IN VIVO ASSESSMENT OF SEROTONERGIC SIGNALING PATHWAYS UNDERLYING THE CORTICOLIMBIC RESPONSE TO THREAT IN HUMANS

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***IN VIVO* ASSESSMENT OF SEROTONERGIC SIGNALING PATHWAYS UNDERLYING THE CORTICOLIMBIC RESPONSE TO THREAT IN HUMANS**

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A corticolimbic circuit including the amygdala and medial prefrontal cortex (mPFC) affects sensitivity to threat, related aspects of personality and risk for psychopathology. Serotonin (5-HT) is a potent neuromodulator of this circuit, however, 5-HT receptors mediating these effects and genetic sources of variability in 5-HT receptor availability are not understood. We determined the association between 5-HT_{1A} and 5-HT_{2A} binding and the response to threat within this corticolimbic circuit using a multimodal neuroimaging strategy in humans *in vivo*. Corticolimbic circuit function was assessed with a threat-related faces matching paradigm using functional magnetic resonance imaging (fMRI). Regional 5-HT_{1A} and 5-HT_{2A} binding was assessed with [^{11}C]WAY100635 and [^{18}F]altanserin PET, respectively. We evaluated the association between receptor binding and common polymorphisms (rs6295, rs6311 and 5-HTTLPR) in 5-HT related genes.

In Study 1 we found that 5-HT_{1A} binding within the dorsal raphe nucleus was inversely associated with threat-related amygdala reactivity. This is consistent with 5-HT_{1A} autoreceptors negatively regulating 5-HT release, which within the amygdala

potentiates its response to threat. In Study 2 we found that mPFC 5-HT_{2A} binding was inversely associated with threat-related amygdala reactivity and positively associated with amygdala habituation and amygdala-mPFC functional connectivity. In Study 3 we found that mPFC 5-HT_{1A} binding significantly moderated the inverse association between mPFC 5-HT_{2A} binding and amygdala reactivity. These findings are consistent with the co-localization of 5-HT_{1A} and 5-HT_{2A} on glutamatergic neurons within mPFC indicating the 5-HT_{2A} receptor is localized to facilitate regulation of the amygdala and the 5-HT_{1A} receptor is localized to moderate its effects within mPFC. In Study 4 we found that 5-HTTLPR genotype predicted 5-HT_{1A} and 5-HT_{2A} binding in brain regions within this circuit such that the S and L_G alleles were associated with reduced 5-HT_{1A} and 5-HT_{2A} binding.

These findings provide novel insight into mechanisms that mediate the effects of 5-HT signaling on the response to threat of a key corticolimbic circuit in humans. Our findings indicate that 5-HT_{1A} and 5-HT_{2A} receptors contribute significantly to threat-related corticolimbic circuit function in humans. Furthermore, the 5-HTTLPR may contribute to individual variability in neural and behavioral sensitivity to threat by biasing 5-HT_{1A} and 5-HT_{2A} availability.

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PREFACE

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1.0 BRIEF INTRODUCTION

Identifying the neural circuitry that contributes to individual variability in aspects of personality such as anxiety is important both for understanding brain-behavior associations among healthy individuals but also for understanding biological sources of risk for affective disorders such as depression or anxiety disorders. A corticolimbic circuit involving aspects of the medial prefrontal cortex (mPFC) and the amygdala plays a key role in identifying threat within the environment and motivating behavioral and physiological responses. Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that modulates the function of this circuit; however, the role of specific 5-HT receptor mechanisms mediating this effect is not fully understood. A multi-modal neuroimaging approach in humans incorporating blood-oxygen level dependent functional magnetic resonance imaging (BOLD fMRI) and positron emission tomography (PET) offers an approach for identifying 5-HT receptor mechanisms that modulate brain function in humans *in vivo*. The objective of this dissertation is to evaluate the association between 5-HT type 1A (5-HT_{1A}) and 5-HT type 2A (5-HT_{2A}) receptor binding and threat-related corticolimbic reactivity and to identify genetic sources of variability in 5-HT_{1A} and 5-HT_{2A} binding.

We begin with a general overview of sensitivity to threat and anxiety disorders, the role of this corticolimbic circuitry in responsiveness to threat and associated risk for affective disorders (Chapter 2). We review evidence from previous studies implicating 5-HT in the modulation of sensitivity to threat through its effects on this circuitry and articulate a model through which 5-HT_{1A} and 5-HT_{2A} receptors mediate the effects of 5-HT signaling on this circuitry based on their cellular localization. Within Chapters 3-6 we present data from four studies examining the association between 5-HT_{1A} and 5-HT_{2A} binding, corticolimbic circuit function and genetic variants that predict variability in 5-HT_{1A} and 5-HT_{2A} binding. First, we assess whether 5-HT_{1A} binding within the dorsal raphe nucleus (DRN), a putative indicator of the capacity to regulate 5-HT release via the 5-HT_{1A} autoreceptor within DRN, is associated with threat-related amygdala reactivity (Chapter 3). Second, we determine the effects of mPFC 5-HT_{2A} binding on amygdala-mPFC corticolimbic circuit function and threat-related amygdala reactivity (Chapter 4). Third, considering the cellular co-localization of 5-HT_{1A} and 5-HT_{2A} receptors within mPFC, we determine whether the interaction between mPFC 5-HT_{1A} and 5-HT_{2A} binding predicts threat-related amygdala reactivity (Chapter 5). Fourth, we determine the association between specific genetic variants within genes related to 5-HT signaling (*HTR1A*, *HTR2A*, *SLC6A4*) and variability in 5-HT_{1A} and 5-HT_{2A} binding within this corticolimbic circuit (Chapter 6). Finally, we consider the general implications of our findings in the context of the model set forth in the General Introduction and discuss future studies that can build upon findings described herein and further benefit our understanding of the mechanisms underlying sensitivity to threat (Chapter 7).

A modified version of Chapter 3 was published as a Brief Communication in *Nature Neuroscience* (Fisher et al., 2006). A modified version of Chapter 4 was published as a Featured Article in *Cerebral Cortex* (Fisher et al., 2009). A modified version of Chapter 5 is currently under review as an Archival Report in *Biological Psychiatry*. Chapter 6 is currently prepared for submission as an Original Article to *Molecular Psychiatry*.

2.0 GENERAL INTRODUCTION

2.1 INDIVIDUAL VARIABILITY IN ANXIETY, SENSITIVITY TO THREAT AND RISK FOR AFFECTIVE DISORDERS

Why is it important to identify biological mechanisms that contribute to inter-individual variability behavior? Identifying biological mechanisms that contribute to individual differences in behavior and related personality allows us to understand how these behaviors emerge. Additionally, identifying mechanisms associated with aspects of personality linked to risk for psychopathology can provide insight into biological mechanisms that confer risk or underlie responsiveness to treatment. Thus, determining biological mechanisms underlying behavior and personality is of great importance both for understanding fundamental brain-behavior relationships as well as mechanisms associated with related psychopathology.

Affective disorders characterized by altered sensitivity to threat (e.g., depression, anxiety disorder and post-traumatic stress disorder) represent a substantial burden on public health that contributes to emotional and financial pressures on affected individuals, their families and society as a whole (Greenberg et al., 1996; Kessler et al., 2005). Despite this prevalence, treatment response rates of around 50% and rates of remission even lower indicate

treatment efficacy can be improved (Trivedi et al., 2006). It would be beneficial to develop our understanding of the neurobiological mechanisms that contribute risk for these disorders given the associated financial and emotional burdens. In addition to improving our ability to identify risk for affective disorders, a more complete understanding of the biological mechanisms that contribute to risk for and onset of affective disorders may facilitate the development of more focused and effective treatment strategies or more efficiently pairing particular treatment strategies with clinical populations likely to respond to such treatments (Holsboer, 2008).

As humans, sensitivity to stimuli within our environment plays a key role in guiding our behavior. As such, our perception of a particular stimulus or set of stimuli contributes to how we navigate our environment and gives rise to aspects of our individual personality. Measures of personality traits provide a way to quantify inter-individual differences in complex behavior reflecting sensitivity to our environment. The capacity to map biological mechanisms onto reliable and meaningful indices of personality benefits our ability to link mechanisms that contribute to personality and may play a role in risk for affective disorders. For example, individual differences in sensitivity to threatening or stressful stimuli and/or the propensity to categorize social and environmental cues as threatening (i.e., an apprehension and feeling of tension toward a specific stimulus) affects our classification of a stimulus as stressful or not. This also affects how stressful we perceive a specific environment to be and represents a core component of the stable personality construct known as trait anxiety (Etkin et al., 2004; Spielberger et al., 1970).

Individuals with high trait anxiety or the related personality measure neuroticism are at relatively greater risk for affective disorders (e.g., depression and anxiety disorders; (Kendler et

al., 2006; Kendler et al., 1999; Kessler, 1997; Kotov et al., 2010). A recent study (Cuijpers et al., 2010) indicates costs associated with health care for individuals with high levels of neuroticism, regardless of clinical diagnosis, exceed those of individual common mental disorders (Lahey, 2009). Thus, identifying biological mechanisms such as genetic or molecular mechanisms and neural circuits that contribute to inter-individual variability in personality constructs like trait anxiety or neuroticism can be a powerful model for understanding mechanisms that contribute to differences in personality and related risk for affective disorders (Hariri, 2009).

Investigating neurobiological mechanisms that contribute to individual differences in personality and related risk for psychopathology within clinical cohorts is difficult because of confounds such as past history of pharmacological treatment or variability in number, duration and severity of previous clinical episodes. Although clinical cohorts are critically important for studies aimed at identifying neurobiological mechanisms associated with treatment response or biomarkers predictive of treatment response, the use of relatively extreme cohorts introduces the possibility that they may differ on other variables. Potential confounds such as co-morbidity and prior drug exposure introduces confounds that are difficult to control for, thus the use of clinical cohorts in the context of understanding neurobiological mechanisms associated with individual variability in personality and related risk for psychopathology is limited. Healthy, non-clinical populations are useful for characterizing normal function of neurobiological mechanisms, which can then be used as a reference for understanding dysfunction within these pathways in clinical populations. Easier acquisition of larger healthy cohorts relative to clinical cohorts offers a better opportunity to effectively identify mechanisms with relatively smaller effects and to model relatively more complex mechanisms

(e.g., moderation or mediation). The capacity to link these behaviors with underlying neural circuits and molecular mechanisms is increased by the existence of a broad range of variability in threat sensitivity, even in healthy cohorts.

In summary, investigating the neural pathways and underlying molecular mechanisms that contribute to inter-individual differences in personality constructs such as trait anxiety within healthy populations provides a useful approach through which we can understand biological sources of individual variability in personality and related risk for affective disorders. Converging evidence across animal models and in humans implicates a corticolimbic circuitry involving the amygdala and medial prefrontal cortex (mPFC) in modulating sensitivity to threat-related stimuli and individual variability in trait anxiety.

2.2 THREAT-RELATED CORTICOLIMBIC CIRCUITRY

A growing corpus of research in humans and non-human animal models clearly implicates a corticolimbic circuitry comprised of structural and functional inter-connections between the amygdala and mPFC including the anterior cingulate cortex (ACC) in generating and regulating both behavioral and physiologic responses to threatening or fearful stimuli (Hariri et al., 2006; Pezawas et al., 2005; Phelps et al., 2004; Quirk et al., 2003).

2.2.1 The amygdala

The amygdala is a bilateral, subcortical structure conserved across mammalian species, located within the medial temporal lobes. The amygdala is activated by diverse stimuli, including those of both positive and negative affective valence. It receives numerous inputs from thalamic and cortical sensory processing streams as well as contextual information from cortical and hippocampal afferents (Davis and Whalen, 2001; Maren and Quirk, 2004). The amygdala is a central brain structure in mediating the expression of responses to salient environmental stimuli, especially those associated with threat. Through its direct output to cortical, subcortical, hypothalamic and brainstem areas, the amygdala precipitates myriad physiologic and behavioral responses to stimuli perceived as threatening (Davis and Whalen, 2001; LeDoux, 2000; Whalen, 2007).

The human amygdala is a diverse structure comprised of at least 10 subnuclei, many of which can be grouped into two main components: the basolateral complex (BLA) and the central nucleus (CeA; (Davis and Whalen, 2001; Sah et al., 2003). The BLA, encompassing the lateral, basolateral and basomedial nuclei, receives sensory information from cortex, thalamus and the hippocampus. Convergence of these inputs within the BLA positions it to integrate sensory information and associate that information with emotional salience. BLA projects back onto many cortical structures, including reciprocal projections to sensory cortex and prefrontal cortex, however, sensitivity to threat-related stimuli and the generation of behavioral, physiologic and autonomic responses relies on a specific BLA efferent to the CeA (Davis and Whalen, 2001; LeDoux, 2000; Maren and Quirk, 2004; Sah et al., 2003).

Generating appropriate behavioral and physiologic responses to threatening and fearful stimuli requires an integral projection from the BLA to the CeA (Pare et al., 1995; Pitkanen et al., 1997). The CeA, encompassing the centromedial and centrolateral nuclei, directly innervates brain regions critical for generating autonomic, physiologic and behavioral responses such as hypothalamus, periaqueductal grey, parabrachial nucleus and other brainstem structures (LeDoux et al., 1988). Thus, sensitivity to threatening stimuli is critically dependent upon inputs to the amygdala via this feed-forward circuit wherein the BLA integrates information from many sensory inputs and contributes to activity within the CeA, which biases physiological responses. Integral to generating appropriate behaviors, however, is the capacity to regulate this system. Aspects of the mPFC play an integral role regulating the response of the amygdala and shaping the response to fearful environmental stimuli through direct anatomical projections to the amygdala including to a population of γ-amino butyric acid (GABA) neurons within the amygdala known as intercalated cells (IC; (Quirk and Mueller, 2008).

2.2.2 The medial prefrontal cortex

Regions of the mPFC and ACC play a critical role in integrating environmental cues and other information producing appropriate, adaptive responses to environmental stimuli including those with emotional salience (Wood and Grafman, 2003). In the context of processing threat and producing both behavioral and physiologic responses this is accomplished, in part, via direct reciprocal anatomical inter-connections with the amygdala. Both the “bottom-up” (i.e., amygdala projections to mPFC) and “top-down” (i.e., mPFC projections to amygdala)

components of this corticolimbic circuit play an integral role in shaping sensitivity to emotionally salient stimuli, emotional state and motivation (Phillips et al., 2003a). Direct projections from medial prefrontal regions including Brodmann Areas (BA) 24, 25 and 32 and orbitofrontal regions (BA 11) play an important role in regulating the response of the amygdala to threat-related stimuli via "top-down" feedback regulation (Amaral and Price, 1984; Barbas and de Olmos, 1990; Ghashghaei and Barbas, 2002; Pandya et al., 1981). Excitatory projections via glutamatergic neurons from mPFC compose the bulk of these projections (Barbas, 1995; Smith et al., 2000). This top-down regulation is thought to emerge from a polysynaptic circuit where pyramidal neurons from regions of mPFC project to BLA and neurons within IC (**Figure 1**; (Likhtik et al., 2005; Pare et al., 2004; Quirk et al., 2003; Sesack et al., 1989). In the context of regulating CeA output, glutamatergic neurons from mPFC synapse onto glutamatergic neurons within BLA, which in turn drive GABAergic neurons within IC, which inhibit CeA activity. A route requiring one fewer synapse circumvents the BLA with mPFC providing excitatory projections to IC neurons which in turn inhibit CeA (Pare and Smith, 1993); also see Figure 7 of (Quirk et al., 2003). Studies in both humans and animal models indicate this corticolimbic circuit is critical for effectively regulating behavioral and physiologic responses to threat-related stimuli.

2.2.3 Corticolimbic circuitry and sensitivity to threat

2.2.3.1 Fear conditioning studies Fear conditioning is a commonly employed model for studying the neural circuitry underlying learned fear and sensitivity to threat. Fear conditioning involves the repeated pairing of an innocuous or "conditioned stimulus" (CS, e.g., a tone) with a

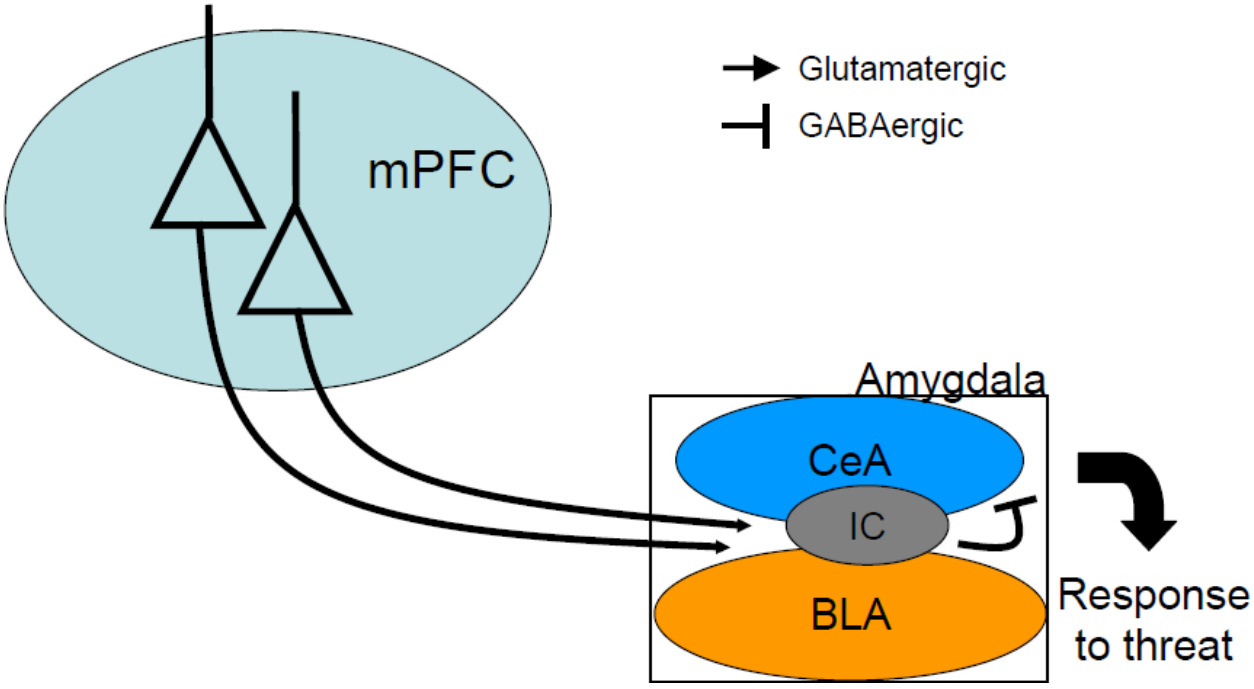

Figure 1. Threat-related corticolimbic regulatory circuitry. Schematic illustrating top-down regulation of the amygdala by medial prefrontal cortex (mPFC). Glutamatergic projection neurons from mPFC provide top-down negative feedback on the central nucleus of the amygdala (CeA) output via projections onto intercalated cells (IC) and the basolateral amygdala (BLA).

noxious or “unconditioned stimulus” (US, e.g., a foot-shock). Following enough trials, sometimes as few as one, presentation of the CS alone is sufficient to elicit an anxiety-related response in the animal (e.g., freezing behavior). Fear extinction is the process by which this anxiety-related response to the CS is extinguished by repeated presentations of the CS without an aversive outcome.

In a series of elegant studies in rodents, LeDoux and colleagues have shown that the expression of a learned “fear memory” and related behavioral response (e.g., freezing in response to the tone) is critically dependent on the amygdala, specifically projections from CeA to key brainstem regions mediating autonomic, physiologic and neuroendocrine responses to threat (LeDoux, 2007; LeDoux, 2000). Recent work, most notably from Quirk and colleagues, indicates that fear extinction involves the development of a second memory; an “extinction memory” that is expressed through the top-down aspect of this corticolimbic circuit in conjunction with the hippocampus and acts to dampen expression of the initial fear memory via regulation of the amygdala (Milad and Quirk, 2002; Morgan et al., 1993; Peters et al., 2010; Quirk et al., 2003; Quirk and Mueller, 2008; Quirk et al., 2000). Stimulation of medial prefrontal cortex produces an inhibition of CeA neurons (Quirk et al., 2003), which when lesioned disrupts the expression of learned extinction (Morgan et al., 1993); Quirk, 2000 #833}. Furthermore, behavioral expression of the learned extinction (i.e., reduced freezing behavior) was greatest in those that exhibited the greatest mPFC tone (Milad and Quirk, 2002) suggesting that function of this corticolimbic circuit is associated with learned threat sensitivity and related behavior.

Recent neuroimaging studies employing BOLD fMRI in humans suggest that fear conditioning paradigms produce activation of this corticolimbic regulatory circuit including the

response of the amygdala during the initial stages of fear learning and the engagement of mPFC during fear extinction accompanied by a relative decrease in amygdala reactivity (Milad et al., 2007; Phelps et al., 2004; Soliman et al., 2010). These findings underscore the capacity to elicit engagement of this corticolimbic circuit in the context of human neuroimaging. Taken together, fear conditioning studies provide support for an integral role played by this corticolimbic circuit in modulating sensitivity to threat and related behavioral and physiologic responses.

2.2.3.2 Corticolimbic response to threat in humans Facial expressions are an essential means through which humans obtain information about their environment regarding threat (Darwin and Ekman, 1998), thus salient facial expressions (conveying fear and anger) represent biologically relevant stimuli for probing engagement of learned threat-related neural circuitry similar to a CS in the context of a fear conditioning paradigm. Numerous neuroimaging studies in healthy human populations have identified the response of this corticolimbic circuit to threatening stimuli, using BOLD fMRI and fearful and angry facial expressions as well as threat-related scenes (Hare et al., 2008; Hariri et al., 2000; Hariri et al., 2003; Heinz et al., 2005; Meyer-Lindenberg et al., 2005; Pezawas et al., 2005; Urry et al., 2006; Whalen et al., 2008).

Variability in the function of this corticolimbic circuitry assessed with BOLD fMRI has been associated with differences in trait anxiety and other related personality measures (Buckholtz et al., 2008; Etkin et al., 2004; Fakra et al., 2009; Haas et al., 2007; Hare et al., 2008; Pezawas et al., 2005; Stein et al., 2007). Etkin and colleagues showed that increased amygdala reactivity to threat-related stimuli is associated with increased trait anxiety (Etkin et al., 2004).

Within a large cohort of healthy volunteers and focusing on a measure of the correlation in BOLD signal between two regions known as functional connectivity, Pezawas and colleagues (Pezawas et al., 2005) found that amygdala-mPFC functional connectivity predicted more than 25% of the variability in harm avoidance, a personality measure related to trait anxiety (Cloninger et al., 1993). Hare and colleagues recently reported an inverse association between habituation of the amygdala response to threat-related stimuli and trait anxiety (Hare et al., 2008). As habituation of the amygdala response may reflect a "bringing on-line" of prefrontal regulatory mechanisms, such as those discussed in the context of fear extinction, this inverse association between amygdala habituation and trait anxiety is consistent with the model that threat-related amygdala reactivity is positively related to anxious personality.

2.2.3.3 Faces matching paradigm with BOLD fMRI Neuroimaging methods for assessing brain activity in humans, *in vivo*, include PET, BOLD fMRI and magnetoencephalography/electroencephalography (MEG/EEG). Among these, BOLD fMRI is the most suitable imaging modality for assessing brain function within our regions of interest (i.e., prefrontal cortex and amygdala). PET is not preferable because of radioactivity exposure and spatial resolution is lower than BOLD fMRI. MEG/EEG possesses superior temporal resolution relative to BOLD fMRI (milliseconds vs. seconds), but lower signal-to-noise necessitates more trials, which is not preferable considering the response of the amygdala to threatening stimuli is known to habituate over time (Breiter et al., 1996; Herry et al., 2007). Additionally, the capacity to image deep brain structures such as the amygdala with MEG/EEG is limited and spatial resolution for that of BOLD fMRI is superior.

The presentation of fearful and angry facial expressions has been shown to elicit robust reactivity within the amygdala and has been used to probe functional connectivity between the amygdala and mPFC (see Section 2.2.3.2). Amygdala reactivity to threat-related facial expressions and amygdala-mPFC functional connectivity has been previously associated with personality measures related to anxiety (see Section 2.2.3.2), thus threat-related facial expressions represent an appropriate stimulus to study corticolimbic circuit function.

Test-retest studies of the specific BOLD fMRI paradigms are sparse, however, two recent studies using very similar threat-related faces matching paradigms indicate that the magnitude of amygdala BOLD response to fearful and angry facial expressions reactivity is a relatively stable metric for up to one year (Johnstone et al., 2005; Manuck et al., 2007). For this reason it is reasonable to consider amygdala reactivity to threat-related facial expression as a stable, trait-like neural marker reflective of sensitivity to threat. As such, we will use a threat-related faces matching paradigm within the current dissertation (for paradigm details, see Sections 3.3.2, 4.3.2-5, 5.3.2-4 and **Figure 2**).

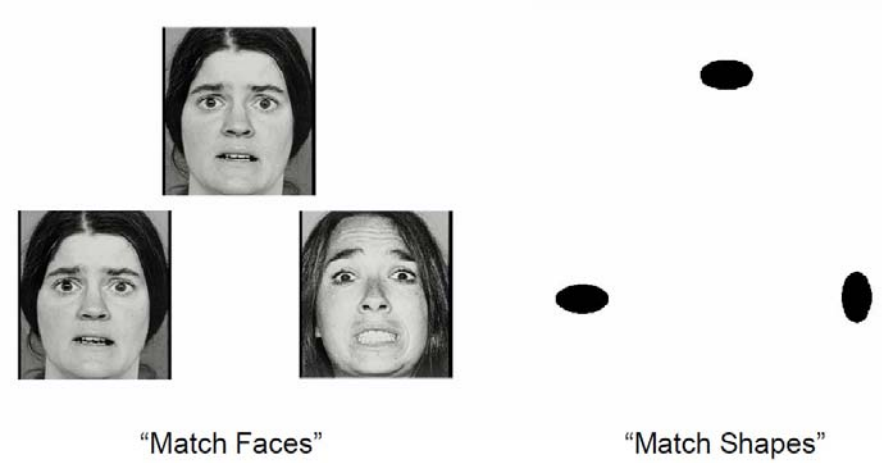

Figure 2. Faces matching paradigm. Example stimuli from threat-related faces matching paradigm. Subjects viewed alternating blocks of a trio of faces (left) or shapes (right) and selected one of two faces (bottom left) or shapes (bottom right) that match the target face or shape. Each faces block contained either fearful or angry facial expressions.

2.2.4 Corticolimbic circuit and serotonin: brain function to brain chemistry

Findings from animal studies, particularly those utilizing fear conditioning paradigms, and human neuroimaging studies strongly link the function of this corticolimbic circuit with sensitivity to threat, anxiety-related behaviors and personality traits and risk for psychopathology. Though our understanding of the specific neural circuits associated with threat and risk for affective disorders is useful, its value in identifying neurobiological mechanisms that contribute to individual differences in personality and related risk for psychopathology is limited. For example, gross manipulation of these circuits via methods such as deep brain stimulation (DBS), although met with some success as a treatment strategy (Lozano et al., 2008), represents an undesirable option for the broad population, thus an understanding of integral molecular mechanisms that modulate this circuitry is essential.

Insight into the molecular mechanisms that shape circuit function can inform the development of pharmacological treatment mechanisms and guide the investigation of genetic variants that may bias brain function by affecting underlying brain chemistry.

Identifying discrete molecular mechanisms that contribute to corticolimbic circuit function and related personality can assist in mapping the basic architecture of brain-behavior links and understanding how brain chemistry contributes to circuit function and affects disease liability. A central goal of the current dissertation is linking 5-HT molecular mechanisms (i.e., 5-HT receptor pathways) with amygdala-mPFC corticolimbic circuit function.

2.3 SEROTONIN SIGNALING AND SENSITIVITY TO THREAT

Serotonin is a neuromodulator with significant effects on emotional behavior, including anxiety and sensitivity to threat (Blier and de Montigny, 1999; Lucki, 1998). Studies that link variability in specific 5-HT receptor mechanisms with variability in related brain function would highlight relevant mechanisms that mediate the effects of 5-HT signaling on this circuitry. Furthermore, the association between 5-HT receptor function and brain function may provide insight into molecular sources of individual variability in personality, risk for psychopathology and responsiveness to specific treatment strategies.

These associations can be investigated in humans through a multi-modal neuroimaging strategy. Specifically, threat-related corticolimbic circuit function can be assayed *in vivo* using BOLD fMRI paradigms described above involving the presentation of fearful and angry facial

expressions. PET receptor imaging offers the opportunity to measure binding of a 5-HT receptor-specific radioligands *in vivo*. Determining associations between outcomes of these two neuroimaging measures in humans provides a unique opportunity to link brain function with underlying 5-HT receptor mechanisms *in vivo*.

2.3.1 General overview of serotonin system

The 5-HT system consists of a multitude of receptor classes (e.g., 5-HT_1, 5-HT_2, etc.) and subtypes within these classes (e.g., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, etc.; (Barnes and Sharp, 1999). Serotonergic innervation to prefrontal cortex and the amygdala originates within midbrain raphe nuclei, emanating primarily from the dorsal and median raphe nuclei (DRN and MRN(Jacobs and Azmitia, 1992). Concentrations of 5-HT within the amygdala are among the highest in the brain (Azmitia and Gannon, 1986; Sadikot and Parent, 1990). Direct mechanisms regulating 5-HT signaling and release include multiple autoreceptor mechanisms and the serotonin transporter (5-HTT; see Figure 1 from both (Hariri and Holmes, 2006) and (Holmes, 2008). Pre-synaptically, the inhibitory 5-HT_{1A} receptor is expressed on the soma and dendrites of serotonergic neurons within the raphe (Blier and De Montigny, 1987; Riad et al., 2000) where it plays a critical role in mediating local negative feedback on serotonergic neurons (Blier et al., 1998). The 5-HT_{1B} receptor, and possibly 5-HT_{5A} and 5-HT_{1D}, serve as terminal pre-synaptic autoreceptors (Morikawa et al., 2000; Stamford et al., 2000; Thomas et al., 2006). Clearance of extracellular 5-HT is accomplished primarily by the 5-HTT (Blakely et al., 1994).

All 5-HT receptors are metabotropic and mediate the effects of 5-HT signaling via G-protein coupled receptor (GPCR) mechanisms, except the 5-HT_3 (Barnes and Sharp, 1999; Maricq et al., 1991). Through their effects on secondary messaging systems, 5-HT receptors can be effectively classified as "excitatory" or "inhibitory" based on whether they bias neuronal membrane potential towards depolarization or hyperpolarization, respectively. Secondary messaging cascades affected by 5-HT receptors include the production of adenylyl cyclase and Protein Kinase A mediated signaling (PKA) or phospholipase-C (PLC) activity, which contributes to receptor depolarization through the release of internal stores of calcium (PKC signaling pathway).

2.3.2 Serotonin and sensitivity to threat: animal studies

In animal models, an array of behavioral paradigms associated with threat and stress have been shown to result in increased activity of 5-HT neurons as indexed by early-gene expression in the DRN as well as increased 5-HT extracellular concentrations within both the amygdala and mPFC (Adell et al., 1997; Amat et al., 2001; Forster et al., 2006; Kirby and Lucki, 1998; Rueter and Jacobs, 1996; Sadikot and Parent, 1990). Dialysis studies in rodents suggest that increases in 5-HT concentrations within the amygdala and mPFC can have opposing effects on behavior. Work from Forster and colleagues found that acute increases in extracellular 5-HT concentrations within the amygdala, specifically the CeA, coincided with expression of anxiety-related behaviors, namely increased freezing behavior (Forster et al., 2006). This finding is consistent with other studies that found increases in 5-HT levels within the amygdala

potentiates amygdala activity and anxiety-related behaviors (Amat et al., 1998; Amat et al., 2004; Burghardt et al., 2007; Burghardt et al., 2004; Maier and Watkins, 2005). Subsequent cessation of freezing behavior has been shown to coincide with both increases in 5-HT concentrations within the mPFC and decreases in 5-HT concentrations within the amygdala (Forster et al., 2006; Hashimoto et al., 1999). These findings suggest an association between 5-HT within this corticolimbic circuit and sensitivity to threat. More interestingly, these findings suggest that local 5-HT signaling within the amygdala and mPFC may have opposing effects on anxiety-related behaviors.

2.3.3 Serotonin and sensitivity to threat: human studies

Consistent with findings in animal studies, neuroimaging studies in humans provide a compelling link between 5-HT signaling and sensitivity to threat. A recent study quantified the effects of acute (30 minutes) serotonin reuptake blockade via intravenous administration of the SSRI citalopram (20 milligrams, mg) on amygdala reactivity in response to emotionally salient faces (Bigos et al., 2008). Using a placebo-controlled, double-blind cross-over design, Bigos and colleagues found that acute citalopram administration resulted in significantly increased amygdala activity in response to emotionally salient faces relative to placebo. Other studies have reported decreases in amygdala response to emotionally salient faces following acute SSRI administration (7.5 mg; (Del-Ben et al., 2005). Receptor occupancy studies at varying doses of citalopram suggest this discrepancy in findings may be due to differences in 5-HTT occupancy (Meyer et al., 2004). SSRI treatment protocols over 7-21 days in healthy populations have

found a decrease in amygdala reactivity (Arce et al., 2008; Harmer et al., 2006). Adaptations of the 5-HT system to long-term alterations in 5-HT signaling (e.g., 5-HT receptor desensitization) likely underlie discrepancies between acute and long-term effects.

A recent study in healthy adults quantified the association between 5-HTT binding within the amygdala, assessed with [^{11}C]DASB PET, and threat-related amygdala reactivity, assessed with BOLD fMRI. Rhodes and colleagues observed a significant inverse correlation wherein greater amygdala 5-HTT binding was associated with reduced threat-related amygdala reactivity (Rhodes et al., 2007). This is consistent with the putative effects of local 5-HT release within the amygdala facilitating amygdala activation in response to emotionally salient stimuli (Bigos et al., 2008; Burghardt et al., 2007; Burghardt et al., 2004; Forster et al., 2006). Furthermore, this finding indicates that individual variability in threat-related amygdala reactivity is inversely related to the capacity to regulate 5-HT signaling within the amygdala. Additional mechanisms that regulate 5-HT signaling (i.e., 5-HT$_{1A}$ autoreceptor feedback) are likely to play a similarly important role in both the regulation of 5-HT release and threat-related amygdala reactivity.Behavioral studies in humans in the context of manipulating 5-HT levels provide important insight into the effects of altered 5-HT signaling on emotional behavior. Manipulation of tryptophan levels, the amino acid precursor to 5-HT, has been used to study the effects of 5-HT signaling on behavioral and physiological measures in humans. For example, tryptophan depletion is associated with reduced 5-HT synthesis (Nishizawa et al., 1997). In a recent study, recognition of fearful faces was impaired in women following acute tryptophan depletion further suggesting that sensitivity to threat-related stimuli is dependent upon 5-HT function (Harmer et al., 2003). Conversely, nutritionally increased tryptophan (putatively

increasing 5-HT synthesis) was associated with increased recognition of fear faces (Attenburrow et al., 2003) providing support for a positive association between 5-HT signaling and sensitivity to threatening stimuli. In humans, blockade of 5-HT reuptake via intravenous administration of the SSRI citalopram produced dose-dependent increases in cortisol and prolactin, physiological features related to threat response, linking altered 5-HT signaling and physiological characteristics of the threat response (Lotrich et al., 2005).

Taken together, these findings indicate 5-HT modulates the response of the amygdala to threat in humans and affects threat sensitivity. The finding from Rhodes and colleagues indicates the use of a multi-modal neuroimaging strategy incorporating BOLD fMRI and PET is a powerful method through which we can assess the association between 5-HT receptor mechanisms and threat-related brain function in humans *in vivo*.

2.3.4 Serotonin receptor mechanisms

The 5-HT system consists of a multitude of different receptor classes (e.g., 5-HT_1, 5-HT_2, etc.) and subtypes within these classes (e.g., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, etc.; (Barnes and Sharp, 1999). It would be beneficial to develop a more complete understanding of the 5-HT receptor mechanisms that mediate the effects of 5-HT signaling on corticolimbic circuit function in humans. This knowledge is important for linking individual variability in sensitivity to threat with specific neurobiological mechanisms, which is critical for understanding biological sources of individual variability in personality and related risk for psychopathology.

The capacity to assess 5-HT receptor binding *in vivo* in humans using PET receptor imaging provides a powerful non-invasive method for assaying regional receptor distribution. *A priori* knowledge regarding the cellular distribution and localization of specific 5-HT receptors can be informative in guiding directionally specific hypotheses about receptor effects on corticolimbic circuit function. As will now be discussed, the 5-HT_{1A} and 5-HT_{2A} receptors are expressed by neurons within this corticolimbic circuitry and localized to aspects of the neuron that indicate these receptors can mediate the effects of 5-HT signaling on this circuitry. The availability of PET receptor imaging agents with both high binding affinity and specificity for the 5-HT_{1A} and 5-HT_{2A} receptors allows for the *in vivo* quantification of these receptors within this threat-related corticolimbic regulatory circuit.

2.3.5 5-HT_{1A} receptor

The inhibitory 5-HT_{1A} receptor is localized as both a pre-synaptic and post-synaptic receptor (Barnes and Sharp, 1999). Pre-synaptically, the inhibitory 5-HT_{1A} receptor is expressed on the soma and dendrites of serotonergic neurons within the raphe where it regulates 5-HT release at downstream targets via negative feedback on 5-HT neurons (Blier and De Montigny, 1987; Riad et al., 2000). Post-synaptically, the 5-HT_{1A} receptor is expressed within both the amygdala and PFC (Barnes and Sharp, 1999) with relatively higher levels in the PFC and lower levels in the amygdala (Hall et al., 1997; Pazos et al., 1987). 5-HT_{1A} receptor expression within the amygdala has been identified extensively on both pyramidal and GABA neurons in rodents (Aznar et al., 2003). Aznar and colleagues reported that 71% of neurons within the amygdala labeled as

pyramidal neurons were also labeled for the 5-HT_{1A} receptor. Similarly, 88% of GABAergic neurons labeled for calbindin or parvalbumin were also positively labeled for the 5-HT_{1A} receptor. This makes it difficult to predict the effects of local 5-HT_{1A} signaling on amygdala function as it is likely dependent upon the neuronal population affected.

Immunolabeling studies in non-human primate and post-mortem human samples of PFC show pronounced 5-HT_{1A} labeling of the initial axonal segment of pyramidal neurons (Azmitia et al., 1996; Cruz et al., 2004). These findings are consistent with studies in rodents showing high levels of 5-HT_{1A} mRNA in pyramidal neurons as well as low levels in GABAergic neurons (Amargos-Bosch et al., 2004; Santana et al., 2004). This localization suggests that 5-HT_{1A} signaling would primarily inhibit PFC pyramidal neurons, putatively including the population of neurons within mPFC and ACC that project to the amygdala (**Figure 3**). Consistent with this, local administration of 5-HT_{1A} agonists within mPFC inhibit spontaneous receptor-mediated firing of projection neurons (Ashby et al., 1994; Cai et al., 2002). This action is reversed by application of a 5-HT_{1A} antagonist (Cai et al., 2002; Casanovas et al., 1999). Conversely, previous studies in rodents suggest 5-HT_{1A} receptor mediated activation may also contribute to excitation of glutamatergic projection neurons (Casanovas et al., 1999; Hajos et al., 1999). Based on the inhibitory nature of the 5-HT_{1A}, this is likely to occur via disinhibition (i.e., 5-HT_{1A} on GABAergic neurons inhibit negative feedback on pyramidal neuron excitability). This would suggest increased prefrontal 5-HT_{1A} receptor function results in decreased amygdala reactivity through disinhibition of prefrontal regulatory circuits. Though conflicting, these findings taken together suggest 5-HT_{1A} signaling modulates PFC function and thus may affect corticolimbic circuit function.

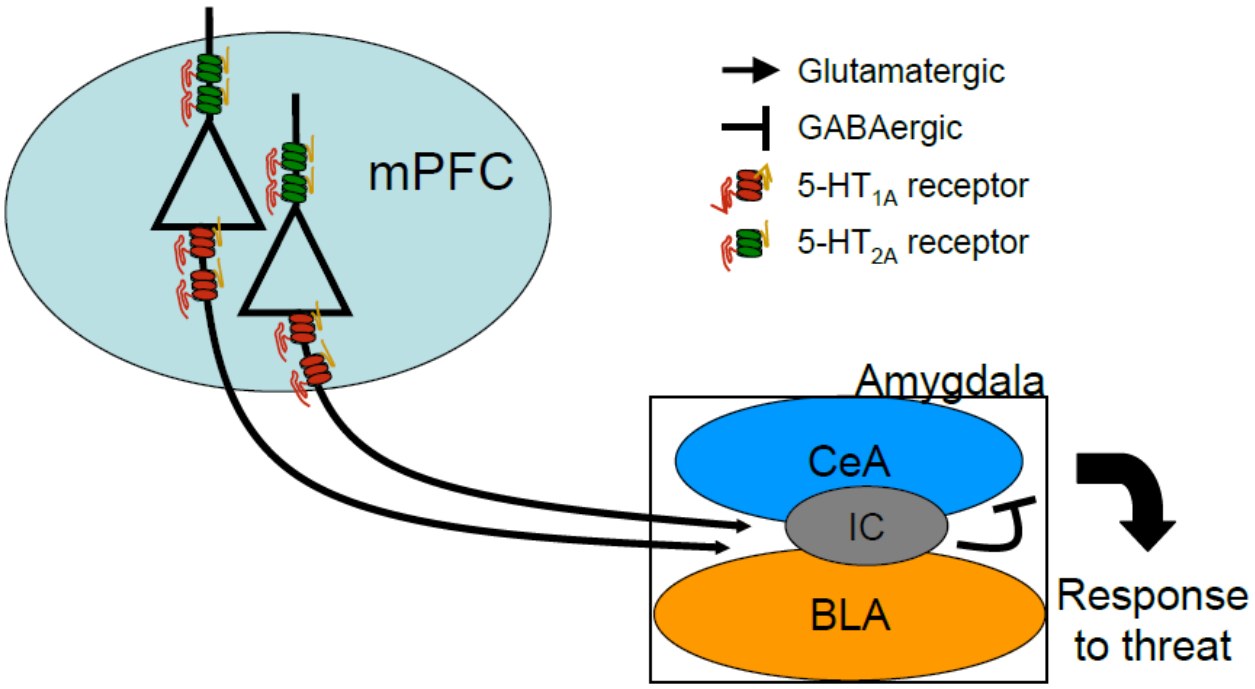

Figure 3. 5-HT_{1A} and 5-HT_{2A} within corticolimbic circuitry. Within medial prefrontal cortex (mPFC), 5-HT_{1A} (red) and 5-HT_{2A} (green) receptors are co-localized to pyramidal neurons suggesting the balance between these two receptors would bias this corticolimbic circuit. Through its excitatory effects, mPFC 5-HT_{2A} facilitates regulation of the amygdala. Through its inhibitory effects, mPFC 5-HT_{1A} effectively moderates 5-HT_{2A} effects. CeA, central nucleus of the amygdala. IC, interacalated cells. BLA, basolateral amygdala.

Behaviorally, inactivation of the *htr1a* gene in mice was associated with increased anxiety and stress responsiveness (Parks et al., 1998). The authors hypothesized this effect was due to reduced capacity for 5-HT_{1A} autoreceptor regulation. Similarly, 5-HT_{1A} autoreceptor agonism in rats blocked the potentiation of fear conditioning following exposure to an inescapable shock (Maier et al., 1995). These findings suggest that greater capacity to regulate 5-HT release via the 5-HT_{1A} autoreceptor is associated with diminished responsiveness to stress. Post-synaptically, 5-HT_{1A} agonism within the amygdala and hippocampus, but not mPFC was associated with diminished fear conditioning (Li et al., 2006). Li and colleagues did not examine whether manipulating regional 5-HT_{1A} signaling modulated fear extinction.

Based on its critical role in negatively regulating 5-HT release at downstream targets, we hypothesize that greater 5-HT_{1A} binding within the DRN will be associated with relatively diminished amygdala reactivity. We will explore the association between amygdala 5-HT_{1A} and amygdala reactivity, but hypothesizing its effects on brain function is limited because of distribution among various neuronal subtypes as described above. Based on its predominant localization, we hypothesize mPFC 5-HT_{1A} will be positively associated with threat-related amygdala reactivity reflecting the inhibitory effects of 5-HT_{1A} on pyramidal neuron function.

2.3.6 5-HT_{2A} receptor

The excitatory post-synaptic 5-HT_{2A} receptor is an extra-synaptic receptor localized within both the mPFC and amygdala (Jakab and Goldman-Rakic, 1998; Leysen, 2004; McDonald and Mascagni, 2007; Miner et al., 2003). Determining the effects of local 5-HT signaling within the

amygdala via 5-HT_{2A} and its effects on threat-related corticolimbic reactivity is of interest, but complicated by inconsistent immunolabeling studies leaving the precise localization of these receptors within the amygdala unclear (Cornea-Hebert et al., 1999; McDonald and Mascagni, 2007; Xu and Pandey, 2000). In PFC of non-human primates, the excitatory 5-HT_{2A} receptor has been localized to multiple neuronal subtypes but its predominant localization appears to be on prefrontal glutamatergic neurons at the proximal portion of the apical dendrite, suggesting it is localized to mediate excitatory effects of 5-HT release on PFC projection neurons (Blue et al., 1988; Jakab and Goldman-Rakic, 1998); **Figure 3**). There is also evidence that the 5-HT_{2A} receptor is expressed by GABAergic neurons (Amargos-Bosch et al., 2004; de Almeida and Mengod, 2007; Jakab and Goldman-Rakic, 1998; Jakab and Goldman-Rakic, 2000; Miner et al., 2003) and possibly at dopamine axon terminals within prefrontal cortex (Miner et al., 2003; Nocjar et al., 2002; Pehek et al., 2001). Despite this, 85-100% of glutamatergic neurons within layers II through V of multiple prefrontal cortical regions express the 5-HT_{2A} receptor (de Almeida and Mengod, 2007; Jakab and Goldman-Rakic, 1998) suggesting it is likely that the dominant impact of the 5-HT_{2A} signaling within PFC is via its excitatory effects on glutamatergic neurons including projection neurons comprising the top-down regulatory component of this amygdala-mPFC corticolimbic circuit. Electrophysiological evidence suggests 5-HT_{2A} agonism within mPFC potentiates pyramidal neuron excitation (Aghajanian and Marek, 1999; Ashby et al., 1994; Puig et al., 2003) and 5-HT_{2A} antagonism diminishes excitation of these neurons (Ashby et al., 1994).

A behavioral study indicated that disruption of *htr2a* expression in mice was associated with diminished anxiety-related behaviors relative to controls, however, learned fear, assessed

with a fear conditioning paradigm, was not altered (Weisstaub et al., 2006). Weisstaub and colleagues went on to show that rescue of *htr2a* expression within excitatory cortical neurons was associated with an increase in anxiety-related behaviors indicating 5-HT_{2A} function within cortex plays a role in anxiety-related behaviors. Another study that induced early stress, via chronic maternal separation, found increased sensitivity of prefrontal pyramidal neurons to 5-HT signaling via 5-HT_{2A} receptors (Benekareddy et al., 2010). Interestingly, 5-HT_{2A} and 5-HT_{2C} mRNA expression and 5-HT_2 binding was not altered in stressed animals. Instead, Benekareddy and colleagues observed alterations in the expression of genes associated with G-protein signaling suggesting the response of secondary message systems underlie the effects of heightened prefrontal 5-HT_{2A} sensitivity. Another study identified an association between 5-HT_2 and anxiety showing that the corticotrophin-releasing factor receptor 1 (CRFR1) modulates 5-HT_2 receptor signaling in cortical neurons subsequently affecting anxiety-related behavior (Magalhaes et al., 2010). A recent study found that 5-HT_{2A} binding is positively associated with the personality trait neuroticism (Frokjaer et al., 2008).

Taken together, these findings provide evidence that 5-HT_{2A} plays an important role in mediating the effects of 5-HT signaling on anxiety-related behaviors through modulation of underlying neural circuitry. Specifically, prefrontal 5-HT_{2A} is localized to facilitate pyramidal neuron excitability and thus modulate sensitivity to threat-related stimuli through its effects on corticolimbic circuit function and threat-related amygdala reactivity. Considering the predominant localization of 5-HT_{2A} on pyramidal neurons within PFC, we hypothesize it will negatively affect threat-related amygdala reactivity related to its effects on corticolimbic circuit function.

2.3.7 Co-localization of prefrontal 5-HT_{1A} and 5-HT_{2A} receptors

Importantly, 5-HT_{1A} and 5-HT_{2A} receptors are co-localized on approximately 80% of glutamatergic neurons within prefrontal cortex (Amargos-Bosch et al., 2004). Consistent with this co-localization, studies have indicated that local 5-HT_{2A} antagonism within prefrontal cortex blocks 5-HT induced excitation of pyramidal neurons (Amargos-Bosch et al., 2004; Puig et al., 2003). Conversely, pyramidal neuron inhibition is diminished upon local 5-HT_{1A} antagonism (Amargos-Bosch et al., 2004; Puig et al., 2005). A study by Ashby and colleagues examining concurrent 5-HT_{1A} and 5-HT_{2A} effects in rat mPFC found that 5-HT_{1A} agonism diminished neuronal basal firing rate. Antagonism of 5-HT_{2A} further reduced this decreased firing while 5-HT_{2A} agonism had an opposite effect, facilitating the capacity for excitation of pyramidal neurons indicating a functional interaction between these two receptors within mPFC (Ashby et al., 1994).

The above findings suggest that interactive effects between mPFC 5-HT_{1A} and 5-HT_{2A} modulate pyramidal neuron excitability within mPFC via their opposing effects on prefrontal neuron excitability. Furthermore, co-expression of 5-HT_{1A} and 5-HT_{2A} suggests molecular interactions between these receptors can moderate the net effect of 5-HT signaling on pyramidal neuron excitability with subsequent effects on an amygdala-mPFC corticolimbic circuit involved in the regulation of sensitivity to threat and amygdala reactivity (**Figure 3**).

2.3.8 5-HT_{1A} and 5-HT_{2A} positron emission tomography radioligands

Positron emission tomography and single-photon emission computed tomography (SPECT) represent the two best methods for measuring specific receptor binding in humans *in vivo*. PET is preferable despite greater costs because PET offers superior spatial resolution (millimeters vs. centimeters) and the radioisotopes used have substantially shorter half-lives (PET: [^{11}C] $t_{1/2}$ ≈ 20 minutes (mins), [^{18}F] ≈ 110 mins; SPECT: [^{123}I] $t_{1/2}$ ≈ 780 mins). We will assess 5-HT_{1A} and 5-HT_{2A} receptor binding *in vivo* within the current dissertation using two well-characterized PET radioligands: [^{11}C]WAY100635 and [^{18}F]altanserin, respectively.

[^{11}C]WAY100635 is a 5-HT_{1A} antagonist radioligand that exhibits both a high affinity (K_i = 0.24 nanomolar, nM) and greater than 100-fold selectivity for the 5-HT_{1A} receptor relative to the 5-HT_{1B}, 5-HT_{1D} as well as noradrenergic and dopaminergic receptors (Fletcher et al., 1996; Forster et al., 1995; Johansson et al., 1997). Test-retest variability of approximately 15% across regions indicates [^{11}C]WAY100635 is a highly reliable measure of *in vivo* 5-HT_{1A} binding (Parsey et al., 2000a). Radiolabeled metabolites of [^{11}C]WAY100635 are not thought to affect PET signal within brain due to high polarity and thus being unlikely to cross the blood-brain barrier (Pike et al., 1996).

The 5-HT_{2A} antagonist radioligand [^{18}F]altanserin shows both a high affinity (K_i = 0.13 nM) and greater than 30-fold selectivity for the 5-HT_{2A} receptor relative to the α_1, 5-HT_{2C} and D_2 receptors (Leysen, 1989; Leysen, 1990; Smith et al., 1998). Test-retest variability of approximately 10-15% indicates it is a highly reliable measure of *in vivo* 5-HT_{2A} binding (Smith et al., 1998). Radiolabeled metabolites of [^{18}F]altanserin have been identified and are capable of

crossing the blood-brain barrier, however, their distribution is uniform throughout the brain such that they do not introduce regional bias in the assessment of volume of distribution (Price et al., 2001b). For additional details regarding PET methodology, see Sections 3.3.3, 4.3.8-9, 5.3.5-7 and 6.3.3-5.

2.3.9 5-HT_{1A}, 5-HT_{2A} and affective disorders

PET receptor imaging studies suggest 5-HT_{1A} and 5-HT_{2A} binding is altered in cohorts with affective disorders, however, the direction remains equivocal. PET studies in humans have reported diminished 5-HT_{1A} binding in depressed populations across multiple brain regions including the midbrain raphe, amygdala and cortical regions (Drevets et al., 1999; Sargent et al., 2000). Parsey and colleagues found increased 5-HT_{1A} binding in medication-naïve depressed individuals relative to controls, but no difference in depressed individuals with a history of antidepressant exposure suggesting alterations in 5-HT_{1A} binding may be dependent upon antidepressant treatment history (Parsey et al., 2006c).

Previous research indicates an association between affective disorders and alterations in prefrontal 5-HT_{2A} receptor availability (Stockmeier, 2003). One study reported decreased 5-HT_{2A} receptor binding in frontal cortex within a cohort of depressed individuals (Yatham et al., 2000). Another study reported decreased subgenual prefrontal cortex (sgPFC) 5-HT_{2A} receptor binding within a population of women recovered from eating disorders, an illness often comorbid with mood disorders such as depression (Bailer et al., 2004). However, a third study in humans reported increased 5-HT_{2A} receptor binding in frontal cortex within a recovered

depressed population (Bhagwagar et al., 2006). A fourth study found no differences in prefrontal 5-HT_{2A} binding between depressed individuals and healthy controls (Meyer et al., 1999). Meyer and colleagues found that 5-HT_{2A} binding in depressed individuals decreased following a 6-week treatment protocol with the SSRI paroxetine (Meyer et al., 2001).

Together these studies do not support a specific directional association. However, they suggest 5-HT_{1A} and 5-HT_{2A} binding within clinical populations differs from that of healthy individuals. Taken together, these studies highlight potential discrepancies across studies in clinical cohorts such as history of medication exposure that undermine the capacity to relate findings across studies. These findings underscore the need to understand how variability in 5-HT_{1A} and 5-HT_{2A} binding maps onto variability in brain function in response to threat, a central aspect of personality measures related to risk for affective disorders.

2.3.10 Other serotonin receptor subtypes

Numerous studies within rodents and humans suggest that 5-HT receptors in addition to the 5-HT_{1A} and 5-HT_{2A} play a role in mediating the effects of 5-HT on anxiety and sensitivity to threat including the 5-HT_{2C} (Burghardt et al., 2007), 5-HT_3 (Yoshioka et al., 1995) and 5-HT_7 (Bonaventure et al., 2007) receptors (Barnes and Sharp, 1999; Holmes, 2008; Sharp et al., 2007). Indeed, it would be naïve to presume the 5-HT_{1A} and 5-HT_{2A} are the exclusive mechanisms mediating the effects of 5-HT signaling on this circuitry. However, the localization of these receptors is well-described and PET receptor imaging offers well-characterized radioligands for imaging these receptors that can be applied towards quantifying 5-HT_{1A} and 5-

HT_{2A} receptor binding *in vivo* using PET imaging in humans. In the context of a multi-modal neuroimaging approach including BOLD fMRI, 5-HT_{1A} and 5-HT_{2A} binding can be associated with of threat-related amygdala reactivity and broader amygdala-mPFC corticolimbic brain function. For these reasons, the studies described here will focus on the association between prefrontal 5-HT_{1A} and 5-HT_{2A} receptor binding and function of a corticolimbic circuit within a population of healthy adults using a multi-modal neuroimaging strategy incorporating receptor-binding PET and BOLD fMRI.

2.4 IMAGING GENETICS

As genes play a fundamental role in our biology, genetic variation plays a critical role in biological sources of individual variability. Imaging genetics has emerged as a commonly applied strategy for mapping effects of specific genetic polymorphisms on to individual differences in complex behaviors in terms of more proximal effects on brain chemistry and brain function in humans using neuroimaging (e.g., PET and BOLD fMRI; (Fisher et al., 2008; Hariri, 2009). Candidate genetic variants can be used to link genetic variation within individual variability in brain function or behavior through putative effects on brain chemistry. Imaging genetics with PET receptor imaging allows for the mapping of common genetic variants onto individual variability in receptor binding, a putative index of receptor availability, which can inform predicted effects of these genetic variants on brain function and behavior. Specific variants have been identified within genes coding for the 5-HT_{1A} (*HTR1A*), 5-HT_{2A} (*HTR2A*)

receptor and 5-HTT (*SLC6A4*) that may contribute to individual variability in 5-HT_{1A} and 5-HT_{2A} receptor binding and subsequently modulate function of this corticolimbic circuitry.

2.4.1 *HTR1A* C(-1019)G polymorphism

Within the promoter region of the *HTR1A* gene, a single nucleotide polymorphism (SNP) wherein a cytosine (C) is replaced by an guanine (G) at the -1019 base-pair (bp) location of the *HTR1A* gene (C(-1019)G; reference SNP: rs6295) has been associated with risk for suicide, depression and responsiveness to antidepressant treatment (Lemonde et al., 2004; Lemonde et al., 2003). The G-allele is associated with relatively increased 5-HT_{1A} transcription through a diminished capacity for the transcription factors NUDR/DEAF-1 and Hes5 to bind and repress transcription (Lemonde et al., 2003). A follow-up study indicates this genetic variant may have a specific effect on serotonergic neurons (Czesak et al., 2006). This suggests the G-allele would be associated with relatively increased midbrain raphe 5-HT_{1A} and subsequently decreased 5-HT release at downstream targets due to increased negative feedback.

Using an imaging genetics approach, we recently observed that the G-allele was associated with significantly reduced threat-related amygdala reactivity and that this effect significantly mediated the association between C(-1019)G genotype and trait anxiety (Fakra et al., 2009). Though the C(-1019)G SNP has been associated with alterations in *HTR1A* transcription *in vitro* (Lemonde et al., 2003); its effects in humans *in vivo* remain equivocal. A previous study found no association between the C(-1019)G genotype and 5-HT_{1A} binding in healthy adults (David et al., 2005), though another did report an association within the

midbrain raphe nuclei consistent with the G-allele showing relatively increased 5-HT_{1A} binding (Parsey et al., 2006c). Although the latter study included a greater sample size, it also included depressed individuals within their analysis. Additional studies investigating whether C(-1019)G status predicts 5-HT_{1A} binding are necessary to fully understand its association with 5-HT_{1A} *in vivo*.

Based on *in vitro* studies, we hypothesize the G-allele will be associated with relatively increased 5-HT_{1A} binding. We are specifically interested in the effects of this genetic variant on 5-HT signaling within the context of this amygdala-mPFC corticolimbic circuit, thus we will focus on its effects within these regions and the DRN where the 5-HT_{1A} autoreceptor modulates 5-HT release at downstream targets.

2.4.2 *HTR2A* G(-1438)A polymorphism

Multiple genetic variants within the *HTR2A* gene have been previously described (Cargill et al., 1999; Myers et al., 2007) and a recent PET study in twins found that 5-HT_{2A} binding in humans *in vivo* is largely genetically determined (Pinborg et al., 2008). However, little is known about specific genetic variants that contribute to inter-individual variability in 5-HT_{2A} binding in humans *in vivo* (Willeit and Praschak-Rieder, 2010). A G(-1438)A SNP (rs6311) within the promoter region of the *HTR2A* gene has been associated with altered transcriptional efficacy such that the A-allele showed increased promoter activity relative to the G-allele (Parsons et al., 2004). A T(102)C SNP (rs6313) within exon 1 of the *HTR2A* gene has been associated with increased 5-HT_{2A} density in post-mortem humans (Turecki et al., 1999). This is a synonymous

coding SNP (i.e., SNP within an exon that does not alter the amino acid sequence of the 5-HT_{2A} receptor) in complete linkage disequilibrium with the G(-1438)A SNP (i.e., the T-allele at rs6313 is always present with the A-allele at rs6311; (Spurlock et al., 1998). It should be noted that other studies have found no association between these variants and 5-HT_{2A} density (Kouzmenko et al., 1999) or mRNA in post-mortem brain (Bray et al., 2004).

A recent study in 21 healthy adults found no association between rs6311 or four other *HTR2A* variants and 5-HT_{2A} binding *in vivo* (Hurlemann et al., 2008). To date, this is the only imaging genetics finding relating *HTR2A* variants to 5-HT_{2A} binding in humans *in vivo*, thus additional studies are necessary to further evaluate genetic variants associated with 5-HT_{2A} binding. Based on findings from *in vitro* studies, we hypothesize the A-allele of rs6311 will be associated with increased 5-HT_{2A} binding within regions of this amygdala-mPFC corticolimbic circuit.

2.4.3 5-HTTLPR

The 5-HTT represents the primary active mechanism for clearing 5-HT from the extracellular space (Blakely et al., 1994; O'Rourke and Fudge, 2006), thus alterations in 5-HTT transcriptional efficacy have implications for regulating 5-HT signaling and 5-HT receptor function. A 44-bp variable nucleotide tandem repeat (VNTR) known as the "5-HTT linked polymorphic region" (5-HTTLPR), within the promoter region of the gene (*SLC6A4*) coding for 5-HTT, has been associated with variation in brain function and risk for psychopathology (Hariri et al., 2002b; Lesch et al., 1996; Munafo et al., 2008). Relative to the "long" (L) allele, the "short" (S) allele of

the 5-HTTLPR has been associated with reduced transcriptional efficacy, *in vitro*, suggesting this polymorphism may affect 5-HT signaling through its effects on 5-HTT levels (Lesch et al., 1996). An additional A/G SNP within the *SLC6A4* promoter (rs25531) moderates the 5-HTTLPR *in vitro* such that the L-allele coupled with the A-allele at rs25531 ("L_A") shows increased transcriptional efficacy while "L_G" allele functions more similar to the S-allele (Hu et al., 2005; Nakamura et al., 2000).

Across numerous cohorts, S-carriers have shown relatively increased threat-related amygdala reactivity (Hariri et al., 2002b; Munafo et al., 2008); increased anxiety (Katsuragi et al., 1999; Lesch et al., 1996; Mazzanti et al., 1998) and risk for depression (Caspi et al., 2003). The 5-HTTLPR polymorphism has also been associated with structural and functional variation in corticolimbic circuit function (Heinz et al., 2005; Pezawas et al., 2005). Studies have identified an association between the 5-HTTLPR polymorphism and 5-HTT binding in humans (Heinz et al., 2000; Kalbitzer et al., 2009; Praschak-Rieder et al., 2007; Reimold et al., 2007), however, others have not (Murthy et al., 2010; Parsey et al., 2006a); for review see (Willeit and Praschak-Rieder, 2010). An explanation for these findings is that the effects of the 5-HTTLPR on 5-HT signaling may be most prominent during the development of structural and functional neural circuits (Hariri and Holmes, 2006; Sibille and Lewis, 2006).

Differences in 5-HTT function related to the 5-HTTLPR variant may contribute to alterations in individual 5-HT receptors via effects on 5-HT signaling and homeostatic mechanisms. In addition to 5-HTT binding in humans, the 5-HTTLPR S-allele has been associated with reduced 5-HT_{1A} binding *in vivo* (David et al., 2005); but see (Borg et al., 2009). To date, no studies have examined the association between the 5-HTTLPR and 5-HT_{2A} binding

using PET receptor imaging in humans, *in vivo*. *In vitro* studies found the 5-HT_{2A} receptor desensitized in response to agonism (Leysen and Pauwels, 1990). Similiarly, 5-HT_{2A} binding decreased following a 6-week paroxetine treatment protocol in depressed individuals (Meyer et al., 2001). Based on these findings, we hypothesize that the S-allele (and L_G-allele) will be associated with relatively reduced 5-HT_{1A} and 5-HT_{2A} binding across regions within this corticolimbic circuit.

2.5 MODEL OF SEROTONERGIC SIGNALING PATHWAYS MODULATING CORTICOLIMBIC CIRCUITRY

In summary, a corticolimbic circuit involving the amygdala and mPFC plays a key role in processing threat within the environment and contributing to complex behaviors and related aspects of personality such as anxiety. Though 5-HT is thought to modulate this circuitry, the specific 5-HT receptor mechanisms mediating these effects are not fully understood. 5-HT_{1A} and 5-HT_{2A} receptors are localized within this circuitry to play an important role in mediating the effects of 5-HT signaling. The 5-HT_{1A} autoreceptor plays a key role in regulating 5-HT release via negative feedback inhibition. Though expressed within the amygdala, the cellular localization of 5-HT_{1A} and 5-HT_{2A} within the amygdala makes their effects on amygdala activation ambiguous and dependent on the specific neuronal populations that are activated. Within mPFC, 5-HT_{1A} and 5-HT_{2A} are co-localized to pyramidal neurons suggesting their functional interactions may play an important role in modulating prefrontal-mediated

regulation of the amygdala (**Figure 3**). Specifically, mPFC 5-HT_{2A} is localized to facilitate regulation of the amygdala via biasing pyramidal neurons towards increased excitation. 5-HT_{1A} is localized to inhibit the excitability of mPFC pyramidal neurons and thus moderate or "gate" the effects of mPFC 5-HT_{2A}. Considering their effects of transcriptional efficacy, common functional polymorphisms within the *HTR1A*, *HTR2A* and *SLC6A4* genes are likely to contribute to individual differences in the availability of 5-HT_{1A} and 5-HT_{2A}. The G-allele of the *HTR1A* C(-1019)G SNP would be hypothesized to be associated with increased 5-HT_{1A} while the 5-HTTLPR S-allele would be hypothesized to be associated with relatively decreased 5-HT_{1A}. The A-allele of the *HTR2A* G(-1438)A SNP would be hypothesized to be associated with relatively increased 5-HT_{2A} while the 5-HTTLPR S-allele would be hypothesized to be associated with relatively decreased 5-HT_{2A}.

3.0 STUDY 1: CAPACITY FOR 5-HT_{1A} MEDIATED AUTO-REGULATION PREDICTS AMYGDALA REACTIVITY[1]

3.1 ABSTRACT

We examined the contribution of 5-HT_{1A} autoreceptors (with [^{11}C]WAY100635 positron emission tomography) to amygdala reactivity (with blood oxygenation level–dependent functional magnetic resonance imaging) in 20 healthy adult volunteers. We found a significant inverse relationship wherein 5-HT_{1A} autoreceptor density predicted a notable 30–44% of the variability in amygdala reactivity. Our data suggest a potential molecular mechanism by which a reduced capacity for negative feedback regulation of 5-HT release is associated with increased amygdala reactivity.

[1]Chapter 3 is a slightly modified version of an article published in *Nature Neuroscience* as a Brief Communication. Full citation: Fisher, P.M., Meltzer, C.C., Ziolko, S.K., Price, J.C., Moses-Kolko, E.L., Berga, S.L., Hariri, A.R. (2006). Capacity for 5-HT_{1A}-mediated autoregulation predicts amygdala reactivity. *Nature Neuroscience*. 9(11), 1362-63.

3.2 MAIN TEXT

Variability in central serotonin or 5-hydroxytryptamine (5-HT) function is associated with individual differences in affect, temperament and risk for developing mood disorders such as depression. A key component of 5-HT regulation is the somatodendritic 5-HT_{1A} autoreceptor. By means of the negative feedback inhibition of serotonergic raphe neurons, which project to corticolimbic targets supporting emotional reactivity, this autoreceptor plays a critical role in regulating 5-HT release. Increased 5-HT_{1A} autoreceptor availability, associated with either genetic or environmental variables, is associated with major depression (Lemonde et al., 2003; Parsey et al., 2006c), and the therapeutic efficacy of many antidepressant drugs may be dependent on down-regulation or desensitization of the 5-HT_{1A} autoreceptor after chronic treatment (Blier and de Montigny, 1999; Parsey et al., 2006c). These observations suggest that diminished 5-HT stimulation of corticolimbic targets, resulting from increased 5-HT_{1A} autoreceptor–mediated negative feedback, may contribute to depressed behavioral and physiologic arousal.

In the current study, we used a multimodal neuroimaging strategy to examine the contribution of somatodendritic 5-HT_{1A} autoreceptors to individual differences in amygdala reactivity, a core component of emotional arousal and attention. During a single visit, 20 healthy adult subjects underwent both positron emission tomography (PET), using the radiotracer [^{11}C] WAY100635, to determine the binding potential of the 5-HT_{1A} autoreceptor and functional magnetic resonance imaging (fMRI), using a well-established emotional faces–matching protocol, to determine the functional reactivity of the amygdala. Using Logan

graphical modeling, we analyzed the PET data and calculated the distribution volume and binding potential of the 5-HT_{1A} autoreceptor in the brainstem region of the dorsal raphe nucleus (DRN). We analyzed the BOLD fMRI data using a general linear model random effects analysis within SPM2 (http://www.fil.ion.ucl.ac.uk/spm/) to determine amygdala reactivity. Then, using linear regression, we examined the relationship between 5-HT_{1A} autoreceptor binding potential in the DRN and BOLD signal change in the amygdala (for additional methods, see Section 3.3).

Consistent with previous studies, [^{11}C]WAY100635 PET revealed high values of mean 5-HT_{1A} binding potential in the DRN (3.35 ± 1.10, mean ± s.d.) and BOLD fMRI revealed strong bilateral amygdala reactivity (right amygdala: (16,–4,–18), t_{19} = 4.60, P = 0.005 (FDR corrected), 61 voxels; left amygdala: (–32,–2,–22), t_{19} = 6.06, P = 0.002 (FDR corrected), 86 voxels). Regression analyses (**Figure 4A**) revealed a significant inverse relationship between DRN 5-HT_{1A} autoreceptor binding potential and bilateral amygdala reactivity. Notably, 5-HT_{1A} autoreceptor binding potential accounted for 44% of the variability in right amygdala reactivity (**Figure 4B**) and 30% of the variability in left amygdala reactivity, effects independent of both age and sex. Moreover, postsynaptic 5-HT_{1A} density in the amygdala explained only an additional 8% of the variance in amygdala reactivity beyond that accounted for by 5-HT_{1A} autoreceptor density. Consistent with the inhibitory effects of postsynaptic 5-HT_{1A} receptors, increasing amygdala 5-HT_{1A} binding potential was associated with decreasing functional reactivity. Our findings, however, indicate that 5-HT_{1A} autoreceptor-mediated effects on net 5-HT release is more important in regulating amygdala reactivity than local postsynaptic 5-HT_{1A} density. We were

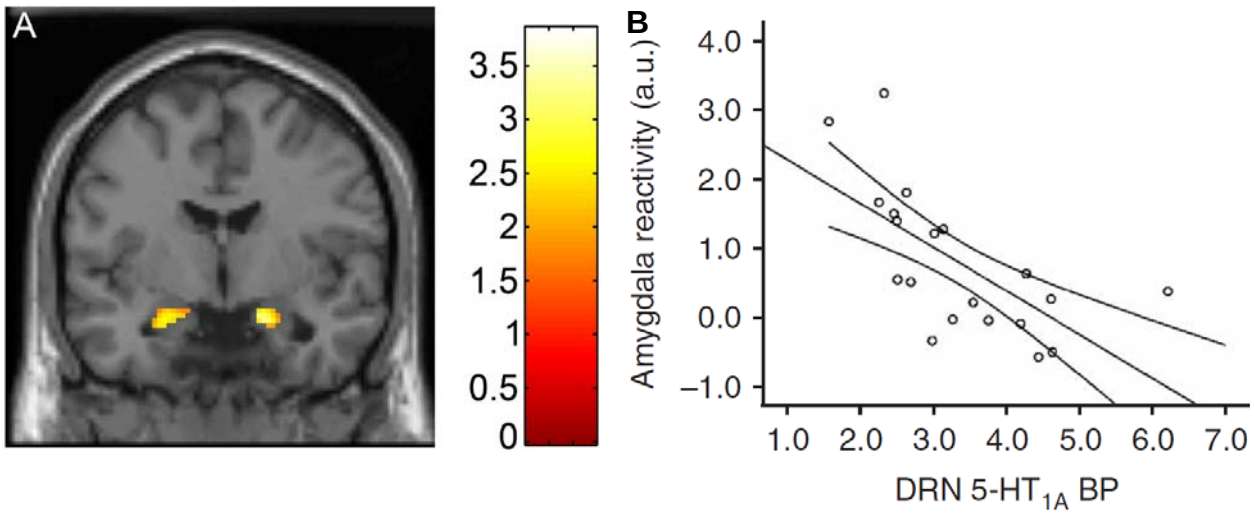

Figure 4. 5-HT_{1A} autoreceptor availability predicts threat-related amygdala reactivity. (**A**) Statistical parametric map representing right (r = -0.66, r^2 = 0.44, p = 0.001) and left (r = -0.55, r^2 = 0.30, p = 0.012) amygdala reactivity, which was negatively correlated with DRN 5-HT_{1A} binding potential in 20 subjects. (**B**) 5-HT_{1A} binding potential plotted against right amygdala activation cluster shown in **A**. Curved lines represent 95% confidence limits on regression line. Color bar in **A** represents t-scores. (Fisher et al., 2006)

surprised to find that 5-HT_{1A} autoreceptor binding potential did not correlate with secondary task-related activations in the hippocampal formation and medial prefrontal cortex. As these regions also receive serotonergic innervation, the specificity of the observed relationship with amygdala reactivity may reflect the targeted engagement of this region by our fMRI task, leading to greater local serotonergic release and modulation.

Our current results highlight a specific molecular mechanism within the serotonergic system that modulates amygdala reactivity and, in turn, may contribute to both the risk for major depression and the therapeutic effects of antidepressant drugs. The somatodendritic 5-HT_{1A} autoreceptor inhibits firing of efferent serotonergic projections to cortical and subcortical structures, thereby decreasing available serotonin in these regions. An increase in 5-HT_{1A} binding potential in the DRN, like that documented in antidepressant-naïve individuals with major depression (Parsey et al., 2006c), indicates a greater capacity for negative feedback inhibition and subsequent reduction in serotonin release at downstream targets, such as the amygdala. Accordingly, relative increases in 5-HT associated with reduced 5-HT_{1A} autoreceptors lead to potentiated amygdala reactivity. Thus, antidepressants targeting the 5-HT system may alleviate the blunting of emotional arousal in depression by effecting an increase in 5-HT stimulation, via 5-HT_{1A} autoreceptor downregulation, which increases the reactivity of the amygdala.

It is worth noting that reduced 5-HT_{1A} receptor density and relatively increased amygdala reactivity have also been documented in depression (Hasler et al., 2004). Although individuals in these studies were typically medicated with antidepressant drugs, including selective serotonin reuptake inhibitors (SSRIs), and suffered from comorbid conditions such as

anxiety, these data suggest that an alternative interpretation of our current finding is that relatively reduced 5-HT_{1A} autoreceptor density and associated increased amygdala reactivity may represent a pre-existing trait-like risk factor for depression. Application of our multimodal imaging protocol in antidepressant-naïve depressed individuals before and after pharmacological intervention would allow for an explicit comparison of these alternative models.

Inactivation of the 5-HT_{1A} autoreceptor in transgenic mice is associated with increased anxiety and stress reactivity (Parks et al., 1998). 5-HT_{1A} knockout mice also exhibit ''hyper-vigilance'' for threat cues, and increased reactivity to ambiguous stimuli and context (Klemenhagen et al., 2006). Moreover, pharmacological antagonism of the 5-HT_{1A} potentiates the antidepressant effects of SSRIs (Arborelius, 1999), and a functional genetic polymorphism associated with relatively increased 5-HT_{1A} autoreceptors is associated with poorer response to antidepressants (Lemonde et al., 2004). Consistent with our findings, these independent observations converge to suggest that increased 5-HT neurotransmission associated with decreased 5-HT_{1A}–mediated negative feedback inhibition results in increased behavioral arousal and reactivity, which are mediated in large part by the amygdala (LeDoux, 2000).

It has been hypothesized that downstream effects on somatodendritic 5-HT_{1A} autoreceptors mediate neural and behavioral changes associated with functional alterations of the 5-HT transporter (5-HTT). For example, antidepressant effects of the SSRIs, which block 5-HTT–mediated transport, typically coincide with downregulation of somatodendritic 5-HT_{1A} autoreceptors (Blier and de Montigny, 1999). In addition, a functional polymorphism resulting in reduced transcriptional efficiency of the human 5-HTT is associated with reduction in 5-HT_{1A}

autoreceptors (David et al., 2005). The less-efficient 5-HTT polymorphism is also associated with increased risk for depression, especially in the context of environmental stressors (Caspi et al., 2003), and greater amygdala reactivity to emotionally provocative stimuli (Hariri et al., 2002b). This raises the possibility that alterations in serotonergic tone associated with 5-HT_{1A} autoreceptor density may be important for regulating the responsiveness of the amygdala and represent a key molecular mechanism for the effects of 5-HT subsystem gene polymorphisms on brain function and emotional behaviors.

3.3 SUPPLEMENTARY METHODS

3.3.1 Subjects

Twenty healthy adult volunteers participated after providing informed consent in accordance with the University of Pittsburgh Institutional Review Board (11M; mean age: 39.2 ± 13.8 (s.d.) years). Subjects were generally healthy and exclusion criteria included: 1) early dementia or mild cognitive impairment according to the Mini-Mental State Examination (scores below 27); 2) sleep disorders assessed by the Pittsburgh Quality Sleep Index; and 3) current or lifetime psychiatric diagnoses assessed by Structured Clinical Interview (DSM-IV; (Buysse et al., 1989; Folstein et al., 1975).

3.3.2 fMRI protocol

The fMRI paradigm consisted of four blocks of an emotional face-processing task interleaved with five blocks of a sensorimotor control task as previously described (Brown et al., 2005). Briefly, our challenge paradigm consists of task blocks wherein subjects match angry or fearful facial expressions with an identical target expression and contrasting control blocks wherein subjects match simple geometric shapes. This is a widely employed and well characterized task, which has consistently elicited robust amygdala and interconnected corticolimbic regional activation in multiple samples and study designs. While this design produces consistent amygdala activation and readily allows for exploration of individual differences in activation magnitude, it does not afford interpretations regarding the response of the amygdala to specific categories of emotional facial expressions as the explicit comparison is between angry and fearful facial expressions and simple geometric shapes.

Blood oxygenation level-dependent (BOLD) functional images were acquired on a GE Signa 1.5T scanner (GE Medical Systems, Milwaukee, WI) using a reverse spiral sequence covering 28 axial slices (3.8mm thick) encompassing the entire cerebrum and the majority of the cerebellum (TR/TE = 2000/35 ms, FOV = 24 cm, matrix = 64 x 64). Scanning parameters were selected to optimize BOLD signal while maintaining enough slices to acquire whole-brain data. Before the collection of fMRI data for each subject we acquired and visually inspected localizer scans for artifacts (e.g. ghosting) as well as good signal across the entire volume of acquisition, including the medial temporal lobes. fMRI data from all 20 subjects included in this study were cleared of such problems. Single subject fMRI data were preprocessed and main

effects of task (face-processing vs. sensorimotor control) were calculated using SPM2 as described previously (Brown et al., 2005).

3.3.3 PET protocol

The radiosynthesis of [^{11}C]WAY100635 was performed as previously described (Cidis Meltzer et al., 2001; McCarron et al., 1996). PET scans were acquired on an ECAT HR+ PET scanner (CTI PET Systems, Knoxville, TN) in 3D imaging mode (63 transaxial planes, 2.4-mm thickness; 15.2-cm field-of-view). A Neuro-insert (CTI PET Systems, Knoxville, TN) in the camera gantry reduced random coincidences. Head movement was minimized by the use of a thermoplastic mask system. The transmission scan was acquired using rotating rods of ^{68}Ge/^{68}Ga for attenuation correction of emission data. PET data were corrected for radioactive decay and scatter using a model-based method (Watson et al., 1997). PET image reconstruction was performed using filtered back-projection (Fourier rebinning, 2D backprojection, Hann filter: 3 mm) for a final reconstructed image resolution of about 6 mm. The PET imaging session was carried out across 90 minutes with arterial blood sampling for all 20 subjects.

Following a transmission scan, intravenous injection of 14.3 ± 1.4 (s.d.) mCi [^{11}C]WAY100635 immediately preceded emission imaging. An 8-mm diameter circular ROI for the dorsal raphe nucleus and the cerebellar reference region were placed on respective planes selected using anatomical landmarks and positioned as previously described (Meltzer et al., 2004). The *in vivo* kinetics were calculated using the Logan graphical method as described previously (Logan et al., 1990; Lopresti et al., 2005). The Logan graphical analyses were applied

over the 25 to 90 min PET scan interval. Regression of Logan variables yields a slope equivalent to the radiotracer distribution volume (DV). Binding potential values were calculated using BP = [(DV_{ROI} / DV_{CER}) – 1]. Partial volume effects due to differences in cerebral volumes were corrected for in calculating regional BP values using a previously validated two-component MR-based atrophy correction algorithm (Cidis Meltzer et al., 2001; Meltzer et al., 1999; Meltzer et al., 1990).

3.3.4 Regression analyses

The relationship between 5-HT_{1A} autoreceptor availability and amygdala reactivity was determined using linear regression analyses of the single-subject amygdala BOLD and DRN 5-HT_{1A} BP values. Age and sex were included in the regression analyses to test for their potential effects on these independent values as well as their interrelationship. To account for the contribution of postsynaptic amygdala 5-HT_{1A} BP to the variance in BOLD assessed amygdala reactivity, independent of 5-HT_{1A} autoreceptor effects, we employed a general linear model analysis including both the postsynaptic 5-HT_{1A} BP measure and DRN 5-HT_{1A} BP. Correlations between 5-HT_{1A} BP and BOLD were restricted to amygdala clusters exhibiting a main effect of task identified using a one sample t-test across all subjects within SPM2 ($P < 0.05$, FDR corrected for multiple comparisons over the volume of the amygdala as defined by the WFU Pickatlas; (Maldjian et al., 2004; Maldjian et al., 2003).

4.0 STUDY 2: MEDIAL PREFRONTAL CORTEX 5-HT_{2A} DENSITY IS CORRELATED WITH AMYGDALA REACTIVITY, RESPONSE HABITUATION, AND FUNCTIONAL COUPLING[2]

4.1 ABSTRACT

Feedback inhibition of the amygdala via medial prefrontal cortex (mPFC) is an important component in the regulation of complex emotional behaviors. The functional dynamics of this corticolimbic circuitry are, in part, modulated by serotonin (5-HT). Serotonin 2A (5-HT_{2A}) receptors within the mPFC represent a potential molecular mechanism through which 5-HT can modulate this corticolimbic circuitry. We employed a multimodal neuroimaging strategy to explore the relationship between threat-related amygdala reactivity, assessed using blood oxygen level-dependent functional magnetic resonance imaging, and mPFC 5-HT_{2A} density, assessed using [^{18}F]altanserin positron emission tomography in 35 healthy adult volunteers. We observed a significant inverse relationship wherein greater mPFC 5-HT_{2A} density was associated with reduced threat-related right amygdala reactivity. Remarkably, 25-37% of the

[2]Chapter 4 is a slightly modified version of an article published in *Cerebral Cortex* as a Featured Article. Full citation: Fisher, P.M., Meltzer, C.C., Price, J.C., Coleman, R.L., Ziolko, S.K., Becker, C., Moses-Kolko, E.L., Berga, S.L., Hariri, A.R. (2009). Medial prefrontal cortex 5-HT_{2A} density is correlated with amygdala reactivity, response habituation and functional coupling. *Cerebral Cortex*. 19(11), 2499-507.

variability in amygdala reactivity was explained by mPFC 5-HT_{2A} density. We also observed a positive correlation between mPFC 5-HT_{2A} density and the magnitude of right amygdala habituation. Furthermore, functional coupling between the amygdala and mPFC was positively correlated with 5-HT_{2A} density suggesting that effective integration of emotionally salient information within this corticolimbic circuitry may be modulated, at least in part, by mPFC 5-HT_{2A}. Collectively, our results indicate that mPFC 5-HT_{2A} is strongly associated with threat-related amygdala reactivity as well as its temporal habituation and functional coupling with prefrontal regulatory regions.

4.2 INTRODUCTION

Regulation of emotional arousal involves coordinated communication between cortical and subcortical structures. Dysfunction within the neural circuitry for emotional arousal and regulation contributes to risk for psychiatric illness, including anxiety disorders and major depression, and represents a pathophysiological marker of these same conditions (Mayberg, 2003; Phillips et al., 2003b). Among areas of the brain implicated in this regulatory network, the medial prefrontal cortex (mPFC) is thought to play a critical role in regulating amygdala-mediated arousal in response to emotionally salient, especially threat-related, environmental cues (LeDoux, 2000; Wood and Grafman, 2003). Recent rodent work suggests that homologous prefrontal regions act to inhibit amygdala output via glutamatergic stimulation of inhibitory γ-amino butyric acidergic (GABAergic) neurons within the amygdala (Quirk et al., 2003). In

humans, variation in amygdala-mPFC functional coupling has been associated with individual differences in behavior and risk for psychiatric illness (Drevets et al., 1992; Pezawas et al., 2005).

The central serotonin (5-HT) system has been linked to variation in neural reactivity within the amygdala and PFC to emotionally salient environmental cues (Bigos et al., 2008; Fisher et al., 2006; Hariri and Holmes, 2006; Hariri et al., 2002b; Harmer et al., 2006; Holmes et al., 2003; Pezawas et al., 2005; Weisstaub et al., 2006). Through negative feedback inhibition, the serotonin 1A ($5\text{-}HT_{1A}$) somatodendritic autoreceptor acts to regulate 5-HT release at corticolimbic targets associated with emotional reactivity. Recently, we reported a significant inverse relationship between $5\text{-}HT_{1A}$ autoreceptor density and threat-related amygdala reactivity in 20 healthy adult volunteers (Fisher et al., 2006). In this sample, 30-44% of the variability in amygdala reactivity was accounted for by $5\text{-}HT_{1A}$ density suggesting that the capacity for regulating 5-HT release is an important modulatory component of the neural circuitry for emotional arousal. More generally, this finding further links increased 5-HT signaling with potentiated amygdala reactivity (Bigos et al., 2008; Burghardt et al., 2007; Forster et al., 2006; Hariri et al., 2002b; Rhodes et al., 2007).

In addition to such autoregulatory serotonergic mechanisms impacting amygdala reactivity, postsynaptic receptors are likely instrumental in determining 5-HT modulation of this circuitry. Of these, excitatory serotonin 2A ($5\text{-}HT_{2A}$) receptors localized in the mPFC may be of particular importance. Glutamatergic neurons represent the predominant neuronal population expressing the $5\text{-}HT_{2A}$ receptor within mPFC (de Almeida and Mengod, 2007; Jakab and Goldman-Rakic, 1998; Leysen, 2004). Furthermore, proximal portions of the apical dendrites of

these glutamatergic neurons may represent a ‘‘hot spot’’ of 5-HT_{2A} localization coincident with relatively dense 5-HT innervation (Blue et al., 1988; Jakab and Goldman-Rakic, 1998). Interestingly, whereas rapid increases in amygdala 5-HT release are associated with the initiation of fear-related behaviors, relatively delayed 5-HT release in mPFC is associated with their attenuation (Forster et al., 2006). Taken together, these data suggest that the 5-HT_{2A} receptor is ideally situated to mediate excitatory effects of 5-HT release on mPFC projection neurons that, in turn, facilitate regulation of amygdala reactivity and associated emotional behaviors.

Previous research in major depression is consistent with this effect of altered prefrontal 5-HT_{2A} receptor density (Stockmeier, 2003). One study reported increased 5-HT_{2A} receptor density in frontal cortex within a recovered depressed population (Bhagwagar et al., 2006). In contrast, a second study reported decreased 5-HT_{2A} receptor density in frontal cortex within a currently depressed population (Yatham et al., 2000). A third study reported decreased subgenual prefrontal cortex (sgPFC) 5-HT_{2A} receptor density within a recovered population of women with eating disorders, which often are comorbid with mood disorders including depression (Bailer et al., 2004). These findings further implicate prefrontal 5-HT_{2A} receptors as a potentially important mechanism in the regulation of corticolimbic circuit function supporting emotional behaviors. To date, however, no studies have explored the relationship between 5-HT_{2A} receptor density in the mPFC and amygdala reactivity in humans. In the current study, we explored the relationship between human mPFC 5-HT_{2A} receptor density and threat-related amygdala reactivity using a multimodal neuroimaging strategy in 35 healthy adult volunteers. Blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI) was used to

measure amygdala reactivity in response to threat-related facial expressions (Brown et al., 2006; Brown et al., 2005; Fisher et al., 2006; Manuck et al., 2007; Zhou et al., 2008). Positron emission tomography (PET) was used to assess 5-HT_{2A} receptor density within 2 subregions of the mPFC, namely the pregenual prefrontal cortex (pgPFC) and sgPFC, using [^{18}F]altanserin, a radioligand with high affinity and specificity for the 5-HT_{2A} receptor. We focused on these 2 mPFC subregions because they are both functionally and structurally interconnected with the amygdala and richly innervated by 5-HT neurons (Blue et al., 1988; McDonald, 1998; Pandya et al., 1981; Pezawas et al., 2005).

Based on the work summarized above illustrating 1) mPFC regulation of amygdala reactivity via feedback (i.e., ''top--down'') inhibition and 2) localization of 5-HT_{2A} receptors to proximal areas of the dendrites of mPFC glutamatergic projection neurons, we hypothesized that greater 5-HT_{2A} receptor density within mPFC (both pgPFC and sgPFC) would be inversely related to amygdala reactivity reflecting a greater capacity for prefrontal regulation. We sought to further characterize the relationship between serotonergic regulation of mPFC and amygdala reactivity using functional connectivity and hypothesized that greater pgPFC and sgPFC 5-HT_{2A} receptor density would be associated with increased functional coupling between these regions and the amygdala. Additionally, we explored the degree to which mPFC 5-HT_{2A} receptor density was associated with habituation of amygdala reactivity, a commonly observed phenomenon similar to extinction and likely to reflect the capacity for prefrontal regulatory control (Breiter et al., 1996; Büchel et al., 1998; Herry et al., 2007).

4.3 METHODS

4.3.1 Participants

Thirty-five healthy adult volunteers participated after providing written informed consent in accordance with the University of Pittsburgh Institutional Review Board (18 males, age: 37.7 ± 12.8 [mean ± standard deviation (SD)] years). Subjects were recruited through local advertisements, referrals, and ongoing studies. Subjects were generally healthy with exclusion criteria including 1) current or lifetime psychiatric diagnoses assessed by Structured Clinical Interview (Diagnostic and Statistical Manual of Mental Disorders, Version IV, DSM-IV; (First et al., 1996), 2) cardiovascular disease or diabetes, 3) history of substance abuse or use of antidepressants, 4) early dementia or mild cognitive impairment according to the Mini-Mental State Examination (scores exceeding 27; (Folstein et al., 1975), and 5) sleep disorders assessed by the Pittsburgh Quality Sleep Index (Buysse et al., 1989). Most subjects (N = 30) completed both fMRI and PET scans on the same day. All subjects completed the fMRI scan in the morning and the PET scan in the afternoon. Those subjects who did not complete both scans on the same day (N = 5) completed the PET and fMRI scans within 1 month. To limit the potential effects of circulating reproductive hormone levels on measures of 5-HT_{2A} binding, all premenopausal women (N = 11) were scanned within days 3 and 7 of their menstrual cycle (Moses et al., 2000). Subjects were paid for their participation in this study.

4.3.2 fMRI protocol

The experimental fMRI paradigm consisted of 4 blocks of a face-processing task interleaved with 5 blocks of a sensorimotor control task as described previously (Brown et al., 2005; Fakra et al., 2009; Hariri et al., 2005; Hariri et al., 2002b). Subject performance (accuracy and reaction time) was monitored during all scans. Stimulus presentation and subject performance was controlled using E-prime (Psychological Software Tools, Inc., Pittsburgh, PA, http://www.pstnet.com/). During the face-processing task, subjects viewed a trio of faces (expressing either anger or fear) and selected one of two faces (bottom) identical to a target face (top; **Figure 2**). Angry and fearful facial expressions can represent honest indicators of ecologically valid threat, especially that related to conspecific challengers (Darwin and Ekman, 1998). Thus, we interpret amygdala activation elicited by our task as being "threat-related".

Each face-processing block consisted of six images (i.e., trio of faces), balanced for sex and representing one target affect (angry or fearful) all derived from a standard set of pictures of facial affect (Ekman and Friesen, 1976). During the sensorimotor control blocks, subjects viewed a trio of simple geometric shapes (circles and vertical and horizontal ellipses) and selected one of two shapes (bottom) identical to a target shape (top). Each sensorimotor control block consisted of six different shapes trios. All blocks were preceded by a brief instruction (''Match Faces'' or ''Match Shapes'') lasting two seconds. In the face-processing blocks, each of the six face trios was presented for four seconds with a variable inter-stimulus interval (ISI) of 2-6 seconds (mean ISI = 4 seconds) for a total block length of 48 seconds. In the sensorimotor control blocks, each of the six shape trios was presented for four seconds with a

fixed inter-stimulus interval of two seconds for a total block length of 36 seconds. Total protocol time was 390 seconds over which 195 whole-brain volumes were collected.

As we were not interested in neural networks associated with face-specific processing per se, but rather in eliciting a maximal amygdala response across all subjects, we chose not to use neutral faces as control stimuli because neutral faces can be subjectively experienced as affectively laden or ambiguous and thus engage the amygdala (Schwartz et al., 2003a; Wright et al., 2003). We chose to use threat-related facial expressions as opposed to threatening scenes (e.g., IAPS pictures) because previous research indicates a more robust amygdala response to threat-related facial expressions (Hariri et al., 2002c).

4.3.3 fMRI data acquisition

Each subject was scanned using a GE Signa 1.5 Tesla head only scanner (GE Medical Systems, Milwaukee, WI) at the University of Pittsburgh Medical Center (UPMC) Magnetic Resonance Research Center (MRRC). BOLD functional images were acquired using a reverse spiral sequence covering 28 slices (3.8 millimeter thickness, no gap) encompassing the entire cerebrum and the majority of the cerebellum (repetition time (TR) = 2000 milliseconds, echo time (TE) = 35 ms, field-of-view [FOV] = 24 centimeter, matrix = 64 x 64, flip-angle = 70°). All scanning parameters were selected to optimize BOLD signal while maintaining enough slices to acquire whole-brain data. An auto-shimming procedure was conducted before the acquisition of BOLD data in each subject to minimize field inhomogeneities. Prior to the acquisition of fMRI data for each subject, we acquired and visually inspected localizer scans for artifacts (e.g.,

ghosting) as well as good signal across the entire volume of acquisition, including the medial temporal lobes. All datasets included in analysis were cleared of any artifacts.

4.3.4 fMRI data pre-processing

Whole-brain image analysis was completed using the general linear model (GLM) of SPM (Statistical Parametric Mapping; http://www.fil.ion.ucl.ac.uk/spm). Images for each subject were realigned to the first volume in the time series to correct for head motion. Data for subjects with greater than 2 mm translational movement in any one direction (x, y or z) or 2° rotational movement along any axis (roll, pitch or yaw) were excluded from analysis. T1-weighted in-plane structural images were used to co-register and normalize functional images into a standard space (37 slices, TR = 500 ms, TE = MF, FOV = 24, slice-thickness = 3.8 mm, no gap, matrix = 256 x 192). Data were spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12-parameter affine model (final voxel resolution of functional images = 2 x 2 x 2 mm). Following normalization, functional imaging data were smoothed to minimize noise and residual difference in gyral anatomy with a Gaussian filter, set at six millimeter full-width at half-maximum (FWHM). Voxel-wise signal intensities were ratio normalized to the whole-brain global mean.

4.3.5 fMRI data analysis – group level analysis

Preprocessed data sets were analyzed using second-level random-effects models that account for both scan-to-scan and participant-to-participant variability to determine task-specific regional responses within SPM. Following preprocessing, our GLM, employing canonical hemodynamic response functions, was used to estimate condition-specific and task-specific BOLD activation for each individual (beta-weights and contrast images, respectively). Individual contrast images (i.e., weighted sum of the beta images) were used in second-level random-effects models to determine mean task-specific amygdala reactivity using one-sample t-tests.

4.3.6 Region of interest analysis

Group-level effects for our contrast of interest (i.e., faces > shapes) were assessed within the amygdala using an ROI constructed from the WFU Pickatlas (v1.04; (Lancaster et al., 2000; Maldjian et al., 2003). All analyses were thresholded at a voxel level statistical threshold of $p < 0.05$, false-discovery rate (FDR) corrected for multiple comparisons (Genovese et al., 2002), within an inclusive mask of activations of interest and an extent threshold of at least 10 contiguous voxels. Because of our *a priori*, directionally specific hypotheses and our use of a rigorous random-effects model, these statistical thresholds effectively control for ‘‘false positives’’ arising from multiple comparisons. All neuroimaging data are reported using the coordinate system of Talairach and Tournoux.

4.3.7 MR structural image acquisition for PET

High-resolution structural MR images were acquired using a GE Signa 1.5 Tesla head only scanner (GE Medical Systems, Milwaukee, WI) at the University of Pittsburgh Medical Center (UPMC) Magnetic Resonance Research Center (MRRC). Images were acquired using a spoiled-gradient (SPGR) recalled sequence with parameters optimized for contrast between gray matter, white matter, and cerebrospinal fluid (124 slices; slice thickness = 1.5 mm, no gap; TR = 25 ms; TE = 5 ms; FOV = 24x18; flip angle = 40°; matrix = 256 x 192).

4.3.8 PET image acquisition

PET scans were acquired using an ECAT HR+ PET scanner (CTI PET systems, Knoxville, TN) in 3D imaging mode (63 transaxial planes, 2.4-mm thickness, 15.2-cm FOV). Catheters were placed in an antecubital vein for radiotracer injection and a radial artery for arterial blood sampling. Head movement was minimized by use of a softened thermoplastic face mask system. A 10-min transmission scan was acquired using rotation rods of $^{68}Ge/^{68}Ga$ for attenuation correction of emission data. A Neuro-insert (CTI PET systems) in the camera gantry was used to reduce random coincidences. The PET data were acquired over 90 min. Blood samples were collected throughout the scan session to determine an arterial input function. Dynamic arterial blood sampling was performed during emission scanning. PET data were corrected for scatter, and image reconstruction was performed using filtered back projection for a final reconstructed image resolution of about 6 mm.

4.3.9 [^{18}F]Altanserin positron emission tomography

[^{18}F]altanserin is a 5-HT_{2A} receptor antagonist with high affinity and specificity for the 5-HT_{2A} receptor relative to other 5-HT receptor subtypes as well as noradrenergic a1 and dopaminergic D2 receptors (Leysen, 1989; Leysen, 1990). The radiosynthesis of [^{18}F]altanserin was performed using a modification of the original method (Lemaire et al., 1991) that has been applied in several studies in our laboratory (Bailer et al., 2007; Bailer et al., 2004; Meltzer et al., 1998; Price et al., 2001b; Smith et al., 1998). Dynamic PET imaging was initiated at the start of the [^{18}F]altanserin injection (slow bolus over 20 s) for which the mean injected dose was 7.23 ± 0.31 mCi (mean ± SD) of high-specific [^{18}F]altanserin. The 90-min dynamic PET acquisition began at injection along with arterial blood sampling for the determination of the arterial input function.

The total plasma radioactivity concentration was corrected for the presence of radiolabeled [^{18}F]altanserin metabolites, and this ‘‘metabolite-corrected’’ arterial input function was used for data analysis. This method has previously been shown to effectively control for regional bias due to radiolabeled metabolites (Price et al., 2001a; Price et al., 2001b).

The Logan analysis was applied over the 12- to 90-min post-injection integrals (10 points) to obtain regional [^{18}F]altanserin distribution volume values within specific regions of interest, V_T, and signal due to free and non-displaceable binding within the cerebellar reference region, V_{ND}. Regional V_T values and V_{ND} were used to determine binding potential, BP_{ND}, a measure of specific non-displaceable receptor binding where $BP_{ND} = (V_T / V_{ND}) – 1 = f_{ND}*B_{avail}/K_d$. BP_{ND} is related to f_{ND}, free-fraction in tissue, and directly proportional to B_{avail}/K_d, where B_{avail} is the concentration of receptors available for radiotracer binding (i.e., not occupied by

endogenous 5-HT) and K_d is the equilibrium dissociation rate constant (i.e., inversely related to binding affinity). Partial volume effects due to differences in cerebral volumes were corrected for in calculating BP_{ND} values using a previously validated 2-component MR-based atrophy correction algorithm (Meltzer et al., 1990).

Regions of interest (ROIs) were drawn on the resliced MR images for each subject and applied to their respective, coregistered PET images. ROIs were identified for the pregenual prefrontal cortex (pgPFC, including portions of Brodmann's Area 24 and 32), subgenual prefrontal cortex (sgPFC, including portions of Brodmann's area 25 and 32), and cerebellum, which was used to estimate nondisplaceable radiotracer uptake (V_{ND}). As we have no *a priori* hypothesis regarding the laterality of our effects, regions-of-interest from the right and left hemisphere were combined to reduce noise (Meltzer et al., 2001). Evidence of possible specific binding within subregions of the cerebellum and spill-in from adjacent cortical regions complicates particular selection of reference region (Gunn et al., 1998; Hirvonen et al., 2006; Parsey et al., 2000b). Our cerebellar ROI did not include white matter and was placed below the occipital cortex.

4.3.10 Regression analyses

The relationship between threat-related amygdala reactivity and mPFC 5-HT_{2A} binding was determined using linear regression analyses between the single-subject amygdala BOLD and ROI-specific 5-HT_{2A} BP_{ND} values. Single subjects with BP_{ND} values < 0.0 within an ROI were excluded because BP_{ND} < 0.0 indicates signal intensity equivalent to free or nonspecific binding.

Previous studies have reported that 5-HT_{2A} BP_{ND} is inversely correlated with age (Bailer et al., 2004; Meltzer et al., 1998). Additionally, previous studies suggest that the amygdala reactivity elicited by our task may decrease with age (Tessitore et al., 2005). To account for age-related variability in these 2 measures, age was included as a covariate in all analyses. Considering the broad age range of participants in this study (20-57 years), we report amygdala reactivity and BP_{ND} values standardized for age effects. These values are the standardized residuals of the respective measurements after accounting for effects of age. This procedure was adopted to more clearly illustrate the relationship between regional 5-HT_{2A} BP_{ND} and amygdala reactivity, independent of age. The statistics reported reflect comparisons between observed fMRI BOLD and BP_{ND} values including age as a covariate. As sex was not significantly correlated with any BOLD fMRI (all r's < 0.23 and p's > 0.18) or 5-HT_{2A} BP_{ND} measures (all r's < 0.12 and p's > 0.50), it was not included in any subsequent analyses exploring the relationship between prefrontal 5-HT_{2A} BP_{ND} and amygdala reactivity, habituation, or functional coupling.

4.3.11 Habituation analyses

To assess the magnitude of amygdala habituation and the degree to which this habituation was related to 5-HT_{2A} BP_{ND}, single-subject amygdala reactivity data were extracted and separated by block type (i.e., Fear block 1, Angry block 1, etc.). We used paired t-tests to examine differences in amygdala reactivity between first and second exposure to fearful (experimental task blocks 1 and 3) and angry (experimental task blocks 2 and 4) expressions. Linear regression analyses were used to examine the relationship between mPFC 5-HT_{2A} BP_{ND} and the magnitude

of amygdala habituation represented as the difference in average signal intensity between first and second presentation blocks for each expression type for each subject.

4.3.12 Functional connectivity analyses

Using functional connectivity, we examined the degree to which threat-related amygdala reactivity was associated with activity in mPFC. Furthermore, we sought to characterize how functional connectivity between these regions varied as a function of mPFC 5-HT_{2A} receptor density. Connectivity estimates reported reflect functionally relevant correlations between components of neural circuits; however, they do not establish the causal or time-lagged nature of regional neural coupling (Friston, 1994). MarsBaR (Brett et al., 2002) was used to extract the mean BOLD time series for each subject from all voxels within a 5-mm radius sphere centered on the voxel exhibiting the maximal main effect of task (right amygdala seed coordinates: 26, –3, –17; left amygdala seed coordinates: –24, –7, –15). Extracted time series were mean centered and drift corrected. Individual values exceeding 3 SDs of the mean of the drift-corrected time series were replaced by the average of the 2 adjacent values. These corrected time series were entered as regressors in individual GLM design matrices also including task condition as an additional regressor. Analyses yielded individual contrast images reflecting the areas wherein BOLD signal changes were temporally coupled with signal changes from each amygdala seed (i.e., left or right).

These individual contrast images were then included in second-level analyses including sgPFC or pgPFC 5-HT_{2A} BP_{ND} and age as covariates to identify regions of mPFC whose functional

coupling with each respective amygdala seed was significantly correlated with 5-HT_{2A} BP_{ND} across individuals. Using the WFU PickAtlas Toolbox, we restricted our connectivity analyses to Brodmann areas (BAs) 24, 25, and 32 that overlap with our PET ROIs. Analyses of positive or negative coupling with amygdala reactivity were thresholded at a voxel level of $p < 0.05$, FDR corrected for multiple comparisons and an extent of at least 10 contiguous voxels within the regions examined. Analyses of these mPFC regions whose coupling with amygdala reactivity was positively or negatively correlated with sgPFC or pgPFC 5-HT_{2A} BP_{ND} were thresholded at a voxel level of $p < 0.05$, uncorrected, and an extent of at least 10 contiguous voxels within mPFC regions examined.

4.4 RESULTS

4.4.1 Amygdala reactivity

Consistent with previous reports, the main effect of task comparison (i.e., task > control) was associated with robust, bilateral amygdala reactivity (right amygdala: [26, –3, –17], $z = 6.22$, 146 voxels, $p_{FDR} < 0.05$; left amygdala: [–24, –7, –15], $z = 5.03$, 130 voxels, $p_{FDR} < 0.05$). The main effect of task activation within the right but not left amygdala was inversely correlated with age (right amygdala: $r = –0.414$, $r^2 = 0.171$, $p = 0.013$; left amygdala: $r = –0.42$, $r^2 = 0.002$, $p = 0.809$). No clusters within our prefrontal ROIs (i.e., BAs 24, 25, and 32) exhibited a statistically significant main effect of task.

4.4.2 Prefrontal 5-HT_{2A} BP_{ND} analyses

Average [^{18}F]altanserin binding across all individuals revealed specific 5-HT_{2A} binding within the sgPFC (BP_{ND} = 1.24 ± 0.45, mean ± SD) and pgPFC (BP_{ND} = 1.10 ± 0.37). Across all subjects, 5-HT_{2A} BP_{ND} values were highly correlated between these 2 regions ($r = 0.761$, $r^2 = 0.579$, $p < 0.0005$). Consistent with previous reports, we found that age was inversely correlated with 5-HT_{2A} BP_{ND} in both prefrontal regions examined (sgPFC: $r = –0.569$, $r^2 = 0.324$, $p < 0.0005$; pgPFC: $r = –0.629$, $r^2 = 0.396$, $p < 0.0005$).

4.4.3 Prefrontal 5-HT_{2A} BP_{ND} and amygdala reactivity

As predicted, 5-HT_{2A} receptor binding within both the sgPFC and pgPFC was inversely correlated with amygdala reactivity across all individuals. 5-HT_{2A} BP_{ND} in the sgPFC predicted 25% of the variability in right amygdala reactivity ($r = –0.497$, $r^2 = 0.247$, $p = 0.002$), whereas pgPFC 5-HT_{2A} BP_{ND} predicted 37% of the variability in right amygdala reactivity ($r = –0.609$, $r^2 = 0.371$, $p < 0.0005$; **Figure 5**). We observed no activation clusters within the left amygdala exhibiting a statistically significant relationship with prefrontal 5-HT_{2A} BP_{ND}.

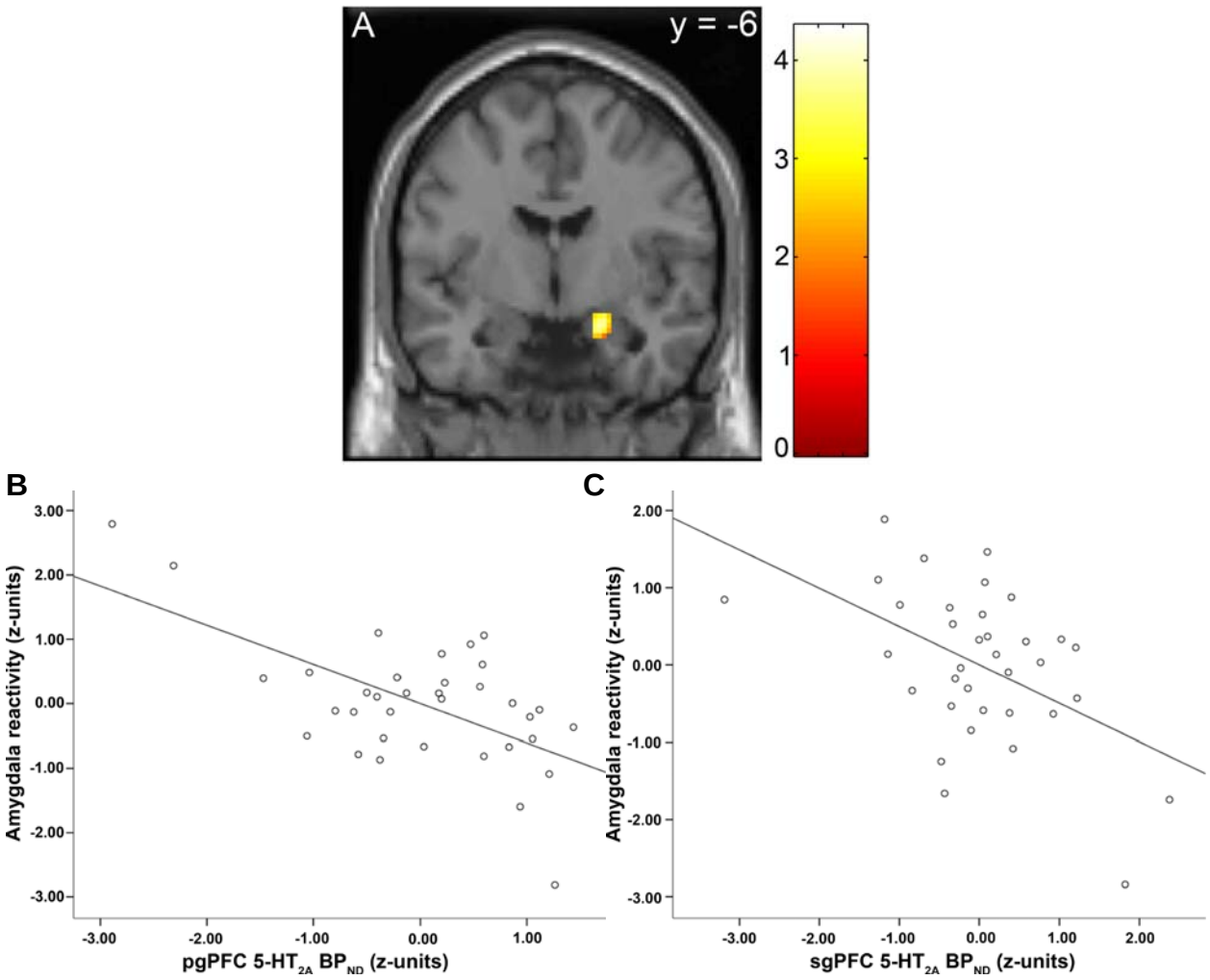

Figure 5. Prefrontal 5-HT_{2A} BP_{ND} is inversely associated with amygdala reactivity. (**A**) Statistical parametric map representing the right amygdala cluster inversely correlated with both sgPFC and pgPFC 5-HT_{2A} BP_{ND}. (**B** and **C**) Plot of inverse correlation between right amygdala reactivity and pgPFC 5-HT_{2A} BP_{ND} (r^2 = 0.37, $p < 0.0005$) and sgPFC 5-HT_{2A} BP_{ND} (r^2 = 0.25 $p = 0.002$). Color bar in **A** represents t-scores. (Fisher et al., 2009).

4.4.4 Habituation of amygdala response

Paired t-tests revealed significant habituation of right amygdala reactivity across blocks of both fearful and angry facial expressions (first vs. second fear blocks: $t_{24} = 3.10$, $p = 0.005$; first vs. second angry blocks: $t_{24} = 2.55$, $p = 0.018$; **Figure 6**). The magnitude of the habituation to fearful expressions (i.e., Fear block 1 > Fear block 2) was positively correlated with mPFC 5- HT_{2A} BP_{ND} (pgPFC: $r = 0.458$, $r^2 = 0.210$, $p = 0.006$; sgPFC: $r = 0.410$, $r^2 = 0.168$, $p = 0.015$; **Figure 7**). However, there was no statistically significant relationship between the magnitude of habituation to angry expressions and mPFC 5-HT_{2A} BP_{ND} (pgPFC: $r = 0.241$, $r^2 = 0.058$, $p = 0.164$; sgPFC: $r = 0.199$, $r^2 = 0.039$, $p = 0.252$). We did not observe significant habituation of left amygdala reactivity across the 2 block types. Though left amygdala reactivity decreased across fearful blocks (first vs. second fear blocks: $t_{24} = 2.77$, $p = 0.001$), we observed a significant increase in left amygdala reactivity across angry blocks (first vs. second angry blocks: $t_{24} = -4.87$, $p < 0.001$). Most importantly, neither pgPFC nor sgPFC 5-HT_{2A} BP_{ND} was significantly correlated with the change in left amygdala reactivity across blocks (all t's < 1.8, p's > 0.08).

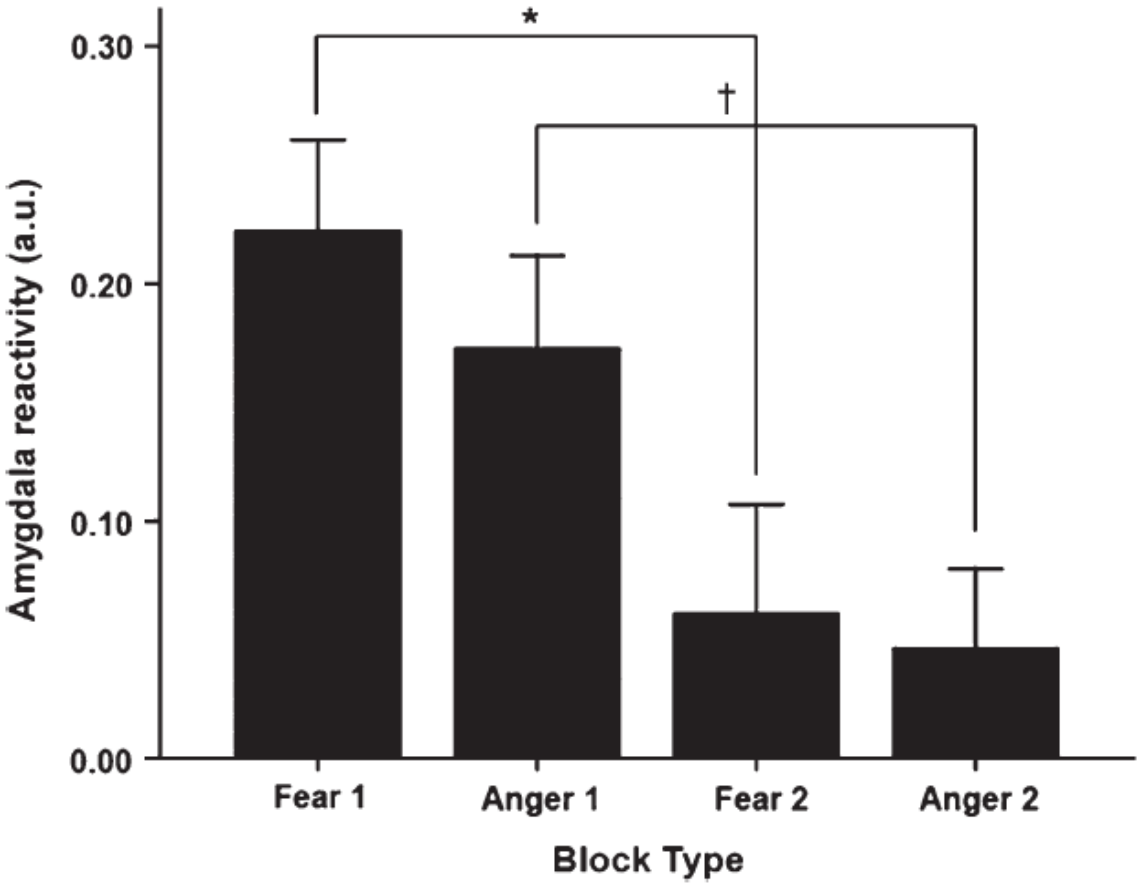

Figure 6. Habituation of amygdala response. Amygdala reactivity is significantly decreased between first and second exposures to both fearful and angry facial expression blocks. *p = 0.005, †p = 0.018, paired t-tests. (Fisher et al., 2009).

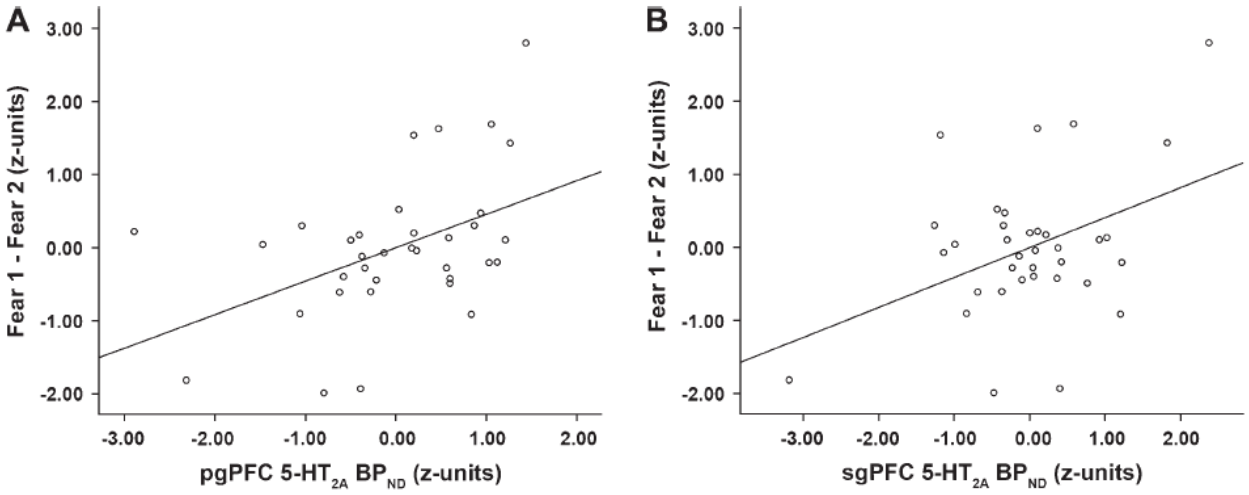

Figure 7. Prefrontal 5-HT_{2A} BP_{ND} is positively associated with the magnitude of amygdala habituation in response to fearful facial expression blocks. (**A**) pgPFC 5-HT_{2A} BP_{ND} is positively correlated with magnitude of amygdala habituation to fearful facial expressions (r^2 = 0.21, P = 0.006). (**B**) sgPFC 5-HT_{2A} BP_{ND} is positively correlated with magnitude of amygdala habituation to fearful facial expressions (r^2 = 0.17, P = 0.015). (Fisher et al., 2009).

4.4.5 Functional connectivity analyses

We observed significant positive correlations between right amygdala reactivity and multiple prefrontal ROIs (**Table 1**). No clusters within our prefrontal ROIs, however, exhibited statistically significant negative correlations with right amygdala reactivity. Both pgPFC and sgPFC 5-HT_{2A} BP_{ND} were positively correlated with the magnitude of functional connectivity between right amygdala reactivity and multiple prefrontal regions (**Table 1**). There were no significant negative correlations between 5-HT_{2A} BP_{ND} and amygdala--prefrontal functional connectivity within our prefrontal ROIs. We observed similar patterns of positive correlation between 5-HT_{2A} BP_{ND} and functional connectivity between the left amygdala and prefrontal ROIs (data not shown).

Table 1.*
Prefrontal regions functionally coupled with the amygdala and their relationship with prefrontal 5-HT_{2A} BP_{ND}

	x,	y,	z[a]	Voxels	z-score	*P* value
Positively Coupled						*FDR corrected*
BA 25	-6,	11,	-11	54	5.85	< .0005
BA 25	4,	9,	-12	57	5.41	< .0005
BA 24/32	-14,	17,	30	934	4.78	< .0005
BA 24/32	18,	45,	7	921	4.74	< .0005
Negatively Coupled						
No regions above threshold						
Positively Correlated with pgPFC 5-HT_{2A} BP_{ND}						*Uncorrected*
Amygdala-BA 32	10,	36,	22	88	3.47	< .0005
Amygdala-BA 24	-6,	26,	17	358	3.44	< .0005
Amygdala-BA 32	4,	6,	44	241	2.93	0.002
Amygdala-BA 24	6,	-14,	34	63	2.88	0.002
Amygdala-BA 32	-10,	41,	3	30	2.77	0.003
Amygdala-BA 24	-16,	-15,	41	24	2.45	0.007
Amygdala-BA 24	12,	-7,	45	13	2.04	0.021
Negatively Correlated with pgPFC 5-HT_{2A} BP_{ND}						
No regions above threshold						
Positively Correlated with sgPFC 5-HT_{2A} BP_{ND}						
Amygdala-BA 24	-4,	4,	33	54	3.09	0.001
Amygdala-BA 24	-6,	26,	15	14	2.93	0.002
Amygdala-BA 32	16,	17,	30	30	2.92	0.002
Amygdala-BA 32	12,	36,	24	41	2.69	0.004
Amygdala-BA 32	16,	12,	47	23	2.68	0.004
Amygdala-BA 24	4,	4,	33	56	2.47	0.007
Amygdala-BA 24	-10,	15,	27	80	2.39	0.008
Amygdala-BA 32	4,	23,	41	14	2.27	0.012
Amygdala-BA 24	6,	28,	15	20	2.21	0.013
Negatively Correlated with sgPFC 5-HT_{2A} BP_{ND}						
No regions above threshold						

[a]Talairach & Tournoux coordinates.

*(Fisher et al., 2009)

4.5 DISCUSSION

Previous neuroimaging studies have identified important functional relationships wherein prefrontal engagement associated with amygdala reactivity contributes to the shaping of complex emotional behaviors (Johnstone et al., 2007; Ochsner et al., 2002). Complimentary studies suggest that this relationship can be, at least in part, modulated by serotonergic function (Buckholtz et al., 2008; Forster et al., 2006; Meyer-Lindenberg et al., 2006; Pezawas et al., 2005; Weisstaub et al., 2006). The results of our current study suggest that mPFC 5-HT_{2A} receptors play an important role in mediating serotonergic modulation of prefrontal-amygdala circuitry. Specifically, we found that mPFC 5-HT_{2A} density in both sgPFC and pgPFC was inversely correlated with the magnitude of threat-related amygdala reactivity. The density of mPFC 5-HT_{2A} was also correlated with increased amygdala habituation to fear-related expressions, a phenomenon likely dependent on top-down prefrontal regulation (Phelps et al., 2004). Finally, mPFC 5-HT_{2A} density was positively correlated with the magnitude of amygdala-mPFC functional coupling, suggesting that 5-HT plays an important role in facilitating the integration of affective information between these brain regions. Importantly, all the observed relationships between mPFC 5-HT_{2A} and amygdala reactivity, temporal habituation, and functional coupling were independent of age and sex suggesting the general importance and potentially broad impact of mPFC 5-HT_{2A} on the regulation of corticolimbic brain function.

The predominant localization of excitatory 5-HT_{2A} receptors to glutamatergic projection neurons in the mPFC (Jakab and Goldman-Rakic, 1998) supports our observed inverse relationship between mPFC receptor density and amygdala reactivity. The mPFC projects

extensively to the amygdala, and these projections are thought to play a key role in regulating amygdala reactivity, specifically in response to emotionally salient environmental cues (Quirk et al., 2003). Given the ubiquitous expression of the 5-HT_{2A} receptor among populations of projection neurons in the mPFC and the extensive connectivity between the mPFC and the amygdala, it is likely that projection neurons targeting the amygdala are characterized by this 5-HT_{2A} distribution pattern, but this has not been specifically confirmed. Consistent with this potential effect, increased 5-HT in the mPFC is associated with decreased fear-related behavior in animals (Forster et al., 2006).

Consistent with previous studies (Breiter et al., 1996; Büchel et al., 1998; Schwartz et al., 2003b; Wright et al., 2003), there was significant habituation of right amygdala reactivity across repeated exposure to both fearful and angry facial expressions. Fear-conditioning studies have indicated that expression of learned extinction involves direct mPFC inhibition of the amygdala (Phelps et al., 2004; Quirk et al., 2003). The similarity in the decreased response of the amygdala during habituation with that documented during fear extinction suggests that capacity for engagement of prefrontal regulatory circuitry may be an important component in more general regulation of amygdala reactivity. Our finding that prefrontal 5-HT_{2A} BP_{ND} is positively correlated with the magnitude of right amygdala habituation to fearful facial expressions supports this possibility and further implicates the 5-HT_{2A} receptor as an important modulator of this circuitry. Although 5-HT_{2A} BP_{ND} did not significantly predict right amygdala habituation to angry expressions, the direction of the effect was consistent with that of fearful expressions. This difference may simply reflect a bias in our paradigm that always presented

fearful before angry expressions thus lessening the overall right amygdala response to angry expressions.

In contrast to the significant relationships observed for the right amygdala, there were no significant relationships between mPFC 5-HT_{2A} BP_{ND} and either left amygdala reactivity or temporal habituation. As we are unaware of any data suggesting hemispheric asymmetry in the expression of 5-HT_{2A} or of serotonergic innervation more broadly, we believe these differences likely reflect relative characteristics of the right and left amygdala precipitated by our fMRI challenge paradigm. The right lateralized relationship between 5-HT_{2A} and amygdala reactivity may reflect a bias in the perceptual processing of faces, such as those employed in our paradigm, to right hemisphere structures, including the amygdala (Farah et al., 1998; Fischer et al., 2003). The specificity of the relationship between 5-HT_{2A} and amygdala habituation may reflect the general tendency for greater temporal habituation in the right amygdala, which may be preferentially involved in stimulus novelty and detection, rather than the left amygdala, which may be preferentially involved in sustained stimulus evaluation (Britton et al., 2008; Wright et al., 2001). Thus, the laterality observed in our data likely reflects the relative functional engagement and dynamics of the amygdala and its subsequent modulation by mechanisms regulated by prefrontal 5-HT_{2A} and not any intrinsic properties of 5-HT_{2A} expression or action.

In addition to mapping the relationship between prefrontal 5-HT_{2A} BP_{ND} and overall amygdala reactivity as well as amygdala habituation, we used functional connectivity to examine the impact of 5-HT_{2A} receptors on the correlated responses of the amygdala and regions of the mPFC. In general, we observed a strong positive correlation between amygdala

reactivity and fMRI BOLD signal in multiple mPFC regions including BAs 24, 25, and 32. The correlations, however, were task independent as none of these mPFC regions exhibited a main effect of task and likely reflect a more general pattern of functional coupling between these regions that is not specific to processing of threat-related stimuli. The task-independent nature of these correlations may explain why, unlike the right lateralized effects observed between 5-HT_{2A} and amygdala reactivity as well as temporal habituation, a bilateral relationship emerged between 5-HT_{2A} and amygdala-prefrontal connectivity. Considering the extensive reciprocal anatomical connectivity between the amygdala and mPFC (Pandya et al., 1981), we believe our observed positive coupling reflects the primarily perceptual processing nature of our simple task and the excitatory drive of the amygdala on these downstream cortical target regions. This is in contrast to paradigms involving active cognitive tasks (e.g., affective labeling or emotion regulation) that explicitly engage prefrontal regulatory inhibitory networks resulting in negative correlations between amygdala and prefrontal activation (Hariri et al., 2000; Hariri et al., 2003; Ochsner et al., 2002). However, positive coupling between the amygdala and PFC, especially its medial regions, likely reflects effective integration of amygdala-mediated arousal by prefrontal circuits that subsequently effect complex, adaptive behavioral responses (Heinz et al., 2005; Pezawas et al., 2005). Thus, our results may reflect the importance of 5-HT signaling via mPFC 5-HT_{2A} receptors in facilitating amygdala drive on PFC.

Additional neural circuitry involving direct projections from mPFC to the dorsal raphe nucleus (DRN), which contains the cell bodies of serotonergic neurons, may contribute to the regulation of threat-related amygdala reactivity via an indirect pathway. Rodent studies have indicated that activation of mPFC neurons inhibit 5-HT neurons possibly via action on

GABAergic populations within the DRN, the neuronal subpopulation primarily targeted by projections from mPFC (Jankowski and Sesack, 2004; Varga et al., 2001). More specifically, the regulation of 5-HT release in the context of stressful and arousing stimuli via mPFC seems to be an important mechanism for effectively dealing with a stressor and is associated with the cessation of fear-related behavior (Amat et al., 2005; Forster et al., 2006). Given our previous neuroimaging findings that a greater capacity for regulation of 5-HT release at downstream targets via 5-HT_{1A} autoreceptor density predicts decreased amygdala reactivity, it may be that greater prefrontal 5-HT_{2A} density reflects, in part, a greater capacity for regulation of 5-HT release via a prefrontal-DRN inhibitory feedback mechanism subsequently resulting in a dampening of amygdala reactivity. Within the current study, however, we are unable to disentangle effects of prefrontal regulation of amygdala reactivity that are the result of direct or indirect feedback.

As our data suggest that individual differences in mPFC 5-HT_{2A} density are correlated with behaviorally relevant amygdala function, identifying factors that contribute to the emergence of such inter-individual variability may inform ongoing efforts to establish biological markers of disease liability. The emerging field of imaging genetics has begun to demonstrate that common functional genetic polymorphisms have the potential to influence variability in behaviorally relevant brain function by affecting the expression and function of specific molecular signaling cascades (Hariri, 2009; Hariri and Holmes, 2006). A recent PET study in monozygotic twins suggests that approximately 40-50% of inter-individual variability in cortical 5-HT_{2A} density is genetically driven (Pinborg et al., 2008). Against this general background of likely heritable variation in 5-HT_{2A} density, *in vitro* studies have identified specific functional

polymorphisms in the human 5-HT_{2A} gene (*HTR2A*) that affect transcriptional regulation and expression (Parsons et al., 2004; Veenstra-VanderWeele et al., 2002). Such genetically driven variability in *HTR2A* gene expression could explain some of the inter-individual variability in mPFC 5-HT_{2A} density observed in our current study. Future studies with larger sample sizes that can more effectively control for non-genotype effects (e.g., age, sex, ethnicity, multiple functional polymorphisms) are necessary to explore potential specific genetic contributions to variability in 5-HT_{2A} density and related brain function.

Extrapolation of our current findings to the understanding of serotonergic regulation of corticolimbic circuit function and related emotional behaviors should be done with caution and attention to several study limitations. First, immunolabeling studies have indicated that the 5-HT_{2A} receptor is localized within the amygdala (McDonald and Mascagni, 2007). We were interested in exploring the degree to which threat-related amygdala reactivity is associated with 5-HT_{2A} BP_{ND} within the amygdala itself, but consistent BP_{ND} values in the amygdala near or below zero (data not shown) did not allow for receptor binding measurements in this region. Future studies employing radiotracers with greater signal-to-noise ratios in the amygdala are needed to evaluate the relationship between amygdala 5-HT_{2A} density and reactivity. Second, our paradigm is best suited to the examination of amygdala reactivity and inter-connected cortical activation associated with behavioral and physiological arousal to salient stimuli. Although we believe that our results strongly support mPFC 5-HT_{2A} receptors as an important component in a putative corticolimbic regulatory network, it may be beneficial to examine relationships between prefrontal 5-HT_{2A} BP_{ND} in the context of a task specifically designed to engage top-down emotional regulatory circuitry. Third, our data only indirectly suggest that

mPFC 5-HT_{2A} density regulates the impact of 5-HT on prefrontal circuitry related to amygdala reactivity. The addition of a pharmacological challenge of the 5-HT system (e.g., acute administration of a selective 5-HT reuptake inhibitor; (Bigos et al., 2008) to our multimodal neuroimaging design would allow for the direct determination of how individual differences in mPFC 5-HT_{2A} density affect the ability of 5-HT to drive this circuitry. On a related note, our findings are correlational in nature and do not imply causality. Though we believe that the underlying biology of this circuitry supports our interpretation, both our metric of amygdala reactivity (BOLD fMRI) and receptor binding (PET BP_{ND}) are indirect, and thus, our findings should be interpreted with caution. Fourth, our findings are within a healthy adult population with no history of psychiatric illness. Although altered prefrontal 5-HT_{2A} density has been reported in patients with mood and anxiety disorders (Yatham et al., 2000), the patterns reported herein may not be related to the pathophysiology of these and related disorders, and comparable multimodal studies in patient populations are needed. Finally, it is highly unlikely that the 5-HT_{2A} receptor represents the exclusive mechanism by which 5-HT signaling in the PFC can modulate this circuitry. Multiple pre- and postsynaptic 5-HT receptors, both excitatory and inhibitory, are likely important in orchestrating signaling patterns that modulate prefrontal circuitry related to regulating arousal (Sharp et al., 2007). Future studies employing multiple PET radiotracers targeting different 5-HT receptor subtypes are needed to better understand these complex signaling pathways.

5.0 STUDY 3: BALANCE OF SEROTONIN 1A AND 2A RECEPTORS IN MEDIAL PREFRONTAL CORTEX PREDICTS THREAT-RELATED AMYGDALA REACTIVITY[3]

5.1 ABSTRACT

Background: The amygdala and medial prefrontal cortex (mPFC) comprise a key corticolimbic circuit that helps shape individual differences in threat sensitivity and related risk for psychopathology. Though serotonin (5-HT) is a key modulator of this circuit, the specific receptors mediating its effects are unclear. The co-localization of 5-HT_{1A} and 5-HT_{2A} receptors on mPFC glutamatergic neurons suggests that their functional interactions may mediate 5-HT effects on this circuit through top-down regulation of amygdala reactivity.

Methods: Using a multi-modal neuroimaging strategy in 39 healthy volunteers we determined whether threat-related amygdala reactivity, assessed with blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI), was significantly predicted by the interaction between mPFC 5-HT_{1A} and 5-HT_{2A} receptor levels, assessed with positron emission tomography (PET).

[3]Chapter 5 is a slightly modified version of an article currently under review at *Biological Psychiatry* as an Archival Report. Full author-listing: Fisher, P.M., Price, J.C., Meltzer, C.C., Moses-Kolko, E.L., Becker, C., Berga, S.L., Hariri, A.R.

Results: 5-HT_{1A} significantly moderated the effects of 5-HT_{2A} on amygdala reactivity wherein mPFC 5-HT_{2A} levels were significantly inversely correlated with amygdala reactivity, but only when mPFC 5-HT_{1A} levels were relatively low.

Conclusions: This interaction effect suggests that mPFC 5-HT_{1A} may play a role in 'gating' the effects of mPFC 5-HT_{2A} on threat-related amygdala reactivity, and that these specific receptors may be critical for mediating serotonergic modulation of this circuitry.

5.2 INTRODUCTION

A growing corpus of research in humans and non-human animal models clearly implicates a corticolimbic circuitry comprised of structural and functional inter-connections between the amygdala and regions of the medial prefrontal cortex (mPFC) including the anterior cingulate cortex (ACC) in generating and regulating both behavioral and physiologic responses to threatening or fearful stimuli (Hariri et al., 2006; Pezawas et al., 2005; Phelps et al., 2004; Quirk et al., 2003). Specifically, regions of the mPFC are critically involved in the integration and subsequent regulation of stimulus-driven amygdala response via glutamatergic projections to populations of GABAergic neurons within the amygdala known as intercalated cell masses (Likhtik et al., 2005; Quirk et al., 2006; Quirk et al., 2003; Sesack et al., 1989). Variability in the structure and function of this corticolimbic circuitry has been associated with inter-individual differences in personality measures reflecting sensitivity to environmental threat and related-risk for affective disorders (Almeida et al., 2009; Buckholtz et al., 2008; Etkin et al., 2004; Fakra

et al., 2009; Pezawas et al., 2005; Shin et al., 2005). Developing an understanding of discrete mechanisms through which such behaviorally relevant inter-individual variability in corticolimbic circuit function emerges is important for mapping the basic architecture of brain-behavior links as well as identifying novel targets for pharmacological intervention in psychopathological states involving abnormal threat and stress responsiveness.

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter that exerts potent modulatory effects on mood and affect as well as responsiveness to stress and threat (Holmes, 2008; Lucki, 1998). Neuroimaging studies in humans have mapped inter-individual differences in amygdala reactivity to biologically salient environmental stimuli (e.g., facial expressions of threat) onto variability in 5-HT signaling within corticolimbic circuitry (Bigos et al., 2008; Fisher et al., 2009; Fisher et al., 2006; Hariri et al., 2002b; Harmer et al., 2006; Heinz et al., 2005; Heinz et al., 2000; Munafo et al., 2008; Pezawas et al., 2005; Rhodes et al., 2007). Although studies have begun to examine the role of specific 5-HT receptor signaling pathways in mediating the modulatory effects of 5-HT on this circuitry (Holmes, 2008; Sharp et al., 2007), these specific pathways and their likely molecular interactions are not fully understood. Previous work in humans using positron emission tomography (PET) has implicated the 5-HT type 1A ($5\text{-}HT_{1A}$) and 5-HT type 2A ($5\text{-}HT_{2A}$) receptors in the modulation of mood, affect, stress and threat responsiveness as well as the corticolimbic circuitry supporting these behaviors (Bhagwagar et al., 2006; Frokjaer et al., 2008; Parsey et al., 2006b; Stockmeier, 2003; Szewczyk et al., 2009; Tauscher et al., 2001). Importantly, the anatomical localization of these two receptors within the prefrontal cortex positions them to effectively mediate the effects of 5-HT signaling on corticolimbic circuit dynamics.

The excitatory 5-HT_{2A} is a hetero-receptor through which 5-HT can drive prefrontal function via depolarization of pyramidal neurons. The predominant localization of the 5-HT_{2A} within mPFC appears to be on proximal portions of the apical dendrites of glutamatergic neurons (Amargos-Bosch et al., 2004; de Almeida and Mengod, 2007; Jakab and Goldman-Rakic, 1998; Wedzony et al., 2008). Though the 5-HT_{2A} receptor is expressed on other neuronal subtypes within mPFC (Amargos-Bosch et al., 2004; Miner et al., 2003), it is expressed on nearly all pyramidal neurons within layers of prefrontal cortex that provide input to the amygdala (de Almeida and Mengod, 2007; Ghashghaei et al., 2007). In a recent multi-modal neuroimaging study incorporating blood-oxygen level dependent functional magnetic resonance imaging (BOLD fMRI) and PET in a population of healthy adults, we identified a significant inverse association between mPFC 5-HT_{2A} receptor binding and threat-related amygdala reactivity (Fisher et al., 2009). Specifically, we found that approximately 25% of the inter-individual variability in amygdala reactivity was predicted by mPFC 5-HT_{2A} binding potential, an index of receptor availability. This suggests that mPFC 5-HT signaling via the 5-HT_{2A} may play an important role in modulating the response of the amygdala to threat via facilitation of prefrontal mediated regulation. Additionally, we found that mPFC 5-HT_{2A} was significantly predictive of amygdala habituation suggesting 5-HT signaling via 5-HT_{2A} receptors may also play an important role in prefrontal-mediated fear extinction circuitry.

The 5-HT_{1A} receptor is also highly expressed within mPFC and may play a role in mediating the effects of 5-HT signaling on this corticolimbic circuit. Like the 5-HT_{2A}, the inhibitory 5-HT_{1A} receptor is expressed on prefrontal pyramidal neurons (Amargos-Bosch et al., 2004; Azmitia et al., 1996; Cruz et al., 2004; de Almeida and Mengod, 2008; Wedzony et al.,

2008). Thus, 5-HT_{1A} signaling is positioned to inhibit prefrontal function via hyperpolarization of pyramidal neurons. Similar to the 5-HT_{2A} receptor, however, evidence suggests the 5-HT_{1A} receptor may be expressed on other neuronal subtypes within cortex including small populations of GABAergic neurons (Azmitia et al., 1996; de Almeida and Mengod, 2008). Interestingly, 5-HT_{1A} receptors within mPFC are localized to initial portions of the axon hillock of pyramidal neurons, the site from which an action potential is generated (Cruz et al., 2004; Czyrak et al., 2003; DeFelipe et al., 2001). This suggests the inhibitory effects of the 5-HT_{1A} may play an integral role in 'gating' the excitability of these neurons and modulating corticolimbic circuit function (Amargos-Bosch et al., 2004; Azmitia et al., 1996; Cruz et al., 2004; de Almeida and Mengod, 2008). Previously, our laboratory reported a significant association wherein threat-related amygdala reactivity was significantly inversely correlated with 5-HT_{1A} autoreceptor binding within the dorsal raphe (Fisher et al., 2006). However, the effects of mPFC 5-HT_{1A} on amygdala reactivity has yet to be explored.

Importantly, 5-HT_{1A} and 5-HT_{2A} receptors are co-localized on approximately 80% of glutamatergic neurons within prefrontal cortex (Amargos-Bosch et al., 2004). Consistent with this co-localization, studies have indicated that local 5-HT_{2A} antagonism within prefrontal cortex blocks 5-HT induced excitation of pyramidal neurons (Amargos-Bosch et al., 2004; Puig et al., 2003). Conversely, pyramidal neuron inhibition is diminished upon local 5-HT_{1A} antagonism (Amargos-Bosch et al., 2004; Puig et al., 2005). Taken together, these findings implicate opposing effects of mPFC 5-HT_{1A} and 5-HT_{2A} on corticolimbic circuit function via their effects on prefrontal neuron excitability. Furthermore, co-expression of 5-HT_{1A} and 5-HT_{2A} suggests molecular interactions between these receptors can moderate the net effect of 5-HT signaling

on pyramidal neuron excitability with subsequent effects on prefrontal-mediated regulation of amygdala reactivity. Building upon previous work from our laboratory, we sought to determine the degree to which prefrontal 5-HT_{1A} moderates the association between prefrontal 5-HT_{2A} and threat-related amygdala reactivity implicating 5-HT_{1A} as effectively gating prefrontal regulatory circuits that are otherwise facilitated by 5-HT_{2A} signaling.

We employed a multimodal neuroimaging strategy in 39 healthy adult volunteers. BOLD fMRI was used to measure threat-related amygdala reactivity in response to an archival challenge paradigm involving the perceptual processing of fearful and angry facial expressions (Fisher et al., 2009; Fisher et al., 2006). 5-HT_{1A} and 5-HT_{2A} receptor binding was assessed with [^{11}C]WAY100635 and [^{18}F]altanserin PET, respectively, which each exhibit high affinity and specificity for their receptor targets. Regional 5-HT_{1A} and 5-HT_{2A} receptor binding was determined in two subregions of the mPFC that are anatomically interconnected with the amygdala and innervated by 5-HT neurons, namely the pregenual prefrontal cortex (pgPFC) and subgenual prefrontal cortex (sgPFC; (Barbas, 1995; Blue et al., 1988; Kim and Whalen, 2009; McDonald, 1998; Pandya et al., 1981).

Building upon previous findings from our laboratory (Fisher et al., 2009), we sought to determine the degree to which mPFC 5-HT_{1A} and 5-HT_{2A} binding was associated with threat-related amygdala reactivity. Considering the localization of the 5-HT_{1A} receptor to initial portions of the axon hillock, we hypothesized that mPFC 5-HT_{1A} binding would be positively associated with threat-related amygdala reactivity reflecting the inhibitory effects of the 5-HT_{1A} on prefrontal pyramidal neurons. In contrast and consistent with our prior report (Fisher et al., 2009), we hypothesized that mPFC 5-HT_{2A} binding would be negatively associated with threat-

related amygdala reactivity reflecting the excitatory effects of the 5-HT_{2A} on prefrontal pyramidal neurons. Finally, given the relative and specific co-localization of the 5-HT_{1A} and 5-HT_{2A} on pyramidal neurons, and the potential molecular interactions such co-localization may mediate, we hypothesized that there would be a significant interaction between mPFC 5-HT_{1A} and 5-HT_{2A} binding such that relatively increased 5-HT_{1A} binding would effectively gate the excitatory effects of the 5-HT_{2A} as reflected by increased amygdala reactivity.

5.3 METHODS

5.3.1 Participants

Thirty-nine healthy adult volunteers participated after providing written informed consent in accordance with the University of Pittsburgh Institutional Review Board (20 males, age: 39.1 ± 12.7 years [mean ± s.d.]). Subjects were recruited through local advertisements, referrals, and ongoing studies. Subjects were generally healthy with exclusion criteria included 1) current or lifetime mood, anxiety and psychotic disorder as assessed by Structured Clinical Interview DSM-IV (First et al., 1996), 2) family psychiatric history, 3) medical or neurological illness likely to affect cerebral physiology or anatomy, 4) cardiovascular disease or diabetes, 5) history of substance abuse or use of antidepressants, 6) early dementia or mild cognitive impairment according to the Mini-Mental State Examination (scores exceeding 27; (Folstein et al., 1975), 7) reversed sleep-wake cycle. The association between mPFC 5-HT_{2A} binding and amygdala

reactivity has been described previously involving a subset of this cohort (35 individuals; (Fisher et al., 2009). Additionally, a subset of this cohort (20 individuals) was included in a previous study examining the association between 5-HT_{1A} binding within the dorsal raphe nucleus and threat-related amygdala reactivity (Fisher et al., 2006).

5.3.2 fMRI protocol

The experimental fMRI paradigm consisted of 4 blocks of a face-processing task interleaved with 5 blocks of a sensorimotor control task as described previously (Fisher et al., 2009; Fisher et al., 2006). Subject performance (accuracy and reaction time) was monitored during all scans. During the face-processing task, subjects viewed a trio of faces (expressing either anger or fear) and selected 1 of 2 faces (bottom) identical to a target face (top). Angry and fearful facial expressions can represent honest indicators of ecologically valid threat, especially that related to conspecific challengers (Darwin and Ekman, 1998). Based on this, we interpret amygdala activation elicited by our task as being threat related.

Each face-processing block consisted of 6 images (i.e., trio of faces), balanced for sex and representing one target affect (angry or fearful) all derived from a standard set of pictures of facial affect (Ekman and Friesen, 1976). During the sensorimotor control blocks, subjects viewed a trio of simple geometric shapes (circles and vertical and horizontal ellipses) and selected 1 of 2 shapes (bottom) identical to a target shape (top). Each sensorimotor control block consisted of 6 different shapes trios. All blocks were preceded by a brief instruction (‘‘Match Faces’’ or ‘‘Match Shapes’’) lasting 2 s. In the face-processing blocks, each of the 6

face trios was presented for 4 s with a variable inter-stimulus interval of 2-6 s (mean ISI=4 s) for a total block length of 48 s. In the sensorimotor control blocks, each of the 6 shape trios was presented for 4 s with a fixed inter-stimulus of 2 s for a total block length of 36 s. Total protocol time was 390 s (195 whole-brain volumes).

As we were not interested in neural networks associated with face-specific processing per se, but rather in eliciting a maximal amygdala response across all subjects, we chose not to use neutral faces as control stimuli because neutral faces can be subjectively experienced as affectively laden or ambiguous and thus engage the amygdala (Schwartz et al., 2003a; Wright et al., 2003).

5.3.3 fMRI acquisition parameters

Acquisition parameters have been described previously (Fisher et al., 2009; Fisher et al., 2006; Schwartz et al., 2003b). Briefly, each subject was scanned using a GE Signa 1.5-T head only scanner (GE Medical Systems, Milwaukee, WI). BOLD functional images were acquired using a reverse spiral sequence covering 28 slices (3.8-mm thick) encompassing the entire cerebrum and the majority of the cerebellum (repetition time=2000 ms, echo time=35 ms, field-of-view [FOV]=24 cm, matrix=64x64). The first 2 functional volumes acquired were discarded to allow the scanner to reach equilibrium. All scanning parameters were selected to optimize BOLD signal while maintaining enough slices to acquire whole-brain data. Prior to the acquisition of fMRI data for each subject, we acquired and visually inspected localizer scans for artifacts (e.g., ghosting) as well as good signal across the entire volume of acquisition, including the medial

temporal lobes. Additionally, an auto-shimming procedure was conducted before the acquisition of BOLD data in each subject to minimize field inhomogeneities. fMRI data for all 39 subjects included in this study were cleared of such problems.

5.3.4 fMRI data analysis

Whole-brain image analysis was completed using the general linear model (GLM) of SPM8 (http://www.fil.ion.ucl.ac.uk/spm). Images for each subject were realigned to the first volume in the time series to correct for head motion, spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12- parameter affine model (resolution of functional images = 2 x 2 x 2 mm), and smoothed to minimize noise and residual difference in gyral anatomy with a Gaussian filter, set at 6-mm full-width at half-maximum. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. These preprocessed data sets were analyzed using second-level random-effects models that account for both scan-to-scan and participant-to-participant variability to determine task-specific regional responses.

Variability in single-subject whole-brain functional volumes was determined using the software program ART (Artifact Recognition Toolbox, http://www.nitrc.org/projects/artifact_detect). Individual whole-brain BOLD fMRI volumes meeting at least one of two criteria were excluded from determination of task-specific effects: 1) significant mean-volume signal intensity variation (i.e., within volume mean signal greater or less than 4 standard deviations of mean signal of all volumes in time series), and 2) individual

volumes where scan-to-scan movement exceeded 2 mm translation or 2° rotation in any direction. On average, 2.1 volumes per subject were excluded due to significant mean-volume signal intensity variation (range of volumes excluded per subject=0-17). Across all subjects, zero volumes were excluded due to excessive motion. Only 1% of all volumes were excluded, thus we believe this approach enhanced our capacity to determine task-specific effects by excluding volumes with substantial variability without compromising our power to detect task-specific effects by excluding a large number of volumes. We believe this method effectively balances the use of available functional neuroimaging data with a reasonable approach towards accounting for effects due to artifact or movement.

Following preprocessing, our GLM, employing canonical hemodynamic response functions, was used to estimate condition-specific and task-specific BOLD activation for each individual (beta-weights and contrast images, respectively). Individual contrast images (i.e., weighted sum of the beta images) were used in second-level random-effects models to determine mean task-specific amygdala reactivity using 1-sample t-tests. Group-level effects for our contrast of interest (i.e., faces>shapes) were assessed within the amygdala using an ROI constructed from the WFU Pickatlas (v1.04; (Lancaster et al., 2000; Maldjian et al., 2003).

To address the issue of multiple voxel-level comparisons, AlphaSim, a software program within AFNI (http://afni.nimh.nih/gov/afni) that utilizes a Monte Carlo simulation method, was used to determine a voxel-wise statistical threshold of $p<0.05$, uncorrected, combined with a cluster extent threshold of $k > 56$ voxels within our amygdala search volume that is sufficiently unlikely ($\alpha<.05$) to have occurred by chance (Forman et al., 1995). Single subject amygdala reactivity values for our contrast of interest were extracted from SPM8 using Marsbar (v0.42;

(Brett et al., 2002). A 5 mm radius sphere was centered on the voxel exhibiting the maximal response to our task across all subjects within both the right and left amygdala. Due to image pre-processing constraints we could not extract mean BOLD response values from mPFC regions defined by our PET ROIs. Instead, mean BOLD response was extracted from spheres (6mm radius) centered on regions that predominantly overlap our two mPFC ROIs (Marsbar, pgPFC ROI center: (0, 38, 9), sgPFC ROI center: (0, 34, -6), MNI coordinates). Regional 5-HT receptor binding and other variables were regressed against these extracted BOLD values. All neuroimaging data are reported using the coordinate system of Talairach and Tournoux.

5.3.5 General PET methods

Technical detail concerning the MR and PET imaging procedures related to both [^{11}C]WAY100635 and [^{18}F]altanserin can be found in previously published work and is described below (Bailer et al., 2007; Cidis Meltzer et al., 2001; Fisher et al., 2009; Fisher et al., 2006; Soloff et al., 2010). See previous reports for discussion regarding limitations, challenges and methodological attempts to minimize potential artifacts and biases related to these radioligands (Meltzer et al., 2004; Parsey et al., 2006b; Parsey et al., 2000b; Price et al., 2001a; Price et al., 2001b; Soloff et al., 2010).

Structural MR images (GE Signa 1.5-T scanner) were acquired for each subject using a spoiled-gradient (SPGR) recalled sequence (TR=25 ms, TE=5 ms, FOV=24 cm, slice thickness=1.5 mm, matrix=256×192) with parameters optimized for contrast between gray matter, white matter, and cerebrospinal fluid.

Catheters were placed in an antecubital vein for radioligand injection and a radial artery for arterial blood sampling. PET scans were acquired using an ECAT HR+ PET scanner (CTI PET systems, Knoxville, TN) in 3D imaging mode (63 transaxial planes, 2.4-mm thickness, 15.2-cm FOV). Head movement was minimized by use of a thermoplastic mask immobilization system. A 10-min transmission scan (rotating ^{68}Ge/^{68}Ga rods) was acquired for attenuation correction of emission data. PET data were further corrected for dead time and scatter.

Each radioligand was administered as a slow bolus over 20 seconds. PET data acquisition and arterial blood sampling was initiated at the start of radioligand injection. The total radioactivity concentration in plasma was determined from approximately 35 0.5-ml hand-drawn blood samples collected over the scanning interval. Larger additional blood samples were acquired at 5-6 times over the study duration for the determination of the fraction of the total radioactivity that resulted from radiolabeled metabolites of the parent radioligand. The total plasma radioactivity concentration was corrected for radiolabeled metabolites and this ‘‘metabolite-corrected’’ arterial input function was used for data analysis (Meltzer et al., 2004; Price et al., 2001b).

Image reconstruction was performed using filtered back projection for a final image resolution of about 6 mm. Regions of interest (ROIs) were drawn on resliced MR images for each subject and applied to their respective, coregistered PET images (ROIs drawn by S.K.Z. and C.B.). ROIs were identified for the sgPFC, pgPFC, amygdala and cerebellum. The cerebellum was used as the reference region for nondisplaceable radiotracer uptake (i.e., free and nonspecific concentrations, V_{ND}) for both [^{11}C]WAY 100635 and [^{18}F]altanserin.

PET data for both radioligands were analyzed using the Logan graphical method (Logan et al., 1990) to obtain regional volume of distribution values (V_T). Regional V_T values were used to determine the nondisplaceable binding potential, BP_{ND}, a measure of specific binding. The BP_{ND} is directly proportional to B_{avail}/K_d, where B_{avail} is the concentration of receptors available for radiotracer binding (i.e., not occupied by endogenous 5-HT) and K_d is the equilibrium dissociation rate constant (i.e., inversely related to binding affinity). The PET binding measures were corrected for partial volume effects that arise from atrophy-related CSF dilution using a previously validated 2-component MR-based atrophy correction algorithm (Cidis Meltzer et al., 2001; Meltzer et al., 1999; Meltzer et al., 1990).

5.3.6 [^{18}F]Altanserin specific methods

The radiosynthesis of [^{18}F]altanserin was performed using a modification of the original method (Lemaire et al., 1991) that has been applied in several studies in our laboratory (Bailer et al., 2004; Fisher et al., 2009; Meltzer et al., 1998; Smith et al., 1998; Soloff et al., 2010). [^{18}F]Altanserin was administered via intravenous injection (7.23±0.31 mCi, mean ± SD) and PET scanning was performed over 90-min. The Logan analysis regression was performed over the 12- to 90-min post-injection integration intervals (10 points) to obtain regional [^{18}F]altanserin V_T and BP_{ND} values.

5.3.7 [^{11}C]WAY100635 specific methods

The radiosynthesis of [^{11}C]WAY 100635 was performed as previously described (McCarron et al., 1996) and has been applied in several studies in our laboratory (Bailer et al., 2007; Fisher et al., 2006; Meltzer et al., 2004). [^{11}C]WAY100635 was administered via intravenous injection (14.01±2.10 mCi) and PET scanning was performed over 90 min. The Logan analysis regressions was performed over the 14 to 90 min PET scan integration interval (13 points) to obtain regional [^{11}C]WAY100635 V_T and BP_{ND} values.

5.3.8 Regression analyses

The relationship between threat-related amygdala reactivity and mPFC 5-HT receptor binding was determined using linear regression analyses between extracted single-subject amygdala BOLD values and ROI-specific 5-HT_{1A} or 5-HT_{2A} BP_{ND} values in SPSS (v17.0). Single subjects with BP_{ND} values < 0.0 within an ROI were excluded because BP_{ND} < 0.0 indicates signal intensity equivalent to free or nonspecific binding. We have previously reported within a subset of this cohort that both amygdala reactivity and 5-HT_{2A} BP_{ND} are inversely correlated with age (Fisher et al., 2009). This is consistent with previous studies (Bailer et al., 2004; Meltzer et al., 1998; Tessitore et al., 2005). To account for age-related variability in these 2 measures, age was included as a covariate in all analyses. Considering this, we report amygdala reactivity values standardized for age effects. These values are the standardized residuals of amygdala reactivity after accounting for effects of age. This procedure was adopted to more clearly illustrate the

relationship between regional 5-HT receptor BP_{ND} and amygdala reactivity, independent of age. The statistics reported reflect regression analysis between observed fMRI BOLD and BP_{ND} values including age as a covariate. As sex was not significantly correlated with our neuroimaging data, it was not included in any analyses determining the relationship between prefrontal 5-HT_{1A} BP_{ND} or 5-HT_{2A} BP_{ND} and amygdala reactivity. The degree to which an interaction between mPFC 5-HT_{1A} and 5-HT_{2A} BP_{ND} was predictive of amygdala reactivity was determined using a linear regression model including 5-HT_{1A} BP_{ND}, 5-HT_{2A} BP_{ND}, age and the interaction term. The significance of simple slopes was determined using both SPSS (v17.0) and a previously validated approach (Preacher et al., 2006).

5.4 RESULTS

5.4.1 Amygdala reactivity

Consistent with previous reports we observed robust mean threat-related reactivity in the bilateral amygdala across all participants (**Figure 8**; (Brown et al., 2005; Hariri et al., 2005; Hariri et al., 2002c). BOLD signal values from a 5mm radius sphere centered on the voxel maximally responsive to the main effects of task (i.e., faces>shapes) in the right and left amygdala were extracted for each single-subject. These task-related functional activation values were entered as dependent measures into regression analyses to test for effects of mPFC 5-HT_{1A} and 5-HT_{2A} binding potential (BP_{ND}) as well as other metrics indicated below. The magnitude of mean right

amygdala reactivity, but not left amygdala reactivity, was inversely associated with age (right amygdala: r^2=0.19, p=0.005; left amygdala: r^2=0.02, p=0.35). Neither right nor left amygdala reactivity were associated with gender ($r^{2,}$s<0.03, p's>0.3).

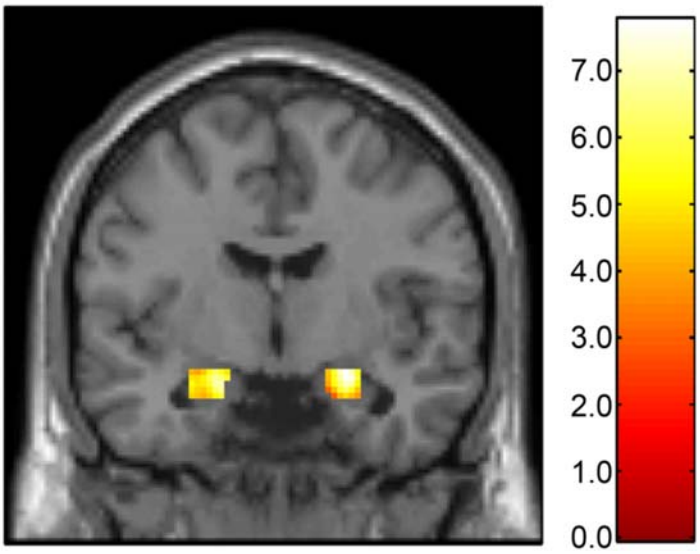

Figure 8. Amygdala reactivity to perceptual processing of fearful and angry facial expressions. Statistical parametric map representing bilateral amygdala clusters exhibiting a significant response to task (i.e., faces > shapes; right amygdala: (24, -6, -11), z = 6.28, k = 145 voxels ($p < 0.05$, corrected); left amygdala: (-18, -7, -15), z = 5.77, k = 146 voxels ($p < 0.05$, corrected). Color bar represents t-scores.

5.4.2 Prefrontal 5-HT receptor binding

Consistent with previous reports, average [^{11}C]WAY100635 binding across all individuals revealed specific 5-HT_{1A} binding within both pgPFC (BP_{ND}=4.32 $\pm$ 1.18, mean $\pm$ s.d.) and sgPFC (BP_{ND}=4.86 $\pm$ 1.41). Likewise, [^{18}F]altanserin revealed specific 5-HT_{2A} binding within both pgPFC (BP_{ND}=1.06 $\pm$ 0.37) and sgPFC (BP_{ND}=1.19 $\pm$ 0.46). 5-HT_{1A} BP_{ND} and 5-HT_{2A} BP_{ND} values were

highly correlated between the two regions examined, namely pgPFC and sgPFC (5-HT_{1A} BP_{ND}: r^2=0.69, p=5.87 x 10^{-11}; 5-HT_{2A} BP_{ND}: r^2=0.63, p=1.80 x 10^{-9}). Within regions, however, 5-HT_{1A} BP_{ND} values were not significantly correlated with 5-HT_{2A} BP_{ND} values (pgPFC: r^2=3.28 x 10^{-5}, p=0.97; sgPFC: r^2=0.001, p=0.88). 5-HT_{2A} BP_{ND} was significantly inversely correlated with age (pgPFC: r^2=0.41, p=1.30 x 10^{-5}; sgPFC: r^2=0.41, p=1.29 x 10^{-5}). 5-HT_{1A} BP_{ND}, however, was not significantly correlated with age (pgPFC: r^2=0.01, p=0.53, sgPFC: r^2=0.003, p=0.73). Neither 5-HT_{1A} nor 5-HT_{2A} BP_{ND} values were significantly correlated with gender (r^2's<0.01, p's>0.5).

5.4.3 Prefrontal 5-HT_{1A} receptor binding and amygdala reactivity

Within both pgPFC and sgPFC, regional 5-HT_{1A} BP_{ND} was not significantly predictive of amygdala reactivity (right amygdala vs. pgPFC 5-HT_{1A} BP_{ND}: t_{36}=-0.76, p=0.94, **Figure 9B**; right amygdala vs. sgPFC 5-HT_{1A} BP_{ND}: t_{36}=0.54, p=0.60; left amygdala vs. pgPFC 5-HT_{1A} BP_{ND}: t_{36}=0.16, p=0.88, **Figure 9A**; left amygdala vs. sgPFC 5-HT_{1A} BP_{ND}: t_{36}=-0.09, p=0.93).

5.4.4 Prefrontal 5-HT_{2A} receptor binding and amygdala reactivity

Consistent with a similar analysis carried out in a sub-sample of this cohort (Fisher et al., 2009), regional 5-HT_{2A} BP_{ND} was significantly inversely correlated with amygdala reactivity. Right amygdala reactivity was inversely correlated with 5-HT_{2A} BP_{ND} within both pgPFC (t_{36}=-3.44, p=0.002, **Figure 9D**) and sgPFC (t_{36}=-2.49, p=0.02). There was no significant correlation

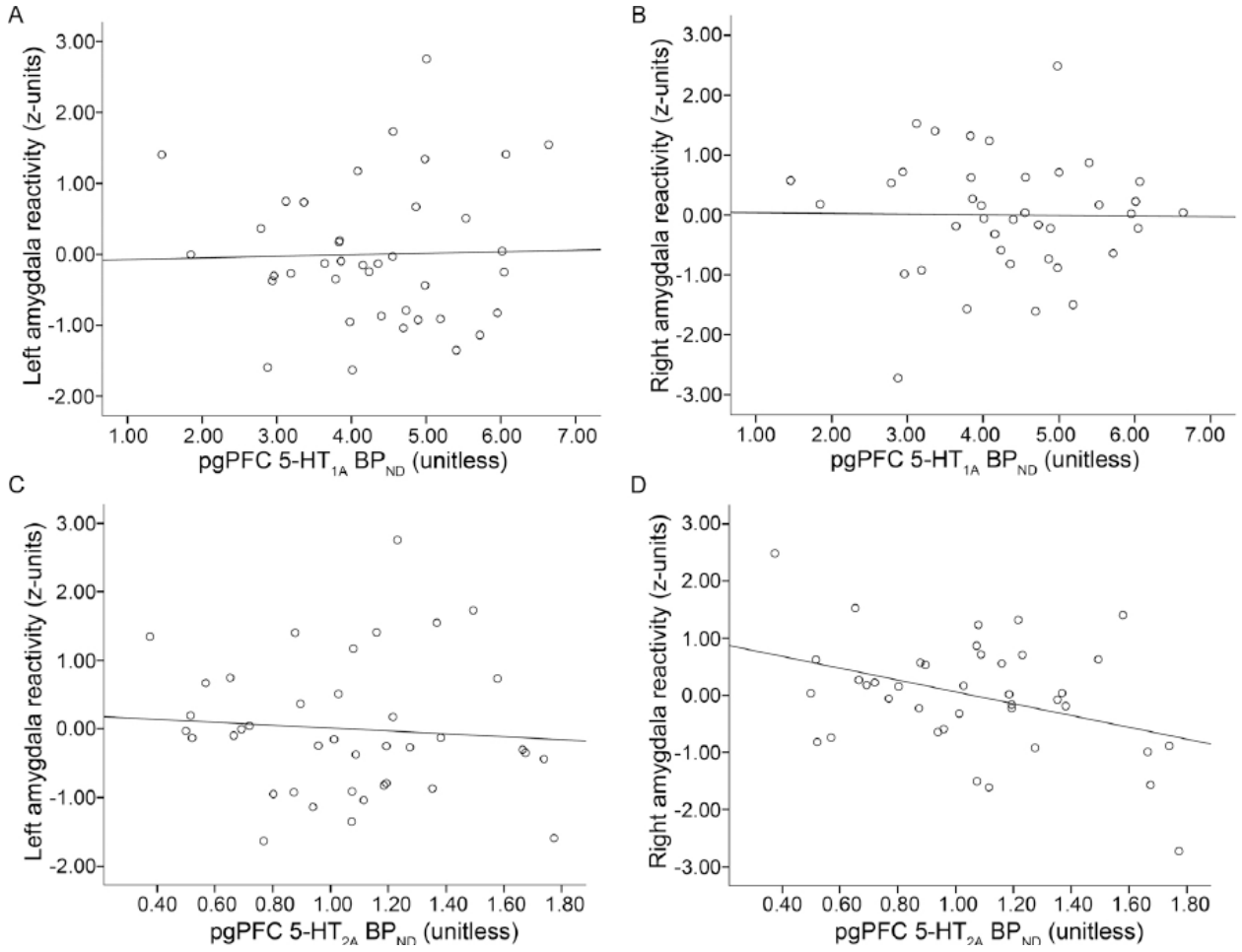

Figure 9. Association between amygdala reactivity and 5-HT_{1A} BP_{ND} and 5-HT_{2A} BP_{ND}. (**A**, **B**) Plot of non-significant correlation between left and right amygdala reactivity and pgPFC 5-HT_{1A} BP_{ND}. (**C**) Plot of non-significant correlation between left amygdala reactivity and pgPFC 5-HT_{2A} BP_{ND}. (**D**) Plot of significant inverse correlation between right amygdala reactivity and pgPFC 5-HT_{2A} BP_{ND}.

between left amygdala reactivity and 5-HT_{2A} BP_{ND} within either pgPFC (t_{36}=-0.61, p=0.55, **Figure 9C**) or sgPFC (t_{36}=0.72, p=.47).

5.4.5 Interaction between 5-HT_{1A} and 5-HT_{2A} BP_{ND} and amygdala reactivity

Consistent with our hypothesis, the interaction between mPFC 5-HT_{1A} BP_{ND} and mPFC 5-HT_{2A} BP_{ND} was significantly predictive of threat-related amygdala reactivity. Specifically, threat-related right amygdala reactivity was significantly correlated with the interaction between pgPFC 5-HT_{1A} BP_{ND} and 5-HT_{2A} BP_{ND} (t_{34}=2.18, p=0.03; **Figure 10B**). Further examination of this interaction effect revealed an association wherein pgPFC 5-HT_{2A} BP_{ND} was significantly inversely correlated with amygdala reactivity, but only at mean or relatively low levels of pgPFC 5-HT_{1A} BP_{ND}. The main effect of pgPFC 5-HT_{2A} BP_{ND} (t_{34}=-3.55, p=0.001) on right amygdala reactivity remained significant even when including 1) pgPFC 5-HT_{1A} BP_{ND}, 2) the interaction term and 3) age in the model.

The interaction between sgPFC 5-HT_{1A} BP_{ND} and 5-HT_{2A} BP_{ND} was also significantly predictive of right amygdala reactivity (t_{34}=2.72, p=0.01; **Figure 11B**). Again, further examination of this interaction effect indicated sgPFC 5-HT_{2A} BP_{ND} was significantly inversely correlated with threat-related amygdala reactivity, but only when sgPFC 5-HT_{1A} BP_{ND} was at mean or relatively low levels. The main effect of sgPFC 5-HT_{2A} BP_{ND} (t_{34}=-2.72, p=0.006) on right amygdala reactivity remained significant even when including 1) pgPFC 5-HT_{1A} BP_{ND}, 2) the interaction term and 3) age in the model.

Threat-related left amygdala reactivity was not significantly associated with the interaction between pgPFC 5-HT_{1A} BP_{ND} and pgPFC 5-HT_{2A} BP_{ND} (t_{34}=1.65, p=0.11). Main effects of pgPFC 5-HT_{2A} BP_{ND} (t_{34}=-0.61, p=0.54) and pgPFC 5-HT_{1A} BP_{ND} (t_{34}=0.25, p=0.80) on left amygdala reactivity remained non-significant including 1) the interaction term and 2) age in the model. Examination of the trend-level interaction effect revealed a pattern of association similar to that of right amygdala reactivity; however, this effect never reached statistical significance (**Figure 10A**). Left amygdala reactivity, however, was significantly correlated with the interaction between sgPFC 5-HT_{1A} BP_{ND} and sgPFC 5-HT_{2A} BP_{ND} (t_{34}=3.42, p=0.002). Examination of this interaction effect revealed that when sgPFC 5-HT_{1A} BP_{ND} is relatively low, sgPFC 5-HT_{2A} BP_{ND} is inversely associated with left amygdala reactivity (**Figure 11A**). Additionally, when sgPFC 5-HT_{1A} BP_{ND} is relatively high, sgPFC 5-HT_{2A} BP_{ND} is positively associated with left amygdala reactivity. Main effects of sgPFC 5-HT_{2A} BP_{ND} (t_{34}=-0.49, p=0.63) and sgPFC 5-HT_{1A} BP_{ND} (t_{34}=0.22, p=0.83) on left amygdala reactivity remained non-significant including 1) the interaction term and 2) age in the model.

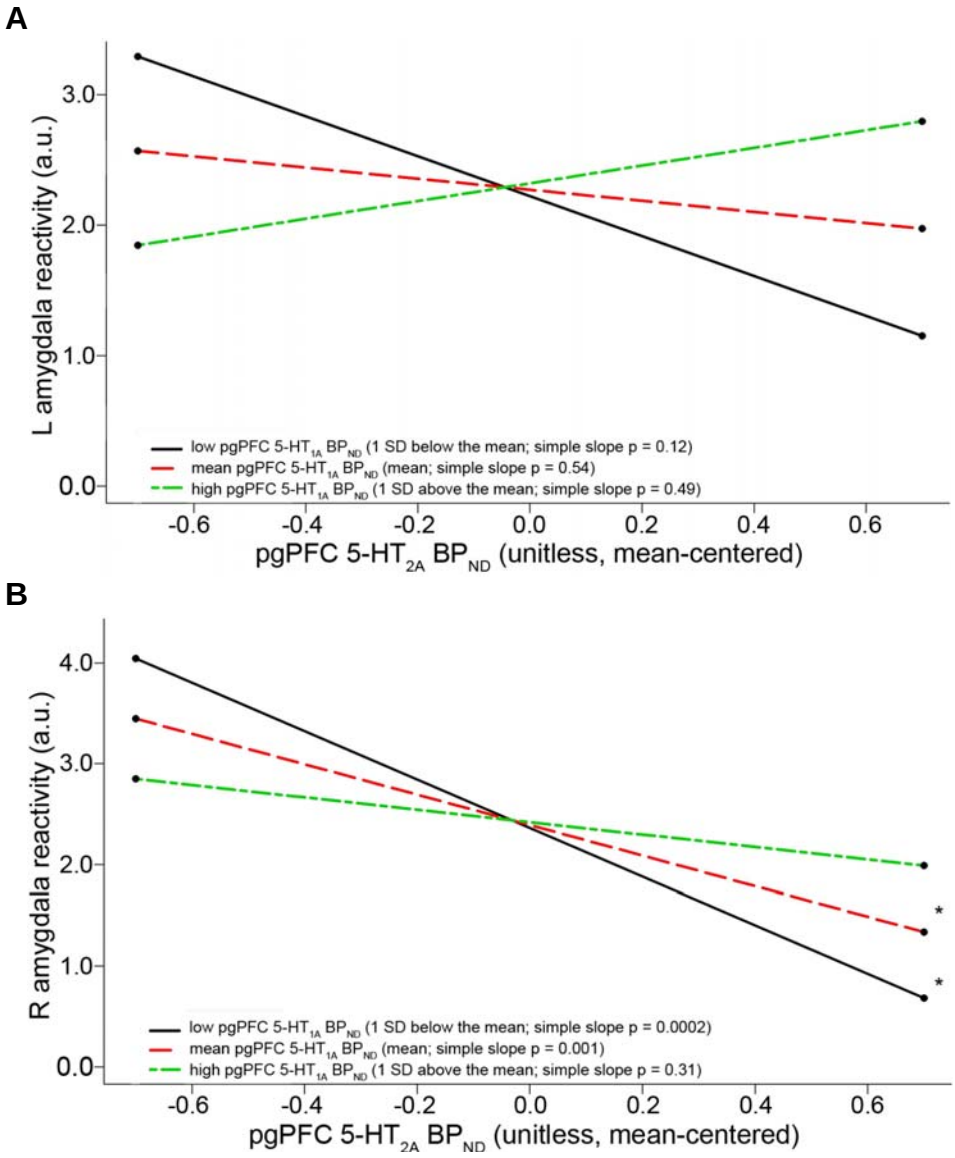

Figure 10. pgPFC 5-HT_{1A} BP_{ND} significantly moderates the association between pgPFC 5-HT_{2A} BP_{ND} and amygdala reactivity. (**A**) pgPFC 5-HT_{1A} BP_{ND} moderates the association between pgPFC 5-HT_{2A} BP_{ND} and left amygdala reactivity (trend). Lines indicate association between pgPFC 5-HT_{2A} BP_{ND} and left amygdala reactivity when pgPFC 5-HT_{1A} BP_{ND} is low (1 standard deviation (SD) below mean [-1 SD], solid black line), mean (equivalent to mean, red dotted line) and high (1 SD above mean[+1 SD], green dotted line). (**B**) pgPFC 5-HT_{1A} BP_{ND} significantly moderates the association between pgPFC 5-HT_{2A} BP_{ND} and right amygdala reactivity. Lines indicate association between pgPFC 5-HT_{2A} BP_{ND} and right amygdala reactivity when pgPFC 5-HT_{1A} BP_{ND} is at low (-1 SD, solid black line), mean (red dotted line) and high (+1 SD, green dotted line). *Indicates simple slope $p<0.05$. a.u., arbitrary units.

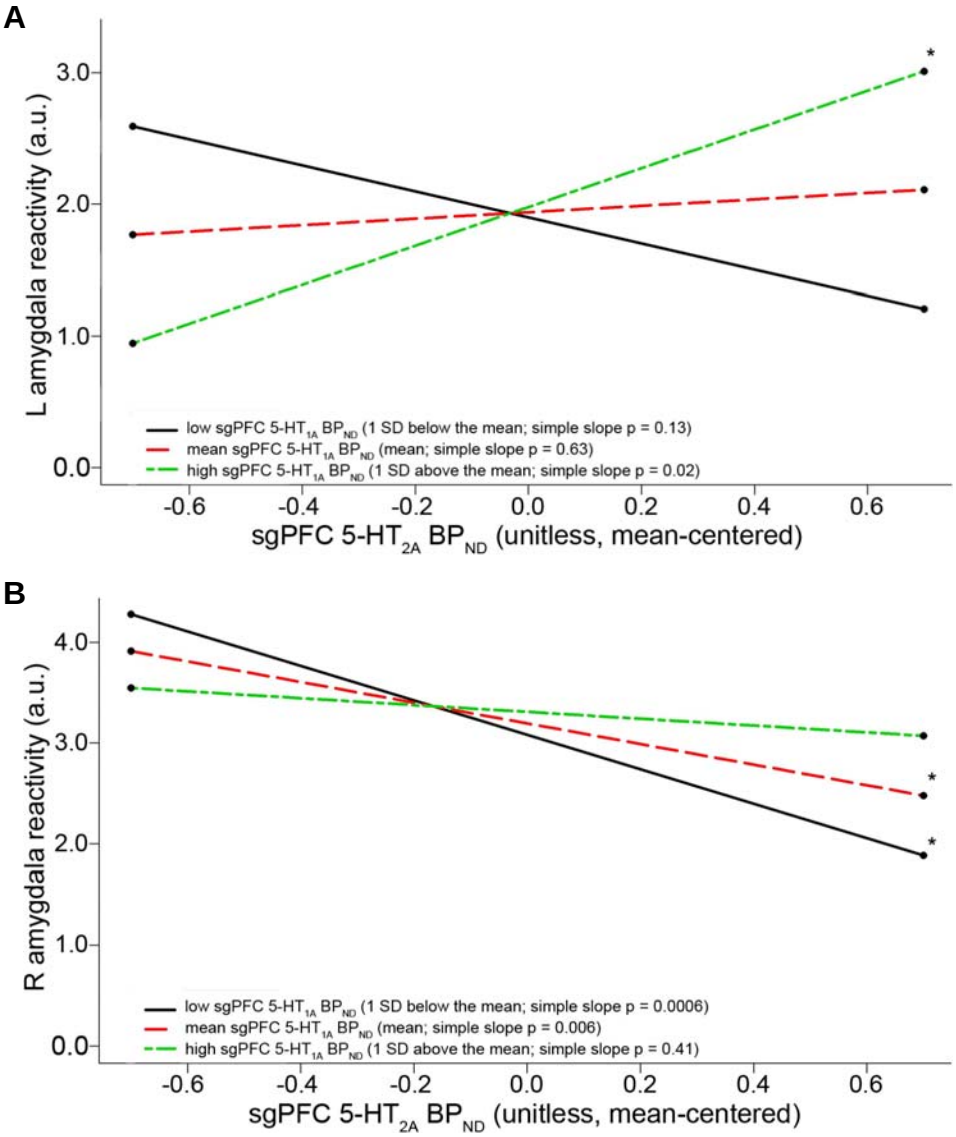

Figure 11. sgPFC 5-HT_{1A} BP_{ND} significantly moderates the association between sgPFC 5-HT_{2A} BP_{ND} and amygdala reactivity. (**A**) sgPFC 5-HT_{1A} BP_{ND} significantly moderates the association between sgPFC 5-HT_{2A} BP_{ND} and left amygdala reactivity. Lines indicate association between sgPFC 5-HT_{2A} BP_{ND} and left amygdala reactivity when sgPFC 5-HT_{1A} BP_{ND} is at low (1 standard deviation (SD) below mean [-1 SD], solid black line), mean (equivalent to mean, red dotted line) and high (1 SD above mean [+1 SD], green dotted line). (**B**) sgPFC 5-HT_{1A} BP_{ND} significantly moderates the association between sgPFC 5-HT_{2A} BP_{ND} and right amygdala reactivity. Lines indicate association between sgPFC 5-HT_{2A} BP_{ND} and right amygdala reactivity when sgPFC 5-HT_{1A} BP_{ND} is at low (-1 SD, solid black line, mean (red dotted line) and high (+1 SD, green dotted line). *Indicates simple slope $p<0.05$. a.u., arbitrary units.

5.4.6 Prefrontal 5-HT_{1A}, 5-HT_{2A} receptor binding and mPFC reactivity

We did not observe a significant main effect of task within mPFC regions corresponding to our PET regions of interest (ROIs; mean pgPFC BOLD response: t_{38}=-0.56, p=0.58; mean sgPFC BOLD response: t_{38}=1.37, p=0.18). Not surprisingly, regional 5-HT_{1A} BP_{ND} was not significantly correlated with mean BOLD signal in these mPFC regions (pgPFC BOLD response vs. pgPFC 5-HT_{1A} BP_{ND}: t_{36}=1.52, p=0.14; sgPFC BOLD response vs. sgPFC 5-HT_{1A} BP_{ND}: t_{36}=0.56, p=0.58). Regional 5-HT_{2A} BP_{ND} also was not significantly correlated with mean BOLD signal in these mPFC regions (pgPFC BOLD response vs. pgPFC 5-HT_{2A} BP_{ND}: t_{36}=-0.39, p=0.70; sgPFC BOLD response vs. sgPFC 5-HT_{2A} BP_{ND}: t_{36}=-0.73, p=0.47).

5.5 DISCUSSION

Previous studies have underscored the importance of a corticolimbic circuit wherein subregions of the mPFC including the pgPFC and sgPFC play a key role in regulating the response of the amygdala to threatening or fearful stimuli (Pezawas et al., 2005; Quirk and Mueller, 2008). Both human and animal studies suggest that 5-HT signaling modulates the response of this circuit and related behaviors (Bigos et al., 2008; Fisher et al., 2009; Fisher et al., 2006; Forster et al., 2006; Forster et al., 2008; Hariri et al., 2002b; Pezawas et al., 2005; Rhodes et al., 2007). Linking discrete serotonergic signaling mechanisms that may mediate sensitivity to threat through their modulation of this corticolimbic circuitry is integral to understanding

neurobiological pathways mediating inter-individual variability in behavior and related risk for psychopathology. Results from our current multimodal neuroimaging study reveal that the balance of 5-HT_{1A} and 5-HT_{2A} receptors in the mPFC is critical for shaping the response of the human amygdala to threat. Specifically, mPFC 5-HT_{2A} binding is inversely correlated with threat-related amygdala reactivity, but only when mPFC 5-HT_{1A} binding is at mean or relatively low levels. Importantly, these patterns are independent of age and gender suggesting the general importance and broad effects of the balance between mPFC 5-HT_{1A} and 5-HT_{2A} on amygdala reactivity.

These findings are remarkably consistent with the anatomical localization of 5-HT_{1A} and 5-HT_{2A} receptors to the axon hillock and apical dendrites of prefrontal glutamatergic pyramidal neurons, respectively. Given its principal localization proximal to the soma on apical dendrites, the excitatory 5-HT_{2A} is situated to mediate 5-HT depolarization of prefrontal glutamatergic neurons. In contrast, the localization of the inhibitory 5-HT_{1A} to the initial portion of the axon hillock positions it to mediate 5-HT hyperpolarization of these same neurons. Considering the high co-expression of 5-HT_{1A} and 5-HT_{2A} on most prefrontal glutamatergic neurons, this arrangement allows the 5-HT_{1A} to effectively (and negatively) gate the depolarizing effects of the 5-HT_{2A} on prefrontal output. In turn, such serotonergic modulation of prefrontal neuron output shapes the capacity of this circuitry to exert an inhibitory effect on amygdala reactivity (**Figure 3**). This gating function is reflected by our finding that mPFC 5-HT_{2A} binding is inversely correlated with decreased amygdala reactivity but only at average or below average levels of 5-HT_{1A} binding. The absence of a main effect of mPFC 5-HT_{1A} binding on amygdala reactivity is further consistent with this gating model wherein the capacity for mPFC 5-HT_{1A} to modulate

threat-related amygdala reactivity is dependent upon additional signaling mechanisms such as, but not necessarily limited to, mPFC 5-HT_{2A}.

The directionality of the interaction effects between mPFC 5-HT_{1A} and mPFC 5-HT_{2A} binding were consistent for both the right and left amygdala; however, there were qualitative differences in the moderation effects. With respect to the right amygdala, only at very high levels of either pgPFC or sgPFC 5-HT_{1A} binding was 5-HT_{2A} binding (in either mPFC subregion) positively associated with amygdala reactivity. In contrast, left amygdala reactivity was significantly positively associated with sgPFC 5-HT_{2A} binding at relatively more moderate mPFC 5-HT_{1A} binding levels. 5-HT_{2A} BP_{ND} within pgPFC was not significantly predictive of left amygdala reactivity at any pgPFC 5-HT_{1A} level; however, this interaction effect was at trend-level. Furthermore, the significant correlation observed between right amygdala reactivity and mPFC 5-HT_{2A} BP_{ND} was not observed for left amygdala reactivity. As we are unaware of any evidence suggesting asymmetry in the expression, distribution or functional effects of the 5-HT_{2A} we speculate that these differences are likely artifacts related to task-specific dynamics of right and left amygdala reactivity, including greater stimulus sensitivity and response habituation in the right amygdala (Farah et al., 1998; Fischer et al., 2003), which biases our ability to detect associations with the right amygdala.

There is strong evidence suggesting that 5-HT signaling within the amygdala plays an important role in modulating threat-related amygdala reactivity (Bigos et al., 2008; Christianson et al., 2010; Rhodes et al., 2007), and both 5-HT_{1A} and 5-HT_{2A} are expressed in the amygdala (Barnes and Sharp, 1999; Kia et al., 1996; McDonald and Mascagni, 2007). However, we did not observe a significant correlation between either amygdala 5-HT_{1A} or 5-HT_{2A} BP_{ND} and amygdala

reactivity (data not shown). Unlike mPFC, the 5-HT_{1A} and 5-HT_{2A} may be expressed on both glutamatergic and GABAergic neurons within the amygdala (Aznar et al., 2003; McDonald and Mascagni, 2007). This potential for both receptor subtypes to effect inhibitory and excitatory modulation of the amygdala complicate efforts to map correlations between estimates of local binding and reactivity in the absence of cell-type specific values, which are beyond the scope of current PET techniques. Finally, additional 5-HT receptor signaling mechanisms within the amygdala, such as the 5-HT_3 and 5-HT_{2C} receptors, have been implicated in anxiety-related behavioral phenotypes in animal models and may have a greater role in mediating the effects of local 5-HT signaling on amygdala function (Bhatnagar et al., 2004; Burghardt et al., 2007; Christianson et al., 2010; Clark et al., 2004).

There are important limitations to our study. First, our BOLD fMRI challenge paradigm is explicitly designed to elicit threat-related amygdala reactivity associated with driving behavioral and physiologic arousal in response to environmental stimulation. Our task does not engage mPFC regions involved in regulating amygdala reactivity and overlapping with our PET ROIs. Thus, we are not able to explore the effects of mPFC 5-HT_{1A} and 5-HT_{2A} on mPFC function related to the top-down regulation of amygdala reactivity. Alternative BOLD fMRI paradigms such as those involving emotion regulation (Ochsner and Gross, 2005) or extinction of conditioned fear responses (Phelps et al., 2004) are necessary to determine effects of 5-HT_{1A} and 5-HT_{2A} signaling on mPFC activity and related amygdala reactivity. Second, our inferences are drawn from correlation analyses and cannot be used to determine causality. BOLD fMRI and PET receptor imaging provide only indirect metrics of amygdala excitation and 5-HT receptor signaling, respectively. Thus, our findings must be interpreted with caution.

Future studies employing pharmacological challenge of specific 5-HT receptor systems or 5-HT reuptake blockade in the context of our multimodal neuroimaging strategy may provide more direct evidence implicating these and perhaps other receptor mechanisms in mediating the effects of 5-HT signaling on corticolimbic circuit function. For example, in addition to their specific cellular expression (i.e., apical dendrites or axon hillock) the 5-HT_{2A} is typically localized extra-synaptically (Miner et al., 2003). Thus, volume transmission of 5-HT is necessary to engage the 5-HT_{2A} while synaptic transmission may be sufficient for the 5-HT_{1A}. Accordingly, experimentally increasing 5-HT neurotransmission (via pharmacologic challenge with a selective serotonin reuptake inhibitor) should increase volume transmission and bias effects toward the 5-HT_{2A} resulting in greater prefrontal drive and lesser amygdala reactivity. Imaging genetics is another potentially useful tool for furthering our understanding of these mechanisms (Hariri and Holmes, 2006; Hariri and Weinberger, 2003; Meyer-Lindenberg and Weinberger, 2006). Common functional genetic polymorphisms can be used to establish relative differences in 5-HT signaling (e.g., 5-HTTLPR) and, against this genetic background, specific models of relative 5-HT receptor effects can be evaluated. A bias towards greater prefrontal drive and reduced amygdala reactivity via 5-HT_{2A} signaling would be predicted in individuals possessing genetic variants associated with relatively increased 5-HT neurotransmission (e.g., 5-HTTLPR Short allele carriers relative to Long allele homozygotes). Unfortunately, we did not have the resources for the addition of a pharmacologic challenge to our paradigm and our sample is underpowered to formally test effects of functional genetic polymorphisms on the observed patterns.

Despite these limitations, our current findings provide unique *in vivo* evidence that 5-HT receptors in the mPFC play an important role in shaping inter-individual variability in amygdala reactivity. Specifically, the data reveal that 5-HT_{1A} may function to effectively gate the capacity of 5-HT_{2A} to drive prefrontal pyramidal neuron excitability related to the regulation of threat-related amygdala reactivity. The current work not only further highlights the effectiveness of multimodal neuroimaging in identifying molecular signaling pathways that modulate neurobiological circuits in humans but also specifically implicates the balance of mPFC 5-HT_{1A} and 5-HT_{2A} in modulating the response of the human amygdala and possibly mediating the effects of altered 5-HT signaling on mood, affect and related psychopathology.

6.0 STUDY 4: ASSOCIATIONS BETWEEN THREE COMMON SEROTONIN GENE POLYMORPHISMS AND *IN VIVO* 5-HT_{1A} AND 5-HT_{2A} BINDING[4]

6.1 ABSTRACT

Using an imaging genetics approach in a population of healthy volunteers, we evaluated the effects of three common polymorphisms in serotonin (5-HT) related genes on *in vivo* regional 5-HT_{1A} and 5-HT_{2A} receptor binding levels using [^{11}C]WAY100635 and [^{18}F]altanserin PET, respectively. We found that 5-HTTLPR genotype predicted 5-HT_{1A} and 5-HT_{2A} binding across multiple brain regions such that the S and L_G alleles were associated with relatively reduced 5-HT_{1A} and 5-HT_{2A} binding. In contrast, *HTR1A* C(-1019)G and *HTR2A* G(-1438)A genotypes did not predict 5-HT_{1A} and 5-HT_{2A} binding, respectively. Our findings suggest the 5-HTTLPR may contribute to individual variability in neural and behavioral sensitivity to threat as well as related risk for psychopathology by biasing the relative levels of 5-HT_{1A} and 5-HT_{2A} and, subsequently, the regional effects of serotonin signaling.

[4]Chapter 6 is a slightly modified version of an article currently prepared for submission to *Molecular Psychiatry* as an Original Article. Full author-listing: Fisher, P.M., Price, J.C., Meltzer, C.C., Kimak, M.A., Moses-Kolko, E.L., Berga, S.L., Ferrell, R.E., Hariri, A.R.

6.2 INTRODUCTION

Recent imaging genetics studies using receptor binding positron emission tomography (PET) have linked common variants in human serotonin (5-hydroxytryptamine, 5-HT) related genes with individual differences in 5-HT receptor and 5-HT transporter (5-HTT) levels *in vivo* (Willeit and Praschak-Rieder, 2010). Such mapping of common genetic polymorphisms onto alterations in molecular signaling pathways may contribute to a more complete understanding of pathways mediating individual differences in brain chemistry, brain circuitry, behavior and, ultimately, risk for related psychopathology (Hariri, 2009). In the current study, we used imaging genetics to map the relationships between three common variants in 5-HT related genes and *in vivo* 5-HT_{1A} and 5-HT_{2A} binding levels in 48 healthy adult volunteers.

Consistent with our prior research (Fisher et al., 2009; Fisher et al., 2006), we focused on 5-HT_{1A} and 5-HT_{2A} because of their importance in mediating serotonergic modulation of behaviorally relevant brain circuits, particularly a corticolimbic circuit including the amygdala and aspects of the medial prefrontal cortex and anterior cingulate cortex. This corticolimbic circuit supports the generation, integration and regulation of threat-related behavioral and physiologic arousal. We targeted three common human genetic polymorphisms with the potential to either directly or indirectly affect 5-HT_{1A} or 5-HT_{2A} receptor binding. The first target variant was a common single nucleotide polymorphism (rs6295) in the promoter region (C(-1019)G) of the human gene (*HTR1A*) encoding 5-HT_{1A}. The second target variant was a common single nucleotide polymorphism (rs6311) in the promoter region (G(-1438)A) of the human gene (*HTR2A*) encoding the 5-HT_{2A}. The final genetic target was a common functional

variable nucleotide tandem repeat polymorphism (5-HTTLPR) in the human gene (*SLC6A4*) encoding 5-HTT.

The *HTR1A* -1019G allele is associated with relatively increased gene transcription and receptor expression (Lemonde et al., 2003), particularly of 5-HT_{1A} autoreceptors (Czesak et al., 2006), *in vitro*. The *HTR2A* -1438A allele has been associated with increased transcriptional activity (Parsons et al., 2004) and post-mortem 5-HT_{2A} density (Turecki et al., 1999). The 5-HTTLPR S allele has been associated with relatively diminished 5-HTT expression *in vitro* (Lesch et al., 1996), which putatively could increase synaptic 5-HT concentrations resulting in down-regulation of pre- and postsynaptic 5-HT receptors (Hariri and Holmes, 2006). An A/G variant (rs25531) within the 5-HTTLPR has been associated with 5-HTT expression *in vitro* such that the L_G allele functions like the S allele (Hu et al., 2005). Based on this evidence, we hypothesized that the *HTR1A* -1019G allele would be associated with relatively increased 5-HT_{1A} binding. Similarly, we hypothesized that the *HTR2A* -1438A allele would be associated with relatively increased 5-HT_{2A} binding. Finally, we hypothesized that the 5-HTTLPR S allele and L_G allele would be associated with relatively decreased 5-HT_{1A} and 5-HT_{2A} binding.

6.3 METHODS

6.3.1 Subjects

Sixty-eight healthy adult volunteers were recruited from the community and participated after providing written informed consent in accordance with the University of Pittsburgh Institutional Review Board (35 males, age: 49.8 ± 18.0 years; mean ± standard deviation; age range: 20-80 years). All subjects in this study self-identified as Caucasian. Subjects were recruited through local advertisements, referrals, and ongoing studies. Subjects were generally healthy with exclusion criteria including 1) current or lifetime psychiatric diagnoses assessed by Structured Clinical Interview (Diagnostic and Statistical Manual of Mental Disorders, Version IV; (First et al., 1996), 2) cardiovascular disease or diabetes, 3) history of substance abuse or use of antidepressants, 4) early dementia or mild cognitive impairment according to the Mini-Mental State Examination (scores exceeding 27; (Folstein et al., 1975), and 5) sleep disorders assessed by the Pittsburgh Quality Sleep Index (Buysse et al., 1989). To avoid confounds related to significant age-related brain atrophy and dilution of PET signal, we considered a subset of the population that was 60 years old and younger for analysis of genetic variants predicting 5-HT_{1A} and 5-HT_{2A} binding (N = 48, 24 males, age: 40.9 ± 13.1).

6.3.2 Genotyping

High–molecular weight DNA was isolated from EDTA-anticoagulated whole blood samples obtained from all participants using a salting-out procedure (Miller et al., 1988). Each sample was genotyped using polymerase chain reaction amplification or fluorescence polarization primers (Chen et al., 1999). Genotyping of the *HTR1A* C(-1019)G has been previously described (Fakra et al., 2009). Genotyping of the *HTR2A* G(-1438)A has been described previously (Halder et al., 2007). Genotyping was performed using the LJL AnalystHT (Molecular Devices, Sunnyvale, California). Genotyping of the 5-HTTLPR (Wendland et al., 2006) and method for visualizing amplification products (Edenberg and Reynolds, 1998) have been described previously. Genotyping of the rs25531 A/G SNP has been previously described (Dombrovski et al., 2010). Only the L allele was subtyped for rs25331. All genotypes were scored by 2 independent readers using sequence-verified standards, and all call rates were greater than 95%.

Across all 68 healthy volunteers, genotypes were determined to be in Hardy-Weinberg equilibrium (**Table 2**).

Table 2. Genotype frequencies

Genotype			χ2	p-value[a]
Bi-allelic 5-HTTLPR				
L/L	L/S	S/S		
18	33	17	0.06	0.81
Tri-allelic 5-HTTLPR				
L'/L'	L'/S'	S'/S'		
15	33	20	--	--
HTR1A C(-1019)G				
C/C	C/G	G/G		
16	30	22	0.83	0.36
HTR2A G(-1438)A				
G/G	G/A	A/A		
16	38	11	2.02	0.16

[a]p-value determined with χ^2-test and 1 degree of freedom

6.3.3 PET methods

Technical detail concerning the MR and PET imaging procedures related to both [^{11}C]WAY100635 and [^{18}F]altanserin can be found in previously published work and is described below (Bailer et al., 2007; Cidis Meltzer et al., 2001; Fisher et al., 2009; Fisher et al., 2006; Soloff et al., 2010). See previous reports for discussion regarding limitations, challenges and methodological attempts to minimize potential artifacts and biases related to these radioligands (Meltzer et al., 2004; Parsey et al., 2006b; Parsey et al., 2000b; Price et al., 2001a; Price et al., 2001b; Soloff et al., 2010).

Structural MR images (GE Signa 1.5 T scanner) were acquired for each subject using a spoiled-gradient recalled sequence (SPGR; TR=25 ms, TE=5 ms, FOV=24 cm, slice thickness=1.5

mm, matrix=256×192) with parameters optimized for contrast between gray matter, white matter, and cerebrospinal fluid.

Catheters were placed in an antecubital vein for radioligand injection and a radial artery for arterial blood sampling. PET scans were acquired using an ECAT HR+ PET scanner (CTI PET systems, Knoxville, TN) in 3D imaging mode (63 transaxial planes, 2.4-mm thickness, 15.2-cm FOV). Head movement was minimized by use of a thermoplastic mask immobilization system. A 10-min transmission scan (rotating $^{68}Ge/^{68}Ga$ rods) was acquired for attenuation correction of emission data. PET data were further corrected for dead time and scatter.

Each radioligand was administered as a slow bolus over 20 seconds. PET data acquisition and arterial blood sampling was initiated at the start of radioligand injection. The total radioactivity concentration in plasma was determined from approximately 35 0.5-ml hand-drawn blood samples collected over the scanning interval. Larger additional blood samples were acquired at 5-6 times over the study duration for the determination of the fraction of the total radioactivity that resulted from radiolabeled metabolites of the parent radioligand. The total plasma radioactivity concentration was corrected for radiolabeled metabolites and this ‘‘metabolite-corrected’’ arterial input function was used for data analysis (Meltzer et al., 2004; Price et al., 2001b).

Image reconstruction was performed using filtered back projection for a final image resolution of about 6 mm. Regions of interest (ROIs) were drawn on resliced MR images for each subject and applied to their respective, coregistered PET images (ROIs drawn by S.K.Z. and C.B.). ROIs were identified for the dorsal raphe nucleus (DRN), subgenual prefrontal cortex (sgPFC), pregenual prefrontal cortex (pgPFC), amygdala and cerebellum. The cerebellum was

used as the reference region for nondisplaceable radiotracer uptake (i.e., free and nonspecific concentrations, V_{ND}) for both [^{11}C]WAY 100635 and [^{18}F]altanserin.

PET data for both radioligands were analyzed using the Logan graphical method (Logan et al., 1990) to obtain regional volume of distribution values (V_T). Regional V_T values were used to determine the nondisplaceable binding potential, BP_{ND}, a measure of specific receptor binding. The BP_{ND} is directly proportional to B_{avail}/K_d, where B_{avail} is the concentration of receptors available for radiotracer binding (i.e., not occupied by endogenous 5-HT) and K_d is the equilibrium dissociation rate constant (i.e., inversely related to binding affinity). The PET binding measures were corrected for atrophy-related CSF dilution using a previously validated 2-component MR-based atrophy correction algorithm (Cidis Meltzer et al., 2001; Meltzer et al., 1999; Meltzer et al., 1990).

6.3.4 [^{18}F]Altanserin PET methods

The radiosynthesis of [^{18}F]altanserin was performed using a modification of the original method (Lemaire et al., 1991) that has been applied in several studies in our laboratory (Bailer et al., 2004; Fisher et al., 2009; Meltzer et al., 1998; Smith et al., 1998; Soloff et al., 2010). [^{18}F]Altanserin was administered via intravenous injection (7.21 ± 0.33 mCi, mean ± SD) and PET scanning was performed over 90-min. The Logan analysis regression was performed over the 12- to 90-min post-injection integration intervals (10 points) to obtain regional [^{18}F]altanserin V_T and BP_{ND} values. [^{18}F]altanserin scans failed in 3 individuals.

6.3.5 [^{11}C]WAY100635 PET methods

The radiosynthesis of [^{11}C]WAY100635 was performed as previously described (McCarron et al., 1996) and has been applied in several studies in our laboratory (Bailer et al., 2007; Fisher et al., 2006; Meltzer et al., 2004). [^{11}C]WAY100635 was administered via intravenous injection (13.76 ± 2.04 mCi) and PET scanning was performed over 90 min. The Logan analysis regression was performed over the 14 to 90 min PET scan integration intervals (13 points) to obtain regional [^{11}C]WAY100635 V_T and BP_{ND} values.

6.3.6 Statistical analysis

Tests of genetic association and all other statistical analyses were performed in PASW (v18.0). Age and gender were evenly distributed across all genotype groups (all p's > 0.3). Subjects with regional binding values < 0.0 (N = 1 for 5-HT_{2A} binding) or greater than 3 standard deviations from the regional mean (N = 2 for 5-HT_{1A} binding) were excluded from analysis. Forty-six participants were included in analyses related to 5-HT_{1A} binding and 44 participants were included in analyses related to 5-HT_{2A} binding. For all analyses, $p < 0.05$ was considered statistically significant and $p < 0.1$ was considered trend-level. Age and gender were included as covariates in all analyses. With respect to the 5-HTTLPR, "bi-allelic" refers to the consideration of only L and S allele genotype, while "tri-allelic" refers to the additional consideration of the rs25531 A/G SNP (L_A = L', L_G or S = S').

Regions included in analyses were the amygdala, DRN, pgPFC and sgPFC. Due to negligible 5-HT_{2A} receptor concentrations and specific binding, DRN was not included as a region in 5-HT_{2A} binding analyses. A repeated-measures ANOVA with genotype group as a between-subjects factor was used to determine a main effect of genotype group on 5-HT_{1A} binding or 5-HT_{2A} binding across regions. For each genetic variant we considered each genotype group separately (e.g., L/L vs. L/S vs. S/S; "3 group") and combining minor allele carriers (e.g., L/L vs. S carriers). *Post hoc* t-tests for individual regions were performed where a significant main effect of genotype group across regions was identified. *Post hoc* regional analyses were corrected for 4 (significance threshold of $p < 0.013$) and 3 ($p < 0.017$) multiple comparisons for 5-HT_{1A} and 5-HT_{2A}, respectively. Previous studies suggest -1019G allele effects on 5-HT_{1A} transcriptional efficacy may be specific to serotonergic neurons (Czesak et al., 2006; Lemonde et al., 2003). For this reason we independently examined effects of *HTR1A* C(-1019)G on DRN 5-HT_{1A} binding.

Considering significant age effects on 5-HT_{2A} binding, we plot 5-HT_{2A} binding values standardized with respect to age and sex. This is to more clearly illustrate the association between genetic variants and 5-HT_{2A} binding. All statistics reported reflect 5-HT_{2A} binding values including age and sex as covariates.

6.4 RESULTS

6.4.1 5-HT_{1A} and 5-HT_{2A} receptor binding

Consistent with previous studies, we observed specific 5-HT_{1A} and 5-HT_{2A} receptor binding within all regions examined (**Table 3**).

Table 3. Mean regional 5-HT_{1A} and 5-HT_{2A} binding

Region	Mean	S.D.[a]
5-HT_{1A} binding (BP_{ND})		
Amygdala	6.89	1.81
DRN	3.44	1.27
pgPFC	4.51	1.11
sgPFC	4.96	1.35
5-HT_{2A} binding (BP_{ND})		
Amygdala	0.32	0.16
pgPFC	1.06	0.35
sgPFC	1.22	0.42

[a]S.D. = Standard Deviation

6.4.2 Genotype effects on 5-HT_{1A} binding

6.4.2.1 5-HTTLPR and 5-HT_{1A} binding 5-HT_{1A} binding across regions was different between 5-HTTLPR groups at a trend-level (3 groups: $F(2,41) = 2.72$, $p = 0.08$; **Figure 12**). Comparing L/L vs. S carriers, 5-HTTLPR significantly predicted 5-HT_{1A} binding across regions ($F(1,42) = 5.57$, $p = 0.02$). *Post-hoc* analysis examining each region independently revealed that S carriers displayed reduced mean 5-HT_{1A} binding relative to L/L individuals, however, individual regional effects did

not survive correction for multiple comparisons (DRN: t_{43} = -2.09, p = 0.04; sgPFC: t_{43} = -2.07, p = 0.04; pgPFC: t_{43} = -1.78, p = 0.08; amygdala: t_{43} = -1.40, p = 0.17; all p's > 0.05 after correction). The association between the tri-allelic 5-HTTLPR genotype and 5-HT_{1A} binding across regions was similar to that of the bi-allelic 5-HTTLPR; however, these effects were weaker (3 groups: F(2,41) = 2.16, p = 0.13; L'/L' vs. S' carriers: F(1,42) = 3.30, p = 0.08; **Figure 13**).

6.4.2.2 *HTR1A* C(-1019)G and 5-HT_{1A} binding We observed no evidence for an association between *HTR1A* C(-1019)G and 5-HT_{1A} binding across regions (3 groups: F(2,41) = 0.11, p = 0.89; C/C vs. G-carriers: F(1,42) = 0.17, p = 0.69; **Figure 14**). DRN 5-HT_{1A} binding was not significantly different across *HTR1A* C(-1019)G groups (3 groups: F(2,42) = 0.07, p = 0.93; C/C vs. G-carriers: F(1,43) = 0.09, p = 0.76).

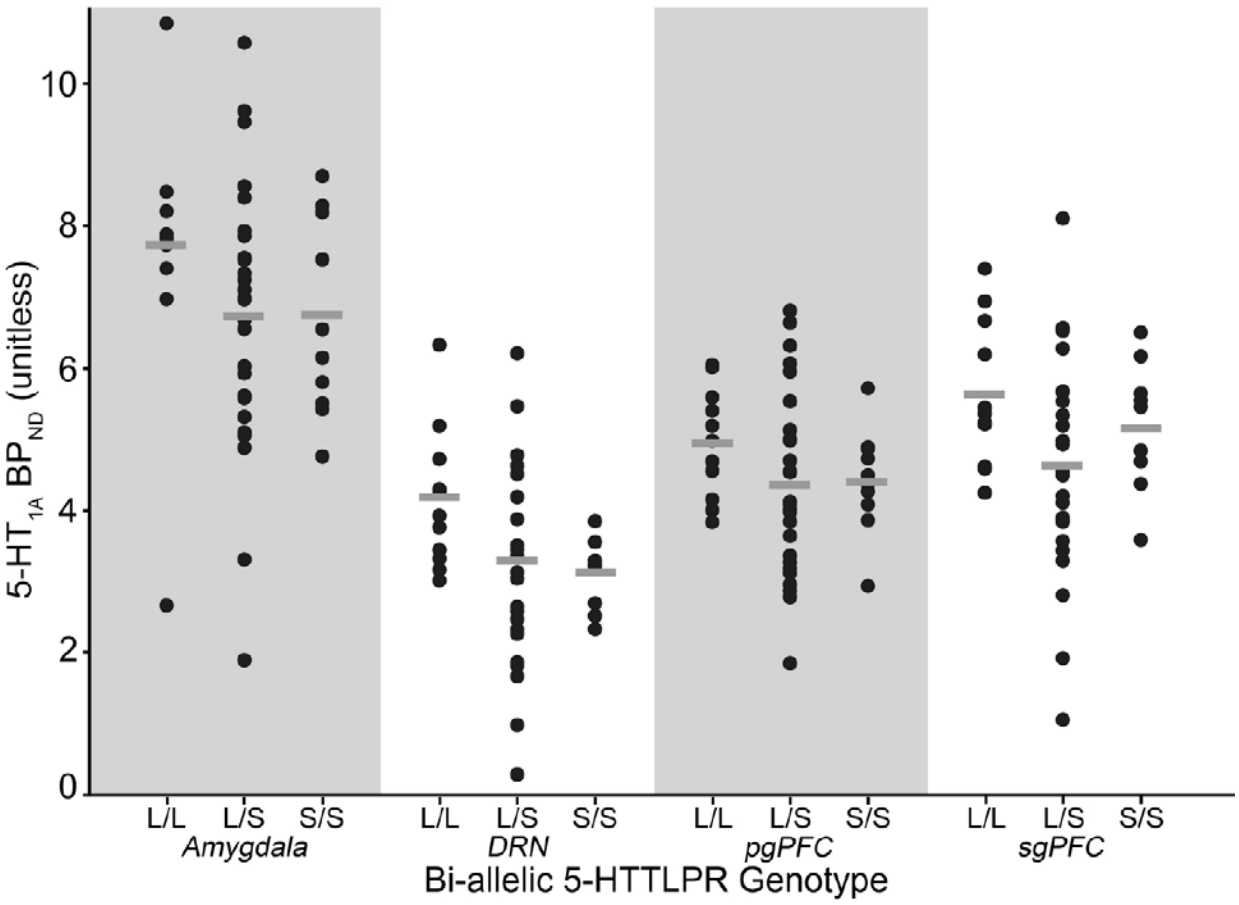

Figure 12. Regional 5-HT_{1A} binding associated with bi-allelic 5-HTTLPR genotype group. Relative to L/L individuals, S carriers displayed significantly reduced 5-HT_{1A} binding across regions (3 groups: $F(2,41) = 2.72$, $p = 0.08$; L/L vs. S carriers: $F(1,42) = 5.57$, $p = 0.02$). Plots indicate single-subject 5-HT_{1A} binding values within the amygdala, DRN, pgPFC and sgPFC grouped by bi-allelic 5-HTTLPR genotype. Gray line indicates mean.

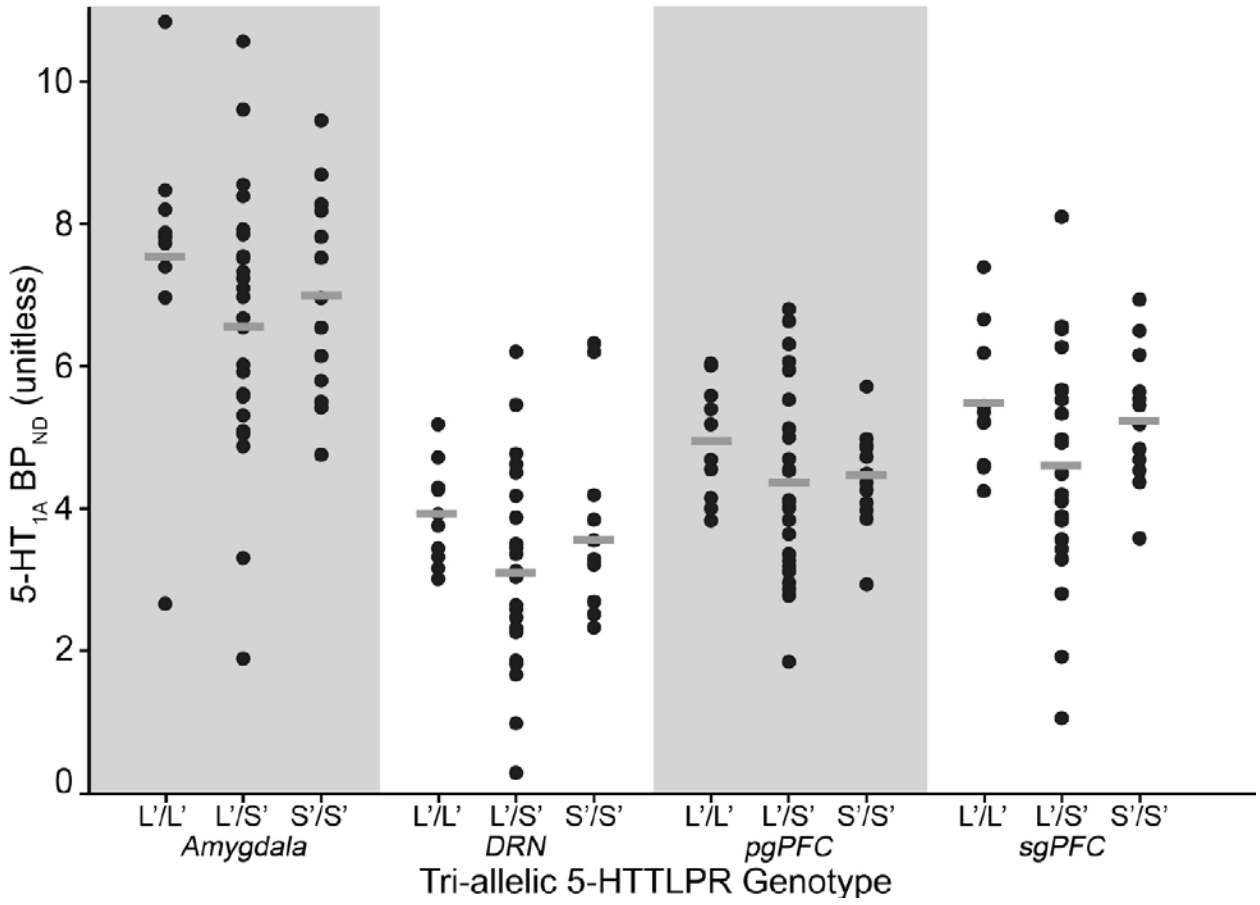

Figure 13. Regional 5-HT_{1A} binding associated with tri-allelic 5-HTTLPR genotype group. Relative to L'/L' individuals, S' carriers displayed reduced 5-HT_{1A} binding across regions at a trend-level (3 groups: $F(2,41) = 2.16$, $p = 0.13$; L'/L' vs. S' carriers: $F(1,42) = 3.30$, $p = 0.08$). Plots indicate single-subject 5-HT_{1A} binding values within the amygdala, DRN, pgPFC and sgPFC grouped by tri-allelic 5-HTTLPR genotype. Gray line indicates mean.

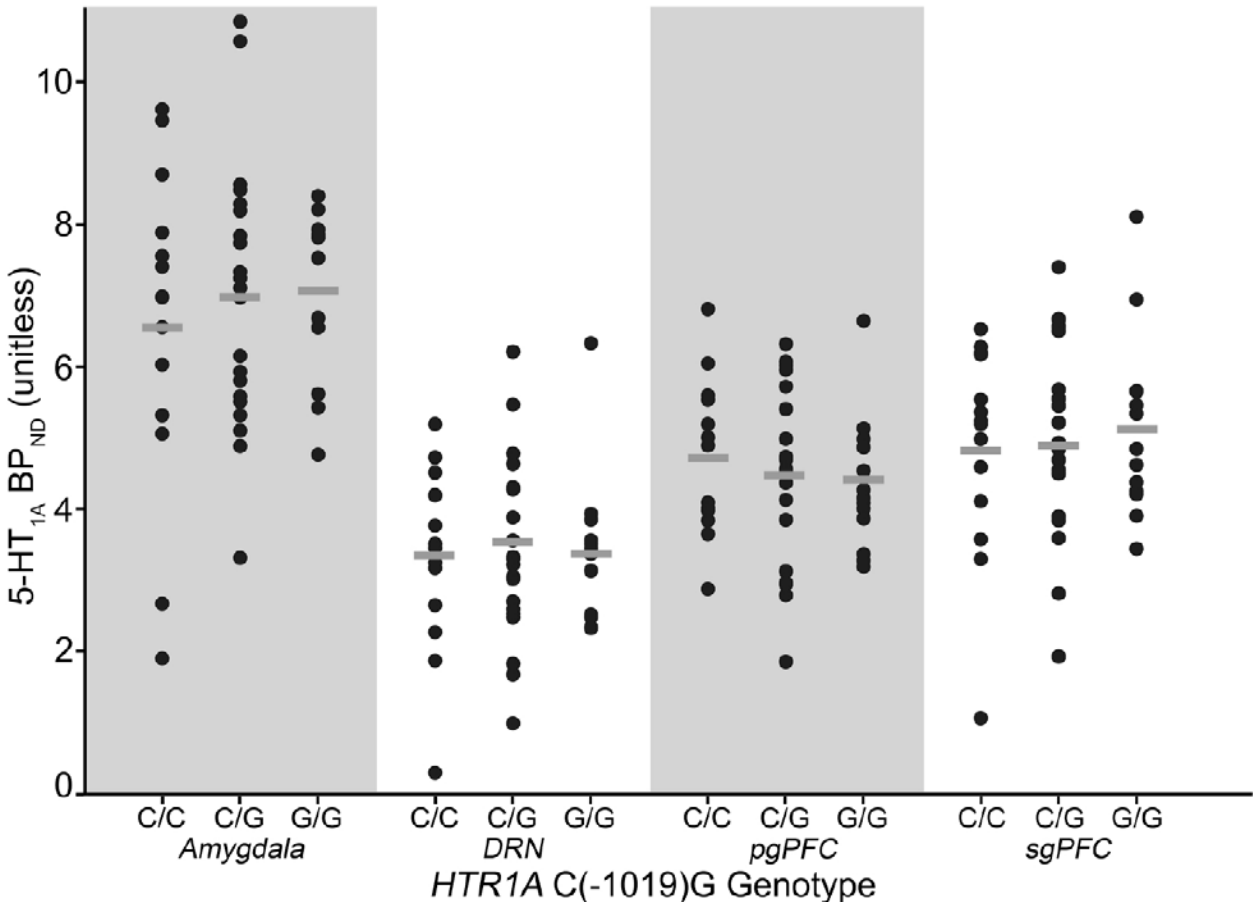

Figure 14. Regional 5-HT_{1A} binding not associated with *HTR1A* C(-1019)G genotype group (3 groups: $F(2,41) = 0.11$, $p = 0.89$; C/C vs. G-carriers: $F(1,42) = 0.17$, $p = 0.69$). Plots indicate single subject 5-HT_{1A} binding values within the amygdala, DRN, pgPFC and sgPFC grouped by C(-1019)G genotype. Gray line indicates mean.

6.4.3 Genotype effects on 5-HT_{2A} binding

6.4.3.1 5-HTTLPR and 5-HT_{2A} binding 5-HT_{2A} binding across regions was not significantly different between bi-allelic 5-HTTLPR groups (3 groups: $F(2,39) = 1.56$, $p = 0.22$; L/L vs. S carriers: ($F(1,40) = 0.03$, $p = 0.96$; **Figure 15**). The tri-allelic 5-HTTLPR genotype, however, was associated with 5-HT_{2A} binding across regions at a trend-level (3 groups: $F(2,39) = 2.87$, $p = 0.07$). We did not observe an effect of the tri-allelic 5-HTTLPR on 5-HT_{2A} binding across regions when comparing L'/L' vs. S' carriers ($F(1,40) = 0.83$, $p = 0.37$; **Figure 16**).

6.4.3.2 *HTR2A* G(-1438)A and 5-HT_{2A} binding We observed no evidence of an association between *HTR2A* G(-1438)A and 5-HT_{2A} binding across regions (3 groups: $F(2,37) = 0.25$, $p = 0.77$; G/G vs. A-carriers; $F(1,38) = 0.09$, $p = 0.77$; **Figure 17**).

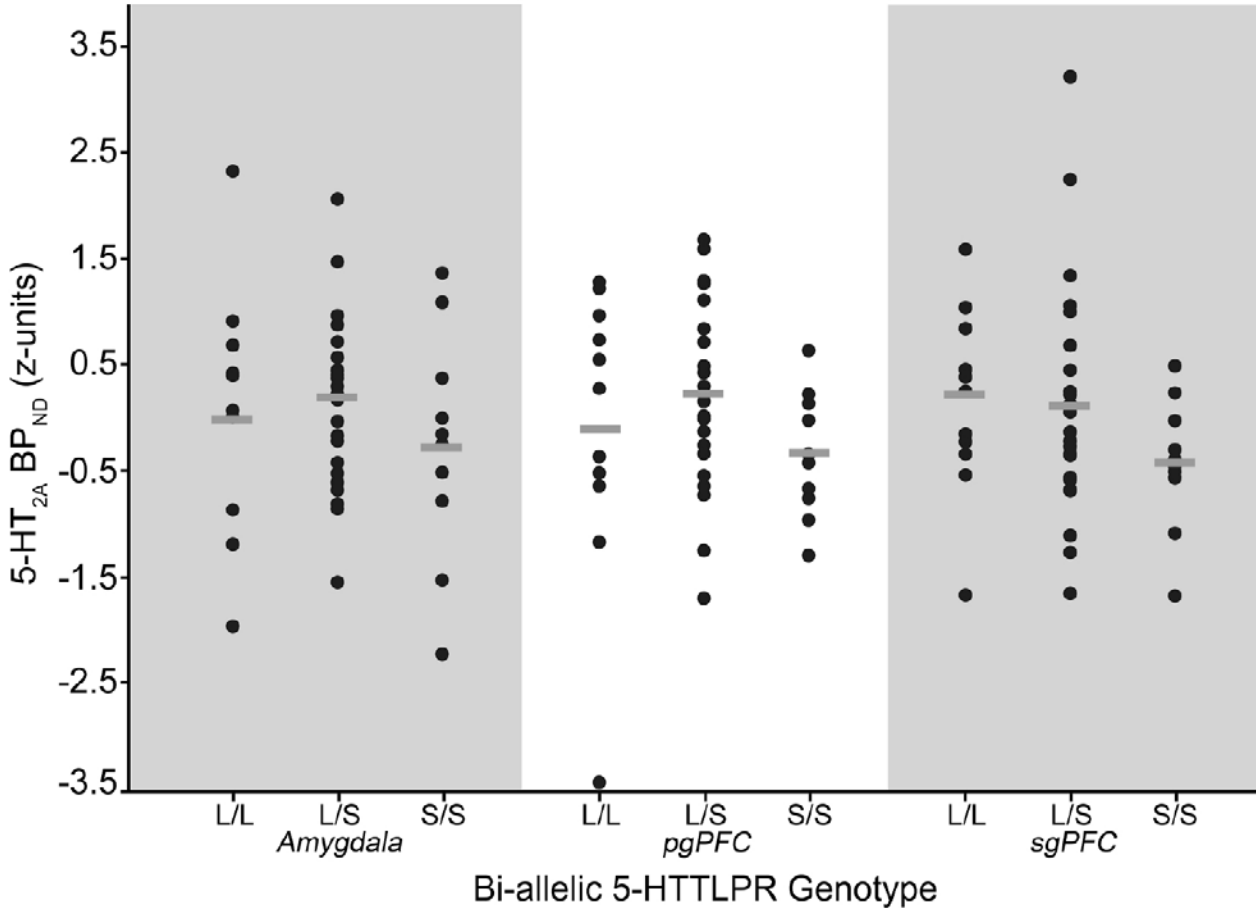

Figure 15. Regional 5-HT_{2A} binding not associated with bi-allelic 5-HTTLPR genotype group (3 groups: $F(2,39) = 1.56$, $p = 0.22$; L/L vs. S carriers: $F(1,40) = 0.03$, $p = 0.96$). Plots indicate single subject 5-HT_{2A} binding values standardized with respect to age and sex within the amygdala, pgPFC and sgPFC grouped by bi-allelic 5-HTTLPR genotype. Gray line indicates mean.

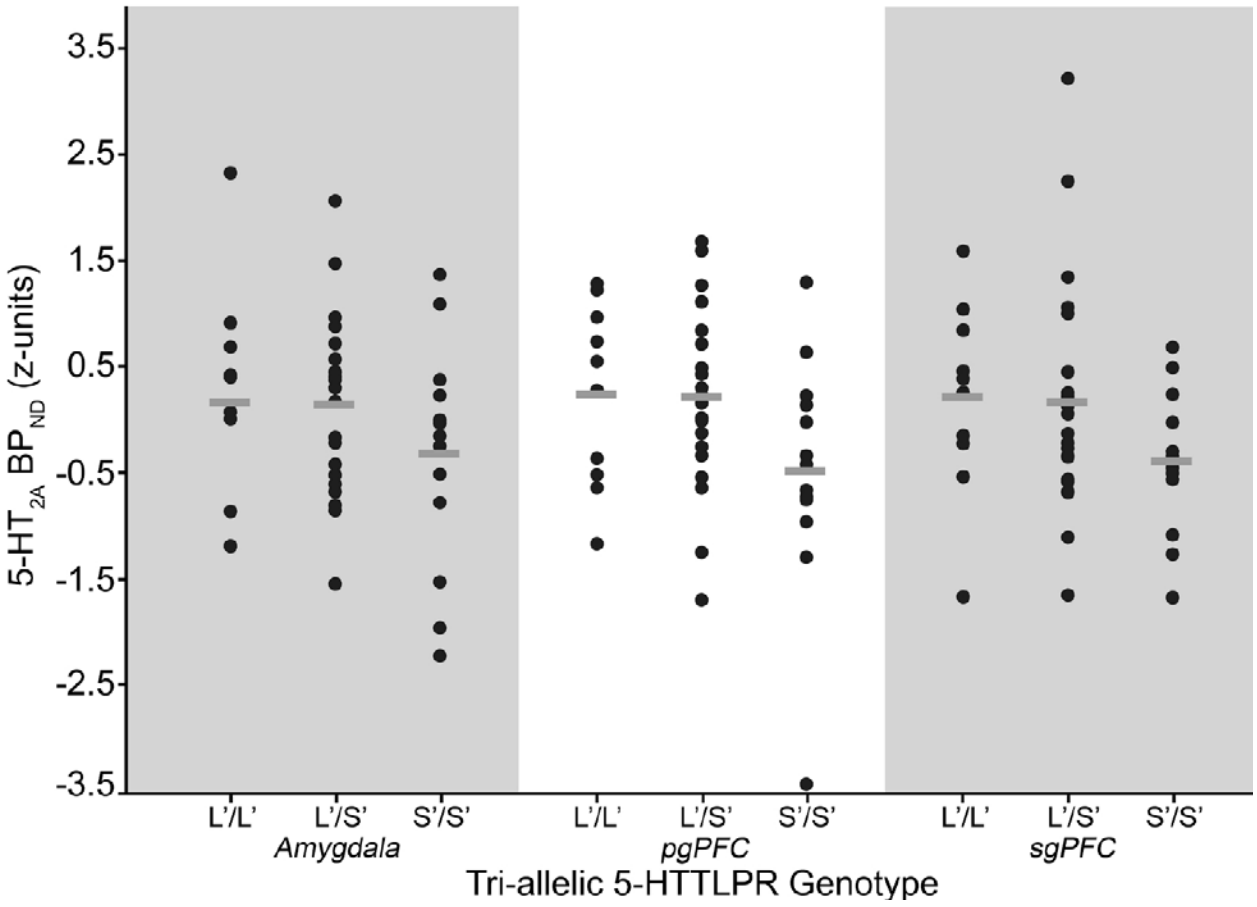

Figure 16. Regional 5-HT_{2A} binding associated with tri-allelic 5-HTTLPR genotype group. Relative to L'/L' individuals, S' carriers displayed reduced 5-HT_{2A} binding across regions as a trend-level (3 groups: $F(2,39) = 2.87$, $p = 0.07$; L'/L' vs. S' carriers: $F(1,40) = 0.83$, $p = 0.37$). Plots indicate single subject 5-HT_{2A} binding values standardized with respect to age and sex within the amygdala, pgPFC and sgPFC grouped by tri-allelic 5-HTTLPR genotype. Gray line indicates mean.

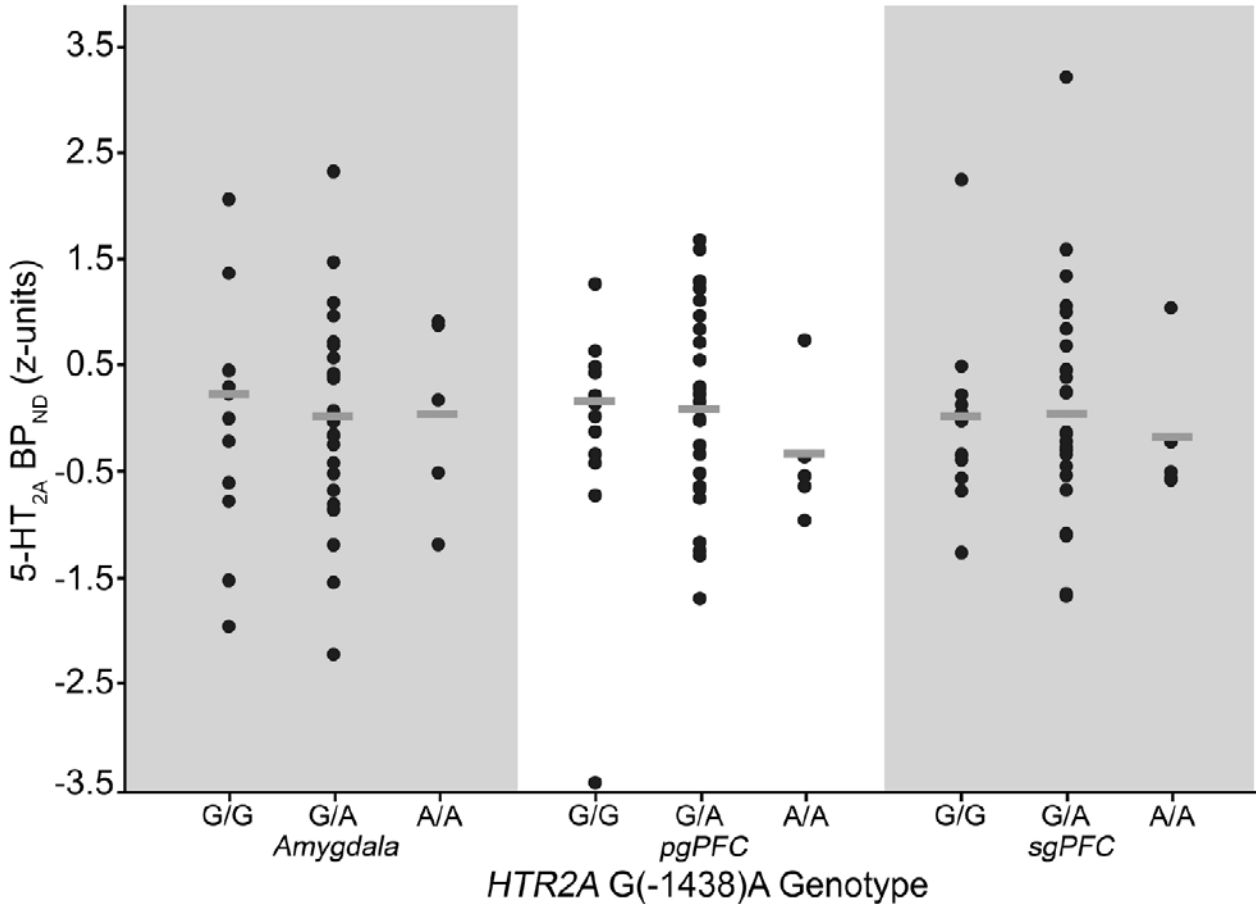

Figure 17. Regional 5-HT_{2A} binding not associated with *HTR2A* G(-1438)A genotype group (3 groups: $F(2,37) = 0.25$, $p = 0.77$; G/G vs. A-carriers; $F(1,38) = 0.09$, $p = 0.77$. Plots indicate single subject 5-HT_{2A} binding values standardized with respect to age and sex within the amygdala, pgPFC and sgPFC grouped by G(-1438)A genotype. Gray line indicates mean.

6.5 DISCUSSION

We used imaging genetics with neuroreceptor PET to test associations between three common serotonin-related gene polymorphisms and *in vivo* 5-HT_{1A} and 5-HT_{2A} binding within a population of healthy individuals with no history of psychopathology or psychotropic medication use. We found evidence for an association between the 5-HTTLPR and differences in both 5-HT_{1A} and 5-HT_{2A} binding across regions within a corticolimbic circuitry. In contrast, we found no evidence of association between *HTR1A* C(-1019)G and 5-HT_{1A} binding or *HTR2A* G(-1438)A and 5-HT_{2A} binding.

In our sample and using the 5-HTTLPR bi-allelic classification, carriers of the 5-HTTLPR S allele exhibited reduced 5-HT_{1A} binding across multiple regions within a corticolimbic circuitry. However, region-specific effects did not remain statistically significant when applying statistical thresholds accounting for multiple comparisons. Consideration of the tri-allelic 5-HTTLPR classification including rs25531 within *SLC6A4*, which putatively modulates the effects of the 5-HTTLPR on transcriptional efficiency (Hu et al., 2005), resulted in similar patterns albeit at trend-levels ($p = 0.08$). In contrast, the tri-allelic but not the bi-allelic 5-HTTLPR was associated with 5-HT_{2A} binding at a trend-level ($p = 0.07$) with S' carriers exhibiting reduced binding relative to L'/L' individuals.

Previous studies indicate the 5-HTTLPR S allele is consistently associated with relatively increased threat-related amygdala reactivity (Hariri et al., 2002b; Munafo et al., 2008). We have shown that 5-HT_{1A} binding within the DRN is inversely associated with threat-related amygdala reactivity (Fisher et al., 2006). Our current observation that 5-HT_{1A} binding is reduced

in S allele carriers is consistent with these findings and suggests that the 5-HTTLPR may bias threat-related amygdala responsiveness through downstream effects on 5-HT_{1A}. Reduced regional 5-HT_{1A} binding in S allele carriers has been reported in another sample of healthy volunteers (David et al., 2005). However, a third study of healthy volunteers did not observe a significant association (Borg et al., 2009), while a fourth study in healthy women reported relatively increased prefrontal 5-HT_{1A} in S allele carriers (Lee et al., 2003). Within all regions examined, including those in the prefrontal cortex, we observed reduced mean 5-HT_{1A} binding in S allele carriers, even when only considering women (data not shown).

We did not observe an association between *HTR1A* C(-1019)G and 5-HT_{1A} binding across regions. Though previous studies suggest the -1019G allele is associated with increased 5-HT_{1A} transcription *in vitro* (Czesak et al., 2006; Lemonde et al., 2003), our study did not reveal similar effects *in vivo*. Our null finding across all regions examined is similar with a previous study in healthy volunteers (David et al., 2005). One previous study identified a positive association between midbrain raphe 5-HT_{1A} binding and the -1019G allele, consistent with reported effects *in vitro* (Parsey et al., 2006c). However, this study included both healthy volunteers and patients with major depressive disorder.

We did not observe an association between *HTR2A* G(-1438)A and 5-HT_{2A} binding across regions. This null finding is consistent with a previous report in a smaller sample of healthy volunteers (Hurlemann et al., 2008). *In vitro* studies have reported both increased transcriptional efficiency and post-mortem 5-HT_{2A} density associated with the -1438A allele (Parsons et al., 2004; Turecki et al., 1999). Though a previous study using twin pairs indicates *in*

vivo 5-HT_{2A} binding is strongly genetically determined (Pinborg et al., 2008), our study does not provide evidence that the *HTR2A* G(-1438)A is source of such genetic variability.

The current lack of support for *in vivo* alterations in receptor binding associated with these two polymorphisms should be interpreted with several important caveats. First, the impact of *HTR1A* C(-1019)G and *HTR2A* G(-1438)A on 5-HT_{1A} and 5-HT_{2A} binding, respectively, has been examined in only a few small samples. Our current sample size limits our power to observe small effects. Unfortunately, this is typical of imaging genetics studies using receptor binding PET (Willeit and Praschak-Rieder, 2010). Second, regulatory variants such as those we considered likely affect 5-HT receptor expression as a function of environmental triggers (e.g., cortisol release after acute stress). Thus, associations between 5-HT_{1A} and 5-HT_{2A} binding and these genetic polymorphisms may be unmasked through gene-by-environment interactions such as those described for behavioral and clinical phenotypes (Caspi et al., 2010; Caspi et al., 2003). A recent study suggests DNA methylation may moderate the functional impact of *HTR2A* G(-1438)A on 5-HT_{2A} expression (Falkenberg et al., 2010). As we did not collect environmental measures (e.g., stressful life events, minutes of daylight) or measures of methylation, we cannot determine whether they moderate genetic effects on receptor binding. Future studies modeling possible gene-by-environment interactions and epigenetic factors are needed to more completely evaluate the impact of these polymorphisms on 5-HT receptor availability.

In contrast to our null findings with the *HTR1A* and *HTR2A* polymorphisms, we did find evidence for an association between polymorphisms of *SLC6A4* and *in vivo* 5-HT_{1A} and 5-HT_{2A} binding. Results from previous studies examining links between the 5-HTTLPR with *in vivo* 5-HTT binding have been inconsistent (Willeit and Praschak-Rieder, 2010) leading to speculation

that the direct effects of the 5-HTTLPR on 5-HTT may be confined to development and normalize into adulthood (Murthy et al., 2010). Our findings suggest that the 5-HTTLPR may contribute to alterations in 5-HT receptor availability and that these effects are present in adults. We considered the tri-allelic 5-HTTLPR classification in light of findings indicating rs25531 may moderate the effects of the 5-HTTLPR (Hu et al., 2005). We found a slightly stronger association between 5-HT_{1A} binding and the bi-allelic 5-HTTLPR classification, while the association with 5-HT_{2A} binding was stronger for the tri-allelic 5-HTTLPR classification.

It is not clear from our data why these associations differ as a function of the bi-allelic and tri-allelic 5-HTTLPR genotypes. We speculate that these differences may reflect cell-type specific ensembles of regulatory transcription factors and their relative sensitivity to rs25531. In other words, regulatory transcription factors in neurons expressing the 5-HT_{2A} but not the 5-HT_{1A} may be particularly affected by rs25531. In fact, similar cell-type specific effects have been reported for *HTR1A* C(-1019)G (Czesak et al., 2006). The presence of few L_G alleles (n = 6) within our current study, however, limits our capacity to establish the degree to which rs25531 moderates the influence of the 5-HTTLPR on 5-HT_{1A} or 5-HT_{2A} binding. Additional studies with larger sample sizes are needed to better determine whether rs25531 moderates the effect 5-HTTLPR on either 5-HT_{1A} or 5-HT_{2A} binding.

Imaging genetics studies combining neuroreceptor PET and BOLD fMRI in larger samples also hold unique promise in mapping links between genetic polymorphisms and individual differences in brain circuitry and behavior through effects on brain chemistry, leading to a deeper understanding of neurobiological mechanisms that shape behavior and related risk for psychopathology.

7.0 GENERAL DISCUSSION

Identifying neural circuitry and underlying molecular mechanisms that contribute to inter-individual variability in sensitivity to threat is important for understanding pathways that shape how we successfully navigate our environment and that contribute to risk for psychopathology. A corticolimbic circuit involving the amygdala and mPFC plays a key role in processing threat and generating behavioral and physiological responses to these stimuli. Serotonin is a key modulator of affect and sensitivity to threat, in part, through its capacity to bias the response of this circuitry. The distribution of the inhibitory 5-HT_{1A} and excitatory 5-HT_{2A} receptors indicate they are localized to mediate the effects of 5-HT signaling on this circuitry in the context of threat. Within the current dissertation, we used a multi-modal neuroimaging strategy in conjunction with an imaging genetics approach to determine whether regional 5-HT_{1A} and 5-HT_{2A} binding was predictive of threat-related corticolimbic reactivity and to identify genetic sources of variability of *in vivo* 5-HT_{1A} and 5-HT_{2A} binding. We assessed threat-related amygdala reactivity and corticolimbic circuit function with BOLD fMRI using a well-studied faces matching paradigm that involved fearful and angry facial expressions. Regional 5-HT_{1A} and 5-HT_{2A} binding was assessed using [^{11}C]WAY100635 and [^{18}F]altanserin PET, respectively.

We found that 5-HT_{1A} autoreceptor binding within the DRN was inversely associated with threat-related amygdala reactivity. This suggests that through the capacity to regulate 5-HT release at downstream targets, 5-HT_{1A} autoreceptors significantly contribute to threat-related amygdala reactivity. We found that mPFC 5-HT_{2A} binding was inversely associated with threat-related amygdala reactivity. Additional analyses indicated mPFC 5-HT_{2A} binding was positively associated with habituation of the amygdala and functional connectivity between the amygdala and mPFC. Medial PFC 5-HT_{1A} binding significantly moderated the negative association between mPFC 5-HT_{2A} binding and threat-related amygdala reactivity. This interactive effect is consistent with the co-localization of 5-HT_{1A} and 5-HT_{2A} receptors within mPFC and indicates this interaction significantly contributes to individual variability in threat-related amygdala reactivity.

We determined the association between three common functional polymorphisms, implicated in modulating 5-HT signaling, and 5-HT_{1A} and 5-HT_{2A} binding within this amygdala-mPFC corticolimbic circuit. We found that the S and L_G alleles of the 5-HTTLPR were associated with relatively decreased 5-HT_{1A} and 5-HT_{2A} binding within this corticolimbic circuit but that variants within both the *HTR1A* and *HTR2A* genes were not significantly predictive of individual variability in 5-HT_{1A} and 5-HT_{2A} binding, respectively. These findings suggest the 5-HTTLPR contributes to variability in 5-HT_{1A} and 5-HT_{2A} receptor availability within this corticolimbic circuit.

7.1 SUMMARY OF FINDINGS

7.1.1 Study 1

Through negative feedback on serotonergic neurons, the somatodendritic 5-HT_{1A} autoreceptor within the midbrain raphe plays a critical role in regulating 5-HT release, thus affecting 5-HT signaling at these downstream targets (Riad et al., 2000). We hypothesized that DRN 5-HT_{1A} binding would be inversely associated with threat-related amygdala reactivity reflecting a negative association between the capacity to regulate 5-HT release and amygdala response to threat. From findings described in Study 1 and consistent with our hypothesis, we found that threat-related amygdala reactivity was significantly inversely correlated with DRN 5-HT_{1A} autoreceptor binding. This suggests that relatively increased capacity for regulation of 5-HT release via DRN 5-HT_{1A} is associated with reduced threat-related amygdala reactivity (Fisher et al., 2006). Our findings are consistent with a subsequent study that found that threat-related amygdala reactivity was inversely associated with local 5-HTT binding within the amygdala (Rhodes et al., 2007). Taken together, these findings are supportive of a model wherein local 5-HT release within the amygdala is associated with potentiating the response of the amygdala to threat (Bigos et al., 2008; Burghardt et al., 2007; Burghardt et al., 2004; Forster et al., 2006).

7.1.2 Study 2

The predominant localization to proximal portions of the apical dendrite of glutamatergic neurons within prefrontal cortex suggests 5-HT_{2A} can facilitate top-down regulation of the amygdala via this amygdala-mPFC corticolimbic circuit. Within Study 2 we determined whether mPFC 5-HT_{2A} binding was significantly associated with threat-related amygdala reactivity, amygdala habituation and amygdala-prefrontal functional connectivity. We hypothesized this would be reflected by an inverse association between mPFC 5-HT_{2A} binding and threat-related amygdala reactivity. Consistent with our hypothesis, mPFC 5-HT_{2A} binding was significantly inversely correlated with threat-related amygdala reactivity. Additionally, habituation of amygdala reactivity to repeated presentation of threatening stimuli may reflect mPFC regulation of the amygdala analogous to that described in fear conditioning studies (Milad et al., 2007; Phelps et al., 2004; Quirk et al., 2006). We found that magnitude of amygdala habituation to fearful facial expressions was positively associated with mPFC 5-HT_{2A} binding. This finding is consistent with mPFC 5-HT_{2A} facilitating regulation of the amygdala via neural circuitry underlying habituation. Functional connectivity between the amygdala and mPFC was positively associated with mPFC 5-HT_{2A} binding suggesting that coordinated communication between these brain areas may be dependent, in part, upon 5-HT signaling via 5-HT_{2A}.

Our findings that mPFC 5-HT_{2A} binding is 1) significantly inversely associated with threat-related amygdala reactivity and positively associated with 2) habituation and 3) amygdala-mPFC functional connectivity are consistent with mPFC 5-HT_{2A} representing an integral receptor through which 5-HT modulates this corticolimbic circuit and sensitivity to threat. The fear

extinction literature implicates mPFC in regulating the amygdala via direct glutamatergic projections (Quirk et al., 2006). The predominant localization of 5-HT_{2A} receptors on pyramidal neurons throughout prefrontal cortex (Jakab and Goldman-Rakic, 1998) suggests 5-HT_{2A} receptors would facilitate this regulatory circuit, thus contributing to greater prefrontal feedback (Jakab and Goldman-Rakic, 1998). This model is consistent with our current findings.

A recent study reported a significant positive association between prefrontal 5-HT_{2A} binding, assessed with [^{18}F]altanserin PET, and neuroticism in healthy individuals (Frokjaer et al., 2008). Considering previous studies have found that neuroticism is positively associated with amygdala reactivity to negative emotional stimuli (Haas et al., 2007) this finding is at odds with our finding that mPFC 5-HT_{2A} binding is inversely associated with threat-related amygdala reactivity. Frokjaer and colleagues measured *in vivo* 5-HT_{2A} binding with a bolus-plus-infusion approach. However, it is not clear how this difference in methodology would contribute to our apparently discrepant findings. We did not collect the personality measure neuroticism, thus we cannot determine its association with 5-HT_{2A} binding within our sample. Future studies replicating either our findings or those of Frokjaer and colleagues are necessary to further evaluate links between prefrontal 5-HT_{2A} binding, brain function and behavior.

7.1.3 Study 3

The predominant localization of 5-HT_{1A} receptors within prefrontal cortex is on the initial axonal segment of glutamatergic neurons (Cruz et al., 2004). In Study 3 we determined whether mPFC 5-HT_{1A} significantly moderated the negative effects of mPFC 5-HT_{2A} on threat-related amygdala

reactivity. Its co-expression with 5-HT_{2A} receptors on glutamatergic neurons within prefrontal cortex (Amargos-Bosch et al., 2004) lead us to hypothesize mPFC 5-HT_{1A} binding would be positively associated with threat-related amygdala reactivity. We also hypothesize that mPFC 5-HT_{1A} would effectively "gate" the impact of mPFC 5-HT_{2A} on threat-related amygdala reactivity reflected by mPFC 5-HT_{1A} binding significantly moderating the association between mPFC 5-HT_{2A} binding and threat-related amygdala reactivity. Inconsistent with our hypothesis, we did not observe a significant correlation between mPFC 5-HT_{1A} binding and threat-related amygdala reactivity. However, mPFC 5-HT_{1A} binding did significantly moderate the negative association between mPFC 5-HT_{2A} binding and threat-related amygdala reactivity such that mPFC 5-HT_{2A} binding was significantly inversely associated with threat-related amygdala reactivity but only among individuals with mean or relatively low levels of mPFC 5-HT_{1A} binding. This moderation effect is consistent with the anatomical and functional organization of the top-down aspect of this corticolimbic regulatory circuitry and the effects of 5-HT_{1A} and 5-HT_{2A} receptors on pyramidal neuron excitability within prefrontal cortex (Celada et al., 2004). We did not identify a significant association between 5-HT_{1A} or 5-HT_{2A} binding within the amygdala and threat-related amygdala reactivity. As was discussed previously, this may be due to a more even distribution of these receptors across various neuronal populations within the amygdala (see Sections 2.3.5 and 2.3.6).

The findings described in Chapters 3-5 highlight the capacity to link receptor mechanisms with brain function through the use of a PET/fMRI multi-modal neuroimaging strategy. Multi-modal PET/fMRI neuroimaging studies are not common, particularly those involving the acquisition of PET receptor binding for two separate radioligands. Despite this,

linking individual variability in PET receptor imaging measures with brain function represents the most viable method for identifying receptor pathways that contribute to variability in brain function in humans *in vivo*. Our finding that the interaction between mPFC 5-HT_{1A} and 5-HT_{2A} binding was predictive of threat-related amygdala reactivity is both consistent with their co-localization and suggests that future studies should consider the use of multiple receptor targets with PET where there is evidence that interactive effects may bias the impact of a single receptor system on brain function.

7.1.4 Study 4

In Study 4 we examined whether common genetic polymorphisms within three 5-HT related genes (*SLC6A4*, *HTR1A* and *HTR2A*) were associated with individual variability in 5-HT_{1A} or 5-HT_{2A} binding within this corticolimbic circuitry.

7.1.4.1 *HTR1A* C(-1019)G polymorphism Previous *in vitro* studies suggest the G-allele of the *HTR1A* C(-1019)G SNP is associated with increased 5-HT_{1A} transcription (Czesak et al., 2006; Lemonde et al., 2003). Thus, we hypothesized that the G-allele would be associated with increased 5-HT_{1A} binding *in vivo*. We found no evidence of an association between *HTR1A* C(-1019)G genotype status and regional 5-HT_{1A} binding. One previous study reported a similar null finding (David et al., 2005) while a third study reported that the G-allele was associated with relatively increased DRN 5-HT_{1A} binding in a cohort of both healthy controls and individuals with major depression (Parsey et al., 2006c). Our null finding may be due to transient effects of

this regulatory variant on transcription that normalize throughout adulthood. The relatively broad age range of participants in our study may introduce additional confounds that vary across the lifespan and may limit our capacity to observe an effect. It should also be noted that our small sample size may have limited our capacity to observe a significant effect.

7.1.4.2 *HTR2A* G(-1438)A polymorphism A previous *in vitro* study suggests the A-allele of the *HTR2A* G(-1438)A SNP is associated with relatively increased 5-HT_{2A} transcription (Parsons et al., 2004). Thus, we hypothesized that the A-allele would be associated with increased 5-HT_{2A} binding *in vivo*. Inconsistent with our hypothesis, this genetic variant was not significantly predictive of regional 5-HT_{2A} binding. A previous study in twins observed that 5-HT_{2A} binding was strongly genetically determined (Pinborg et al., 2008). Despite this, our finding suggests *HTR2A* G(-1438)A does not contribute to individual variability in 5-HT_{2A} binding. The genetic variant we examined is in linkage disequilibrium with a significant block of the *HTR2A* promoter region proximal to the transcription start site (Spurlock et al., 1998). This suggests the *HTR2A* G(-1438)A variant identifies genetic variation within a large section of the proximal promoter region. Additional variants within the 3' portion of the large 2nd intron of the *HTR2A* gene and within the 3' untranslated region may provide additional insight into genetic sources of variability in 5-HT_{2A} binding. A study found that response to SSRI treatment within a large cohort of depressed individuals was predicted by genetic variants within this tail-end region of the *HTR2A* gene (McMahon et al., 2006). Future studies are necessary to determine whether genetic variation within this region contributes to variability 5-HT_{2A} receptor function or broader aspects of 5-HT signaling.

7.1.4.3 5-HTTLPR The 5-HTTLPR has been associated with alterations in 5-HT signaling and effects on function and structure of this amygdala-mPFC corticolimbic circuit. However, its effects on specific 5-HT receptors are not fully understood. We hypothesized that S-carriers would exhibit relatively diminished regional 5-HT_{1A} and 5-HT_{2A} binding on the basis that increased 5-HT signaling would contribute to desensitization and relative down-regulation of both the 5-HT_{1A} and 5-HT_{2A} receptor. Consistent with our hypothesis, we identified a significant association wherein S-carriers exhibited relatively reduced 5-HT_{1A} binding compared to L/L individuals. We observed a similar effect when considering the rs25531 A/G SNP. However, this effect did not reach significance. Our findings are consistent with a previous study (David et al., 2005) though other studies have reported no association with 5-HT_{1A} binding (Borg et al., 2009). Consistent with our hypothesis, we found 5-HT_{2A} binding across regions was relatively diminished in L_G/S allele carriers relative to L_A/L_A homozygotes at a trend-level. We found no effect of bi-allelic 5-HTTLPR group on 5-HT_{2A}. To our knowledge, this is the first study to consider the effect of the 5-HTTLPR polymorphism on 5-HT_{2A} binding. Future studies are necessary to determine whether these effects are maintained across similar cohorts or within a larger sample. However, our findings provide evidence for 5-HT receptor mechanisms through which 5-HTTLPR status biases 5-HT signaling and may contribute to previously observed associations with brain function, personality and related risk for psychopathology.

7.1.5 5-HTTLPR, 5-HT receptor binding and amygdala response to threat

Results from the multi-modal neuroimaging studies outlined in Chapters 3-5 provide novel evidence of specific molecular mechanisms through which 5-HT modulates the function of a corticolimbic circuit in response to threat. Linking genetic variants with individual variability in these pathways benefits our capacity to leverage genetic information in the context of understanding sources of risk for psychopathology (Hariri, 2009). Findings from Study 4 provide support for the well-studied 5-HTTLPR contributing to individual variability in 5-HT_{1A} and 5-HT_{2A} binding within this corticolimbic circuitry, which based on Studies 1-3, has clear implications for variability in threat-related brain function. This represents novel insight into the receptor pathways through which 5-HTTLPR may bias brain function, personality and related risk for psychopathology.

7.2 ADDITIONAL SEROTONERGIC AND MONOAMINERGIC MECHANISMS

As was briefly discussed earlier (see Section 2.3.9), there is compelling reason to consider the 5-HT_{1A} and 5-HT_{2A} receptors in modulating this corticolimbic circuitry, but it is likely that additional 5-HT receptor mechanisms play an important role. A regulator of volumetric 5-HT neurotransmission is the capacity for local reuptake via 5-HTT. Thus, mPFC 5-HTT may play an important role in moderating the effects of 5-HT signaling on extra-synaptic receptor systems within mPFC. Our findings suggest that an interaction between mPFC 5-HT_{1A} and 5-HT_{2A}

receptors contributes to variability in threat-related amygdala reactivity (Chapter 5). Similarly, mPFC 5-HTT may play a significant role in moderating the effects of 5-HT signaling within mPFC. Well-characterized radioligands exist for quantifying 5-HTT binding *in vivo* (e.g., [^{11}C]DASB), however, low 5-HTT concentrations and low specific binding within cortical regions precipitate the need to improve 5-HTT PET imaging technology before considering the *in vivo* study of such interactions (Frankle et al., 2004).

We found evidence of an inverse association between threat-related amygdala reactivity and 5-HT_{1A} (see Section 3.2). However, threat-related amygdala reactivity was not significantly associated with 5-HT_{1A} or 5-HT_{2A} binding within the amygdala. As was discussed earlier, 5-HT_{1A} receptors within the amygdala have been observed distributed evenly across GABAergic and glutamatergic populations (Aznar et al., 2003). Study of 5-HT_{2A} immunolabeling within the amygdala has reported inconsistent localization (McDonald and Mascagni, 2007). Low specific 5-HT_{2A} binding with PET hinders the capacity to identify associations between amygdala 5-HT_{2A} binding and brain function. A recent study in rodents found that 5-HT_{2C} signaling within the amygdala contributed to increased responsiveness to stress (Christianson et al., 2010). This finding is consistent with another study in rodents showing that 5-HT_{2C} antagonism blocked increased fear expression related to acute increases in 5-HT release (Burghardt et al., 2007). Though a 5-HT_{2C} PET radioligand with favorable *in vivo* imaging characteristics is not currently available, these findings suggest this receptor mechanism may plan an integral role in the capacity for 5-HT to potentiate the response of the amygdala to threat. Functional genetic variants within the *HTR2C* gene may provide insight into its role in threat-related amygdala reactivity within humans.

Dopaminergic and noradrenergic projections to both the amygdala and prefrontal cortex indicate they likely also play a role in modulating corticolimbic circuit function. In a recent PET/fMRI multi-modal neuroimaging study in a healthy adult cohort, dopamine D_1 but not D_2 receptor binding within the amygdala was found to be positively associated with threat-related amygdala reactivity (Takahashi et al., 2010). Another PET/fMRI study in humans found that the capacity for dopamine storage within the amygdala, assessed with [^{18}F]DOPA PET, was positively correlated with BOLD response to negative emotional scenes in both the amygdala and ACC (Kienast et al., 2008). Additionally, a previous BOLD fMRI study using a pharmacological challenge indicated threat-related amygdala reactivity is relatively increased following acute amphetamine administration (Hariri et al., 2002a). Lastly, a study in rodents found that the capacity for mPFC to affect behavioral responses to conditioned stimuli was dependent upon dopamine D_4 receptor signaling within mPFC (Laviolette et al., 2005).

7.3 mPFC REGULATION OF MIDBRAIN RAPHE: AN INDIRECT PATHWAY

A topic briefly mentioned within the Discussion of Study 2 (see Section 4.4) was the possibility that 5-HT_{1A} and 5-HT_{2A} receptor function modulates 5-HT signaling and threat-related amygdala reactivity via an alternative indirect pathway. Electrophysiological studies in rodents indicate that mPFC 5-HT_{1A} and 5-HT_{2A} signaling can facilitate prefrontal-mediated regulation of 5-HT release via mPFC projections to the DRN (Boothman et al., 2003; Casanovas et al., 1999; Celada et al., 2004; Hajos et al., 1999; Peyron et al., 1998). This is consistent with an anatomical study

in rodents that identified projections from mPFC to the midbrain raphe, synapsing predominantly onto GABAergic neurons, which in turn provide local negative feedback on 5-HT neurons (Jankowski and Sesack, 2004). This represents an indirect mechanism by which mPFC 5-HT signaling can modulate 5-HT signaling within the amygdala via effects on the excitability of serotonergic neurons within the DRN. Additional findings in rodent studies found that this mPFC-DRN pathway affected the classification a particular stressor as "controllable" or "uncontrollable", which impacted subsequent anxiety- and depressive-related behaviors (Amat et al., 2005; Maier and Watkins, 2005). Evaluating the effects of manipulating local 5-HT_{1A} and 5-HT_{2A} signaling in the context of these types of behavioral paradigms would reveal their role in modulating this circuitry.

The net effect of this indirect mechanism would be consistent with mPFC 5-HT_{2A} receptors facilitating regulation of the amygdala. The net effect of this indirect mechanism would be inconsistent with our hypothesized role of mPFC 5-HT_{1A} receptors (i.e., via the indirect mechanism 5-HT_{1A} would contribute to decreased amygdala reactivity via negative regulation of 5-HT release). These conflicting effects may explain our finding that mPFC 5-HT_{1A} was not significantly predictive of threat-related amygdala reactivity.

7.4 FUTURE CONSIDERATIONS

The results reported here provide novel evidence associating 5-HT_{1A} and 5-HT_{2A} binding with amygdala-mPFC corticolimbic circuit function and genetic sources of variability in 5-HT_{1A} and 5-

HT_{2A} binding. Additional studies including those suggested below can extend the findings reported here toward further understanding the role of 5-HT receptor mechanisms in modulating the neural circuitry underlying sensitivity to threat.

7.4.1 Pharmacological challenge

Previous studies employing pharmaco-fMRI protocols have reported SSRI exposure contributes to significant alterations in amygdala reactivity to emotionally salient faces (Bigos et al., 2008; Harmer et al., 2006). Pharmacological challenge of specific 5-HT receptors or 5-HTT blockade in the context of a multi-modal neuroimaging strategy (pharmaco-PET/fMRI) may provide more direct evidence implicating 5-HT_{1A}, 5-HT_{2A} or other receptors in mediating the effects of 5-HT signaling on corticolimbic circuit function. Though speculative, our current findings suggest that 5-HT_{1A} and 5-HT_{2A} binding would predict individual variability in changes in brain function following antidepressant exposure. For example, we would predict that lower DRN 5-HT_{1A} binding would be associated with diminished capacity for negative feedback following short-term 5-HT reuptake blockade contributing to increased threat-related amygdala reactivity compared to individuals with higher DRN 5-HT_{1A} binding. The extra-synaptic localization of the 5-HT_{2A} receptor suggests volume transmission is necessary for signaling. Accordingly, experimentally increasing 5-HT neurotransmission (via pharmacologic challenge with an SSRI) in the context of relatively higher mPFC 5-HT_{2A} binding should bias effects toward greater prefrontal drive and lesser amygdala reactivity relative to individuals with less mPFC 5-HT_{2A} binding.

The use of a pharmacological challenge can also inform our understanding of neurobiological mechanisms that mediate responsiveness to treatment strategies in clinical cohorts (i.e., "response to treatment" or "remission"). Despite being commonly prescribed, sensitivity to SSRI treatment for depression is low (Trivedi et al., 2006). The capacity to identify individuals who may be more or less responsive to SSRI treatment is critical for the development of more effective strategies and may substantially diminish financial costs related to unsuccessful treatment protocols. Additionally, pharmacological challenge paradigms may identify populations of individuals for whom specific treatment strategies are effective. Linking changes in brain function following pharmacological challenge (e.g., SSRI administration) with pre- and/or post-treatment 5-HT receptor binding with PET would implicate 5-HT receptors in predicting treatment-related alterations in brain function and behavioral response.

Imaging genetics is another potentially useful tool for furthering our understanding of these mechanisms (Hariri and Holmes, 2006; Hariri and Weinberger, 2003; Meyer-Lindenberg and Weinberger, 2006). Common functional genetic polymorphisms can be used as putative indicators of relative differences in 5-HT signaling (e.g., 5-HTTLPR) and, against this genetic background, specific models of relative 5-HT receptor effects can be evaluated. A bias towards greater prefrontal drive and reduced amygdala reactivity via 5-HT_{2A} signaling would be predicted in individuals possessing genetic variants associated with relatively increased 5-HT neurotransmission (e.g., 5-HTTLPR S-carriers relative to L/L individuals).

7.4.2 Epigenetics

Epigenetic mechanisms modify DNA through mechanisms that do not involve alterations in the underlying DNA sequence. For example, methylation of nucleotides within or surrounding a particular gene can modify gene transcription (Sharma et al., 2010). Environmental factors may contribute to differences in behavior by affecting epigenetic modifications such as methylation which in turn moderate gene expression or the functional effects of genetic polymorphisms (Weaver et al., 2004). A recent study suggests DNA methylation may moderate the functional impact of *HTR2A* G(-1438)A on 5-HT_{2A} receptor expression (Falkenberg et al., 2010). Traumatic life events may contribute to alterations in underlying neurobiology by facilitating or disrupting methylation at specific loci which subsequently affects transcriptional efficacy of a particular gene or set of genes (Meaney, 2010). This can also be important for contextualizing the "functional" effects of a specific polymorphism. Linking environmental factors that contribute to these epigenetic mechanisms may provide insight into neurobiological mechanisms through which gene-by-environment interaction effects modulate risk for psychopathology (Caspi et al., 2003).

7.4.3 Genetic epistasis

Imaging genetics has primarily focused on leveraging knowledge regarding the functional effects of single, relatively common genetic variants to make hypotheses about how a variant may predict differences in receptor binding, brain function, personality or risk for

psychopathology. Genetic epistasis (i.e., gene-gene interactions) may facilitate the identification of genetic sources of variability in underlying neurobiology. For example, Pezawas and colleagues found that *BDNF* G(196)A genotype (more commonly referred to as *BDNF* Val66Met) moderated the effects of 5-HTTLPR status such that differences in ACC volume between L/L and S-carriers were more pronounced in BDNF Met-carriers (Pezawas et al., 2008). Many candidate polymorphisms, including those considered herein (e.g., 5-HTTLPR, *HTR1A* C(-1019)G, *HTR2A* G(-1438)A) are variants within the promoter region of genes. As such, their functional effects are likely through modulation of transcriptional efficacy. However, the extent to which these variants modulate transcriptional efficacy may be moderated by independent genetic variants that in turn affect the expression of the relevant transcription factors. Future studies are likely to benefit from considering such effects.

Investigating gene-gene interactions, however, requires larger sample sizes to account for the expectedly small group-size of individual's homozygote for the minor allele at two independent loci. For example, two independent genetic variants each with a minor allele frequency of 0.4 would predict "double minor-allele homozyogotes" with a frequency of 0.03. In the case of our current imaging genetics study, it is possible that gene-gene effects or epigenetic factors may have diminished our capacity to observe a direct association between *HTR1A* C(-1019)G and *HTR2A* G(-1438)A status and 5-HT_{1A} or 5-HT_{2A} binding.

7.4.4 Mediation analysis

Associating genetic polymorphisms with individual differences in personality through effects on underlying molecular mechanisms (e.g., 5-HT_{1A}) or brain function (e.g., threat-related amygdala reactivity) provides a compelling link through which we can interpret the contributions of genetic variants to differences in personality. In a recent study we identified an association wherein *HTR1A* C(-1019)G SNP contributed to individual variability in trait anxiety but only via its indirect effects through threat-related amygdala reactivity (Fakra et al., 2009). This finding suggests that the amygdala response to threat-related stimuli represents a neurobiological mechanism affected by this genetic variant and in turn is predictive of individual variability in trait anxiety. Similar models can be applied to identify 5-HT receptor mechanisms through which specific genetic variants contribute to individual variability in brain function or personality. These complex statistical models, however, require much larger cohorts than those available here. Simulation studies suggest sufficient power to detect these types of effects require a sample size of 100 or greater where direct effect sizes are moderate to large (MacKinnon et al., 2002).

The S-allele of the 5-HTTLPR polymorphism has been repeatedly associated with relatively increased threat-related amygdala reactivity (Hariri et al., 2002b; Munafo et al., 2008). In light of our findings that the 5-HTTLPR is associated with 5-HT_{1A} and 5-HT_{2A} binding, the effects of the S-allele on threat-related amygdala reactivity may be mediated, in part, through its effects on 5-HT_{1A} and 5-HT_{2A} binding. Identifying these types of links are critical for

understanding the mechanisms through which genetic variants contribute to variability in brain function and related personality.

7.4.5 BOLD fMRI: paradigm shift

The BOLD fMRI faces matching paradigm used within this dissertation is designed primarily to elicit maximal response of the amygdala with sufficient inter-individual variability (Hariri et al., 2000; Hariri et al., 2002c). Studies determining the reproducibility of amygdala reactivity using similar paradigms indicated stable amygdala reactivity up to 1-2 years following the initial scan (Johnstone et al., 2005; Manuck et al., 2007). Previous associations between threat-related amygdala reactivity and trait anxiety support the amygdala response as a potential neurobiological correlate of sensitivity to threat and thus a very useful metric for probing genetic and molecular mechanisms underlying sensitivity to threat. Despite this, we did not observe top-down regulation of the amygdala via mPFC (i.e., negative functional connectivity) or task-dependent variation in functional connectivity (i.e., psychophysiological interactions; (Friston, 1994) within our BOLD fMRI analyses. Future studies employing alternative or additional paradigms perhaps better suited to elicit measurable engagement of this distributed circuit may complement the capacity to link 5-HT receptor binding and the distributed response of this corticolimbic circuit. Paradigms such as “matching vs. labeling” (Hariri et al., 2000), emotional regulation (Ochsner and Gross, 2005) or fear conditioning paradigms (Phelps et al., 2004) may complement the faces matching paradigm used herein through elicitation of

measurable engagement of this distributed circuit and significant task-dependent variation in functional connectivity in a manner that may also map onto PET receptor binding measures.

7.5 FUTURE RESEARCH

The current set of studies provides novel evidence for 5-HT_{1A} and 5-HT_{2A} receptors mediating the effects of 5-HT signaling on the response of a corticolimbic circuit to threat-related stimuli. Future studies, including those outlined below, can build on these findings to more completely understand how 5-HT mechanisms modulate threat-related corticolimbic circuit function, personality and related risk for affective disorders. Complementary neuroimaging techniques can be used to further determine the association between specific 5-HT mechanisms and threat-related corticolimbic circuit function. Additional paradigms can more thoroughly model brain function and behavior in response to threat offering the opportunity to more effectively model 5-HT mechanisms associated with inter-individual differences in threat-related corticolimbic circuit function. Modifying inclusion/exclusion criteria would further maximize the capacity to isolate variation in threat-related brain function specific to 5-HT mechanisms and relate it to variation in personality that is associated with risk for affective disorders.

7.5.1 Modeling additional serotonin mechanisms

Of the many mechanisms regulating 5-HT neurotransmission, the 5-HT_{1B} and the 5-HT_{2C} are of particular importance as they govern local pre-synaptic release of 5-HT and post-synaptic stimulation, respectively, at multiple corticolimbic targets including the amygdala (Hariri and Holmes, 2006; Holmes, 2008; Sharp et al., 2007). Future studies aimed at elucidating the association between 5-HT_{1B} and 5-HT_{2C} signaling and threat-related corticolimbic circuit function would further develop our understanding of 5-HT mechanisms that bias sensitivity to threat. Reflecting the capacity for 5-HT_{1B} to negatively regulate 5-HT release, we would hypothesize that greater amygdala 5-HT_{1B} binding assessed with PET would be associated with reduced threat-related amygdala reactivity. Future hypotheses can be developed using pharmacological challenge paradigms (e.g., receptor-specific antagonism) and imaging genetics to model 5-HT mechanisms that are without suitable PET radioligands (e.g., 5-HT_{2C} receptor). Beyond considering 5-HT mechanisms individually, modeling the capacity for function of many 5-HT mechanisms within a single cohort offers the opportunity to quantify effects of interactive 5-HT mechanisms and potentially develop individual-specific models of distributed 5-HT signaling and its effects on threat-related corticolimbic circuit function and aspects of personality related to threat sensitivity.

7.5.2 Additional paradigms for modeling corticolimbic brain function

Future hypotheses can also target behavioral paradigms outside the fMRI scanning environment to potentiate and stratify inter-individual differences in threat sensitivity, broadening variation in threat-related brain function that can be associated with 5-HT mechanisms. For example, a behavioral stressor (e.g., sleep deprivation, pharmacological stressor) prior to a PET/fMRI neuroimaging session (e.g., fMRI and 5-HTT PET) can assess how stressor exposure moderates the association between 5-HTT binding and threat-related corticolimbic circuit function. We can also assess the association between changes in threat-related amygdala reactivity between pre- and post-stressor exposure and pre-stressor assessed 5-HTT binding. Related findings would provide insight into how stress-exposure moderates the association between underlying brain chemistry (e.g., capacity for 5-HTT function) and brain function (e.g., threat-related amygdala reactivity).

Similarly, components of corticolimbic circuit function can be more effectively modeled using fMRI paradigms such as fear conditioning and fear extinction, complementing the threat-related faces matching paradigm employed in the current set of studies. Future hypotheses can determine whether particular 5-HT mechanisms are more strongly associated with distinct aspects of threat-related corticolimbic circuit function. For example, *HTR2C* variants would by hypothesized to be significantly associated with amygdala reactivity during fear conditioning trials reflecting the effect of amygdala 5-HT_{2C} signaling on amygdala reactivity (Christianson et al., 2010). Conversely, mPFC 5-HT_{2A} binding would be hypothesized to be associated with task-related amygdala-mPFC functional connectivity reflecting its role in facilitating prefrontal-

mediated regulation of amygdala reactivity (Fisher et al., 2009). Despite this, complementary fMRI paradigms can more effectively model aspects of threat-related corticolimbic circuit function, which can be related to individual differences in 5-HT mechanisms.

7.5.3 Natural sources of variability

Modifying the inclusion/exclusion criteria used in the current set of studies can increase variability in relevant aspects of behavior and personality while limiting variation of potential confounding variables maximizing the capacity to isolate specific effects of 5-HT mechanisms on threat-related corticolimbic circuit function and personality. A broader personality phenotype can be obtained by removing the exclusion criterion of lifetime history of psychiatric illness. This would improve the capacity for this type of study to effectively model how the association between 5-HT receptor availability and threat-related corticolimbic circuit function varies as a function of risk for affective disorders based on measures of personality.

Future studies aimed at modeling how age-related effects on threat-related brain function (Fisher et al., 2009; Tessitore et al., 2005), 5-HT_{2A} binding (Meltzer et al., 1998) and other neuronal processes (Morrison and Hof, 1997) may provide unique insight into how risk for affective disorders and sensitivity to threat varies with age. The impact of age-related effects on how 5-HT mechanisms shape corticolimbic circuit function is not a central focus of the current body of research. As such, narrowing the age-range into early to middle adulthood (e.g., 22-40 years old) would serve to constrain its potential confounding effects and increase the signal-to-noise of effects of interest. Broadening and narrowing specific inclusion/exclusion

criteria would benefit the ability to model the associations between 5-HT receptor binding and threat-related brain function more effectively across a broader spectrum including individuals at greater risk for affective disorders.

7.5.4 Limitations of future research

Modeling the function of multiple receptor systems within a single cohort is limited by the need to consider radiation exposure regulations. Additionally, the usefulness of measuring changes in brain function in response to pharmacological challenge is limited by BOLD being an indirect measure of neural activity and systemic effects of the pharmacological challenge that may confound findings. A limitation of using additional stressor paradigms within and outside the scanning environment is it may precipitate the need for scans over multiple days which could negatively affect participant recruitment and increase participant attrition. Additionally, habituation of brain response to multiple emotionally-salient paradigms within a single scanning session may diminish variation in observed effects. A limitation of this approach is that removing the exclusion criterion of individuals based the DSM-IV may lead to inclusion of individuals with current clinical illness.

7.6 FINAL SUMMARY

In conclusion, through the use of a combined multi-modal neuroimaging and imaging genetics approach the findings from our current set of studies provide novel evidence for 5-HT_{1A} and 5-HT_{2A} as mechanisms through which 5-HT modulates the function of a corticolimbic circuit critical for processing threat in humans. Our finding that 5-HTTLPR genotype is associated with both 5-HT_{1A} and 5-HT_{2A} binding within this corticolimbic circuit implicates its effects on these receptor pathways as molecular mechanisms through which it contributes to individual variability in brain function, personality and related risk for psychopathology.

8.0 BIBLIOGRAPHY

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