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Anxiety and how to control it: the functional role of the bed nucleus of the stria terminalis.

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ANXIETY AND HOW TO CONTROL IT: THE FUNCTIONAL ROLE OF THE BED NUCLEUS OF THE STRIA TERMILAS

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A Dissertation Approved on

April 13, 2020

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DEDICATION

This dissertation is dedicated to my mentor and friend, Brendan Depue.

Congratulations on raising your first PhD.

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ABSTRACT

ANXIETY AND HOW TO CONTROL IT: THE FUNCTIONAL ROLE OF THE BED NUCLEUS OF THE STRIA TERMILAS

Lindsay K. Knight

April 13, 2020

Anxiety disorders afflict up to one third of the population. Research to date has primarily focused on the amygdala, however, new perspectives suggest that a tiny basal forebrain region known as the bed nucleus of the stria terminalis (BNST) may hold key insights into understanding and treating anxiety disorders. Therefore, my first aim was to empirically investigate the importance and influence of the BNST in anxiety processing. Using fearful faces and human screams as aversive stimuli, two threat conditions were created: one in which threats were certain and predictable (fear) and another in which threats were uncertain and unpredictable (anxiety). Results indicated that the amygdala showed preferential engagement during fear and displayed functional connectivity with regions involved in stimulus processing and motor response. By contrast, the BNST preferentially responded during anxiety and exhibited functional connectivity with prefrontal regions underlying interoception and rumination. Together, this suggests that the amygdala and BNST play distinct but complementary roles during threat processing, with the BNST specializing in the detection of potential threats to promote hypervigilant monitoring.

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CHAPTER I: INTRODUCTION

Chapter 1 is a slightly modified version of "New Frontiers in Anxiety Research: The Translational Potential of the Bed Nucleus of the Stria Terminalis" published in Frontiers in Psychiatry, Mood and Anxiety Disorders, and has been reproduced here. Under the Frontiers Terms and Conditions, authors retain the copyright to their work. Frontiers permits the use, distribution and reproduction of material from published articles, provided the original authors and source are credited.

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Background

Anxiety disorders are currently the most prevalent subgroup of mental disorders in most western societies, with nearly a 1 in 3 lifetime incidence in the United States (Craske et al., 2017; Kessler et al., 2012). These disorders are not only pervasive, but are frequently chronic and a leading cause of disability worldwide (Griebel & Holmes, 2013). While significant progress has been made in understanding the neural circuitry of threat processing in preclinical studies, these mechanistic advances have not translated to widely efficacious therapies. Promising new treatments either have turned out to be only

moderately effective, or have induced adverse side effects, limiting applicability in clinical practice (Griebel & Holmes, 2013; Hyman, 2013; LeDoux & Pine, 2016).

To date, anxiety disorder research has primarily fixated on the amygdala, with nearly 5000 human neuroimaging studies alone detailing its central role in emotion processing and threat detection (Avery et al., 2016). This line of work has led to well-supported conclusions that anxiety disorders can in part be attributed to hyper-responsivity of the amygdala to perceived threat (Etkin & Wager, 2007), as well as dysregulated prefrontal control over amygdala reactivity due to altered structural or functional connectivity (Quirk & Beer, 2006). Yet discouragingly, this same ventromedial prefrontal (vmPFC) to amygdala circuit dysfunction has also been proposed as a model for many other disorders ranging from depression (Johnstone et al., 2007) to psychopathy (Blair, 2007). While many psychiatric and mood disorders undoubtedly share some semblance of dysregulated emotion processing, explaining this common finding, it is unlikely that this single pathway represents such a broad etiology that could account for the heterogeneous symptomatology and phenotypic dysfunction seen across disorders, or even within a single disorder. Though revolutionary in its initial discovery, this explanation of anxiety disorders now stands as an oversimplification that is ultimately hindering our understanding. The field is in need of the next iteration of specificity. Fortuitously, emerging research suggests that a tiny and lesser-known basal forebrain region may bring about a new wave of insights and opportunities for the development of novel therapeutics. Enter: the bed nucleus of the stria terminalis (BNST).

Distinguishing Anxiety from Fear

Anxiety can be defined as a prolonged state of apprehension brought on by an uncertain or unpredictable prospective threat. In rodents, anxiety-like behaviors can be elicited by physically distant threats such as a predator in the environment, or diffuse contextual threats like a brightly lit open space. While comparable situations can indeed be anxiety-provoking for humans (e.g., dark enclosed spaces), in general, humans are much more prone to encounter *psychological* stressors. Thus an anxious emotional state can be triggered by ambiguously threatening stimuli, or even by internally generated thoughts of real or imagined prospective threats. While the term "anxiety" is often colloquially used interchangeably with "fear", more precisely, fear describes a phasic response to the presence of an immediate and identifiable threat (Avery et al., 2016). However, it should be noted that perception is critical, as a threatening stimulus that is perceived as present or even imagined can activate a fear response.

Corresponding to this psychological dissociation between fear and anxiety, converging evidence suggests that two partially segregated neural circuits support these divergent responses (Davis et al., 2010; Naaz et al., 2019). Spearheaded by Davis and Walker, a highly influential model theorizes that the amygdala underlies phasic responses to fears, supporting feelings of fear, while the BNST, considered part of the "extended amygdala", is thought to mediate more sustained responses to unpredictable, ambiguous or diffuse threats, thus underlying persistent states of anticipation or hypervigilance and promoting feelings of anxiety (Davis et al., 2010). In further support of these distinct functional roles, studies in rodents show that lesioning the amygdala eliminates conditioned fear to auditory (Zimmerman et al., 2007) and visual stimuli (Walker & Davis, 1997) and reduces fear-potentiated startle (Ventura-Silva et al., 2013), but does

not alter anxiety-like behavior in an elevated plus maze (Ventura-Silva et al., 2013) or anxiety-like responses to bright light or corticotropin-releasing hormone (CRH) injection (Walker & Davis, 1997). Conversely, lesioning the BNST attenuates anxiety-like responses (Fendt et al., 2003; Goode et al., 2019; Hammack et al., 2004; Waddell et al., 2006; Zimmerman & Maren, 2011) and alters cortisol release (Sullivan et al., 2004), but importantly, does not affect conditioned fear (Goode et al., 2019; Waddell et al., 2006; Walker & Davis, 1997; Zimmerman & Maren, 2011).

Neurochemical Profile of the BNST

While there is a general consensus for the involvement of the BNST in anxiety processing, the mechanisms are less well understood due to the complexity of the BNST structure and the wide variety of the neurotransmitters it expresses, including GABA, glutamate, noradrenaline (NA), serotonin (5-HT), and CRH, among others (Forray & Gysling, 2004). The literature suggests that glutamatergic and GABAergic neuronal populations have opposing influences, with glutamate promoting anxiogenic effects, whereas GABA reduces anxiety (Gungor et al., 2018). To reinforce this assertion, optogenetic activation of glutamatergic BNST cells projecting to the ventral tegmental area (VTA) were found to be anxiogenic and aversive, while activation of GABAergic BNST cells projecting to the VTA were anxiolytic and rewarding (Jennings et al., 2013b). Moreover, though the GABAergic population dominates in the BNST (Kash et al., 2015), in many cases, the glutamatergic subpopulation exerts a greater overall influence, in part due to higher intrinsic excitability and altered responsivity to NA (Gungor et al., 2018).

The interaction between NA and 5-HT is also believed to contribute to anxiety, with the majority of evidence suggesting that anxiety disorders are characterized by underactivation of serotonergic function and overactivation or complex dysregulation of noradrenergic function (Ressler & Nemeroff, 2000). In adaptive anxiety, release of CRH is met by inhibition via 5-HT, which aids in decreasing reactivity of the BNST and regulating the stress response. Furthermore, while NA ramps up autonomic arousal, raising heart rate and increasing memories of aversive contexts, 5-HT acts to decrease such memories. Thus, dysregulation of this mutually inhibitory system can lead to increased vigilance and aversive behavior due to overactive NA (Ashwani et al., 2011), and decreased inhibition of stress reactivity due to a hyporesponsive 5-HT system (Ressler & Nemeroff, 2000).

The BNST and the Stress Response

CRH has repeatedly been identified as an important contributor to fear and anxiety, and is largely expressed in stress-related brain regions, including the amygdala and BNST. Once more, this points to the BNST as not only a mediator of anxious feelings and behaviors, but a central modulator of the stress response (Lebow & Chen, 2016). The BNST is ideally situated in the brain to stimulate allostatic changes through its dense connections with the paraventricular nucleus (PVN) of the hypothalamus, the primary node of the hypothalamic-pituitary-adrenal (HPA) axis that initiates the stress response and ultimately regulates cortisol release. Perhaps even more compelling, evidence suggests that the BNST's position is important for coordinating neuroendocrine and behavioral responses (Radley & Sawchenko, 2011; Radley & Johnson, 2017). Very few limbic forebrain regions provide direct innervation to the PVN, but the BNST

appears to serve as a point of convergence between these higher-order regions and HPA effector neurons. Furthermore, rather than merely relaying these signals, the BNST has been shown to dynamically integrate information from multiple upstream sources, including the medial prefrontal cortex and hippocampus, to modulate the downstream neuroendocrine and behavioral responses during stress (Radley & Sawchenko, 2011; Radley & Johnson, 2018). Thus, differences in the structural or functional connectivity of the prefrontal-BNST or hippocampal-BNST pathways could bias an individual towards different coping styles or alter susceptibility toward anxiety and other stress-related disorders. With this understanding of the BNST's role in mediating anxiety and the stress response, a renewed emphasis has been placed on the investigation of the human BNST throughout the past decade, although research in humans, and specifically in relation to anxiety and other stress-related disorders, is still in its infancy.

The Human BNST

The human BNST is a small medial basal forebrain structure, about 1/10 the size of the amygdala. On human MRI images, the BNST sits posterior to the nucleus accumbens, inferior to the lateral ventricles, and medial to the internal capsule and caudate, and just anterior to the crossing fibers of the anterior commissure (Avery et al., 2016; Theiss et al., 2017; Figure 1). Two major white matter tracts are known to emanate from the BNST. Most prominently, a white matter bundle known as the stria terminalis extends superiorly and the anteriorly from the amygdala, wrapping around the thalamus in a C-shape before descending to the BNST (Price & Amaral, 1981). The second and lesser studied, but more direct connection, is the ventral amygdalofugal pathway, which consists of a group of fibers that provide a direct dorsal-ventral link between the

amygdala and the BNST (Porrino et al., 1981). Diffusion tensor imaging (DTI) studies have suggested an additional and novel structural connection in human from the BNST to the temporal pole (Avery et al., 2014).

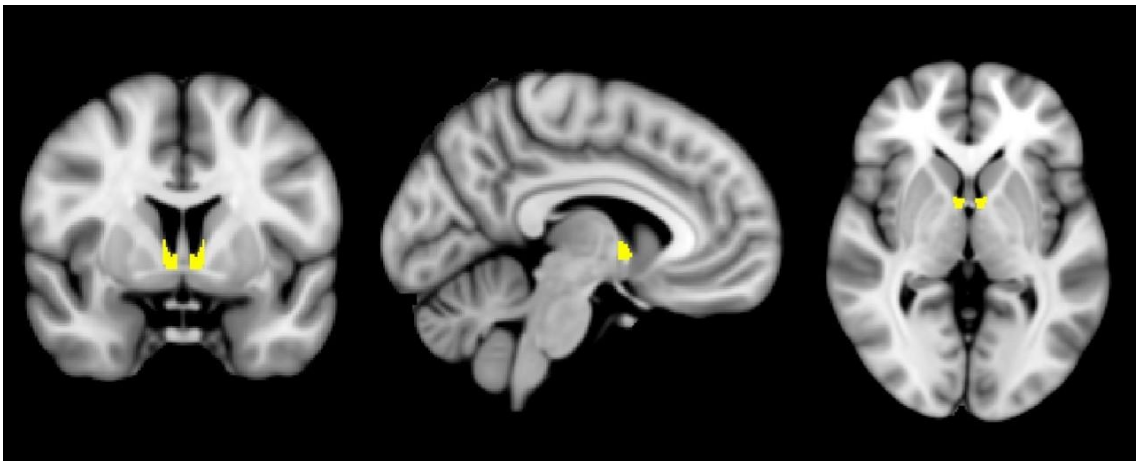

Figure 1. The Human Bed Nucleus of the Stria Terminalis (BNST). A mask of the BNST (from Avery et al., 2014) is shown highlighted in yellow, overlaid on a standardized average brain.

Functionally, even less is known about the BNST in humans, in part due to the combination of its small structure size and the relatively low spatial resolution of standard functional MRI (fMRI). With just 12 to 18 sub-nuclei comprising the BNST (Lebow & Chen, 2016) and at approximately 190 mm^3 – the size of a sunflower seed – the BNST is so small that many human neuroimaging studies have qualified their reported results with statements such as "a region overlapping" or "consistent with" the BNST (Avery et al., 2016). However, with recent advances in neuroimaging technology, including improvements that permit a 27x increase in spatial resolution (e.g. $3mm^3$ to $1mm^3$), new opportunities await to reinvigorate the investigation about the distinction between fear and anxiety in humans, and the relative importance and influence of the BNST in cognitive health and dysfunction.

Studies that have begun to approach these questions in humans have described complementary findings to the pioneering work of Davis and Walker. For example, work by Alvarez and colleagues (2011) reported a similar dissociation in the functional roles of the amygdala and BNST using a combination of cued and contextual threats. During fMRI scanning, participants were placed in three pre-recorded virtual reality environments: a restaurant, casino or bank. One environment served as a predictable threat context in which electric shocks were consistently delivered following an auditory tone. In the other two contexts, the tone was meaningless, with shocks being administered in an un-signaled or semi-random manner in one environment (unpredictable threat), and no shocks being delivered in the control context. Results showed that amygdala activity transiently increased at the onset of both threat contexts, but only the unpredictable threat context yielded sustained activity in the BNST, supporting previous animal models of phasic and sustained fear. Additional investigations in humans have helped uncover a more nuanced role for the BNST, suggesting that rather than simply mediating sustained responses to threats, the BNST appears to exhibit a specialized role in detecting potential threats when the specifics of the threat are uncertain. In another study, participants viewed videos of a line fluctuating in height over time and were told that each time the line exceeded a certain threshold, they would accumulate an electric shock to be delivered after the task (but in fact, participants were never actually shocked). During this time of anxiously anticipating future shocks, there was robust BNST activity, but the amygdala showed minimal task-modulated activity even at exploratory statistical thresholds (Somerville et al., 2010). As a result, the BNST was given a new title of "threat monitoring", and in support of this notion, further reports demonstrated the

BNST's ability to track threat proximity in both the physical sense (e.g., monitoring the distance of an approaching tarantula; Mobbs et al., 2010) and the psychological sense (e.g., tracking the likelihood of threat occurrence; Somerville et al., 2010).

Studies subsequently sought to separate out the responses related to the anticipation or monitoring of a prospective threat, relative to actual threat confrontation (i.e., presentation of aversive stimulus). In two closely related but independent studies, BNST activity was found to be significantly elevated during uncertain threat anticipation, while it was the amygdala that exhibited a significant response during the aversive outcome (Klumpers et al., 2017; Naaz et al., 2019). In sum, these findings suggest that a regional dissociation can be attributed to the BNST playing a role in helping to detect a potential threat and maintain hypervigilance until threat encounter or situational resolve, while the amygdala preferentially responds to the actual presence of an aversive stimulus, mediating instantaneous responses during acute danger. Therefore, given that human anxiety is largely driven by future-oriented hypothetical threats that may never occur, studies involving the BNST stand at the forefront of essential future research.

The BNST and Clinical Anxiety

Anxiety disorders are characterized by both excessive fear and anxiety. However, elucidating the mechanisms of sustained anxious states and regulation of the stress response, both processes mediated by the BNST, appear to be especially relevant, and not just in the case of generalized anxiety disorder (GAD). For example, individuals with posttraumatic stress disorder (PTSD) not only suffer from conditioned fear to cues that evoke traumatic memories, but they also exhibit persistent symptoms of sustained anxiety (e.g., hypervigilance). Similarly, in panic disorder (PD), though a hallmark is the

experience of panic attacks, another key element is anxiety caused by persistent apprehension and continuous worry about the recurrence of future panic attacks (Grillon, 2008). Even specific phobia, the prototypical "fear disorder", involves episodes of sustained anxiety when anticipating a future confrontation with their phobic fear (Grillon, 2008). Finally, intolerance of uncertainty, or an inability to cope with potential negative outcomes, is an established hallmark of GAD, but may also be a transdiagnostic feature of Obsessive-Compulsive Disorder (OCD), such that compulsions and ritualistic behaviors are performed as a means to reduce this distress (Holaway et al., 2006).

Evidence from human neuroimaging studies reinforces the role of the BNST across anxiety disorder subtype. One study in GAD patients found higher arousal and increased activation in the BNST when exposed to a gambling game with high monetary uncertainty (Yassa et al., 2012). Similarly, relative to healthy controls, GAD patients exhibited enhanced phasic activity in the amygdala and heightened sustained activity in the BNST when faced with a temporally unpredictable threat exposure involving human screams (Buff et al., 2017). Utilizing the same experimental paradigm, Brinkmann and colleagues (2017a, 2017b) found corresponding results in both PTSD and PD, with patients displaying sustained activation in the BNST during unpredictable anticipation of aversive sounds, relative to controls. Human neuroimaging investigations have additionally explored the role of the BNST in patients with specific phobia when anxiously anticipating the presentation of phobogenic stimuli (e.g., spiders). Under conditions of unpredictable sustained anticipation, patients showed increased activation in anterior cingulate cortex and once more, the BNST (Münsterkötter et al., 2015; Straube et al., 2007), while the predictable phasic fear condition was associated with elevated

amygdala activity (Münsterkötter et al., 2015). Together, these studies further strengthen the case for distinct functionality of the amygdala and BNST, and indicate heightened and prolonged reactivity of the BNST may be a contributing factor to clinical anxiety disorders.

Oversights and Opportunities

Although this relationship between uncertainty about future adverse events and anxiety makes intuitive sense, this conceptualization of anxiety has not been reflected in many neuroimaging investigations aimed at elucidating the neurocircuitry of clinical anxiety disorders. This is principally true in studies investigating how emotion is regulated. Dysregulated emotion is a hallmark of many psychiatric disorders including anxiety disorders, and consequently, a strong focus has been placed on uncovering the neural mechanisms supporting effective emotion regulation (ER) due its significance and potential applicability transdiagnostically. Typically, ER is studied in the context of individuals attempting to volitionally control their emotional response to explicitly cued and overtly displayed pictorial stimuli (negative scenes or faces), through reappraisal or distancing/suppression strategies (Depue et al., 2015; Ochsner et al., 2002; Ochsner et al., 2004). This work indicates that the degree of regulating subjective negative emotion is dependent upon the strength of functional and structural connections between the vmPFC and the amygdala, which is likely mediated by higher-order lateral prefrontal regions to ultimately downregulate amygdala activity through top-down goal-directed behavior.

However, three critical barriers arise when this line of research is intended to specifically elucidate ER mechanisms in the context of anxiety disorders. First, many ER studies utilize stimuli meant to induce disgust or general negative affect rather than

simulate ecologically relevant threats. Secondly, because the predominant focus of ER research has been centered on emotion control during the *overt* display of such aversive stimuli, these tasks do not capture the psychological processes at the heart of anxious pathology – namely, anticipatory cognitive and affective processes in the face of uncertain or unpredictable threats – and instead essentially uncover mechanisms needed to regulate general negative affect or disgust *after* a concrete stimulus has been presented. In light of this, recent studies have attempted to model threat anticipation more precisely to explicate the complex underlying neural circuitry (Grupe & Nitschke, 2013). Furthermore, other lines of research are deriving more nuanced views of how attentional control may modulate anxiety-potentiated coupling between medial prefrontal and amygdala circuitry (Robinson et al., 2016). Nevertheless, the field remains in critical need of work that definitively targets *anxiety* regulation. Finally, despite growing research demonstrating that the BNST is a primary mediator of both anticipatory anxiety and the stress response, the BNST is essentially absent from ER literature. As a result, many crucial outstanding questions remain: How does the brain regulate thought and feeling in anticipation of uncertain and unpredictable threats? If the amygdala can be downregulated after a stimulus has been presented, can the BNST also be downregulated prior to stimulus presentation? If so, what are the mechanisms and does this downregulation reduce subjective feelings of anxiety? Does this then subsequently change processing of the overt stimulus?

Study Motivation

It is well known that dysregulated emotion is a primary contributor to impaired functioning in anxiety disorders, however, studies to date have only investigated the

mechanisms of effective ER in the context of controlling one's emotional response to the presence of explicitly cued emotional stimuli, when anxiety by definition, is a sustained response to an uncertain or unpredictable threat. Therefore, current ER paradigms are only investigating the mechanisms underlying the phasic response to overt fearful or disgusting stimuli. No study to date has investigated the neural mechanisms underlying the down regulation of anxious feelings, which is arguably central to the majority of symptoms in anxiety disorders and a primary purpose for seeking treatment. Furthermore, despite evidence suggesting the BNST's involvement in anxiety and the stress response, the BNST is frequently overlooked in human anxiety literature and has never been studied in the context of emotion regulation. Therefore, the primary goals of this dissertation is to investigate the relative importance and influence of the BNST in generating anxiety (Chapter 3), and moreover, to elucidate the neural mechanism supporting *anxiety regulation* (Chapter 4).

To effectively investigate these topics, these projects took a three-pronged revised approach. The first factor is the use of a novel study design, developed to specifically elicit anticipatory anxiety in an ecologically valid and socially relevant manner using fearful human faces and human screams as aversive stimuli (Chapter 3). This paradigm was then modified and combined with a standard ER paradigm to specifically target *anxiety regulation* (Chapter 4). The second component is the use of improved technology, including high-resolution fMRI imaging (1.5-2.0 mm^3) and careful delineation of the BNST and amygdala nuclei groups (basolateral amygdala – BLA; central amygdala – CEA) through ultra-high-resolution anatomical masks (.65mm). Finally, these investigations take both a focused region of interest (ROI) approach, along with a whole-

brain network-level approach to characterize how large-scale networks that support diverse cognitive processes (e.g., attentional networks, somatomotor networks) are modulated and integrated with these ROIs to support anxiety and its regulation. Thus, through this work, this dissertation aims to answer: 1) Is there evidence that the BNST is preferentially involved in anxiety processing in humans (i.e., responsive to uncertain and unpredictable prospective threat? 2) Can BNST activity be volitionally downregulated (in a similar manner to work that has shown downregulation of the amygdala)? 3) If so, what are the prefrontal control mechanisms? 4) Does downregulation of the BNST correspond to decreased feelings of anxiety? and 5) How do large-scale networks subserving other cognitive functions contribute to increased anxiety as well as support the regulation of anxious feelings? In this way, this work will help to develop models of anxiety regulation in cognitively healthy individuals. Following these foundational studies, future work can subsequently refine models for how these BNST-mediated circuits may be altered in specific clinical populations, and additionally explore how therapeutic and pharmacological interventions may strengthen BNST-regulatory networks.

CHAPTER II: GENERAL METHODS

In the current studies, many methodological details were consistent across studies. They are briefly introduced here.

Participants

Participants were recruited through on-campus flyers and an online research participation system (SONA Systems), and were paid for their participation. All participants were required to answer an MRI screening questionnaire to ensure their safety in an MR environment. In addition, participants were at least 18 years of age, right-handed, native English speakers, with normal or corrected-to-normal vision and hearing, and had no disclosed history of neurological or psychiatric disorders. These exclusion criteria are standard in neuroimaging research to reduce potential confounds due to handedness, differences in perceptual abilities, or effects of psychiatric drugs. Participants are screened for being a native English speaker as there may be difficulty in interpreting task instructions as a result of language. Every effort was made to recruit an equal number of male and female subjects in each study, and to ensure that minorities were represented in proportion to the composition of the local community.

Recruited participants were fully informed and made as comfortable as possible in order to maximize retention rates. Candidate subjects responding to these notices received a brief description of the research and completed prescreening questions over

the phone. When arriving to participate in a study, participants were familiarized with the protocol by the experimenter, including risks and benefits of the research. In the case of fMRI sessions, participants also completed a detailed screening form to indicate any contraindications based on a superset of the Society for Magnetic Resonance in Medicine standardized MRI screening protocol (absolute exclusions for ferrous metal in any part of body, such as pacemakers, cochlear implants, surgical clips or metal fragments, serious medical conditions, claustrophobia). To protect against potential risks of boredom, fatigue, or frustration, participants were allowed rest breaks as needed. Participants' comfort levels were monitored throughout the session. Participants could communicate with the experimenter at all times. It was made clear that participation was voluntary and that participants could withdraw from the study at any time without penalty or prejudice. Any questions that the subjects had were answered by the experimenter. After testing, participants were debriefed as to the purpose and predictions of the experiments. Written informed consent was obtained prior to all experimental sessions, and experimental protocols were approved by University of Louisville's Institutional Review Board prior to data collection.

Scanning Methods

Stimuli

Images of fearful and neutral faces (White and Black, male and female faces) were acquired from the Chicago Face Database (Ma et al., 2015). Audio clips of aversive human screams were used for threat conditions. Additionally, multitalker babble (neutral human sounds) and nature sounds of a flowing river and chirping birds (neutral nature sound) were used for control conditions. All audio clips were edited to 2 seconds in

length and normalized for loudness with MP3Gain. During scanning, visual stimuli were displayed through ePrime onto an Invivo Esys LCD TV monitor at the back of the scanner bore, which was viewed by participants through a mirror on the head-coil. Auditory stimuli were present binaurally through headphones at a predetermined constant level.

Imaging Data Acquisition

Structural Images

All structural MRI images were acquired using a Siemens 3-Tesla Skyra MR scanner located at the University of Louisville, School of Medicine. A 20-channel head coil was used for radiofrequency reception. Participants were given earplugs to reduce scanner noise, and were additionally given headphones to receive instructions and auditory stimuli. Foam padding was added to limit motion if additional room remained within the head coil, and a piece of folded tape was placed over the participant's forehead as a reminder to remain still throughout the scan. Structural images were obtained via a T1-weighted magnetization-prepared rapid gradient-echo sequence (MPRAGE) in 208 sagittal slices. Imaging parameters were as follows: echo time (TE) = 2.26 ms, repetition time (TR) = 1700 ms, flip angle = 9.0°, field of view (FoV) = 204 mm, 208 sagittal slices, and voxel size = 0.8 x 0.8 x 0.8 mm. Scan parameters were consistent for all imaging sessions associated with these studies.

Functional Images

Functional blood oxygenation level-dependent (BOLD) images were collected using gradient-echo T2*-weighted echoplanar imaging (EPI). Parameters were optimized

for individual studies and are discussed within the respective methods sections of each study.

Imaging Data Analysis

Image processing was implemented using the FSL package (Analysis group, FMRIB, Oxford, UK, http://www.fmrib.ox.ac.uk/fsl/). A standard pre-processing pipeline was applied: MCFLIRT – linear slice-time correction/motion correction, optiBET – brain extraction (Lutkenhoff et al., 2014), time-series prewhitening, high pass filter (0.01 Hz), and registration and spatial normalization to the Montreal Neurological Institute (MNI) 152-T1 1-mm template. Individual's functional images were first registered to their high-resolution MPRAGE scans via a 6 parameter linear registration, and the MPRAGE images were then in turn registered to the MNI template via 12 parameter non-linear registration (Andersson et al, 2007). These registrations were combined in order to align the functional images to the 1-mm isotropic voxel standard space template. Functional images were smoothed with a Gaussian kernel of 6-mm full-width at half-maximum (FWHM) for whole-brain analyses and 3-mm FWHM for ROI analyses.

Following preprocessing, lower-level statistics were be implemented in fMRI Expert Analysis Tool (FEAT). Using multiple regression analysis, statistical maps representing the association between the observed time series (e.g., BOLD signal) and one or a linear combination of regressors for each subject were constructed. For each regressor, a double-gamma HRF was convolved with an event vector starting at the stimulus onset with an appropriate trial duration for each condition. Contrasts of interest were formulated as linear combinations of the main regressors. Lower-level models were then passed to group-level analyses using mixed effects models (FLAME 1+2) and

outlier de-weighting to combine and spatially normalize all subjects. The higher level models employed non-parametric permutation methods through FSL's randomise function (Nichols & Holmes, 2002) using the Threshold-Free Cluster Enhancement (TFCE) method, which detects clusters of contiguous voxels without first setting an arbitrary statistical cut-off (e.g., $Z > 2.58$), and controls the family-wise error (FWE) rate at $p < .05$ (Smith & Nichols, 2009). Each contrast underwent 5000 permutations. Randomise produces corrected 1-*p* statistical maps, which were used for all statistics in figures and tables. Fslview was used to produce brain images for figures.

Region of Interest (ROI) Masks

Ultra-high-resolution anatomical masks (normalized to MNI space) were acquired to accurately delineate the BLA (0.65 mm^3; Leal et al., 2014, 2017) and the BNST (0.60 mm^3; Avery et al., 2014) for ROI analyses, kindly shared by the authors. To ensure optimal regional alignment for veritable signal extraction, two types of registration were explored: the Advanced Normalization Tool (Avants et al., 2009) and FSL's three-stage registration. Two individuals viewed and compared each mask on participants' EPI images relative to a standard brain and independently confirmed the use of FSL's registration. Following masking of these regions, FSL's featquery was used to extract percent signal change (PSC) from each ROI. Factorial ANOVAs or follow-up *t*-tests, as appropriate, were then performed to assess differences in functional activation.

CHAPTER III: EXPERIMENT 1 – FEAR VS. ANXIETY

Chapter 3 is a slightly modified version of "Explicit and Ambiguous Threat Processing: Functionally Dissociable Roles of the Amygdala and Bed Nucleus of the Stria Terminalis" published in the Journal of Cognitive Neuroscience. This article has been reproduced here with permission granted by the copyright holder. My role in this work included developing the novel fMRI paradigm, recruiting participants, and contributing to data collection and statistical analysis. I created all figures and tables and all writing is my own.

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Aims

One approach researchers have taken to more specifically investigate anxiety, is to differentiate anxiety from fear. Psychologically, anxiety can be defined as a prolonged state of apprehension elicited by an uncertain or unpredictable prospective threat. While the term "anxiety" is often used interchangeably with "fear," more precisely, fear describes the phasic response to an immediate and identifiable threat. Correspondingly, converging evidence has suggested that this subtle psychological distinction between fear

and anxiety is paralleled by partially segregated neural circuits (Avery et al., 2016). Spearheaded by Davis and Walker, this highly influential model theorizes that responses to phasic and sustained threats are mediated, respectively, by the amygdala and the bed nucleus of the stria terminalis (BNST) — a basal forebrain region considered part of the "extended amygdala." (Davis, 1998; Davis et al., 2010; Walker et al., 2003). In early versions of this hypothesis, a strict double dissociation was proposed, suggesting that the amygdala mediates phasic responses to Fear (fear), while the BNST responds gradually and displays more sustained responses to unpredictable, ambiguous or diffuse threat (anxiety). This hypothesis has since been revised to suggest a more subtle functional segregation, proposing that the amygdala contributes to both phasic and sustained fear, with the medial division of the central nuclei mediating phasic fear, while the lateral nuclei and its projections to the BNST underlie sustained anxious responses (Davis et al., 2010).

Several neuroimaging investigations in humans have further supported a functional dissociation between the amygdala and BNST during threat processing. In one study, three distinct virtual reality contexts were used to indicate safety, predictable threat of shock or unpredictable threat of shock, respectively. In line with previous animal literature, transient activity in the amygdala was found to be greatest during predictable threat, while the BNST showed a positive linear trend in both transient and sustained activity from safety, to predictable threat, to peak responsivity in unpredictable threat contexts. These results were interpreted to suggest that a phasic fear responses are mediated by transient activity in the amygdala, but that in situations of prolonged exposure to threat, this transient amygdala response may give way to activation of the

BNST in order to maintain anxiety (Alvarez et al., 2011). Years later, Klumpers and colleagues presented complementary results using a shock paradigm with cues signaling safety or potential threat. Comparing the anticipatory period waiting period following a threat cue to the moment of shock confrontation, no evidence for amygdala involvement was found during shock anticipation, but robust amygdala activation was observed during the actual aversive outcome (shock). In comparison, the BNST was found to be significantly elevated during shock anticipation. Though the findings generally support a similar regional dissociation, due to the nature of the study design, these results indicate that the BNST may instead give way to the amygdala, with the BNST playing a role in helping to predict potential outcomes, while the amygdala mediates instantaneous responses during acute danger (Klumpers et al., 2017).

However, still others advocate a different view. Contrary to the notion that the amygdala is primarily involved in phasic fear, sustained changes in amygdala activation and connectivity have been observed during extended periods of anticipatory threat (McMenamin et al., 2014). Furthermore, in a recent review, Shackman and Fox (2016) amalgamated work, which suggests that both the amygdala and BNST exhibit similar functional profiles in response to a variety of aversive threats. Many of the studies reviewed demonstrate that both the amygdala and BNST display phasic responses to immediate and short-lived threat, both regions are engaged by uncertainty or anxiety, and both show heightened activity during sustained exposure to threat (Shackman & Fox, 2016). This suggests that the prominent view of a strict functional dissociation warrants reevaluation, and additional thorough investigation examining the specific nature of the differential contributions of the amygdala and BNST is needed.

The lack of consensus in the field regarding the roles of the amygdala and BNST in threat processing may in part stem from differences in how paradigms separate aspects of threat to psychologically elicit both fear and anxiety. Furthermore, much of the work of Davis and colleagues was drawn from animal studies, which typically evaluate defense behaviors, while human studies, and the human experience, incorporate subjective feelings. Finally, the combination of the very small size of the BNST and the relatively low spatial resolution of standard fMRI presents an obstacle, one which may cause a false assumption of BNST activation or misattribution of activity to another region, and thus discrepancies in reported results.

Hypotheses

Therefore, in the present study I aimed to further empirically test and delineate the neurobiological mechanisms underlying these theoretical models using high-resolution fMRI (1.5 mm^3), as well as employing careful delineation of basolateral amygdala nuclei group (BLA) and the BNST using ultra high-resolution anatomical masks (Avery et al., 2014; Leal et al., 2014; Leal et al., 2017). To investigate the functional activation and connectivity profiles of the amygdala and BNST during threat processing, the Threat Anticipation Task was designed to vary threat on two key dimensions: certainty/uncertainty of threat occurrence, and immediacy/temporal unpredictability of an aversive outcome. From this, two threat conditions were created, one in which threat was certain and predictable (Fear), and another in which threat was uncertain and unpredictable in order to elicit anxious anticipation (Anxiety). I hypothesized that, in line with the newer proposed models, both the amygdala and BNST would show heightened responses to Fear and Anxiety, but would display functional dissociations in their degree

of activation, with the amygdala responding more to Fear and the BNST to Anxiety, in the manner proposed by Davis and Walker. Similarly, although some degree of overlap in the connectivity profiles of the BLA and BNST was anticipated, I hypothesized that relative to the BNST, the BLA would show increased connectivity with stimulus processing and motor response regions (Klumpers et al., 2017), supporting the notion that the amygdala is more closely tied to phasic responses to immediate and identifiable threats (fear), while the BNST would show relatively increased connectivity to medial prefrontal regions (Klumpers et al., 2017), supporting its role in more prolonged states of apprehension (anxiety) through worry and rumination. Finally, using self-report questionnaires to measure state and trait anxiety, and worry and rumination, I hypothesized that higher scores in anxiety-related traits would mirror the group analyses during threat, relating to increased activity in the BLA and BNST, increased connectivity between the BLA and sensorimotor processing regions, and increased connectivity between the BNST and higher-order medial prefrontal regions. Finally, if differences were to emerge between anxiety-like traits, I would hypothesize that worry and rumination, the more cognitive aspects of anxiety, would be most closely linked to connectivity between the BNST and the medial prefrontal cortex (Paulesu et al., 2010).

Methods

Participants

A total of 20 healthy young adults (16 females) participated in the study (mean age = 20.2, SD = 1.88). Written informed consent was obtained prior to experimental sessions, and experimental protocols were approved by University of Louisville's

Institutional Review Board prior to data collection. No participants were excluded from any analyses.

Procedure

The study was divided into two consecutive days. On the first day, participants visited the laboratory to provide consent, read through task instructions and complete self-report questionnaires measuring personality traits: State-Trait Anxiety Inventory – STAI (Spielberger et al., 1970), Penn State Worry Questionnaire – PSWQ (Meyer et al., 1990), and Rumination Response Scale – RRS (Treynor et al., 2003). Importantly, participants were also instructed to complete a short practice round of the Threat Anticipation Task in order to become familiar with the paradigm and to reduce any startle response that would not be amenable to scanning. The practice round of the Threat Anticipation Task was composed of 10 trials, organized in mini-blocks of two successive trials of the same condition to simulate the actual task, with the order of condition blocks pseudorandomized. The number of practice trials per condition was: 2 Fear, 2 Neutral, 4 Anxiety (2 with an aversive outcome and 2 with a neutral outcome), and 2 Wait. On the second day, participants completed the functional magnetic resonance imaging portion of the study at the University of Louisville, School of Medicine, followed by post-scan ratings of visual stimuli.

Scanning Paradigm

In the Threat Anticipation Task (Figure 2), participants were presented with human faces paired with different sounds (human screams, multitalker babble, or nature sounds). The task contained four conditions: Fear, Anxiety, Neutral and Wait. A cue (500 ms) was presented at the beginning of each trial to indicate condition. To evoke threat

processing that elicited imminent fear (Fear) or anticipatory anxiety (Anxiety), the likelihood of aversive outcome (fearful face + human scream) and onset time of aversive stimulus presentation were manipulated.

Fear trials were cued by a "100%" circumscribed by a red triangle (500 ms). Participants were informed that a red triangle indicated a potential threat, and the probability within the red triangle signaled the likelihood that the aversive outcome would occur (i.e., for the Fear condition, there was a 100% certainty that the fearful face + human scream would be presented). Immediately following the cue in Fear trials, a fearful face and human scream were presented (2000 ms). Aversive stimuli were followed by an inter-trial interval (ITI) of 1500 ms (total trial length = 4000 ms; 24 trials). Thus, in the Fear condition, threats were both certain and predictable (Figure 2).

The Anxiety condition simulated uncertain and unpredictable threats by varying the likelihood of aversive outcome and onset time of aversive stimuli presentation. Anxiety trials were cued with a red triangle containing probabilities of 80%, 60%, 40% or 20% that a fearful face and scream would occur, creating event uncertainty. Additionally, cues were followed by a variable delay period during which a black screen was shown. Participants were informed that a fearful face and scream could occur at any time, creating temporal unpredictability (in actuality: range of 500-5000 ms). On trials when aversive outcomes did not occur, a neutral face and nature sounds were instead presented (2000 ms). Thus, in the Anxiety condition, threats were both uncertain and unpredictable (anxiety). Anxiety trials were formulated such that within each cue probability, aversive stimuli did occur that percentage of the time (e.g., the 60% cue was used 12 times and aversive stimuli were presented following 7 of those 12 trials, or 58.33% of the time).

Across all probability conditions of Anxiety trials, aversive stimuli occurred 50% of the time (24 aversive trials and 24 neutral trials). Stimulus presentation was followed by a variable ITI (500 – 5000 ms, total trial length = 8000 ms; 48 trials) (Figure 2).

Each of the threat conditions was matched with a control condition, cued by blue squares. Participants were instructed that a blue square signaled safety. Neutral trials were cued with a blue square containing 100% probability, which was immediately followed by a neutral face paired with multitalker babble (total trial length = 4000 ms; 24 trials). Wait trials were cued with a blue square containing either a 60% or 20% and were followed by the same variable waiting period as Anxiety trials. However, event outcomes were either a neutral face and multitalker babble, or a neutral face and nature sounds (total trial length = 8000 ms; 24 trials). Participants were informed that aversive stimuli (fearful face + scream) would never occur during Neutral or Wait conditions (Figure 2).

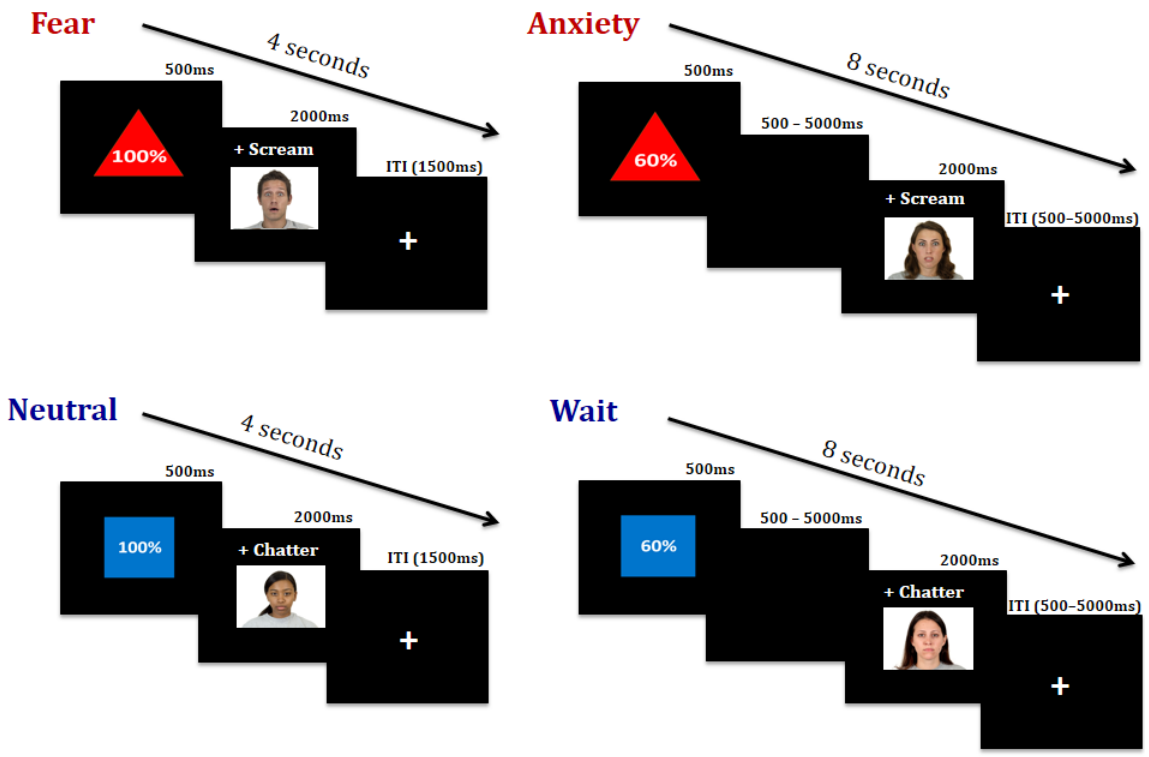

Figure 2. Scanning Paradigm for Experiment 1. Example trials for the Fear, Anxiety, Neutral and Wait conditions. In trials where cued stimuli did not occur (Anxiety: fearful face and human scream; Wait: neutral face and multitalker babble), a neutral face and nature noises where instead presented.

The Threat Anticipation Task employed a hybrid event-related design that contained mini-blocks of 16 seconds (4 Fear/Neutral trials, or 2 Anxiety/Wait trials per mini-block). Mini-blocks were presented in a pseudorandom order. This design was chosen to balance considerations for the psychological state of the participant with statistical power. Each condition consisted of 24 trials, with the exception of the Anxiety condition where 48 trials were presented (24 aversive outcome and 24 neutral outcome), to ensure the estimation of activation was equal across threat conditions when analyzing trials in which aversive stimuli were presented [Fear (100% aversive occurrence x 24

trials) and Anxiety (50% overall aversive occurrence x 48 trails)]. Following each mini-block, an additional pseudorandom variable ITI (jitter) was incorporated to increase design efficiency for hemodynamic response estimation (0 - 14000 ms). Finally, a fixation cross was presented for 30 seconds in the beginning and end of the task, which was utilized for additional low-level baseline estimation for the fMRI analysis.

After scanning, participants rated all faces, presented in a pseudorandom order, using a seven-point Likert scale to assess valence (1 = Extremely Pleasant, 4 = Neutral, 7 = Extremely Unpleasant). A unique face was presented on every trial (total = 120). Behavioral data were analyzed using SPSS (Version 25.0.0; SPSS, INC.). A probability level of $p < 0.05$ was considered statistically significant.

Neuroimaging Methods

Functional Imaging Data Acquisition

Functional blood oxygenation level-dependent (BOLD) images were collected using gradient-echo T2*-weighted echoplanar imaging [(EPI); TR = 3000 ms; TE = 30 ms; multi-band accelerated factor 2; FoV = 192 mm; 78 transverse slices with whole-brain coverage, 1.5mm^3 voxels, flip angle = 90°]. Slices were oriented obliquely along the AC–PC line. An additional high contrast full-head BOLD image was obtained to facilitate three-stage registration (TR = 7390 ms; TE = 30 ms; FoV = 192 mm; 100 transverse slices, 1.5 mm^3 voxels, flip angle = 90°).

Functional Analyses

Image preprocessing and data analysis were implemented using the FSL package (version 5.0.9, Analysis group, FMRIB, Oxford, UK http://www.fmrib.ox.ac.uk/fsl/) as described in Chapter 2. Following preprocessing, Lower-level statistics were

implemented in FEAT. Using multiple regression analysis, statistical maps representing the association between the observed time-series (e.g., BOLD signal) and one or a linear combination of regressors for each subject were constructed. Regressors for the main effects were constructed by modeling each of the conditions versus low-level fMRI baseline (ITI, Jitter, and fixation): Fear, Anxiety (only trials with an aversive outcome), Neutral, Wait, and a dummy variable modeling the Anxiety trials in which neutral stimuli were presented. The contrasts of interest were created by comparing threat conditions against one another: Fear > Anxiety, and Anxiety > Fear. For each regressor, a double-gamma hemodynamic response function (HRF) was convolved with an event vector starting at the cue onset through stimulus presentation (duration of 2500ms for Fear and Neutral; duration of 3000-7500ms for Anxiety and Wait). In addition to modeling the whole trial of the Fear and Anxiety conditions, individual trials epochs were evaluated. For Fear, one model contained the Cue epoch (500 ms) and another model examined the Stimulus epoch (2000 ms). For Anxiety, the first model assessed the Cue+Delay epoch (1000-5500 ms) and a separate model contained the Stimulus epoch (2000 ms). Higher-level analyses were conducted using FLAME 1+2 to combine and spatially normalize all subjects. The Higher-level models employed nonparametric permutation methods through FSL's randomize function (Nichols & Holmes 2002). Paired-sample t-tests for each contrast of interest were performed using the Threshold-Free Cluster Enhancement (TFCE) method, which detects clusters of contiguous voxels without first setting an arbitrary statistical cutoff (e.g., $Z > 2.58$), and controls the family-wise error (FWE) rate at $p < .05$ (Smith & Nichols, 2009). Each contrast underwent 5000 permutations. Randomize produces corrected 1-p maps, which were used for all figures and tables. A

conjunction analysis was additionally conducted by thresholding TFCE corrected maps ($p < .05$) for Fear and Anxiety main effects and then combining these maps to visualize commonalities between Fear and Anxiety processing. Figures of statistical brain maps were created using FSLview.

Ultra high-resolution anatomical masks (normalized to MNI space) were acquired to accurately delineate the BLA (.65 mm^3; Leal et al., 2014; Leal et al., 2017) and the BNST (.60 mm^3; Avery et al., 2014) for ROI analyses. As stated in the introduction, Davis has shown that the medial division of the central nucleus of the amygdala (CEA) may mediate fear, while the lateral portion of the CEA mediates anxiety. Since this level of resolution could not be achieved, only the BLA was selected in an attempt to cleanly dissociate between the roles of the amygdala and BNST. Figure 3 shows these registered masks on the MNI 152 T1-1mm standard brain and a representative subject. Following masking of these regions, FSL's featquery was used to extract percent signal change (PSC) from each ROI. Only main effects were modeled for this analysis to associate discrete HRF responses for the conditions of interest vs. low-level baseline. A 2x2 factorial ANOVA and follow-up t-tests were then performed to assess differences in functional activation across regions by condition.

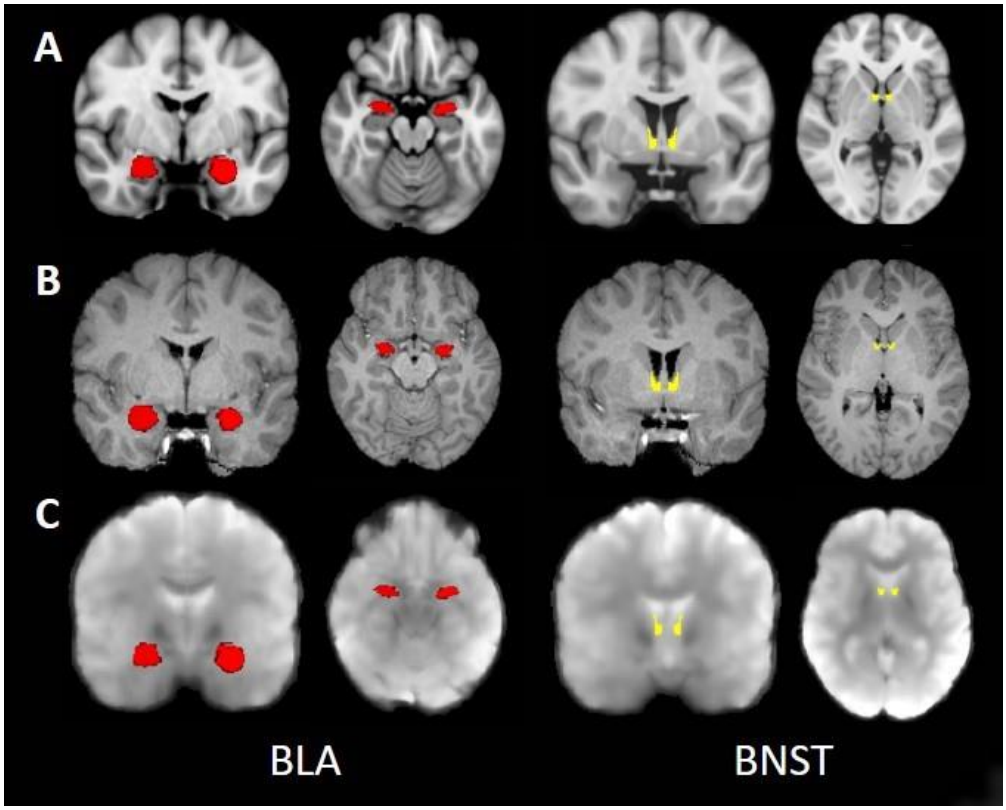

Figure 3. Basolateral Amygdala (BLA) and Bed Nucleus of the Stria Terminalis (BNST) Masks. Row A) Overlaid on the MNI 152 T1-1mm standard brain; Row B) Overlaid on a representative subject's structural image; Row C) Overlaid on a representative subject's EPI image. The BLA is shown in red, and the BNST is shown in yellow.
BLA = basolateral amygdala nuclei group, BNST = bed nucleus of the stria terminalis

Functional Connectivity

A priori seed regions were selected for this analysis: BLA and BNST. Whole-brain seed-based functional connectivity was performed by using following steps: 1) lower-level subject specific models (FSL's FEAT) were run by applying high-pass filtering (100 secs), subsequently the residuals and mean functional output were added together (FSL's res4d and mean_func), 2) the average time course was also extracted over 3 brain masks: ventricles, white matter and subject space whole brain (FSL's meants, 3) a second lower-level subject specific model combined the two previous

outputs, to remove the signal from the ventricles and white matter and to globally normalize the functional signal, 4) subsequently, the residuals and mean functional output were again added together (FSL's res4d and mean_func) to produce a preprocessed subject specific time series that was highpass filtered, controlled for white matter and ventricle signals and was globally normalized, 5) this subject specific time series was then used with regressors for the conditions of interest and masked for specific seed ROIs, 6) finally, higher-level group models combining all subjects were run for each seed (FSL's FEAT). Regions displaying significant functional connectivity were then masked using a 10mm radius sphere centered around the peak voxel, and PSC was extracted from each ROI across conditions. T-tests were performed to compare differentiation in degree of connectivity from each seed. Reported brain regions were required to meet two criteria to be considered functionally connected: 1) display connectivity significantly different from zero in either threat condition, and 2) reveal a significant differentiation between the BLA and BNST as determined by t-tests between parameter estimates.

Questionnaires

Three questionnaires associated with personality traits and affective style were administered to participants: 1) State-Trait Anxiety Inventory (Spielberger et al., 1970), 2) Penn-State Worry Questionnaire (Meyer et al., 1990), and 3) Ruminative Response Scale (Treynor et al., 2003).

State-Trait Anxiety Inventory (STAI)

This is a commonly used self-report measurement of state anxiety (anxiety in the present moment) and trait anxiety (anxiety level as a personal characteristic). The STAI can be used in a clinical setting to diagnose anxiety and to distinguish it from depressive

syndromes, and is also often used in research as an indicator of distress. The STAI has 20 items for assessing trait anxiety and 20 items for state anxiety. State anxiety items including statement like “I am tense; I feel secure” while trait anxiety items include statements such as “I worry too much over something that really doesn’t matter; I am a steady person.” All items are rated on a 4-point scale from 1 (*not at all*) to 4 (*very much so*) for state items, and from 1 (*almost never*) to 4 (*almost always*) for trait items. Scores range from 20 to 80, with higher scores indicating greater anxiety. Considerable evidence attests to the construct and concurrent validity of the scale.

Penn-State Worry Questionnaire (PSWQ)

This is a self-administered 16-item scale designed to measure worry. It is the most common self-report measure of worry and is considered by many to be the “gold-standard”. The items on the scale assess the occurrence, intrusiveness, pervasiveness and other characterizing features of an individual’s experience with worry. The scale has also been shown to identify worry, over and above anxiety and depression. Items such as “My worries overwhelm me” are rated on a five-point scale from 1 (*not at all typical of me*) to 5 (*very typical of me*). Possible score range from 16-80 with 16-39 signifying “low worry,” 40-59 indicating “moderate worry,” and 60-80 suggesting “high worry”. The PSWQ shows high internal consistency and good test-retest reliability (Meyer et al., 1990).

Ruminative Response Scale (RRS)

The scale consists of 22 items, comprising three subscales: (1) reflection — turning inward to engage in cognitive problem solving; (2) brooding — comparing one’s current situation with some unachieved standard; and (3) depressive rumination.

Participants responded to items such as “How often do you think ‘Why do I always act this way?”). Responses range from 1 (*almost never*) to 4 (*almost always*). Subscale totals of the RRS can be individually utilized, or all items can be summed together for a composite total rumination score, indicative of one’s propensity to engage in repetitive and passive self-focused attention.

Individual Differences Analyses

Exploratory analyses of functional brain data was conducted using individual differences from questionnaires measuring personality and affective style. Questionnaire responses were used as predictors of functional activity and functional connectivity. These analyses were included to demonstrate generalizability of the experimental findings beyond the utilized tasks, and allow characterization of how differences in these measures (trait anxiety, levels of worry and rumination) predicted degree of anxiety response, anxiety regulation ability, and variance in functional activity/connectivity across participants.

Both the State and Trait scales from the STAI were used. First, parameter estimates of average BOLD activation were extracted from each ROI (BLA and BNST) for Fear and Anxiety conditions, and questionnaires were correlated by condition. Additionally, regions displaying functional connectivity with ROIs were masked using a 10mm radius centered around the peak voxel, and mean functional connectivity parameter estimates between regions were extracted for Fear and Anxiety and correlated with scores from the questionnaires of interest. To correct for multiple comparisons, the Benjamini-Hochberg procedure was implemented to control the false discovery rate (FDR) at a level $< .05$ (Benjamini & Hochberg, 1995).

Results

Behavioral Results

Analysis of post-scan face ratings were initially conducted using all fearful faces associated with Fear/Anxiety (and excluding the 24 neutral faces in the Anxiety condition, corresponding to fMRI analyses) and all neutral faces associated with Neutral/Wait (collapsed across multitalker babble and nature sounds in Wait). Results revealed a significant main effect of Threat (Fearful, Neutral; $F(1,19) = 160.64$, $p < .001$), with fearful faces being rated as significantly more negative than neutral faces, but no main effect was found for Certainty (Certain, Uncertain; $F(1,19) = 3.58$, $p = .07$). Additionally, there was no significant interaction of Threat x Certainty ($F(1,19) = 3.83$, $p = .07$), as fearful faces associated with Fear and Anxiety were rated as equally negative (Fear: $M = 5.65$, $SD = 0.57$; Anxiety: $M = 5.66$, $SD = 0.59$; $t(19) = -0.51$, $p = 0.61$), despite faces associated with Wait being rated as less pleasant than faces associated with Neutral (Wait: $M = 3.71$, $SD = 0.43$; Neutral: $M = 3.61$, $SD = 0.43$; $t(19) = -2.23$, $p = 0.04$).

Following inclusion of neutral faces associated with the Anxiety condition (from trials when aversive stimuli did not occur and a neutral face and nature noises were instead presented), importantly, these were rated as significantly less pleasant than faces associated with Wait trials (Anxiety (neutral faces): $M = 3.82$, $SD = 0.37$; Anxiety (neutral faces) vs. Wait: $t(19) = 3.08$, $p < 0.001$). Thus, a clear grading was present in the behavioral data: neutral faces associated with the Neutral condition were rated as the most pleasant, while neutral faces associated with the Wait condition were rated as less

pleasant, and neutral faces associated with the Anxiety condition were rated as least pleasant of all.

Neuroimaging Results

Task related whole-brain activity

Group level GLM analysis was performed to examine the neural circuits recruited for certain and predictable, and uncertain and unpredictable threats. Because one aim was to investigate both the neural similarities and differences between fear and anxiety, I first assessed the commonalities between Fear and Anxiety conditions by conducting a conjunction analysis. Significant clusters were observed in bilateral amygdala, bilateral primary and secondary visual areas (inferior to the calcarine fissure/BA17/18/19, and fusiform gyrus/BA37), bilateral auditory processing (superior and middle temporal gyri/BA22/21, respectively), and bilateral sensory input relay centers in visual and auditory pathways (lateral geniculate nucleus; LGN, medial geniculate nucleus; MGN), as well as the right inferior frontal gyrus (rIFG) extending to the anterior portion of pars-opercularis and pars-triangularis (BA44/45, respectively; Figure 4). While these TFCE corrected conjunction results did not reveal involvement of the BNST, the voxelwise (uncorrected) conjunction showed bilateral BNST (p = .001). Furthermore, a conjunction analysis was conducted comparing all threat (Fear, Anxiety) versus all neutral (Neutral, Wait) to assess regions involved in threat processing after contrasting against conditions that elicit similar levels of visual and auditory processing. This analysis showed very similar results (amygdala, rIFG, enhanced auditory processing), with the exception that significant differences in visual cortical activation no longer emerged (Supplementary

Figure 2). The voxelwise (uncorrected) conjunction for all threat compared to all neutral additionally revealed activity in the right BNST (p = .01).

Conversely, to investigate the differences in neural regions recruited by Fear and Anxiety, these conditions were contrasted directly. The contrast Fear > Anxiety revealed greater activation in bilateral primary visual areas and bilateral auditory regions. In comparison, the contrast Anxiety > Fear was associated with greater activation in medial prefrontal cortex (mPFC), extending from the pre-supplementary motor area (preSMA/medial BA6/8) rostrally towards the dorsal anterior cingulate cortex (dACC/BA32) (Figure 4; Table 1). An exploratory voxelwise (uncorrected) analysis additionally revealed bilateral amygdala activation for Fear > Anxiety (p = .005). Finally, the left BNST was observed in the whole-brain voxelwise analysis for Anxiety > Fear (p = .03), but no amygdala activation was found for this contrast.

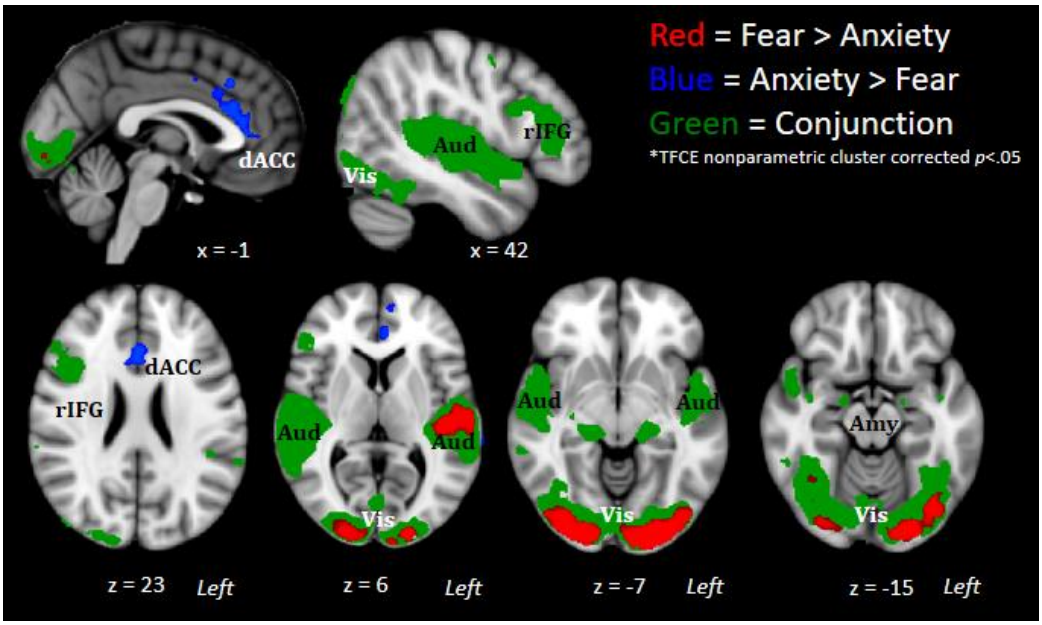

Figure 4. Experiment 1: Whole-Brain Functional Activation. Conjunction analysis displaying commonalities between Fear and Anxiety (green), Fear > Anxiety (red), and Anxiety > Fear (blue).

Aud = auditory cortex, dACC = dorsal anterior cingulate, Vis = visual cortex, rIFG = right inferior frontal gyrus, Amy = amygdala.

Table 1. Cluster and peak report for whole-brain TFCE corrected analyses for Fear > Anxiety, Anxiety > Fear and the conjunction analysis between Threat conditions, as shown in Figure 3.

Region	**X**	**Y**	**Z**	**Cluster Size**
Fear > Anxiety				
Ventral Visual Processing Stream (VVPS)	-35	-87	-21	45666
Left Auditory Cortex	-44	-22	4	5225
Right Auditory Cortex	55	-18	5	1797
Anxiety > Fear				
Dorsal Anterior Cingulate Cortex (dACC)	-11	33	20	5837
Conjunction – All Threat				
Ventral Visual Processing Stream (VVPS)	38	-35	-32	86665
Left Auditory Cortex/Lateral Geniculate Nucleus (LGN)	-40	1	-24	64608
Right Auditory Cortex/Lateral Geniculate Nucleus (LGN)	33	1	-26	29841
Right Inferior Frontal Gyrus (rIFG)	46	32	0	7824
Right Inferior Frontal Gyrus (rIFG)	39	26	-12	1926
Right Temporal Pole	-29	12	-33	1080
Right Amygdala	-18	-6	-28	515
Left Temporal Pole	-31	-1	-44	370
Left Amygdala	18	-5	-20	267

Coordinates in MNI space.

Region of Interest Analyses

Based on previous literature and recent theoretical models, one primary impetus for this study aimed to compare and contrast the relative contributions of two key regions underlying threat processing: the amygdala and BNST. Regions of interest (ROIs) were masked, and percent signal change was extracted across the time series by condition. When assessing Region (BLA, BNST) x Condition (Fear, Anxiety) effects, no significant main effects were noted for Region, ($F(1,76) = 3.68$, $p = .06$), or Condition, ($F(1,76) = 1.37$, $p = .25$), but a significant Region x Condition interaction was found, in a pattern consistent with my hypothesis, ($F(1,76) = 141.54$, $p < .0001$). One-sample t-tests indicated that both the amygdala and the BNST were significantly elevated above baseline across Threat conditions (BLA Fear: $t(19) = 6.68$, $p < 0.0001$; BLA Anxiety: $t(19) = 4.97$, $p < 0.0001$; BNST Fear: $t(19) = 3.85$, $p = 0.001$; BNST Anxiety: $t(19) = 9.53$, $p < 0.0001$). Follow-up pairwise comparisons revealed that while the BLA showed a more elevated response in Fear relative to Anxiety, this difference was not statistically significant ($t(38) = 1.48$, $p = 0.15$, Cohen's $d = .47$). However, the BNST showed increased activity during the Anxiety condition compared to Fear ($t(38) = 3.95$, $p < 0.001$, $d = 1.25$; Figure 5). Analyses were additionally conducted in two control ROIs directly above and below the BNST in the head of the caudate and ventral striatum, respectively, to support that the extracted signal was reliably related to the BNST and that reported results were not contaminated by signal from nearby structures (Supplementary Figure 3).

Next, to further understand the roles of Fear and Anxiety, I separated trials into Cue, Delay and Stimulus presentation epochs. This approach allowed us to examine what

occurs during the Delay epoch of Anxiety trials, the epoch that specifically differentiates Fear and Anxiety. Of note, only Anxiety trials in which the aversive stimulus occurred were analyzed. Analysis of Region (BLA, BNST) x Condition (Fear, Anxiety) effects during the Cue (+ Delay) epoch revealed no significant main effect of Region ($F(1,76) = 0.61$, $p = .44$) and no significant main effect of Condition ($F(1,76) = 0.05$, $p = .82$), however, there was a significant Region x Condition interaction ($F(1,76) = 20.94$, $p < .0001$). Similarly, during the aversive Stimulus epoch, no main effects were found for Region ($F(1,76) = 1.02$, $p = .35$) or Condition ($F(1,76) = 3.67$, $p = .06$), but again a significant Region x Condition interaction emerged ($F(1,76) = 66.77$, $p < .0001$). Pairwise comparisons revealed that the BLA showed increased activity to the certain Cue in the Fear condition (100%) relative to the uncertain Cue+Delay in the Anxiety condition (80-20%) ($t_{(38)} = 2.30$, $p < .05$, $d = .72$), but interestingly, no difference was observed in BLA activity between the Fear and Anxiety conditions during the aversive Stimulus epoch ($t(38) = 0.43$, $p = 0.67$, $d = .14$). The BNST, by comparison, demonstrated an elevated response to the uncertain Cue and anticipatory Delay in the Anxiety condition ($t(38) = 3.17$, $p < 0.01$, $d = 1.00$), and showed a further potentiated response during the aversive stimulus presentation ($t(38) = 5.23$, $p < 0.0001$, $d = 1.66$; Figure 5). Furthermore, a repeated measures analysis was conducted to reinforce that these trial epochs could be reliably separated (Supplementary Figure 4).

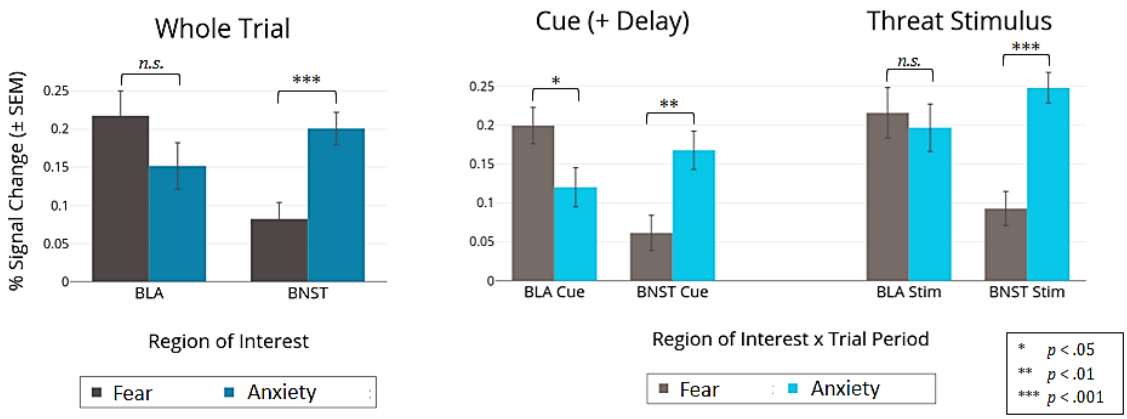

Figure 5. Experiment 1: Region of Interest Analysis. Percent signal change (PSC) extracted from ROIs across the Fear and Anxiety conditions. The figure on the left depicts PSC by region for both threat conditions, across all trial epochs. The right figure displays regional PSC during each trial epoch: Cue (Fear), Cue+Delay (Anxiety), and Stimulus (Fear, Anxiety). Across the whole trial period and in discrete trial epochs, significant Region x Condition interactions were found.

BLA = basolateral amygdala nuclei group, BNST = bed nucleus of the stria terminalis. Error bars represent standard error of the mean (SEM).

Functional Connectivity

To assess brain regions that represent possible coherence across the time-course of activation during Fear and Anxiety, seed-based functional connectivity from the BLA and BNST was analyzed. Brain regions were required to meet two criteria to be considered functionally connected: 1) display connectivity significantly different from zero in either threat condition (as determined by a one-sample t-test on the extracted parameter estimates), and 2) reveal a significant differentiation between the BLA and BNST (as determined by a t-test between parameter estimates extracted for each region).

When comparing connectivity with the BLA relative to the BNST, significant results were found in regions supporting stimulus perception (ventral visual processing stream - VVPS), emotion detection and processing (ventral insula, ventromedial

prefrontal cortex – vmPFC, dorsomedial PFC – dmPFC), and motor preparation and execution (medial and lateral primary motor cortex - PMC). Connectivity between the BLA and VVPS was significantly elevated across both threat conditions (including all trial epochs when the Anxiety condition was subdivided). Nevertheless, there was a significant difference in the degree of connectivity between the BLA and VVPS relative to the BNST (Fear: d = 6.86, Anxiety: d = 8.81). These same findings were also observed between the BLA and emotional detection and processing regions (ventral insula, vmPFC, dmPFC), with significantly increased connectivity being found across both threat conditions and all trial epochs (BLA > BNST) (ventral insula - Fear: d = 2.92, Anxiety: d = 5.84; vmPFC - Fear: d = 4.94, Anxiety: d = 14.65; dmPFC - Fear: d = 6.73, Anxiety: d = 5.84). Finally, enhanced functional connectivity was observed between the BLA and PMC in the Fear condition, and during the Stimulus epoch of Anxiety trials (Fear: d = 8.44, Anxiety: d = 3.20; Figure 6).

In contrast, the BNST revealed increased functionally connectivity, and significantly greater connectivity relative to the BLA, with the dorsal anterior insula during both threat conditions and across all trial epochs (Fear: d = 6.69, Anxiety: d = 5.57). Additionally, a significant functional relationship was found between the BNST and sgACC during Fear. Interestingly, both the BLA and BNST exhibited elevated connectivity with the subgenual anterior cingulate cortex (sgACC) during the Anxiety condition across the whole trial, but a significant difference emerged during the Stimulus epoch (BNST > BLA: d = 3.96; Figure 6).

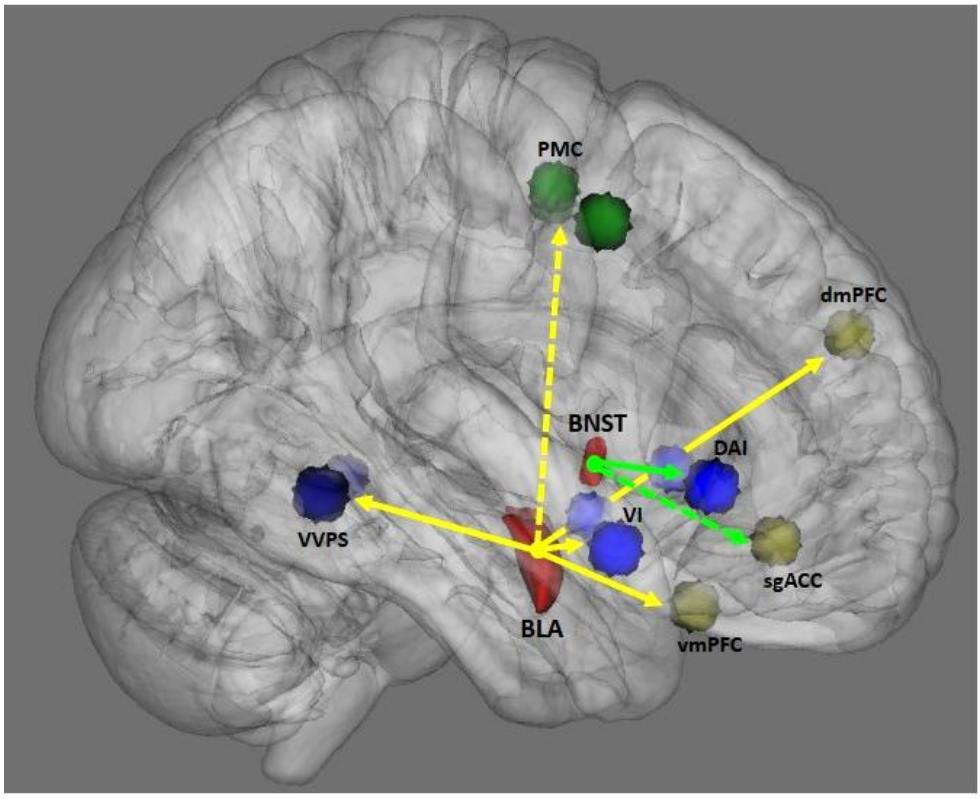

Figure 6. Experiment 1: Seed-based functional connectivity. Brain regions were required to meet two criteria to be considered functionally connected: 1) displayed connectivity significantly different from zero in either threat condition, and 2) revealed a significant differentiation between the BLA and BNST. Yellow arrows depict significantly greater connectivity from the BLA compared to the BNST. Green arrows show significantly greater functional connectivity with the BNST versus the BLA. Solid lines represent significant connectivity across both threat conditions and all trial epochs, while dashed lines signify significant connectivity only during the Stimulus epoch.

BLA = basolateral amygdala nuclei group, BNST = bed nucleus of the stria terminalis, VVPS = ventral visual processing stream, PMC = primary motor cortex, dmPFC = dorsomedial prefrontal cortex, sgACC = subgenual anterior cingulate cortex, vmPFC = ventromedial prefrontal cortex, DAI = dorsal anterior insula, VI = ventral insula.

Questionnaire Correlations

To examine whether individual differences in anxiety-related personality traits were associated with brain activation and connectivity, questionnaires (STAI, PSWQ, RRS) were correlated with subject-level parameter estimates derived from the functional activation ROI analyses and from parameter estimates of functional connectivity between

seeds and ROIs that resulted from functional connectivity analyses. After correcting for multiple comparisons (FDR < .05), results emerged exclusively within the Stimulus epoch of Anxiety trials. Increased connectivity between the BLA and PMC was negatively correlated with state anxiety (r = -.756, p < .001) and trait anxiety (r = -.599, p = .005). Furthermore, results revealed that increased functional connectivity between the BNST and sgACC was negatively related to worry (r = -.620, p = .004) and total rumination (r = -.630, p = .003; Figure 7).

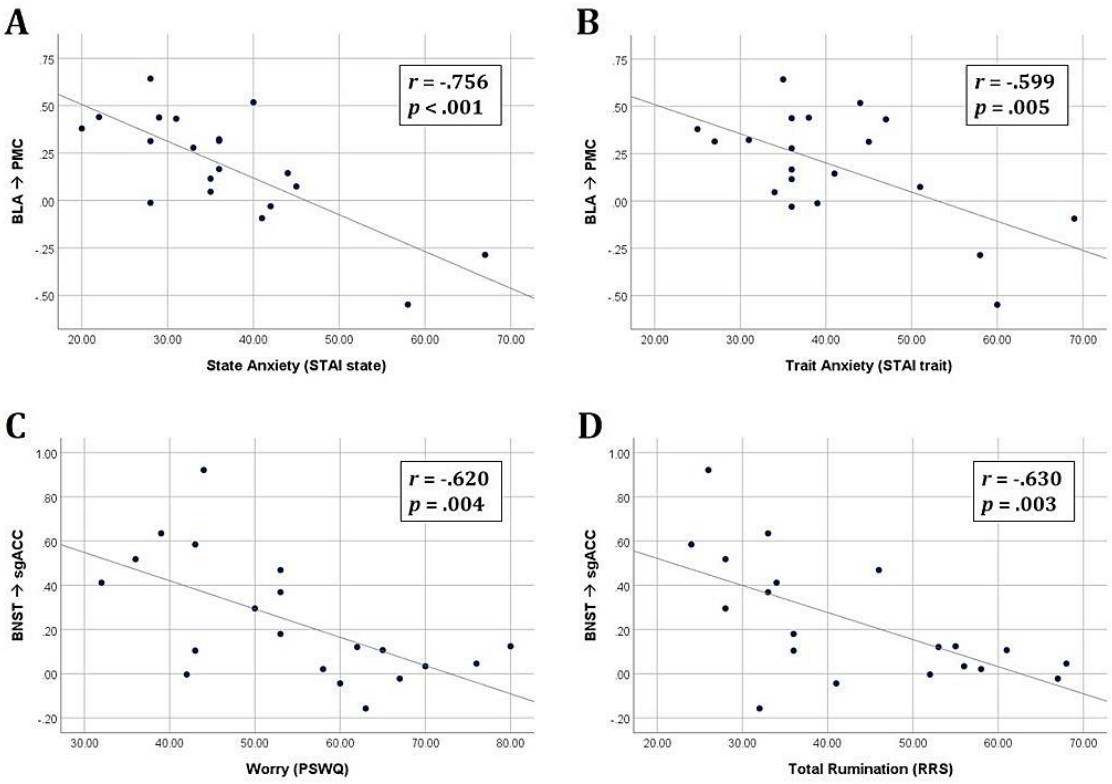

Figure 7. Experiment 1: Questionnaire Correlations. Correlations between questionnaires and parameter estimates extracted from functional connectivity analysis. All results shown are within the Anxiety condition, during the Stimulus epoch. A) Negative correlation between state anxiety (STAI) and functional connectivity parameter estimates between the BLA and PMC. B) Negative correlation between trait anxiety (STAI) and functional connectivity parameter estimates between the BLA and PMC. C) Negative correlation between worry (PSWQ) and functional connectivity between the BNST and sgACC. D) Negative correlation between total rumination (RRS) and functional connectivity between the BNST and sgACC.

BLA = basolateral amygdala nuclei group, BNST = bed nucleus of the stria terminalis, bilat. PMC = bilateral primary motor cortex, dmPFC = dorsomedial prefrontal cortex, sgACC = subgenual anterior cingulate cortex, STAI = State-Trait Anxiety Inventory, PSWQ = Penn State Worry Questionnaire, RRS = Ruminative Response Scale.

Discussion

To the best of my knowledge, this study is the first to investigate the differential contributions of the amygdala and BNST to Fear and Anxiety processing, using task-based high-resolution fMRI ($1.5mm^3$) and precise delineation of these brain structures via ultra-high-resolution anatomical masks. This paradigm employed a multimodal stimulus task intended to psychologically elicit feelings of fear or anxiety through cues signaling certain and predictable threats, or uncertain and unpredictable threats, respectively. While the BLA and BNST both displayed heightened activity to Fear and Anxiety, important distinctions were noted in degree of recruitment, temporal activation profiles and functional connectivity. Specifically, the BLA showed preferential involvement in Fear processing, responding to the certain cue and to the presence of the threatening stimulus across both conditions. The BNST, by contrast, indicated biased engagement during Anxiety, showing significantly increased activity at the uncertain cue, and exhibited distinct patterns of functional connectivity relative to the BLA. Notably, the current findings additionally present valuable insight into how alterations in this network activity and connectivity may relate to individual differences in anxiety-related personality traits.

Behavioral Findings

Immediately following scanning, participants rated all fearful and neutral face stimuli on a 7-point Likert scale to assess perceived valence. As anticipated, all fearful

faces associated with threat conditions were rated as significantly more negative than neutral faces when compared with their respective control conditions. Furthermore, there were no differences when ratings of fearful faces were compared between the Fear and Anxiety conditions. However, ratings of neutral faces associated with the Wait condition were rated as significantly less pleasant than faces associated with the Neutral condition, suggesting that simply waiting for the arrival of an unpredictable stimulus, despite knowing that the stimulus would be neutral, may put individuals in a mildly anxious state and consequently alter processing of the stimulus itself (Somerville et al., 2012). Importantly, this effect was seen to a greater degree with the neutral faces associated with Anxiety trials. Neutral faces that were presented after anticipation of a potential threat were rated as significantly less pleasant than faces in Wait trials, indicating that the anxious state induced by a cued threat led to a more negative association of neutral faces in Anxiety. These findings suggest that manipulation of Fear and Anxiety induced negative affect equally, but more importantly, that the manipulation of Anxiety vis-à-vis the unpredictable probability of threat occurrence and temporal nature of the threat during the delay period induced negative affect before the stimulus was presented (i.e., Anxiety [neutral faces] vs. Wait).

Commonalities and Functional Dissociations

Whole Brain Functional Activation

To characterize the neural mechanisms associated with Fear and Anxiety, the results will be discussed in terms of commonalities and differences observed in threat processing. The initial conjunction analysis assessing similarities across both threat conditions revealed activation in the amygdala, primary visual and auditory cortices,

sensory thalamic relay centers (MGN, LGN), and rIFG. These finding provide an initial overview of common neural correlates recruited across a broad spectrum of threat. Given the current paradigm and stimuli presented, it is unsurprising that an upregulation in visual and auditory cortices was seen across both threat conditions. Furthermore, it is well known that the amygdala plays a key functional role in detecting salient and novel cues in the environment that predict affective or threatening events (Adolphs, 2008; Blackford et al., 2010). Increased activation of the rIFG suggests enhanced negative context monitoring and rapid surveillance of the environment for potential danger, along with a general withdrawal response (Banich & Depue, 2015; Corbetta & Shulman, 2002; Depue et al., 2015; Hampshire et al., 2010). Taken together with the behavioral results, the observed upregulation of regions related to sensory modalities, in combination with increased amygdala and rIFG response, highlight the common neural mechanisms for general threat detection, and support the validity of the current paradigm.

Directly contrasting the Fear and Anxiety conditions revealed differences associated with certain and predictable versus uncertain and unpredictable threat processing. Greater activation was observed in visual and auditory cortices for the Fear versus Anxiety contrast. One interpretation of this is that, while both types of threat recruited these sensory regions, Fear is more stimulus bound and exemplifies a stronger representation of the stimulus. However, it is also possible that due to the nature of the study design, an initially strong response in these regions during the Anxiety condition may be diminished due to the inclusion of the Delay epoch, or that the aversive Stimulus epoch in the Fear condition may be exhibiting a stronger relative influence due to the lack of a Delay epoch between the Cue and aversive outcome. In the opposing contrast,

Anxiety versus Fear revealed greater activation in the dACC extending to the dmPFC, suggesting higher-level detection of emotion and conflict monitoring (Egner et al., 2008; Eisenberger & Leiberman, 2004), likely in anticipation of threat. Recent research highlights the ACC as a central locus for signaling outcome uncertainty in a valence-specific manner. Through a Pavlovian procedure in monkeys investigating the certainty versus uncertainty of punishments and rewards, one study identified a novel punishment uncertainty signal in the ACC, demonstrating that some neurons are selectively excited by the prospect of uncertain punishment, and are most strongly activated during greatest uncertainty (50% probability of an aversive outcome; Monosov, 2017).

ROI Analysis

Focusing on the a priori ROIs, I next assessed the differential contributions of these regions to Fear and Anxiety. Across the whole trial period, I found that both regions were significantly activated across threat conditions, but importantly, found a significant region by condition interaction in a pattern consistent with my hypothesis. Globally, the BLA displayed preferential responsivity to certain and predictable threats relative to the BNST, as shown by qualitatively increased activity in Fear relative to Anxiety, although this finding was not statistically significant. An opposing pattern of activity was observed in the BNST, with significantly more activation being found in Anxiety compared to Fear. These findings indicate that while a partial functional dissociation was observed, in the manner proposed by Davis and colleagues, both regions displayed elevated activity across conditions, lending support to perspectives outlined by Shackman and Fox (2016).

Additional insight was uncovered following division of trials into epochs. For Fear, the Cue epoch and aversive Stimulus epoch were modeled separately, and for

Anxiety, trials were separated into the Cue+Delay epoch and the Stimulus epoch. While a significant region by condition interaction was still present in both the Cue (+Delay) and aversive Stimulus epochs, subsequent pairwise comparison revealed additional insights into the functional roles of each region. First, a significant difference was noted when comparing the Fear Cue to the Anxiety Cue+Delay epoch, with the BLA exhibiting increased responsivity to the concretely paired certain cue in Fear, at a magnitude comparable to BLA's response to the aversive Stimulus. However, when comparing the two threat conditions during the Stimulus epoch, no difference was observed in BLA activity (due to the elevated BLA response in Anxiety during the Stimulus epoch). Together, this explains why a significant difference did not emerge between conditions across all epochs of the trial. However, more importantly, this demonstrates that the BLA preferentially responds to the threatening stimuli's overt display, regardless of whether the onset of a stimulus is immediate (Fear) or temporally delayed (Anxiety).

In contrast, the BNST showed increased activity to the uncertain cue and unpredictable anticipatory delay (Cue+Delay) in the Anxiety condition, and continued to display an elevated response throughout the stimulus presentation. While both the BLA and BNST displayed heightened activity at all threat cues, plausibly serving as an alerting system to potential danger, the magnified response of the BNST in the Anxiety condition suggests that the BNST may underlie increased vigilance when the specifics of a threat are unknown.

These results complement previous work by Somerville (2010), which showed that the BNST continuously tracks threat proximity (low, medium, or high risk of receiving a shock), and that this threat monitoring was exaggerated in individuals with

high trait anxiety. In this paradigm, participants never actually received a shock while being scanned, and notably, the study reported that the amygdala showed minimal task-modulated activity even at exploratory thresholds. Klumpers et al. (2017) reports a similar dissociation in the roles of the amygdala and BNST using a shock paradigm. During the presentation of a cue signaling potential shock (16% or 33% reinforcement rate), significant activation was noted in the BNST in two independent samples. In contrast, no evidence was found for amygdala involvement during uncertain shock anticipation, but robust amygdala activation was exhibited during the actual aversive outcome (with high probability for localization in the BLA; Klumpers et al., 2017). Thus, taken together, these results help clarify the functional roles of the amygdala and BNST: a regional dissociation can be attributed to the BLA preferentially responding the *actual presence* of an aversive stimulus or a concretely paired cue, while the BNST exhibits a functional specialization for the detection of a potential threat and maintains hypervigilance until threat arrival or situational resolve.

Functional Connectivity

Analysis of seed-based functional connectivity was assessed from these two ROIs: the BLA and BNST. Results revealed increased functional connectivity between the BLA and bilateral VVPS across Fear and Anxiety, with the strongest connectivity being observed during the Stimulus epoch of the Anxiety condition (Klumpers et al., 2017). These findings build on previous results, which demonstrated that the amygdala responded to the aversive stimulus itself across both types of threat, and thus more strongly in the Fear condition, on average, as it contained only a certain cue and stimulus presentation (no delay epoch). Moreover, whole-brain results showed that Fear was more

stimulus bound when contrasted directly with Anxiety. Added evidence from functional connectivity then suggests that the stimulus bound nature of Fear may be mediated through the BLA and its back projections to upregulate visual processing (Amaral et al., 2003; Pessoa & Adolphs, 2010). In addition, the BLA displayed increased functional connectivity with cortical motor areas indicating a role in preparation for and executing a motor response (Avendan et al., 1983; Llamas et al., 1977). A similar temporal pattern of results was observed, with enhanced connectivity being observed in the Fear condition, and during the Stimulus epoch of the Anxiety condition. Together, these results suggest that in the face of threat, the amygdala may facilitate coordinated activity between sensory processing areas and motor control, so as to afford quick and adaptive behavioral changes.

Finally, the BLA showed increased functional connectivity with the emotional detection and processing regions (vmPFC, dmPFC, and ventral insula) across the whole trial for both threat conditions. It is well known that the amygdala shares extensive connections with the mPFC (Phan et al., 2002), whose activity is thought to underlie many facets of cognitive and emotional processing including emotional detection, appraisal, self-monitoring, and emotion regulation (Etkin et al., 2011), while the insula has additionally been implicated in general affective processing and integration of body state representations (Craig, 2002; Craig 2009; Critchley et al., 2004). This indicates that in addition to facilitating gross motor movement planning for defensive behaviors or escape, the BLA may contribute to specific motor selection in concert with changes in emotional and body state, especially when that state is representative of discomfort, thought to be represented in the ventral insula (Jezzini et al., 2012).

In both Fear and Anxiety, the BNST was found to exhibit extensive functional connectivity with the insula, specifically in the most dorsal anterior portions. In addition to underlying integration of body states, the anterior insula in particular has been hypothesized to play a role in the *perception* of subjective interoceptive states (Grupe & Nitschke, 2013). Thus this metacognitive aspect of interoception may in part underlie feelings of anticipatory anxiety when a potential threat is detected, through increased awareness and interpretation of physiological arousal (Damasio & Carvalho, 2013; Herrmann et al., 2016). The BNST additionally displayed increased functional connectivity with the sgACC in Fear and during the Stimulus epoch of Anxiety, a prefrontal region putatively involved in internal mentation. Together, these results suggest greater connectivity of the BNST to regions supporting higher-level perception of interoceptive state, as well as prefrontal regions that may modulate these responses through reflection and rumination, suggesting a role of the BNST in the more psychological aspects of anxiety (Andrews-Hanna, 2012; Klumpers et al., 2017; Mobbs et al., 2007; Torrisi et al., 2018). Finally, the sgACC is additionally known to be highly involved in communication with the amygdala for downregulation of negative affect (Banks et al., 2007; Connolly et al., 2013), bringing up the intriguing question of whether this region has the same top-down control over the BNST.

Relationships with Questionnaires

In order to evaluate the generalizability of the neural findings beyond the utilized paradigm, personality questionnaires measuring anxiety, worry and rumination were collected, and relationships between individual differences in personality and alterations in ROI recruitment and network connectivity were assessed during threat processing.

However, given that the sample size was modest (N = 20), the results should be interpreted with care as I do not want to over-speculate on relationships with individual differences. As such, the relationships with questionnaire scores are primarily presented as broad support for the task results, rather than claims on how individual traits specifically modulate threat processing.

After correcting for multiple comparisons, no significant results were found when questionnaires were correlated with parameter estimates in ROI activity across threat conditions. However, correlations with functional connectivity parameter estimates revealed two functional pathways that exhibited relationships with anxiety-like personality traits, suggesting that functional connectivity may be a better predictor of behavior than regional activation alone. First, connectivity between the BLA and bilateral PMC was found to be negatively related to state and trait anxiety during the Stimulus epoch of Anxiety. Initially, this may seem counterintuitive. However, these results likely indicate better integration of emotional and motor responses for individuals with lower trait anxiety. Since these findings were specific to the stimulus period of Anxiety, this implies that the anticipatory delay influenced responses to the stimulus (otherwise, the same results would be seen in Fear). Therefore, these results suggest that individuals with lower anxiety have increased connectivity between the amygdala and cortical motor systems, which may reflect enhanced motor planning during the anticipatory delay in preparation for the arrival of an aversive stimulus. Thus, this increased communication and better preparedness for protective or defensive motor behaviors may reduce anxiety, or conversely, individuals who are less anxious may have better ability to prepare an appropriate motor response in the face of a potential threat. Additionally, increased

functional connectivity between the BNST and sgACC, likely an index for communication between emotional and regulatory systems, was found to be related to reduced worry and total rumination. These findings are supported by the functional connectivity results, which demonstrated a dissociation between the connectivity profiles of the BLA and BNST, with the BLA showing increased connectivity to stimulus processing and motor response regions, while the BNST showed enhanced communication with medial prefrontal regions putatively involved in internal mentation. Worry and rumination are processes more closely tied to anticipation and as opposed to reactivity, and the BNST was likewise preferentially involved in Anxiety processing, suggesting that this functional pathway may underlie some of the more cognitive aspects of anxiety (Muris et al., 2005).

Limitations and Future Directions

Limitations of the present study should be acknowledged. First, the sample size was modest, using only 20 healthy participants. As previously stated, results should be interpreted with care given the limited sample size, and future studies will be needed to investigate how individual differences in personality specifically modulate components of threat processing. Second, all participants were considered psychologically healthy, and while this study indicates that differences in personality may alter regional activity and connectivity, investigation of clinical populations is needed to specifically elucidate the neural underpinnings of anxiety disorders. The sample was also predominantly female, and given that the BNST is known to be a sexually dimorphic region (Hines et al., 1985), it is unknown how these mechanisms may vary by gender. Additionally, the analyses only focused on the functional role of the BLA relative to the BNST, and

therefore it is possible that another picture may have emerged for other amygdala regions such as the CEA. Finally, in order to maximize statistical power, trial lengths were brief (max = 5500 ms until stimulus presentation), and I therefore could not confidently address the phasic versus sustained response profile debate for the amygdala and BNST.

Therefore, future studies would benefit from larger sample sizes with roughly equal numbers of males and females to assess gender differences, and longer trial lengths to investigate phasic and sustained responses of these ROIs under Fear and Anxiety conditions. In addition, while this study used 80-20% probabilities of an aversive outcome in the Anxiety condition, individual probability conditions had too few trials to be able to assess how different levels of threat likelihood affected processing. Therefore, future studies should expand on the current design, including more trials to evaluate parametric modulation of threat likelihood. This could provide insight into whether the BNST tracks these different probabilities of occurrence, and how these mechanisms differ in individuals with high trait anxiety. Finally, while this study only included a threat condition that was both certain *and* predictable, additional studies could explore conditions in which threat is certain but delayed, or where threat is cued as certain but never arrives. In the first scenario, I would hypothesize that the BNST would respond at the cue and show a sustained response until stimulus arrival, while the latter may serve as a model for generalized anxiety through a simulated state of perpetual anticipation for a fear that may never occur.

Summary

In summary, results from functional activation contrasts revealed that Fear engages more stimulus bound processing, as evidenced by increased activation in visual

and auditory cortices. By contrast, Anxiety processing involves the dmPFC and dACC, suggesting higher-level emotional detection. These results were further supported by analysis of ROIs, which showed that the BLA exhibited preferential involvement in Fear, as measured by percent signal change, and displayed heightened responsivity to the presence of aversive stimuli presentation across conditions. These findings demonstrate that activity of the amygdala is more concretely tied to the threatening stimulus itself, or a concretely paired cue, putatively mediating feelings of fear. The BNST, by comparison, showed preferential involvement during Anxiety processing, and exhibited significantly elevated activity at the uncertain cue and showed a potentiated response to the aversive stimulus presentation. This further supports that BNST activity may predominantly serve as an alerting system, responding as soon as a prospective threat is detected, and putatively mediating feelings of anticipatory anxiety. However, as these analyses did not include a mediation model with subjective feelings, the precise relationships between these regional dissociations and the feelings of fear and anxiety will have to be explored in future work. In addition, functional connectivity results demonstrated that, on a whole, the BLA display increased connectivity with regions supporting stimulus processing and gross motor response, while the BNST was more functionally related to anterior prefrontal regions that underlie interoception, internal mentation and rumination. Importantly, these findings were strengthened by relationships with individual differences in personality trait and mood state, which further emphasized these partial functional dissociations, and suggested that differences in individual affective state may play a modulatory role in these key networks during threat processing.

Based on the current results, I believe that both proposed models on threat detection, as they relate to fear and anxiety, have validity. These results support that the BLA is more involved in fear processing, while the BNST shows preferential engagement to anxiety, as proposed by Davis and colleagues. However, contrary to this model, these results indicated that all regions respond to both threat conditions, lending support to the perspectives of Shackman and Fox. Therefore, I instead propose an alternative idea that amends these disparities. Over and above the type of threat being processed, the BNST appears to exhibit a functional specialization for the detection of a potential threat, putatively serving as an alerting system to maintain hypervigilance and thus, worry and rumination, until the arrival of a threat or resolution of the threatening situation. In complement to the BNST, the BLA preferentially responds to the certainty of threat occurrence or the actual presence of a threatening stimulus, regardless of whether that threat is immediate or occurs after an anticipatory delay. Together, these results and this altered view of threat processing may help explain the inconsistencies that currently exist in the literature and inform future research.

CHAPTER IV: EXPERIMENT 2 – ANXIETY REGULATION

Aims

Anxiety disorders are additionally characterized by maladaptive patterns of ER, including experiencing emotions suddenly and with high intensity, while having difficulties understanding those emotions or implementing goal-directed behaviors when distressed (Cisler et al., 2010). Moreover, dysregulated emotion is a common feature among many psychiatric disorders. Consequently, a strong focus has been on uncovering the neural mechanisms of ER due to its significance and potential applicability transdiagnostically. ER is typically studied in the context of individuals attempting to volitionally control their emotional response to explicitly cued pictorial stimuli (negative scenes or faces), either through reappraisal or distancing/suppression strategies (Depue et al., 2015; Ochsner et al., 2002; Ochsner et al., 2004). Many ER paradigms make use of images acquired from the International Affective Picture System (IAPS), a widely used normative database most prominently known for its images that induce disgust. Several lines of work (Depue et al., 2015; Naaz et al., 2019; Ochsner et al., 2002; Ochsner et al., 2004) indicate that the degree of regulating subjective negative emotion is mediated through the strength of functional and structural connections between the middle frontal gyrus (MFG) and the amygdala. This model posits that emotional responses are effectively reduced through goal-directed inhibitory control implemented by the MFG, which downregulates

amygdala activity via connectivity through the ventrolateral (inferior frontal gyrus [IFG] and orbital frontal cortex [OFC]) and ventromedial PFC (vmPFC).

Indeed, numerous studies have used this as a working model of ER in healthy individuals, derived from similar ER paradigms. However, critical barriers arise when this line of research is intended to specifically elucidate ER mechanisms in the context of anxiety disorders. First, as previously described, feelings of anxiety are related to uncertain and unpredictable threats. The use of negative scenes (not usually "threats"), and the participants' regulation during the image presentation, not in the anticipation of it, suggests that existing ER paradigms truly measure the regulation of general negative affect, or feelings more akin to fear (i.e. related to an explicit stimulus). Therefore, no ER study has definitively targeted *anxiety regulation*. Secondly, despite mounting research demonstrating that the BNST is a primary mediator of anxiety and a critical node of stress response neurocircuitry (Avery et al., 2016), it is all but absent in ER literature.

Hypotheses

Therefore, this work modified existing ER paradigms to specifically induce anticipatory anxiety, in order to investigate the mechanisms underlying volitional anxiety regulation. Despite strong evidence supporting the BNST as a key mediator in anxiety, it is currently unknown even *if* the BNST can be downregulated and how this relates to subjective feelings of anxiety. Thus, this work aimed to characterized these regulatory circuits using high-resolution fMRI ($2mm^3$) and careful delineation of amygdala nuclei groups and the BNST through ultra-high-resolution anatomical masks (Avery et al., 2014; Leal et al., 2014; Leal et al., 2017).

I hypothesized that comparison of the Feel Anxiety > Suppress Anxiety will show increased BOLD activation in the BNST and amygdala nuclei groups, as well as increased activation within visual, saliency (e.g. insula) and supplementary motor areas. Comparison of Suppress Anxiety > Feel Anxiety is expected to show relative decreases in activation of ROIs, and increased activation in and functional connectivity with prefrontal regions (MFG/IFG/vmPFC), signifying a hierarchical functional network for anxiety regulation and a mechanism for downregulation of the BNST.

Although analyses that focus on the role of the BNST are an important first step, I did not want to trade one narrow (amygdala-centric) view for another (BNST-centric). Therefore, additional analyses investigated how the BNST and amygdala interact within larger-scale brain network in order to better understand how anxiety modulates communication with higher-order regions subserving processes such as attention, inhibitory control, motor preparation, and memory. Here, I hypothesized that Suppress Anxiety would be associated with increased connectivity within attentional and inhibitory control regions (MFG/IFG), and decreased connectivity between these prefrontal control regions and memory and motor systems (hippocampus and supplementary motor area [SMA], respectively) denoting decreased need for motor and memory processes in this relatively reduced state of anxiety via top-down guidance.

Through this, I aimed to provide a better understanding of the relative importance and influence of the BNST in generating anxiety, the top-down mechanisms regulating its response, and large-scale network-level alterations that subserve goal-driven behavior. Through these foundational studies, future work can subsequently refine models for how these networks may be altered in specific clinical populations, determine whether anxiety

regulation is amenable to training, and explore how therapeutic and pharmacological interventions may strengthen BNST-regulatory networks.

Methods

Participants

A total of 32 adults were recruited for the study. Two participants were excluded from analyses (one due to excessive motion, and one participant that did not complete the scanning session), leaving a final sample of 30 healthy adults (21 females, 9 males, mean age = 22.63, S*D* = 7.54).

Procedure

The study was divided into two consecutive days. On one day, participants completed the Threat Anticipation Task in the fMRI scanner. The other day was devoted to an in-lab behavioral session, during which participants completed behavioral questionnaires and provided a saliva sample (the results of which will be reported in a subsequent study). Session order was determined by scanner and subject availability (16 participants completed the scan on the first day, and 14 participants completed the scan following their behavioral session).

To optimize statistical power, the scanning task was run as a within-subjects design, such that participants completed two runs of the Threat Anticipation Task with opposing instructions. During one run, participants were instructed to “feel” and “experience” the emotional anticipation, and during a second run, participants were asked to “suppress” and “decrease” the intensity of their emotional experience (with order of runs counterbalanced across participants). Intermittent subjective anxiety ratings, and behavioral ratings of all visual/audio stimuli pairs were obtained.

Scanning Paradigm

In the Threat Anticipation Task (Figure 8), participants were presented with human faces paired with different sounds (human screams, multitalker babble, or a flowing river). The task contained four conditions presented as mini-blocks in a pseudorandom order with no more than two sequential mini-blocks of the same condition. In total: Fear (20 trials), Anxiety (30 trials), Neutral (16 trials), Wait (16 trials). Conditions were indicated with a cue word (1s) to inform participants what might be coming.

Fear trials were cued with the word "THREAT!" After cue presentation, a black screen briefly appeared for .5s, which was then *always* followed by the presentation of a fearful face and human scream (2s) and an inter-trial interval (ITI) of .5 seconds (total trial length = 4s). With this design, threats were certain and predictable in fear trials. Anxiety trials were cued with "THREAT?" and were followed by black screen, after which a fearful face and scream *could* occur. Participants were instructed that black screens would be presented for up to 10 seconds and face/screams could occur at any time, creating temporal uncertainty (in actuality: range of 3-9s, avg 6s). Additionally, threats only occurred 66% of the time (20 trials), creating event uncertainty. On trials when threats did not occur, a neutral face and nature sounds were instead presented. Thus, anxiety trials were both uncertain and unpredictable. Stimulus presentation was followed by a variable ITI (0-6s, avg 3s; total trial length = 12s).

Neutral trials were cued with "SAFE!" and were immediately followed by a neutral face and multitalker babble. Wait trials were cued with "SAFE?" and contained the same variable waiting period and event occurrence as Anxiety trials. However, event

outcomes were either a neutral face and multitalker babble, or a neutral face and nature sounds. Participants were instructed that threatening stimuli would never occur during Neutral and Wait trials.

All faces repeated twice per condition, and screams repeated up to four times (with faces always being paired with the same scream and assigned to the same condition). After each mini-block (4 Fear/Neutral trials or 2 Anxiety/Wait trials), participants rated their current level of anxiety (1 = *no anxiety*, up to 10 = *extremely anxious*; 4s to rate, followed by a pseudorandomised jitter 1-5s).

The task was run twice, with a "Feel" run and a "Suppress" run (16 mins for each run, a 15 min break between runs, and order of runs counterbalanced across participants). In the "Feel" run, "THREAT" cues were displayed in *green* and participants were instructed to actively "feel the emotional anticipation, engage in the emotional content of the pictures and sounds, and become aware of any sensations in your body (heart rate, breathing, sweating and/or tension)." "SAFE" cues were presented in *blue* and participants were instructed to "view and respond naturally" to the stimuli. In the "Suppress" run, "THREAT" cues were displayed in *red* and participants were instructed to "decrease the intensity of your emotion, detach from body sensations and passively view the faces." "SAFE" cues were again be presented in *blue* and participants were instructed to "view and respond naturally."

Unique faces and screams were used in each run. After completing one run, participants rated the face/audio pairs from that run using a Likert scale to indicate the pleasantness/unpleasantness of each stimuli pair. Face/audio pairs were shown for 2s (as

in the task), after which participants had 4s to give their response (1 = *extremely pleasant*, 10 = *extremely unpleasant*). Each rating was followed by a 0-2s pseudorandomized jitter.

Importantly, prior to completing the full Threat Anticipation Task, participants were instructed to complete a short practice round of the task in order to become familiar with the paradigm and to reduce any startle response that would not be amenable to scanning. The practice round of the Threat Anticipation Task was composed of 32 trials (16 Practice Feel trials and 16 Practice Suppress trials), organized in mini-blocks of two or four successive trials of the same condition to simulate the actual task, with the order of condition blocks pseudorandomized. The number of practice trials per condition was as follows: 8 Fear, 8 Neutral, 8 Anxiety (6 with an aversive outcome and 2 with a neutral outcome), and 8 Wait.

All behavioral data were analyzed using SPSS (Version 26.0.0; SPSS, INC.). A 2 (Run) x 4 (Condition) factorial ANOVA and follow-up t-tests were performed to assess differences in subjective anxiety during the task and ratings of face/audio stimuli pairs. A probability level of $p < 0.05$ was considered statistically significant for all analyses. Mauchly's test indicated that the assumption of sphericity was violated for the interaction term (Run x Condition: $\chi^2(5) = 15.68$, $p = .008$) and the main effect of Condition ($\chi^2(5) = 20.85$, $p = .001$) in the subjective anxiety ratings, as well as in the stimuli ratings (Run x Condition: $\chi^2(5) = 46.267$, $p < .001$; Condition $\chi^2(5) = 92.241$, $p < .001$). Therefore, *F* ratios were adjusted for these effects using the Greenhouse-Geisser correction. Bar plots of behavioral results were created using R version 3.5.0.

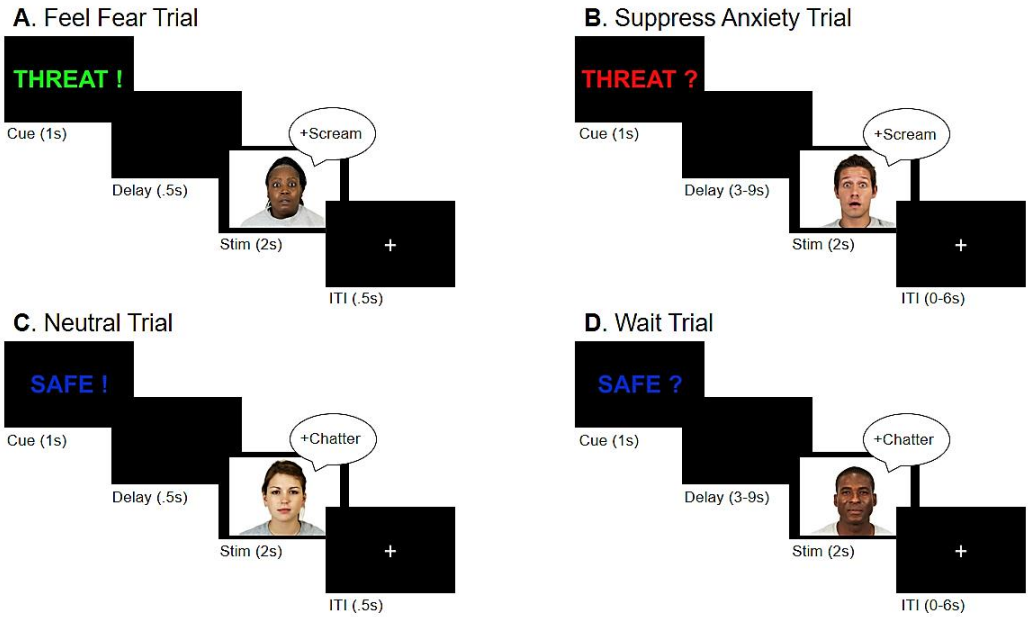

Figure 8. Scanning Paradigm for Experiment 2. Example trials from the Threat Anticipation Task. In Fear and Neutral trials, occurrence of cued stimuli was certain and predictable (fearful face and human scream in Fear; neutral face and multitalker babble in Neutral). In Anxiety and Wait trials, occurrence of cued stimuli was uncertain and unpredictable. During trials in which cued stimuli did not occur (Anxiety: fearful face and human scream; Wait: neutral face and multitalker babble), a neutral face and nature noises where instead presented. In the "Feel" run, "THREAT" cues were displayed in *green* and participants were instructed to actively "feel the emotional anticipation and engage in the emotional content of the pictures and sounds." In the "Suppress" run, "THREAT" cues were displayed in *red* and participants were instructed to "passively view the faces and decrease the intensity of their emotion." "SAFE" cues were always presented in *blue* and participants were instructed to "view and respond naturally".

Neuroimaging Methods

Functional Imaging Data Acquisition

Functional blood oxygenation level-dependent (BOLD) images were collected using gradient-echo T2*-weighted echoplanar imaging [(EPI); TR = 2000 ms; TE = 29 ms; multi-band accelerated factor 3; FoV = 250 mm; 78 transverse slices (interleaved) with whole-brain coverage, flip angle = 62°, 2 x 2 x 2mm voxels]. Slices were oriented

obliquely along the AC–PC line. Scanning parameters were consistent between the two runs of the task (Feel Task and Suppress Task).

Functional Activation

Image preprocessing and data analysis were implemented using the FSL package (version 5.0.9, Analysis group, FMRIB, Oxford, UK http://www.fmrib.ox.ac.uk/fsl/) as described in Chapter 2. Following preprocessing, lower-level statistics were implemented in FEAT. Using multiple regression analysis, statistical maps representing the association between the observed time-series (e.g., BOLD signal) and one or a linear combination of regressors for each subject were constructed. Regressors for the main effects were constructed by modeling each of the conditions versus low-level fMRI baseline (ITI, Jitter, and fixation) for both the Feel Run and the Suppress Run: Fear, Anxiety (only trials with an aversive outcome), Neutral, Wait, and dummy variables modeling the Anxiety trials in which neutral stimuli were presented, and the Rating Period. The contrasts of interest were created by comparing threat conditions against their respective control conditions (e.g., Feel Anxiety > Feel Wait) as well comparisons of the threat conditions across runs (e.g., Feel Anxiety > Suppress Anxiety). For each regressor, a double-gamma hemodynamic response function (HRF) was convolved with an event vector starting at the cue onset through stimulus presentation (duration of 3500ms for Fear and Neutral; duration of 3500-12000ms for Anxiety and Wait with and average duration of 6600ms). Higher-level analyses were conducted using FLAME 1+2 to combine and spatially normalize all subjects. The Higher-level models employed nonparametric permutation methods through FSL's randomize function (Nichols & Holmes 2002). Paired-sample t-tests for each contrast of interest were performed using

the Threshold-Free Cluster Enhancement (TFCE) method, which detects clusters of contiguous voxels without first setting an arbitrary statistical cutoff (e.g., $Z > 2.58$), and controls the family-wise error (FWE) rate at $p < .05$ (Smith & Nichols, 2009). Randomize produces corrected 1-p maps, which were used for all figures and tables. Figures of statistical brain maps were created using FSLview.

Ultra high-resolution anatomical masks (normalized to MNI space) were acquired to accurately delineate the BLA, CEA (.65 mm^3; Leal et al., 2014; Leal et al., 2017) and the BNST (.60 mm^3; Avery et al., 2014) for ROI analyses. Following masking of these regions, FSL's featquery was used to extract percent signal change (PSC) from each ROI. Bar plots of PSC results were created using R version 3.5.0. Follow-up t-tests were then performed to assess significant involvement within a condition, as well as differences in functional activation across condition by region.

Functional Connectivity

Functional connectivity analyses were conducted using the CONN toolbox 18.b (Whitfield-Gabrieli & Nieto-Castanon, 2012) based on SPM12 (Friston et al., 2011) in the 2019a version of MATLAB. CONN's default functional and anatomical preprocessing pipeline was utilized, which included: functional realignment and unwarping (Andersson et al., 2001), slice-timing correction (Henson et al., 1999), outlier identification (using 99% liberal setting), coregistration, direct segmentation and normalization (Ashburner & Friston, 2005), and functional smoothing (6 mm FWHM). Following preprocessing, CONN's default denoising pipeline was applied, which combines two general steps: linear regression of potential confounding effects in the BOLD signal using Ordinary Least Squares (OLS), and temporal band-pass filtering.

Potential confounding effects were accounted for through implementation of an anatomical component-based noise correction procedure (aCompCor), which includes noise components from cerebral white matter (five components) and cerebrospinal fluid (CSF) areas (Behzadi et al., 2007), estimated subject-motion parameters (Friston et al., 1995), and identified outlier scans or scrubbing (Power et al., 2014). The resulting residual BOLD time series were then band-pass filtered (0.008 – inf Hz), as this filter benefits from keeping higher-frequency information fitting event-related tasks (Whitfield-Gabrieli and Nieto-Castanon, 2015). Stimuli onsets and duration were specified in the toolbox, so that BOLD time series could be appropriately divided into task-specific blocks. Block regressors were then convolved with a canonical hemodynamic response function, resulting in weighted connectivity metrics, by condition or contrast.

Functional Connectivity

ROI-to-ROI connectivity metrics were used to characterize the connectivity between all pairs of ROIs among a pre-defined set of regions. ROI-to-ROI connectivity (RRC) matrices represent the level of functional connectivity between each pair of ROIs. Each element of an RRC matrix is defined as the Fisher-transformed bivariate correlation coefficient between a pair of ROI BOLD timeseries. Weighted seed-based connectivity (wSBC) maps were then generated to characterize condition-specific functional connectivity strength. wSBC maps were computed using a weighted Least Squares (WLS) linear model with temporal weights identifying each experimental condition (i.e., condition-specific boxcar timeseries convolved with a canonical hemodynamic response function).A standard second-level GLM analysis of functional connectivity matrices was

utilized to produce a single statistical matrix of T- or F- values, characterizing the effect of interest (e.g., difference in connectivity between two conditions) among all possible pairs of ROIs. FDR-corrected p-values were then computed using the standard Benjamini and Hochberg's algorithm.

14 a priori ROIs were selected from CONN's atlas (Harvard Oxford) for this analysis, based on previous research demonstrating their involvement in a canonical emotion regulation network (Depue et al., 2016; Kohn et al., 2014). These included bilateral middle frontal gyrus (R MFG, L MFG), bilateral inferior frontal gyrus, triangularis and opercularis subregions (R IFG tri, L IFG tri, R IFG oper, L IFG oper), subcallosal prefrontal cortex (which I term here as vmPFC), bilateral supplementary motor areas (R SMA, L SMA), bilateral hippocampi (R HIPP, L HIPP), and the three primary ROIs – BNST, BLA and CEA. In this way, I aimed to investigate how anxiety regulation modulates communication between large-scale brain networks underlying attention, executive function memory, and motor processes, and to explore how these higher-order regions interact with the BNST and amygdala nuclei groups.

Results

Behavioral Results

Analysis of subjective anxiety ratings during the Threat Anticipation Task (Figure 9A) revealed that there was a significant Interaction between Run (Feel, Suppress) and Condition (Fear, Anxiety, Neutral, Wait), $F(2.24, 65.02) = 38.36$, $p < .001$. In addition, there was a significant main effects of Run ($F(1, 29) = 54.53$, $p < .001$), such that ignoring Condition effects, subjective anxiety ratings were higher during the Feel Run compared to the Suppress Run. A significant main effect was also found for

Condition ($F(2, 57.89) = 105.138$, $p < .001$). Follow-up simple effect t-tests showed that participants reported significantly higher anxiety when actively anticipating an uncertain threat (Feel Anxiety trials) compared to waiting for a neutral face/audio stimuli pair (Feel Wait trials; Feel Anxiety vs. Feel Wait: $p < .001$). Across all anxiety trials, participants reported feeling significantly less anxious when actively suppressing their emotional response (Suppress Anxiety vs. Feel Anxiety: $p < .001$), although reported feelings associated with Suppress Anxiety trials were still significantly different from Wait trials in that run (Suppress Anxiety vs. Suppress Wait: $p < .001$). This same pattern followed for Fear trials. Participants reported significantly higher anxious feelings following Feel Fear trials relative to Feel Neutral trials ($p < .001$). When participants were instructed to suppress this emotional response, they reported significantly less anxiety (Suppress Fear vs. Feel Fear: $p < .001$). However, this reduced level of subjective anxiety was still significantly greater than Suppress Neutral trials (Suppress Fear vs. Suppress Neutral: $p < .001$).

Analysis of face/audio stimulus ratings (Figure 9B) were conducted using all fearful faces associated with Fear and Anxiety (excluding the 10 neutral faces per Run in the Anxiety condition, corresponding to the fMRI analyses), and all neutral faces associated with Neutral and Wait (collapsed across multitalker babble and nature sounds in the Wait condition). Results indicated that there was no significant Run x Condition Interaction ($F(1.44, 41.85) = 2.24$, $p = .13$). However, main effects were found for both Condition ($F(1.25, 36.20) = 981.18$, $p < .001$) and Run ($F(1, 29) = 6.73$, $p = .02$). Post-hoc simple effect analyses confirmed that stimuli associated with threat conditions were rated as significantly more unpleasant than their neutral counterparts (Feel Anxiety vs.

Feel Wait: $p < .001$; Feel Fear vs. Feel Neutral: $p < .001$), and this pattern held during the Suppress Run (Suppress Anxiety vs. Suppress Wait: $p < .001$; Suppress Fear vs. Suppress Neutral: $p < .001$). Finally, ratings were compared across runs. Results showed that face/audio stimuli pairs associated with the Suppress Fear condition were rated as significantly less negative than those associated with the Feel Fear condition (Suppress Fear vs. Feel Fear: $p = .002$). A similar trend was found for stimuli associated with Suppress Anxiety trials, although this reduction in negative ratings was not significant (Suppress Anxiety vs. Feel Anxiety: $p = .18$).

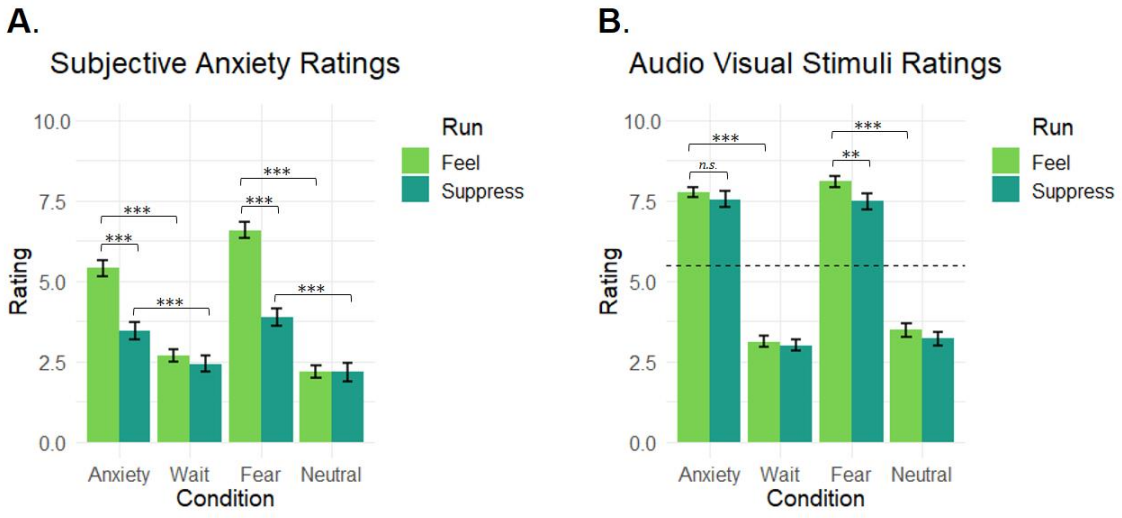

Figure 9. Experiment 2: Behavioral Results. (**A**) Analysis of subjective anxiety ratings from the Threat Anticipation Task revealed a significant interaction between Run (Feel, Suppress) and Condition (Fear, Anxiety, Neutral, Wait), $F(2.24, 65.02) = 38.36$, $p < .001$, as well as significant main effects of Run ($F(1, 29) = 54.53$, $p < .001$), and Condition ($F(2, 57.89) = 105.138$, $p < .001$). Higher ratings indicate more anxiety. (**B**) No significant interaction was found for face/audio stimulus ratings ($F(1.44, 41.85) = 2.24$, $p = .13$). However, main effects were found for Run ($F(1, 29) = 6.73$, $p = .02$) and Condition ($F(1.25, 36.20) = 981.18$, $p < .001$). A rating of "10" indicates "*extremely unpleasant*," while a rating of "1" represents "*extremely pleasant*." The dashed line designates a neutral rating.

Neuroimaging Results

Task Related Whole-Brain Activity

Group level GLM analyses were carried out to examine the neural circuits recruited when feeling and suppressing anxiety, and similarly, when feeling and suppressing fear. Because a primary aim at this phase was to get a broad overview of the neural correlates associated with anxiety regulation, I first generated whole-brain statistical maps of Feel Anxiety, Suppress Anxiety, Feel Fear and Suppress Fear, contrasted against their respective control controls. This approach allowed us to visually inspect neural similarities and differences between fear and anxiety. Additionally, I aimed to assess the specific contributions of the key subcortical regions: BNST, BLA, and CEA. Therefore, I also extracted PSC from these three ROIs in each contrast.

Surprisingly, following cluster-level multiple comparisons corrections, no whole-brain differences emerged between Feel Anxiety and Feel Wait. Lowering the threshold to $p = .20$ (as shown in Figure 10) the strongest differences between the conditions were seen in primary visual areas, brainstem nuclei and the cerebellum. Upon further investigation, supplementary analyses comparing Feel Wait > Suppress Wait revealed whole-brain activation differences, namely in the dorsomedial PFC, visual cortices, and brainstem nuclei (Supplementary Figure 5). Thus, this upregulated activity in visual and brainstem regions during Feel Wait compared to Suppress Wait — likely due to a mild anxiety brought on by simply waiting for a stimulus presentation — resulted in non-significant differences in the Feel Anxiety > Feel Wait contrast. A comparable analysis comparing Feel Neutral > Suppress Neutral only revealed one significant cluster in the supramarginal gyrus, suggesting that the Neutral condition remained a more stable

baseline across the Feel and Suppress Runs (Supplementary Figure 5). Nevertheless, despite these weakened results in light of upregulated activation in the Feel Wait condition, findings showed significantly elevated BNST activation during Feel Anxiety > Feel Wait ($t(29) = 11.74$, $p < .0001$), and lesser but significant involvement of the BLA ($t(29) = 4.63$, $p < .0001$) and CEA ($t(29) = 7.16$, $p < .0001$).

Suppress Anxiety > Suppress Wait revealed a shift from stimulus process and physiological output regions to prefrontal monitoring and control regions. Specifically, this contrast showed increased activation in bilateral inferior frontal gyrus (IFG) encompassing the pars-opercularis and pars-triangularis (BA 44/45, respectively), bilateral putamen, and right middle frontal gyrus (rMFG; Figure 10). Focusing in on the specific ROIs, the BNST still showed a significantly elevated response ($t(29) = 5.00$, $p < .0001$), however activation profiles for the BLA and CEA fell below levels associated with Suppress Wait, although not to a significant degree in the case of the CEA (BLA: $t(29) = -3.20$, $p = .003$; CEA: $t(29) = -1.31$, $p = .20$). Moreover, activation of the BNST was significantly greater than the CEA (BNST vs. CEA: $t(29) = 5.06$, $p < .0001$).

Comparison of Feel Fear > Feel Neutral revealed similar but more robust results to the Feel Anxiety > Feel Wait contrast (again, see Supplementary Figure 5 for differences in control conditions). When participants were actively feeling fear, increased activation was found in primary visual cortices, the cerebellum, primary auditory cortices extending into bilateral IFG, primary motor areas, bilateral amygdala, and brainstem nuclei (Figure 10). Once more, the BNST, BLA and CEA were all significantly elevated (BNST: $t(29) = 11.68$, $p < .0001$; BLA: $t(29) = 10.27$, $p < .0001$; CEA: $t(29) = 13.57$, $p < .0001$). Contrary to expectations (and to the previous study in Chapter 3), the BNST

showed extensive involvement in Feel Fear > Feel Neutral. One hypothesis is that this may be due to chronic activation of the BNST across the entire Feel Run, indicating an elevated general stress response. Therefore, a supplementary analysis was conducted, comparing the rating periods of the Feel and Suppress Runs, a four-second frequently occurring time period that followed every trial type and contained no aversive images or sounds. This rating period additionally appeared in a consistent manner, such that participants likely began to predict its appearance (indicating that uncertainty was very low). Results showed that the BNST was indeed significantly elevated in Feel Rating > Suppress Rating, likely indicating increased general stress associated with the entire Feel Run, on top of a phasic fear response (Supplementary Figure 6).

Finally, Suppress Fear > Suppress Neutral again showed a shift toward more prefrontal recruitment, albeit predominantly in the right hemisphere. This contrast showed increased engagement of the right pars-opercularis and pars-triangularis (rIFG), along with the right posterior superior temporal sulcus (pSTS). The CEA remained significantly elevated ($t(29) = 7.82$, $p < .0001$), however, the BNST was no longer significantly elevated ($t(29) = 2.00$, $p = .06$) and the BLA showed decreased activation to below Suppress Neutral levels ($t(29) = -2.51$, $p = .02$). In this contrast, the CEA showed the greatest involvement (CEA vs. BNST: $t(29) = 2.11$, $p = .04$).

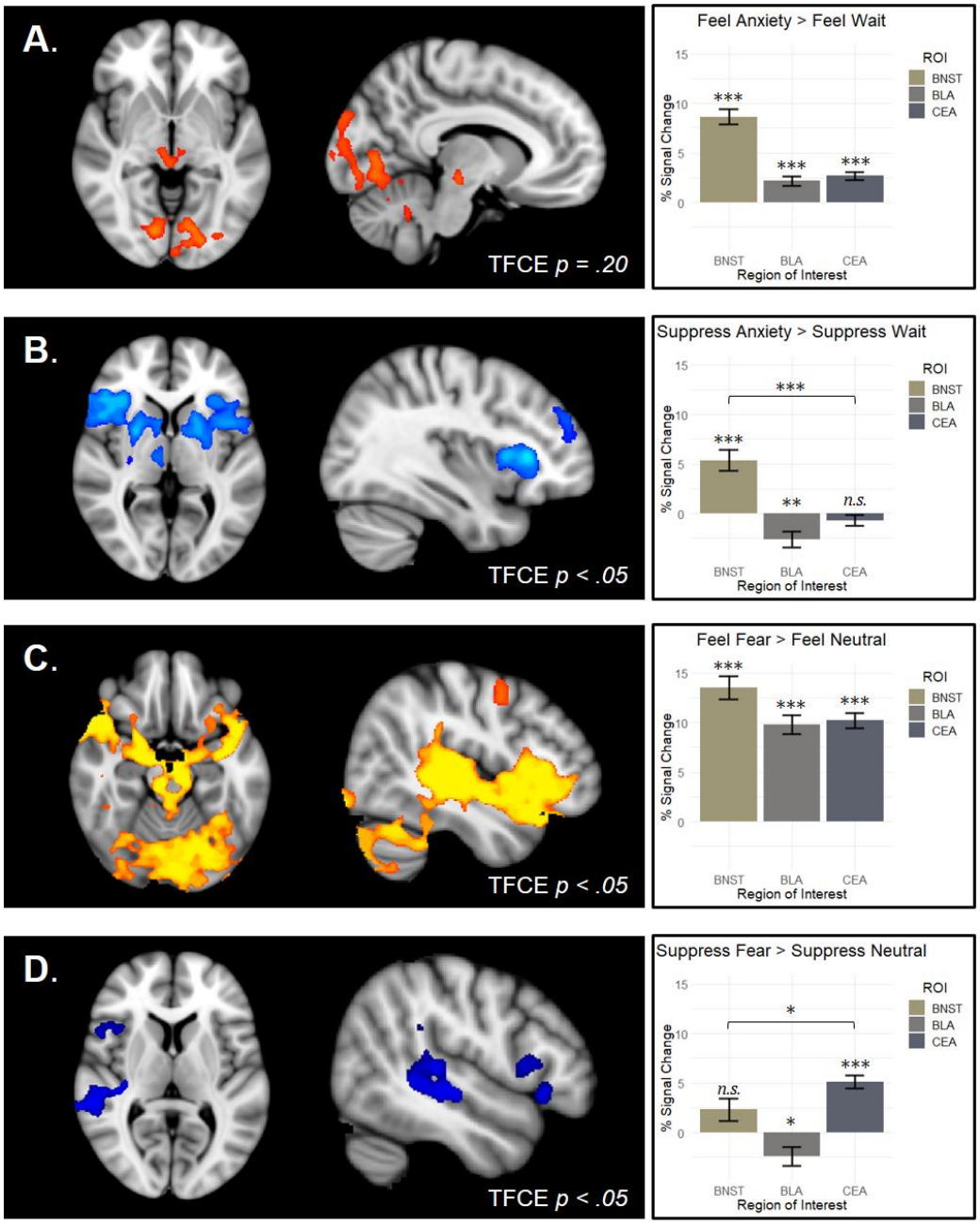

Figure 10. Experiment 2: Whole Brain Functional Activation. (**A**) Feel Anxiety > Feel Wait did not show significant differences (but see also Supplementary Figure 5). However, the strongest voxel-level (uncorrected) differences emerged within primary visual areas, brainstem nuclei and the cerebellum. Significant BNST was found, along with elevated response in the BLA and CEA. (**B**) Suppress Anxiety > Suppress Wait showed a shift to prefrontal monitoring and control regions (bilateral IFG and right MFG). BNST was still significantly elevated, but both BLA and CEA were suppressed below baseline. (**C**) Feel Fear > Feel Neutral showed increased activation in primary visual, primary auditory, amygdala, cerebellum and brainstem. BNST, BLA and CEA were all significantly elevated. (**D**) Suppress Fear > Suppress Neutral again showed a shift toward prefrontal recruitment, predominantly in the right hemisphere. CEA

remained significantly elevated, but both the BNST and BLA showed significantly reductions.

BNST = bed nucleus of the stria terminalis; BLA = basolateral amygdala; CEA = central amygdala; IFG = inferior frontal gyrus; MFG = middle frontal gyrus

Region of Interest Analyses

Focusing on the a priori ROI, I next assessed the direct effects of anxiety regulation by concentrating the analysis on the Suppress Anxiety > Feel Anxiety contrast and the Suppress Fear > Feel Fear contrast. In Suppress Anxiety > Feel Anxiety, results showed significant suppression of BNST ($t(29) = 14.54$, $p < .0001$) and the BLA ($t(29) = 4.49$, $p < .0001$), but not the CEA ($t(29) = 1.43$, $p = .16$; Figure 11). Furthermore, there was significantly greater suppression of the BNST relative to the BLA ($t(29) = 4.28$, $p < .0001$). Supplementary analyses were conducted to investigate if the degree of BNST downregulation between Feel and Suppress runs was related to individual differences in behavioral reports of anxiety regulation (i.e. difference in reported anxiety from the Feel to Suppress run across anxiety trials). A moderate positive correlation was found, such that greater BNST downregulation was associated with a larger drop in reported anxious feelings, however, this correlation did not reach significance ($r = .30$, $p = .11$; Supplementary Figure 7).

For Suppress Fear > Feel Fear, significant suppression was demonstrated in the BLA ($t(29) = 6.31$, $p < .0001$), CEA ($t(29) = 4.76$, $p < .001$), as well as the BNST ($t(29) = 12.41$, $p < .0001$; Figure 11). Moreover, Suppress Fear > Feel Fear was more markedly associated with amygdala downregulation, such that the difference between BLA and BNST suppression was no longer significant ($t(29) = .96$, $p = .34$).

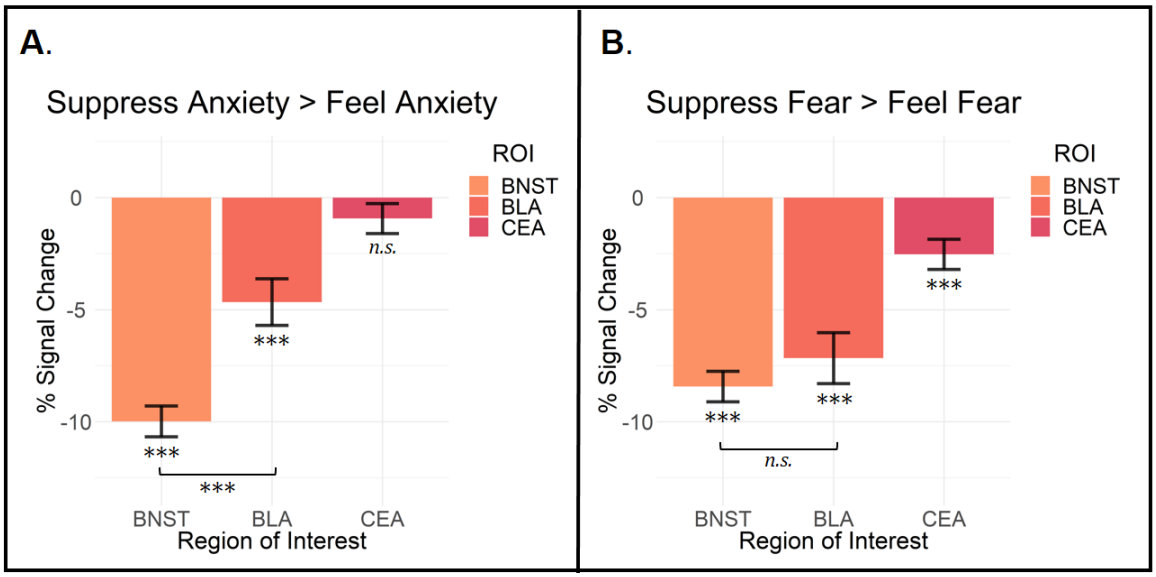

Figure 11. Experiment 2: Region of Interest Analysis. (**A**) Suppress Anxiety > Feel Anxiety showed significant suppression of the BNST and BLA, but not the CEA. BNST suppression was also significantly greater than BLA suppression. (**B**) Suppress Fear > Feel Fear demonstrated significant suppress of the BNST, BLA and CEA. The degree of BLA suppression was enhanced in this contrast such that the difference in BLA and BNST suppression was no longer significant.

BNST = bed nucleus of the stria terminalis; BLA = basolateral amygdala; CEA = central amygdala

Functional Connectivity

ROI-to-ROI connectivity was performed to assess brain regions that display coherence across the time-course of activation when actively suppressing anxiety and fear. This was done to investigate how anxiety regulation modulates communication between large-scale brain networks underlying attention, executive function, memory, and motor processes, and how these higher-order regions interact with the BNST and amygdala nuclei groups.

The comparison of Suppress Anxiety > Suppress Wait revealed significantly decreased connectivity among several higher-order prefrontal regions, namely between

right and left MFG (R MFG – L MFG: $t(29) = -2.96$, $p < .05$), right and left IFG pars triangularis (R IFG tri – L IFG tri: $t(29) = -3.79$, $p < .01$), left MFG and left IFG pars triangularis (L MFG – L IFG tri: $t(29) = -3.44$, $p < .05$), and left IFG pars triangularis and both right and left IFG pars opercularis (L IFG tri – R IFG oper: $t(29) = -2.81$, $p < .05$; L IFG tri – L IFG oper: $t(29) = -2.64$, $p < .05$). Notably, the right IFG pars triangularis was shown to have increased positive connectivity with the BNST (R IFG oper – BNST: $t(29) = 2.93$, $p = .057$) during Suppress Anxiety > Suppress Wait (Figure 12).

In comparison, Suppress Fear > Suppress Neutral showed increased connectivity between the right MFG and vmPFC (R MFG – vmPFC: $t(29) = 3.43$, $p < .05$). In addition, the CEA showed decreased connectivity with the left supplementary motor area (CEA – L SMA: $t(29) = -3.57$, $p < .05$) and the BNST showed decreased connectivity with the left hippocampus (BNST – L HIPP: $t(29) = -3.74$, $p = .01$; Figure 12). A similar trend of decreased connectivity between the BNST and right hippocampus was found for Suppress Anxiety > Suppress Wait as well, however this did not reach significance following FDR correction for multiple comparisons (uncorrected: $p = .03$, corrected: $p = .15$).

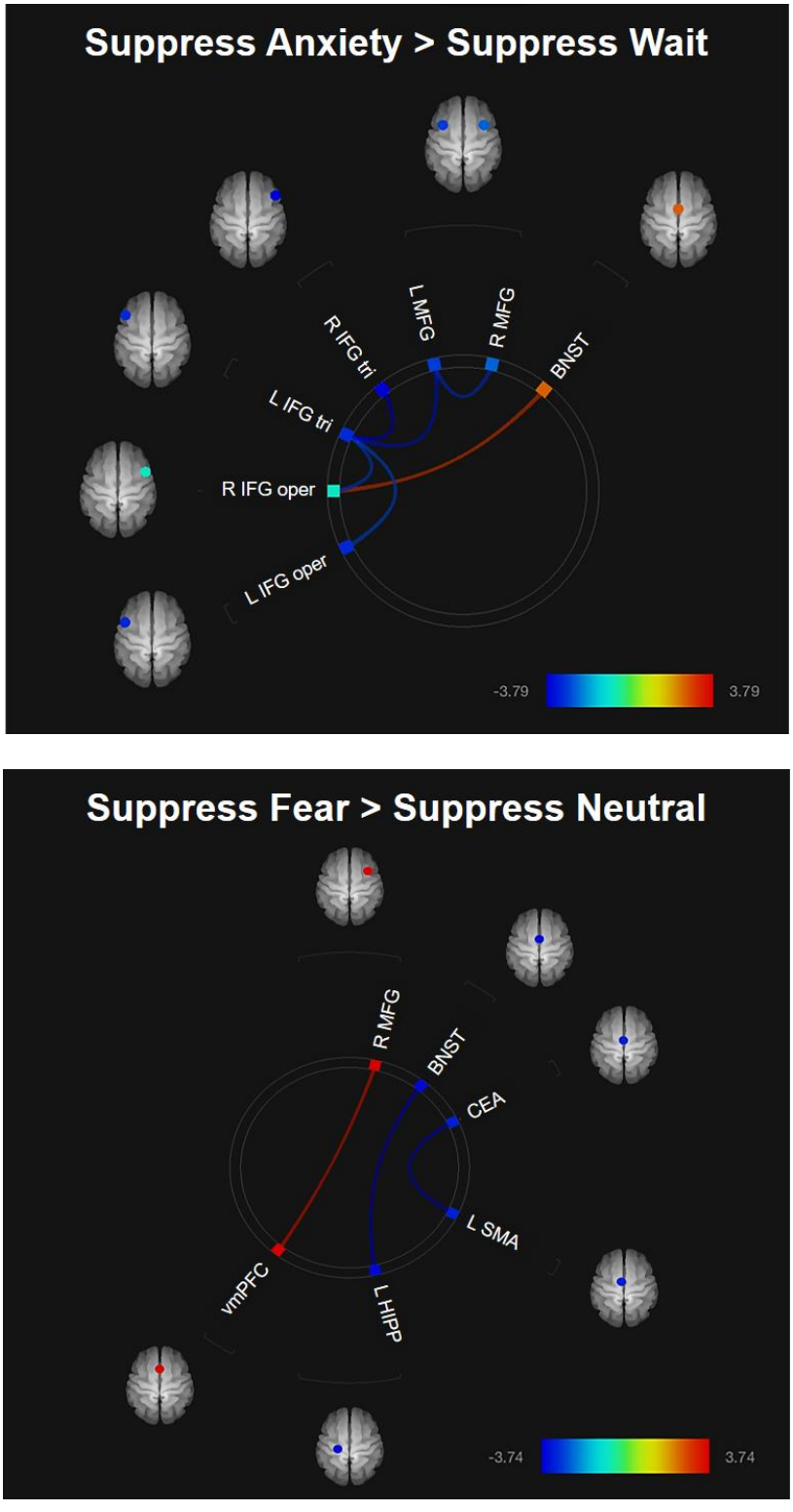

Figure 12. Experiment 2: Functional Connectivity Analysis. Suppress Anxiety > Suppress Wait revealed significantly decreased connectivity among several higher-order prefrontal regions, while the right IFG pars triangularis was shown to have increased positive connectivity with the BNST. Suppress Fear > Suppress Neutral showed

increased connectivity between the right MFG and vmPFC, and decreased connectivity between the CEA and left SMA, and the BNST and left hippocampus.

IFG = inferior frontal gyrus; BNST = bed nucleus of the stria terminalis; MFG = middle frontal gyrus; vmPFC = ventromedial prefrontal cortex; CEA = central amygdala; SMA = supplementary motor area

Discussion

To the best of my knowledge, this is the first study to specifically evaluate the neural mechanism supporting anxiety regulation (i.e., volitional emotional control during uncertain and unpredictable prospective threat), and to investigate whether the BNST can be downregulated. The Threat Anticipation Task employed a multimodal stimulus (fearful human faces and screams) to psychologically elicit feelings of fear or anxiety through cues signaling certain and predictable threats (fear), or uncertain and unpredictable threats (anxiety). This task was then run twice with opposing instructions. During one run, participants were instructed to actively feel and engage with their emotion (Feel Run), whereas in the other run they were told to decrease the intensity of their emotions and passively view threatening stimuli (Suppress Run). Participants were able to successfully modulate their emotional responses, as documented through significant differences in subjective anxiety ratings across runs. Corresponding to these differences in subjective feelings, the BNST and BLA showed significant downregulation in Suppress Anxiety > Feel Anxiety, while the BNST, BLA and CEA were all significantly downregulated in Suppress Fear > Feel Fear; once more indicating a partial dissociation between fear and anxiety (as in Chapter 3). Whole brain functional activation results revealed general commonalities between fear and anxiety, with Feel Anxiety and Feel Fear showing upregulation in stimulus processing regions and psychological output

structures, as well as corresponding general shifts toward stronger prefrontal engagement when suppressing these emotions. However, functional connectivity results indicated differences in network communication. Suppress Anxiety showed increased connectivity between the right IFG and BNST, and decreased connectivity among higher-order attentional circuits. In comparison, Suppress Fear showed increased connectivity between right MFG and vmPFC, and decreased connectivity between BNST and left hippocampus, and CEA and premotor cortex. These findings replicate previous work that indicates partially dissociated functional roles of the amygdala and BNST, and importantly, extends this previous work by showing that the BNST can be downregulated, and that this is done through a combination of increased prefrontal recruitment for regulatory control, and decreased connectivity among attentional circuits that may promote unwanted vigilance.

Behavioral Findings

After each mini block (4 Fear/Neutral trials or 2 Anxiety/Wait trials), participants rated their current level of anxiety on a 10-point scale. Results showed that participants were significantly more anxious when actively anticipating an uncertain threat (Feel Anxiety) compared to waiting for a neutral face/audio stimuli pair to arrive (Feel Wait). Though this finding was expected, this initial comparison was important to confirm that feelings of anxiety were in fact induced. The same pattern held for Fear trials, with participants reporting significantly higher anxiety following Feel Fear trials, relative to its neutral counterpart (Feel Neutral). Average subjective anxiety ratings for Feel Anxiety and Feel Fear were 5.41 and 6.58, respectively, indicating a moderate level of anxiety (slightly surpassing the halfway point between “no anxiety” and “extremely anxious”).

Following anxiety regulation, participants reported feeling significantly less anxious, demonstrating that anxious feelings were successfully downregulated to a degree (average change from Feel Anxiety to Suppress Anxiety = 2.0). Still, reported feelings associated with Suppress Anxiety trials were significantly different from Wait trials in that run, illustrating that Anxiety trials were still more anxiety provoking, even when participants attempted to volitionally control their emotional responses. Once more, Fear trials followed this same pattern. Participants reported significantly higher anxiety following Feel Fear trials relative to Feel Neutral trials. When participants were instructed to suppress this emotional response, they reported significantly less anxiety, however, this reduced level of subjective anxiety was still significantly greater than Suppress Neutral trials. Together, this demonstrates that the stimuli and trial design were indeed anxiety provoking, but that participants were able to volitionally decrease their emotional responses from moderate anxiety to a mildly anxious state.

Following each run, participants rated all face/audio stimuli pairs. Analysis of stimulus ratings were conducted using all fearful faces associated with Fear and Anxiety (excluding the 10 neutral faces per Run in the Anxiety condition, corresponding to the fMRI analyses), and all neutral faces associated with Neutral and Wait (collapsed across multitalker babble and nature sounds in the Wait condition). Here, main effects were found for both Condition and Run, again indicating that on the whole, stimuli were rated as less negative in the Suppress Run compared to the Feel Run. Post-hoc simple effect analyses confirmed that stimuli associated with threat conditions were rated as significantly more unpleasant than their neutral counterparts, and this pattern held in both runs. Ratings were additionally compared across Runs, which revealed that face/audio

stimuli pairs associated with the Suppress Fear condition were rated as significantly less negative than those associated with the Feel Fear condition. This suggests that following emotion regulation and a reduction in anxious state, the stimuli themselves were perceived as less threatening. A similar trend was found for stimuli associated with Suppress Anxiety trials, although this reduction in negative ratings was not significant.

Functional Activation

Group-level whole-brain functional activation analyses were carried out in order to investigate the neural correlates associated with feeling anxiety and fear, as well as suppressing these emotions. This wide-angle approach additionally allowed us to visually inspect neural similarities and differences between upregulating and downregulating fear and anxiety. Finally, within these whole brain contrasts, I extracted out PSC from the three apriori ROIs to assess the specific contributions of these key subcortical regions.

Similarities between Feel Anxiety and Feel Fear included upregulation in visual processing regions and brainstem output nuclei. Given the stimuli presented and the current contrast (threatening stimuli compared to neutral ones), it is unsurprising that an upregulation in visual cortices was seen across both threat conditions. It is well known that emotional significance, specifically for fear-associated stimuli, can boost neural responses in the visual cortex (Vuilleumier & Driver, 2007). Moreover, attention can have an additive modulatory effect on visual processing of the same stimuli, simply by altering one's own internal attentional state (Vuilleumier & Driver, 2007). Thus, the combination of negatively valenced emotional stimuli, and instructions for participants to "actively engage in the emotional content of the pictures and sounds" resulted in the increased activation seen in visual cortices. Coinciding upregulation of brainstem nuclei

likely reflects increased engagement of downstream targets that mediate many common behavioral and autonomic responses to fear and anxiety, such as increased respiration, perspiration and pupil dilation (Walker et al., 2003).

Though Feel Anxiety and Feel Fear displayed several similarities, one noticeable difference was that fear clearly demonstrated more extensive visual and auditory upregulation during the Feel Run. While it is difficult to tease apart whether this discrepancy between fear and anxiety was due to differences in their respective control conditions (see Supplementary Figure 6) one interpretation suggests that, while both types of threat recruited these sensory regions, fear is more stimulus bound and exemplifies a stronger representation of the stimulus. This notion is supported by my previous work (see Chapter 3), which showed very similar results when contrasting Fear with Anxiety directly. Furthermore, the Feel Fear > Feel Neutral contrast showed robust bilateral amygdala activation, even following whole-brain correction, replicating the previous work that the amygdala is most responsive to the presentation of an aversive stimulus, and is thus more strongly associated with Fear. Added evidence from the previous connectivity analyses further suggested that the stimulus-bound nature of Fear may be mediated through the BLA and its back projections to upregulate visual processing (Amaral et al., 2003; Pessoa & Adolphs, 2010). The present analysis additionally showed increased activation in the primary motor cortex, which may also be mediated through increased activity in and connectivity with the BLA (Chapter 3; Avendan et al., 1983; Llamas et al., 1977). Taken all together, these results corroborate previous findings and reiterate that in the face of threat, the amygdala may facilitate

coordinated activity between sensory processing areas and motor control, so as to afford quick and adaptive behavioral changes.

Similarities were also noted when comparing the Suppress Anxiety > Suppress Wait and Suppress Fear > Suppress Neutral contrasts. When volitionally regulating both types of threat, a shift toward prefrontal activation was apparent – specifically to the rIFG in both contrasts, and the previously seen upregulation in visual and brainstem areas were no longer detected. Increased engagement of the rIFG has been implicated in several cognitive processes, including: enhanced negative context monitoring and rapid surveillance of the environment for potential danger, integration of top-down and bottom-up information, and a general withdrawal response (Banich & Depue, 2015; Corbetta & Shulman, 2002; Depue et al., 2015; Dodds et al., 2011; Hampshire et al., 2010). In addition, activation in the IFG has been interpreted as a gating mechanism that inhibits responses to stimuli that are irrelevant to current goals (Frank & Sabatinelli, 2012). Greater IFG recruitment has been demonstrated when monitoring stimulus changes across multiple modalities (Downar et al., 2001), during successful working-memory trials in the presence a negative distractor (Shafer et al., 2012), and in the realm of response inhibition such as with the go/no-go task (Chikazoe et al., 2007). Lastly, greater IFG activation has been found to be negatively correlated with anxiety in a sample of anxious adolescents, and moreover, treatment with cognitive behavioral therapy (CBT) and selective serotonin reuptake inhibitors (SSRIs) was found to greater increases in IFG activation among successfully treated patients (Maslowsky et al., 2010; Shechner et al., 2012). These data, and the noted shift toward greater rIFG recruitment when participants attempted to cognitively regulate their emotional responses, are consistent with the

hypothesized role of the IFG as a flexible change detector that promotes the continued processing of a primary task while inhibiting potentially distracting or threatening stimuli (Corbetta & Shulman, 2002).

Subtle differences between conditions also emerged, with results showing that Suppress Anxiety additionally recruited the rMFG. In concert with the rIFG, the rMFG has been shown to orchestrate goal-directed inhibitory control across cognitive, emotional and memory domains (Depue et al., 2015). It has been suggested that the central role of the MFG during inhibitory regulation is to update and maintain higher-order goal representations, which then subsequently influence communication within and between other network regions – such as the rIFG — to accomplish the task at hand (e.g. reduce emotional reactivity; Depue et al., 2016). Anatomically, the MFG lacks direct connections to subcortical limbic regions, while the posterior region of the IFG is the only lateral PFC region with significant direct input to the amygdala. This suggests that while the MFG represents the highest-order goal-directed behavior, the IFG is optimally positioned to integrate information from other prefrontal areas and regulate subcortical activity (Ray & Zald, 2012).

The accompanying ROIs results provide additional insight into how fear and anxiety are processed and regulated, and furthermore, partially replicates my previous work. As in Chapter 3, the BNST and amygdala nuclei groups were all found to display heightened activity in Feel Anxiety > Feel Neutral, but the BNST showed the most heightened response in this contrast, as hypothesized. Furthermore, in the Suppress Anxiety > Suppress Wait, the BNST still showed a significantly elevated response, while activation profiles for the BLA and CEA fell below levels associated with Suppress Wait.

Together, this demonstrates that, even when volitionally suppressing anxiety (i.e. in a mildly anxious state), the BNST showed the greatest involvement in anticipating an uncertain and unpredictable aversive threat compared to waiting for a neutral one.

The fear condition exhibited a similar pattern with regard to the amygdala nuclei group: the BLA and CEA displayed significantly responses in the Feel Run (also significantly higher than in Feel Anxiety), and both showed significantly reduced activation profiles in Suppress Fear > Suppress Neutral, although the CEA still remained significantly elevated in this contrast. As before, this suggest that, even when volitionally regulating fear, the CEA still shows the greatest involvement during the processing of an explicit threat compared to a neutral image and sound.

Contrary to expectations, the BNST was also highly elevated in Feel Fear > Feel Neutral. However, a supplementary analysis points to chronic BNST activation, due to increased general stress associated with the entire Feel Run, on top of a phasic fear response (Supplementary Figure 6). This interpretation is additionally supported by recent animal work that has investigated the role of the BNST in phasic fear. In a preliminary rat study, electrolytic post-training lesions of the BNST were shown to significantly impair cued fear. However, based on very high contextual freezing level in this experiment, the authors hypothesized that the entire paradigm may have been too aversive, leading to higher levels of general stress and thus confounding their results relating to the BNST. Therefore, a second experiment was conducted after developing an amended protocol that elicited cued fear in a lower general stress environment, which revealed that the BNST did not mediate the expression of cued fear under lower stress conditions (Luyck et al., 2020). Therefore, I suspect that the fifteen-minute Feel Run,

during which participants were frequently enhancing their emotional responses and becoming aware of their physiological sensations, may have incited an elevated stress response. These findings highlight the importance of paradigm design considerations and validation, as subtle changes may influence behavioral and neural responses and lead to flawed interpretations. It may also be worthwhile to retrospectively review existing literature to evaluate whether papers that find strong BNST involvement in phasic fear could be explained by an activated general stress response.

Region of Interest Analysis

Based on previous literature and recent theoretical models implicating the BNST in anxiety processing, one primary impetus for this study was to investigate whether the BNST could be volitionally downregulated in a similar manner as has been previously demonstrated in the amygdala. Therefore, I additionally assessed the direct effects of anxiety regulation by focusing in on the Suppress > Feel contrasts for both anxiety and fear, and extracted PSC from the three regions of interest: BNST, BLA, CEA.

In Suppress Anxiety > Feel Anxiety, results showed significant suppression of BNST and the BLA, but not the CEA, once more showing a partial dissociation between the functional roles of the BNST and amygdala nuclei groups. Furthermore, there was significantly greater suppression of the BNST relative to the BLA. Supplementary analyses additionally displayed a moderate but non-significant positive correlation between BNST downregulation and behavioral anxiety regulation, indicating that a reduction in BNST activity may play a role in reducing anxious feelings, but is likely not the sole contributor.

For Suppress Fear > Feel Fear, significant suppression was demonstrated in the BLA and CEA, as well as the BNST. To reiterate, I believe that the involvement of the BNST in the Fear condition was at least in part due to chronic activation of the BNST across the whole Feel Run, thus resulting in this suppression effect when contrasted with the Suppress Run. Nonetheless, the Suppress Fear > Feel Fear contrast was more notably associated with amygdala downregulation, such that CEA was significantly suppressed, and the difference between BLA and BNST suppression was no longer significant due to increased downregulation of the BLA.

Together, this reinforces a partial dissociation between the functional roles of the BNST and amygdala, with the BNST modulating states of apprehension in the face of an uncertain prospective threat, and the amygdala being more closely associated with responsivity to threat encounter. It is important here to emphasize that I do not support the view of a strict double dissociation between the amygdala and BNST, but rather one of partially segregated information processing in the midst of a highly interconnected system. This view is strengthened by recent work that used spectral dynamic causal modeling (DCM), which demonstrated interconnectivity among all amygdala nuclei groups and the BNST at rest, but with an asymmetric connectivity structure (Hofmann & Straube, 2019). These results indicated that while activity flow within the amygdala is highly correlated and informed by the BNST, activity flow in the BNST seems to be partially separated from the amygdala, likely mediated by integration into different cortical and subcortical networks. However, the authors note that there also existed periods of time where both the BNST and amygdala were activated together, showing that these regions naturally flow in and out of phase with one another at baseline. When

DCM models were manipulated so that effective connectivity strength between one or several amygdala nuclei was increased, this resulted in heightened initial amygdala amplitude as well as increased and longer lasting BNST amplitude in response to a simulated stimulus (Hofmann & Straube, 2019). This example underscores the complex and dynamic system at hand, but simultaneously bolsters confidence that the functional data gathered from the amygdala and BNST best reflects a partially segregated information processing system.

Functional Connectivity

We additionally assessed ROI-to-ROI functionally connectivity as a means to evaluate how anxiety regulation modulates communication within and between large-scale brain networks underlying cognitive processes such as attention, executive function, motor response and memory, and to uncover how these higher-order brain regions interact with the BNST and amygdala nuclei groups.

Results revealed that Suppress Anxiety > Suppress Wait was associated with increased connectivity between the rIFG and BNST. An amalgamation of data has indicated that the rIFG is crucial to the integration between bottom-up sensory information and top-down response-related information, due to its extensive anatomical connections with prefrontal, sensory and motor regions, and demonstrated involvement in both attention and inhibition (Diquattro & Geng, 2011; Dodds et al., 2011). Increased connectivity between the IFG and amygdala has been shown to relate to improved control over emotional distractibility during ongoing cognitive behavior (Dolcos et al., 2006), but this relationship has never before been demonstrated with the BNST. Speculatively, I suggest that connectivity between the rIFG and BNST may serve a similar purpose,

helping to reduce vigilant anticipation and reactivity to the aversive stimuli, and promoting control over emotional and downstream physiological responses.

Furthermore, decreases in connectivity were noted among several other prefrontal regions, namely between right and left MFG, right and left IFG pars triangularis, left MFG and left IFG pars triangularis, and left IFG pars triangularis with both right and left IFG pars opercularis. One of the most consistent conclusions to emerge from theoretical models of anxiety is that anxiety is characterized by an attentional bias to threat, which consists of vigilance for threat (i.e., rapid orienting to threat) and attentional maintenance on threat (i.e., delayed disengagement from threat) (Richards et al., 2013). Two systems in the brain are known to modulate attention: the dorsal attentional network (DAN) – of which the MFG is a constituent, and the ventral attention network (VAN) – to which the IFG belongs. While DAN supports goal-directed attention, VAN underlies stimulus-driven attention reorienting, acting as a "circuit breaker" to interrupt ongoing processing and shift attention toward a behaviorally-relevant stimulus. Attentional Control Theory suggests that rapid orienting to threat (i.e., vigilance) occurs as a result of increased influence of VAN, while Attentional Maintenance Theory suggests that increased anxiety is due to difficulties inhibiting and shifting attention away from threat, which may additionally involve DAN (Richard et al., 2013). In support of this, resting state functional connectivity analyses found that stressed participants (relative to controls) showed increased connectivity within DAN and VAN (and also sensorimotor [SM] and primary visual [VN] networks). Furthermore, when these participants were then asked to perform a simple decision-making task, stressed participants showed relatively weaker deactivation, suggesting greater difficulty in tuning down these networks, which may

reflect difficulties filtering sensory information (Soares et al., 2013). Relative suppression of attentional networks has indeed been shown to be beneficial, and has been interpreted as a filtering mechanism, gating sensory responses by behavioral relevance. For example, suppression in VAN has been noted when stimuli that are considered behaviorally irrelevant are presented (Corbetta et al., 2008). Finally, while transient increases in vigilance have been shown to improve attention and perception (Robertson, 2001), I suggest that decreases in connectivity between attentional circuits reduce vigilance and perception, and may then subsequently aid in reducing anxious feelings. Taken all together, this suggests that decreased connectivity between several nodes of DAN and VAN may represent enhanced sensory filtering, so as to reduce vigilance and protect the system from involuntarily reorienting to the environment when task demands require detaching from threatening stimuli and decreasing emotional reactivity.

It should be pointed out, that rMFG → rIFG (triangularis and opercularis) were two of the only pathways that did *not* exhibit decreased connectivity, suggesting this communication between these regions remained intact, or at comparable levels as in Suppress Wait. It has been suggested that attentional control is initiated via the rMFG, which putatively links DAN and VAN and funnels down attentional biases (Corbetta et al., 2008). Together, the functional activation and functional connectivity results suggest a hierarchical regulatory network between the rMFG, rIFG and BNST. With the primary role of the rMFG being to initiate goal-directed behavior, this suggests that increased engagement of the rMFG represents a stronger task representation to reduce emotional reactivity, which was then implemented through modulation of attentional processing and inhibitory control via the rIFG.

Suppress Fear > Suppress Neutral indicated slightly different regulatory mechanisms, including decreased connectivity between the CEA and left SMA, and between the BNST and left hippocampus. In Chapter 3, analyses revealed increased connectivity between the amygdala and cortical motor areas during the Fear condition, as well as during the stimulus presentation of the Anxiety condition, indicating a role in the preparation for a motor response in the face of threat (Avendan et al., 1983; Llamas et al., 1977). Here, I show the opposite pattern through decreased connectivity between the CEA and PMA in relation to fear suppression, likely indicative of decreased need for a preparatory fight or flight response. Regarding decreased connectivity between the BNST and hippocampus, hippocampal activity has been reported in fear conditioning as it is integral to association learning and emotional memory formation (Knight et al., 2004), and so the same logic holds that fear suppression may lead to disrupted hippocampal connectivity and an intentional downregulation of memory processing. Nevertheless, BNST—hippocampal connectivity has not previously been demonstrated in the context of ER.

Suppress Fear > Suppress Neutral additionally exhibited increased connectivity between the right MFG and vmPFC. This finding is in line with several other reports on ER neural mechanisms, which posit that amygdala activity can be effectively downregulated through goal-directed inhibitory control implemented by the MFG via connectivity through the vmPFC (Delgado et al., 2008; Levesque et al., 2003; Ochsner et al., 2002; Ochsner et al., 2004; Phan et al., 2005). Though these results did not provide a direct link between the vmPFC and amygdala, the role of the vmPFC in fear extinction and regulation of amygdala activity is well supported (Delgado et al., 2008; Motzkin et

al., 2015; Phelps et al., 2004), and coincides with the ROI results that demonstrated significantly decreased BLA and CEA activity in Suppress Fear > Feel Fear. Jointly, these results support the canonical ER network (rMFG → vmPFC → amygdala), and moreover, demonstrate that this canonical circuitry is most directly associated with ER over fear processing. Together, this supports the notion that existing ER studies to date have likely been measuring regulation over fear or general negative affect, as the Suppress Anxiety results revealed divergent mechanisms.

Limitations and Future Directions

Limitations of the present study should be acknowledged. To begin, the sample size was modest, at 30 healthy adults. Therefore, results should be interpreted cautiously and future studies should be conducted to replicate and extend these findings. Furthermore, all recruited participants were psychologically healthy. While this was my aim — to first understand if the BNST can be downregulated in a cognitively healthy sample and delineate what these putative mechanisms are — I can only speculate as to how these neural circuits may be altered in clinical populations. Additionally, the sample was predominantly female, and given that the BNST is known to be a sexually dimorphic region (Hines et al., 1985), it is unknown how these mechanisms may vary by gender.

Regarding the paradigm, this study was designed with only two runs: all conditions in a Feel Run and all conditions in a Suppress Run. One unintended consequence of this, was that it appeared the BNST was chronically active across the entire Feel Run (Supplementary Figure 6). Therefore, future work could benefit from separating out Feel Fear, Feel Anxiety, Suppress Fear, and Suppress Anxiety into four separate runs to better parse out the role of the BNST in phasic fear, independent from an

elevated stress response. Finally, while this study only included threat conditions that were either both certain *and* predictable, or uncertain *and* unpredictable, additional studies could explore conditions in which threat is certain but significantly delayed, or where threat is cued as certain but never arrives. The latter in particular may serve as a model for generalized anxiety through a simulated state of perpetual anticipation for a fear that may never occur.

Summary

Through this work, I have attempted to uncover the neural correlates of anxiety regulation and to assess the similarities and differences with suppressing fear in terms of the neural mechanisms recruited. The results showed that anxiety regulation is associated with pronounced BNST downregulation and modest BLA suppression, and deactivation of visual regions and brainstem output nuclei. Activation and connectivity analyses added that suppressing anxiety recruits prefrontal regions (rMFG, rIFG), and increases connectivity between the rIFG and BNST, while simultaneously disconnecting from attentional circuits. Together, this suggests that suppressing anxiety is a coordinated response that downregulates emotional, sensory and physiological processing through increased recruitment of the rMFG and rIFG, and a reduction in communication between higher-order attentional networks that may drive unwanted hyper-vigilant monitoring and reorienting.

In comparison, regulation of fear likewise downregulated the BNST and BLA, but also the CEA. Fear regulation similarly recruited the rIFG, and accompanying reductions in visual and physiological regions were seen. However, connectivity results showed that regulating fear is associated with increased connectivity in the canonical emotion

regulation circuit (rMFG → vmPFC), in addition to decreased connectivity between the CEA and SMA, and BNST and hippocampus. In sum, this indicates that fear suppression – like anxiety regulation – is associated with downregulation of emotional, sensory and physiological processes, but is additionally characterized by disconnection from motor and memory circuitry.

Two novel findings resulted from this work: 1) I provide the first evidence that the BNST can be volitionally downregulated, and 2) I suggest that anxiety regulation in part stems from modulating attentional systems. How these processes are accomplished, appear to be through enhanced recruitment of the rMFG and rIFG, which then disconnect from other attentional regions (but not each other) in order to disrupt communication in stimulus-driven attentional circuits, reduce vigilance and allow passive viewing of the threatening stimuli. The concurrent increased connectivity between the rIFG and BNST may represent directed regulatory control over BNST responsivity, or may alternatively indicate more frequent monitoring and communication of the current context, allowing the BNST to relax, knowing that the rIFG will "break the circuit" and provide an update should there be a sudden change in the threatening landscape.

CHAPTER V: GENERAL DISCUSSION

Overview and Recap of Results

In these studies, I utilized high-resolution fMRI to investigate the differential contributions of the amygdala and BNST in the processing and regulation of fear and anxiety. In Experiment 1, I demonstrated that the amygdala shows preferential involvement in fear processing, and exhibited heightened responsivity to the overt presentation of the threatening stimulus. Additionally, this study highlighted that fear engaged more stimulus-bound processing (visual and auditory cortices), and displayed increased connectivity between the amygdala and regions supporting stimulus processing and gross motor response. Together, these findings suggest that fear – and the amygdala – facilitate coordinated activity between sensory processing and motor control areas, so as to afford quick and adaptive behavioral changes in the face of an explicit threat. By comparison, the BNST showed preferential involvement in anxiety processing, indicating a functional specialization for detection and monitoring of an uncertain and unpredictable prospective threat. This was further supported by increased connectivity between the BNST and anterior prefrontal regions underlying interoception, internal mentation and rumination. This work therefore leads to the conclusion that the BNST appears to exhibit a functional specialization for the detection of a potential threat, putatively serving as an alerting system to maintain hypervigilance, and thus worry and rumination, until the arrival of a threat or resolution of the threatening situation.

In Experiment 2, I investigated the regulation of fear and anxiety. This work showed that regulating fear is associated with increased connectivity in the canonical emotion regulation circuit (rMFG → vmPFC), which putatively downregulates amygdala activity and subsequent physiological output. Parallel to this enhanced connectivity, I found a corresponding reduction in amygdala activity (both BLA and CEA), decreases in visual processing, and disconnections in motor and memory circuits. Anxiety regulation, on the other hand, was associated with pronounced BNST downregulation, in addition to moderate BLA suppression. It too showed relative deactivations in visual processing and physiological output regions, but was uniquely associated with modulation of higher-order attentional circuits. I propose that suppressing anxiety is accomplished through decreased connectivity among attentional circuits in order to decrease hypervigilant monitoring, but with simultaneous specific recruitment of the rMFG and rIFG, and increased communication between the rIFG and BNST to provide directed inhibitory control.

In summary, both studies provide evidence that the BNST is more intimately associated with anxiety, while the amygdala predominantly underlies fear. Moreover, fear appears to be more stimulus bound, supporting a response to an immediate and identifiable threat through modulation of sensorimotor regions, while anxiety incorporates higher-order cognition: interoception and rumination when processing anxiety (Experiment 1) and disengagement from attentional systems when controlling anxiety (Experiment 2).

Clinical Implications and Future Directions

Following these novel findings, the natural next step would be to extend this paradigm to specific clinical populations, to better understand how these neural mechanisms are altered in individuals with anxiety disorders. Given these results and what is known about Attentional Control and Maintenance Theories of anxiety, I would expect that individuals with clinical anxiety would show relatively weaker decreased connectivity in attentional circuitry when attempting to regulate anxiety. Dynamic connectivity (i.e. network connectivity across time) could be also be used to assess latency in connectivity alterations, which could provide neural evidence for the Attentional Maintenance Theory that anxiety is additionally associated with slower disengagement from threat. Other avenues of research stemming from this work could investigate how different interventions may train attention and BNST-regulatory circuits (e.g. rIFG—BNST). Below, I briefly discuss several future directions for research and application development that may prove beneficial for understanding and treating anxiety disorders, given the renewed appreciation for the involvement of the BNST.

Cognitive Training

Attentional training may be one route toward ameliorating anxiety in patients. Using a modified dot-probe task to facilitate attentional disengagement, one study found that 72% of patients in the treatment group no longer met diagnostic criteria for social anxiety disorder following training, relative to 11% of controls (Schmidt et al., 2009). Studies have additionally shown that attention modification programs can be effective even when delivered through the internet (Kuckertz et al., 2014), maximizing potential applicability for diverse populations. Moreover, research indicates that training may not only ameliorate attentional biases toward threat, but can also reduce emotional

vulnerability to subsequent stressors (Amir et al., 2009; Heeren et al., 2012; See et al., 2009). Whether the therapeutic benefits of attentional training are a result of better disengagement from threat cues or increased control over attentional deployment remains unclear, however some reports suggest that training most directly modulates top-down processes of disengagement, rather than alters attention orienting (Eldar & Bar-Haim, 2009; Heeren et al., 2012). Regardless, these findings provide evidence that attentional training may be a viable option to promote better recruitment of the rIFG and/or enhanced disengagement of attentional circuits in those suffering from anxiety.

Deep Brain Stimulation

Although very preliminary, small clinical case studies suggest that being able to selectively regulate BNST activity could have profound effects on anxious propensities and predispositions. In a single-patient case study, BNST deep brain stimulation (DBS) was used in a woman who had battled remitting and relapsing anorexia nervosa since adolescence (over 40 years in total), as well as concurrent major depressive disorder (MDD). Following bilateral BNST implantation, improvement was gradual, but incredibly profound. Nine months after surgery, the patient was released from the psychiatric ward after nearly a four-year stay, and tube feeding for her eating disorder was discontinued. The patient reported that all of her anxiety concerning food and eating had essentially vanished and her food intake had become more stable. In the patient's own words, despite the absence of anxious or obsessive thoughts, she continued to eat just enough to keep her weight stable out of habit, although she was now motivated to begin behavioral training to break this pattern (Blomstedt et al., 2017).

More commonly, DBS has also been used to treat OCD. Initially, DBS for OCD targeted the entire length of the anterior limb of the internal capsule (ALIC). However, long-term outcomes published from a multi-site study (Greenberg et al., 2006; Nuttin et al., 2003) reported that as the stimulation site moved posteriorly along the ALIC (approaching the BNST), clinical improvement was seen with lower stimulation amplitudes, suggesting closer proximity to the optimal target site. The BNST then came to the forefront of OCD literature following a double-blind, randomized crossover trial that effectively reduced obsessions and compulsions in patients with intractable OCD. In this report, it was noted that beneficial effects on mood and anxiety were observed first, before apparent changes in obsessions or compulsions, suggesting that these initial anxiolytic effects may subsequently drive the attenuation of OCD symptoms (Luyten et al., 2016). Post hoc analyses comparing electrode placement in this group revealed that only one out of six ALIC-stimulated patients showed a clinical response, while twelve out of fifteen BNST-stimulated patients showed a favorable outcome. These findings led the authors to conclude that the BNST might be a better stimulation target to alleviate anxiety and consequential obsessions and compulsions (Luyten et al., 2016; Raymaekers et al., 2017). Together, these findings suggest that DBS in the BNST could provide a safe, last-resort treatment option for severely affected, treatment- resistant anxiety patients (Karas et al., 2019).

Beyond DBS

DBS offers certain advantages, including its adaptability (stimulation parameters can be adjusted until satisfactory) and its reversibility (stimulation can be switched off at any time). Still, DBS has its own disadvantages: an invasive surgical procedure, a

permanent implant and associated hardware-related inconveniences such as the need to remain near a clinical site for DBS. Furthermore, recent investigations have shown that targeting such a deep-brain structure as the BNST has proven difficult for DBS (Nuttin et al., 2013). In patients with OCD who had undergone DBS in the BNST, every implanted lead deviated *at least* 1.3 mm from its intended position. In comparison, when a group of patients who had received DBS for movement disorders was analyzed (subthalamic nucleus (STN) or ventral intermediate nucleus (VIM) of the thalamus), the *maximal* deviation of *all* implanted leads was 1.3 mm (Nuttin et al., 2013).

To combat these downfalls of DBS, even newer non-invasive and highly-accurate methods are emerging that may provide similar relief. On such treatment on the horizon is the use of MRI-guided focused ultrasound (mgFUS; Insightec, www.insightec.com). mgFUS can be used to deliver pulsated sound wave energy (or sonications) through the skull to the targeted region, creating a small thermal lesion with sub-millimeter accuracy. During this single-day outpatient procedure, MR-thermometry provides real-time changes in tissue temperature and treatment volume, which can be used to monitor treatment progress. Moreover, the applied energy can be increased gradually, allowing for identification of any unwanted side effects before a permanent therapeutic ablation is made. This procedure has now been FDA approved to treat Essential Tremor and Tremor-Dominant Parkinson's Disease and is currently in clinical trials for OCD and depression. Whether novel treatments like this one will become mainstream for psychiatric disorders remains to be seen, but evidence of such innovation — and particularly a novel technique able to target a centrally-located, tiny brain structure such as the BNST — provides hope for those suffering from these debilitating disorders.

Sex Differences

Continued research on the BNST may additionally uncover insights into the onset and prevalence of anxiety disorders. Stress-related psychiatric disorders are known to occur more frequently in woman than men. Woman are, in fact, twice as likely to suffer from depression and several anxiety disorders, including PTSD (Tolin & Foa, 2006). While this disparity is often attributed to gender differences in psychological factors such as affective style, biological factors also undoubtedly play a role (Bangasser, 2013). Psychiatric disorders linked to CRH dysregulation occur more frequently in women, and indeed, sex differences in CRH expression have been observed in the amygdala and BNST (Sterrenburg et al., 2012). Emerging research also suggests that sex differences in receptors for CRH and glucocorticoids (GR) may additionally contribute to this disparity. Following HPA activation, GRs provide critical negative feedback to inhibit additional glucocorticoid release. However, studies have shown that compared to males, female rats have fewer GRs, which is linked to slower negative feedback, suggesting that females may shift more easily into a dysregulated state of stress reactivity (Bangasser, 2013).

To reiterate, the BNST is ideally situated in the brain to modulate downstream neuroendocrine and behavioral responses during stress due to it dense projections to the primary node of the HPA axis. Anatomically, the BNST itself is a sexually dimorphic structure (Allen & Gorski, 1990, Hines et al., 1985). Although this adds a layer of complexity to research, these sex differences in BNST structure, CRH expression, and receptors for CRH and glucocorticoid may help explain the gender disparity that exists in the prevalence of anxiety disorders and other stress-related psychiatric disorders. Interestingly, however, the BNST does not show strong sexual differentiation at birth, but

rather appears to develop sexual dimorphism around puberty (Chung et al., 2002), and animal studies have shown that sex differences in CRH receptors also emerge around puberty, implicating gonadal hormones in both of these effects (Weathington et al., 2012). This late divergence in BNST volume between males and females may be a general characteristic of the BNST, and if so, curiously coincides with the earliest onset of many anxiety disorders. Together, these observations offer yet a few more motivations for continued investigation of the BNST structure and function in humans.

Pharmaceutical Development

Further investigation of these sex differences will not only contribute to our understanding of the pathogenesis and prevalence of anxiety disorder, but may also have important implications for pharmaceutical development. For example, one pipeline in development is the use of CRF antagonists to treat stress-related disorders (Kehne, 2007, Million et al., 2003). CRF is known to bind differently in males versus females, suggesting differences in this receptor conformation. This may in turn affect binding of pipeline CRF antagonists and thus result in altered efficacy between men and women. Conversely, understanding the mechanisms that differentially regulate these receptors in males versus females may promote novel anxiety treatments.

Increased visibility of these sex differences is imperative to promote the use a female animals in preclinical research. A review of animal studies showed a large sex bias in neuroscience and biomedical research, with a 5:1 ratio of all male to all female animal studies (Beery et al., 2011). Given evidence of sex differences at the structure-level and receptor-level, it is reasonable to believe that some pharmaceuticals may work well in one sex and not the other. Moreover, if new drugs appear to ineffective in all-male

studies, they may never move past the preclinical phase, despite the fact they could prove beneficial for women (Bangasser, 2013). As future investigations continue to investigate the BNST and its complex connectivity and neurochemical composition, it will be essential to consider the ways in which these features differ between the sexes.

General Limitations

Human neuroimaging has shown great technological advances in the recent years, allowing us to investigate small regions like the BNST that were previously elusive and inaccessible. Yet despite vast improvements, current tools only allow us to confidently investigate the BNST as a singular unit and to measure the output as a global signal. Anatomists have long recognized that the BNST is composed of several sub-nuclei, which differ in anatomical and neurochemical features and likely reflect functional differentiation between these sub-nuclei. For example, one study in mice found that two BNST subregions modulated anxiety in opposing directions: while the oval nucleus promoted anxiety, the anterodorsal BNST appeared to mediate anxiolytic effects (Kim et al., 2013). Furthermore, research in rodents suggests that the regulatory influence the BNST has on HPA axis activity (see Chapter 1) emanates from the anteroventral BNST (Radley & Johnson, 2018). Together, these examples and several others underscore the fact that subregion specificity is an import aspect to consider when studying the BNST — a level of specificity that fMRI cannot currently address — but a point that should continue to motivate coordinated bi-directional translational research.

Conclusions

From these foundational studies, future work can characterize how specific BNST-mediated pathways and whole-brain networks may be altered in clinical populations, and determine whether anxiety regulation is amenable to training. Furthermore, given the sexual dimorphism of the BNST, this work may be fundamental for understanding the gender disparity in the prevalence of anxiety and stress-related disorders. The BNST represents a novel target, and thus through this work, our enhanced understanding of BNST connectivity during anxiety regulation may facilitate new understanding of how current therapeutics and pharmacological interventions may strengthen BNST-regulatory networks, and aid in the development of novel therapeutic strategies for anxiety disorders, and transdiagnostically.

Progress in understanding the pathogenesis of anxiety and in identifying neural signatures that differentiate affected vs. non-affected individuals is critically dependent upon our ability to develop relevant models of anxiety. The crux of anxiety concerns uncertain and unpredictable threats, and therefore the first essential step is to develop lab paradigms that psychologically elicit anxiety in an ecologically valid manner, which I set out to do in this set of studies. At the same time, while the segregation between fear and anxiety is important in our theoretical approach to parse out the specific roles of regions such as the BNST, it is hard to image a real-life threatening scenario that solely depends on the actions of a single structure. Thus in our continued effort to uncover the relative importance and influence of the BNST, I must also continue to explore the intricacies in which regions dynamically communicate within larger circuits and networks. Higher-order cognition undoubtedly requires cooperative activity from disparate regions and integration between distributed brain networks (Medaglia et al., 2015). Moreover,

network organization is known to be temporally dynamic, whereby some regions may flexibly shift their functional connectivity to affiliate more strongly with some networks than others depending on the emotional state and current task demands (McMenamin et al., 2014; Pessoa, 2018). The approach of cognitive network neuroscience, therefore, aims to reconcile the seemingly opposing perspectives of functional segregation and functional integration, by investigating how networks, and regions within networks, dynamically communicate to support optimal processing (Sporns, 2014). Understanding how the BNST flexibly shifts its alliances to dynamically communicate with cognitive, affective and motoric networks will be the next frontier in understanding the contribution of the BNST to human anxiety. Nevertheless, many avenues of research suggest I are on our way to untangling these intricacies, and I can be optimistic that the next decade of research will bring great strides in anxiety research and the neural bases of psychopathology, in part thanks to the untapped potential of the BNST.

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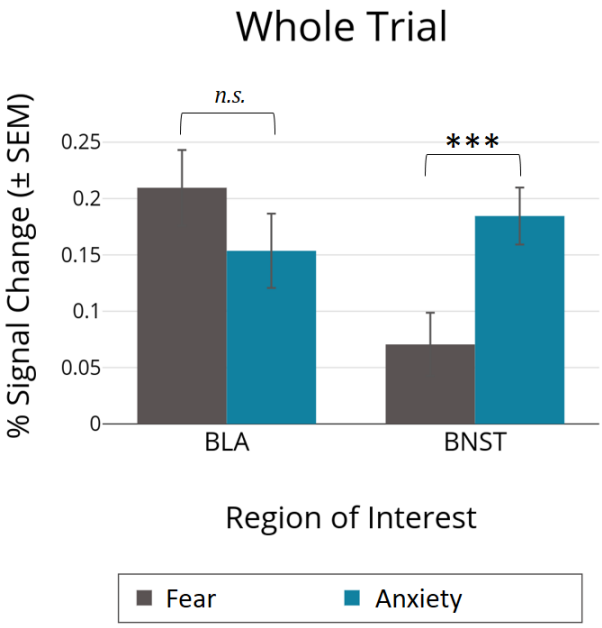
APPENDICES

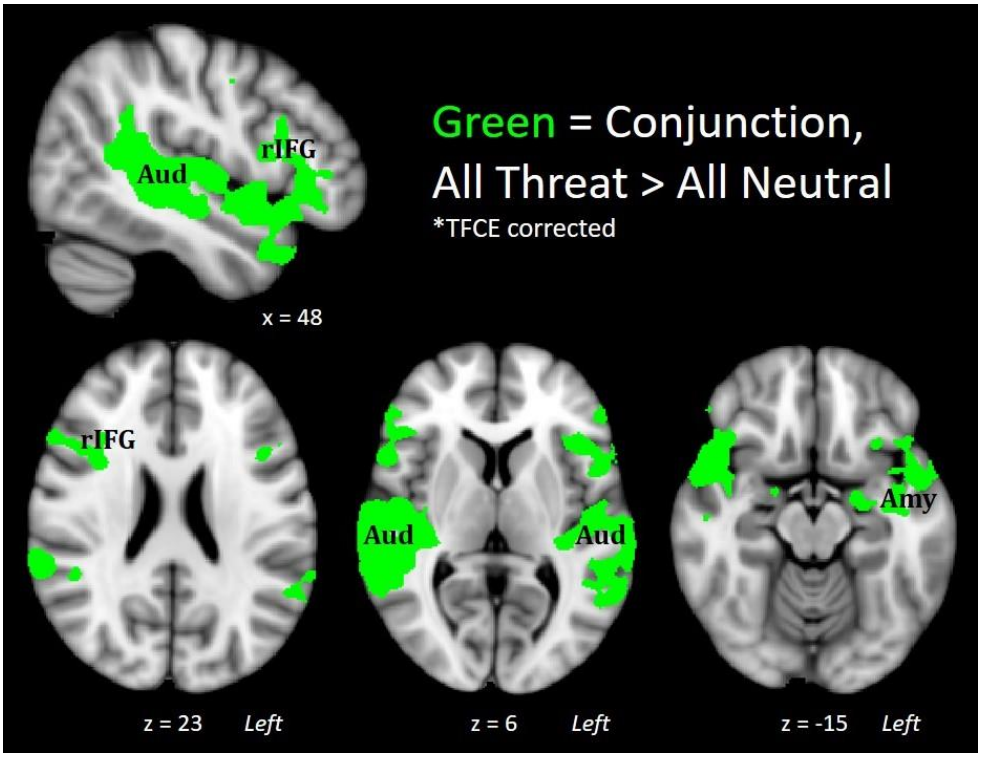
Appendix A: Supplementary Figure 1



Supplementary Figure 1. 1.5 mm smoothing. Percent signal change (PSC) extracted from ROIs across the Fear and Anxiety conditions across trial epochs. An identical pattern of results was found when compared to 3 mm smoothing (Figure 4). The BLA exhibited a qualitatively elevated but non-significantly different response in the Fear compared to Anxiety condition ($t_{(38)}$ = 1.19, p = .24), while the BNST showed increased activity during the Anxiety condition relative to Fear ($t_{(38)}$ = 3.01, p = .005).

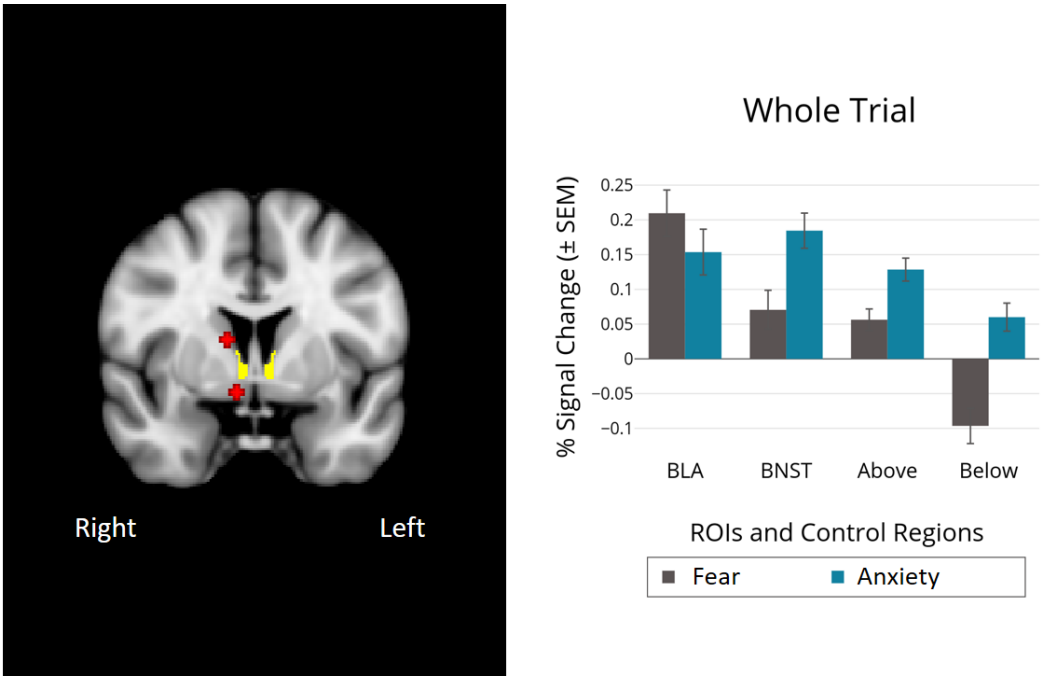
BLA = basolateral amygdala nuclei group, BNST = bed nucleus of the stria terminalis. Error bars represent standard error of the mean (SEM).

Appendix B: Supplementary Figure 2



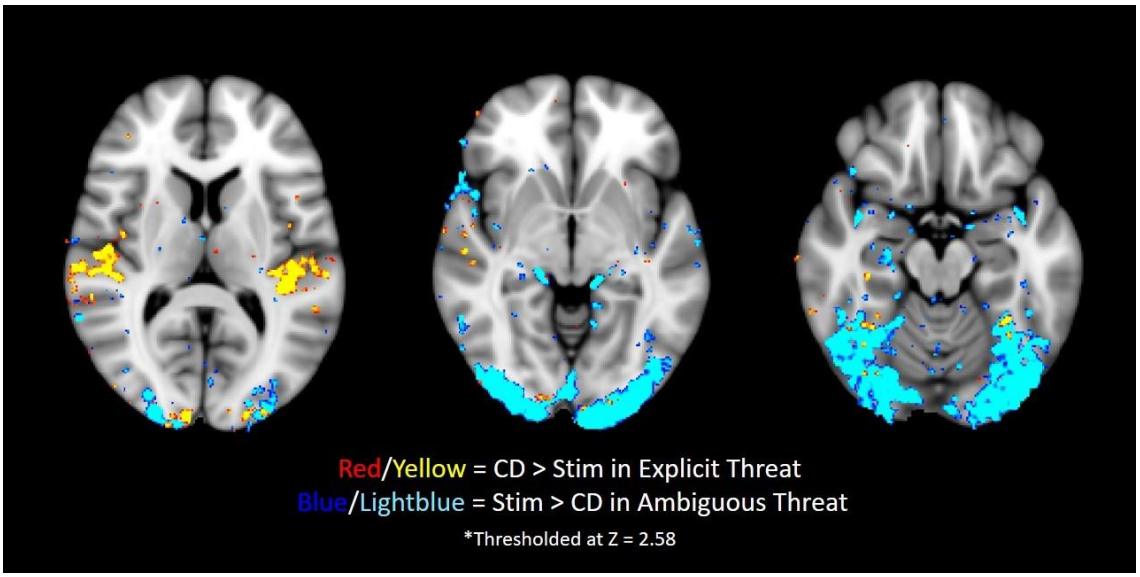
Supplementary Figure 2. An additional conjunction analysis was conducted to compare all threat (Fear, Anxiety) versus all neutral (Neutral, Wait) to assess regions involved in threat processing after contrasting against conditions that elicit similar levels of visual and auditory processing. This analysis showed many similarities to threat vs. baseline (Figure 3; e.g. amygdala, rIFG), with the exception of reduced visual cortical activation. Of note, even after contrasting again the neutral conditions, greater activity is seen in auditory processing regions across threat conditions. The voxelwise (uncorrected) conjunction for all threat compared to all neutral additionally revealed activity in the right BNST (p = .01).

Appendix C: Supplementary Figure 3



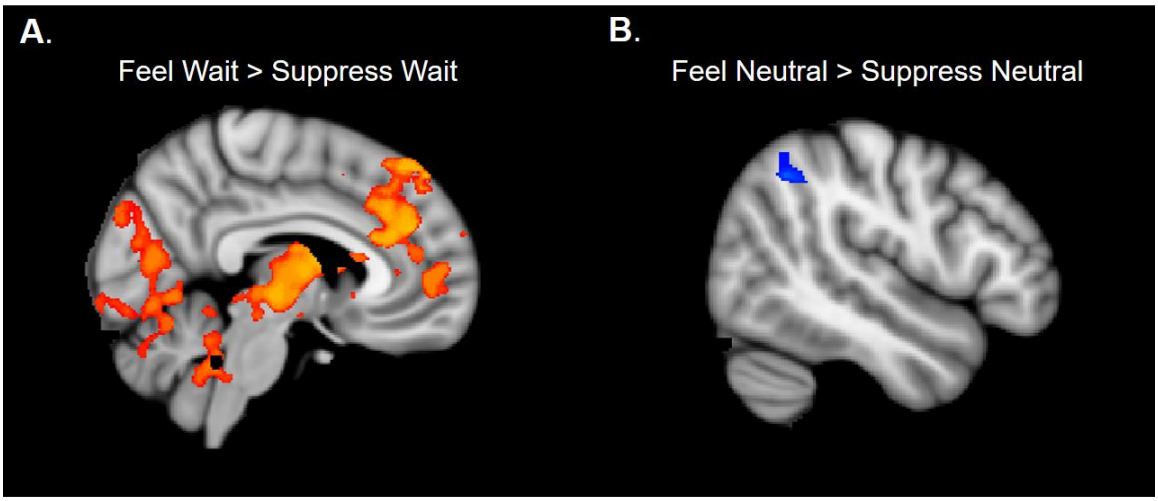
Supplementary Figure 3. **Left:** Control regions of comparable size to the BNST were drawn directly above and below the BNST in the head of the caudate and the ventral striatum, respectively. BNST is shown in yellow, while control regions are shown in red. **Right:** Percent signal change was extracted from these regions and compared to the BNST. Both the mask above and below the BNST exhibited greater activity in the Anxiety condition, relative to Fear, aligning with the fact that these regions share structural and functional connections with the BNST (Avery et al., 2014; Torrisi et al., 2018). However, the pattern and the magnitude of these responses revealed dissociations from the BNST's activation profile. Signal from the ventral striatum showed the most distinct pattern, with a negative PSC in Fear and a significantly reduced response in Anxiety relative to the BNST (Below vs. BNST in Anxiety: $t_{(38)} = 3.85$, $p < .001$). The caudate exhibited a pattern globally more similar to the BNST, but still with relatively reduced activation in Anxiety (Above vs. BNST in Anxiety: $t_{(38)} = 1.85$, $p = .07$), comparable to the magnitude of the BLA's response in Anxiety (Above vs. BLA in Anxiety: $t_{(38)} = .68$, $p = .50$). Given the findings of a recent meta-analysis demonstrating that threat anticipation reliably engages the caudate nucleus (Avery et al., 2016), it makes conceptual sense that this region would show the most similar pattern of activity to the BNST. Nevertheless, the combination of the ventral striatum showing a distinct pattern of activity, indicating that the ROI results are not contaminated by nearby structures, with the BNST showing the highest magnitude response, suggests the activation resulting from Anxiety is most reliably centered in the BNST.

Appendix D: Supplementary Figure 4



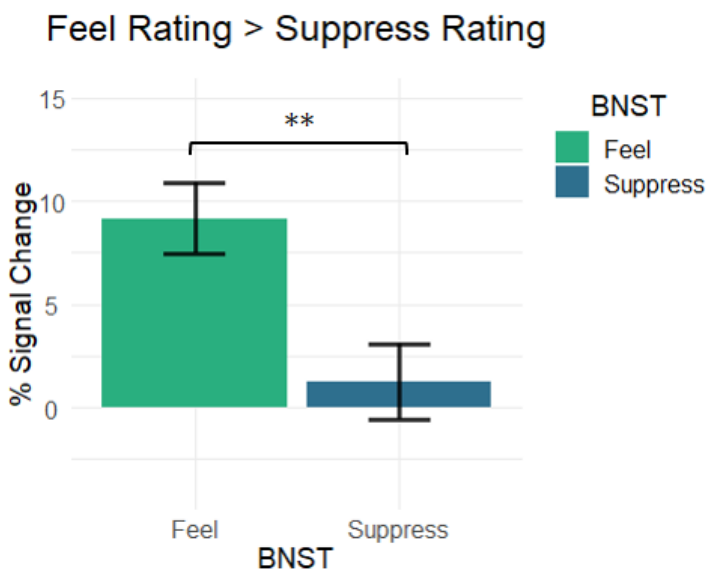
Supplementary Figure 4. Repeated measures analysis contrasting the Cue (+ Delay) epoch and the aversive Stimulus epoch within each Threat condition. In Fear, no significant differences were found in amygdala activation between the Cue and aversive Stimulus epochs, however, greater activity in auditory and visual cortices was found for the Cue > Stimulus epoch. This lack of differentiation in amygdala activity between Cue and Stimulus epochs in this repeated measures analysis corroborates our ROI findings (Figure 4). While it is not immediately clear why increased auditory activity was observed at Cue, speculatively, this may represent preparatory response for the auditory stimulus, given that the cue in Fear signals the immediate presentation of the aversive Stimulus with 100% certainty. Conversely, Anxiety showed greater activity in the amygdala and visual cortices for Stimulus > Cue+Delay. Again, increased activity in the amygdala during the aversive Stimulus in Anxiety is in line with our reported ROI results (Figure 4). Moreover, significant differences between trial epochs across threat conditions using this repeated measures design indicates that these trial periods can be reliably separated. Thus the Fear results that show a similar response of the amygdala from Cue to Stimulus (Figure 4) can be interpreted as a strong and consistent response to both the Cue and aversive Stimulus.

Appendix E: Supplementary Figure 5



Supplementary Figure 5. Feel Wait Versus Suppress Wait and Feel Neutral Versus Suppress Neutral. Significant whole brain activation difference were found between Feel Wait and Suppress Wait, namely in the dorsomedial prefrontal cortex, paracingulate cortex, visual cortices, and brainstem output regions. Conversely, comparison between Feel Neutral and Suppress Neutral only revealed one significant cluster in the supramarginal gyrus. Together, these results show that while the Neutral condition remained a stable baseline across the Feel and Suppress Runs, the Wait condition showed significantly increased activation during the Feel Run in similar regions as the Feel Anxiety condition, thus resulting in non-significant differences in the Feel Anxiety > Feel Wait contrast.

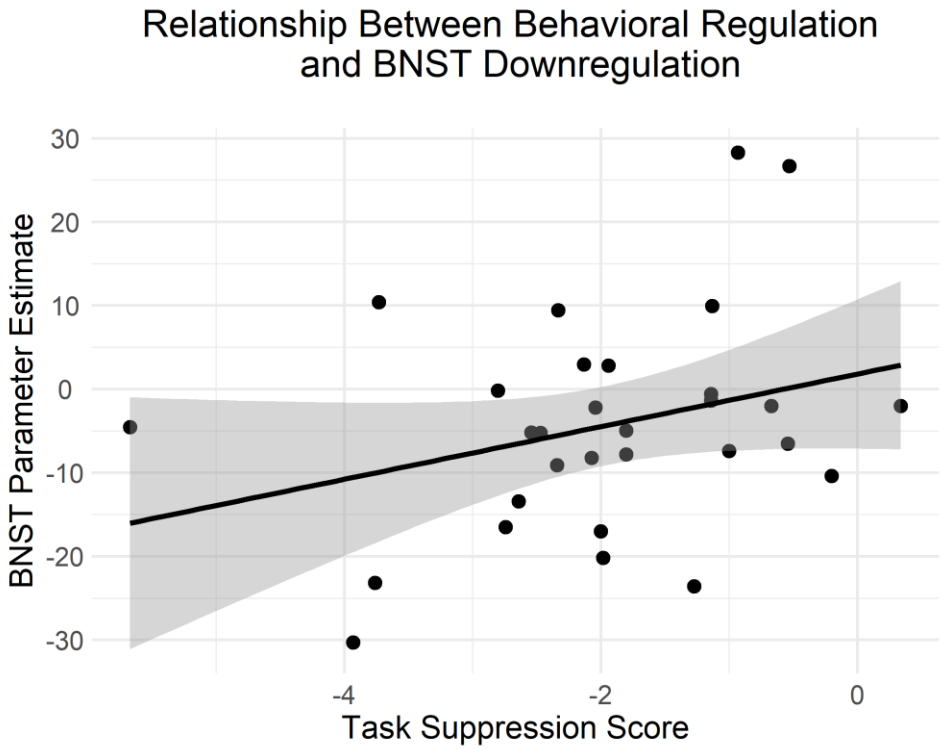
Appendix F: Supplementary Figure 6



Supplementary Figure 6. BNST Activation during Feel Run Rating Period Versus Suppress Run Rating Period. A significant difference in BNST activation was found between the rating periods of the Feel and Suppress Runs. Rating periods followed every two Anxiety and Wait trials and every four Fear and Neutral trials. These four second rating screens reflect a frequently recurring time period that contained no aversive images or sounds. Additionally they followed every trial type, and appeared in a consistent manner such that participants likely began to predict its appearance. Thus, this increased BNST activity found in the FEEL run during the Rating period likely reflects chronic BNST activation across the entire run.

BNST = bed nucleus of the stria terminalis. Error bars represent standard error of the mean (SEM).

Appendix G: Supplementary Figure 7



Supplementary Figure 7. Relationship between Behavioral Task Suppression Scores and BNST Downregulation. A correlation between task suppression scores (change in ratings from anxiety trials in Feel Run to Suppress Run) and BNST parameter estimates from the Suppress Anxiety > Feel Anxiety contrast. Results demonstrate a positive but non-significant relationship such that greater BNST downregulation was associated with larger different in reported behavioral suppression (r = .30, p = .11).

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Publications

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Stoica, T., **Knight, L.K.**, Naaz, F. Ramic, M., Depue, B.E. (2019) "Cortical Morphometry and Structural Connectivity Relate to Executive Function and Estradiol Level in Healthy Adolescents" *Brain and Behavior, ePub Early View.* https://doi.org/10.1002/brb3.1413

Knight, L.K., Stoica, T., Fogleman, N.D., Depue, B.E. (2019) "Convergent Neural Correlates of Empathy and Anxiety during Socioemotional Processing" *Frontiers in Human Neuroscience,* 13(94), 1-15. https://doi.org/10.3389/fnhum.2019.00094

Naaz, F.*, **Knight, L.K.***, Depue, B.E. (2019) "Explicit and Ambiguous Threat Processing: Functionally Dissociable Roles of the Amygdala and Bed Nucleus of the Stria Terminalis" *Journal of Cognitive Neuroscience*, 31(4), 543–559. https://doi.org/10.1162/jocn_a_01369

Knight, L.K., Naaz, F., Stoica, T., Depue, B., (2017). "Lifetime PTSD and Geriatric Depression Symptomatology Relate to Abnormal Dorsomedial Frontal and Amygdala Morphometry" *Psychiatry Research: Neuroimaging*, 267, 59-68. https://doi.org/10.1016/j.pscychresns.2017.07.003

Fogleman, N.D., Naaz, F., **Knight, L.K.**, Stoica, T., Patton, S.C., Brenner, L.A., Banich, M.T. & Depue, B.D., (2017). "Reduced Lateral Prefrontal Cortical Volume is associated with Performance on the Modified Iowa Gambling Task: A Surface Based Morphometric Analysis of Previously Deployed Combat Veterans with PTSD, Mild Traumatic Brain Injury (TBI), or Co-occurring PTSD/Mild TBI" *Psychiatry Research: Neuroimaging*, 267, 1-8. https://doi.org/10.1016/j.pscychresns.2017.06.014

Petrov, R.R., **Knight, L.K.**, Chen, S.R., Wager-Miller, J., McDaniel, S.W., Diaz, F., Barth, F., Pan, H.L., Mackie, K., Cavasotto, C.N., and Diaz, P., (2013). "Mastering tricyclic ring systems for desirable functional cannabinoid activity." *European Journal of Medicinal Chemistry*, 69, 881-907. https://doi.org/10.1016/j.ejmech.2013.09.038

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Manuscripts in Preparation

Faul, L.*, **Knight, L.K.***, Espay, A.J., Depue, B.E., LaFaver, K. (*Under Review*) "Neural Activity Changes After Inpatient Rehabilitation for Functional Movement Disorders"

Stoica, T., Patton, S., **Knight, L.K.**, Depue, B.E. *(Under Review)* "Gender Differences in Brain Networks Associated with Bottom-up and Top-down Influences on Emotion Relate to Personality Traits"

Knight, L.K., Depue, B.E. (*In Preparation*) "Anxiety and How to Control It: The Functional Role of the Bed Nucleus of the Stria Terminalis"

Knight, L.K., Naaz, F., Stoica, T., Depue, B.E. (*In Preparation*) "Higher Order Functional Changes Following Emotion Regulation Training"

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2019 – 2020	Arts & Sciences Research and Creative Activities Grant *"Neural Circuitry Underlying Imaginal Exposure Therapy for Eating Disorders"*
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Summer 2019	Graduate Student Council Travel Award
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Fall 2018	GRADtalks *"Neuroimaging of Functional Movement Disorders"*

	University of Louisville
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Fall 2015	Guest Lecture – Honors Lifespan Developmental Psychology *"Language and Intelligence"* University of Louisville

Conference Presentations

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Brosof, L.C., **Knight, L.K.**, Hunt, K.J., Levinson, C.A., Depue, B.E. (March, 2019) Imaginal exposure eating disorder fear scripts are associated with increased activation related to threat and internally processed thought compared to neutral scripts: A proof-of-concept fMRI study. Presented at the International Conference of Eating Disorders in New York, NY

Knight, L.K., Wells, E., Faul, L., Jacob, A., Mohanty, D., LaFaver, K., Depue, B.E. (October, 2018) Neuroimaging of Functional Movement Disorders (FMD) Before and After a Multidisciplinary Rehabilitation Program. Presented at Research!Louisville at the University of Louisville in Louisville, KY

Hunt, K., **Knight, L.K.**, Naaz, F., Depue, B.E. (October, 2018) Common Neural Networks Involved in the Regulation of Emotion and Memory. Presented at Research!Louisville at the University of Louisville in Louisville, KY

Cook, O., **Knight, L.K.**, Depue, B.E. (October, 2018) Induced Forgetting: Dissociations between Memory Suppression and Fear Extinction. Presented at Research!Louisville at the University of Louisville in Louisville, KY

Knight, L.K., Stoica, T., Naaz, F., Depue, B.E. (April, 2018) Common Neural Correlates of Empathy and Anxiety during Socioemotional Processing. Presented at Neuroscience Day at the University of Louisville in Louisville, KY

Knight, L.K., Stoica, T., Naaz, F., Depue, B.E. (March, 2018) Common Neural Correlates of Empathy and Worry when Processing Fearful Human Faces. Presented at Cognitive Neuroscience Society (CNS) in Boston, MA

Stoica, T., **Knight, L.K.,** Naaz F., Depue, B.E. (March, 2018) Gender Differences in Engagement of Negative Stimuli during Emotion Regulation and Processing Tasks relates to Personality/Affective Style. Presented at Cognitive Neuroscience Society (CNS) in Boston, MA

Naaz, F., **Knight, L.K.,** Siers, B., Depue, B.E. (March, 2018) Episodic Memory Training Induces Functional Plasticity in PFC - Hippocampal Neural Circuitry. Presented at Cognitive Neuroscience Society (CNS) in Boston, MA

Knight, L.K., Naaz, F., Siers, B., Depue, B.E. (June, 2017) Explicit and Ambiguous Threat Processing Evoke Functionally Dissociable Activation Profiles in the Amygdala and Bed Nucleus of the Stria Terminalis. Presented at Organization for Human Brain Mapping (OHBM) in Vancouver, BC, Canada

Naaz, F., **Knight, L.K.,** Stoica, T., Siers, B., Depue, B.E. (June, 2017) Neural Changes Related to the Training of Emotion Regulation. Presented at Organization for Human Brain Mapping (OHBM) in Vancouver, BC, Canada

Stoica, T., **Knight, L.K.,** Naaz, F., Faul, L. Depue, B.E. (June, 2017) Gender Differences in the Neural Substrates of Down-Regulating Negative Emotion and Social Threat. Presented at the Organization for Human Brain Mapping in Vancouver, BC, Canada

Knight, L.K., Naaz, F., Siers, B., Depue, B. (April, 2017) Neural Differences between Fear (Certain Threat) and Anxiety (Uncertain Threat). Presented at Neuroscience Day at the University of Louisville in Louisville, KY

Stoica, T., **Knight, L.K.**, Naaz, F., Depue, B.E. (April, 2017) Functionally Connected Brain Regions Down-Regulate Negative Emotion and Social Threat. Presented at Neuroscience Day at the University of Louisville in Louisville, KY

Knight, L.K., Naaz, F., Siers, B., Depue, B. (March, 2017) Does Immediate versus Diffuse Threat Evoke Dissociable High-Resolution Functional Imaging Activation Profiles from Amygdala and Bed-Nucleus of the Stria Terminalis? Presented at Cognitive Neuroscience Society (CNS) in San Francisco, CA

Stoica, T., **Knight, L. K.**, Naaz, F., Depue, B.E. (March, 2017) Common Neural Substrates of Down-Regulating Negative Emotion and Social Threat. Presented at Cognitive Neuroscience Society (CNS) in San Francisco, CA

Naaz, F., **Knight, L.K.,** Stoica, T., Faul, L., Siers, B., Depue, B.E. (March, 2017) Detecting Neural Correlates of Autobiographical Memory for Recent and Remote Memories through High-Resolution fMRI. Presented at Cognitive Neuroscience Society (CNS) in San Francisco, CA

Knight, L.K., Naaz, F., Depue, B. (April, 2016) Increased PTSD Symptomatology and Geriatric Depression Related to Decreased Volume in Cortical Areas Associated with

Self-Referential and Working Memory. Presented at Neuroscience Day at the University of Louisville in Louisville, KY

Knight, L.K., Naaz, F., Depue, B. (April, 2016) Increased PTSD Symptomatology and Geriatric Depression Related to Decreased Volume in Cortical Areas Associated with Self-Referential and Working Memory. Presented at Cognitive Neuroscience Society (CNS) in New York, NY

Knight, L.K., Stoica, T., Fogleman, N.D., Patton, S.C., Naaz, F., Depue, B. (October, 2015) Compromised Frontoparietal Attentional Network in PTSD Veterans Relates to Poorer Performance during Risky Decision-Making. Presented at Research!Louisville at the University of Louisville in Louisville, KY

Fogleman, N.D., Stoica, T., **Knight, L.K.**, Patton, S.C., Naaz, F., Depue, B.E. (October, 2015) Surface-based Morphometry in Lateral Prefrontal Cortex is Associated with Reward Processing and Impulse Inhibition in Combat Deployed Veterans with Post-Traumatic Stress Disorder. Presented at the Society for Neuroscience (SFN) in Chicago, IL

Leadership & Mentorship

2019 – 2020	Series in Statistics *Planning Committee* University of Louisville
2018 – Present	Cognitive Neuroscience Journal Club *Organizer and Facilitator* University of Louisville
2018 – Present	Graduate Network – College of Arts & Sciences *Department Representative for Psychology* University of Louisville
2018 – 2019	Cognitive Neuroscience Society Trainee Association *Representative* International Association
2015 – Present	Research Mentor *Undergraduate Research Assistants (N > 10)* University of Louisville
2015 – 2016	Research Mentor *Senior Honors Thesis (N = 1)* University of Louisville

Summer 2013	Research Mentor *Undergraduate Summer Research Scholar (N = 1)* Indiana University

Community Outreach

Spring 2019	Brain Days! *Science Outreach – Volunteer* A two day annual event in Louisville, KY. This collaborative effort between Kentucky Science Center, the Society for Neuroscience, and faculty and graduate students at the University of Louisville brings hands-on learning activities for kids of all ages to learn about the brain!
Spring 2019	Louisville Regional Science and Engineering Fair *Science Outreach – Judge* An annual science and engineering fair for students grades 6-12 held at the Kentucky Science Center. In addition to winning trophies, cash and other awards, top middle school winners may compete at the national Broadcom MASTERS competition, while top high school students win a trip to compete in the Intel International Science and Engineering Fair.
Fall 2016	Get Psyched! *Science Outreach – Volunteer* A free fun learning day for kids ages 6-12, hosted by the Psychology department at the University of Louisville.
2013 – 2015	Fund for the Arts / Louisville Ballet *Dance Outreach – Instructor* Part of the 5x5 program dedicated to exposing all Louisville students to at least 5 arts and culture experiences by the 5th grade.

Graduate Coursework

Fundamentals of Neuroscience, Neuroanatomy Lab, Intro to fMRI Analysis, Topics in Neuroimaging, Advanced Statistics I & II, Functional Neuroanatomy, Research Ethics, Cognitive Neuroscience, Translational Neuroscience, Developmental Neurobiology, Advanced Behavioral Endocrinology, MATLAB Programming, Multivariate Statistics, Clinical Trials: Planning & Design

Professional Memberships

2018 – Present	American Psychological Association
2015 – Present	Cognitive Neuroscience Society
2015 – Present	Organization for Human Brain Mapping
2013 – Present	Society for Neuroscience