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The response of neurons in the bed nucleus of the stria terminalis to serotonin: Implications for anxiety

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ABSTRACT

Substantial evidence has suggested that the activity of the bed nucleus of the stria terminalis (BNST) mediates many forms of anxiety-like behavior in human and non-human animals. These data have led many investigators to suggest that abnormal processing within this nucleus may underlie anxiety disorders in humans, and effective anxiety treatments may restore normal BNST functioning. Currently some of the most effective treatments for anxiety disorders are drugs that modulate serotonin (5-HT) systems, and several decades of research have suggested that the activation of 5-HT can modulate anxiety-like behavior. Despite these facts, relatively few studies have examined how activity within the BNST is modulated by 5-HT. Here we review our own investigations using in vitro whole-cell patch-clamp electrophysiological methods on brain sections containing the BNST to determine the response of BNST neurons to exogenous 5-HT application. Our data suggest that the response of BNST neurons to 5-HT is complex, displaying both inhibitory and excitatory components, which are mediated by 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors. Moreover, we have shown that the selective activation of the inhibitory response to 5-HT reduces anxiety-like behavior, and we describe data suggesting that the activation of the excitatory response to 5-HT may be anxiogenic. We propose that in the normal state, the function of 5-HT is to dampen activity within the BNST (and consequent anxiety-like behavior) during exposure to threatening stimuli; however, we suggest that changes in the balance of the function of BNST 5-HT receptor subtypes could alter the response of BNST neurons to favor excitation and produce a pathological state of increased anxiety.

Anxiety disorders affect more than 40 million Americans annually (DuPont et al., 1996), and changes in serotonin (5-HT) functioning have been linked to both their etiology and treatment. Although there is substantial evidence that changes in 5-HT functioning can modulate fear and anxiety-like states in humans and animals alike, the literature is unclear regarding the valence of this modulation, such that treatments that deplete central 5-HT have been shown to produce both anxiolytic or anxiogenic behavioral changes (see (Handley, 1995) for review). This paradox is highlighted by the observation that selective serotonin reuptake inhibitors (SSRIs), which currently are the most-prescribed medication for the treatment of anxiety disorders, produce therapeutic reductions in anxiety only after several weeks of treatment, and yet the acute effects of SSRI treatment often are associated with increases in anxiety-like behavior in both humans and animals alike (Burghardt et al., 2004; Grillon et al., 2007).

Substantial evidence suggests that the activity of the bed nucleus of the stria terminalis (BNST) mediates many forms of anxiety-like behavior in humans and animals (Straube et al., 2007; Walker et al., 2003; Walker and Davis, 2008), leading investigators to suggest that abnormal processing within this nucleus may underlie anxiety disorders in humans, and effective anxiety treatments may depend, in part, on restoring normal BNST functioning. As mentioned above, the most effective treatments for anxiety disorders are drugs that modulate 5-HT systems, either by blocking the reuptake of 5-HT or by modulating the activity of specific 5-HT receptor subtypes. As we will show, the BNST receives a reasonably dense innervation by serotonergic afferents (Commons et al., 2003; Phelix et al., 1992), and multiple 5-HT receptor subtypes are expressed within this region (see below). Despite these important observations,

Abbreviations: 4-AP, 4-aminopyridine; 5-CT, 5-carboxyamidotryptamine; 5-HT, serotonin; 5-HTT, serotonin transporter; 8-OH-DPAT, R(±)8-hydroxydipropylaminotetralin hydrobromide; ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; CRF, corticotropin-releasing factor; DRN, dorsal raphe nucleus; EPSC, excitatory postsynaptic current; GABA, gamma aminobutyric acid; I_A, voltage-dependent A-type potassium current; ICV, intracerebroventricular; I_h, hyperpoarization-activated cation current; $I_{K(IR)}$, inwardly rectifying potassium current; I_{NaP}, persistent sodium current; I_T, low-threshold T-type calcium current; mCPP, metachlorophenylpiperazine; mRNA, messenger ribonucleic acid; RT-PCR, reverse transcriptase polymerase chain reaction; SSRIs, selective serotonin reuptake inhibitors; Ucn II, urocortin II.

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surprisingly few studies have directly examined how neural activity within the BNST is modulated by 5-HT, or how this modulation may be affected by stress. Here, we review our ongoing behavioral, immunohistochemical, genetic, and whole-cell patch-clamp investigations into the effects of 5-HT on BNST function. Our data suggest that the response of individual BNST neurons to 5-HT is complex, and can display both inhibitory and excitatory components, and that this response itself depends on both cell type and the prior stress history of the animal. Postsynaptic 5-HT responses are mediated primarily by activation of one or more of 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_7 receptors. Specifically, we have shown that the activation of $5HT_{1A}$ receptors mediates an inhibitory response to 5-HT in the majority of BNST neurons, and reduces anxiety-like behavior (Levita et al., 2004). Conversely, we describe data suggesting that the activation of 5-$HT_{2A/2C/7}$ receptors mediates an excitatory response to 5-HT, which may be anxiogenic. Moreover, we propose that in the normal state, the net effect of 5-HT release in the BNST is to dampen neural activity via postsynaptic 5-HT_{1A} receptor activation, which would consequently act to reduce anxiety-like behavior. However, during exposure to threatening/stressful stimuli we suggest that changes in the functional expression of 5-HT receptor subtypes in BNST neurons alters their 5-HT response in favor of excitation. This may represent the normal adaptive transient response to changes in environmental pressure. However, prolonged exposure to threatening/stressful stimuli may result in a pathological state of persistent anxiety.

We argue that the complex 5-HT response profile observed in the BNST may 1) help to explain some of the confusion regarding the role of 5-HT in modulating anxiety, and 2) may be altered by stress. Moreover, we argue that changes in the BNST response to 5-HT could represent an important mechanism underlying anxiety disorders in humans. Novel treatment strategies could therefore be designed to reverse stress-induced changes in BNST 5-HT responding and/or make it less likely that these alterations could occur.

1. The bed nucleus of the stria terminalis and anxiety

Studies investigating the neurobiology of fear- and anxiety-like behavior have utilized fear-learning procedures combined with brain-lesion techniques to determine the brain pathways that ascribe affective behavioral responses to neutral stimuli. Within these pathways, these studies have implicated the amygdala as a critical structure in which neutral stimuli are associated with affective behavioral states, and in particular, the amygdala has been shown to be critical for fear responding (see (Davis et al., 1993; Fanselow and LeDoux, 1999; LeDoux, 1993; Maren and Fanselow, 1996)). Importantly, the amygdala has been divided into several subnuclei, including the basolateral amygdala (BLA), which receives thalamic and cortical input from sensory regions, and the central nucleus of the amygdala (CeA), which projects to brain regions that mediate the individual behaviors associated with fear responding. Sensory information from a stimulus with an affective component (i.e. electric shock or a cue previously paired with shock) has been argued to activate the BLA, which in turn activates the CeA to produce a coordinated fear response (see (Davis et al., 1993) for review). Because many anxiety disorders seem to involve exaggerated or inappropriate fear responding, many studies have suggested that malfunction of BLA and CeA neural circuits underlie these disorders in humans. While it is likely that changes in these regions contribute to the etiology of some anxiety disorders, an increasing body of data has implicated the BNST in the mediation of certain fear and anxiety-like behaviors that are not mediated by the anatomically-related CeA pathway (Davis et al., 1997; Davis, 1998; Davis and Shi, 1999; Walker and Davis, 1997, 2008; Walker et al., 2003).

Davis et al. (1997) initially found that excitotoxic lesions of the BNST blocked the enhanced startle response (a behavioral measure of anxiety) observed after central administration of the stress hormone, corticotropin-releasing factor (CRF), whereas CeA lesions did not (Lee and Davis, 1997). Other anxiety-like behaviors that have subsequently been shown to be mediated by the BNST (and not the CeA) include an unconditioned enhancement of the startle response by a prolonged bright light (Walker and Davis, 1997), freezing behavior induced by predator odor (Fendt et al., 2003), fear responding to long-duration conditioned stimuli previously paired with shock (Waddell et al., 2006), and the anxiogenic behavioral changes observed after uncontrollable stress (Hammack et al., 2004). Importantly, the BNST and CeA both receive substantial afferent information from the BLA, and both project to many of the same subcortical regions involved in mediating individual fear responses (see (Davis et al., 1997) for review). Based on these data, Walker and Davis (2003, 2008) suggested that the BNST mediates a sluggish fear response system that controls behavioral responding to diffuse long-duration stimuli and continues to influence behavior long after the stimulus has terminated, while the CeA mediates a rapid response system to specific threat that terminates when the threat is removed ((Walker et al., 2003; Walker and Davis, 2008); Also see Walker, Miles and Davis in this volume). Davis and colleagues initially likened the former response system to "anxiety" and have suggested that maladaptive responding of the BNST may underlie some forms of anxiety disorders in humans. Consistent with this argument, Waddell et al. (2006) found that BNST lesions blocked fear responding to a 10-min tone that was previously paired with shock, but not a 1-min tone previously paired with shock. This group argued that an anxiety state was conditioned to the 10-min tone that was dissociable from the fear state conditioned to the shorter tone (Waddell et al., 2006). Importantly, the BNST has also been linked to fear behavior in nonhuman primates, where BNST activity was positively correlated with individual differences in rhesus monkey fear responding (Kalin et al., 2005). More recently, activity within the BNST has been associated with the anticipatory anxiety experienced prior to the presentation of a phobic stimulus in humans (Straube et al., 2007).

Consistent with a role for the BNST in anxiety-like responding, electrical stimulation of the anterolateral region produces many of the endocrine, cardiovascular and respiratory responses that are normally elicited by anxiogenic stimuli (Casada and Dafny, 1991). Moreover, anxiogenic pharmacological agents increase the expression of transcription factors, such as the immediate early gene, c-fos, in the anterolateral BNST (Singewald et al., 2003). Hence, activation of BNST neurons is thought to be associated with the expression of anxiety-like behavior. Significantly, the anterolateral BNST and CeA, are two extrahypothalamic regions that display high levels of CRF-immunoreactivity (Sawchenko and Swanson, 1985; Swanson et al., 1983) and CRF mRNA (Makino et al., 1994). The dense expression of CRF in cell bodies and fibers in these brain regions suggest that CRF plays an important role in the modulation of neural activity within the BNST, CeA and their projection regions. Extrahypothalamic CRF has been implicated heavily in mediating many of the behavioral responses to stressful stimuli, including increases in anxiety-like behavior (see (Davis et al., 1997; Koob et al., 1993; Koob and Heinrichs, 1999; Schulkin et al., 1998)), and CRF_1 receptor knockout mice exhibit an anxiolytic behavioral profile (Smith et al., 1998). Significantly, local infusion of CRF receptor antagonists into the BNST acts to reduce the anxiogenic response elicited by intracerebroventricular (ICV) CRF injection (Lee and Davis, 1997). Together, these results suggest that release of CRF and/or activation of CRF receptors in the BNST mediate anxiety-like behavior, and further suggest that the anterolateral BNST may be a particularly important subregion for mediating anxiety-like behavioral states.

2. 5-HT and anxiety

A substantial body of literature has implicated 5-HT systems in the modulation of fear and anxiety behaviors (see (Graeff et al., 1996; Handley et al., 1993; Handley, 1995; Lowry et al., 2005, 2008)). However, as mentioned above, the nature of the relationship between 5-HT receptor activation and anxiety-like behavior is still unclear. Hence, 5-HT receptor activation can be anxiogenic or anxiolytic depending on the dosage of the 5-HT agonist used, the brain region targeted, and the method of testing