**STUDY INFORMATION**

Title: Relationship between elevated depressive symptoms, negative attention bias and brain volume.

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Description: Depression is the leading cause of disability worldwide and is associated with significant physical health problems, interpersonal discord, and other mental health challenges. Cognitive models suggest that depression may be maintained by a preferential bias to attend to negative stimuli and reduced ability to disengage from negative stimuli leading to sustained negative affect and a persistent depressive episode. This study examines structural MRI data from a cohort of individuals with elevated depressive symptoms in comparison to a non-depressed healthy sample. We outline our plan to test for differences in brain volumes in depressed individuals compared to healthy young adults. We will also examine if observed differences in brain volumes correspond to mood scores or biases in negative attention.

Hypotheses: 1) We hypothesize that participants with elevated depressive symptoms (EDS) will have reduced gray matter volumes (GMV) in comparison to healthy controls regarding the amygdala, hippocampus, dorsolateral prefrontal cortex (DLPFC), cingulate gyrus/cortex (CC), inferior frontal gyrus, superior temporal gyrus (STG), insula, orbital frontal cortex (OFC), and thalamus (Gray et al., 2020).

2) We predict there will be a negative correlation between Hamilton Depression Rating Scale-17 (HDRS-17) and GMV of the previously mentioned brain regions in the EDS group. Mood and Anxiety Symptom Questionnaire- anhedonia depression (MASQ-ad) scores, in the EDS group, are expected to negatively correlate with GMV of hippocampus, amygdala, and thalamus.

3) With respect to attention bias, we predict sad bias negatively correlates with GMV of the hippocampus, amygdala, and cingulate cortex. Lastly, we expect that the number of trials that participants avoid looking at happy stimuli, (i.e., longer neutral stimulus gaze time than the happy stimulus gaze time) in the dot probe task used, will negatively correlate with GMV in the orbital frontal cortex (Grieve et al., 2013).

**DESIGN PLAN**

Study Type: Retrospective Case Control Study

Blinding: Individuals with elevated depressive symptoms were enrolled in a double-blind clinical treatment trial using an attention bias modification (ABM) aimed to reduce negative attention bias (NIH R33 MH109600). Healthy Control subjects were enrolled in a separate study with no blinding (NIH R01 AG043425).

Study Design: Planned regression analyses will be performed between participants with elevated depressive symptoms (EDS) and healthy controls to compare gray matter volumes in the regions of interest and ANOVA will be used to identify EDS brain volume relationships with mood.

**SAMPLING PLAN**

Existing Data: As of the date of submission, the structural MRI data of individuals with elevated depressive symptoms have not been accessed by this research team or any other collaborators. Behavioral data, such as demographic information and clinical assessments, have been previously accessed by project collaborators in relation to separate research assessing the efficacy of attention bias modification treatment delivered to participants over the course of one month.

Structural MRI data from healthy controls have been previously assessed for the purpose of serving as a healthy control sample in a study examining the relationship between sleep and cognition in aging.

Explanation of Existing Data: Both datasets (EDS and HC) used in these planned analyses were collected as part of larger projects funded by the NIH. Volumetric MRI data of depressed individuals have not been examined in any way. Volumetric MRI data of healthy controls have been assessed only in relation to analyses compared to an aging population.

Data Collection Procedure:

*EDS Data Collection*: Participants were recruited using paper flyers across the Austin, Texas community and from advertisements posted on social media and advertising websites. Candidate participants were invited if they had the following inclusion criteria: (1) scored 13 or above on the Quick Inventory of Depression Severity- Self Report version (QIDS-SR), (2) have at least moderate negatively attentional bias, and (3) are between 18-40 years of age. Participants were excluded from the study if they had the following exclusion criteria: (1) current substance use disorder, (2) current or past psychotic disorder or bipolar disorder, (3) are MRI compromised, (4) have changes in psychiatric or neurological medication 12 weeks prior to study, (4) receiving psychotherapy or electroconvulsive therapy, (5) report current opioid analgesics or systemic corticosteroid use for an acute medical condition or taken as needed, (6) or have had suicidal behaviors or suicidal ideation within the last six months.

Imaging data was collected using a 3T Siemens Skyra (TIM Systems, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel coil at the Biomedical Imaging Center at The University of Texas at Austin. Anatomical MRI volumes were acquired using a T1-weighted MPRAGE with the following parameters: (TR = 2.3 s, TE = 3.08 ms, flip angle = 9 degrees, slice thickness = .9 mm, 192 slices, FOV = 22 cm and matrix size = 256 × 256 mm). To ensure that no artifacts were present in these anatomical images, all MRI data had passed a visual inspection at the time of data collection. If poor data quality was observed in the first anatomical scan, a second MPRAGE was acquired during the same scanning session.

An eye tracking dot-probe task was also used to measure negatively biased attention in this sample. The dot-probe task utilizes affective stimuli not used during ABM. Positive images are also used to determine whether any observed effects of ABM on attention bias transfer to another stimulus category. In this task, two images depicting an emotional (sad, happy) or neutral facial expression from the Karolinska Directed Emotional Faces (KDEF) stimuli collection are presented concurrently on the left and right side of visual field. Each trial consists of a fixation cross for 500 ms followed by the stimulus pair for 1000ms. The location of the emotion and neutral stimulus varies randomly. Following stimuli offset, a target probe appears on screen (either the letter “O” or “Q”) in the same location as one of the images, randomized to appear behind the emotional and neutral image with equal frequency.

The dot probe task includes 192 trials (96 trials per block) with 12 pairs of sad and neutral images and 12 pairs of happy and neutral images randomly presented four times each within each block of trials. Stimuli will be matched for actor so that the only difference between stimuli pairs is emotion expression. Eye tracking data will be recorded during this task.

The study was done in a four-week period in which an in-clinic assessments of attentional bias, general sustained attention, and depression severity were completed weekly including at baseline (week 0). Attentional bias modification (ABM) completed interventions in-clinic and at home 5 times per week within the four-week period. Functional MRI assessments were completed at pre-ABM (baseline, week 0), week 2, and post-ABM (week 4). Planned analyses only include data collected at baseline.

*HC Data Collection*: While only healthy younger adults are used in the proposed analyses, participants from this sample included healthy older and younger adults from central Texas, in and around Austin. The study was advertised through posters, online forums, and recruitment events at aging conferences and senior recreation centers. Candidate participants were invited to participate in the study if they met the following inclusion criteria: (1) endorsed fewer than 8 items on the Pittsburgh Sleep Quality Inventory (PSQI), (2) endorsed fewer than 16 items on the Center for Epidemiological Studies Depression Scale (CESD) or fewer than 15 items on the Geriatric Depression Scale (GDS), (3) did not meet criteria for significant sleep disturbance or disorder, neurological or psychiatric disorders, or cardiovascular disease, including uncontrolled hypertension and diabetes, (4) were not currently taking sleep medication or psychoactive substances, and (5) were right handed. Candidate participants were administered an abbreviated neuropsychological battery assessing executive function and memory, and those who scored below two standard deviations from the age-adjusted norm were excluded from the study.

Imaging data was collected using a Siemens Skyra 3T scanner (TIM Systems, Siemens Medical Solutions, Erlangen, Germany) with a 32-channel coil at The University of Texas at Austin Biomedical Imaging Center. Anatomical MRI volumes were acquired using a 3D multi echo MPRAGE T1-weighted sequence with the following parameters: TR = 2530.0 ms, TE = 1.69, 3.55, 5.41, and 7.27 ms, T1 = 1100 ms, FOV = 256 mm2, 176 coronal slices, isotropic voxel size 1.0 mm3. To ensure that no artifacts were present in these anatomical images, all MRI data had passed a visual inspection at the time of data collection. If poor data quality was observed in the first anatomical scan, a second MPRAGE was acquired during the same scanning session.

Participants in both samples were compensated for their participation in the study. Ethical approval was received from The University of Texas at Austin Institutional Review Board and prior written consent was obtained from all participants.

Sample Size: As noted, MRI data for these analyses have been previously collected. Sample size for the EDS group was determined based on power calculations needed to detect a difference in depression symptom severity change in an ABM treatment trial (Hsu et al., 2018) and reflect the number of participants in which baseline structural MRI data is available. The HC sample in these analyses were recruited as part of a separate study and reflect the subset of participants that provided usable structural MRI data. Group demographics for both subject groups are summarized in the table below:

|  |  |  |
| --- | --- | --- |
|  | EDS | HC |
| Sample Size | 129 | 55 |
| Gender (M/F/Other) | 27/100/2 | 19/36/0 |
| Age (yrs +/- stdev) | 25.16 ± 5.64 | 21.38 ± 3.66 |
| Medication Status (T/F) | 32/97 | 10/45 |

**VARIABLES**

We will examine the bilateral volumes of nine cortical and subcortical brain regions which have been identified from a previous meta-analysis of altered brain functional and structure in MDD (Gray et al., 2020). These brain regions include the amygdala, hippocampus, dorsolateral prefrontal cortex, cingulate cortex, inferior frontal gyrus, superior temporal gyrus, insula, orbital frontal cortex, and thalamus. Studies supporting our hypotheses for each of the nine ROIs are listed in the table below.

|  |  |  |
| --- | --- | --- |
| Brain ROIs | Studies Supporting Hypothesis for ROI | Total Studies |
| Amygdala | Alemany et al., 2013; Frodl et al., 2008; Lee et al., 2011; Li et al., 2010; Redlich et al., 2014; Scheuerecker et al., 2010; Tang et al., 2010; Wagner et al., 2011 | 8 |
| Hippocampus | Arnone et al., 2013; Bergouignan et al., 2009; Chaney et al., 2014;Cheng et al., 2010; Frodl et al., 2008; Lee et al., 2011; Redlich et al., 2014; Shah et al., 1998; Soriano-Mas et al., 2011; Wagner et al., 2011; Zou et al., 2010 | 11 |
| DLPFC | Frodl et al., 2008; Grieve., 2013; Li et al., 2010; Perico et al., 2011; Shad et al., 2012; van Tol et al., 2014; Vasic et al., 2008 | 7 |
| Cingulate Gyrus/Cortex | Cai et al., 2015; Hwang et al., 2010; Lee et al., 2011; Machino et al., 2014; Mak et al., 2009; Perico et al., 2011; Redlich et al., 2014; Salvadore et al., 2011; Serra-Blasco et al., 2013; van Eijndhoven et al., 2013; van Tol et al., 2010; van Tol et al., 2014; Vasic et al., 2008 | 13 |
| Inferior Frontal Gyrus | Cai et al., 2015; Fang et al., 2015; Lai et al., 2015; Peng et al,, 2011; Salvadore et al., 2011; Scheuerecker et al., 2010; Serra-Blasco et al., 2013; van Tol et al., 2010; Vasic et al., 2008; Wagner et al., 2011 | 10 |
| Superior Temporal Gyrus | Fang et al., 2015; Guo et al., 2014; Inkster et al., 2011; Lai et al., 2015; Lee et al., 2011; Machino et al., 2014; Mak et al., 2009; Peng et al., 2011; Salvadore et al., 2011; Shad et al., 2012; Stratmann et al., 2014; van Tol et al., 2014; Vasic et al., 2008 | 13 |
| Insula | Hwang et al., 2010; Lai et al., 2015; Lee et al., 2011; Liu et al., 2014; Peng et al., 2011; Redlich et al., 2014; Serra-Blasco et al., 2013; Soriano-Mas et al., 2011; Stratmann et al., 2014; Vasic et al., 2008 | 10 |
| Orbital frontal Cortex | Chaney et al., 2014; Grieve et al., 2013; Mak et al., 2009; Scheuerecker et al., 2010; Shad et al., 2012; van Eijndhoven et al., 2013; Wagner et al., 2011 | 7 |
| Thalamus | Kim et al., 2008; Lee et al., 2011; Li et al., 2010; Redlich et al., 2014; Shad et al., 2012; Soriano-Mas et al., 2011; Vasic et al., 2008; Zhang et al., 2012 | 8 |

Further, we will examine the relationship between these brain volumes and the following mood scores in EDS participants: Hamilton Depression Rating Scale-17 (HDRS-17), Quick Inventory of Depression Severity- Self Report version (QIDS-SR), Mood and Anxiety Symptom Questionnaire (MASQ) scores, and negative attention bias data from the dot probe task.

**ANALYSIS PLAN**

Imaging data have been formatted according to BIDS (brain imaging data structure) standards. fMRIprep will be used for preprocessing anatomical and functional data. fMRIprep’s freesurfer capability will be used for measuring cortical and subcortical brain volumes.

First, we will perform a number of data quality assessments regarding group demographics and regional brain volume distributions. We will test for differences in age or gender between EDS and HC groups. If there are significant differences in age between groups, age will be used as a covariate for subsequent analyses comparing EDS and HC. We will determine if there are differences in gender between groups using a chi-squared test, and samples will be adjusted to eliminate differences in gender ratios. In addition, brain volumes of ROIs that are three standard deviations from the mean will be considered outliers and removed from analysis. Notably, participants with more than one half of their brain volumes If more than half of a participant’s brain volumes are outliers, they will be removed from all analyses.

Planned multivariate analysis of variance (MANOVA) and regression analyses will be conducted to assess differences in gray matter volume (GMV) between depressed (EDS) participants and healthy controls (HC). Specifically, we will use MANOVA to determine differences in GMV between the EDS participants and HC in the following regions of interests (ROIs): the amygdala, hippocampus, dorsolateral prefrontal cortex (DLPFC), cingulate gyrus/cortex, inferior frontal gyrus, superior temporal gyrus, insula, orbital frontal cortex, and thalamus. Right and left ROIs will be tested as separate variables in the MANOVA. If MANOVA findings support our hypothesis that brain volumes in HC participants are greater than EDS participants, we will perform post-hoc t-tests on individual ROIs. Significance for post-hoc t-tests will be corrected for multiple comparisons using the with the Family-wise Error Rate (FWE) at p<0.05.

Regression analyses will be performed to examine the relationship among behavioral assessments including Hamilton Depression Rating Scale (HDRS) and Mood and Anxiety Symptom Questionnaire- anhedonia depression (MASQ-ad) and brain volumes in the EDS group. Data from attentional bias modification (ABM) baseline line assessments in which emotion-inducing pictures were presented to EDS participants will also be used to analyze using regression analysis. Based on our hypotheses, data from attentional bias measures at baseline line, in which emotion-inducing pictures were presented to EDS participants, will negatively correlate with GMVs in the OFC, hippocampus, amygdala, and CC. We will test this hypothesis using regression analysis.

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