inhibitory and excitatory connections, respectively. For inter-regional connections, the thickness of the arrows was adjusted to represent the relative incidence of connections. Data from Turesson et al. (2013).

2013). On the other hand, BNST-AL's influence on BNST-AV will depend on the transmitter content (GABA vs glutamate) of the targeted BNST-AV neurons, which is unknown at this time. Similarly, the significance of the mixed glutamatergic and GABAergic connections between BNST-AM and AV (Turesson et al., 2013) (Fig. 5) is currently unclear.

Amygdala-BNST interactions

According to the model proposed by Walker et al. (2009a), BL would send threat signals to CeA and BNST. In turn, neurons in CeM would respond immediately, activating downstream fear effectors. By contrast, BNST activation would not only depend on BL activity, but also on CRF inputs from CeL. As a result, BNST's activation would be delayed relative to that of CeM, leading to more slowly developing and longer-lasting anxiety-like states in response to sustained but diffuse threats. This model also proposes that BNST, once active, inhibits CeM, preventing its recruitment during the generation of anxiety-like states. Below, we review empirical findings for and against this model.

CeA involvement in generating responses to long threat-signaling cues. According to Walker and Davis (1997), CeA would not be involved in modulating anxiety-like responses to diffuse and uncertain threats because BNST, once activated, suppresses CeA neurons. Although this prediction found experimental support for unconditioned threats, such as bright lights (Walker and Davis, 1997) or predator odors (Fendt et al., 2003; Li et al., 2004; Rosen, 2004), it did not for the fear of open spaces. Indeed, CeA lesions reduce anxiety-like behavior in the EPM (Möller et al., 1997; Moreira et al., 2007). Similarly, contradictory results were reported for the impact of CeA lesions on conditioned negative associations to long cues or contexts. Although CeA lesions do not block fear-potentiated startle to long cues (Walker et al., 2009a), many found that they reduce freezing to an aversive context (Goosens and Maren, 2001, 2003; Sullivan et al., 2004). However, some failed to find an effect of CeA lesions (Fanselow and Kim, 1994) or concluded that CeA is not involved in the expression but in the consolidation of contextual fear memories (Pitts et al., 2009).

Relative timing of BNST versus CeM activation. Central in the Walker et al. (2009a) model is the notion that BNST activation is delayed relative to that of CeM. However, accumulating data show that the firing rates of BNST neurons are rapidly altered by short and long cues, appetitive or aversive (Haufler et al., 2013; Jennings et al., 2013) (Fig. 6*A*,*B*). Moreover, Hammack et al. (2015) showed that, during exposure to a threatening context, the difference in freezing between sham and BNST-lesioned animals is constant for the duration of the context exposure when the model predicts increasing differences with time. Together, these results demonstrate that BNST responses to threatening stimuli or environments are nearly immediate and not necessarily more important the longer the animal is exposed.

BNST involvement in the processing of short-lasting cues. Despite earlier studies showing that BNST does not regulate fear responses to discrete threatening stimuli, accumulating evidence indicates otherwise. During the recall of classically conditioned fear responses, ~25% of neurons in BNST-AL and AM displayed short-latency alterations in firing rates in response to discrete CSs (Haufler et al., 2013). Consistent with this, muscimol injections in BNST were found to enhance fear-potentiated startle (Meloni et al., 2006), suggesting that BNST exerts tonic inhibitory effects in CeA or their common targets. Support for this notion was obtained by examining the effects of BNST lesions on the CS specificity of conditioned fear responses (Duvarci et al., 2009). In this study (Fig. 6*C*), rats were subjected to a differential auditory fear conditioning paradigm where a 30 s auditory CS (CS^+) was paired to footshocks, whereas another (CS^-) was not. Although BNST-lesioned and sham rats acquired similarly high levels of conditioned fear to the CS^+, rats with BNST lesions froze less than sham rats to the CS^-, again indicating that BNST activity does affect the processing of short cues.

Additional evidence of short cue processing by BNST comes from the addiction literature. Indeed, a large body of work indicates that BNST plays a critical role in various aspects of addiction, including the dysphoria that follows the pleasurable effects of drug consumption (Wenzel et al., 2011, 2014), in the stress associated with drug withdrawal, and in the reinstatement of drug-seeking (Erb and Stewart, 1999; Aston-Jones and Harris, 2004; Koob, 2009, 2010). In such experiments, animals are trained to lever-press for drug self-administration when presented with a short cue. After an extinction period where lever responses have no effect, reintroduction of cues results in reinstatement of drug seeking behavior. Critically, BNST inactivation interferes with this cue-induced reinstatement (Buffalari and See, 2010).

To summarize this section, although the Walker et al. (2009a) model offers an attractive and parsimonious explanation for the functional dissociation between the amygdala and BNST, some of its key postulations are not supported by available experimental findings. Interestingly, the companion perspective paper (Shackman and Fox, 2016) reached the same conclusion based on an entirely different set of data: functional imaging studies in humans. Thus, although it appears definite that BNST is not required for the genesis of defensive behaviors triggered by discrete threatening cues, equally incontrovertible evidence indi-

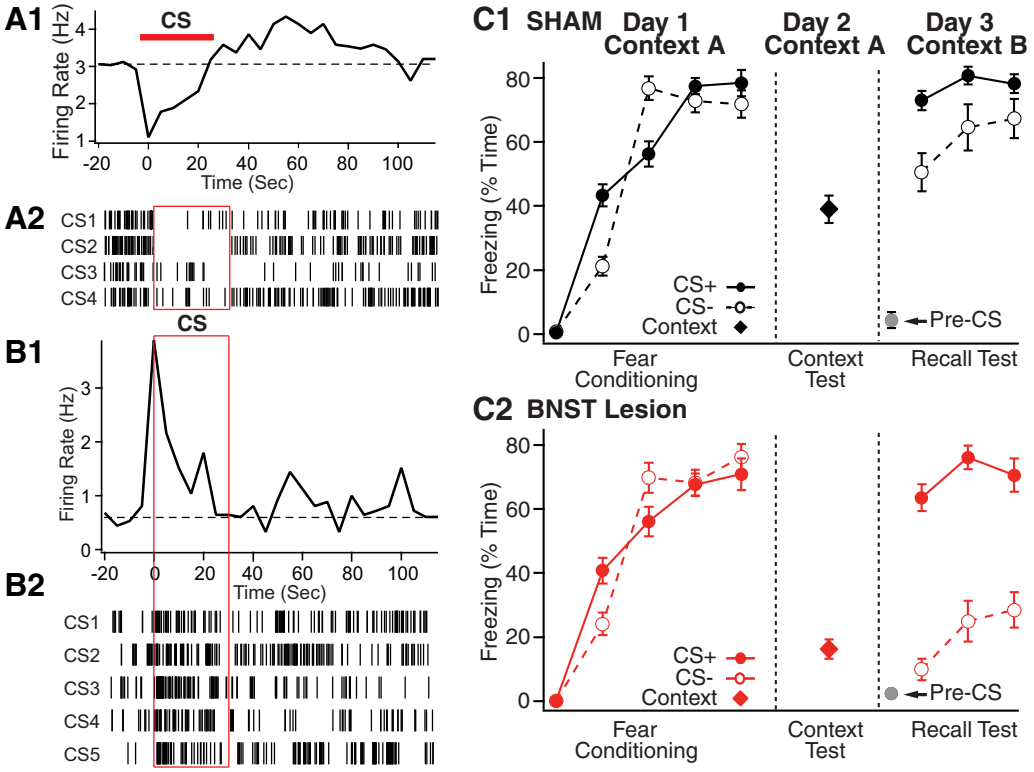

Figure 6. BNST activity alters the processing of discrete threatening stimuli. ***A***, ***B***, Two examples of anterior BNST neurons with short-latency responses to discrete threatening cues. These cells were recorded extracellularly in rats that had been subjected to a classical auditory fear conditioning protocol (CS, conditioned stimulus, pure tone). We show CS-evoked activity during the recall test, conducted 1 d after conditioning. ***A1***, ***B1***, Peri-CS histograms of neuronal discharges. ***A2***, ***B2***, Rasters where each tick represents an action potential. Cells with inhibitory responses prevailed in BNST-AL, whereas cells with excitatory responses were concentrated in BNST-AM. ***C***, Excitotoxic BNST lesions enhance the stimulus specificity of conditioned fear responses. The two graphs compare percentage time freezing to the CS^+ (filled symbols and solid lines), CS^- (empty symbols and dashed lines), or conditioning context exposure in sham (***C1***, black) or BNST-lesioned (***C2***, red) rats. There is markedly reduced freezing to the CS^- with no change in behavior to the CS^+ in BNST-lesioned rats. ***C***, Modified from Duvarci et al. (2009).

cates that it can modulate the processing of such cues. Because BNST projections to the amygdala constitute a likely neuronal substrate for this modulation, the next section describes these connections.

Connections between BNST and CeA. Whereas BNST-AM contributes negligible projections to CeA (Bienkowski and Rinaman, 2013), BNST-AL and BNST-AV project strongly to CeM, and lightly to CeL (Sun and Cassell, 1993; Dong et al., 2001b). BNST to CeA projections prevalently arise from GABAergic neurons, although a few glutamatergic neurons also contribute (Gungor et al., 2015). In the opposite direction, CeA projections to BNST mostly originate in CeL and mainly target BNST-AL, sparring the juxtacapsular region (Dong et al., 2001a). CeM contributes less to BNST's innervation (Sun and Cassell, 1993; Bienkowski and Rinaman, 2013) and BNST-AM receives far weaker inputs from CeA than BNST-AL (Krettek and Price, 1978a; Weller and Smith, 1982; Sun et al., 1991).

Given the asymmetry between BNST to CeA versus CeA to BNST connections (Fig. 2, blue), it is difficult to determine the net impact of their interactions. However, it was reported that CeA axons elicit IPSPs in a higher proportion of BNST-AL cells (~80%) (Li et al., 2012) than BNST inputs to CeM neurons (~60%) (Gungor et al., 2015). Furthermore, the GABA-A reversal potential is more negative in BNST than CeA neurons by ~10 mV (Gungor et al., 2015). Together, these differences should conspire to give CeA the upper hand in reciprocal BNST-CeA interactions.

Complicating matters further, however, is the possibility that the impact of BNST inputs to CeM is altered via their actions in CeL. Indeed, different subsets of CeL neurons reciprocally inhibit each other and form different connections with CeM (Ciocchi et al., 2010; Haubensak et al., 2010; Viviani et al., 2011; Li et al., 2013). In particular, CeL cells that do not express somatostatin (SOM^-) send GABAergic projections to CeM, whereas SOM^+ neurons do not (Li et al., 2013). Thus, depending on whether BNST axons contact SOM^+ or SOM^- CeL cells, the impact of BNST inputs in CeM might be dampened or increased, respectively. Given that these two types of CeL cells are thought to show opposite responses to threatening CSs in Pavlovian fear conditioning paradigms (Ciocchi et al., 2010; Haubensak et al., 2010), identifying which one receives inputs from, and projects to, BNST will be key to understand CeA-BNST interactions.

In conclusion, overall, the data reviewed here suggest that BNST's role is not limited to the generation of aversive responses to diffuse threats but that it also shapes the impact of discrete threatening stimuli. In threatening conditions, antagonistic interactions between BNST and CeA likely determine the intensity and specificity of aversive responses. However, BNST-AL and CeL cells express a variety of peptides that might affect how these two regions interact. In addition, much evidence indicates that BNST's influence over anxiety depends on several functionally distinct cell groups and BNST regions. Within BNST-AL, CRF-expressing cells in the oval nucleus are recruited by threats and stressors, but it remains unclear how they alter the activity of neurons in the rest of BNST and in its targets. Non-CRF BNST-AL cells might exert an anxiolytic influence, but their interaction with CRF cells remains largely uncharacterized. Similarly, GABAergic and glutamatergic BNST-AV neurons regu-

late their targets (e.g., PVN, VTA) in opposite ways, but we know little about how they influence each other. Last, recent data suggest that BNST-AM is also involved in generating defensive behaviors. To shed light on how BNST contributes to anxiety, we need to characterize the interplay between these different BNST subregion and the various cell types therein.

Response from Dual Perspective Companion Authors–Alexander J. Shackman and Andrew S. Fox

Anxiety disorders impose a staggering burden on public health, existing treatments are inconsistently effective, and the development of new therapeutics has stalled (Hyman, 2014). The central extended amygdala, including the central nucleus of the amygdala (Ce) and bed nucleus of the stria terminalis (BST), plays a pivotal role in contemporary models of fear and anxiety (Fox et al., 2015; Tovote et al., 2015). Yet, key aspects of its functional architecture have only recently come into focus. Gungor and Paré provide an insightful review of recent progress, focusing on work in rodents.

Gungor and Paré make it clear that both the Ce and BST are involved in modulating phasic and sustained responses to threat. For example, Gungor and Paré show that a sizable number of BST neurons rapidly respond to punctate threat and safety cues. This contradicts the hypothesis that the BST is a "sluggish" system and only responds to persistent threat (Davis, 2006). Building on this observation, they highlight evidence showing that the BST plays a crucial role in shaping phasic responses to acute cues when they are encountered in potentially dangerous contexts and contributes to the "overgeneralization" of fear and anxiety (Kheirbek et al., 2012; Lissek, 2012). As noted in our companion review, other work suggests that the lateral Ce also contributes to overgeneralization. These observations are particularly important because, in humans, overgeneralization marks populations at risk for developing anxiety disorders (e.g., Barker et al., 2014; Gazendam et al., 2015), promotes maladaptive avoidance (Grillon, 2002), predicts the future emergence of anxiety disorders (e.g., Craske et al., 2012), and distinguishes anxiety patients from controls (Duits et al., 2015).

Gungor and Paré emphasize that the BST can be partitioned into subregions, each containing intermingled cell types with distinct, even opposing, functional phenotypes. This indicates that inferences drawn from excitotoxic lesion, pharmacological inactivation, or neuroimaging studies will necessarily reflect a mixture of cellular signals. At present, the tools required to parse these signals do not exist for use in humans. Conversely, there is no guarantee that the mechanisms identified in animal models are evolutionarily conserved and will translate to humans. Understanding the relevance of these intermixed signals to the subjective feelings that define neuropsychiatric disease will therefore require coordinated cross-species research and the development of bidirectional translational models combining precise mechanistic techniques with whole-brain imaging. Inconsistent nomenclature is another important barrier. For example, in our companion review, we argue that researchers should refrain from using the words "fear" and "anxiety" to refer to phasic and sustained responses to threat because it is inconsistent with everyday usage of these terms.

As outlined in the two *Dual Perspective* reviews, there is compelling evidence that the central extended amygdala plays a key role in orchestrating phasic and sustained responses to threat. The development and refinement of integrated bidirectional models would open the door to identifying the specific molecules, cells, and circuits that mediate effects detected in human imaging studies (compare Ferenczi et al., 2016) and accelerate the of development of improved treatments for pathological fear and anxiety.

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