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Influence of a Serotonin Transporter Promoter Polymorphism (5-HTTLPR) on Corticolimbic Abnormalities in Bipolar Disorder An Integrated Genetics and Functional MRI Study

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An Integrated Genetics and Functional MRI Study

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Abstract

INFLUENCE OF A SEROTONIN TRANSPORTER PROMOTER POLYMORPHISM ON CORTICOLIMBIC ABNORMALITIES IN BIPOLAR DISORDER

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Bipolar disorder (BD) is associated with abnormalities of the subgenual anterior cingulate cortex (sgACC) and the amygdala, components of a corticolimbic neural system that subserves emotional regulation. The short *s* allele—as opposed to the long *l* allele—of a serotonin transporter promoter (5-HTTLPR) polymorphism is associated with more severe course features of BD and impaired functional connectivity between the sgACC and amygdala in healthy control (HC) individuals. This study tests the hypothesis that the *s* allele influences the dysfunction in the sgACC-amygdala neural system in BD. Twenty-six euthymic BD participants (17 *s* carriers, 9 *ll*) and 43 HC participants (31 *s*, 12 *ll*) completed an event-related functional magnetic resonance imaging scan while processing fearful, happy, or neutral faces. During fear and happy face processing, sgACC activation was significantly lower ($p < 0.05$) in the BD versus the HC group, and in HC and BD *s* carriers compared to HC and BD *ll* individuals respectively. In the sgACC region where BD activation was less than HC, response to emotional faces was lowest in the BD *s* group, suggesting that the *s* allele may contribute to more severe sgACC dysfunction in a subset of individuals that represent a distinct genetically-derived subtype within the heterogeneous BD clinical phenotype. Thus, sgACC dysfunction may be an endophenotype of BD, and the *s* allele appears to influence this dysfunction in a subset of BD individuals. Future treatment may be optimized for this subset, by targeting treatments to affect this system, and by further study of treatment response amongst those who carry the *s* allele.

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Introduction

I. Bipolar Disorder

The lifetime prevalence of bipolar disorder is estimated to be around 1.4%, with a mean and median onset of 18 years of age (1). There is an exceptionally high rate (10 – 15%) of completed suicide amongst this population (1), a rate that is the highest among individuals with any psychiatric illness. In addition, bipolar disorder is the sixth leading cause of disability worldwide (2), with an estimated economic burden of greater than $20 billion in the United States per year, in terms of both the direct costs of treatment and indirect costs of lost wages and productivity (3). Clearly, there is a large societal burden associated with bipolar disorder, a reflection of its profound effect on not only the individual but also his or her family and surrounding community.

The hallmark of bipolar disorder is severe emotional dysregulation, and the disorder is defined, in the Diagnostic and Statistical Manual of Mental Disorders (4), by a clinical course of at least one manic, hypomanic, or mixed episode, often with intermittent major depressive episodes. Mania, seen in bipolar disorder type I, is characterized by at least one week (or any time course if hospitalization is required) of extreme mood elevation, exhibited as euphoria or irritability, and symptoms including grandiosity, decreased need for sleep, pressured speech, racing thoughts, distractibility, psychomotor agitation, impulsivity, increased goal-directed activity, and excessive involvement in pleasurable activities. Hypomania, seen in bipolar disorder type II, has similar symptoms, but of lesser duration and/or severity relative to mania. A mixed episode is one in which the individual experiences both manic and depressive symptoms every day for at least one week. To meet diagnostic criteria, episodes cannot be the direct

result of a general medical condition or exposure to substances of abuse or medications (4). Within the spectrum of the disorder, there are also subsets associated with distinct clinical features; these include individuals with pediatric-onset bipolar disorder, those who experience psychotic symptoms as part of the disorder, and those with rapid-cycling—having four or more distinct mood episodes in one year (4).

Unfortunately, despite potentially devastating consequences, there is usually a delay in diagnosing and treating individuals with bipolar disorder (5-6) and often treatment and medication strategies are changed due to side effects, ineffectiveness, and compliance difficulties. Thus, there is a vital and increasing interest in studying the pathophysiology of bipolar disorder with the hopes that research may better elucidate the molecular and cellular mechanisms that underlie the disorder, and therefore guide future treatment and management modalities. The goal of this work is to better understand how genetic variation may influence the development of neurobiological abnormalities in bipolar disorder and may contribute to and underlie the clinical variation within the spectrum of the illness.

II. Neuroimaging in Bipolar Disorder

Recent advances in the sophistication and availability of neuroimaging technology has dramatically improved the manner in which researchers study the structure and function of the human brain. Psychiatry in particular has benefited from the advent of these tools, as they have permitted non-invasive examination of neurobiological abnormalities amongst individuals with psychiatric illness (and comparison with healthy volunteers), thus increasing understanding and acceptance of the idea that neural circuitry dysfunction underlies the development of these illnesses (7).

Prior to current methodologies, studies of neural function were limited to ablation and electrical stimulation strategies in animal models, or evaluation of patients with discrete neurological deficits and attempts to correlate these with structural changes; innovations in technology, including functional magnetic resonance imaging (fMRI) as well as electroencephalography, magnetic resonance spectroscopy (which allows relative quantification of neuronal biochemicals), cerebral perfusion imaging, and nuclear medicine imaging modalities, dramatically changed the field by offering a window into the dynamic functioning of neural circuits. In particular, fMRI provides spatial and temporal resolution of brain structure and function, and, as a non-invasive instrument that does not involve administration of any dyes or radioactive tracers, allows for repeated longitudinal scanning without long-term risks to the participant (8).

Functional Magnetic Resonance Imaging

The current work employs fMRI to examine neural function in individuals with bipolar disorder and healthy comparison participants. FMRI is able to reveal changes in regional brain activity by highlighting differences in blood oxygenation with a temporal resolution on the order of seconds. During response to an fMRI task, neurons, and in turn local neural networks, expend energy in order to fire action potentials and signal other cells via neurotransmission across synapses. Thus, an increase in neural activity—in a region that is particularly relevant to the task—over time creates a local energy demand, and this is met by recruiting additional oxygen and glucose in the form of increased blood flow (9). This is known as the *hemodynamic response*. This relationship was first noted visually after scientists noted a 'flush of red blood' following exogenous electrical stimulation of an exposed area of a cat's brain in the late 19th century (10). The differing

magnetic signal properties between oxygenated and deoxygenated blood enable fMRI to non-invasively highlight changes in blood flow. This produces the blood oxygen level dependent (BOLD) contrast, and serves as a surrogate measure of neural activation (8).

BOLD contrast maps are derived from subtracting the response to a 'baseline' component of a task (e.g. presentation of a fixation cross-hair) from the experimental condition (e.g. presentation of an emotionally-salient stimulus), such that the resulting difference in activation can be attributed to the specific component of interest (i.e. processing of the emotional stimulus). An 'event-related' design is often used to present the stimuli to the participant in the scanner, with the condition(s) of interest presented repeatedly and as isolated events between varying periods of the baseline condition. This protocol decreases the likelihood of habituation from responding to a stimulus that appears at set-intervals, and allows modeling of the hemodynamic BOLD response over several data points (8).

In sum, fMRI is an important instrument in determining the neurobiological abnormalities associated with psychiatric illness, and, by keeping the above principles in mind, tasks can be designed to reliably identify areas in which the BOLD response varies in association with the stimulus of interest. Below, we review the insights fMRI and other imaging tools have revealed about neural processing in bipolar disorder, with particular focus on two key structures that are involved in the processing of emotions: the anterior cingulate cortex (ACC) and the amygdala.

Anterior Cingulate Cortex

The cingulate cortex is a structure that is located in the midline of the brain, superior to and surrounding the corpus callosum and extending anteriorly and wrapping

around the genu of the corpus callosum. It has long been considered part of the classic limbic system (11) and the historic Papez circuit (12) that anatomically defined the brain structures—and the relationships between the structures—in a neural circuit involved in emotion regulation. Ablative lesions of the cingulate lead to a variety of symptoms including emotional instability, dramatic mood and personality changes, and distractibility (13-14). Similarly, phenomenological descriptions of patients with known cingulate cortex epileptic seizure foci include alterations in mood state ictally and impulsive behaviors interictally (15).

Based on structural cytoarchitectural and functional distinctions, the cingulate cortex has been divided into the anterior cingulate cortex (ACC) that is involved with 'executive' functions and a posterior part that is 'evaluative.' The ACC consists of Brodmann areas 24, 25, 32, and 33 (16). The ACC has been further divided into a supragenual *cognitive* division that is situated superior to the anterior genu of the corpus callosum and is involved in allocating attention, executive functioning, and monitoring task completion; and the subgenual ACC (sgACC), an *affective* division that abuts the genu and extends ventrally and is involved in "assessing the salience of emotional and motivational information and the regulation of emotional responses" (17). The affective division is connected to several subcortical structures, including the amygdala, and an important function of the sgACC is the regulation of these subcortical structures.

These divisions of the ACC can also be distinguished by fMRI: within a sample of healthy volunteers, a cognitive version of a scanner task elicited activation in only the cognitive portion of the ACC (18) while an emotionally-valenced version of the same task led to increased activation in the affective division alone (19). In addition, tasks

designed to simulate emotional conflict, via presentation of emotionally-incongruent stimuli, have demonstrated the role of the sgACC in recognizing and then suppressing amygdala hyperactivation during emotional conflict (20). Thus, researchers have come to appreciate the central role of the sgACC in emotional regulation and processing, and have also designed fMRI probes that effectively interrogate this structure.

Given the profound emotional dysregulation that defines bipolar disorder, it follows that sgACC dysfunction has been associated with the illness. Multiple structural imaging studies of adults with bipolar disorder have demonstrated overall gray matter ACC volume reductions in comparison to healthy volunteers (21-22) and specifically within the sgACC (23). Interestingly, volumetric studies of the ACC in pediatric bipolar samples have yielded inconsistent results, with some groups reporting decreases (24-26) and others no difference (27-28), suggesting that structural changes in the ACC develop as a consequence of aberrant neurodevelopment during adolescence (29) or earlier (30).

FMRI and metabolic studies of individuals with bipolar disorder have been consistent with these structural studies, and have afforded the ability to assess differences across mood state. Decreased activation in the dorsal ACC during a cognitively-demanding fMRI task (31) and the sgACC in response to faces exhibiting emotional expressions (32) have been described in individuals with bipolar disorder. Blood flow and metabolism in the sgACC also appears to be abnormal in the depressive state (23). In addition, proton magnetic resonance spectroscopy of the ACC in participants with bipolar disorder revealed abnormal levels of choline-containing compounds thought be involved in neuronal signaling mechanisms, the levels of which correlated with depression severity at the time of scan (33); and myo-inositol—a compound that is part of

a biochemical pathway that may be targeted by lithium treatment— abnormalities in juveniles with pediatric-onset bipolar disorder (34).

In sum, sgACC abnormalities have been consistently reported in bipolar disorder across several neuroimaging modalities, and given its role in emotional processing, is likely associated with the profound mood dysregulation that defines the disorder.

Amygdala

The amygdala is a subcortical and evolutionarily important structure with significant reciprocal connections to the sgACC that is essential in processing emotional stimuli. The almond-shaped structure is located bilaterally in the anteromedial temporal lobe, anterior to the hippocampus (35). Amygdala pathology has been described in patients with elevated mood, grandiosity, distractibility, and psychosis as part of an epileptic syndrome (36-37). Similarly, animal models have revealed the importance of the amygdala in emotional memory and learning (38) and ablative lesions of the amygdala are associated with impulsivity, fearlessness, aggression, excessive involvement in pleasurable activities, and an inability to recognize familiar objects (39).

The role of the amygdala in emotional processing can be successfully interrogated by fMRI tasks; specifically, the amygdala is activated when presented with either positive or negative emotionally-valenced stimuli (40), with greatest activation seen in response to negative stimuli such as fearful or angry faces (40). Highlighting its role in social cognition, there is greater activation to pictures of human faces depicting negative emotions relative to non-face negatively-valenced images (41). The amygdala is also hyperactivated by subliminal presentation of negatively valenced stimuli (42), perhaps indicative of its evolutionarily important task of initiating rapid response to a possible

threat. Of note, patients with bilateral amygdala lesions lose the capacity to recognize the emotional valence of faces (43) and the amygdala also displays adaptive capacity: it exhibits response habituation and decreased responsivity when stimuli are presented repeatedly (44) without the development of an actual threat.

Given that there is evidence of impaired processing of emotional faces (45) and affective cues in bipolar disorder, especially during mania (46), it is not surprising that imaging research has found consistent evidence of amygdala abnormalities amongst this psychiatric population.

Structural imaging has shown reduced amygdala volumes in adolescents with bipolar disorder (26, 47-49), and this abnormality appears to remain stable throughout this neurodevelopmental epoch (50), until adulthood. Studies of amygdala size in adults with bipolar disorder have yielded highly variable results including decreased (47, 51), increased (52-54), and no difference (55) in comparison to matched healthy adults. The reason for this variability is unclear but may suggest that amygdalar reductions are specific to pediatric populations or subsets of the broader bipolar spectrum, and/or that the accumulation of neurotoxic exposures and medication effects may lead to changes in amygdala structure over time. Alternatively, the variability may reflect the heterogeneity of the adult population and MRI methodologies in general (56). Of note, this is the opposite of the ACC findings, where there was greater consistency in the adult populations compared to the pediatric populations, suggesting differential effects of neurodevelopment on these structures.

Functional imaging differs from structural results in that amygdala dysfunction has been described across bipolar populations and subsets. Specifically, adolescents (57-

58) and adults with bipolar disorder exhibited a heightened amygdala response to positively- and negatively-valenced emotional faces, and this was seen in manic, mixed, depressed, and euthymic participants (32, 59-62). Metabolic function and cerebral blood flow is also consistently elevated in the amygdala of individuals with bipolar disorder (63-64). In addition, medication appears to blunt and 'normalize' this hyperactivation in individuals with bipolar disorder (32). Lastly, preliminary results also reveal functional connectivity deficits between the amygdala and the ACC specifically, suggesting that communication between the two structures may be impaired in bipolar disorder (65).

In sum, there is a wealth of evidence suggesting that amygdala abnormalities are consistent across groups of individuals with bipolar disorder and neuroimaging modalities, and that this is likely associated with specific deficits in emotional processing, particularly the processing of facial expressions. The amygdala and sgACC form a connected circuit that appears to be functionally and structurally abnormal in the disorder. In addition, the findings of sgACC structural changes consistently seen in adults and not in pediatric samples, suggest that bipolar disorder is a neurodevelopmental disorder (56), whereas consistent amygdala volume changes and dysfunction in pediatric populations suggests that subcortical dysfunction may predate cortical abnormalities (66). These studies also highlight the potential role of functional assessment tools in the detection of early markers of bipolar disorder.

Other Cortico-Limbic Regions

Neurobiological dysfunction in bipolar disorder is not limited to the amygdala and ACC.

Functional and metabolic imaging has implicated abnormalities in subcortical structures such as the ventral striatum and thalamus (61, 67) as well as higher-order cortical structures such as the ventral prefrontal cortex (68-69). Thus, it is clear that aberrancy is present in several nodes of a network involved in emotion processing, of which the amygdala and ACC circuit appears to be particularly salient and the most relevant to bipolar disorder, and is the focus of this work.

III. Genetics and Bipolar Disorder

While neuroimaging has afforded 'visualization' of neural activity in individuals with bipolar disorder, the integration of genetics with neuroimaging has allowed insight into the molecular and cellular pathways that underlie these neural circuitry abnormalities. Bipolar disorder is highly heritable with estimates of heritability of 80% (70). There is an increased risk of development of disease in the relatives of affected probands (71), and greater concordance in monozygotic versus dizygotic twin pairs (70, 72). While several genetic loci have been linked to bipolar disorder, many results have failed replication attempts, and there has not been as much success as there has been in schizophrenia (73). A potential reason for this is that the variability within the spectrum of bipolar disorder (i.e. bipolar I vs. II, pediatric vs. adult-onset, cycling differences, presence of psychosis) suggests overlapping but possibly differentiable entities, and that different sets of genes may predispose to a particular phenotype within the spectrum (74).

To deal with this issue of phenotypic heterogeneity amidst a polygenic background, there has been a paradigm shift in the field, with a new focus on identifying and investigating *endophenotypes* within bipolar disorder. Endophenotypes are considered intermediate, internal phenotypes that are associated with the illness,

heritable, increased in frequency amongst relatives of probands, and state-independent. The genes that influence the heritability of these endophenotypes are assumed to be fewer in number than for the overall illness itself, and the endophenotype is meant to serve as a mechanistic bridge between the biochemical and cellular consequence of genes and the features of the clinical phenotype. Endophenotypes that have been evaluated in psychiatric illness including bipolar disorder include behavior traits, response to psychotropic medications and neurotransmitter depletion, and neurobiological abnormalities (75).

Initial studies associating a functional polymorphism within the promoter region of a serotonin transporter gene (SLC6A4) with anxiety-related personality traits (76-77) was of immediate interest to research in psychiatry as a potential substrate for an endophenotype model. The transporter protein regulates reuptake and transmission of serotonin in the synapse (78). The polymorphism (5-HTTLPR) consists of two common alleles that differ in length by 44 base pairs, with the dominant shorter *s* allele having decreased *in vitro* transcriptional activity compared to longer *l* allele, and this difference was seen in those with one or two copies of the *s* allele, suggesting that the *s* allele acts dominantly (76-77). The lack of a consistent *in vivo* demonstration of differential serotonin transporter binding and concentration in adults as a function of variation at the locus, however, suggests that the polymorphism may exert its effects on neurodevelopment early in life rather than directly altering binding (79). In addition, the phenotypic expression of the gene may also be related to specific environmental exposures, as carrying one or two copies *s* allele has been associated with increased incidence of depression and suicidality in relation to life stress (80-81).

The findings of decreased levels of serotonin metabolites and impaired turnover in individuals with bipolar disorder (82), plus the fact that the serotonin system is the target of many pharmacological therapeutics in bipolar disorder, suggests that influence and variation of the 5-HTTLPR polymorphism may explain some of the variation in the phenotypic expression of the disorder. Although genetic linkage studies have not definitively related this 5-HTTLPR polymorphism with bipolar disorder (83) nor found a susceptibility locus near the serotonin transporter gene (84), there is some evidence for an association of the *s* allele with younger age of bipolar onset (85), and differences in mood-state cycling (86), suggesting that it may be related to specific genetic subsets within the disorder spectrum.

In sum, while no specific genes have been definitively linked to bipolar disorder despite its high heritability, there is a growing interest for endophenotypes within the spectrum that may be more readily associated to a subset of genes, of which the 5-HTTLPR is a strong candidate.

IV. Imaging Genomics: Focus on the 5-HTTLPR Polymorphism

Given the associations of the 5-HTTLPR polymorphism with specific psychological traits and risk for development of psychiatric symptoms, the concept of *imaging genomics* was developed to attempt to bridge the mechanistic gap between genes and behavior by revealing the effect of genetic variation on neurobiological functioning. Building on the concept of endophenotypes, imaging genomics posits that neurobiological function is a more proximal consequence of genetic variation than behavior, and provides a quantifiable effect of this variation. Similarly, dysfunction in neural circuits may then be linked to behavior and clinical differences in participants. In

addition, while gene-behavior associations often require evaluation of thousands of subjects, the proximity of neurobiological function to gene effects implies that fewer subjects are required to achieve statistical power (87-88).

Using functional imaging to evaluate neural function, a seminal work by Hariri and colleagues found that healthy volunteers carrying the *s* allele had greater activation in the amygdala in response to fearful faces in comparison to *ll* homozygous individuals, suggesting that the *s* allele may mediate amygdala hyperreactivity which in turn contributes to the anxiety-related traits that have been linked to the locus (89). This intriguing result has been replicated in other healthy volunteer samples (90-92) and has been reported amongst participants with depression (93), social phobia (94), and as a trend in panic disorder (95)—individuals for whom amygdala hyperreactivity may indeed contribute to the development of psychopathology. Further, it follows that amygdala hyperreactivity is a plausible endophenotype—perhaps not specific to a single psychiatric illness, but associated with specific genes and clinical symptoms.

Of particular relevance to the current work, the *s* allele has also been associated with reductions in the gray matter volume of both the amygdala *and* the ACC in healthy volunteers (96). In addition, the *s* allele was associated with impaired structural and functional connectivity between the amygdala and the sgACC. There is evidence of coupling between the amygdala and the sgACC, suggesting a circuit whereby amygdala activation is transmitted to the sgACC, whose reciprocal connections in turn respond and suppress the amygdala (96). Given that the ACC contains the richest concentration of serotoninergic neurons of any cortical structure (97), it is not surprising that this structure is particularly influenced by variation in the serotonin transporter gene.

In sum, the field of imaging genomics has allowed identification of neural circuitry dysfunction as the results of variation at specific gene loci. In particular, the 5-HTTLPR polymorphism appears to influence the structure, function, and inter-structure connectivity of the ACC and the amygdala, and may be associated with distinct genetic subsets within the overall bipolar disorder phenotype.

Statement of Purpose and Hypothesis

Previous studies implicate aberrancy in both the (sgACC) and the amygdala in bipolar disorder, structures that share reciprocal connections and are prominently involved in affective processing. Furthermore, there is convergent evidence that the *s* allele of the 5-HTTLPR polymorphism is associated with abnormalities in these same regions. Thus, the aim of this project is to investigate whether variation in the 5-HTTLPR polymorphism influences dysfunction in the sgACC and the amygdala in bipolar disorder.

To achieve this end, healthy control participants (HC) and individuals with bipolar disorder (BD) were recruited to participate in fMRI scanning during processing of emotionally salient stimuli, namely a series of emotional faces exhibiting fearful, happy, and neutral expressions. Genotyping for the 5-HTTLPR polymorphism of the SLC6A4 gene allowed for comparison of BOLD fMRI signal within the sgACC and amygdala between diagnostic and genotypic groups.

It was specifically hypothesized that in the sgACC, response to fearful faces in particular would be 1) decreased in BD participants compared to HC individuals, and 2) decreased amongst *s* allele carriers compared to *ll* homozygotes within each diagnostic group, such that 3) within the area of decreased BD activation, the BD *s* carriers would have the greatest magnitude of dysfunction. Similar differences were predicted in response to happy faces, but of lesser magnitude, and no difference is predicted for neutral faces.

In addition, it is hypothesized that activation in response to fearful faces in the amygdala will be increased in 1) BD participants relative to the HC group and 2) in both

BD and HC carrying the *s* allele compared to *ll* homozygous individuals, such that 3) BD *s* carriers will exhibit the greatest magnitude of amygdala response. Similar relationships were expected for happy faces but of lesser magnitude; no difference was predicted for neutral faces.

Secondary analyses explored the potential influence of demographic (age and gender) and clinical factors on dysfunction in these regions. Clinical factors included presence of rapid cycling, history of psychosis, age of bipolar disorder diagnosis (all of which have been associated with 5-HTTLPR), as well as history of alcohol or substance abuse/dependence, and medication status and medication subclass.

Methods

Participants

The HC group included 43 participants, 60% of whom were female, with mean age and standard deviation of 28.8 ± 10.8 years (range 19 – 54), while the BD group included 26 participants, 65% female, age of 31.7 ± 13.2 yrs (range 18 – 60). Fifty-six percent of the HC group were Caucasian, 23% were African American, 9% were Asian American, and 12% were of other ethnicity, while 69% of the BD group were Caucasian, 26% were African American, and one individual (6%) was of other ethnicity, but not Asian American (see Table 1 for further details). Participants were recruited from the Yale School of Medicine Medical Center in New Haven, CT, the Veteran Affairs Connecticut Healthcare System in West Haven, CT, and their surrounding communities. Common exclusion criteria for both groups included: any history of neurological disease, loss of consciousness for greater than or equal to five minutes, any medical condition that may potentially affect neurovascular function including hypertension, or current alcohol or substance abuse or dependence. Presence or absence of psychiatric illness was assessed by trained evaluators using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) Version 2.0 (98). Exclusion criteria for the HC group included any personal history of DSM-IV Axis I diagnosis or known first-degree family member history of such illnesses. Written informed consent was obtained prior to participation in accordance and with approval of the human investigation committees of the Yale School of Medicine and the Veteran Affairs Connecticut Healthcare System.

BD diagnosis was confirmed via the SCID, which also allowed collection of relevant clinical history. Only euthymic BD participants were included to remove the

potential confound of state-related effects; mood state at the time of scanning was assessed via the SCID as well. Forty-six percent (12 of 26) of the BD participants met criteria for rapid-cycling and 27% (7) had a history of psychosis, but none were psychotic at time of scan. Twelve percent (3) were off medications at time of scan, all for greater than one year prior to scan, with the remaining participants taking psychotropic medications at time of scan, including lithium (27%, 7 of 26), anticonvulsants (62%, 16), atypical antipsychotics (38%, 10), antidepressants (38%, 10) and benzodiazepines (12%, 3). There were seven (27%) BD participants with a history of alcohol abuse or dependence and eight (31%) with history of substance abuse or dependence; in all instances, individuals had been in remission for greater than six months at time of scan. The average age at diagnosis for the BD group was 23.2 ± 9.7 yrs.

Genotyping

Ten milliliter blood samples were drawn from participants to allow genotyping at the SLC6A4 locus. PCR amplification was completed using an MJR tetrad cycler. Amplification was done using PCR primers previously designed by Gelernter and colleagues (78) based on reported sequences of the two polymorphisms (76): specifically, a 419 base pair product corresponding to *l* allele and a 375 base pair product corresponding to the *s* allele. PCR was done with Klen*Taq* polymerase with standard buffer PC2 and the addition of 5% glycerol. Cycling temperatures for 30 cycles and 30 seconds at each point were 98/66/72°C. Since the *s* allele has been found to be dominant in transcriptional activity versus the *ll* genotype (76), genotyping led to the creation of two genotype comparison groups, *ll* individuals and *s* carriers (*ls* or *ss*).

MRI Data Acquisition and Emotional Face Paradigm

Participants were scanned using a 3-Tesla Siemens Trio MR scanner (Siemens, Erlangen, Germany) at the Yale Magnetic Resonance Research Center in New Haven, CT. FMRI data were acquired with a single-shot echo planar imaging (EPI) sequence in alignment with the anterior commissure-posterior commissure (AC-PC) plane to create 32 three-mm thick slices without gap with the following parameters: TR = 2000 ms, TE = 25 ms, matrix = 64 x 64, FOV = 240 x 240 mm^2, and flip angle = 80°.

During the functional runs, an event-related emotional face task was completed by each participant. Faces from the Ekman series (99) depicting expressions of fear, happiness, or neutrality were shown to the participants via the PsyScope software package (100) on a computer attached to a projector. Participants were asked to make a male-female discrimination via a two-response button box that they were oriented to prior to scanning. This task was chosen to induce implicit rather than explicit processing of the emotional face as the former has been found to activate the emotional processing neural circuit more readily (42). Each face was presented for two seconds and separated by four, eight, or twelve second intervals during which time a cross-hair fixation point was displayed. Face stimuli included images of ten actors (five of each gender), with each individual exhibiting all three of the expressions for a total of 30 faces per run. Ordering of face stimuli was varied systematically to control for sequential dependencies and counterbalanced for facial expression, sex, the identity of the face, and the length of the interval between stimuli. Each run lasted 4 minutes and 50 seconds and data was compiled and averaged over four runs.

fMRI Data Processing

Raw data pre-processing was completed using Statistical Parametric Mapping (SPM99) software (101). Two images at the beginning of each fMRI run were discarded to account for the approach of the hemodynamic response to steady-state. The functional scans were realigned to the first volume to correct for inter-scan movements. After motion correction, the functional data were spatially normalized to a standard EPI template from the Montreal Neurological Institute (MNI). FMRI data was resampled to 4 mm^3 voxels during normalization. Lastly, they were spatially smoothed with a 12 mm FWHM Gaussian kernel.

SPM99 was also used for the model specification and estimation. At the individual subject level, event-related response amplitudes were estimated using the general linear model for each of the three event types: fearful, happy, and neutral expression. This created statistical images of the BOLD contrast response of each face type versus the baseline fixation cross-hair control for each individual subject.

Anatomical Structures of Interest Definition

Anatomy-based masks were created to limit voxel-by-voxel comparison to the hypothesized structures of interest. Bilateral amygdala regions were defined by the WFU toolbox (102), each 1920 mm^3 in volume (30 voxels). Similarly, the sgACC region was defined by dividing the WFU-defined anterior cingulate cortex region at the axial plane of z = 0, creating a ventral region that was 5560 mm^3 (87 voxels) in volume with center at MNI coordinates of x = 0 mm, y = 38 mm, z = -4 mm. This division approximates the sgACC, the region of the ACC most associated with emotional regulation (17) and with strong reciprocal connections with the amygdala whose disruption is associated with the *s* allele (96). See Figure 1 for visualization of these regions.

SPM Group Level Analysis

Individual BOLD contrast maps of response to fear faces were computed in SPM99 for each subject to use in comparisons between diagnostic groups (HC versus BD). Groups were compared with t-tests at each voxel within the sgACC and amygdala with significance considered at a p-value < 0.05 and a cluster extent threshold of five voxels. This combined application of a statistical threshold and cluster size filter has been shown to reduce the identification of false-positive activation clusters (103). Using the MarsBar toolbox for SPM (104), clusters of significance were used to create binary images of regions with differential activity due to the effect of diagnosis. Next, comparisons were done in an identical manner comparing genotype within a diagnostic category, namely HC *ll* vs HC *s* carriers, and BD *ll* vs BD *s* carriers. Significant clusters were similarly used to create binary images of regions with differential activity due the effect of genotype. These between-diagnosis and between-genotype clusters were then compared visually in SPM99 to assess degree of overlap, and their binary images combined by a function in MarsBar producing an output image containing only the voxels common to the input images. These same analyses were then completed for happy and neutral face contrasts. Thus, these analyses allowed assessment of response to a positive stimulus (happy faces), a negative stimulus (fear faces), and a non-emotional face stimulus.

Mean BOLD signal change values for each subject were then extracted from these clusters of differential activity to allow for analysis of the effects of demographic factors including age and gender on the above comparisons. Exploratory analyses were performed on these single subject values as well to assess for potential effects of clinical

variables among BD participants, including presence of rapid cycling, medication subclass (lithium, anticonvulsants, atypical antipsychotics, and antidepressants), history of psychosis, history of substance or alcohol abuse/dependence, and age at time of bipolar diagnosis.

Description of Student's Role in Project

I helped in the interview for assessment of diagnosis and symptoms of participants under the supervision of faculty and fellows in the Mood Disorders Research Program Laboratory at Yale, and helped to organize the clinical and demographic data that was gathered during these interviews. I was involved in the development and programming of the emotional face task and its timing parameters, and was involved in supervising and attending the scans of participants in the study. In addition, I supervised and assisted in the acquisition of images from the fMRI scanner, and in the completion and review of all imaging data preprocessing and contrast map formation, and then completed the group level analysis using the software tools described above. I consulted with statisticians to help organize and complete the post-hoc analyses of the individual subject mean signal values, and was able to build off of my years of fMRI experience to interpret the results of these analyses and evaluate their potential clinical implications. Also, while I helped in coordinating the transport of blood samples and organizing the genetic data in relation to this study, all genotyping was done in the laboratory of Dr. Joel Gelernter as described above, under the supervision of Dr. Gelernter and Ms. Ann Marie Lacobelle, MS.

Results

Participants

The HC and BD groups did not differ significantly in age ($T = 0.99$, $p = 0.33$) or gender distribution ($Z = 0.40$, $p = 0.69$). Genotyping at the SLC6A4 locus led to the formation of four diagnosis-genotype groups: 12 HC *ll* participants, 31 HC *s* carriers (26 *ls* and 5 *ss*), 9 BD *ll*, and 17 BD *s* carriers (15 *ls* and 2 *ss*). The frequency of the *l* allele in this sample was 60.1%, and the frequency of the *s* allele was 39.9%. Clinical characteristics for these groups are summarized in Table 1. There was no difference in age between HC and BD, and no statistically significant difference in age between HC *ll* and HC *s* carriers, as well as BD *ll* and BD *s* carriers. There was not a statistically different distribution of Caucasians and African Americans between HC and BD groups ($p = 0.91$); there were too few non-Caucasian, non-African American participants in the study for statistically meaningful analysis of distribution of all ethnicities. There was a trend for a difference in the percentage of participants with substance abuse history, with BD *ll* having a greater percentage than BD *s* carriers ($p = 0.051$).

In the scanner, the groups did not have significantly different physical head motion in terms of translational (with HC *ll* having 0.61 ± 0.29 mm, HC *s* car having 0.56 ± 0.27 mm, BD *ll* having 0.69 ± 0.28 mm, and BD *s* car having 0.68 ± 0.27; $F = 0.88$, $p = 0.45$; values given as mean ± standard deviation) and rotational movement (with HC *ll* having 0.49 ± 0.36 degrees, HC *s* car having 0.46 ± 0.27 degrees, BD *ll* having 0.53 ± 0.22 degrees, and BD *s* car having 0.57 ± 0.50 degrees; $F = 0.35$, $p = 0.79$). Of note, the magnitude of movement overall was well below standards in the literature (105).

Fear Faces

The BD group had significantly decreased BOLD activation compared to the HC group

Table 1. Clinical Features of the Bipolar Disorder and Healthy Control Groups

	HC *ll* **n = 12**	**HC *s* car** **n = 31**	**BD *ll*** **n = 9**	**BD *s* car** **n = 17**	Test for Significance
Gender					
Female	6 (50)	20 (65)	5 (55)	12 (71)	p = 0.69
Male	6 (50)	11 (35)	4 (45)	5 (29)	
Age, in yrs	24.5 ± 6.4	30.4 ± 11.7	38.1 ± 16.1	28.2 ± 10.3	HC, p = 0.11 BD, p = 0.07 [a]
Ethnicity					
Caucasian	5 (42)	19 (61)	5 (55)	13 (76)	
African American	5 (42)	5 (16)	4 (45)	3 (18)	
Asian American	1 (8)	3 (10)	0	0	
Other	1 (8)	4 (13)	0	1 (6)	
Presence of Rapid Cycling	-	-	3 (33)	9 (53)	p = 0.35
Hx of Psychosis in Past	-	-	1 (11)	6 (35)	p = 0.20
On Medication at Scan Time	-	-	9 (90)	15 (88)	p = 0.96
Lithium at Scan Time	-	-	1 (11)	6 (35)	p = 0.20
Anticonvulsant at Scan Time	-	-	6 (67)	10 (59)	p = 0.70
Atyp. Antipsychotic at Scan Time	-	-	2 (22)	8 (47)	p = 0.23
Antidepressant at Scan Time	-	-	3 (33)	7 (41)	p = 0.70
Benzodiazepine at Scan Time	-	-	2 (22)	1 (6)	p = 0.22
History of Alcohol Abuse/Dependence	-	-	4 (44)	3 (18)	p = 0.15
History of Substance Abuse/Dependence	-	-	5 (55)	3 (18)	p = 0.051
Age at Diagnosis of BD, in yrs	-	-	26.8 ± 12.1	21.1 ± 7.8	p = 0.17 [b]

BD, Bipolar Disorder; HC, Healthy Control; CARS-M = Clinician-Administered Rating Scale for Mania
Values as whole number and (percentage), except in case of continuous variable, given as mean ± standard deviation
Statistical tests: continuous variable: Student's t-test, categorical variable: nonparametric test, Mann-Whitney
[a] Age: compared across genotype within diagnostic category: HC *ll* vs HC *s* car: p = 0.11; BD *ll* vs BD *s* car: p = 0.07
[b] Age of BD onset not available for one subject, n = 25 of 26

in response to fear faces in a sgACC cluster of 38 voxels (T = 2.21, p_{uncorr} = 0.015, nearest MNI maxima at x = 8 mm, y = 36 mm, z = -4 mm) that includes part of Brodmann Area 24 and crosses the midline. In the between genotype comparisons, the

HC *s* carriers had significantly decreased activation compared to the HC *ll* individuals in a sgACC cluster of 84 voxels that extended bilaterally (T = 2.81, p_{uncorr} = 0.003, with MNI maximum at x = 8 mm, y = 36 mm, z = -4 mm) and the BD *s* carriers had significantly decreased activation in a cluster of 73 voxels that also extended bilaterally, including parts of BA 32 (T = 2.81, p_{uncorr} = 0.003,with MNI maximum at x = -12 mm, y = 36 mm, z = -8 mm). By visualization, the cluster of difference between-diagnostic groups was almost entirely within the larger between-genotype clusters, and this was confirmed by the MarsBar function that created a 35 voxel (2240 mm^3) region representing the area in which all three clusters shared the same voxel. Using the extracted mean signal change values, it was found that there was no effect of age or gender in any of the comparisons.

See Figure 2 for visualization of these regions.

There were no significant differences detected between HC and BD or between genotype in either the left or right amygdala in response to fear faces.

Happy Faces

The BD group had significantly decreased BOLD activation compared to the HC group in response to happy faces in a sgACC cluster of 7 voxels that was primarily in the left hemisphere near BA 32 (T = 1.88, p_{uncorr} = 0.032, MNI maxima at x = -4 mm, y = 32 mm, z = -8 mm). The HC *s* carriers had significantly decreased activation compared to the HC *ll* individuals in a sgACC cluster of 73 voxels that extended bilaterally (T = 3.02, p_{uncorr} = 0.002, MNI maxima at x = -4 mm, y = 28 mm, z = -4 mm), while the BD *s* carriers had significantly decreased activation compared to BD *ll* individuals in essentially the same region (73 voxels, T = 3.02, p_{uncorr} = 0.002, MNI maxima at x = -8

mm, y = 28 mm, z = -4 mm). The seven voxel cluster of difference between diagnostic groups was entirely within the between genotype clusters by visualization and this was confirmed by the creation of a 7 voxel (440 mm^3) region that represented the overlapping voxels. There was no effect of age or gender on these differences based on the extracted mean values. See Figure 3 for visualization of these regions.

Similar to the fear faces, there were no significant differences detected in any of the comparisons within the right or left amygdala in response to happy faces.

Neutral Faces

There was not a significant difference between HC and BD groups in BOLD response to neutral faces. There was an 8 voxel cluster extending bilaterally in the sgACC representing decreased activation in the HC *s* carriers versus the HC *ll* group (T = 2.05, p_{uncorr} = 0.022, MNI maxima at x = -4 mm, y = 24 mm, z = -8), and an 86 voxel cluster extending bilaterally in which BD *s* carriers had decreased activation in BD *ll* (T = 2.98, p = 0.002, nearest gray matter maximum at x = -4, y = 40, z = 0). See Figure 4 for visualization of these regions; the graph displays the mean BOLD signal change from an eight voxel region presenting the overlap of these two regions. There was a significant effect of age on these between-genotype comparisons, with BOLD activation in the both between-genotype clusters being negatively correlated with age (r = -0.34, p = 0.004). There was no effect of gender.

There were no significant differences in any comparison within the left and right amygdala in response to neutral faces.

Clinical Factors

Amongst BD participants, no significant effects on response in the sgACC and amygdala to any of the face types were detected for presence of rapid cycling, history of psychosis, or history of alcohol abuse/dependence. A history of substance abuse/dependence did affect the neutral face response, with BD individuals who did have such history having increased activation compared to those who did not ($T = 3.50$, $p = 0.002$); it did not affect response to fear or happy faces. There were not enough unmedicated participants ($n = 3$) to allow for statistically meaningful comparisons between medicated and unmedicated participants. Amongst those who were on medication there was no effect of medication subclass for the BD comparisons. Age at diagnosis of bipolar disorder did not significantly correlate with activation in any of the clusters.

Discussion

In this study, fMRI analyses demonstrated decreased response in the sgACC amongst BD participants compared to the HC group in response to fearful faces, and, to a lesser extent but still statistically significant, to happy faces. In addition, for both fear and happy faces, sgACC activation was decreased amongst carriers of the *s* allele of the 5-HTTLPR polymorphism in both the HC and BD groups compared to the *ll* individuals within the respective diagnostic group. Furthermore, via an overlay method revealing common voxels, it was shown that the aberrant functioning in the BD group compared to a group of healthy volunteers was influenced by variation in the 5-HTTLPR polymorphism, such that BD participants carrying the *s* allele had the greatest magnitude of dysfunction in the region.

The results of this study showed that in response to emotionally valenced stimuli (but not to neutral faces) there was a reduction in response of the sgACC amongst participants with BD. This is consistent with previous reports of sgACC functional and metabolic abnormalities in BD (23, 32-34), and may suggest, given that this sample included only euthymic participants, that this sgACC dysfunction is a trait-feature of BD. That is, there is evidence that sgACC dysfunction is a key component to the neurobiological pathophysiology of the disorder, and that it exists during euthymia with possible exaggerations during alterations of mood state (23). This dysfunction may develop during adolescent neural maturation, as previous work has shown structural changes in amongst adults with BD (21-23) but not consistently in pediatric BD populations (25-29).

Differences in sgACC activation were also seen amongst HC *s* carriers versus HC *ll* individuals; in fact, the HC *s* carriers decrease was highly significant and involved a majority of the sgACC total voxels (as per our anatomical definition). While previous studies in healthy volunteers reported an association of the *s* allele with dysfunction of the amygdala and disruption of functional connectivity between it and the sgACC (96), the current study suggests functional abnormality in the sgACC itself. Thus, the *s* allele may be associated not only with aberrancy in the reciprocal connections specific to the amygdala, but also impaired processing ability in the sgACC overall. In addition, it is important to note that the HC group was screened for potential psychiatric disease and were also excluded for any such familial history. This suggests that the influence of the 5-HTTLPR polymorphism on emotional processing neural circuitry does not necessarily lead to the development of psychiatric illness. It is part of a balance of genes and environmental exposures whose combined influences and interactions may contribute to the expression of clinical pathology.

The finding of a difference within the sgACC between BD *s* carriers and BD *ll* individuals in response to both fearful and happy faces helps to link the results of the two comparisons already discussed. While function in the sgACC was found to be impaired in BD participants as a group overall, those carrying one or two copies of the *s* alleles exhibited the greatest magnitude of impairment in this region. This suggests that the BD *s* carriers may represent a biologically salient subtype within the spectrum that is distinct in its degree of sgACC-amygdala circuitry dysfunction, with similarly distinct clinical features that future work with larger samples and statistical power may be able to elucidate and define. In addition, the BD *s* carriers may be the subset of individuals with

BD that most benefit from treatments targeting serotoninergic transmission or treatments that target the sgACC. This finding also adds to the argument that there are genetically-derived subsets within the disorder, each linked to a different set of genes (74), with the 5-HTTLPR polymorphism being one such genetic locus.

The mean BOLD signal change values that were extracted from the clusters of differential activity in the sgACC included group means that were *negative*, especially for BD subgroups carrying the *s* allele (see Figures 2-4). These negative values were derived from the subtraction of the baseline from the face conditions. Thus, it is possible that the value indicates a failure of recruitment of the sgACC during face processing due to abnormalities in the region. It is also possible that additional structures are recruited during the task, leading to an inhibition of sgACC (106). Alternatively, the decreases may represent a suppression of high tonic activation in these regions in resting conditions (106). Metabolic studies in healthy volunteers have reported heightened resting-state activity in ventral and medial prefrontal cortices (107-108), and 'deactivations' during task performance in proportion to task difficulty (109). Thus, while exact interpretation of negative values is not possible, there is evidence that it may represent a suppression of increased tonic resting activity during emotional face processing, especially in BD *s* carriers.

In the amygdala comparisons, there were no significant differences seen across diagnosis or across genotype. This was not expected, as it was predicted that the BD group would have increased amygdala reactivity compared to the HC group for both the fearful and happy faces (32, 59-62) and that the *s* carriers would have increased activity compared to *ll* individuals in response to fearful faces in particular (89-92). Mean signal

change values were extracted for individual subjects to assess the level of BOLD response, and it was found that the mean BOLD signal change ± standard error for left amygdala activation was 0.37 ± 0.04 and for right amygdala was 0.31 ± 0.03. These values suggest that the task was successful in activating the amygdala, but that there were no significant detected differences across groups.

One possible reason for this result is the fact that a majority of the BD group (88%) were on psychotropic medication at the time of scan, and prior work has shown that individuals on medication do not exhibit the degree of amygdalar hyperreactivity that is typically seen in unmedicated individuals or even those in a manic or depressive mood state (32). Thus, there may have been sufficient 'normalization' of amygdala response due to medication in the BD group so as to preclude detection of a difference with the HC group and even between the BD *ll* and BD *s* carrier groups.

However, medication status does not explain the lack of difference between the HC *ll* and *s* carrier groups. One possibility for *this* result may be related to aspects of the fMRI analyses used in the current study, namely the use of the crosshair fixation point as the subtraction baseline from the response to the emotional faces to form the contrast maps for each individual subject. Canli and colleagues have reported that increased amygdala activation in healthy volunteers carrying the *s* allele carriers may be driven by relative deactivation when viewing neutral faces (91) compared to hyperactivation in the amygdala at rest—that is, when *not* focusing on a task-relevant stimuli (110). Thus, subtraction of the response to a non-face, non-active baseline, during which time there may be resting amygdala hyperactivation, from the response to an emotional face may lead to a perceived blunting of response to the emotional stimuli of interest. Indeed,

some studies have used a non-emotional version of the task (e.g. activation response during a matching task of non-face stimuli subtracted from activation response during a matching task of emotional face stimuli) as the baseline subtraction condition (89). To this end, a secondary analysis in SPM99 was done using response to neutral faces as the subtracted baseline from the fear and happy conditions to look for potential differences in the amgydala across genotype. There was a trend for increase in the HC *s* carriers greater than HC *ll* carriers in the happy – neutral contrast in the amygdala bilaterally and in the right amygdala for the fear – neutral contrast (see Figure 5).

In relation to the overall aims of the current study, however, it was decided that while subtracting neutral from the other emotion types may be an appropriate contrast for a HC group, this would be problematic in BD. Specifically, there are studies showing impairments in BD in emotional affect recognition (45), as well as limbic hyperactivation in pediatric BD populations responding to neutral faces (58), thus calling to question the validity of a 'neutral' face when perceived by a BD individual, as they may attribute an emotional valence to an ambiguous expression. Thus, the subtraction of the response to neutral faces from the fear and happy face conditions would make meaningful interpretation of results extremely challenging, and so the cross-hair fixation point baseline was used in the analyses of this study.

Another pertinent consideration in this study was the relative ethnic heterogeneity within the overall participant sample. While the overall measured 5-HTTLPR allele frequency in our sample overall was 60.1% for the *l* allele and 39.9% for the *s* allele—which is nearly identical to frequencies reported for European American populations—looking specifically at the African Americans in the sample revealed a frequency of

76.5% for the *l* allele and 23.5% for the *s* allele within this subgroup. This difference highlights the variability in the frequency of these alleles across ethnic populations (78), and in fact the measured African American frequencies in this sample are quite similar to reports of frequencies in African American populations (111). This is important to note because any imbalance in distribution of ethnicities across genotype groups may make it difficult to disentangle the effect of a specific allele versus the effect of ethnicity. Unfortunately, while secondary analyses involving only the Caucasians (the largest represented group in this sample) in the study may have adjusted and accounted for this potential contributing factor, this left too few subjects for statistically meaningful between genotype comparisons; however, this will be a focus of future work, and the accumulation of a larger samples will allow for validation of results within a more ethnically homogeneous subset of participants.

Recently, there has been a new *tri*allelic classification of the 5-HTTLPR polymorphism, with description of two *l* allele variants, L_A and L_G, with the latter having *in vitro* transcriptional functioning more similar to the *s* allele (112). This has led some to suggest a reclassification of the allelic system, with L_G aligned with the *s* allele to form a 'risk' allele subset; in addition, some studies have suggested that African Americans are more likely to carry the L_A variant of the *l* allele (113) and thus a lower proportion of *both* risk alleles. Studies using this triallelic classification system have found differences in metabolic function after tryptophan depletion in the amygdala and sgACC in individuals with remitted major depressive disorder (114), suggesting that there may be a gradient of effects on function across the three genotype groups. However, although triallelic characterization was available for many of the participants in the current sample,

it was found that reclassification would yield a L_AL_A group with sample size too small for statistically powered group comparisons, and, so the original classification scheme was used in this work. It is hoped that these relationships can be further explored in future studies, in which larger sample sizes will allow for comparison across groups with sufficient statistical power to detect differences.

Associations between features of BD such as age of bipolar diagnosis onset, presence of rapid cycling, and history of psychosis, with the *s* allele were not seen. Similarly, there was no effect of these clinical features with BOLD response in the sgACC or amygdala, perhaps due to insufficient power. Thus, future studies will continue to assess possible associations with the *s* allele in BD participants with specific clinical features that may only be appreciated with the accumulation of large samples. This will allow characterization of a 'gene expression' to 'neural circuit dysfunction' to 'clinical phenotype' mechanistic bridge, and further characterize the BD *s* carrier subgroup.

In comparison to previous work showing medication subclass effects on activation and neurochemical metabolism (32, 34) within the sgACC and amygdala, this study did not reveal any medication subclass effects on the comparisons within BD. However, medication may, as discussed above, have blunted some of the response in these structures, and the continued scanning of unmedicated individuals with BD in the future will also help clarify these potential effects in relation to the *s* allele. Similarly, in the future, adding participants who are in an active mood state would allow assessment of how the 5-HTTLPR may influence state-related activation changes associated with either depression or mania.

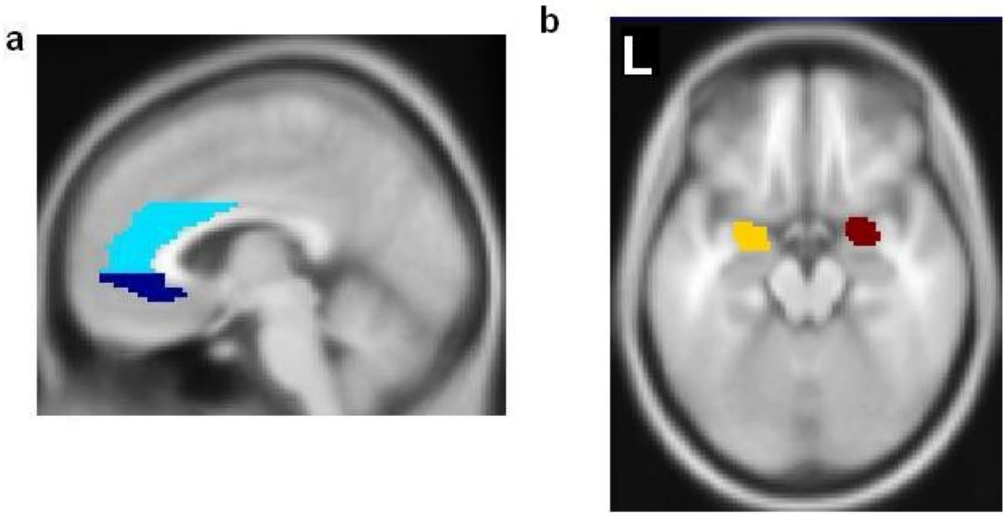
Future directions also include completing functional connectivity analyses to assess for an effect of the *s* allele on possible sgACC-amygdala communication impairment in BD. In addition, studies of adolescents with BD would allow examination of a group less likely to be affected by medications and perhaps more phenotypically (and thus genetically) homogenous. Similarly, longitudinal studies of both HC and BD participants may reveal how the 5-HTTLPR influences neurodevelopmental changes over time. Also, results from microarray and genome-wide studies of BD may serve as the next set of candidate genes to investigate within the imaging genomics field, allowing assessment of how these genes may affect neural function and contribute to the BD phenotype. Lastly, studies examining the additive or synergistic effects of two or more genes will allow insight as to how a subset of genes may define a larger phenotypic subset in BD.

In conclusion, the present work provides evidence for an association of the *s* allele of the 5-HTTLPR with dysfunction in the sgACC—a key structure in processing and regulation of emotion—in comparison to the *ll* genotype. This difference was also seen in a sample of euthymic individuals with BD, an illness that is characterized by profound emotional dysregulation, with dysfunction greatest in those in the BD group carrying the *s* allele. In combination, these findings suggest that the *s* allele is associated with abnormal functioning of the sgACC, and that this dysfunction may be a trait-related, heritable neurobiological endophenotype, that underlies a distinct subtype within the more heterogeneous clinical phenotype. Clinically, knowledge of this molecular mechanism may in fact guide therapeutic treatment strategies for the individual patient,

as there is evidence of influence of this polymorphism on response to medications (115), and allow for individualized assessment and selection of treatment options. In addition, it may guide selection of therapeutic targets, such as the growing interest in deep brain stimulation modulation of the ACC (116), and may in the future also allow clinicians to distinguish, based on genetic profile, which patients may or may benefit from these more invasive treatments. The combination of multimodal neuroimaging research and the exponentially increasing wealth of genetic information will continue to yield greater insights into the pathophysiology of BD—and provide hope to those who live with it every day.

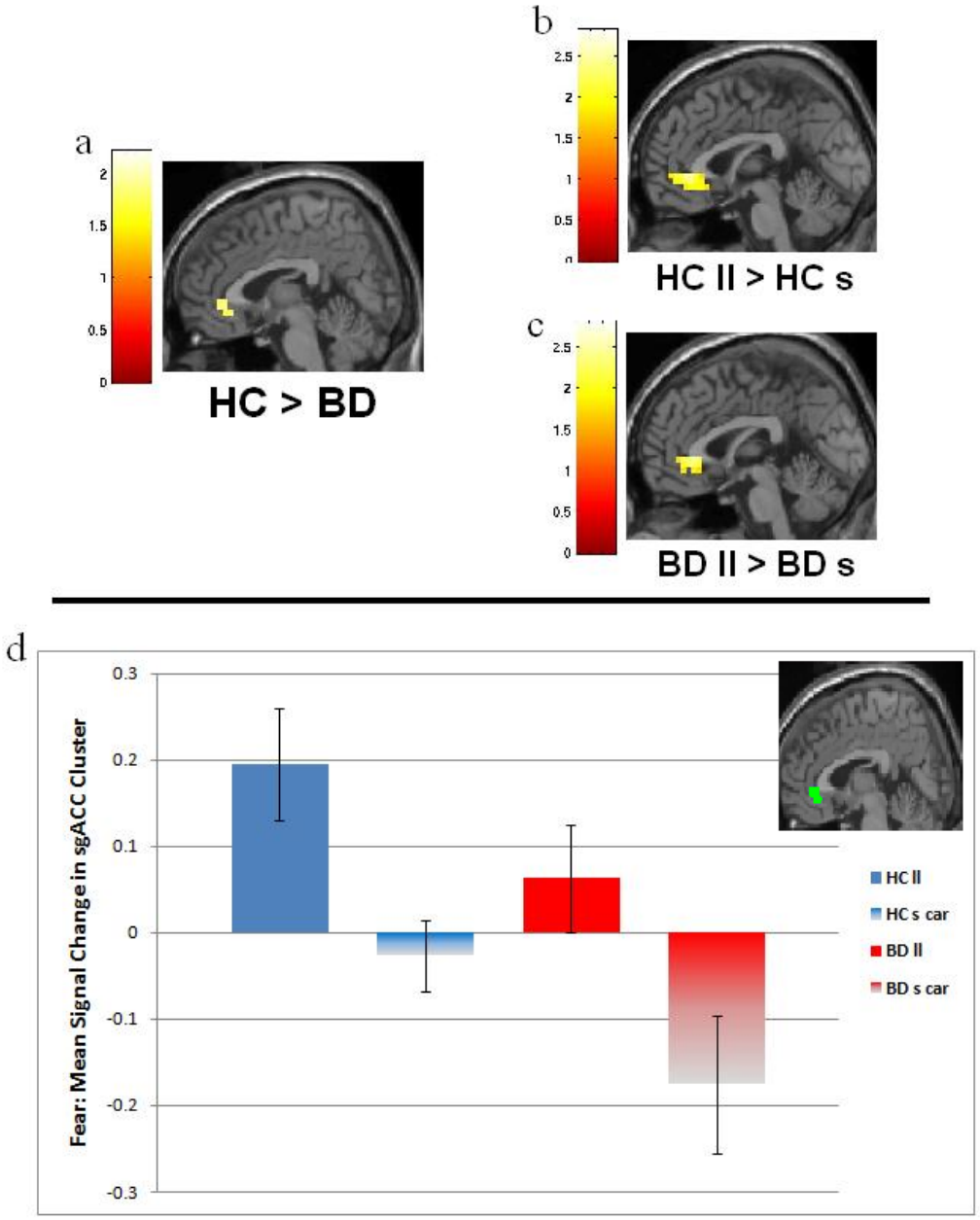
Appendix

Figure 1.



Anatomical Structures of Interests, Defined for Between Group Analyses
Images depicting the masks used to limit analyses to voxels within our hypothesized regions
a. Image shows WFU definition of total ACC (both blue structures); **dark blue** region represents our division of structure to create subgenual ACC at z = 0; created based on functional subdivision with sgACC representing affective division
b. WFU definition of **left** and **right** amygdala regions of interest
L = left, ACC = anterior cingulate cortex, sgACC = subgenual ACC

Figure 2.



Fear Faces: Between group Comparisons of BOLD Response within sgACC

a. Sagittal image at x = 4 displaying the sgACC subregion where activation to fear faces was significantly greater in the HC compared to the BD group (38 voxel cluster, $p_{uncorr} = 0.015$)

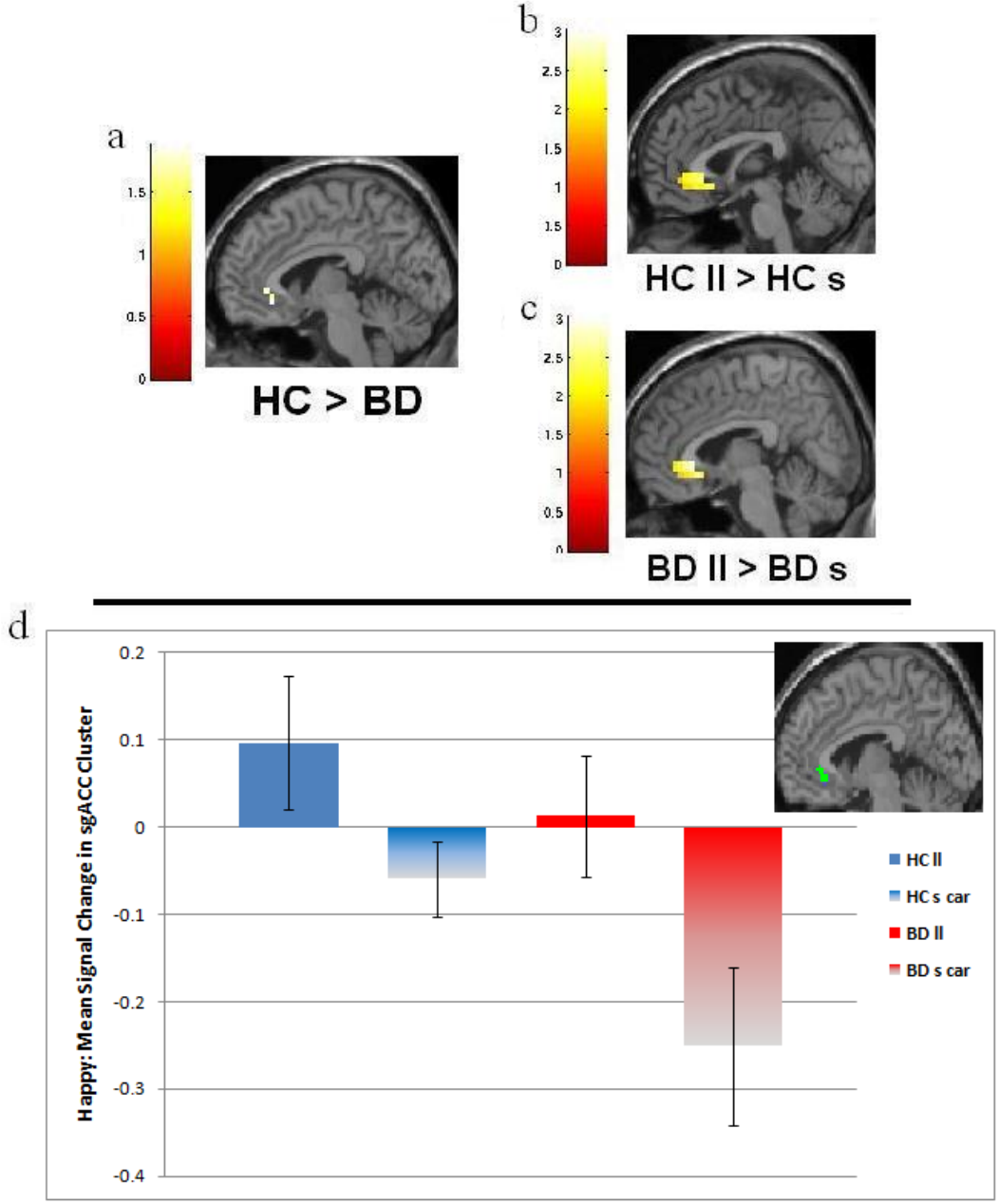
b. Image at x = 0 displaying the sgACC subregion where activation was significantly greater in the HC *ll* homozygotes compared to the HC *s* carriers (84 voxel cluster, $p_{uncorr} = 0.003$)

c. Image at x = 0 displaying the sgACC subregion where activation was significantly greater in the BD *ll* compared to the BD *s* carriers (73 voxel cluster, $p_{uncorr} = 0.003$)

d. Image at x = 0 displaying region of common voxels from clusters seen in a through c with graph depicting mean signal change ± standard error of BOLD response to Fear faces in this region; HC *ll* > HC *s* carriers; BD *ll* > BD *s* carriers; HC > BD; greatest decrease in BD *s* carriers

sgACC = subgenual anterior cingulate cortex

Figure 3.



Happy Faces: Between Group Comparisons of BOLD Response within sgACC

a. Sagittal image at x = -4 displaying the sgACC subregion where activation to happy faces was significantly greater in the HC compared to the BD group (6 voxel cluster, $p_{uncorr} = 0.032$)

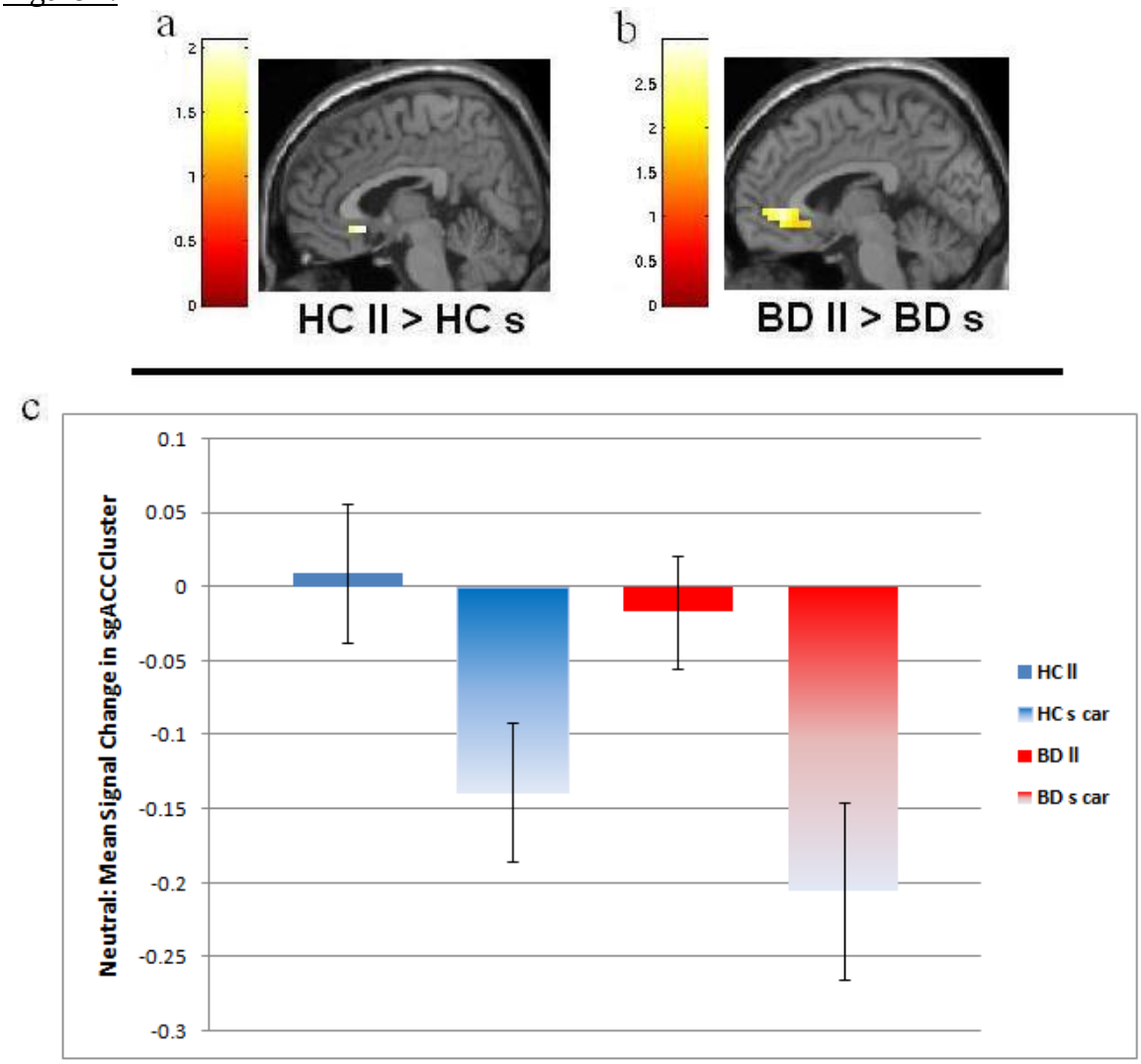
b. Image at x = 0 displaying the sgACC subregion where activation was significantly greater in the HC *ll* homozygotes compared to the HC *s* carriers (73 voxel cluster, $p_{uncorr} = 0.003$)

c. Image at x = -8 displaying the sgACC subregion where activation was significantly greater in the BD *ll* compared to the BD *s* carriers (73 voxel cluster, $p_{uncorr} = 0.003$)

d. Image at x = -4 displaying region of common voxels from clusters seen in a through c with graph depicting mean signal change ± standard error of BOLD response to Happy faces in this region; HC *ll* > HC *s* carriers; BD *ll* > BD *s* carriers; HC > BD; greatest decrease in BD *s* carriers

sgACC = subgenual anterior cingulate cortex

Figure 4.



Neutral Faces: Between Group Comparisons of BOLD Response within sgACC

There were no significant differences between HC and BD in response to neutral faces

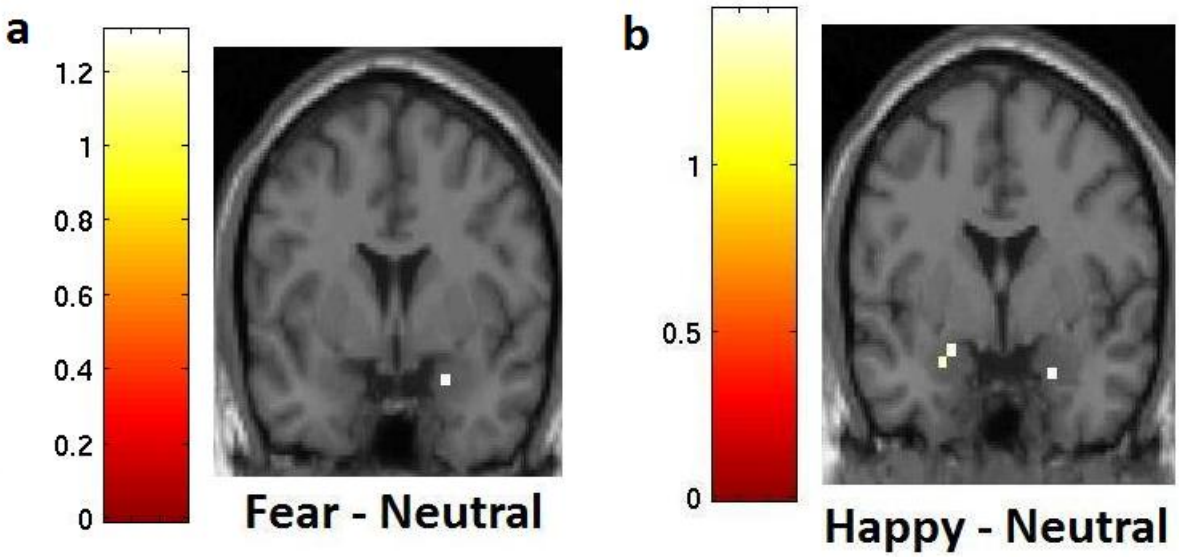
a. Sagittal image at x = 4 displaying the sgACC subregion where activation to neutral faces was significantly greater in the HC *ll* group compared to the HC *s* carriers (8 voxel cluster, p_{uncorr} = 0.022)

b. Image at x = -2 displaying the sgACC subregion where activation was significantly greater in the BD *ll* compared to the BD *s* carriers activation (86 voxel cluster, p_{uncorr} = 0.002)

c. Graph depicting mean signal change ± standard error response to Neutral faces; values derived from a region of shared voxels from a and b; HC *ll* > HC *s* carriers; BD *ll* > BD *s* carriers

sgACC = subgenual anterior cingulate cortex

Figure 5.



Amygdala Differences: HC Genotype Differences using Neutral Face Reponse as Baseline

These images depict clusters in the amygdala where activation was greater in HC *s* carriers compared to HC *ll* individuals

a. Fear – Neutral contrast; coronal image at y = 0

-R Amygdala: 1 voxel, p_{uncorr} = 0.093; MNI at x = 24 mm, y = 4 mm, z = -20 mm

b. Happy – Neutral; coronal image at y = 0

-R amygdala: 3 voxels, p_{uncorr} = 0.071, MNI maximum at x = 24 mm, y = 0 mm, z = -24 mm

-L amygdala: 4 voxels, p_{uncorr} = 0.080, MNI maximum at x = -20, y = 0, z = -16

In images, Left is Left

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