GENETIC, HEMODYNAMIC, AND ELECTROPHYSIOLOGICAL CORRELATES OF CORTICO-LIMBIC FUNCTION IN CLINICALLY DEPRESSED INDIVIDUALS

by

Jayanta Hegde

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Jayanta Hegde entitled GENETIC, HEMODYNAMIC, AND ELECTROPHYSIOLOGICAL CORRELATES OF CORTICO-LIMBIC FUNCTION IN CLINICALLY DEPRESSED INDIVIDUALS and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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SIGNED: Jayanta Hegde
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DEDICATION

I dedicate this dissertation to my parents Narayan and Rita Hegde.
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ABSTRACT

Resting frontal electroencephalographic (EEG) asymmetry has been hypothesized to be a biological marker of clinical depression but may reflect an endophenotype specific to women. Frontal EEG asymmetry was assessed in individuals (22% male) with \( n = 12 \) and without \( n = 21 \) a DSM-IV diagnosis of lifetime Major Depressive Disorder (MDD) or Dysthmic Disorder on 4 occasions within a two-week period. Depressed women exhibited greater relative right frontal activity at rest than never-depressed women across occasions. In contrast, depressed men displayed greater relative left frontal activity than never-depressed men. The same participants engaged in a Passive Viewing Face task while undergoing functional magnetic resonance imaging (fMRI). The present study did not replicate previous findings which show a hyperactive hemodynamic response in the amygdalae among depressed individuals. Mixed linear models indicated a lifetime depression by biological sex by amygdala activation interaction. For never-depressed control participants, frontal asymmetry is unrelated to the level of emotion-related amygdalae activation, but for lifetime depression spectrum participants, in both men and women, relatively greater amygdalae activation to emotional faces is associated with less left frontal activity as compared to those with less amygdalae activation to emotional faces. Also, when activation to emotionally expressive faces was closer to the levels of activation observed in the neutral face condition, the predicted pattern of association between frontal EEG asymmetry and depression based on the above findings was disrupted in men, but preserved in women. When levels of activation to emotion faces was considerably lower than that to neutral faces, the pattern was generally preserved for
men, but not for women. Preliminary tests were also conducted in an attempt to replicate previous reports that document a positive correlation between the risk allele of the serotonin transporter gene and amygdala activation. The present study failed to replicate this pattern, perhaps on account of the relatively small sample size available when non-Caucasian participants were excluded from the analysis.
Genetic, Hemodynamic, and Electrophysiological Correlates of Cortico-Limbic Function in Clinically Depressed Individuals

INTRODUCTION

One of the most important observations in the neuroscientific literature on emotion is that higher cortical areas tend to exert an inhibitory influence on sub-cortical areas, and that this inhibition is related to a variety of extinction responses in humans, non-human primates, and rats. Inhibitory theories of cortical function were first motivated by brain injury evidence in the middle of the 19th century. Emotion-related problems were linked, even at that time, to “over-action of the lower centers as a consequence of loss of control from inaction of higher centers” (Jackson 1884, sited in Quirk 2007). More recently, aberrant functioning in the cortico-limbic system, in the form of deficient top-down inhibition of the amygdalae as well as excessive bottom-up stimulation of prefrontal structures, has been linked to several syndromes, including specific phobias, post-traumatic stress disorder, and depression. The present paper constitutes an attempt to advance our understanding of the cortico-limbic dynamic, as it manifests in the context of clinical depression, by drawing on electroencephalographic (EEG), functional magnetic resonance imaging (fMRI), psychological, and genetic data.

There are numerous ways to operationalize cortico-limbic interactions, and specifically the functional relationship between the prefrontal cortex and the amygdalae, but first it is worth summarizing the literature that has implicated these areas in the “neuropathology” of depression. Furthermore, it is also prudent to explain why neuroimaging data can be fruitfully examined in light of genetic data, and specifically
why the prefrontal-amygdalae system can be illuminated through consideration of the serotonin transporter gene (5-HTT).

*Individual Differences in the Prefrontal Cortex and Depression*

Studies using amytal injections (Alema, Rosadini, & Rossi, 1961; Perria, Rosadini, & Rossi, 1961; Terzian, 1964) support well replicated evidence from lesion studies (i.e. Goldstein 1939, Robinson 1983, Robinson, Kubos, Starr, Reo, & Price 1984) that damage to the left-hemisphere, particularly when close to the frontal pole, results in depressive symptomatology, whereas comparable damage to the right-hemisphere produces undue cheerfulness or emotional apathy. While lesion studies cannot conclusively determine that a given locus subserves any particular emotional response in intact brains, such research strongly implicates diminished function of the left prefrontal cortex (PFC) in sad mood and depression.

A related literature documents decreased activation in both the dorsolateral (dl) and dorsomedial (dm) PFC as well as the pregenual region of the ACC in patients with major depressive disorder (MDD; see Drevets 1998 for comprehensive review). A generally diminished pattern of activation in the dlPFC and the pregenual ACC are frequently accompanied by increases in the ventrolateral and orbital (lateral and medial) regions of the PFC. Activation in the dlPFC, particularly on the left side, has also been demonstrated to increase with successful pharmacological treatment (Kennedy et al., 2001, see also Bruder et al. 2001). While there is a clear picture of overall diminished dlPFC activity, a number of findings spanning various paradigms (e.g. PET, repetitive
transcranial magnetic stimulation, fMRI) can be taken in support of a hemispheric “imbalance hypothesis” in the functioning of the dlPFC in depressed individuals. Across studies, there is reason to believe that the left dlPFC is hypoactive while the right dlPFC is hyperactive (e.g. Phillips, Drevets, Rauch, & Lane 2003, Mayberg 2003, Burt, Lsanby, & Sackeim 2002, Gershon, Dannon, & Grunhaus 2003, Fitzgerald, Brown, Marston, Daskalakis, de Castella, & Kulkarni 2003, Bermpohl et al 2006, Sackeim, Greenberg, Weiman, Gur, Hungerbuhler, & Geschwind 1982, Maeda, Keenan, & Pascual-Leone 2000, Davidson & Irwin 1999, Grimm et al., 2008).

Another body of literature has demonstrated clear anatomical differences in frontal regions in depressed samples. Coffey et al., (1993) found that depressed inpatients have frontal lobe volumes that were 7% smaller than non-psychiatric controls. Drevets et al., (1997) also reported that unipolar and bipolar depressives with a family history of mood disorder show a 48% and 39% reduction in subgenual PFC volume, respectively. In a postmortem study, Öngür, Drevets, & Price (1998) found that glial cell count was significantly reduced in the subgenual PFC in both MDD patients (24%) and bipolar (41%) patients with a family history of MDD. Interestingly, no significant effects were found for non-familial MDD or bipolar disorder patients. In another study of neuronal and glial histopathology in postmortem brains of patients who suffered from MDD, Rajkowska (2000) found decreases in cortical thickness, neuronal size, and neuronal and glial densities in the left PFC. Morphological discrepancies of these sorts may underlie the frontal alpha asymmetry differences that have been shown in depressed and formerly depressed populations. It may also be the case that the inferred right hemispheric
hyperactivity may be a consequence of some third factor which is more directly related to structural differences in the frontal region.

*Frontal EEG Asymmetry*

One way to examine prefrontal areas is to use EEG to measure hemispheric differences in alpha activity (8-13 Hz). Herein, a putative inverse relationship between alpha power and neural activity is assumed. All discussions of asymmetry, unless otherwise qualified, will be in terms of the inferred construct (“activity”) rather than the observed measure (alpha power).

Frontal EEG asymmetry is a reliable individual difference variable that appears to moderate emotional and motivational constructs in human infants and adults, as well as non-human primates (for a review see Coan & Allen 2004). Findings also suggest that a particular pattern of resting frontal EEG activity may indicate a trait-like disposition towards emotional negativity and behavioral withdrawal. Moreover, frontal EEG asymmetry can also distinguish depressed individuals, both those currently symptomatic as well as those in remission, from never-depressed individuals. When taken together, a substantial body of literature suggests that frontal EEG asymmetry may serve as a liability marker for depression (Allen, Urry, Hitt, & Coan, 2004) and possibly anxiety.

While numerous studies implicate the right-hemisphere in the processing of emotional information, evidence also suggests that the more anterior regions of the right and left hemispheres play different roles in the production of emotion. Subsequent to Schneirla’s (1959) original proposal that a propensity to approach and withdraw underlie
all motivated behavior across phylogeny, Davidson and others (e.g. Davidson & Tomarken 1989; Kinsbourne, 1978) have argued that the anterior regions of the left and right hemispheres are specialized for approach and withdrawal behavior respectively.

Gray’s (1994) influential bio-behavioral temperament constructs, the behavioral activation and inhibition systems (BAS/BIS), are conceptually similar to Davidson’s approach/withdrawal model on a psychological level, but differ with respect to the hypothesized functional neuroanatomy. Independently, Watson and Tellegen (1985) have done factor analytic work on mood which has procured two orthogonal factors, positive affect (PA) and negative affect (NA), which also appear to theoretically overlap with the approach/withdrawal dimensions. Several studies using these affect dimensions have shown that the loss of PA differentiates depression from other negative affect states such as anxiety (e.g. Watson, Clark, & Carey 1988). It is unclear whether relative right greater than left activity in the frontal regions, as inferred through metrics of relative electrical activity in the alpha band, is specifically a predisposition for diminished approach affect, increased withdrawal affect, or some combination. Harmon-Jones and Allen (1997) and Coan and Allen (2003) both found that BAS, but not BIS, is related to frontal asymmetry. These findings can be taken as somewhat contradictory to other papers which report a relation between negative or withdrawal emotion-related disorders (i.e. depression, anxiety) and frontal asymmetry.

While there is preliminary evidence to show that frontal EEG asymmetry mediates experimentally manipulated affect states in approach- and withdrawal-related emotion conditions (Coan, Allen, Harmon-Jones, 2001), these changes in relative asymmetry are
superimposed on individual variations in tonic asymmetry. Davidson (1998) has predicted that this tonic asymmetry reflects ‘affective style’, which can contribute to an individual’s vulnerability for particular types of psychopathology. Consistent with this theory, studies have shown that individuals with relatively greater left-sided frontal tonic activity, as inferred by relatively greater alpha activity on the right side, are characterized by high levels of PA (Jacobs & Snyder, 1996; Tomarken, Davidson, Wheeler, Kinney, 1992) and self-reported activity in the BAS (Harmon-Jones & Allen, 1997; Sutton & Davidson, 1997). Also within this model, relatively greater right-sided frontal tonic activity is characterized by high levels of NA (Tomarken et al., 1992) and self-reported activity in the BIS (Sutton & Davidson, 1997), although the latter has not been consistently found (Harmon-Jones & Allen 1998, Coan & Allen 2003). By the approach/withdrawal model, tonic asymmetry by itself is not understood to produce a particular pattern of emotional experience or psychopathology; but instead, it is seen as a diathesis for various behaviors given appropriate elicitation. For Davidson and other proponents of the affective style view, sadness and depression are associated with decreased PA, and therefore decreased left frontal activity. Trait left frontal hypoactivity should leave one more susceptible to the elicitation of sad mood and at increased risk for various affective disorders. To date, over 70 published studies have examined emotion or emotion-related constructs in terms of frontal EEG asymmetries.

*Individual Differences in the Amygdala and Depression*
Drevets, Bogers, & Raichle (2002) has observed elevated baseline amygdalae activity using positron emission tomography (PET) in a depressed sample. Similar hyperactivations have been shown to correlate with measures of depression severity. Moreover, after successful pharmacological treatment with anti-depressants, a reduction to baseline in amygdala activation has been observed (Davidson, Pizzagalli, Nitschke, & Putnam, 2002).

Others have shown that in some depressed individuals, especially those who are characterized by high levels of dispositional negative affect and anxiety, the amygdalae are hyperactive in response to negative emotional stimuli (e.g. Sheline et al., 2001). Amygdalae patterns in response to positive stimuli have been less consistent, however, with some groups reporting hyperactivation (Sheline et al., 2001) while others report hypoactivation (Canli et al., 2004) in the context of depression.

Perhaps the most reliable and widely used method of eliciting amygdalae response is to employ behavioral tasks with neutral and emotionally expressive faces in conjunction with fMRI (e.g. Thomas et al., 2001, Somerville, Wig, Whalen, & Kelley, 2006). Of particular relevance to the present paper, Face tasks have also been used to highlight functional connectivity between limbic regions and higher cortical areas, in the context of memory, emotional perception, fear conditioning, and other behavioral paradigms (e.g Hariri, Drabant, & Weinberger, 2006). Face-fMRI paradigms also allow researchers to consider focal amygdalae activations in the context of morphological differences between clinical and control groups. The literature on amygdalae morphology and depression has been inconsistent, however. One finding that seems to replicate is that
Amygdala volume seems to decrease with successive episodes of depression (Gotlib & Hamilton, 2008). Recently, van Eijndhoven et al., (2009) have observed that patients who are currently suffering from a major depressive episode show enlarged amygdala on the right and left sides as compared to remitted patients as well as never-depressed controls, suggesting a state related volume change.

5-HTTLPR and Imaging Genetics of Amygdala Reactivity

When the effects of specific genes have been described from the cellular level up to the level of brain circuits and regions, neuroimaging paradigms can be useful in further illuminating the influence of these genes on local information processing (Hariri & Forbes, 2007). In studies relating genetics to brain activation, it is very important that both groups be well matched on other characteristics (e.g. gender, race, level of education, task performance). It is also helpful if the behavioral paradigms used to elicit neural response have been well-characterized in the literature and shown to produce robust and focal activations.

The 5-hydroxytryptamine transporter gene (5-HTT) facilitates the reuptake of serotonin (5-HT) from the synaptic cleft. A relatively common polymorphism, 5-HTTLPR, has recently been identified (Heils et al., 1996) and found to result in a short (S) and long (L) variant. The frequency of the S variant, in samples of European decent, is approximately 0.40 and the genotype frequencies reflect a Hardy-Weinberg equilibrium (L/L = 0.36; L/S = 0.48; S/S = 0.16). These relative allele frequencies can vary
considerably across populations (Hariri & Forbes, 2007). Lesch et al. (1996) has shown in vivo that the 5-HTTLPR alters both gene transcription and level of 5-HTT function.

Lesch et al. (1996) also found that individuals carrying the S allele are somewhat more likely to display abnormal levels of anxiety in comparison to L/L homozygotes. Others have subsequently confirmed this association (Du, Bakish, Hrdina 2000, Katsuragi et al., 1999, Mazzanti et al., 1998, Melke et al., 2001). Possession of the S allele has also been associated with ease of fear conditioning (Garpenstrand, Annas, Ekholm, Oreland, & Fredrikson, 2001) and propensity to develop a mood disorder (Lesch & Mossner, 1998). Reduced 5-HTT accessibility, as indexed by the 5-HTTLPR S allele, has also been associated with major depression (Caspi et al., 2003, Malison et al., 1998) and the severity of depression and anxiety in various psychiatric syndromes (e.g. Eggers, Hermann, Barthel, Sabri, Wagner, & Hesse, 2003). Caspi et al. (2003) has also demonstrated that exposure to stressful life events moderates the impact of the 5-HTTLPR for the development of depression, consistent with diathesis-stress models of mood disorder.

It has also now been shown and replicated, across multiple subject populations and experimental paradigms, that carriers of the 5-HTTLPR S allele, whether homo- or heterozygous, exhibit increased amygdala activation compared to subjects who are homozygous for the L allele (Hariri, Drabant, Weinberger, 2006, Heinz et al., 2005, Bertolino et al., 2005, Canli 2005, Hariri 2005, Brown 2005). Importantly, this finding has been demonstrated in German, American, and Italian populations. It has been shown several times that effects are equivalent if subjects carry one or two S alleles. Also,
equivalent effects have been found in both sexes, suggesting that the 5-HTTLPR polymorphism will not explain differences in depression prevalence between men and women.

Cortico-Limbic Function in Depression

Given the patterns of connection among the various structures implicated in depression, it is reasonable to expect that the various functional abnormalities that have been observed in different structures are in fact dynamically linked. Among the neural systems that have been identified in depression, cortico-limbic interactions have been particularly emphasized (e.g. Mayberg 1997, Gotlib & Hamilton, 2008). In her influential model, Mayberg (1997) has suggested that the normal reciprocal relationship between dorsal and ventral regions of the brain become disrupted in depression. Mayberg describes a feedback loop, whereby hyperactivation in limbic structures like the subgenual ACC and amygdalae attenuate activation in dorsal structures like the dlPFC, which in turn reduces the ability of the latter to regulate limbic activation.

A number of findings support this general model. Siegle, Steinhauer, Thase, Stenger, & Carter (2002) have found an inverse relation between amygdala and dlPFC activation in depressed individuals who are confronted with affectively meaningful words. Others have established a link between loss of top-down inhibition of the amygdala by the vmPFC and both mood and anxiety disorders (Drevets 2001, Cannistraro & Rauch 2003). Some have speculated that the consequent elevations in amygdalae stimulation of cortical areas could explain the patterns of rumination on guilt-provoking
and otherwise painful memories that have been repeatedly observed in depressed samples (Cahill 2000). Furthermore, in a study of deep brain stimulation with severely depressed patients, Mayberg et al. (2005) has shown that stimulation near the subgenual ACC attenuates overactivity in this structure while stimulating activity in the previously underactive dLPFC region.

Specific Aims

The present attempt to characterize cortico-limbic functioning is unique in that a metric of frontal EEG asymmetry in the alpha band is examined in relation to amygdala activation as assessed using fMRI. Whereas the vast majority of studies of cortico-limbic connectivity employ a single imaging modality, usually fMRI or PET, we have combined EEG and fMRI methods in the same sample to draw on two mutually relevant parts of the literature on the neuroscience of emotion and emotion-related disorders. In the present paper, neuroimaging data from EEG and fMRI is also examined in light of the 5-HTT gene.

The following questions are addressed here:

1. Is depression associated with greater left relative to right frontal alpha activity?

2. Do depressed individuals show an elevated blood-oxygen-level dependent response in the amygdalae while viewing neutral and various emotionally expressive Ekman faces?
3. Is frontal EEG asymmetry systematically related to activation patterns in the amygdalae? More specifically, is a right-biased EEG pattern (left greater than right alpha) associated with greater amygdalae activation? If yes, does this pattern hold across the three emotional face conditions?

4. Does the risk conferring variant of the 5-HTT gene relate with increased amygdalae response to various emotionally laden?

Finally, it will also be important to investigate the role of biological sex on the various relationships between depression, EEG activity, and amygdala activation.
METHODS

Participants

The present study sample, a subset of a larger investigation into the stability and predictive validity of frontal EEG asymmetry metrics, were selected for the presence or absence of current depression and personal history of depression. Participants were sampled from a college-aged population (18-35) to represent a broad continuum of depression, from virtually no depressive symptoms to full clinical levels of depression. Participants were primarily recruited from introductory Psychology classes at the University of Arizona, but also from advertisements placed in a local newspaper.

Following an intake session where present clinical status as well as personal and family history of psychopathology was assessed using the Structured Clinical Interview for DSM-IV (SCID; Spitzer, Gibbon, & Williams, 1995) and Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960) as well as questionnaires such as the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), participants underwent four EEG assessment sessions within a two week period, each session at least two days apart. Thirty-three individuals were recruited to participate in the fMRI portion of the study. Never-depressed (n=21) and depression history positive (n=12, with n=9 both currently and previously depressed, n=2 only previously depressed, and n=1 currently dysthymic; see Table 1) subjects were closely age- and sex-matched and very similar in terms of level of education (see Table 2). The presence of any DSM-IV TR Axis I or II disorder other than depression resulted in exclusion from the study, as did uncontrolled medical conditions, medical basis for depression, history of head trauma or
central nervous system lesions, left handedness, history of receiving electroshock therapy, and current use of psychotropic medications and recreational drugs. In addition, any individual who displayed active suicidal potential necessitating immediate treatment was disqualified due to the ethical concerns of delaying pharmacotherapy until after the completion of the EEG assessments.

Subjects were compensated $200 for their participation in the EEG study and an additional $40 dollars for their participation in the fMRI study. Those subjects who were undergraduate students were also offered credit counting towards their grade in an introductory Psychology class, and all subjects were offered pictures of their brain.

Twelve participants of the total sample (N=33) were characterized by a present and/or past diagnosis of depression; mostly current and past Major Depression Disorder, but in one case current Dysthymic Disorder (see Table 1). Twenty-one of the remaining participants reported no current or past psychopathology as determined
Table 1: Demographics

<table>
<thead>
<tr>
<th>Lifetime Depression Spectrum (12)</th>
<th>Lifetime MDD (11)</th>
<th>Current and Past MDD (9)</th>
<th>Past MDD Only (2)</th>
<th>Current Dysthymia (1)</th>
<th>No Hx of Psychopathology (21)</th>
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<tr>
<td>Ss</td>
<td>Sex</td>
<td>Age</td>
<td>Race</td>
<td>HRSD</td>
<td>BDI-II</td>
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<tr>
<td>148</td>
<td>F</td>
<td>19</td>
<td>W</td>
<td>15</td>
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<td>M</td>
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<td>W</td>
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| Ss | Sex | Age | Race | HRSD | BDI-II | 5-HTT |
| 169 | F  | 23  | W    | 5    | 6      | ll    |

| Ss | Sex | Age | Race | HRSD | BDI-II | 5-HTT |
| 202 | F  | 19  | W    | 6    | 2      | sl    |
| 204 | M  | 24  | A    | 1    | 2      | sl    |
| 207 | F  | 18  | B    | 0    | 0      | ll    |
| 208 | F  | 18  | W    | 2    | 10     | ll    |
| 211 | F  | 18  | B    | 0    | 2      | ?     |
| 218 | F  | 19  | O    | 0    | 0      | ll    |
| 226 | F  | 18  | ?    | 6    | 6      | sl    |
| 236 | F  | 20  | W    | 4    | 1      | sl    |
| 238 | M  | 20  | W    | 4    | 2      | ll    |
| 239 | M  | 19  | W    | 2    | 0      | sl    |
| 247 | M  | 20  | W    | 2    | 2      | sl    |
| 305 | F  | 18  | W    | 0    | 0      | ll    |
| 307 | F  | 20  | W    | 1    | 4      | ll    |
| 316 | F  | 34  | W    | 0    | 0      | sl    |
| 317 | F  | 20  | A    | 1    | 1      | ss    |
| 318 | M  | 20  | B    | 0    | 0      | sl    |
| 319 | F  | 19  | W    | 1    | 0      | ss    |
| 320 | M  | 19  | W    | 0    | 1      | sl    |
| 321 | M  | 19  | W    | 1    | 0      | sl    |
| 322 | F  | 19  | O    | 3    | 6      | ss    |
| 323 | F  | 20  | W    | 5    | 3      | sl    |
Table 2: Demographics: Between-Group Similarities

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<th>Lifetime Depression Spectrum</th>
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<td><strong>N</strong></td>
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<tr>
<td><strong>% Total N</strong></td>
<td>64%</td>
<td>36%</td>
</tr>
<tr>
<td><strong>% Female</strong></td>
<td>67%</td>
<td>67%</td>
</tr>
<tr>
<td><strong>Age M (SD)</strong></td>
<td>20 (3.4)</td>
<td>22 (4.5)</td>
</tr>
<tr>
<td><strong>BDI-II M (SD)</strong></td>
<td>2 (2.5)</td>
<td>23.25 (9.6)</td>
</tr>
<tr>
<td><strong>HRSD M (SD)</strong></td>
<td>1.85 (1.98)</td>
<td>18.66 (7.8)</td>
</tr>
<tr>
<td><strong>Caucasian</strong></td>
<td>62%</td>
<td>83%</td>
</tr>
<tr>
<td><strong>5-HTT (% with at least one risk allele)</strong></td>
<td>67%</td>
<td>50%</td>
</tr>
<tr>
<td><strong>% Right Handedness</strong></td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Procedure - EEG Assessments**

EEG signals were recorded on four occasions over two weeks from 64 sites on the scalp, according to the International 10-20 System, in addition to the three ocular sites (nasion, left inferior orbit, right inferior orbit). Impedances at all sites were required to be less than 10 K ohms. All EEG and ocular sites were referenced online to a site just posterior to Cz and recorded with DC amplifiers (lowpass filter of 200 Hz). While the primary hypotheses concern the frontal regions, all scalp sites were being recorded for the purpose of computing an offline average reference, as per the recommendations of Davidson, Jackson, and Larson (2000). Data were continuously digitized at 2000 Hz.
The average length of time for each recording session was approximately 8 minutes (in one of two counter balanced orders of instructed eyes open (O)/eyes closed (C): OCCOCOOC or COOCOCOCCO). The average of four assessments (with two sessions per assessment) was taken as the index of trait frontal EEG asymmetry and used for examining relationships with fMRI data.

A directed facial action task was also administered, where EEG was recorded while participants were systematically directed by an experimenter to manipulate their facial expression in ways that are consistent with prototypical emotional expression. These data will not be considered in this paper.

**Procedure – fMRI Acquisition, Data Reduction, ROI analysis**

Images were acquired on a General Electric 3 Tesla whole-body-echo-speed MRI system. A set of 3-plane localizer images were collected first to align a set of T1-weighted images tilted about 280 degrees from the anterior-posterior commissural plane covering most of the whole brain. Participants engaged with two behavioral tasks in the scanner, a modified version of the Emotional Counting Stroop used by Whalen et al. (1998) and a Passive Viewing Face task. Only results from the Passive Viewing Face task will be reported on here. Functional images were collected in two scans (5 minutes 45 second each, 90 second break in between) for the Passive Viewing Face task (TR=3000ms, TE=30ms, 35 slices, 3mm slice thickness, 180 temporal frames, skip = 0, FOV=24). For functional neuroimaging, stimuli were presented using a goggle system
from Resonance Technologies. After completion of the functional scans, a high resolution SPGR volume was acquired (1.5 mm sections, covering whole brain, matrix=256x256, flip angle=30, TR=7.4ms, TE=3ms, TI=1ms, FOV=25.6mm x 25.6mm). SPGR data will be used for future comparisons of the clinical and non-clinical groups on morphometric features, but these data are not reported on here.

fMRI images were reconstructed offline and then analyzed using Statistical Parametric Mapping 2 (SPM2). All volumes were aligned to the tenth volume to correct for movement. Spatial normalization parameters were estimated by warping each participant’s mean functional image to the standard MNI (Montreal Neurological Institute) EPI template. Normalized images were resliced to 3x3x3 mm voxels. Images were left unsmoothed in order to preserve maximal resolution on amygdalae activation. The timeseries in each voxel was highpass-filtered at 1/128Hz and then scaled to a grand mean of 100, and then averaged over all voxels and scans within a session. Functional scans were examined for unambiguous signs of signal drop off. Based on this evaluation of the scans, data collected from a single subject was excluded from further consideration.

For region of interest (ROI) analyses in the left and right amygdala, an MNI template was used to anatomically delineate voxels. Effect sizes (% signal change) for the various contrasts between conditions (i.e. H>N, F>N, A>N) were extracted for each subject separately and compared between groups using follow-up tests in SPSS. Marsbar (http://www.sourceforge.net/projects/marsbar) was used for extraction of effect sizes.
Functional ROIs were also conducted for only the subset of fixation-based contrasts that showed amygdala activation on the whole brain level. Since neutral-based contrasts did not yield any significant activations in the amygdale on the whole brain level, functional ROIs could not be conducted. For this reason, no functional ROI data are considered here.

Passive Viewing Face Task

Participants were presented with 24 second epochs of fearful face stimuli (F), angry face stimuli (A), neutral face stimuli (N), happy face stimuli (Ekman & Friesen 1976), or a single cross on an otherwise blank screen that served as a fixation condition (+). All emotion conditions were comprised of face pictures from the same six individuals (3 women, 3 men). Each participant passively viewed two different runs which took the following form:

N+H+A+F+A+H+F+N+ (Run #1)

break,

+N+F+H+A+H+F+A+N+. (Run #2)

Each face item was presented for 1 second, each block lasting approximately 24 seconds. Each fixation cross lasted 10.4 seconds and the single break between runs lasted 90 seconds. Before image acquisition, participants were told they would be presented with
pictures of faces and that they should simply pay attention to each item. Upon exiting the scanner, participants were asked to describe the stimuli to confirm that they were in fact attending to the pictures.
RESULTS

Aim 1 – Is depression associated with greater left relative to right frontal alpha activity?

The larger study of EEG asymmetry and depression, from which the present study sample was drawn, replicated two findings in the literature (Miller & Cohen 2002 and Knott, Mahoney, Kennedy, & Evans, 2001) which suggest a sex by depression history interaction with respect to frontal alpha asymmetry. This prompted a similar test, by linear mixed model (SPSS 17), to determine whether the present subset of the larger sample also exemplified the same sex by depression interaction across all four frontal site pairs (F8/F7, F6/F5, F4/F3, F2/F1). Lifetime Depression status (past and/or current MDD or Dysthymia = lifetime depression +, never depressed = lifetime depression -) and biological sex (male, female) were included in these models as between-subject variables, while session day (4), resting session within each day (2), and reference scheme (2; AVG, LM) were taken as within-subjects variables. Only interactions involving lifetime depression status and sex were examined. The dependent variable was an EEG asymmetry score based on total alpha power (8-13Hz).

Table 3: Sex x Lifetime Depression History

<table>
<thead>
<tr>
<th>DV</th>
<th>df</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8/F7</td>
<td>1, 663</td>
<td>7.782</td>
<td>0.005</td>
</tr>
<tr>
<td>F6/F5</td>
<td>1, 678</td>
<td>18.497</td>
<td>0.000</td>
</tr>
<tr>
<td>F4/F3</td>
<td>1, 690</td>
<td>20.334</td>
<td>0.000</td>
</tr>
<tr>
<td>F2/F1</td>
<td>1, 689</td>
<td>5.760</td>
<td>0.017</td>
</tr>
</tbody>
</table>
The expected lifetime depression by sex interaction was found at each frontal channel pair (see Table 3). Women who did not have a history of depression showed relatively greater activity in the left hemisphere as compared to women who did have a positive history for depression. Men without a history of depression, on the other hand, showed greater right relative to left activity, as compared to men who had a positive history for either MDD or dysthmia. The pattern among women is consistent with the most prominent theories which have been put forth to explain the relationship between frontal alpha asymmetry and depression (Davidson et al., 2002), whereas the male pattern seems contrary and requires further theoretical and experimental elaboration in the literature.
Aim 2 – Do depressed individuals show an elevated blood-oxygen-level dependent response in the amygdalae while viewing neutral and various emotionally expressive Ekman faces?

One sample t-tests of whole brain activation using Statistical Parametric Mapping 2 (SPM2) in control and lifetime depressed groups were conducted to show whether particular contrasts were able to evoke significant levels of brain activation in the right and left amygdalae. The depressed group showed significant activations in N > Fix, H > Fix, and F > Fix on the right side, as well as N > Fix and F > Fix on the left, while the control group only showed significant activations in the N > Fix condition on left and right sides (see Table 4). No significant activations were found in either depressed or control groups using neutral faces as a baseline contrast for the three emotional face conditions on the uncorrected p=.05 whole brain level. However, these results do not speak to the question of whether there was a relatively stronger hemodynamic response in the amygdalae in the depressed group as compared to the control group.

Table 4: One-Way T-Test on Whole Brain Level with SPM2

<table>
<thead>
<tr>
<th></th>
<th>Control Left Amygdala</th>
<th>Control Right Amygdala</th>
<th>Lifetime Depressed Left Amygdala</th>
<th>Lifetime Depressed Right Amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Happy &gt; Neutral</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Fear &gt; Neutral</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Anger &gt; Neutral</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td><strong>Neutral &gt; Fixation</strong></td>
<td><strong>Y (0.05)</strong></td>
<td><strong>Y (0.001)</strong></td>
<td><strong>Y (0.001)</strong></td>
</tr>
<tr>
<td>Happy &gt; Fixation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y (0.05)</td>
</tr>
<tr>
<td>Fear &gt; Fixation</td>
<td>N</td>
<td>N</td>
<td>Y (0.05)</td>
<td>Y (0.05)</td>
</tr>
<tr>
<td>Anger &gt; Fixation</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>
In order to address the question of whether control and lifetime depressed participants differ in amygdale response (Aim 2), two separate repeated measures GLMs were conducted using SPSS 17; the first to examine the neutral based contrasts (H>N, F>N, A>N) and the second to examine the fixation based contrasts (N>Fix, H>Fix, Fear>Fix, A>Fix).

In the first test, Lifetime Depression status was entered as a dichotomous between-subjects variable, while the three emotion conditions (F>N, H>N, A>N) and the two hemispheres (L, R) were taken as within-subjects variables. Multivariate and within-subjects tests failed to demonstrate any significant effect of emotion condition and between-subject tests failed to show the predicted main effect of Lifetime Depression status. Moreover, there was no significant emotion condition by Lifetime Depression interaction.

In the second test using fixation based contrasts, Lifetime Depression status was again considered a between-subjects variable, while the four face conditions (N>Fix, F>Fix, H>Fix, A>Fix) and the two hemispheres (L,R) were taken as within-subjects variables. Multivariate and within-subjects tests both showed a main effect of face condition (Huynh-Feldt, F=9.426, p=.000) while the between-subjects test failed to show the predicted main effect of Lifetime Depression status. Tests of within-subjects contrasts showed that the neutral face condition was significantly different from each of the three emotional face conditions (p=.000), but the emotional conditions did not differ from one another. Moreover, there was no significant face condition by Lifetime Depression interaction.
Aim 3 – Is frontal EEG asymmetry systematically related to activation patterns in the amygdalae? More specifically, is a right biased EEG pattern associated with greater amygdalae activation? If yes, does this pattern hold across emotional conditions?

Four linear mixed models, corresponding to the four frontal EEG site pairs (F8F7, F6F5, F4F3, F2F1), were conducted for each of the six neutral based anatomical ROI contrasts (H>N, F>N, A>N, each for the right and left amygdala). Lifetime Depression status (past and/or current MDD or Dysthymia = lifetime depression +, never depressed), biological sex (male, female), and z-transformed ROI activations for right and left amygdala were included in these models as between-subject variables, while session day (4), resting session within each day (2), and reference scheme (2; AVG, LM) were taken as within-subjects variables. Interactions involving lifetime depression status and sex as well as a three way interaction between lifetime depression, sex, and amygdalae activation were examined and are of primary interest. The dependent variable was the EEG asymmetry score based on total alpha power (8-13Hz). EEG asymmetry was taken as the dependent measure and amygdala activation as the independent variable, in light of the fact that the EEG asymmetry measures involved multiple observations and because a mixed model procedure was used.

When the above 3-way interaction was found to be significant, a similar mixed model was recomputed for each sex separately, to determine the presence and direction of a 2-way lifetime depression by amygdala activation interaction. These follow-up mixed models were identical to the calculations described above, except that biological sex was completely removed from the model. Estimated means were computed for one standard
deviation above and below the mean. Tables 5 through 10 contain graphs which depict the results of these estimated means, which were only computed in cases where the initial 3-way interaction (lifetime depression by sex by amygdala activation) and the follow-up 2-way interaction (lifetime depression by amygdala activation) were found to be significant. Blank areas of the table indicate that a 3-way interaction was found for the given EEG frontal pair, but the follow-up 2-way for that particular sex was not found.

Overall, the results for these tests suggest that for never-depressed participants, frontal asymmetry is unrelated to the level of emotion-related amygdalae activation, but for lifetime depression spectrum participants, in both men and women, relatively greater amygdalae activation to emotional faces (plus 1 standard deviation; SD) is associated with less left frontal activity as compared to those with less amygdalae activation to emotional faces (minus 1 SD). Plus 1 SD constitutes an activation that is more similar to the neutral activation, because an examination of mean activation levels showed neutral faces to be evocative of significantly more hemodynamic response as compared to each emotion condition.

Moreover, when levels of amygdalae activation to emotional faces are more similar to neutral (plus 1 SD), the pattern described in Aim 1 is altered for men but remains consistent for women. When the emotion-related activation is much less than neutral (minus 1 SD), then the pattern previously seen in the sex by depression interactions appears to be largely present for men, but altered for women.

A GLM was conducted with hemisphere (L, R) and Face Emotion (H>N, F>N, A>N; each for the left and right hemispheres) taken as within-subject factors and
biological sex and lifetime depression status taken as between-subjects factors. The test failed to show a significant main effect for sex or lifetime depression.

Identical models were conducted to investigate the fixation based contrasts. Only the neutral-based analyses are reported here, because fixation-based contrasts did not procure any significant main effects or interactions. Moreover, the consideration of neutral based contrasts is theoretically preferable, because neutral faces constitute a superior contrast condition to the fixation cross, since activations that are related to perception of faces are presumably subtracted, leaving only activations that are specific to the emotional character of the face conditions (i.e. neutral, anger, fear, happiness).
Table 5: Graphs from Linear Mixed Models, Happy > Neutral (Right)

**Men**

<table>
<thead>
<tr>
<th>ROI</th>
<th>ln(R/ln(L)) Total Alpha Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8_F7</td>
<td>-0.300</td>
</tr>
<tr>
<td>F4_F3</td>
<td>-0.200</td>
</tr>
<tr>
<td>F2_F1</td>
<td>-0.100</td>
</tr>
</tbody>
</table>

**Women**

![Graphs showing data for women](image)

<table>
<thead>
<tr>
<th>ROI</th>
<th>ln(R/ln(L)) Total Alpha Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8_F7</td>
<td>-0.200</td>
</tr>
<tr>
<td>F4_F3</td>
<td>-0.100</td>
</tr>
<tr>
<td>F2_F1</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Legend:**
- **Lifetime MDD+**
- **Lifetime MDD-**
Happy > Neutral (Left)

Men

Women

F8_F7

ln(R)-ln(L) Total Alpha Power

Amygdala ROI (z-scored)
Anger > Neutral (Right)

<table>
<thead>
<tr>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
</table>
Anger > Neutral (Left)

Men

Women

F8_F7

Amygdala ROI (z-scored)

\( \ln(\text{R} - \ln(\text{L})) \) Total Alpha Power

- -1 SD

+ 1 SD
Fear > Neutral (Right)

Men

Women

F8_F7

\[ \frac{\ln(R)}{\ln(L)} \] Total Alpha Power

Amygdala ROI (z-scored)

Minus 1 SD Plus 1 SD

F4_F3

\[ \frac{\ln(R)}{\ln(L)} \] Total Alpha Power

Amygdala ROI (z-scored)

Minus 1 SD Plus 1 SD

F2_F1

\[ \frac{\ln(R)}{\ln(L)} \] Total Alpha Power

Amygdala ROI (z-scored)

Minus 1 SD Plus 1 SD
Fear > Neutral (Left)

Men

Women

F2_F1

Amygdala ROI (z-scored)
Aim 4 – Does the presence of one or more short alleles of the 5-HTT gene predict increased amygdalae response to various emotionally laden stimuli among Caucasian participants?

Six univariate ANOVAs were conducted to examine whether the 5-HTT gene (homozygous non-risk vs. combined hetero- and homozygous risk) and/or lifetime depression spectrum (positive for depression history vs. never-depressed) could predict activation to the three emotional face conditions (H>N, A>N, F>N) in either the right or left amygdalae. These analyses were conducted using only the Caucasian subset of the total sample (N=23, 10 lifetime depression +) in order to avoid potentially confounding differences in gene frequency across individuals with different ethnic and racial backgrounds. These tests failed to show significant main effects for either lifetime depression or the 5-HTT gene. Lifetime depression by 5-HTT gene interactions were also found to be statistically non-significant.
DISCUSSION

The present paper reflects at least two important trends in the search for the physiological correlates of the depressive syndromes. The first trend is a movement from single to multi-method studies. The utilization of EEG, fMRI, behavioral, and genetic measures in a single sample has allowed for the integration of several disparate but mutually relevant aspects of the literature. To date, frontal EEG asymmetry and hemodynamic activation in the amygdalae have not been examined in the same sample. The present study also reflects a second trend to characterize the functioning of neural networks, as opposed to individual brain structures. In many areas of cognitive neuroscience, inquiry into the functioning of discrete structures is being supplanted by investigations into “functional connectivity” and “neural systems” (see Mayberg 1997 and Grecious et al. 2007 for examples of this trend in the depression literature). This shift in emphasis marks a natural progression in the field.

Synopsis

In the present sample, we have replicated a finding that has been shown twice before in the literature on resting frontal EEG asymmetry which suggests a depression by sex interaction (Knott et al., 2001; Miller et al., 2002). Depressed women showed the hypothesized right greater than left bias in activity over medial and lateral frontal sites. Depressed men, on the other hand, exhibited greater relative left frontal brain activity at rest than never-depressed men. This finding was also found in the much larger study from which the present sample was drawn (Stewart et al., 2009). The finding in women is
consistent with research suggesting that frontal EEG asymmetry is a liability marker, identifying a vulnerability to develop depression as well as a tendency to experience heightened negative affect and reduced approach motivation (e.g., Allen et al., 2004b; Davidson, 1998; Davidson et al., 2002; Dawson et al., 1997; Gotlib et al., 1998; Henriques & Davidson, 1990, 1991; Jones et al., 2000). The pattern observed in men is more puzzling. These observed sex differences may reflect distinct causal pathways for depression, they may reflect differences in symptom constellations, or they may reflect some confounding third factor. They are, however, consistent with findings that have shown frontal EEG asymmetry in women, but not men, to be predictive of future response to selective serotonin reuptake inhibitors (Bruder et al. 2001). Also, in the larger study of frontal EEG stability (Stewart et al., 2009), never-depressed women with a family history of depression showed a right biased pattern of activity as compared to women without a family history. Interestingly, a similar relationship was not found among male participants.

The present study did not replicate previous findings of hyperactivation in the amygdalae in depressed samples as compared to never-depressed controls. This is unlikely to be an artifact of the passive face viewing task, which has been used in a number of studies to reliably elicit amygdala activation. The failure to replicate may reflect the particular symptom configurations within our depressed sample. It is interesting to consider that neutral faces tended to elicit an appreciably larger hemodynamic response as compared to emotionally expressive faces, among both control and depressed participants.
Mixed linear models show that for never-depressed control participants, frontal asymmetry is unrelated to the level of emotion-related amygdalae activation, but for lifetime depression spectrum participants, in both men and women, relatively greater amygdalae activation to emotional faces (plus 1 SD) is associated with less left frontal activity as compared to those with less amygdalae activation to emotional faces (minus 1 SD). If frontal EEG asymmetry corresponds in someway to cortical inhibitory influence upon the limbic system, and the associated regulatory effect on emotional experience, then it is theoretically consistent for relatively greater amygdalae activation to be associated with relatively less left frontal activity. The fact that this pattern only bares out in the depressed group is puzzling, however. Frontal EEG asymmetry may also reflect a state of prefrontal activity that is partially determined by bottom-up influence from the amygdalae. If this is the case, then these findings may indicate that amygdalae activation to emotional stimuli among depressed individuals, whether high or low, has more of a bottom-up influence on prefrontal activity, as compared to healthy comparison participants. This potentially exaggerated bottom-up influence in the depressed group may be moderated by aberrant function in another structure, like the subgenual anterior cingulate, which has been identified as a potential “gateway” between cortical and amygdalar communication (Carhart-Harris, Mayberg, Malizia, & Nutt 2008).

When ROI activation in the amygdalae, in the context of the various emotional face conditions, was examined in light of the frontal EEG asymmetry data, another interesting pattern was observed. When activation to emotionally expressive faces was closer to the levels of activation observed in the neutral face condition (plus 1 SD), the
predicted pattern of association between frontal EEG asymmetry and depression based on the findings from Aim 1 was disrupted in men, but preserved in women. When levels of activation to emotion faces was considerably lower than that to neutral faces (minus 1 SD), the pattern was generally preserved for men, but not for women. This suggests the possibility that greater amygdalae activation to emotional faces disrupts the EEG asymmetry by depression by sex relationship.

The hypothesized link between risk alleles of the 5-HTT gene and amygdalae activation were not found. This failure to replicate previous studies (for review see Hariri et al., 2006) is quite possibly attributable to the relatively small sample size left to analyze when non-Caucasian participants were excluded from the statistical analyses. Previous studies have had sample sizes of 28 (Hariri 2007) and 26 (Rao 2006) with short and long allele subgroups matched on characteristics of race, personality, intelligence quotient, age, and biological sex.

The results of the present study mirror the present state of the literature on the neural correlates of depression, in the sense that they “do not cohere to tell as clear a story as we would like” (Gotlib & Hamilton 2008). This highlights the fact that the “depression spectrum”, as it is currently circumscribed by DSM-IV-TR diagnosis, does not neatly correspond to a well demarcated underlying neurobiology. The field is faced with the task of working towards depression subtypes and symptom profiles which systematically relate to specific social, physiological, and genetic patterns. The reported sex differences in frontal functioning and their interaction with amygdalae response may constitute a step towards this end.
APPENDIX A

Table 6: Whole Brain SPM Maps for Fixation Based Contrasts (p=0.05, 5 voxel cluster threshold)

<table>
<thead>
<tr>
<th>No Lifetime Hx of Depression</th>
<th>Lifetime Hx +</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anger &gt; Fix</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Fear &gt; Fix</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Happy</td>
<td>Neutral</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>Fix</td>
<td>Fix</td>
</tr>
</tbody>
</table>

Table showing brain images for Happy and Neutral conditions compared to Fix condition.
**APPENDIX B**

Table 7: ANCOVA (Lifetime Depression Status x EEG asymmetry at F8_F7) on Whole Brain Level for Men and Women Separately in SPM (p=0.05, 5 voxel cluster threshold)

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left Amygdala</td>
<td>Right Amygdala</td>
</tr>
<tr>
<td>Neutral &gt; Fixation</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Happy &gt; Fixation</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Fear &gt; Fixation</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Anger &gt; Fixation</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>
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