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

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
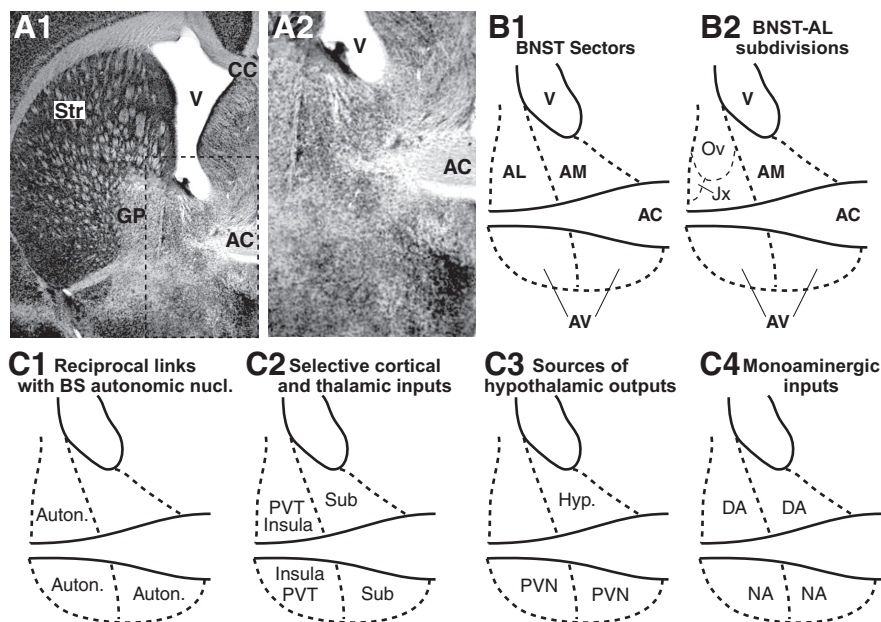
Figure 1. Structure and main connections of BNST. *A*, Anterior BNST at low (1) and high (2) magnification. Coronal sections processed to reveal NeuN immunoreactivity. *B*, Nomenclature. *C*, Connections. Two major fiber bundles, the intra-BNST segment of the stria terminalis (ST) and the anterior commissure (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.

(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 2A 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. 2B) 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. 2B). 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;

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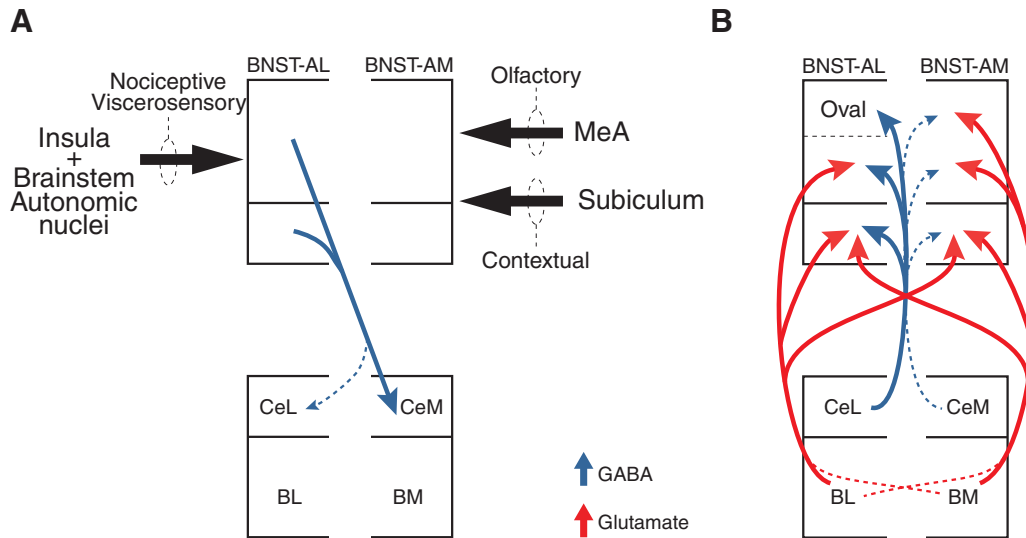
Figure 2. Reciprocal connections between the amygdala and the anterior part of BNST. *A*, BNST projections to the amygdala. Black arrows indicate dominant sensory inputs. MeA, Medial nucleus of the amygdala. *B*, Amygdala projections to BNST.
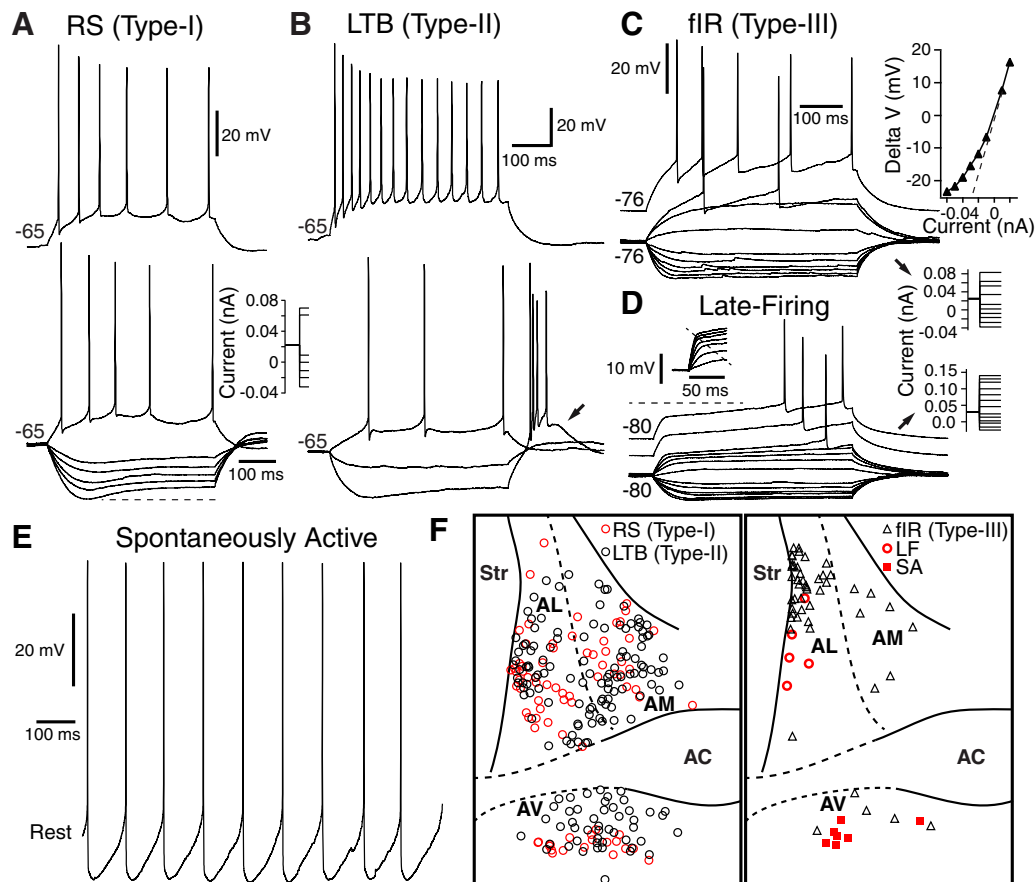

Figure 3. Physiological properties of BNST neurons. Five types have been described (*A–E*). In decreasing order of incidence, they are low-threshold bursting (LTB; Type II; *B*), regular spiking (RS, Type I; *A*), with a fast inward rectifying K⁺ conductance (fIR; Type III; *C*), late-firing (*D*), and 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. 3C) (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. 4B,D) (Haufler et al., 2013). Interestingly, BNST-AM cells show the opposite trend (Fig. 4A,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.
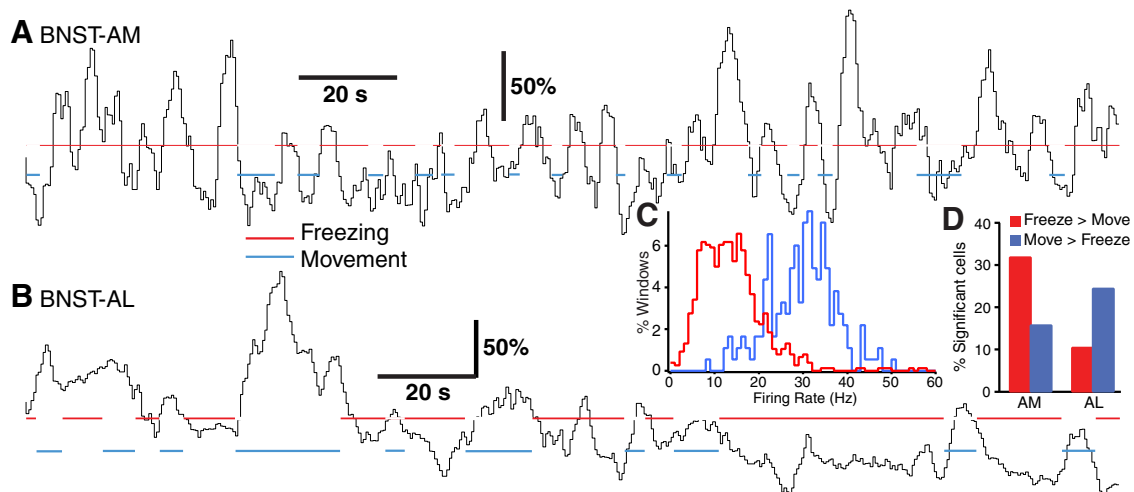
Figure 4. Inverse fluctuations in the firing rate of BNST-AM and BNST-AL neurons in relation to contextual freezing. Rats were subjected to a classical auditory fear conditioning protocol. The next day, while recording BNST neurons, rats were exposed to the conditioning context in the absence of CS. 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.,
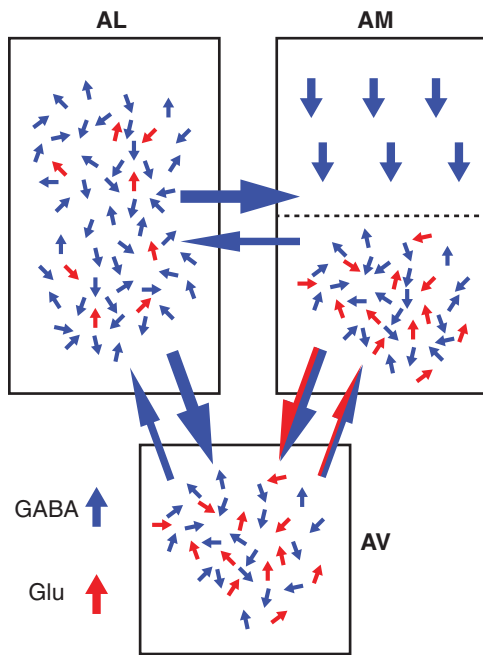
Figure 5. Intrinsic BNST connections. Pattern of intrinsic connections in the anterior BNST, as revealed with glutamate uncaging. Neurons were recorded with the whole-cell method in slices *in vitro*. Glutamate was uncaged by applying brief flashes of ultra-violet light to a circumscribed region (250 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. 6A,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. 6C), 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-
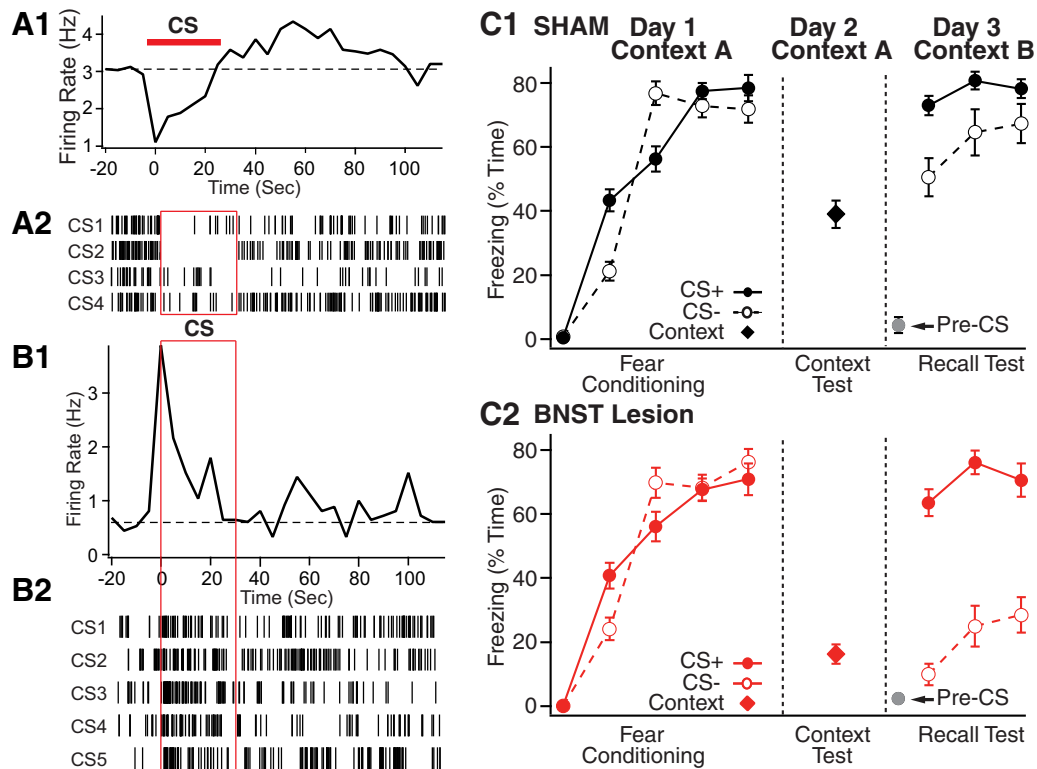
Figure 6. BNST activity alters the processing of discrete threatening stimuli. *A, B*, Two examples of anterior BNST neurons with short-latency responses to discrete threatening cues. These cells were recorded extracellularly in rats that had been subjected to a classical auditory fear conditioning protocol (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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