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Functional Connectivity in Youth at Risk for Depression

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A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science with Honors in Brain, Behavior and Cognitive Science from the College of Literature, Science, and the Arts in the University of Michigan

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Abstract

Offspring of parents with Major Depressive Disorder (MDD) have a three-fold higher risk of developing depression than offspring with no family history of psychopathology. Nevertheless, researchers still know very little about the mechanisms that impart a risk for depression. Previous studies found that relative to those with a low-risk for depression, high-risk individuals show greater activation in the amygdala (Monk et al., in press). In addition, Monk and colleagues found that the high-risk group showed increased activation to fearful faces in the medial prefrontal cortex during attention-constraining tasks. Thus, the present study predicted that the prefrontal cortex plays a role in mediating the increased subcortical response in high-risk individuals. In agreement with the hypothesis, the functional connectivity analysis revealed two regions in the right middle frontal gyrus that showed greater negative connectivity with the amygdala in high-risk individuals. The negative feedback in neural activation between the amygdala and the right middle frontal gyrus appear to be connected to a risk for depression in young adults.

Functional Connectivity in Youth at Risk for Depression

Depression affects about 14 million adults yearly in the United States and is the leading cause of disability in North America (Kessler et al., 1994). People diagnosed with major depressive disorder (MDD) have a pervasive mood disturbance associated with a loss of concern with daily activities. Marked changes in eating, sleeping patterns, concentration and self-image are common with the condition (American Psychiatric Association, 2000). Offspring of parents with MDD have a three-fold higher risk of developing depression than offspring with no family history of mental disorders. Though MDD has been studied extensively in adults, researchers still know very little about the mechanisms that impart a risk for depression, which typically begins in youth.

Prior studies have found abnormalities in the amygdala response in depressed subjects. Found in the medial temporal lobe, the amygdala is a brain structure highly associated with the processing of emotion (Phelps, 2004). S.M., a patient with selective bilateral damage to the amygdala, rated expressions of surprise, fear and anger as less prominent than did brain-damaged controls. This has established the central role of the amygdala in recognizing emotion (Adolphs, Tranel, Damasio, & Damasio, 1994). Patients with MDD show left amygdala hyperactivity in response to fearful and sad faces measured as blood oxygen level dependent (BOLD) activation. In addition, the right amygdala activity did not differ significantly between the MDD subjects and their matched controls (Sheline et al., 2001). Furthermore, depressed subjects have more difficulty in classifying emotions from facial expressions and, in contrast to S.M, tend to judge them more negatively (Gur et al., 1992; Sheline et al., 2001).

The genetic aspects of the amygdala have also been studied. Subjects with two short alleles on the serotonin transporter gene (*SLC6A4*) have an increased risk for depression (Caspi et al.,

2003; A. R. Hariri et al., 2002). The fMRI analysis on subjects with the two short alleles showed heightened amygdala activity in response to fearful faces when compared with individuals that were homozygous for the long allele (Hariri et al., 2002). Treatment using serotonin selective serotonin uptake inhibitors (SSRI) has resulted in a decrease of MDD symptoms and decreased bilateral amygdala activation (Sheline et al., 2001). The serotonin transporter thus appears to partly explain the familial nature of MDD and the increased amygdala response.

Other mood disorders have also implicated the amygdala as a major player in affective disturbances. A study on posttraumatic stress disorder (PTSD) shows that individuals with the condition have heightened activity in the amygdala along with reduced medial frontal gyrus activation (Rauch et al., 2000; Shin et al., 2005). The amygdala has also been shown to have increased activation in anxiety-prone subjects (Stein, Simmons, Feinstein, & Paulus, 2007). Furthermore, those with generalized social phobia (GSP), which is associated with a fear of social interactions, exhibit greater amygdala response to contemptuous and angry facial expressions (Stein, Goldin, Sareen, Zorrilla, & Brown, 2002). It seems likely that the amygdala's association to the emotional response puts it at the focus for many mood disorders, including MDD.

In addition to functional differences, prior research has also found significant physical changes in the amygdala associated with MDD. In one study, depressed subjects had bilaterally reduced amygdala core volumes with no significant disparity in the total amygdala volumes or in the whole brain volumes (Sheline, 1998). In contrast, another researcher reported the enlargement of the amygdala during the first episode of major depression (Frodl et al., 2002). Frodl reconciles this by noting the greater age of that study's participants and hypothesizes that depression progresses through enlarging and eventual shrinking of the amygdala with respect to

its volume. MDD usually occurs in early adulthood (Pine, Cohen, Gurley, Brook, & Ma, 1998), so the further study on the neuroimaging of youth at high-risk for depression before they have a chance to develop the disorder might further explain the progression of the disorder.

The studies on youth with MDD have provided some interesting but conflicting results. A small scale report showed that young girls with MDD had relatively less amygdala activation compared to their non-MDD counterparts (Thomas et al., 2001). A larger study (Roberson-Nay et al., 2006) found that youth with MDD show heightened left amygdala activation when viewing faces, consistent with the adult response. Previous research on the development of MDD found that relative to low-risk for depression, high-risk individuals show greater activation in the amygdala, where risk is determined by familial history of MDD (Monk et al., in press). Thus, the amygdala hyperactivation in youth could be a predisposing factor for clinical depression, partly explained by the aforementioned genetic factors. In addition, Monk et al. (in press) found that the high-risk group had increased activation to fearful faces in the medial prefrontal cortex during attention-constraining activity. Understanding this result could give some insight into how the high-risk youth are able to compensate for their heightened subcortical response and still maintain emotional equilibrium.

The activation of the prefrontal cortex has been of recent interest in unlocking the continuing mysteries of depression, as the higher brain regions are believed to regulate the emotional activity of the amygdala (Phelps, 2004). In explaining MDD, Drevets (1999) focuses on the prefrontal cortex rather than an amygdala abnormality in MDD, since depressive symptoms are commonly associated with diseases of the prefrontal cortex. He also identifies the limbic-thalamic-cortical circuit involving the amygdala, the mediodorsal nucleus, and ventrolateral and medial prefrontal cortex as part of this regulation (Drevets et al., 1992). Another study has

hypothesized that an insufficiency of the prefrontal cortex in modulating the amygdala is responsible for emotional disorders, specifically PTSD (Shalev, Rogel-Fuchs, & Pitman, 1992). Accordingly, two studies have found that cerebral blood flow in the right prefrontal cortex is negatively correlated with the blood flow in the amygdala (Garavan, Ross, & Stein, 1999; A. R. Hariri, Bookheimer, & Mazziotta, 2000). Drevets also suggests that a positive feedback loop between the prefrontal cortex and the amygdala due to rumination could be also a factor. In this case, excessive negative thoughts could elicit an amygdala response and consequently negative mood. Such studies have yet to identify a primary culprit, either the amygdala or the connectivity between the prefrontal cortex and the amygdala, in the development of MDD.

Neuroimaging studies typically look at blood oxygen level dependent (BOLD) activation in response due to different stimuli rather than inter-structural interactions. As an extension, functional connectivity has arisen as a way to analyze the interactions between neural structures. Functional connectivity is defined as the linked neural activation between spatially remote structures (Friston, 2003). This technique analyzes how the activation of individual voxels across the entire brain correlates to the activation of a specific seed region over time. Positive and negative correlations, known as connectivity, in activation between two regions could indicate positive and negative feedback between a given region of interest and the seed region provided that the regions are neutrally connected. Hariri et al. (2000) studied psychophysiological (PPI) interactions, a form of functional connectivity, as part of the analysis to reveal the regulatory relationship between the prefrontal cortex and the amygdala in ordinary human subjects. Functional connectivity would thus allow a researcher to test whether the strength of the connection between the amygdala and prefrontal cortex was related to the risk of

depression. It may also attribute the abnormal activity of either the prefrontal cortex or the amygdala as the primary disposing factor of MDD.

Hypothesis

When group differences in functional connectivity are considered in the fMRI analysis, subjects at high-risk for MDD would show a greater negative connectivity between the amydala and prefrontal cortex.

Method

Participants

The sample size of this study consisted of 22 low-risk and 17 high-risk individuals between 10 and 18 years of age and IQs greater than 70. The low-risk and high-risk participants were matched on age and IQ. The National Institutes of Health and the New York University School of Medicine's Institutional Review Board approved all procedures involved. Informed consent was obtained from parents and offspring aged 18 and older.

The high-risk group included 9 males and 8 females with a mean age of 14.35 (SD = 2.06) and mean IQ of 102.9 (SD = 13.4). Subjects were classified high-risk if they had a parent with a lifetime history of MDD evaluated through a Structured Clinical Interview for DSM-III-R (SCID) by trained clinicians (Spitzer, 1992). Parents had been patients at mood/anxiety disorder clinics. Interviews took place in the New York City area. The offspring were evaluated to be free from current psychopathological disorders through direct interviews and the Parent as Respondent Informant Schedule (PARIS).

The low-risk group had 10 males and 12 females with a mean age of 13.86 (SD = 2.49) and a mean IQ of 105.8 (SD = 9.97). Using the SCID interview, both parents were found to be free of anxiety, mood, & psychotic disorders. The offspring were also evaluated to be free from

current psychopathological disorders through PARIS and direct interviews. Interviews took place with blind evaluators in the New York City area or in Bethesda, MD. Thirteen of these subjects were recruited from the Washington, D.C., metropolitan area while the others were drawn from the study in New York City.

Task

For the task of this study, participants viewed 80 different pictures 3 times each, in random order, one in each of three attention conditions. The 80 pictures comprised 8 different models each portraying 10 levels of intensity of emotion. As shown in Figure 1, the faces were produced by morphing happy, fearful and neutral faces with the following compositions: 100% happy, 75% happy/25% neutral, 50% happy/50% neutral, 25% happy/75% neutral, 100% neutral, 25% fearful/75% neutral, 50% fearful/50% neutral, 75% fearful/25% neutral, 100% fearful, and exaggerated fear (150%) .

The full set of faces was split up into blocks of 10 images, each using a different attention condition. The order of the blocks and the images were randomized across subjects and blocks. Each face was shown for 3 seconds with an inter-trial interval lasting between 750 and 1250 milliseconds. One attention condition required subjects to simply view the faces. The second attention condition asked the subjects about an emotional aspect of the face, whereas the final attention condition asked the subjects about a non-emotional aspect. Figure 2 lists the questions used for each attention condition. Subjects were given a keypad to indicate their response. Participants were trained before hand and told they would see faces of different expressions. They were given an oral explanation of the procedures and allowed to practice on a laptop showing the same instructional screen but a separate set of faces. The training took no longer than five minutes.

While subjects performed the task, their brain function was scanned using fMRI. Scans consisted of 29 interleaved 3.3 mm axial slices covering the entire brain, parallel to the AC-PC on a General Electric Signal 3-tesla scanner. The research group used an echo planar single shot gradient echo T2 weighting (TR=2300 ms; TE=23 ms; FV=240 mm; 64 x 64 matrix; 3.3 x 3.75 x 3.75 mm voxel). High resolution T1-weighted volumetric scans incorporated a magnetization prepared gradient echo sequent (MP-RAGE) [180.10 mm axial slices; FOV=256 mm, NEX=1, TR=11.4 ms, TE=4.4 ms; matrix=256x256; TI=300 ms, bandwidth 130 Hz/pixel=33 kHz for 256 pixels in-plane resolution = $1mm^3$]. Data were adjusted for temporal differences in slice acquirement using slice timing correction. Using SPM2 (Wellcome Department of Neuroscience, University College London), scans were motion corrected and spatialized to a Montreal Neurological Institute (MNI) T1-weighted template image and smoothed with a 6 mm full-width at half-maximum Gaussian kernel. This procedure is known to be valid in children and adolescents (Burgund et al., 2002). The first six scans were discarded to allow magnetization to reach equilibrium.

Functional connectivity of the brain was analyzed by measuring the activation of each voxel across the brain associated with a particular seed region. The seed regions used in this analysis included the left and right amygdalae. The mean echo planar imaging (EPI) time series for the seed region was extracted over all the time points, mean-centered and root-mean-square (RMS) normalized. The analysis involved RMS normalization to be consistent with the psychophysiological interaction (PPI) analysis, the standard procedure of the SPM software package and previous studies (Friston et al., 1997; A. R. Hariri et al., 2000; Rich et al., in press). The ensuing time series was used in a subject-level general linear model as the only regressor of interest against activation throughout the brain. The computer analysis then involved a high-pass

(128 sec cut-off) and a low-pass filtering (SPM-provided canonical hemodynamic blood flow (HRF)) applied to the seed region of interest. An ANOVA test was used to compare the activation of each voxel of the brain between the low risk and high risk groups. Finally, a threshold of $p \leq .001$ (uncorrected) was used to determine significance, and the analysis of related activation was limited to the prefrontal cortex.

Results

Group Similarities

Negative Connectivity

As illustrated in Table 1, the results revealed negative connectivity between the left amygdala and the left middle frontal gyrus in the low-risk group (xyz: -36, 22, 34; $t(3.51) = 3.24$; $p = 0.001$) and the high risk group (xyz: -30, 24, 40; $t(5.58) = 4.72$; $p < 0.001$).

Positive Connectivity

The left amygdala showed positive connectivity between the left amygdala and the right orbitofrontal cortex in the low-risk (xyz: 22, 4, -12; $t(6.99) = 5.55$; $p < 0.001$) and the high-risk (xyz: 22, 6, -12; $t(9.83) = 6.85$ $p < 0.001$) group. The results revealed no positive connectivity between the right amygdala and the prefrontal cortex.

Group Differences

Negative Connectivity

Compared to the low-risk group, the high-risk group exhibited greater negative connectivity between the left amygdala and the right middle frontal gyrus at two locations (xyz: 20, 28, 10; $t(37) = 3.42$; $p < 0.001$ & xyz: 38, 22, 46; $t(37) = 3.69$; $p < 0.001$ (uncorrected)). The same regional activation was found in the high-risk group (xyz: 40, 24, 34; $t(7.90) = 6.01$; $p < 0.001$) but was missing from the low-risk group. This is illustrated in Figures 3 and 4.

Positive Connectivity

Between the amygdala and the prefrontal cortex, the data revealed no significant positive connectivity differences between the two groups.

Discussion

The resulting data were consistent with the overall model of cortical inhibition of the amygdala used as the basis for the hypothesis. As with Sheline (2001), these data revealed an activation difference in the left amygdala but not the right amygdala. Like Hariri's (2000) study on MDD subjects, this study found negative connectivity primarily between the left amygdala and the right prefrontal cortex. However the center of this connectivity (40, 28, 0) differed from the two coordinates found in the right prefrontal gyrus: (20, 28, 10) and (38, 22, 46). Whether the difference is attributable to the onset of MDD or differences in methodology is unclear from available data. Additionally, the data obtained for the low-risk subjects differed from Garavan's (1999) which found contiguous activation associated with response inhibition in four regions in the frontal gyri. The coordinate (36, 23, 33) in the right middle frontal gyrus was closest to the present study's coordinate at (38, 22, 46) in the same region associated with negative connectivity in high-risk individuals. Once more, Garavan's (1999) methodology greatly differed from that of this study and predated functional connectivity using SPM. Thus, no clear conclusions can be made regarding connectivity differences between high-risk for MDD and MDD subjects.

Consistent with the hypothesis, the results have demonstrated greater negative connectivity between the left amygdala and two regions in the right middle frontal gyrus in youth at high risk for depression. Taking previous findings into account, these data suggest that in reaction to a greater amygdala-related emotional response, high-risk individuals compensate by

exerting more conscious effort to maintain an emotional equilibrium. This also supports Monk's (in press) findings where the high-risk group had greater prefrontal activity compared to the low-risk group when they were engaged in cognitive tasks. A risk for depression may therefore be associated with a greater than average amygdala response and greater negative connectivity between the prefrontal cortex and the amygdala. As previously mentioned, the hyperactive amygdala may be explained by having the two short alleles of the serotonin transporter gene, which thus convey a risk for depression.

This study found no evidence of any significant difference in positive connectivity between the amygdala and prefrontal cortex when comparing the high-risk group to the low-risk. Therefore, rumination, as part of a positive feedback loop between structures does not appear to characterize a preceding mechanism in MDD. Similar findings characterized other studies as well (Garavan et al., 1999; A. R. Hariri et al., 2000; Sheline, 1998). Negative connectivity rather than positive connectivity involving the prefrontal cortex significantly differed between the two groups, suggesting that amygdala abnormality rather than faulty connectivity could work as a predisposing factor.

Furthermore, the data revealed some regions that exhibited similar connectivity with the amygdala in both groups. Activation in the left middle frontal gyrus and the left supramarginal gyrus negatively correlated with amygdala activation. The other regions in the gyri likely correspond to conscious activity as with the right middle frontal gyrus occurring as part of the task. A high risk for MDD is not associated with changes in connectivity in these regions.

The orbitofrontal cortex was the only region that demonstrated positive connectivity with the amygdala in both groups. This region has previously been associated with decision making and emotional processing (Bechara, Damasio, & Damasio, 2000), activity which is associated

with the fMRI task. These regions seem to have been activated by the amygdala during the task in order to have the brain decide on how to respond to the stimuli, in this case, the faces. This is another region whose connectivity with the amygdala appears to be unaffected by a risk for depression.

Though the prefrontal cortex appears to modulate the amygdala in the task, it remains unclear whether this is simply due to the cognitive task or actual conscious thoughtful inhibition. Any type of prefrontal cortex activity may naturally inhibit amygdala activation in order to divert further blood flow to cognitive processing. Further experimentation on resting connectivity on high-risk versus low-risk individuals could elucidate this matter and reveal the brain mechanisms in the absence of stimulation.

Additionally, further studies could reveal the effectiveness cognitive relaxation and stress-relief strategies compared to the SSRIs used in Sheline's study (2001). This would also address whether a hyperactive amygdala becomes desensitized to modulation, resulting in depression. An overactive amygdala response combined with the failure to continue to regulate could lead to the depressive symptoms.

Another possible future direction would be to perform longitudinal studies on youth at risk for depression and analyze the connectivity differences between those that later develop MDD and those who do not. The ability to detect a connectivity difference that correlates with the later development of MDD would be useful as a preventative diagnostic tool.

The use of RMS normalization poses a methodological limitation in that the numerical value of the predictor may affect the scale of its coefficient. Thus, this analysis of group differences may overrate the difference in connectivity levels (Rich et al., in press). However, as

previously discussed, this procedure is consistent with previously published studies using functional connectivity.

Overall, the presence of greater negative connectivity occurring in youth at high-risk for depression suggests that that risk is associated with higher amygdala response and modulation of that response by the prefrontal regions of the brain. Functional connectivity analysis on high-risk youth before they have had a chance to develop depression has helped to identify the negative connectivity between the brain and the amygdala as a possible preceding mechanism. However, researchers have only begun to understand the steps leading to depression. Future studies will be necessary that document the changes in connectivity over time to fully understand the development of MDD. Other techniques such as Diffusion Tensor Imaging (DTI) could further examine the structural connections between the prefrontal cortex and the amygdala. The research techniques developed and their findings will only continue to help scientists understand more complex psychological disorders and treat them with minimal side effects.

Acknowledgements

I would like express my sincere thanks to Dr. Christopher Monk who has entrusted and challenged me with some of the most difficult projects available in the field today. I also appreciate his patience and flexibility with my ever-changing responsibilities and schedules. Secondly, I wish to thank Adam Bernstein for aiding in my smooth initial transition into the lab and helping to learn SPM and functional connectivity. Dr. Stephen Fromm of NIH has also earned my appreciation for promptly answering my emails even when he was extremely busy. The next thanks go to Lauren Dayton and Jillian Lee for their time in running analyses for my projects and also to my labmates Jen Weng, Joan Zaccagnini, Hugo Louro, and Julie Maslowsky for providing enjoyable conversation and company during those long hours in the lab. Last but not least, I would like to thank my mom, dad and sister who, without them, my life would not seem complete.

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Figure Captions

Table 1. The table lists all the regions associated with connectivity that either appear in both groups or show a significant difference between the groups. The table is color coded by region to compare connectivity between groups. The coordinates are listed in Montreal Neurological Institute (MNI) format. The table also includes significant connectivity found outside the prefrontal cortex for reference.

Figure 1. This composite image illustrates the ten levels of emotion displayed during the fMRI task.

Figure 2. This figure presents the questions asked of the subjects during the fMRI task for each condition.

Figure 3. This figure highlights the exact region in the right frontal gyrus centered at xyz: 20, 28, 10 that exhibited greater negative connectivity in the high-risk compared to low-risk individuals. This connectivity is prominent in the high-risk group but absent in the low-risk group. The "high-risk minus low-risk" graph illustrates the connectivity difference between the two.

Figure 4. This figure highlights the exact region in the right frontal gyrus centered at xyz: 38, 22, 46 that exhibited greater negative connectivity in the high-risk compared to low-risk individuals. This connectivity is prominent in the high-risk group but absent in the low-risk group. The "high-risk minus low-risk" graph illustrates the connectivity difference between the two.

Passive Viewing Attention Condition:

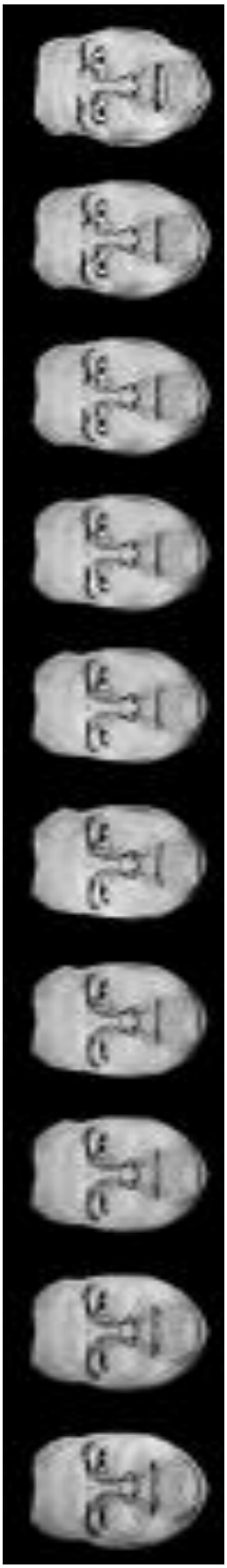
"Just look straight ahead. Do not rate the next set of faces."

Subjective Fear Attention Condition:

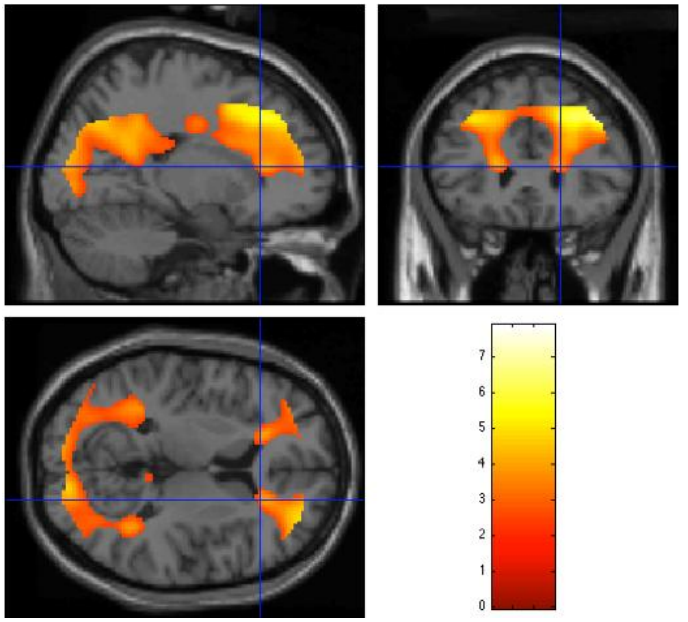
"How afraid are you? 1. Not at all. 2. Just barely. 3. A little. 4. Very. 5. Extremely."

Non-emotional Attention Condition:

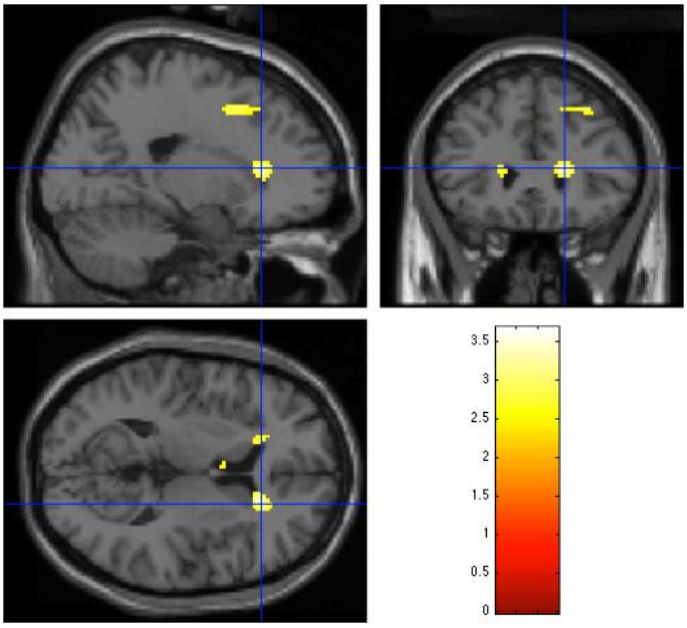
"How wide is the nose? 1. Not at all. 2. Just barely. 3. A little. 4. Very wide. 5. Extremely wide."



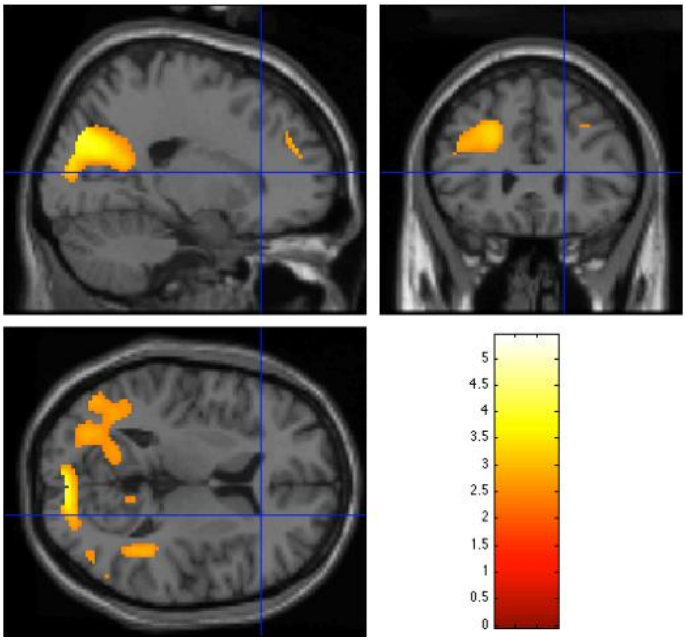
seed stucture	connectivity	group	x	y	z	p	region
left amygdala	negative	low-risk group	54	-54	28	0.000	right inferior parietal lobe
left amygdala	negative	low-risk group	-36	22	34	0.001	left middle frontal gyrus
left amygdala	negative	low-risk group	-34	16	29	0.001	left middle frontal gyrus
left amygdala	negative	high-risk group	40	24	42	0.000	right middle frontal gyrus
left amygdala	negative	high-risk group	58	-50	36	0.000	right parietal lobe
left amygdala	negative	high-risk group	-30	24	40	0.000	left middle frontal gyrus
left amygdala	negative	high-risk - low risk	38	22	46	0.000	right middle frontal gyrus
left amygdala	negative	high-risk - low risk	20	28	10	0.001	right prefrontal cortex
left amygdala	positive	low-risk group	-24	0	-14	0.000	left amygdala
left amygdala	positive	low-risk group	22	4	-12	0.000	right orbitofrontal cortex
left amygdala	positive	low-risk group	-50	2	-12	0.000	left superior temporal sulcus
left amygdala	positive	high-risk group	-22	2	-14	0.000	left amygdala
left amygdala	positive	high-risk group	22	6	-12	0.000	right orbitofrontal cortex
left amygdala	positive	high-risk group	-52	2	-12	0.000	left superior temporal sulcus
left amygdala	positive	high-risk group	56	-4	40	0.001	right precentral gyrus
left amygdala	positive	high-risk group	50	-10	46	0.001	right precentral gyrus
left amygdala	positive	high-risk - low risk	66	-4	12	0.001	right superior temporal gyrus
right amygdala	negative	high-risk group	-36	-60	36	0.000	Left supramarginal gyrus
right amygdala	negative	low risk group	-36	-64	34	0.000	Left supramarginal gyrus
right amygdala	positive	low-risk group	26	-4	-20	0.000	right amygdala
right amygdala	positive	low-risk group	12	-14	-22	0.000	right amygdala
right amygdala	positive	high-risk group	24	-4	-16	0.000	right amygdala
right amygdala	positive	high-risk group	12	-12	-22	0.000	right amygdala



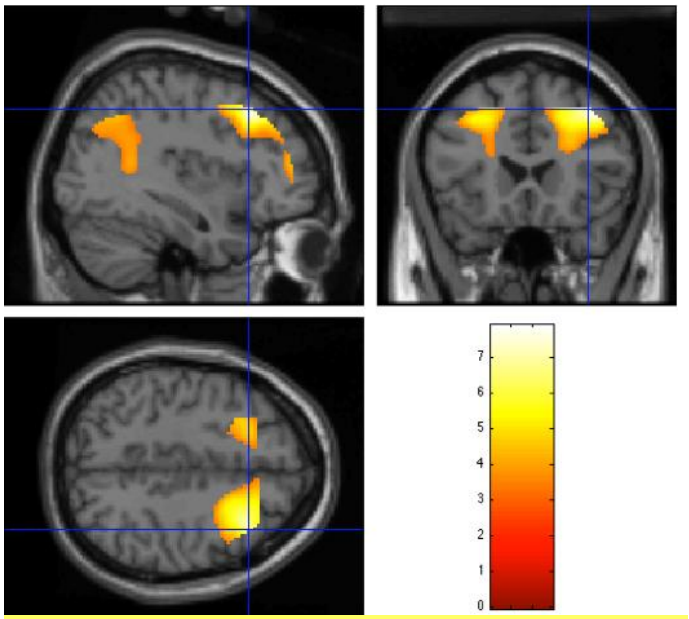
High-Risk Group



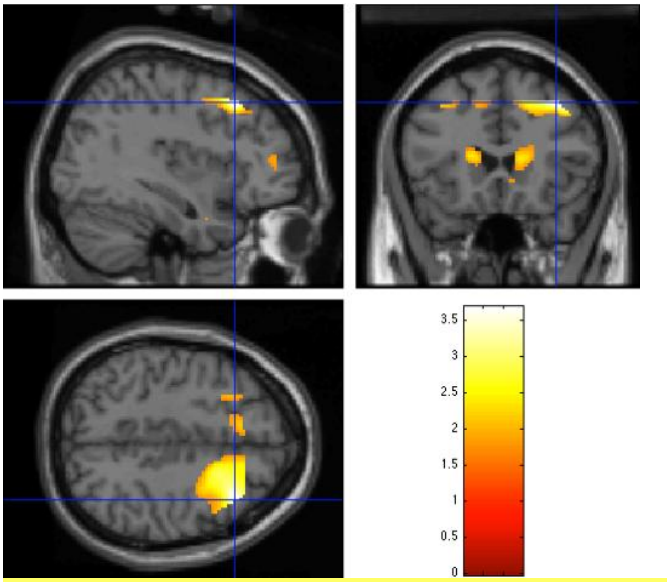
High-Risk Minus Low-Risk



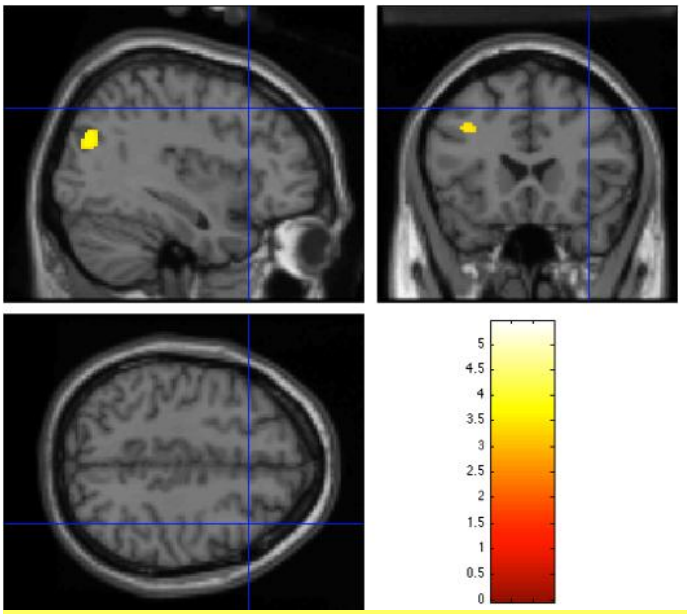
Low-Risk Group



High-Risk Group



High-Risk Minus Low-Risk



Low-Risk Group