Sustained Neural Processing in Affective Regions Predicts Efficacy of a Computer-Based Intervention Targeting Attentional Patterns in Transdiagnostic Clinical Anxiety

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Abstract

Research suggests that individuals with clinical anxiety demonstrate an attention bias toward threatening information in their environment. Attention Bias Modification (ABM) is a computer-based treatment that trains attention towards non-threatening stimuli over threatening stimuli. While alterations in initial processing of threat have been linked to responses to ABM, the impact of sustained processing in the aftermath of neutral and threatening information upon outcomes following this targeted intervention has not been well studied. Our study analyzed how sustained activity in brain regions related to cognitive and affective processing can predict who is a good candidate for ABM. Unmedicated anxious individuals assigned to the ABM condition (n=38) underwent fMRI during performance of a novel task sensitive to sustained emotional information processing. Afterward, they underwent eight ABM treatment sessions. Participants whose sustained reactivity to neutral stimuli was high in the amygdala, the left BNST, the left VLPFC, and the pgACC displayed the least improvement with ABM. These results suggest that certain anxious individuals may have difficulty distinguishing between neutral and threatening information due to an overly threat-oriented appraisal of their environment, and would thus benefit less from ABM. By studying neural predictors of success in ABM treatment and focusing on the individual differences in neural-attentional dimensions within a transdiagnostic sample of anxiety patients, we can help identify which subset of anxious patients would be good candidates for this intervention in the clinical setting.

Introduction

Anxiety disorders are the most prevalent class of mental illness. A recent meta-analysis of many research studies revealed that the current global prevalence of anxiety disorders is around 7.3% (Baxter et al., 2013). In the United States alone, anxiety affects roughly 18% of the nation's population (Kessler et al., 2005). For those affected, the excessive worry, fear, and other psychological symptoms can greatly decrease quality of life, and can cause consequential medical morbidity and disability. Clinical and subclinical forms of anxiety also represent a significant public health burden, costing the U.S. more than $42 billion a year (Greenberg et. al, 1999). Anxiety disorders share features of both fear and anxiety and can manifest as many different types. Fear is an emotional response to a perceived threat and often associated with fight or flight or escape responses. Anxiety is the anticipation of a future threat and vigilance in preparation for this future threat, which may or may not include avoidance behaviors (American Psychiatric Association, 2015). Common anxiety disorders include generalized anxiety disorder, panic disorder, specific phobias, social phobia, post-traumatic stress disorder, separation anxiety disorder, and agoraphobia. Response rates for current first-line treatments stand at only 50-70% with high rates of relapse and low rates of remission (Ballenger, 2004; Barlow et al., 2004, Hofmann and Smits, 2008; McEvoy, 2007). Only 12.7% of patients affected by an anxiety disorder receive adequate treatments such as cognitive-behavioral therapies (CBT) or pharmacotherapy, and disorder prevalence rates remain high (Wang et al., 2015). Given the prevalence of these disorders, these observations emphasize the need to continue developing new and refining old treatment approaches to increase patient access and reduce costs.

Attention Bias Modification (ABM) is a novel computer-based treatment approach that

offers several benefits over current first-line treatments including cost-effectiveness, ease of dissemination, and low patient burden (Price et al., 2016). ABM is designed to target a well-replicated and studied observation in anxiety: selective attention to threat. A large body of research has established that anxious individuals as a trans-diagnostic group exhibit an attentional preference toward threatening information, or an attentional bias (AB). For example, researchers have demonstrated AB in anxious patients by using an emotional version of the Stroop task. Participants are asked to name the color in which words are printed, and the results show that anxious individuals are slower to name colors of words associated with concerns relevant to their clinical condition, indicating AB to the threatening content of the words (Williams, Matthews, & MacLeod, 1996). The most frequently used method that has been used to study anxiety-linked AB has been the attentional probe assessment task (MacLeod, Mathews, & Tata, 1986). In this visual attention task, threatening and neutral stimuli in the form of words or images are briefly and simultaneously presented in two different areas on a screen. This is followed by a small probe in the location of either of the two prior stimuli, and the participant's speed to make a response to the probes in each location is recorded. Anxious individuals demonstrate a quicker response to probes appearing in the location of the threatening stimuli than the neutral stimuli, indicating AB towards threatening stimuli (Bar-Haim et al, 2007; MacLeod et al., 1986).

The observation that AB towards threat was present in those suffering from anxiety disorders invited speculation on the potential causal role of AB on anxiety. ABM treatment was developed to therapeutically exploit the potential causal role of AB upon anxiety. ABM treatment seeks to modify AB and train patients to attend to non-threatening stimuli

preferentially over threatening stimuli in the initial stages of threat processing. The treatment includes variants of the original attentional probe assessment task reconfigured to encourage attentional change (MacLeod, Clark, 2015). In ABM, across repeated training sessions, a probe is systematically placed in the location of neutral stimuli to shape attention through practice, thereby training participants to selectively attend away from threatening information.

If AB does have a causal role in anxiety, then reduction of AB towards threat should also reduce anxious symptoms. In two foundational ABM studies conducted in 2002, MacLeod et al. found that ABM was successful in both modifying attentional bias and that this modification of attentional selectivity did influence stress reactivity (MacLeod et al., 2002). After these studies, a continually growing literature suggests that attention modification interventions are effective in reducing anxiety symptoms. In a study in 2009, individuals with generalized anxiety disorder (GAD) were recruited to complete ABM or a sham/control version of the training, in which attentional patterns are not shaped either toward or away from threat. Participants who completed ABM (but not sham) reported a decrease in anxiety both through self-reports and interview measures (Amir et al, 2009). Another study in 2011 used ABM to train attention away from threat among anxious youth or children. After only 4 weeks, many youths reported a significant decrease in anxiety and no longer met criteria for an anxiety diagnosis (Rozenman, Weersing, & Amir, 2011). In another study in 2012 with an attention training program on participants with generalized social phobia, those who were trained to attend to nonthreatening cues demonstrated reductions in self-reported and physiological measures such as skin conductance of social anxiety (Heeren et al, 2015). These results are consistent with the hypothesis that attention bias plays a causal role in anxiety. However, subsequent meta-analyses studying ABM treatment

show that its potential beneficial effects on anxiety are inconsistent across individuals and studies (Price et al., 2016). Thus more research is necessary to understand *which* anxious patients will most likely benefit from ABM and *why* they do, since anxiety disorders affect a heterogeneous group of individuals.

To understand which anxious patients are most likely to benefit, one factor that may be important to consider is the timeframe over which a given anxious individual exhibits attentional bias towards threat. As a group, anxious individuals exhibit threat vigilance during initial stages of processing (e.g. 16-500 ms after stimulus onset) (Bar-Haim et al., 2007). However, some anxious patients also exhibit sustained threat processing involving perseverative attention—worry and rumination— about the information even after the stimulus has been removed. The sustained or perseverative processing in the aftermath of neutral and threatening information may be important and potentially impact outcomes following ABM. Thus while alterations in initial processing of threat have been linked to responses to ABM (Amir et al, 2011; Kuckertz et al., 2014; Price et al., 2016), we currently know nothing about how sustained patterns of threat processing might impact outcomes following this targeted intervention.

To date, ABM studies have largely focused on group-level observations of whether anxious patients, as a group, benefit from ABM, which can mask considerable within-group heterogeneity linked with ABM treatment outcome. Thus an individual differences approach that examines initial as well as sustained threat processing mechanisms may capture critical, clinically relevant information. Additionally, previous studies have largely focused on establishing ABM efficacy in narrow diagnostic categories. A transdiagnostic approach where

patients across multiple diagnostic categories are recruited to empirically derive common psychological mechanisms behind anxiety disorders has become increasingly viewed as important in advancing the field of psychiatry by better representing the real-world clinical patient population (Insel et. al, 2010). Consistent with this viewpoint, an important question for ABM research is whether transdiagnostic dimensions of threat processing can be used to classify patients according to certain mechanisms and address these mechanisms directly. Although initial and sustained bias are present simultaneously in many patients, we hypothesized that patients relatively high on a sustained or perseverative processing dimension in the aftermath of neutral and threatening information would not be ideal candidates for ABM, which targets initial bias only.

Our study asked specifically whether sustained activity in a relative network of cognitive and affective brain regions related to cognitive and affective processing could predict who would be a good candidate for ABM. The regions chosen were the left and right amygdala, left and right bed nucleus stria terminalis (BNST), left dorsolateral prefrontal cortex (DLPFC), left and right ventrolateral prefrontal cortex (VLPFC), dorsal anterior cingulate cortex (dACC), pregenual anterior cingulate cortex (pgACC), and subgenual anterior cingulate cortex (sgACC). The brain areas chosen were those known to be involved in the neural circuitry both for the initial processing in guiding attention to threat and for sustained processing after the threat has been removed. The amygdala receives fear signals from cortical sensory processing regions and the thalamus that allow for bottom-up responses that encode the affective properties of the stimuli, thus promoting initial bias and the rapid orientation towards fear (LeDoux 2000). The prefrontal cortex, which includes the DLPFC and the VLPFC, is known to be involved in higher

executive functions such as decision-making and emotion regulation (Phillips, Ladouceur, & Drevets, 2009). In threat processing, the top-down ventral prefrontal signals are capable of modulating amygdala activity and biasing visual attention selectively towards threatening stimuli (Desimone & Duncan, 1995). As further support for the top-down mechanism of attentional control, connectivity analyses have revealed a circuit connecting from the ventromedial through dorsolateral prefrontal cortices onto the amygdala (Mohlman et al, 2009). The amygdala also mediates sustained fear processing through its downstream effects on the hypothalamus, the brainstem, and other regions (Davis et al., 2010). Specifically, the amygdala releases corticotropin-releasing factor, a stress hormone, that acts upon the BNST. The BNST subsequently targets many areas such as the hypothalamus, brain stem, and the hypothalamic-pituitary-adrenal axis, playing a crucial role in facilitating a sustained state of anxious apprehension (Davis et al., 2010; Walker, Toufexis & Davis, 2003). The anterior cingulate cortex regions including the dACC, pgACC, and sgACC are regions also implicated in emotion regulation and attentional control. The anterior cingulate cortex has been shown to modulate the thalamus-amygdala pathway involved in processing fear signals (Das et al., 2005). A magnetic resonance imaging study in 2010 for sustained threat processing in generalized anxiety disorder (GAD) patients demonstrated that GAD patients exhibited dysregulation in the anterior cingulate regions, providing further support for the relevance of the anterior cingulate cortex regions in the processing of threat stimuli (Paulesu et al, 2010).

In summary, this study is one of the first to study neural predictors of success in ABM treatment, and extends the literature to a novel sustained attention task. In a randomized controlled design, individuals with transdiagnostic clinical anxiety were allocated to receive

either active ABM or a sham/control variant of the same task, and completed a battery of clinical, behavioral, and neural measures designed to capture both initial and sustained forms of threat processing. The present analyses focus on testing whether sustained neural activation patterns can be used to predict outcome following active ABM. By focusing on individual differences in neural-attentional dimensions within a transdiagnostic sample of anxiety patients, we hope to help identify which subset of anxious patients would be good candidates for ABM in the clinical setting.

Methods

Participants

Adults age 18-55 who often feel "anxious, shy or worried" were recruited using advertisement, the WPIC adult outpatient service clinics, and a local registry of interested research participants. These ages were chosen to limit the heterogeneity of brain structure and function related to development and cognitive aging. After an initial phone interview to determine their eligibility, potential participants completed a battery of assessments including a structured clinical interview administered by a clinical assessor (the MINI International Neuropsychiatric Interview). Participants were eligible if they scored >45 on the Spielberger State-Trait Anxiety Inventory—trait form (STAI-T), a 20-item inventory of trait anxiety well known to be able to assess the severity of habitual anxiety. Additionally, participants were required to exhibit clinically significant impairment as indicated by score at or above 75th percentile on the WHO Disability Assessment Schedule (WHODAS) 2.0. Above 75th percentile is characteristic of those individuals who are affected by one or more mental disorders. These two criteria were designed to get an appropriate distribution of individuals with clinical levels of transdiagnostic anxiety. Participants were then included in the study if they (a) passed the first two criteria; (b) were not currently participating in cognitive behavioral therapy (CBT) since these may be a confounding variable in our study; (c) were not taking any psychotropic medications since they may alter the fMRI BOLD signal; (d) met standard fMRI inclusion criteria, (e) had no evidence of bipolar, psychotic, autism spectrum, substance dependence, or primary depressive disorder; (f) showed no evidence of acute suicidality; (g) scored >20/40 on the Snellen test indicating normal or corrected-to-normal vision; and (h) had a reading level >6th grade as per the WRAT-R reading scale. In cases of comorbid anxiety and depression, the

clinical interviewer determined that the anxiety was primary in all cases. Comorbid depressive diagnoses secondary to anxiety were allowed as in previous ABM research because their inclusion is a more realistic sampling of the anxious population. A total of 70 treatment-seeking adults fulfilled these criteria and participated in the study.

Ethics

This study and the parent trial were approved by the University of Pittsburgh Institutional Review Board (IRB). After being fully informed regarding the nature of the study, all participants gave written consent to participate in the study by signing University of Pittsburgh IRB-approved consent forms.

Procedures

Individuals were interviewed in their first visit by a clinical assessor to ensure qualification for the study, and completed several assessments and self-report measures. Self-report measures included the Mood and Anxiety Symptoms Questionnaire (MASQ): Anxious Arousal subscale, a well-validated questionnaire that assesses the severity of anxious symptoms where higher scores reflect greater levels of symptomatology. Additionally, they provided a list of self-generated personally relevant negative words. In the second visit, all participants underwent a baseline 1.5 hour fMRI session in which they completed several tasks including the Alternating task (described below) in the scanner. Participants were then randomly assigned to one of two conditions: ABM (n=49) or a matched control condition (n=21). Uneven allocation was used to maximize sample size and statistical power in the active ABM group, given that the primary hypotheses were concerned with mechanistic predictors of ABM response. Both patients

and clinical assessors were blinded to treatment allocation and the study hypotheses. In visits 3-10, the participants completed a total of eight 15-minute computerized training sessions in the laboratory twice weekly over four weeks for either the ABM training or the matched control task. Visit 11 was the post-intervention clinical assessment where they were interviewed again by a clinical assessor to reassess anxiety symptoms to evaluate the efficacy of ABM in reducing anxiety symptoms.

Of the 70 participants who qualified for the study, 94.3% of the participants (n=66) completed their assigned treatment condition and returned for post-treatment assessment. After preprocessing the fMRI data, and excluding data exhibiting excessive motion, 57 of these participants (n=38 in the ABM group) had usable fMRI data at baseline on the analyzed Alternating task and were thus included in the analyses.

Stimuli

Words rather than pictorial stimuli were used since words have been associated with increased ABM effect sizes. Following the initial clinical interview, participants collaborated with the clinical assessor to select ten self-relevant threat words related to their specific anxiety domains reported during the clinical interview. All self-relevant threat words chosen were rated by the participant as a -2 or -3 on a scale ranging that rates the word on a range from +3 (very pleasant) to 0 (neutral) to -3 (very unpleasant). The ten total self-relevant threat words were chosen to encompass a range of concepts most relevant to the participant's daily experience of anxiety. Ten idiographic neutral words were chosen from a word list used previously in ABM research that the participant rated neutral on a 1-7 scale ranging from "very unpleasant" to "very

pleasant", and also rated at the highest familiarity level on a 1-7 scale. 20 additional normative threat words and 20 additional normative neutral words were used uniformly for all participants to supplement the idiographic word lists during ABM or control training, but were not used during the Alternating task.

ABM

ABM Condition. Each session of the ABM treatment consisted of 300 trials of a dot-probe task designed to train attention away from threatening stimuli. Participants were seated approximately 30 cm from the computer screen. In this task, each trial began with focusing on a central fixation cross for 500 ms. Following that, a word pair was presented for 500 ms. The words were presented vertically in the center of the screen approximately 1.5 cm from one another (with an approximate 2-degree visual angle) for 500 ms. There were two kinds of word pairs: threat-neutral and neutral-neutral. Participants were presented with 80% threat-neutral word pairs and 20% neutral-neutral word pairs during each visit.

In a threat-neutral trial, each word pair consisted of a threat and neutral word presented together as shown in Figure 1. In the active ABM condition, immediately following the word pair, a probe letter (E or F) was placed in the previous location of the neutral word for 1500 ms 100% of the time. The neutral word position was randomized to either the upper or lower word location, and the probe remained on-screen until the participant responded via button press to indicate the probe letter displayed. In a neutral-neutral trial, each word pair consisted of two neutral words and the probe was shown behind either neutral word with equal probability. Word pairs were presented in a random order.

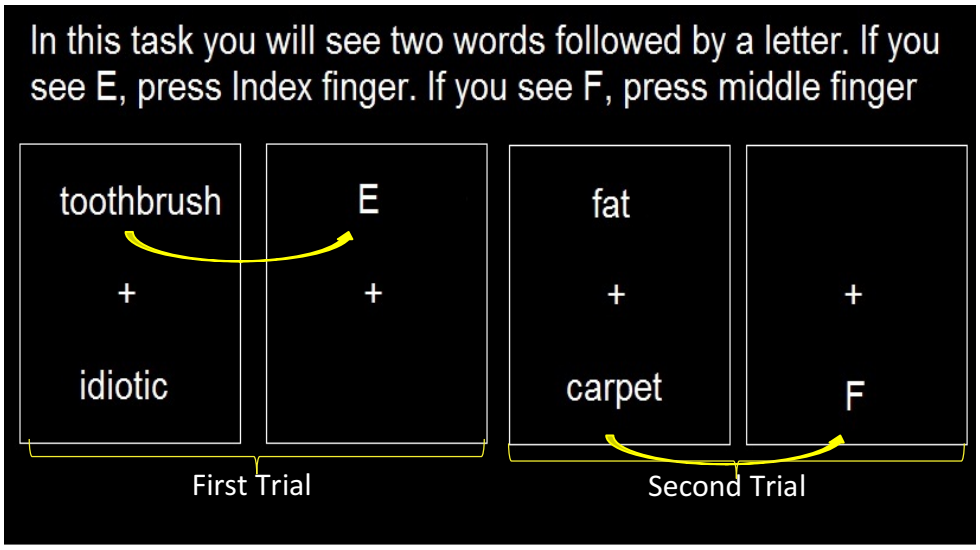


Figure 1 Two Trials of Threat-Neutral Word Pairs in ABM Condition. In the first trial, the threat word is "idiotic" and the neutral word is "toothbrush." "E" is the probe behind the neutral word. In the second trial, the threat word is "fat" and the neutral word is "carpet." "F" is the probe behind the neutral word.

Matched Control Condition. The sham condition was identical to the ABM condition except for the relationship between the probe location and the threat word in the threat-neutral trials. As in the ABM condition, in the control condition, 80% threat-neutral pairs and 20% neutral-neutral pairs were presented in each trial. While in ABM, for 100% of threat-neutral trials, the probe appeared in the previous location of the neutral word, in the control condition, the probe appeared with equal likelihood (50/50) in the position of the threat or neutral word. Thus the position of the neutral word did not predict where the probe would be.

fMRI Data Acquisition and Preprocessing

T2*-weighted images depicting BOLD contrast (TR=2000ms; TE=27ms; flip angle=80°; 38 slices; 3.125x3.125x3.2mm voxels) were acquired on a 3T Siemens Trio at the Magnetic

Resonance Research Center. Visual stimuli were presented on a rear projection screen connected to a computer running E-Prime and viewed through a mirror attached to a head coil. The participant responded to stimuli using a 5-button glove connected to the computer. 38 axial slices (3.125 x 2.125 x 3.2mm voxels) were acquired every 2 seconds parallel to the AC-PC line using a T2* weighted EPI sequence depicting BOLD contrast. Standard preprocessing steps were applied using Analysis of Functional Neuroimaging (AFNI) including slice time correction, motion correction, linear detrending to correct drift, outlier rescaling, temporal smoothing, spatial smoothing, and nonlinear warping to the Montreal Neurological Institute Colin-27 brain set.

Alternating Task. The alternating task was performed in the initial fMRI scan in visit 2, and involved 30 trials total with each trial consisting of both an emotional, worry prompt task (12s) and a non-emotional digit memory task (12s) (See Figure 2). The worry prompt task consisted of the prompt "Does it worry you?" displayed for 1 second; followed by a fixation cue (a row of X's flanked by vertical lines) for 1 second, followed by presentation of a word for 300 ms, followed by a backward mask (row of X's) for the remainder of the 12s period. The participants were instructed to rate their worry of the presented word using buttons for "Yes," "Somewhat," and "No." In negative trials, the presented word was selected from the participant's chosen threat words (See Stimuli). In neutral trials, the presented word was a matched neutral word (See Stimuli). On every trial, the emotional task was then immediately followed by the non-emotional digit memory task.

The digit memory task contained a cue "Did you see it?" (1 second), a fixation cue (1 second), a series of 3 digits displayed for 1 second each in quick succession, a backward mask (1 second), and a probe digit (target or nontarget) that remained onscreen for the remainder of the 12 second task. Participants were told that when the series of 3 digits appeared, they should try to remember each digit in the series. When the probe digit appeared, they should push buttons for "Yes" or "No" to indicate whether the probe digit was present in the target set.

Figure 2 Alternating Task. The experimental protocol showing the time-course for the alternating task. Each trial was 24 seconds, with the emotion processing and digit memory task lasting 12 seconds each.

Statistical Associations of fMRI BOLD Responses in Brain Regions with Residual Anxiety Scores

ROIs. We chose ten brain regions of interests (ROIs) known to be involved during emotional processing and attentional control based off of past research studies. The ten brain regions chosen were the left and right amygdala, the left and right bed nucleus of the stria terminalis (BNST), the left dorsolateral prefrontal cortex (DLPFC), the left and right ventrolateral prefrontal cortex (VLPFC), the dorsal anterior cingulate cortex (dACC), the perigenual anterior cingulate cortex (pgACC), and the subgenual anterial cingulate cortex

(sgACC). Nine of these ROIs were defined using anatomical masks based on standardized (MNI and Talairach) atlases. In addition, we used one functional ROI (the left DLPFC) implicated during a nearly identical task in previous studies of depression (Siegle et al, 2007). Anatomically identified DLPFC was not used because it encompasses a large region of functional heterogeneity (Siegle et al., 2007).

Statistical Association. We employed fMRI data to examine whether activity in these ROIs could predict whether or not participants improved post-ABM treatment. During the 24 second alternating task, brain samples were taken every 2 seconds by the scanner, so the individual subject's degree of BOLD response at each ROI was measured for a total of 12 time points. BOLD responses for each ROI at each of the 12 time points were averaged across all alternating task trials of a given emotional type (neutral and negative). In other words, BOLD signals from all trials presenting a neutral stimulus during the worry prompt were averaged together (See Figure 2). Similarly, all trials presenting a negative stimulus during the worry prompt were averaged together.

As mentioned previously, participants completed a Mood and Anxiety Symptoms Questionnaire (MASQ): Anxious Arousal subscale, that assesses the severity of anxious symptoms before and after the ABM treatment. To compare their MASQ-Anxious Arousal scores before and after treatment, we calculated a residual anxiety score where a lower number indicates a decrease in anxious symptoms, indicating that the ABM treatment was more effective in helping their anxiety relative to other individuals in that treatment group. Conversely, a higher score indicates anxiety symptoms remained high after ABM treatment relative to other

individuals in the ABM group. BOLD responses at each time point across the time series for each participant were then correlated with his or her residual MASQ-Anxious Arousal scores. A correlation value of above 0.32 corresponds to a significance level (p-value) of $p<.05$ for each ROI at each point in time. These correlation coefficients were plotted over time and marked for time points where correlation coefficients were significantly different from zero according to Pearson's correlation tests. Correlations were considered robust if they persisted for at least two consecutive time points.

Results

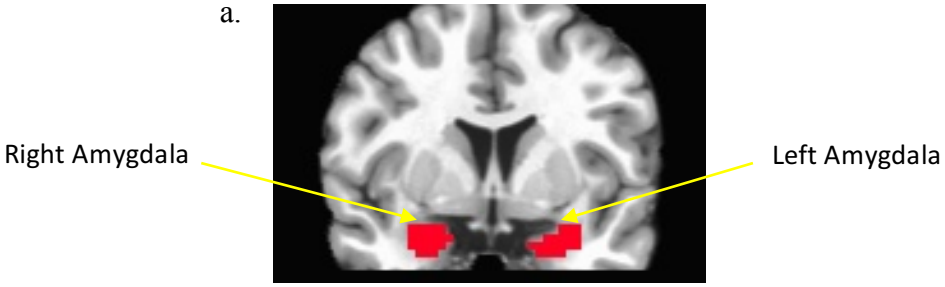
We employed fMRI data to examine whether persistent brain activity across a relevant network of cognitive and affective brain regions selected *a priori* could predict who would benefit the most from ABM. The regions chosen were the left and right amygdala, left and right bed nucleus stria terminalis (BNST), left dorsolateral prefrontal cortex (DLPFC), left and right ventrolateral prefrontal cortex (VLPFC), dorsal anterior cingulate cortex (dACC), pregenual anterior cingulate cortex (pgACC), and subgenual anterior cingulate cortex (sgACC).

Figures 3-9 show the average correlation coefficients between all ABM participants' blood oxygen level– dependent (BOLD) responses (n=38) in the a priori regions during the fMRI alternating task and their individual residual scores from the MASQ (anxious-arousal subscale) after ABM treatment during the negative and neutral trials. Each alternating trial contained a series of 12 time points of 2 seconds each, so 24 seconds total. The vertical red line at 12 seconds separates the worry prompt part of the alternating task from the digit memory portion. Time points at which correlation coefficients were considered significant according to the Pearson's correlation tests were highlighted in red and yellow on the x axis. Yellow indicates a correlation value of $R>0.27$ corresponding to a significance level of $p<0.10$, while red indicates a stronger correlation value of $R>0.32$ corresponding to a significance level of $p<0.05$. Correlations were considered robust if they persisted for at least two consecutive time points, and were indicated by a horizontal black line underneath the red or white area. Scatterplots for the scans with the strongest correlations are also shown.

Left Amygdala. In the left amygdala, significant correlations (R>0.32) can be seen in the neutral trials after the onset of the digit memory task from 12-20 seconds (Fig 3b). The scatter plot depicts the strongest correlation (R=0.35) during the neutral trial at 14 seconds (Fig 3c). In addition, for the neutral trials, there were near-significant correlations as shown in yellow during the worry prompt task from 4-7 seconds (Fig 3b). There were no significant correlations between left amygdala activity and MASQ residual scores across ABM participants during the negative trials (Fig 3d).

Right Amygdala. In the right amygdala, significant correlations (R>0.32) can be seen in the neutral trials after the onset of the digit memory task from 14-22 seconds (Fig 3e). The scatter plot depicts the strongest correlation (R=0.40) during the neutral trial at 22 seconds (Fig 3f). There were no significant correlations between right amygdala activity and MASQ residual scores across ABM participants during the negative trials (Fig 3g).

a.



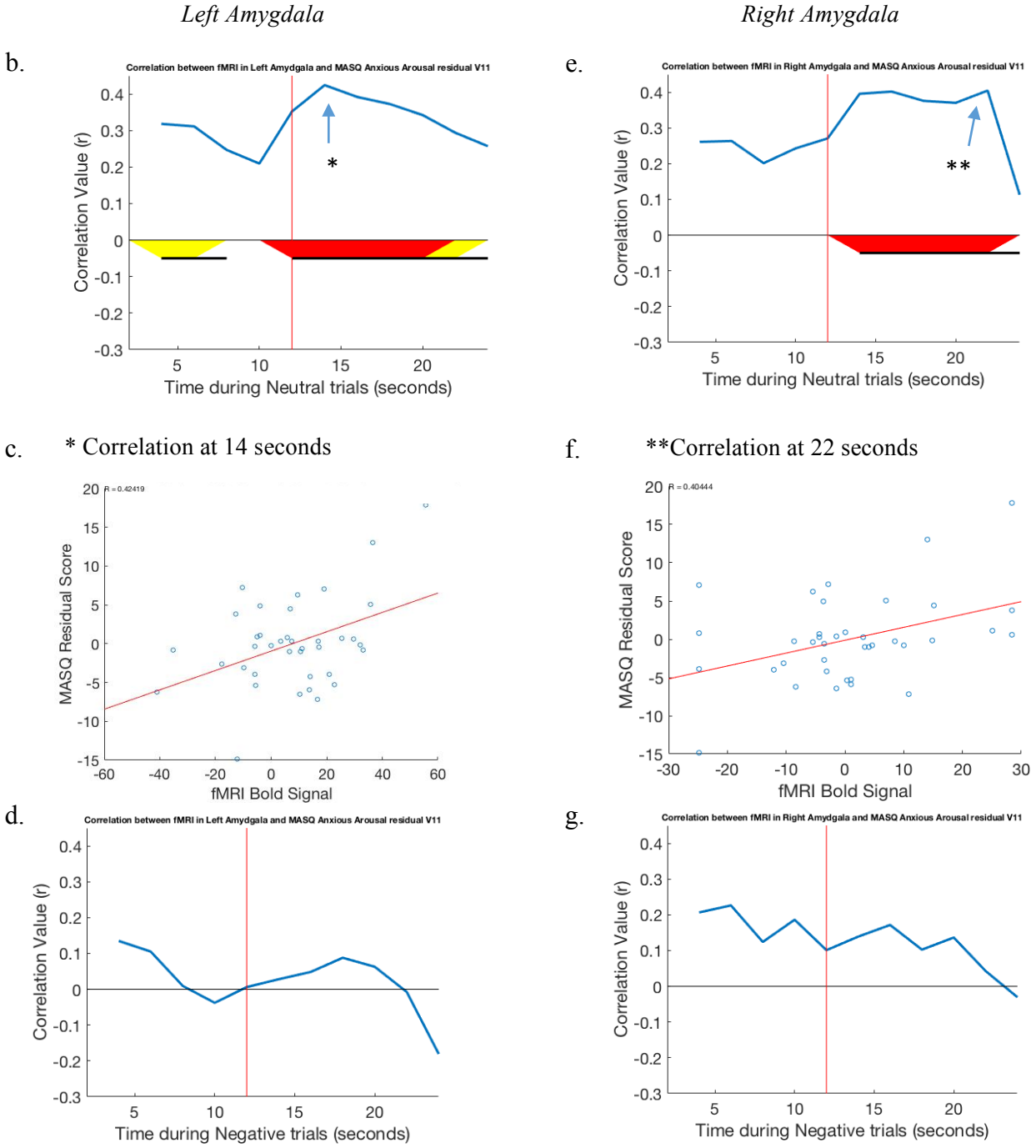
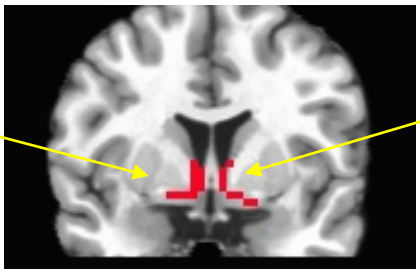


Figure 3: (a). Coronal view of left and right amygdala analyzed in fMRI alternating task. (b). Correlation between BOLD activity in the left amygdala for neutral trials with MASQ residual scores. (c). Scatter Plot of highest correlation coefficient from b. (d). Correlation between BOLD activity in the left amygdala for negative trials with MASQ residual scores. (e). Correlation between BOLD activity in the right amygdala for neutral trials with MASQ residual scores. (f). Scatter Plot of highest correlation coefficient from e. (g). Correlation between BOLD activity in the right amygdala for negative trials with MASQ residual scores.

Left BNST. In the left BNST, significant correlations (R>0.32) can be seen in the neutral trials during the worry prompt task and the digit memory task from 4-8 seconds and 12-22 seconds, respectively (Fig 4b). The scatter plot depicts the strongest correlation (R=0.44) during the neutral trial at 18 seconds (Fig 4c). There were no significant correlations between left BNST activity and MASQ residual scores across ABM participants during the negative trials (Fig 4d).

Right BNST. In the right BNST, significant correlations (R>0.32) can be seen in the neutral trials during the worry prompt task from 6-10 seconds (Fig 4e). The scatter plot depicts the strongest correlation (R=0.40) during the neutral trial at 8 seconds (Fig 4f). For the negative trials, there was a near-significant correlation (0.32>R>0.27) at 4 seconds during the worry prompt task and a significant correlation at 6 seconds. Additionally, from 14-18 seconds during the digit memory task in negative trials, there were near-significant correlations between right BNST activity and MASQ residual scores (Fig 4g).

a.



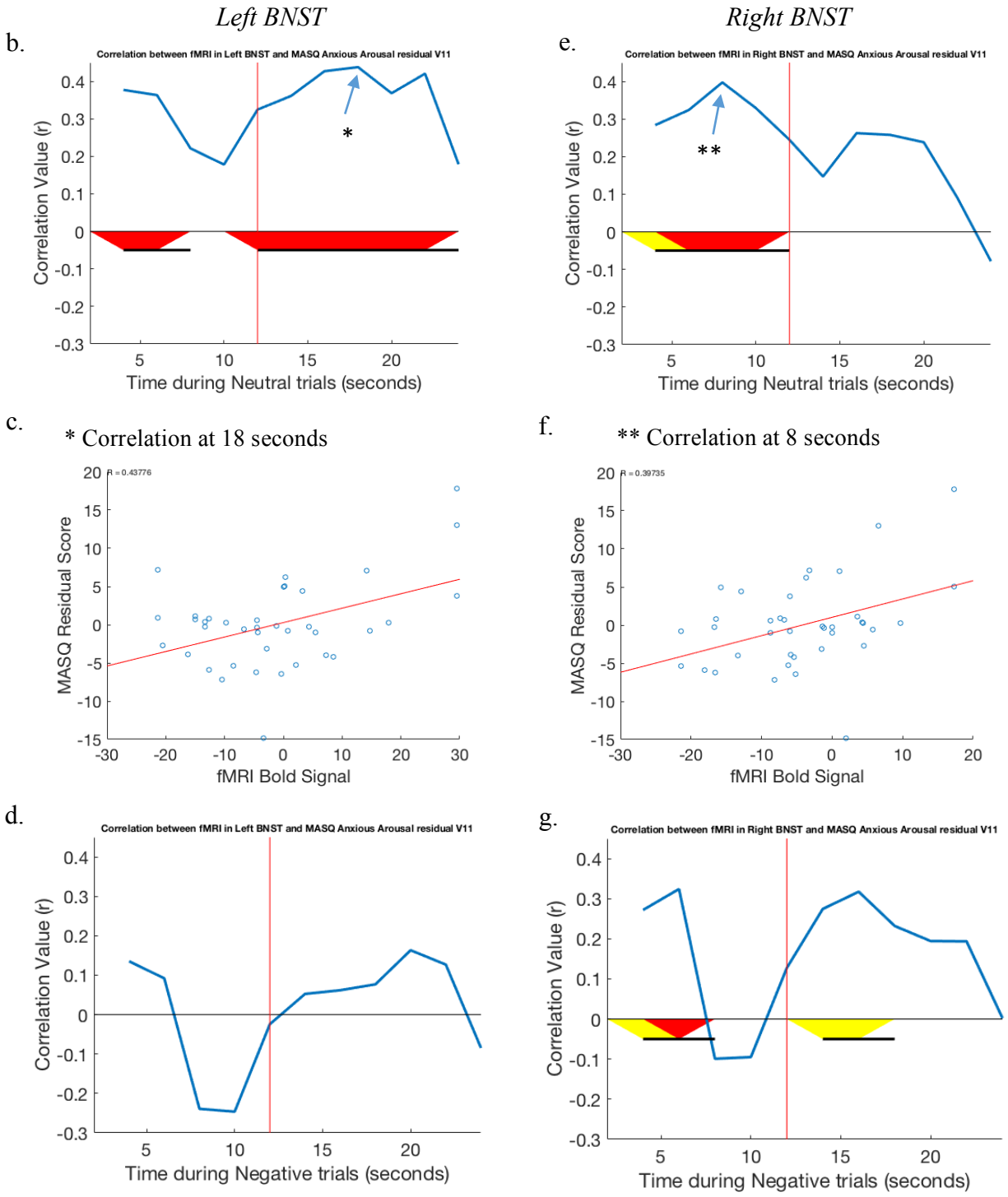
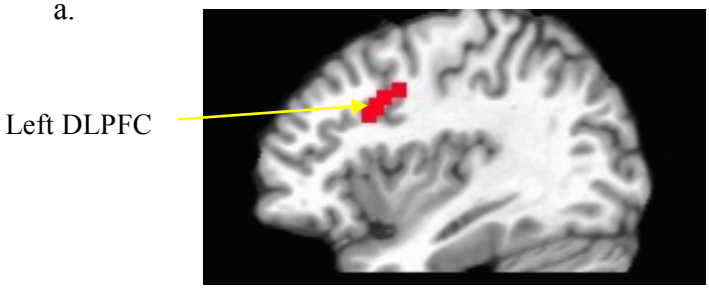


Figure 4: (a). Coronal view of left and right BNST analyzed in fMRI alternating task. (b). Correlation between BOLD activity in the left BNST for neutral trials with MASQ residual scores. (c). Scatter Plot of highest correlation coefficient from b. (d). Correlation between BOLD activity in the left BNST for negative trials with MASQ residual scores. (e). Correlation between BOLD activity in the right BNST for

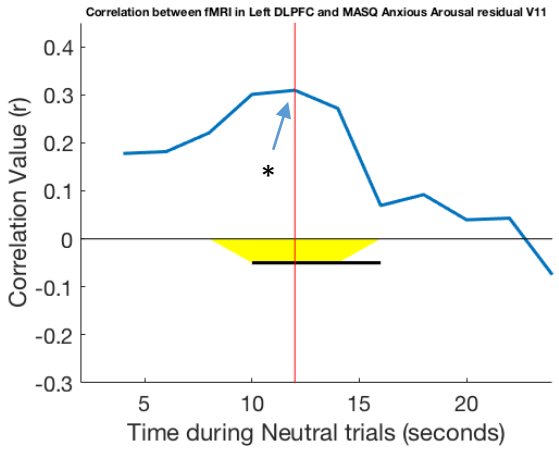
neutral trials with MASQ residual scores. (f). Scatter Plot of highest correlation coefficient from e. (g). Correlation between BOLD activity in the right BNST for negative trials with MASQ residual scores.

Left DLPFC. In the left DLPFC, near-significant correlations (0.32>R>0.27) can be seen in the neutral trials from 10-14 seconds (Fig 5b). The scatter plot depicts the strongest correlation (R=0.31) during the neutral trial at 12 seconds (Fig 5c). There were no robust correlations across two consecutive time points between the left DLPFC and MASQ residual scores across ABM participants during the negative trials (Fig 5d).

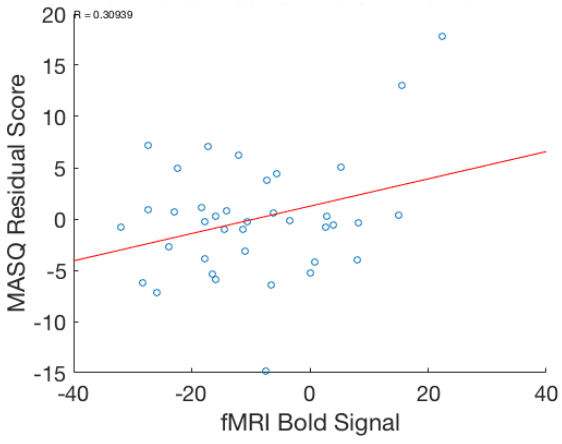
a.



b.



c. * Correlation at 12 seconds



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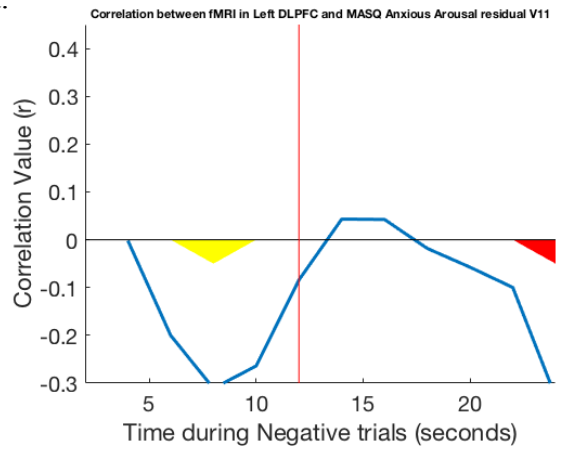
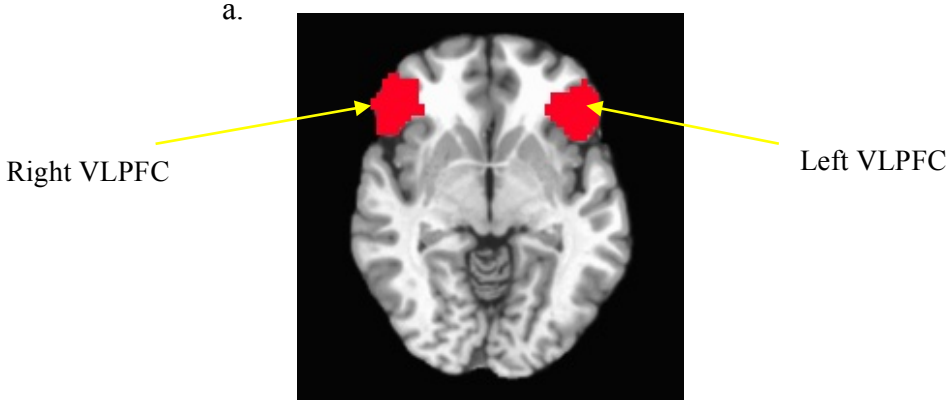


Figure 5: (a). Sagittal view of left DLPFC analyzed in fMRI alternating task. (b). Correlation between BOLD activity in the left BNST for neutral trials with MASQ residual scores. (c). Scatter Plot of highest correlation coefficient from b. (d). Correlation between BOLD activity in the left BNST for negative trials with MASQ residual scores.

Left VLPFC. In the left VLPFC, significant correlations (R>0.32) can be seen in the neutral trials from 4-6 seconds during the worry prompt task and from 14-20 seconds after the onset of the digit memory task (Fig 6b). The scatter plot depicts the strongest correlation (R=0.39) during the neutral trial at 16 seconds (Fig 6c). There were no significant correlations between left VLPFC activity and MASQ residual scores across ABM participants during the negative trials (Fig 6d).

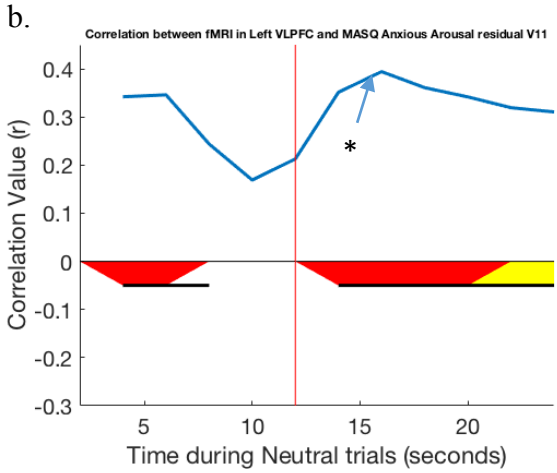
Right VLPFC. In the right VLPFC, significant correlations (R>0.32) were seen in the neutral trials at 4 and 8 seconds, and near-significant correlations were seen at 6 seconds (Fig 6e). The scatter plot depicts the strongest correlation (R=0.35) during the neutral trial at 8 seconds (Fig 6f). There was a near-significant correlation between the right VLPFC and MASQ residual scores across ABM participants during the negative trials at 6 seconds, but it was not considered robust since it did not persist over two consecutive time points (Fig 6g).

a.



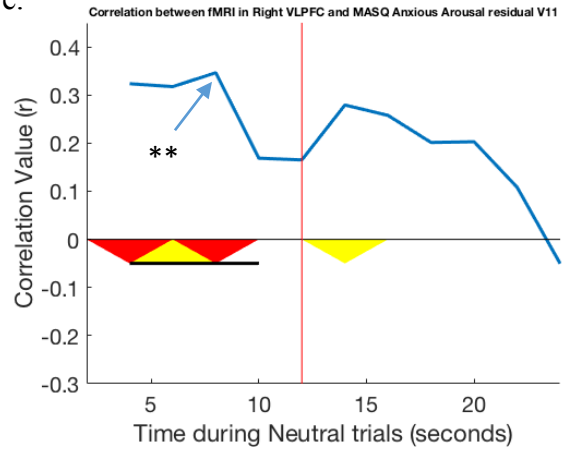
Left VLPFC

b.

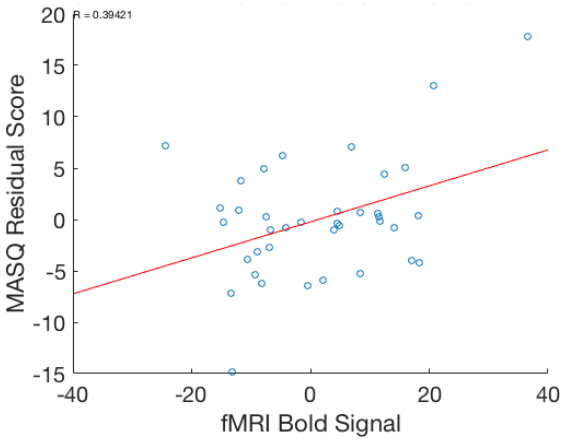


Right VLPFC

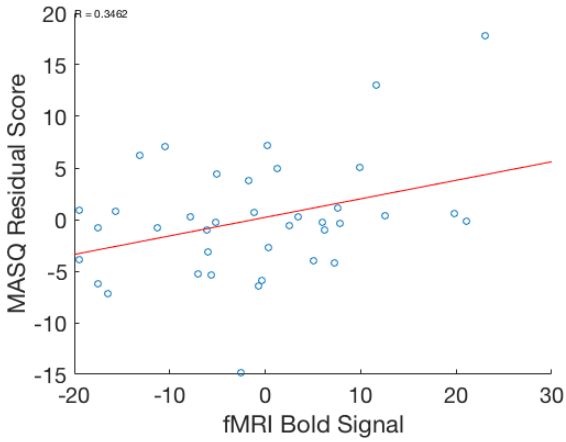
e.



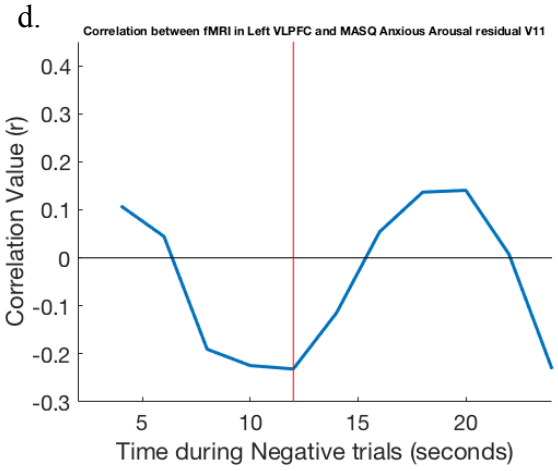
c. * Correlation at 16 seconds



f. ** Correlation at 8 seconds



d.



g.

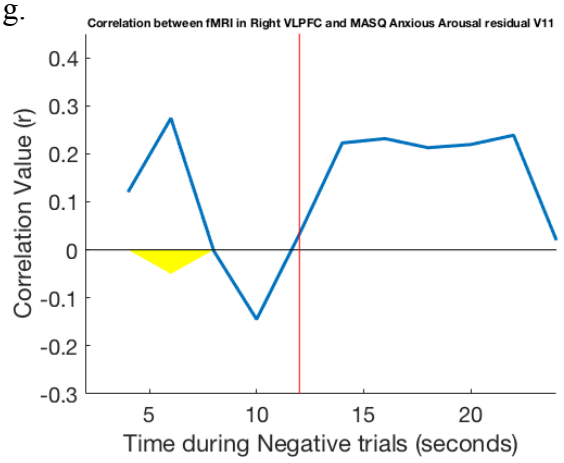
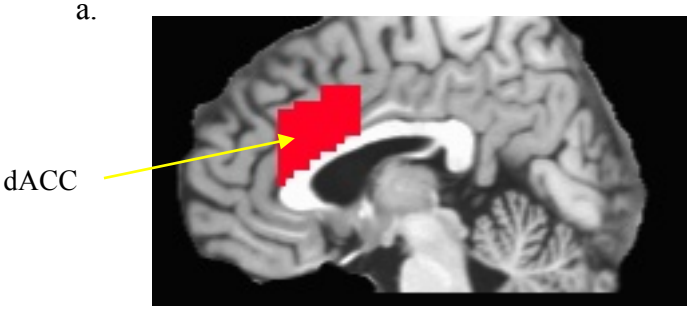


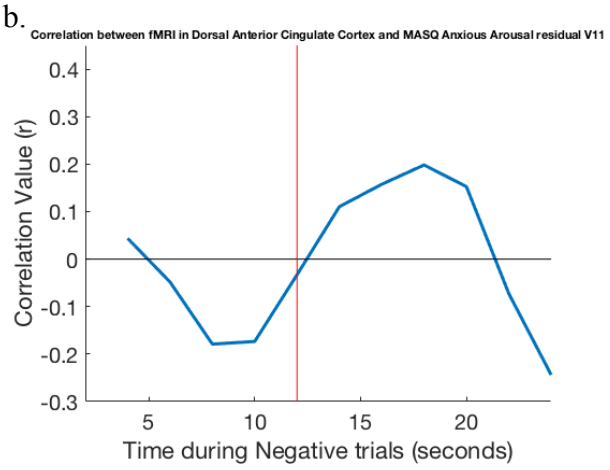
Figure 6: (a). Axial view of left and right VLPFC analyzed in fMRI alternating task. (b). Correlation between BOLD activity in the left VLPFC for neutral trials with MASQ residual scores. (c). Scatter Plot of highest correlation coefficient from b. (d). Correlation between BOLD activity in the left VLPFC for negative trials with MASQ residual scores. (e). Correlation between BOLD activity in the right VLPFC for neutral trials with MASQ residual scores. (f). Scatter Plot of highest correlation coefficient from e. (g). Correlation between BOLD activity in the right VLPFC for negative trials with MASQ residual scores.

dACC. In the dACC, there were no significant correlations between dACC activity and MASQ residual scores across ABM participants during the negative or neutral trials (Fig 7b, Fig 7c).

a.



b.



c.

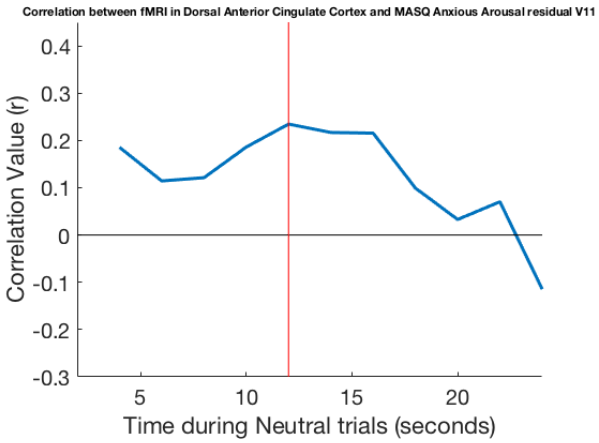
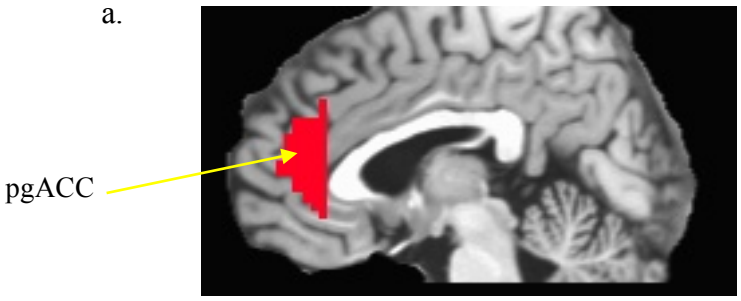


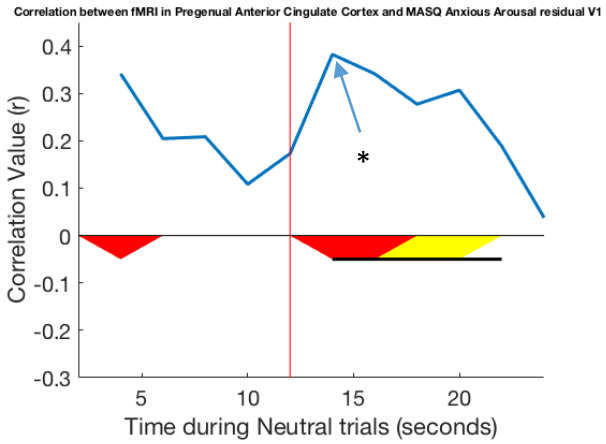
Figure 7: (a). Sagittal view of dACC analyzed in fMRI alternating task. (b). Correlation between BOLD activity in the dACC for neutral trials with MASQ residual scores. (c). Correlation between BOLD activity in the dACC for negative trials with MASQ residual scores.

pgACC. In the pgACC, significant robust correlations (R>0.32) could be seen in the neutral trials after the onset of the digit memory task from 14-16 seconds (Fig 8b). Near-significant robust correlations could be seen in neutral trials from 16-20 seconds. The scatter plot depicts the strongest correlation (R=0.38) during the neutral trial at 14 seconds (Fig 8c). There were no significant correlations between pgACC activity and MASQ residual scores across ABM participants during the negative trials (Fig 8d).

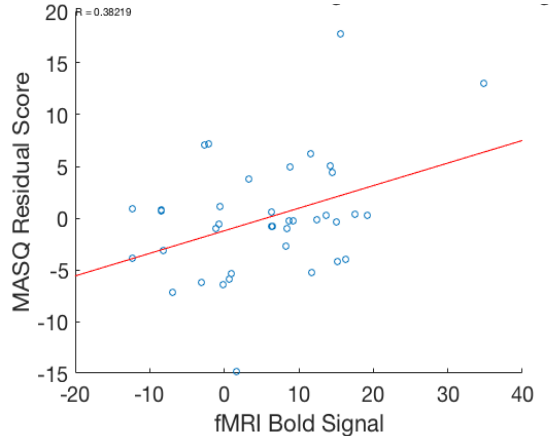
a.



b.



c. * Correlation at 14 seconds



d.

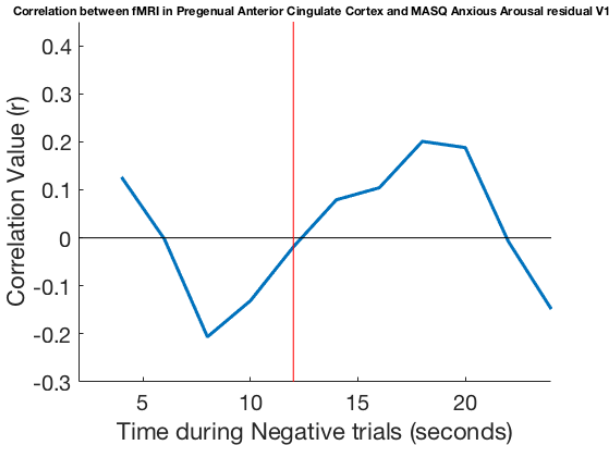


Figure 8: (a). Sagittal view of pgACC analyzed in fMRI alternating task. (b). Correlation between BOLD activity in the pgACC for neutral trials with MASQ residual scores. (c). Scatter Plot of highest correlation coefficient from b. (d). Correlation between BOLD activity in the pgACC for negative trials with MASQ residual scores.

sgACC. In the sgACC, there were no significant correlations between sgACC activity and MASQ residual scores across ABM participants during the negative or neutral trials (Fig 9 b,c).

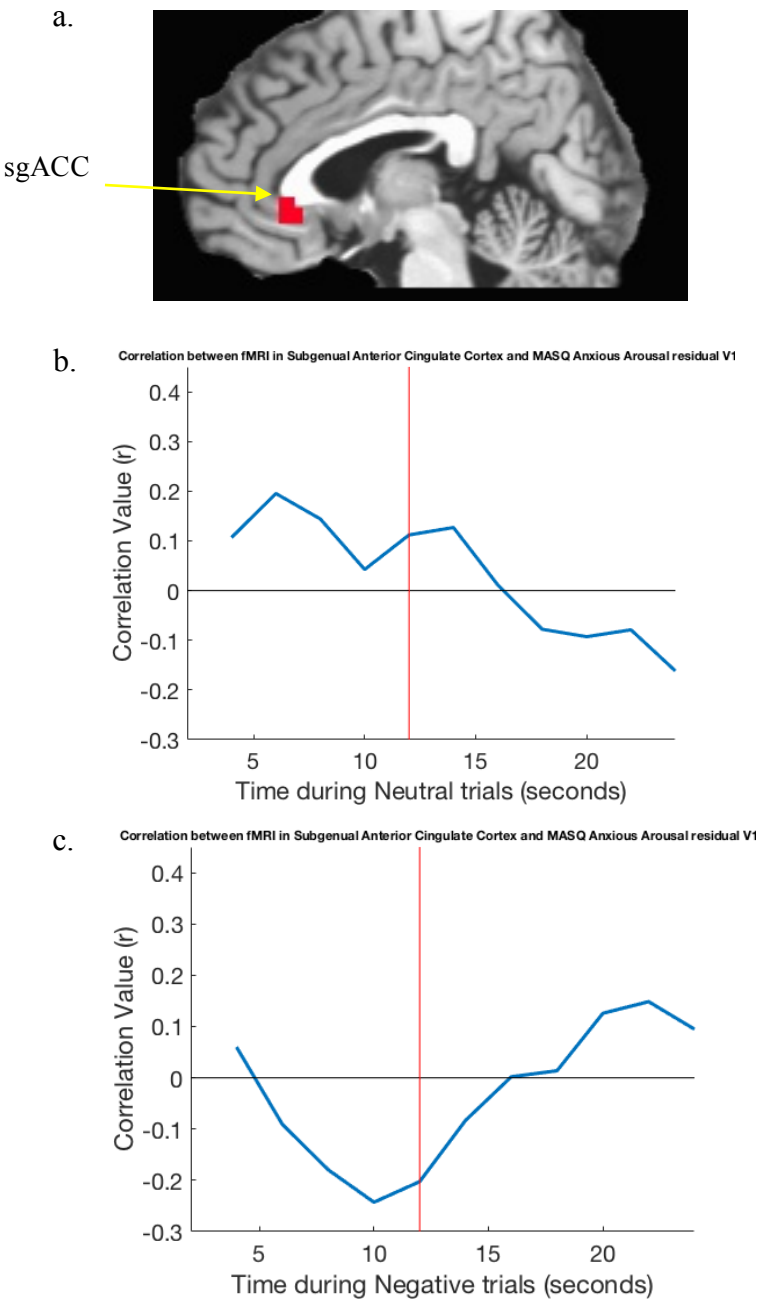


Figure 9: (a). Sagittal view of sgACC analyzed in fMRI alternating task. (b). Correlation between BOLD activity in the sgACC for neutral trials with MASQ residual scores. (c). Correlation between BOLD activity in the sgACC for negative trials with MASQ residual scores.

Below is a summary table of all the ROIs and their correlation values in significant temporal windows.

Table 1. Summary of Temporal Windows with Significant Correlation Values between BOLD signals and MASQ residual scores for all ROIs[a]

ROI[b]	Trial Type	Time Points (seconds)
Left Amygdala	Neutral	12-20
	Negative	-[c]
Right Amygdala	Neutral	14-22
	Negative	-
Left BNST	Neutral	4-6, 12-22
	Negative	-
Right BNST	Neutral	6-10
	Negative	-
Left DLPFC	Neutral	-
	Negative	-
Left VLPFC	Neutral	4-6, 14-20
	Negative	-
Right VLPFC	Neutral	-
	Negative	-
dACC	Neutral	-
	Negative	-
pgACC	Neutral	14-16
	Negative	-
sgACC	Neutral	-
	Negative	-

a. Only time points with correlation values r>0.30 across at least two consecutive time points were considered significant in this table.

b. ROI = Region of Interest

c. - = No significant correlation values with r>0.30 across two consecutive time points were seen in this region.

Discussion

Contrary to initial expectations, sustained responses in **negative** trials of the alternating task did not predict ABM outcome in any of the brain regions examined. The preceding data showed instead hyperactivity in a number of brain regions in **neutral** trials predicted poorer outcomes following ABM treatment in anxious patients. Specifically in the alternating task, when a neutral word was presented briefly for 300 ms, a subset of anxious individuals appeared to continue processing that information for up to 10 seconds, even when they were given a subsequent non-emotional digit memory task. Moreover, sustained processing in these brain regions was correlated with higher residual symptoms following ABM treatment (r's>.3, p's<.05), suggesting that sustained activity patterns in these regions were neural predictors of poorer ABM outcomes. The brain regions in which this pattern was seen included affective regions widely implicated in threat processing (the left and right amygdala; the left BNST), and regions implicated in emotional and cognitive control (left VLPFC and the pgACC). Since ABM focuses on training attention away from threatening stimuli and towards neutral stimuli, anxious individuals who exhibit a sustained neural response in these affective processing regions when only a benign stimulus has been presented may view neutral information in their environment also as a worrisome threat due to an overly threat-oriented appraisal of their environment. Since ABM's goal is to train individuals to attend to neutral information preferentially over threatening information, anxious individuals displaying sustained activity after presentation of neutral words may have difficulty distinguishing between the types of information used in ABM training at the neural level. Therefore these individuals may benefit less from this form of learning, providing clinically relevant information on which anxious patients are ideal candidates for ABM.

Other fMRI research in anxious populations has indicated dysfunction in circuits involving periamygdala regions, the ventral prefrontal cortex (vPFC), and the anterior cingulate cortex (ACC) (McClure et al., 2007). Consistent with these studies, the left and right amygdala, the left VLPFC, the left BNST, and the pgACC all showed hyperactivity in some anxious participants after the onset of the digit memory task in neutral trials, displaying sustained processing that correlated significantly with poorer ABM outcome.

Left and Right Amygdala

One of the most consistent findings in anxiety studies is the hyperactivity of the amygdala, a group of nuclei located in the medial temporal lobe, towards fear-relevant stimuli (Holzschneider & Mulert, 2011). As a key player in the neural circuitry implicated in anxiety and attention to threat, the amygdala acts as the central fear processor of the brain, and initiates many downstream physiological and behavioral responses (Hariri et al., 2001). The amygdala receives fear signals from cortical sensory processing regions and the thalamus that allow for bottom-up emotional responses that encode the affective properties of the stimuli, thus promoting rapid orientation towards threat (LeDoux, 2000). Increased activation of the amygdala can reflect an exaggerated bottom-up threat response to stimuli in the environment and promote attentional bias towards threat among anxious individuals (Britton et al., 2011; Mogg & Bradley, 2016). Research has consistently shown that hyperactivity in the amygdala increases emotional reactivity, and functional neuroimaging studies have confirmed that exaggerated amygdala activation to specific stimuli is seen in many anxiety disorders including social phobia, specific phobia, PTSD, and GAD (Monk et al., 2006; Tillfors et al., 2001; Stein et al. 2002; Ekin and

Wager, 2007; Shin & Liberzon, 2010). Relating to sustained processing of negative information, researchers found that after being shown negative stimuli, depressed individuals showed sustained activity in the amygdala that lasted through to the subsequent non-emotional processing trials (Siegle et al., 2002). The sustained processing in the amygdala to negative words was related to self-reported rumination, and thus provided evidence that sustained bias such as rumination was also associated with hyperactivity in the amygdala (Siegle et al., 2002). Mood disorders are highly comorbid with anxiety, suggesting that abnormalities in neurocircuitry seen in depression may also be present in anxiety. This was consistent with results from our study; anxious individuals who exhibited continued hyperactivity in the left and right amygdala even after the non-emotional digit memory task, had a poorer clinical outcome post-ABM treatment. This hyperactivity may be indicative of rumination about the neutral stimuli. Heightened amygdala activation in anxiety disorders is also thought to generate fear responses to innocuous stimuli misperceived as threatening (Guyer at al., 2009). The observed pattern of amygdala activation during neutral trials provides support that the anxious patients who are a poor fit for ABM may be encoding the neutral stimuli as emotionally threatening information.

Left and Right BNST

The BNST, also known as the extended amygdala, is located in the limbic forebrain and has become a central component for anxiety circuitry. This is because anxiety describes a persistent mood state marked by sustained arousal, and the BNST plays a key role in sustained anxious apprehension and vigilance (Davis et al, 2010). Research studies using animals and humans have shown that the BNST is engaged when processing uncertain threats in the environment, and is the center of integration for negative information (LeDoux & Pine, 2016;

Lebow & Chen, 2016). The BNST receives heavy projections from the amygdala, which releases corticotropin-releasing factor, a stress hormone, that acts upon the BNST. The BNST subsequently targets many areas such as the hypothalamus, brain stem, and the hypothalamic-pituitary-adrenal axis, affecting autonomic and behavioral systems involved in facilitating a sustained state of fear (Davis et al., 2010; Walker, Toufexis & Davis, 2003). Davis et al. used a sustained fear model in rats where a phasic cue was arranged such that the subject does not know when the aversive stimuli will occur. This model was able to elicit a longer-lasting state of fear that demonstrated the necessary activation of the BNST (Davis et al., 2010). Other studies in anxious humans have supported these findings, and additionally showed that activity in the BNST is involved with hypervigilance, an enhanced state of arousal to threats in the environment (Somerville, Whalen & Kelley, 2010). Thus in our experiment, the increased activation of the BNST results support the idea that neutral stimuli may be seen by certain anxious individuals as threatening, and caused continued sustained threat processing even after the onset of the nonemotional digit memory task. In our experiment, sustained processing in both left and right BNST during the non-emotional digit memory task in neutral trials predicted poor outcomes following ABM. These results make sense with the literature since anxious patients who exhibit sustained processing should show hyperactivity in the BNST. Higher activation of BNST correlates with higher sustained arousal, leading to a poorer outcome for ABM.

Left VLPFC

In addition to the bottom-up processing of emotional stimuli, another important aspect to consider is the influence of top-down processing on attention towards threat. The prefrontal cortex is known to be involved in higher executive functions such as decision-making and

emotion regulation (Phillips, Ladouceur, & Drevets, 2008). Research in both animals and humans implicate a circuit connecting the amygdala and the ventral prefrontal cortex in threat processing (Guyer et al., 2009). The left VLPFC specifically is thought to be involved in the control of attention and can be involved in vigilance to the environment (D'Esposito, Postle, & Rypma, 2000; Bechara, Tranel & Damasio, 2000). In research studies, anxiety, which is often characterized by hypervigilance to threat in the environment, tends to activate the VLPFC (Monk et al., 2006). This makes sense because the ventral pathway attempts to characterize the features of the stimuli in the environment, and can attend to affective aspects of the stimuli (O'Reilly, 2010). Ventral prefrontal signals from this pathway are capable of modulating the amygdala and subsequently biasing the amygdala's many downstream effects such as the visual cortex, which is relevant to visual attention. In healthy individuals, instructing them to shift attention away from emotional stimuli results in a corresponding downregulation of amygdala-frontal coupling (Robinson et al., 2016). This is consistent with the idea that healthy individuals are able to use attentional control to downregulate anxious responses. It is thought that anxious individuals have poor attentional control, and are unable to as effectively shift their attention away from emotional stimuli (Grillon et al., 2016). In fact, in anxious individuals, top-down processes can bias visual attention selectively toward threatening stimuli through effects on the amygdala, increasing sensitivity to threat-related information (Desimone & Duncan, 1995, Bishop et al., 2004). In our experiment, participants underwent alternating emotional and non-emotional tasks. The non-emotional digit memory task was designed to shift their attention away from emotional stimuli through activation of their prefrontal cortices. Subsequent activation after the onset of the non-emotional task would indicate sustained processing in this region. As seen from the results,

anxious individuals who respond poorly to ABM exhibited continued hyperactivity in the left VLPFC even after the non-emotional digit memory task during neutral trials. Although functional connectivity across regions was not assessed here, potential co-activation of the amygdala and the left VLPFC during the non-emotional task could suggest an upregulation of amygdala-frontal coupling associated with poor attentional control in the subset of anxious patients who did poorly on ABM (Robinson et al., 2016). If these anxious individuals are perceiving the neutral stimuli as worrisome or threatening, then this observation in the left VLPFC could potentially be caused by two factors. First, because the amygdala is hyperactive, the greater activation of this cognitive control region could be to compensate for the exaggerated bottom-up threat response to the neutral stimuli. Second, it could be a result of decreased neural efficiency of top-down attentional control to orient attention away from threatening stimuli. This result is consistent with previous studies that show that dysfunction in the VLPFC can be present in patients with anxiety (Yokoyama et al., 2015).

pgACC

The anterior cingulate cortex (ACC) is a part of the brain's limbic system, and has been classically shown to be engaged in both cognitive and emotional processing based off studies in both humans and animals (Bush, Luu & Posner, 2000). The ACC is divided into two portions, the dorsal-caudal and ventral-rostral subdivisions. The ventral-rostral subdivision of the ACC, which includes the pgACC, is thought to play a regulatory role with respect to limbic regions involved with generating emotional responses (Etkin et al. 2011). Because of the pgACC's strong connections to affective regions such as the amygdala and the hypothalamus, the pgACC plays an important role for self-conscious emotional reactivity (Sturm et al., 2013). This region

thus processes emotion through regulation of both the autonomic and endocrine system activity (Devinsky et al., 1995; Bush et al., 2000; Kober et al., 2008). Research suggests that top-down emotional regulation employs ventral ACC areas such as the pgACC in order to inhibit negative emotional processing within the amygdala and the limbic regions (Etkin et al., 2007). In a study on emotional conflict, subjects were asked to categorize faces according to their emotional expression, but the faces had congruent or incongruent affective labels written on it (Egner et al., 2007). Activity in the amygdala and other limbic regions was found to reflect the amount of emotional conflict (Etkin et al., 2007). More interestingly, the resolution of these emotional conflicts was associated with activation of the ventral-rostral ACC (Etkin et al., 2007). Activation of the ventral-rostral ACC was predictive of a decrease in amygdala activity, which suggests a top-down inhibition of amygdala response to emotional distractors (Etkin et al., 2011). In our study, we saw that anxious individuals who showed sustained hyperactivity of the pgACC even after the start of the non-emotional digit memory task had worse ABM outcomes. This provides support that those anxious individuals may be perceiving the neutral stimuli as worrisome or threatening, since we would expect pgACC to be active when attempting to inhibit the amygdala response to emotional stimuli or "distractor" during the nonemotional digit memory task. The observation that higher sustained activity in the pgACC in anxious participants is correlated with poorer ABM outcomes could potentially be a result of decreased neural efficiency in top-down emotional regulation from the ACC to the limbic regions, resulting in a failure in this circuitry to effectively decrease amygdala response to the neutral stimuli in these patients.

Non-predictive Task Conditions and Brain Regions

We originally hypothesized that sustained processing in negative trials of the alternating task in the analyzed brain regions would predict poor ABM outcomes to a greater extent than neutral trials. This was because the negative stimuli were self-relevant to the anxious participants, and were designed to be encoded as emotionally threatening information. This emotionally threatening stimuli would subsequently lead to activation of sustained processing substrates such as worry and rumination, that could interfere with the efficacy of the ABM intervention given that ABM targets initial (but not sustained) threat processing. Interestingly, we did not see neural predictors in negative trials of the alternating task, but instead saw a consistent pattern of activation across a network of brain regions that were neural predictors during neutral trials. Neural predictors from negative trials may not have been found because the entire group of anxious patients may have exhibited more uniform sustained processing of the threat words. Therefore, if all anxious patients exhibited similar high sustained activity in the amygdala, BNST, VLPFC, etc., then no significant correlation would be seen when comparing ABM outcomes across individuals. In contrast, only a subset of anxious patients may view neutral stimuli as worrisome or threatening. These anxious patients exhibit high sustained activity across many brain regions, unlike other anxious patients who may not view neutral stimuli as worrisome or threatening and so exhibit less activation. Thus these individual differences in activation allow us to predict individual differences in ABM response in neutral trials.

There were several brain regions studied that did not demonstrate the common observed pattern of hyperactivity that was predictive of ABM outcomes. Regions where no significant

correlations were found across all time points included the right VLPFC, the dACC, and the sgACC, although patterns in the right VLPFC during neutral trials were near-significant and highly suggestive of a similar effect. The right VLPFC and the right BNST regions have analogous functions as their left counterpart, and the sgACC, like the pgACC, also belongs the ventral portion of the ACC. With a larger sample size and subsequent greater statistical power, these regions may also show similar patterns of hyperactivity predicting ABM outcomes during neutral trials. The dACC in contrast to the sgACC and the pgACC belongs to the dorsal region of the ACC. The dorsal region of the ACC is thought to more involved with cognition and motor control due to its strong connections to areas such as the dorsal prefrontal cortex, including the left DLPFC, which also did not predict outcomes in the present analysis. While it is possible that significant correlations could be seen in the dACC or DLPFC with a larger sample size, these regions may not be as relevant to this alternating task as the ventral regions that deal with emotional processing (Etkin at al., 2007). Interestingly, the left BNST, the right BNST, and the left VLPFC also showed activation with significant correlation to ABM outcome during the emotional worry prompt task, or the initial stages of threat processing. Sustained processing was the focus in this study, but the significance of the correlations in these regions with initial processing and ABM outcome could be a future area to study.

Limitations and Future Directions

The present study was optimized for identifying transdiagnostic neurocognitive predictors of ABM response, and so analysis focused only on the active ABM group (n=38). One of the strengths of the study was that participants were randomized to the control condition, but in the current analysis, the control group was not included. Thus the improvements observed in

anxious patients may not be specific to the active ABM group, but could be due to placebo effects or spontaneous recovery. The sample size of the study was also small due to exclusion of data based off of excess movement in the scanner. Finally, we selected and analyzed only ten brain regions a priori, and therefore may be overlooking parts of the brain where there could be significant correlations. While this approach preserves power by limiting the number of multiple comparisons, future studies could also analyze exactly which anxious patients showed sustained hyperactivity across all regions of the brain, to more conclusively identify dysregulated anxiety circuits. Other studies should include larger ABM and control groups to not only replicate these findings in a larger sample, but also analyze other brain regions whose sustained activity may also show significant correlations with ABM outcomes. While the current study was designed to find neural predictors for ABM activity, future work should focus on the translation and dissemination of these findings into the clinical setting.

Conclusions

The goal of this study was to understand which anxious patients are most likely to benefit from ABM. We considered the effect of sustained threat processing, including perseverative cognitions such as worry and rumination, on ABM outcome. Sustained threat processing, in contrast to initial stages of processing, includes processing that persists even after the stimulus has been removed. We found that in a number of brain regions, following a briefly presented neutral word and a prompt to consider whether the word “worries” you or not, sustained activity that persisted after the onset of a subsequent non-emotional task did in fact interfere with successful outcome in ABM. Those with higher activity in these regions had higher residual symptoms post-ABM treatment. These regions included the left and right amygdala, the left

VLPFC, the left and right BNST, and the pgACC, regions known to be involved in cognitive and affective processing. These results have both clinical and research implications. From a precision medicine standpoint, this type of individual mechanistic assessment may allow for identification of specific subsets of anxious patients who would be a good candidate for ABM. This would be particularly useful if fMRI predictors can be translated into a clinically available form (Siegle et al., 2011). Alternatively, existing cognitive intervention approaches could be supplemented or refined to target sustained forms of processing, increasing the percentage of successful response rates, improving care for anxious populations.

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