ARTICLE

Effects of acute stress and depression on functional connectivity between prefrontal cortex and the amygdala

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Stress is known to be a significant risk factor for the development of Major Depressive Disorder (MDD), yet the neural mechanisms that underlie this risk are poorly understood. Prior work has heavily implicated the corticolimbic system in the pathophysiology of MDD. In particular, the prefrontal cortex (PFC) and amygdala play a central role in regulating the response to stress, with dorsal PFC and ventral PFC exhibiting reciprocal excitatory and inhibitory influences on amygdala subregions. However, it remains unclear how best to disentangle the impact of stress from the impact of current MDD symptoms on this system. Here, we examined stress-induced changes in resting state functional connectivity (rsFC) within an a priori corticolimbic network in MDD patients and healthy controls (total $n = 80$) before and after an acute stressor or a "no stress" control condition. Using graph theoretic analysis, we found that connectivity between basolateral amygdala and dorsal prefrontal nodes of the corticolimbic network had a negative association with individual differences in chronic perceived stress at baseline. Following the acute stressor, healthy individuals showed a reduction of the amygdala node strength, while MDD patients exhibited little change. Finally, dorsal PFC–particularly dorsomedial PFC– connectivity to the basolateral amygdala was associated with the strength of the basolateral amygdala responses to loss feedback during a reinforcement learning task. These findings highlight attenuated connectivity between basolateral amygdala and prefrontal cortex in patients with MDD. In healthy individuals, acute stress exposure was found to push the corticolimbic network to a "stress-phenotype" that may be chronically present in patients with current depression and high levels of perceived stress. In sum, these results help to identify circuit mechanisms underlying the effects of acute stress and their role in mood disorders.

Molecular Psychiatry; https://doi.org/10.1038/s41380-023-02056-5

INTRODUCTION

Stress is a significant risk factor for many psychiatric disorders, particularly Major Depressive Disorder (MDD) [1–3]. It is often one of the strongest proximal risk factors for MDD, with periods of chronic stress preceding up to 80% of the first lifetime major depressive episodes [1, 2, 4]. Moreover, after the first lifetime episode of MDD, the risk of future episodes increases following less severe life stressors [2, 4, 5]. It remains unclear, however, how stress impacts neural circuitry to confer risk for the onset and maintenance of depressive episodes [6].

The corticolimbic system, including medial prefrontal areas, amygdala, and the basal-ganglia, has been shown to be affected by both chronic and acute stress in animal and human studies [7, 8]. Broadly speaking, this system coordinates autonomic, neuroendocrine, metabolic and immune system response to stress, and also functions to return the body to homeostasis after acute stress removal [7, 9–11]. Within this system, the amygdala has been indicated as a network "hub", given its high connectedness with multiple cortical areas in primates [12, 13]. Hub regions in brain networks may be particularly relevant for understanding the impact of stress on mood disorders, given their potential to exert influence across multiple functional networks [14, 15]. Consistent with this, the amygdala exhibits reciprocal connections with multiple cortical areas that have been implicated in social, cognitive and affective processing [16].

In addition to these functions, the amygdala plays a key role in coordinating responses to stress [7]. It exerts a strong regulatory influence over the hypothalamic-pituitary-adrenal (HPA) axis through its projections to intermediate nuclei such as the bed nucleus of the stria terminalis (BNST) [17], as well the prefrontal cortex (PFC) and hypothalamus [18]. Owing to its extensive afferent and efferent connections to other regions, preclinical and clinical findings highlight the importance of the amygdala and its functional connectivity within corticolimbic networks in the development of stress-related disorders such as PTSD and MDD [19–23]. In particular, a substantial literature suggests that emotional behaviors and decision-making rely heavily on the bidirectional connections between the amygdala and multiple regions within PFC [24–29]. One recent review [30] identified two distinct PFC-amygdala circuits for regulating responses to threat and stress: the first involved the use of dorsolateral prefrontal areas involved in cognitive regulation to control amygdala threat learning via projections through ventromedial prefrontal cortex,

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Received: 8 June 2022 Revised: 17 March 2023 Accepted: 24 March 2023
Published online: 19 April 2023

and the second involved direct projections to amygdala from dorsomedial PFC areas involved in threat regulation. Preclinical studies have also shown that the functional connectivity of central nuclei of the amygdala with the BNST is correlated with the anxious temperament, a risk factor for anxiety disorders and depression [31].

While this prior work has heavily implicated these corticolimbic circuits in patients with MDD, it remains unclear how best to disentangle the impact of stress from the impact of current MDD symptoms [6]. Here, we examined the resting state functional connectivity (rsFC) within an a priori corticolimbic network as a function of varying levels of recent perceived stress and exposure to either an acute stressor or a no-stress manipulation. If the alterations in corticolimbic networks are driven in part by the accumulated impact of chronic stress, we would predict differences in the baseline rsFC between the healthy control and MDD groups as a function of chronic perceived exposure. Moreover, we would predict dissimilar responses to the effect of the acute stress manipulation in these two groups.

To test these hypotheses, we first characterized the associations between amygdala rsFC within the corticolimbic network and the experience of recent chronic stress across all participants (healthy controls and MDD patients). Second, given prior work suggesting that individual differences in rsFC reflect a mix of stable, trait-like patterns as well as variable, state-like patterns [32], we identified potential changes in rsFC following an acute stress manipulation as compared to a no-stress control manipulation in healthy controls. We compared stress-evoked changes in rsFC between healthy controls and a sample of unmedicated patients with a current diagnosis of MDD. In particular, we focused on the changes in the connectivity of the amygdala and the prefrontal cortex (PFC) nodes within the defined corticolimbic network. Additionally, given the heterogeneity of the PFC and amygdala structural and functional properties [26, 33–41] we focused on two main subregions of PFC, ventral PFC (vPFC) and dorsal PFC (dPFC), and two primary nuclei of amygdala, centromedial (CMA) and basolateral (BLA).

To interpret changes in rsFC between the amygdala and PFC nodes of the corticolimbic network post stress, we also examined the amygdala response amplitude to negative feedback during a monetary reinforcement learning task [42] completed at the same session as the resting scans and its association with the amygdala rsFC. Prior evidence suggests that amygdala activity is often increased in response to negative stimuli in patients with MDD relative to healthy individuals [43–45]. Moreover, it has been suggested that hyperactive amygdala responses may be partly due to insufficient inputs from various prefrontal areas–particularly dorsal prefrontal cortex–that are believed to dampen these responses [24, 46, 47]. Therefore, we examined the association between amygdala rsFC to PFC nodes and amygdala responses to monetary loss to further characterize the functional significance of individual differences in amygdala rsFC.

Finally, we sought to explore the extent to which brain states following an acute stressor were influenced by different components of stress in patients with MDD versus healthy controls. As with many acute stress manipulations, the stressor used here involved a cold pressor task that induces a multidimensional experience involving components of nociceptive processing [48–51], effortful cognitive control (e.g., the inhibition of a prepotent response to withdraw the hand from cold water), and negative emotion (e.g., valuation of the stimulus and experimental context as negative or unpleasant). We quantified the engagement of these three brain states by applying previously validated multivariate predictive models to brain activity acquired during the resting state [52]. If there are significant altered effects of stress on the connectivity of the corticolimbic network in MDD patients, and if these effects are due to the processes related to pain, cognitive control, or negative affect, we would expect to see altered diagnostic-group-related effects in these processes as well (results and methods for multivariate signature analysis are reported in the Supplementary material).

METHODS

Participants

Adults (age 18–60) participated in this study across two samples of healthy controls and a third sample of unmedicated patients meeting criteria for current Major Depressive Disorder. The three samples were all recruited at the Facility for Education and Research in Neuroscience (FERN) neuroimaging center at Emory University in Atlanta, GA: healthy controls who were not exposed to an acute stressor ("No Stress Controls"; NSC), healthy controls who were exposed to an acute stressor ("Healthy Control Stress"; HCS), and individuals with Major Depressive Disorder who were exposed to an acute stressor (MDD Stress). *Eligibility Criteria:* For healthy control subjects in all samples, participants were excluded for any current or past psychiatric disorder, with the exception of specific phobia, or past alcohol abuse, as assessed by the Structured Clinical Interview for the DSM-IV (SCID) [53] administered by a trained master's-level clinician. For participants in the MDD group, diagnosis of current MDD was confirmed using the SCID. Additional exclusion criteria for participants with MDD included current substance abuse or dependence, obsessive-compulsive disorder, bipolar disorder, active suicidal ideation as assessed by the Columbia-Suicide Severity Rating Scale (C-SSRS; [54]), or any form of psychotic disorder. All participants with MDD were also required to be free of any anti-depressant medication for at least 2-weeks (6-weeks for fluoxetine) prior to the study. Participants with MDD and comorbid anxiety disorders or post-traumatic stress disorder were not excluded from the study. Participants in all samples were excluded for recent use of illegal drugs or any psychotropic medications, which was confirmed using a urine drug screen immediately prior to scanning. All participants provided written informed consent and all study procedures were reviewed and approved by the Emory University IRB.

In total, 88 participants met inclusion criteria and participated in the MRI visit (HC, NSC $n = 30$; HC, MAST $n = 29$; MDD, MAST $n = 29$). Seven participants did not finish the scan visit due to time constraints, scanner malfunction, or did not want to continue the study. One additional participant was excluded due to poor fMRI data quality, resulting in a final sample size of 80. Sample demographics for study completers in each group are provided in Table 1. This table also shows the available data for the two clinical measures of recent stress (PSS) and depression severity (BDI-II). Comorbidities for participants with MDD are included in Supplementary Table s1.

Study design

All recruitment and testing procedures were approved by the Emory University Institutional Review Board. During an initial study visit and after informed consent, participants were interviewed using the DSM-IV SCID [53] to confirm eligibility criteria and completed self-report questionnaires. During the second visit, participants completed an initial scan consisting of structural scan, Magnetic Resonance Spectroscopy (MRS), resting state scan (6 min), and tasks including a reinforcement learning task and an acute stress or no stress control task (described below). This was followed by secondary resting state data collection (6 min), MRS, and reinforcement learning task (Fig. 1A). Saliva samples were collected before and after the stress (or no stress) manipulation to determine cortisol response. MRS results from this sample were published previously [55].

Acute stress manipulation. To induce stress during the scanning session, participants completed the Maastricht Acute Stress Task [56]. The MAST is a laboratory stress paradigm that combines alternating periods of well-validated stress-inducing procedures, specifically a cold pressor and performance of serial subtraction in front of evaluators. During the cold pressor, participants were instructed to immerse their hand up to and including the wrist into ice water (1–8 °C). Water immersion occurred 5 times for varying time intervals of [30s–90s] using a fixed randomized sequence that was unknown to participants so as to create a sense of unpredictability. Between water immersion periods, participants were

Table 1. Demographics.

	Healthy control no stress ($n = 27$)	Healthy control stress ($n = 26$)	Major depressive disorder stress ($n = 27$)
Sex (% Female)	19 (70.4%)	18 (69.2%)	19 (70.4%)
Race (Asian)	9 (33.3%)	8 (30.8%)	2 (7.4%)
Race (Black/African American)	5 (18.5%)	3 (11.5%)	4 (14.8%)
Race (White/Caucasian)	13 (48.1%)	15 (57.7%)	21 (77.8%)
Age	23.33 ± 3.96	28.62 ± 8.22	29.56 ± 10.53
PSS	($n = 27$) 11.15 ± 5.43	($n = 26$) 11.27 ± 6.88	($n = 25$) 27.36 ± 5.96
BDI-II	($n = 26$) 3.65 ± 4.5	($n = 23$) 4.48 ± 6.56	($n = 23$) 32.57 ± 12.44

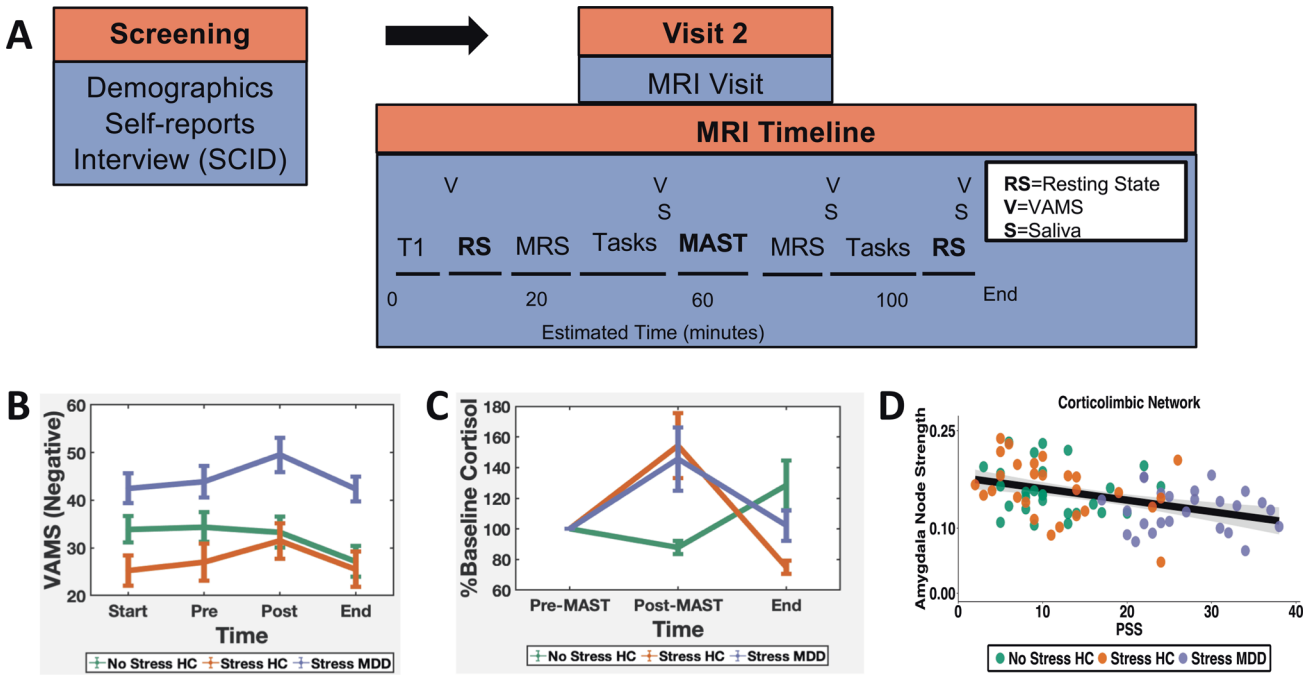

Fig. 1 Study design, effects of stress on mood and salivary cortisol and associations with the Perceived Stress Scale (PSS). A Schematic diagram of the study visits and timing of resting state (RS), Visual Analog Mood Scales (VAMS), saliva and resting-state fMRI (RS) and Magnetic Resonance Spectroscopy (MRS) measurements. **B** Effect of MAST acute stress manipulation and No Stress Control (NSC) on mood ratings. Items are coded such that higher scores indicate greater negative emotional experience and averaged across items. **C** Salivary cortisol response to acute stress manipulation and NSC. Graph depicts percent change in salivary cortisol from the time-point immediately prior to the onset of the MAST stressor (Pre-MAST). All error bars indicate standard error of the mean. **D** Association between Pre-MAST amygdala centrality and the PSS $*p < .001$.

asked to perform serial subtraction starting from 2043 and counting down by 17; with every mistake, a neutral evaluator instructed the participant to re-start from 2043. There were 4 serial subtraction blocks, varying in duration between 30s–90s. Although the MAST protocol we followed was not originally developed for the scanner environment, all procedures were completed while the participant remained in position in the scanner. The scanner bed was moved out part way to facilitate access of the participant's hand to a container of cold water. We note that this protocol represented our own MRI-related adaptation of the MAST, and is slightly distinct from the fMRI adaptation developed by Smeets and colleagues (the "iMAST" [57]) though both procedures are highly similar to the original MAST protocol.

No stress control manipulation. Participants in the NSC condition were instructed to complete a task that followed the same design and timing as the MAST, but used water at a comfortable temperature (26–36 °C) instead of cold water and were asked to count aloud starting from one instead of serial subtraction. Frequency and duration of immersion and counting were determined by computer in the same manner as the MAST. This manipulation was designed to be as similar to the MAST stressor as possible without inducing a stress response.

Functional magnetic resonance imaging

Image acquisition. Imaging data were acquired on a Siemens 3 T TIM Trio scanner with a 32-channel, phased-array head-coil. Each session began with a three-plane localizer scan for slice alignment, and a single-shot, high-resolution structural MPRAGE sequence (repetition time (TR) = 1900, echo time (TE) = 2.27 ms, flip angle (FA) = 9°, field of view (FoV) = 250 mm, 192 × 1.0 mm slices). BOLD functional images were acquired with T2*-weighted echo-planar imaging sequence with the following parameters: 3 mm isotropic voxels, TR = 1000 ms, TE = 30 ms, FA = 65°, FoV = 220 mm, 52 interleaved slices, anterior to posterior phase encoding and a multiband acceleration factor of 4. These acquisition parameters were the same for both resting state and task data.

Image preprocessing—rsFC. Functional and structural images were analyzed using CONN toolbox (version v.20.b; [58]), an SPM-based software

for analysis of functional connectivity for resting state data. Data were preprocessed using a standard pipeline in the CONN toolbox. This preprocessing pipeline included slice timing correction, realignment, segmentation, normalization to the MNI152 template, and smoothing (8 mm FWHM). To reduce the effects of noise, the CONN toolbox has two steps, preprocessing and denoising. In the preprocessing step, an SPM ART based outlier detection is used. Acquisitions with framewise displacement (FD) above 0.9 mm or global BOLD signal changes above 5 SD are flagged as potential outliers. Framewise displacement is computed at each timepoint by considering a 140 × 180 × 115 mm bounding box around the brain and estimating the largest displacement among six control points placed at the center of this bounding-box faces. Global BOLD signal change is computed at each timepoint as the change in average BOLD signal within SPM's global-mean mask scaled to standard deviation units. The denoising step combines the temporal band-pass filtering and linear regression of the potential confounding effects in the BOLD signal. Potential confounding effects are estimated and removed for each voxel and for subject and functional session using Ordinary Least Squares regression. Potential confounding effects used in CONN's denoising pipeline were estimated with an anatomical component-based noise correction procedure (aCompCor) and included noise components from cerebral white matter and cerebrospinal areas, and estimated subject-motion parameters. Temporal frequencies below 0.008 Hz or above 0.09 Hz are removed from the BOLD signal in order to focus on low-frequency fluctuations while minimizing the influence of physiological, head motion and other noise sources. Filtering is implemented using a discrete cosine transform windowing operation to minimize border effects, and performed after regression to avoid any frequency mismatch in the nuisance regression procedure [58]. All the three groups show FD and global BOLD change that are below the conservative thresholds of 0.5 mm and 3 SD respectively both pre and post stress manipulation, and there were no significant differences across the three groups pre or post stress manipulation ($ps > 0.07$).

In order to investigate rsFC, an ROI-to-ROI based correlational analysis was performed using the CONN toolbox on Harvard-Oxford atlas cortical and subcortical ROIs. We chose an a priori corticolimbic network with nodes that are known to be part of stress-sensitive circuitry [59, 60]. This corticolimbic network included 25 nodes, where each node is a region of interest (ROI) drawn from the Harvard-Oxford cortical and sub-cortical atlas (see Supplementary Table s2). When studying the connectivity of the nuclei of the amygdala, we used probabilistic cytoarchitectonic maps of amygdala provided as part of CANlab combined atlas (see https://github.com/canlab/Neuroimaging_Pattern_Masks/tree/master/Atlases_and_parcellations/2018_Wager_combined_atlas) based on the work of [61].

The mean time series of each ROI was calculated by averaging the time series of all the voxels within each ROI. Static functional connectivity was calculated for each subject by computing Pearson's correlation coefficient between the mean time series of each ROI and transforming coefficients into *z* values using the Fisher transform to normalize their distribution. These z-transformed correlations were then used as the edge weights of the corticolimbic network for the rest of the analyses.

To assess Amygdala's importance in the corticolimbic network, we calculated its *node strength*, a common measure of node centrality [62, 63] defined as the sum of the edge weights of all the nodes that exhibit nonzero functional connectivity with amygdala.

For all of our analyses, the right and left amygdala showed similar functional connectivity results; therefore, all the results reported below include their average results.

Self-report ratings questionnaires

To measure affective responses to the acute stress paradigm, all participants completed mood ratings using an adapted version of the visual analogue mood scale (VAMS; [64]). This scale presents participants with five horizontal lines, each representing a bipolar dimensional mood state: Happy-Sad, Relaxed-Tense, Friendly-Hostile, Sociable-Withdrawn, Quick Witted-Mentally Slow. Participants were instructed to move a cursor on each line to the point that best described their current mood state. This VAMS scale was administered before and after the MAST acute stress manipulation. All VAMS ratings were scaled so that higher scores indicated greater negative emotional experience and averaged for each subject to represent negative emotional experience for each timepoint. Following the completion of the MRI scan, participants were asked to rate the stress (or no stress) manipulation on difficulty, stress, and unpleasantness on a scale from 1 (Not at All) to 5 (Extremely).

To assess perceptions of chronic stress, participants were administered the Perceived Stress Scale (PSS; [65]). The PSS is a 10-item questionnaire that asks participants about their perceptions of stress over the last month.

To measure the severity of depressive symptoms, all participants completed The Beck Depression Inventory (BDI-II; [66]). BDI-II is a 21-item self-report questionnaire commonly used to assess for depression severity.

Salivatory cortisol analysis

Salivary cortisol samples were stored at −20 °C until they were assayed in duplicate for cortisol using a commercially available chemiluminescence immuno assay (CLIA) from IBL-International, Hamburg, Germany (Cortisol Luminescence Immunoassay). Cortisol from saliva samples were assayed at the Laboratory for Biological Health Psychology at Brandeis University (Directors: Dr. Nicolas Rohleder and Dr. Jutta Wolf). Inter- and intra-assay coefficients were below 10%.

Statistical analysis

Change in self-report ratings and salivary cortisol were analyzed using separate repeated measures ANOVAs. For cases that violated the sphericity assumption, a Greenhouse-Geisser correction was used. Analyses were performed using MATLAB 2013B (Mathworks, Natick, MA), SPSS v25 (IBM, Armonk, NY), and R (RStudio, Boston, MA). All analyses controlled for sex and age as covariates unless stated otherwise. Neuroimaging multivariate pattern analyses were conducted using CANlab neuroimaging analysis tools, which is an open-source toolbox written for MATLAB (see https://canlab.github.io/) that extends the functionality if SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/).

Reinforcement learning task

Participants completed two runs of a well-validated instrumental conditioning paradigm [42] involving trials with monetary wins and losses. On each trial, subjects viewed two stimuli from one of three pairs and were instructed to choose one of the stimuli, receiving a monetary win, loss, or neutral outcome. In one pair of stimuli (gain pair), one stimulus was associated with an 80% probability of receiving a monetary gain and a 20% chance of receiving nothing, while the other stimulus was associated with a 20% probability of receiving a gain and an 80% chance of receiving nothing. In the second pair (loss pair), the two stimuli were similarly associated with an 80% or 20% chance of a receiving a monetary loss or nothing, respectively. In the third pair (neutral pair), the two stimuli were associated with an 80% or 20% chance of receiving two neutral outcomes, viewing a grey square or the word "nothing", respectively. One RL run was collected at baseline and the second RL run was collected post-stress/NSC manipulation. Each run lasted approximately 10 min and consisted of 72 trials (24 per outcome condition).

The contrast of interest was the difference between feedback in the loss condition relative to neutral condition (feedback for loss pairs > feedback for neutral pairs), where the loss relative to neutral contrast was considered as a proxy for negative stimuli. We additionally analyzed the win condition contrast (feedback for win pairs > feedback for neutral pairs) to establish the specificity of the amygdala response to the loss condition. Given the focus of the current work on amygdala connectivity, analysis of this task was restricted to signals extracted from the anatomically defined basolateral nuclei of the amygdala during the win > neutral and loss > neutral contrasts.

RESULTS

As previously reported [55], we confirmed that our stress vs NSC manipulations produced the expected effects on subjective mood ratings and in salivary cortisol across groups and established the validity of the acute stress manipulation (Fig1B, C). The details of the effects of acute stress manipulation on mood and salivary cortisol is reported in the Supplementary text.

Effects of perceived stress on amygdala functional connectivity

The perceived stress scale (PSS) characterizes how unpredictable and uncontrollable respondents find their lives (Cohen et al.,

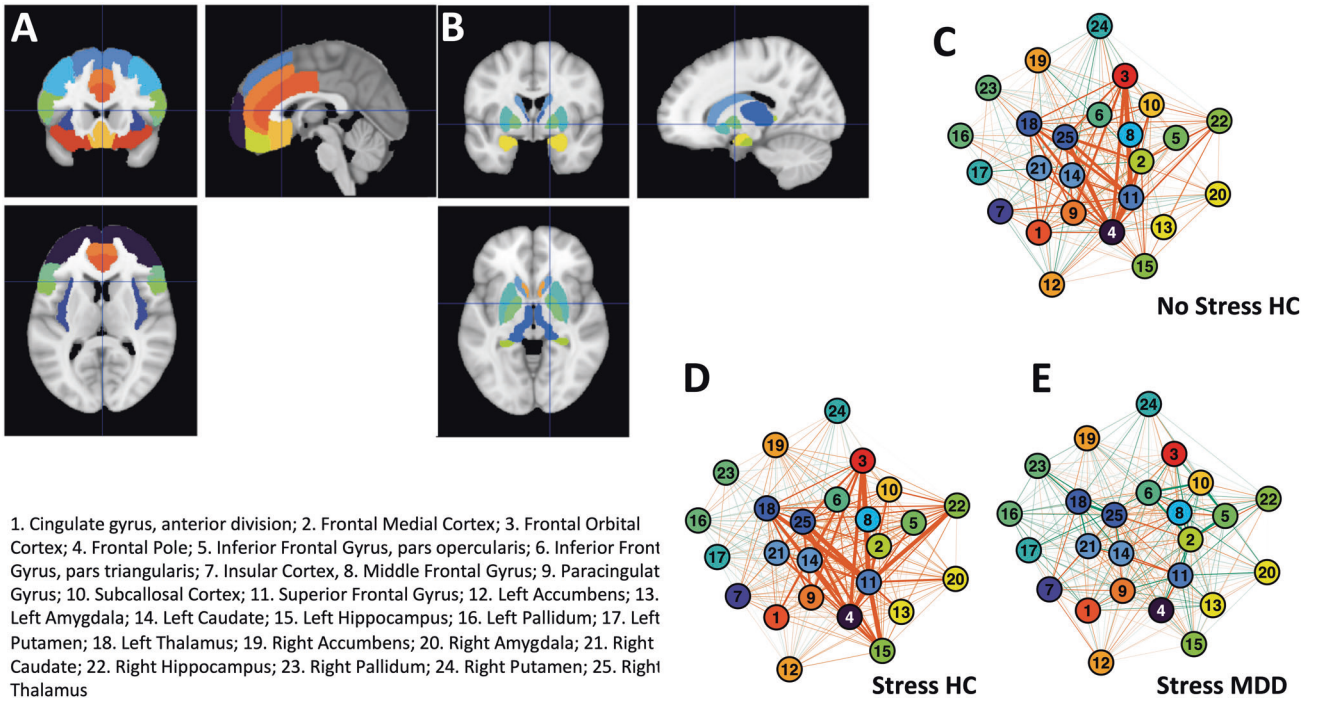

Fig. 2 Regions of interest and changes in network connectivity following acute stress or the NSC condition. A Cortical regions of interest. **B** Subcortical regions of interest. **C–E** Average functional connectivity difference networks. Edges of these networks show the difference between post -stress/NSC FC and pre -stress FC averaged for each of the groups (No Stress HC, Stress HC, and Stress MDD). The Fruchterman-Reingold algorithm, which has been implemented in the qgraph package, was used to create a force-directed layout [82]. The No Stress Healthy Control group network layout has been imposed on the other two group's networks so that the networks are visually comparable. Green edges show positive values and orange edges depict negative values and their thickness corresponds to the magnitude of the change. Each node shows an ROI. The colors of the nodes are matched with the ROIs shown in **A** and **B** to identify the regions easily: 1. Cingulate gyrus, anterior division; 2. Frontal Medial Cortex; 3. Frontal Orbital Cortex; 4. Frontal Pole; 5. Inferior Frontal Gyrus, pars opercularis; 6. Inferior Frontal Gyrus, pars triangularis; 7. Insular Cortex, 8. Middle Frontal Gyrus; 9. Paracingulate Gyrus; 10. Subcallosal Cortex; 11. Superior Frontal Gyrus; 12. Left Accumbens; 13. Left Amygdala; 14. Left Caudate; 15. Left Hippocampus; 16. Left Pallidum; 17. Left Putamen; 18. Left Thalamus; 19. Right Accumbens; 20. Right Amygdala; 21. Right Caudate; 22. Right Hippocampus; 23. Right Pallidum; 24. Right Putamen; 25. Right Thalamus.

1983), and elevations in PSS score have been linked to depressive symptoms in patients with MDD [67]. We hypothesized that recent perceived stress as measured by the PSS would predict amygdala node strength at baseline (prior to the stress manipulation). Consistent with this hypothesis, baseline amygdala node strength in the corticolimbic network was negatively associated with participants' PSS scores before ($r_{partial} = -0.417$, $p < 0.001$) and after controlling for age, sex, depression severity (BDI-II) and diagnostic group ($r_{partial} = -0.266$, $p = 0.031$) (Fig. 1D). We note, however, that the high collinearity of PSS and depressive symptoms [4] limits our ability to attribute this association exclusively to stress.

Effects of acute stress manipulation on amygdala functional connectivity

Global changes in functional connectivity following stress or the NSC condition are presented in Fig. 2. We first assessed whether there were overall differences in amygdala's node strength over time within the corticolimbic network as a function of stress group using a 2 (*Time*) x 3 (*Group*) repeated-measures ANOVA for all participants with useable scans for both pre-stress/NSC and post-stress/NSC time points ($N = 80$). *Time* included pre and post stress or NSC and *Group* included No Stress Control (NSC), Healthy Control Stress (HCS), and MDD stress group. After controlling for age and sex, we found a significant *Time x Group* interaction ($F_{(2,75)} = 6.647$, $p = 0.002$, $\eta_p^2 = 0.151$) indicating that the amygdala node strength has changed differently over time for the three groups. Main effects of group and time were also observed. In a follow-up analysis restricted to only those participants who had completed the acute stress manipulation (i.e., excluding the NSC group), controlling for age and sex, we observed significant effect of *Time* x *Group* interaction ($F_{(1,49)} = 12.179$, $p = 0.001$, $\eta_p^2 = 0.199$) (Fig. 3A). Whereas healthy control participants who completed the stress manipulation showed a significant decrease in amygdala node strength following the MAST stressor ($t_{25} = -4.008$, $p < 0.001$, $d = -0.786$, $M_{pre} = 0.162$, $SD_{pre} = 0.044$, $M_{post} = 0.121$, $SD_{post} = 0.048$), amygdala node strength for the MDD group did not change ($t_{26} = 0.841$, $p = 0.408$, $d = 0.162$, $M_{pre} = 0.125$, $SD_{pre} = 0.032$, $M_{post} = 0.133$, $SD_{post} = 0.040$).

Additionally, we compared healthy control participants who completed the stressor vs no stress control. We observed a significant effect of *Group* ($F_{(1, 51)} = 11.831$, $p = 0.001$, $\eta_p^2 = 0.188$) and a significant effect of *Time* x *Group* interaction ($F_{(1,51)} = 5.856$, $p = 0.019$, $\eta_p^2 = 0.103$) such that healthy controls exposed to the MAST exhibited a greater decrease in amygdala node strength as compared to healthy controls exposed to the NSC. The effect of *Time* x *Group* interaction dropped to marginally significant level after controlling for age and sex ($F_{(1,49)} = 3.774$, $p = 0.058$, $\eta_p^2 = 0.072$).

Owing to the differences in connections of the ventral and dorsal regions of PFC to the amygdala [68, 69], we next examined whether dorsal and ventral PFC nodes of the corticolimbic network contribute differentially to the changes in amygdala node strength following the MAST stressor. Dorsal and ventral PFC nodes of the corticolimbic network are reported in the Supplementary Table s2. A 2 (PFC *Area*) x 3 (*Group*) x 2 (*Time*) repeated-measures ANOVA was used to assess whether there was a difference between the changes in the amygdala node strength

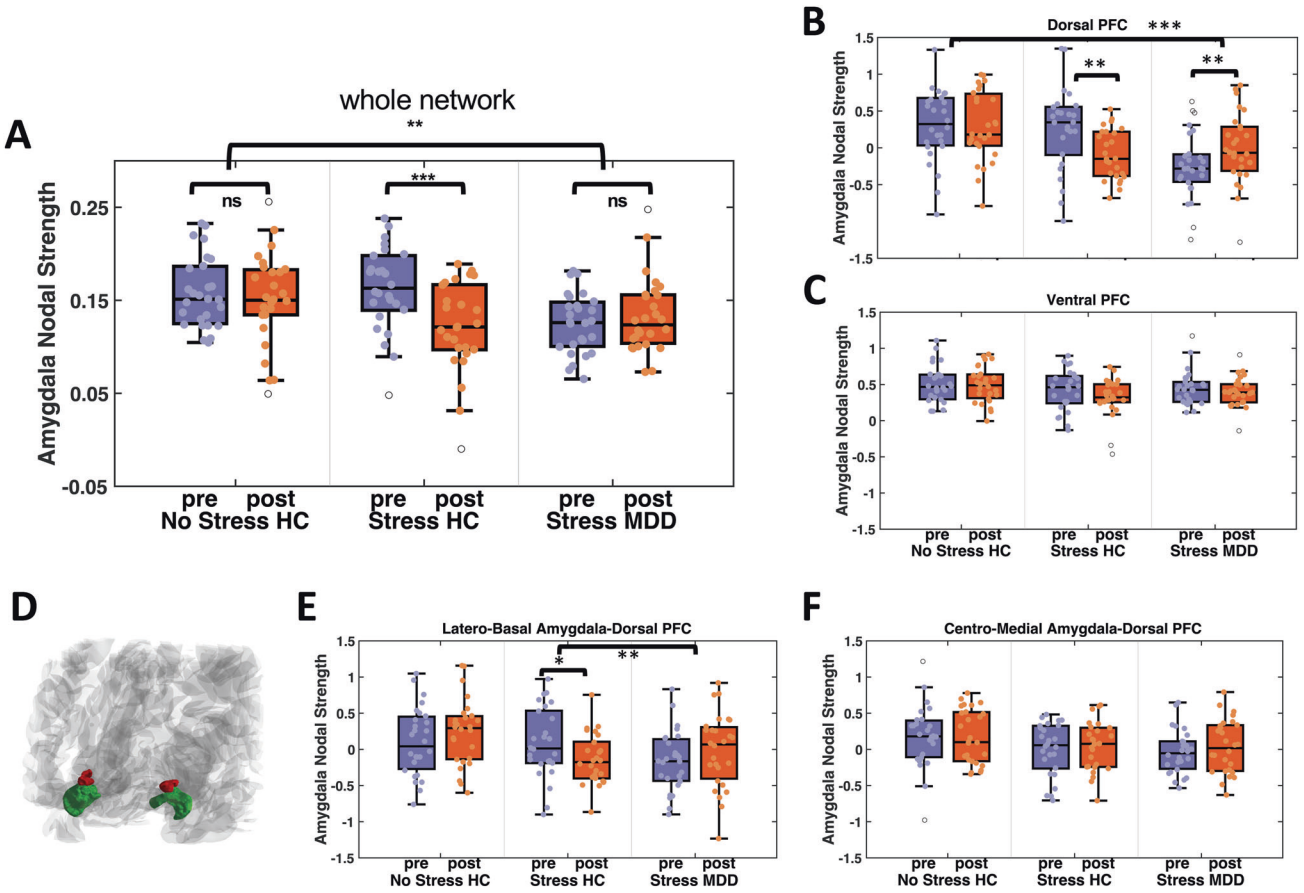

Fig. 3 Amygdala node strength changes following acute stress or the NSC condition. A Healthy control participants who completed the stress manipulation showed a significant decrease in their amygdala node strength following the MAST stressor (***$p < 0.001$), while amygdala node strength for the MDD group and NSC group did not change. **B**, **C** Amygdala connectivity to dorsal and ventral PFC nodes. Amygdala node strength to dorsal–but not ventral–PFC nodes changed significantly for healthy controls following acute stress. **D** BL and CM amygdala nuclei. **E**, **F** Amygdala nuclei connectivity to dorsal PFC nodes. While CM amygdala connectivity to dorsal PFC nodes did not change significantly for either of the two stressed groups, changes in BL amygdala connectivity to dorsal PFC nodes showed a different pattern in HCS and MDD groups. The BL amygdala connectivity to dorsal PFC nodes decreased post stress, whereas it did not change in the MDD group. (*$p < 0.05$, **$p < 0.01$). The shaded areas show %95 CI for the fitted lines.

to the ventral versus dorsal PFC nodes after the acute stress manipulation (Fig. 3B, C). We observed a significant 3-way *Area x Group* x *Time* interaction ($F_{(2,\ 75)} = 9.219$, $p < 0.001$, $\eta_p^2 = 0.197$) controlling for age and sex. In particular, comparing the two stressed groups (HCS and MDD), there was also a significant 3-way *Area x Group* x *Time* interaction ($F_{(1,\ 49)} = 21.493$, $p < 0.001$, $\eta_p^2 = 0.305$) controlling for age and sex. While amygdala connectivity to ventral PFC did not change significantly for either of the two stressed groups ($ps > 0.11$), changes in amygdala connectivity to dorsal PFC showed a different pattern in HCS and MDD groups. In particular, we observed that healthy control participants who completed the stress manipulation showed a significant decrease in their amygdala node strength to dPFC nodes following the MAST stressor ($t_{25} = -3.354$, $p = 0.003$, $d = -0.658$). The amygdala node strength to dPFC nodes showed a significant increase for the MDD group ($t_{26} = 2.88$, $p = 0.008$, $d = 0.55$).

While rsFC cannot provide information about the direction of the information flow between amygdala and other nodes of the network, known anatomical connections between dPFC and different amygdala nuclei can aid in the interpretation of these results (Fig. 3D). In particular, it has been suggested that basolateral amygdala nuclei (BLA) integrate sensory inputs and current physiological state, while the centromedial amygdala (CMA) nucleus sends widespread outputs to direct appropriate behavioral and physiological responses [61, 70, 71]. Consequently, a 2 (*Amygdala Nuclei-dPFC*) x 3 (*Group*) x 2 (*Time*) repeated-measures ANOVA was used to assess whether there is a difference between the change in the CMA-dPFC connectivity and BLA-dPFC connectivity after the acute stress manipulation. We observed a marginally significant 3-way *Amygdala Nuclei-dPFC x Group* x *Time* interaction ($F_{(2,\ 75)} = 2.736$, $p = 0.071$, $\eta_p^2 = 0.068$) controlling for age and sex. This suggests a trend towards differential connectivity for centromedial and basolateral nuclei of the amygdala to dPFC post stress/NSC manipulation. Moreover, when focusing our analysis on only the two groups exposed to the acute stressor (HCS and MDD), there was a significant 3-way *Amygdala Nuclei-dPFC x Group* x *Time* interaction ($F_{(1,\ 49)} = 5.032$, $p = 0.029$, $\eta_p^2 = 0.093$) controlling for age and sex. There were no effects of acute stress on CMA-dPFC connectivity for either sample (paired-*t* $ps > 0.35$). However, when looking at the effects of acute stress on BLA-dPFC connectivity, while MDD group did not show any changes post stress ($t_{26} = 1.517$, $p = 0.141$, $d = 0.292$), the HCS group showed a reduction in BLA-dPFC functional connectivity after the stress manipulation ($t_{25} = -2.25$, $p = 0.033$, $d = -0.441$) (Fig. 3E, F).

Because of the reduced size of these amygdala subregions, we repeated these analyses using smaller smoothing kernels, as smoothing could reduce the spatial precision of these effects. Consistent with the results described above, a significant *Group* x

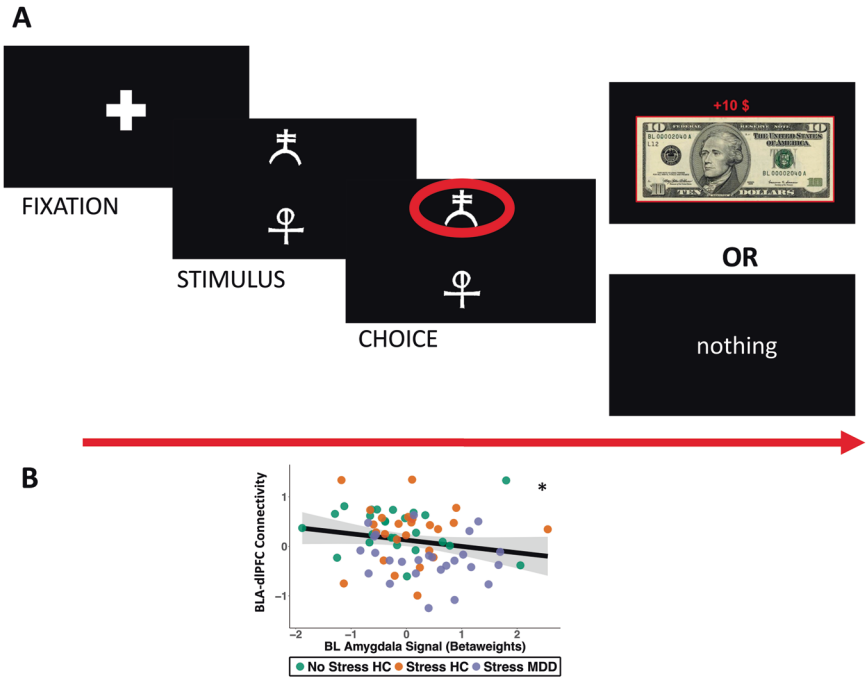

Fig. 4 Associations between amygdala connectivity and amygdala activity during a reinforcement learning task. A Participants chose between two visual stimuli. The stimuli pairs were associated with three possible outcomes (Gain, Loss, Neutral). After making a selection, feedback was shown. Probabilities for each pair varied between 80/20% and 20/80% for the Gain and Loss trials and the monetary outcomes were fixed at + /- $10 and $0. **B** Association between the BLA β estimates for the loss contrast and the BLA-dPFC functional connectivity pre stress manipulation (*$p < 0.05$). The shaded areas show %95 CI for the fitted lines.

Time interaction was observed for BLA-dPFC connectivity when using both a 6 mm and 4 mm smoothing kernel ($F_{(2,75)} = 4.831$, $p = 0.011$, $\eta_p^2 = 0.114$). For additional discussion of the impact of different smoothing kernels on obtained results, please see Supplementary Materials.

The results of the acute stress manipulation together with the baseline relationship between perceived stress and the amygdala nuclei node strength suggest that perceived stress predicts pre-stress BLA-dPFC functional connectivity. Acute stress in healthy controls pushes the corticolimbic network to this stress-phenotype that is already present in individuals reporting higher levels of recent perceived stress [72].

Effects of acute stress manipulation on other networks

To further assess the specificity of the stress effects to the corticolimbic network, we compared the changes in the node strength of amygdala in the corticolimbic network with the node strength of amygdala in a known network involved in face recognition [73]. While we did not predict that this network would be involved in stress response, prior MR spectroscopy work has identified differences in occipital cortex glutamate between healthy controls and MDD patients, highlighting this as a reasonable choice for a positive control [74]. This network was defined anatomically based on pre-defined regions from Juelich and Harvard-Oxford atlases (see Supplementary Table s3 for the names of these regions). A 2 (*Network*) x 2 (*Group*) x 2 (*Time*) repeated-measures ANOVA was used to assess whether there is a difference between the change in the amygdala node strength in the corticolimbic network versus the defined face recognition network after the acute stress manipulation. We observed a 3-way *Network x Group* x *Time* interaction ($F_{(1, 51)} = 6.674$, $p = 0.013$) such that the effects of stress in the corticolimbic network were significantly different from the effects in the face recognition network. This remained significant after controlling for age and sex. Moreover, there was no significant effect of *Time* for either sample (paired-*t ps* > 0.3) in the face recognition network. Taken together, this analysis highlights the specificity of our observed stress effects in corticolimbic areas as compared to other brain networks.

Association between amygdala node strength and neural responses to negative feedback

In order to interpret the findings of the BLA connectivity within the context of the existing literature on increased amygdala response to negative stimuli in patients with MDD [43–45], we examined loss-related amygdala activity in a reinforcement learning task performed between the resting state scans. We compared amygdala activation in response to losses between diagnostic groups and additionally assessed its relationship with the BLA rsFC. We extracted beta weights from an anatomically defined BLA [61] for the contrast of interest (feedback for loss pairs>feedback for neutral pairs). There was a significantly larger response of the BLA, as indexed by its beta weights, in MDD patients ($M = 0.374$, $SD = 0.785$) compared to the two healthy control groups ($M = -0.063$, $SD = 0.814$); t [73] = 2.251, $p = 0.014$ prior to the stress manipulation. This effect was absent in the win contrast (feedback for win pairs > feedback for neutral pairs), t [73] = 0.372, $p = 0.71$, suggesting similar BLA response to positive stimuli in both patients and healthy control groups at baseline (Fig. 4). To assess the specificity of the BLA response to the negative stimuli, we used a 2 (*Group*) x 2 (*Contrast*) repeated measure ANOVA. *Group* included patients with MDD and healthy groups (HCS and NSC). *Contrast* included loss and win contrasts. We observed a marginally significant *Group x Contrast* interaction ($F_{(1, 72)} = 2.836$, $p = 0.09$, $\eta_p^2 = 0.038$) after controlling for age and sex.

Moreover, we examined the relationship between BLA response for the loss contrast and the BLA-dPFC functional connectivity at baseline for all the groups. After controlling for age and sex, we observed a negative association between them ($r_{partial} = -0.262$, $p = 0.024$) that was significantly stronger than the relationship between the BLA beta weights for the win

contrast and the BLA-dPFC functional connectivity at baseline using Steiger's Z test ($r_{partial} = -0.031$; $Z = -1.744$, $p = 0.041$). These results indicated weaker rsFC between BLA and dPFC nodes was associated with stronger BLA responses to negative stimuli. The association between beta weights for the win contrast and rsFC between BLA and dPFC nodes was not significant. Moreover, the strength of the association with losses was significantly greater than the strength of association with wins. This suggests that the amygdala node strength was associated with an exaggerated amygdala response to negative–but not positive–feedback. This association was not better explained by impaired learning in the RL task in MDD patients (see Supplementary Materials). As above, these analyses were repeated using alternative smoothing kernels, and note that we did not observe the same associations between task-based amygdala activity and BLA-dPFC connectivity when using smaller smoothing kernels (see Supplementary Table s4).

These results are consistent with prior work suggesting impaired top-down regulation of amygdala responses to negatively valanced information via dorsal prefrontal areas [24, 46]. Moreover, our work suggests that this may reflect a stress-based phenotype that is exhibited in healthy controls after an acute stressor, but may be chronically engaged in patients with MDD.

Sex effects

Given well-known sex-differences in the prevalence of mood disorders, a final set of analyses tested for effects of sex on amygdala connectivity changes to dorsal PFC. When considering sex as a between subject factor we observed a 3-way 3 (*Group*) x 2 (*Time*) x 2 (*Sex*) interaction ($F_{(2,\ 73)} = 3.92$, $p = 0.024$, $\eta_p^2 = 0.097$). Although both male and female participants showed the same pattern of behavior post stress, the amygdala connectivity changes were stronger in female participants. Because the number of males in our samples was approximately half that of females, these results warrant further investigation in balanced designs optimized to test for sex differences. We did not observe any other sex effects in the analyses presented above.

DISCUSSION

In this study, we examined acute stress induced changes in resting state functional connectivity within a corticolimbic network in healthy participants and patients with MDD. At baseline, we found that recent levels of perceived stress were associated with reduced amygdala node strength. When a group of healthy individuals was exposed to an acute stressor, they exhibited a clear reduction in amygdala node strength as compared to healthy controls who were exposed to a no-stress control condition. Importantly, this effect was absent for unmedicated individuals with current MDD, suggesting that the absence of this response may be a contributor to stress-related psychiatric diseases. More specifically, amygdala node strength in healthy controls exposed to stress reached the levels observed among participants with MDD prior to stress exposure. Taken together, these results suggest that acute stress in healthy controls pushes the corticolimbic network toward a "stress-phenotype" that is already present in depressed individuals reporting higher levels of recent perceived stress.

One caveat to this interpretation was observed when testing alternative smoothing kernels. The application of a spatial smoothing kernel enhances statistical sensitivity and is common in fMRI data analysis [75, 76], but comes at the cost of anatomical precision. Our initial results were obtained using an 8 mm smoothing kernel, which is a common default setting [58]. We additionally tested our analyses using 6 mm and 4 mm smoothing kernels. At these smaller kernels, we continued to observe a significant reduction of centrality within the amygdala network for healthy control participants following acute stress, as well as a lack of change for MDD patients. However, the originally observed patterns of baseline differences for amygdala subregions across groups as well as associations with task-based amygdala responses were not observed when using smaller smoothing kernels (See Supplementary Materials for full results). This could reflect lower statistical power or may suggest that signals from neighboring regions (extended amygdala, BSNT, hippocampus or ventral striatum) were contributing to our pattern of results when using an 8 mm kernel. As such, caution is warranted in the interpretation of our baseline group differences. That said, the differential impact of acute stress on healthy control and MDD connectivity was largely insensitive to smoothing kernel size, and suggests a true lack of network adaptability among MDD patients.

To further understand the changes of the corticolimbic network in response to acute stress, we studied how dorsal and ventral prefrontal areas of the corticolimbic network contributed separately to the changes in amygdala node strength following the acute stressor. While amygdala connectivity to ventral PFC did not change significantly for either of the two stressed groups and was unrelated to perceived stress, changes in amygdala connectivity to dorsal PFC showed a different pattern in HCS and MDD groups. This suggests that among the heterogenous PFC nodes, it is the dorsal PFC nodes (and in particular dorsal medial areas as shown in the Supplementary Fig. s1) of the corticolimbic network that are driving the reduction in amygdala node connectivity to PFC. Since the activity of dorsal PFC regions are most frequently associated with cognitive dimensions of stimuli rather their emotional aspects [77], this result might hint at a regulatory deficit in patients in MDD.

The reduced change in the node strength of amygdala to the dPFC nodes of the corticolimbic network might be due to a top-down influence from the dPFC nodes to amygdala or vice versa. While, our rsFC measures cannot give information about the direction of this influence, evaluating changes of the rsFC within amygdala sub-nuclei can aid interpretation. Importantly, we found that there was no significant change in the centromedial amygdala connectivity with dPFC between groups or in response to stress. This is consistent with known anatomical connections between centromedial amygdala nucleus and PFC [61]. On the other hand, there was a significant 2-way *Group* x *Time* interaction in basolateral amygdala connectivity with dPFC. While the HCS group showed reduction in BLA-dFC functional connectivity, MDD patients showed an increase in FC post stress and reached those of HCS groups post stress. Given that there are bidirectional pathways from dPFC to basolateral amygdala [71], this result could suggest altered reciprocal communication between amygdala and dPFC following acute stress.

It is also noteworthy that while we observed the reduced connectivity to the amygdala within this corticolimbic network, task-evoked amplitude effects for amygdala activity are typically *elevated* in MDD patients in response to negative stimuli in patients with MDD [78, 79]. We hypothesized that elevated amygdala reactivity to negative events may be linked to attenuated BLA-dPFC connectivity. To examine this possibility, we measured the amygdala activity in a reinforcement learning task while participants received negative feedback, performed during the same scanning session as the resting state scans. We observed a significantly larger response of the BLA to negative (loss) feedback in MDD patients compared to the two healthy control groups at baseline. Moreover, there was a negative association between the extracted BLA β weights and the BLA-dPFC functional connectivity at baseline for all the groups that was specific to negative feedback relative to positive feedback. This result suggests that the reduction in the amygdala node strength and increased amygdala activity are not in contradiction

and are different manifestations of attenuated involvement of the amygdala in the corticolimbic network.

Finally, given that many acute stress paradigms incorporate painful stimuli into the stress-induction procedure, we sought to determine if multivariate neural signatures of pain changed due to acute stress. Pain and stress are two distinguished yet overlapping processes presenting multiple conceptual and physiological overlaps. Both phenomena challenge the body's homeostasis and necessitate decision-making to help navigate and adapt to the environment [80]. However, the relationship between them is not clear. We did not detect any significant changes in measures of pain and related processes (i.e. cognitive control and negative affect which are often engaged concurrently in a typical experimental manipulation of pain) for the models used here [52] post stress in either of the stressed groups, suggesting that pain is unlikely to play a significant role in the observed amygdala connectivity effects. It is important to note, however, that the pain signatures have been developed to capture neural activity during the momentary experience of pain, whereas our post stress resting data was collected about 40–60 min post-acute stress manipulation.

Taken together, these findings point at the lack of amygdala response to acute stress manipulation in patients with MDD as compared to healthy controls. These results might hint that acute stress in healthy controls pushes this network to a "stress-phenotype" that is already present in MDD individuals reporting higher levels of recent perceived stress.

Limitations

While the current work has a number of strengths, there are some important limitations. First, patients in our depressed group reported significantly higher levels of recent perceived stress than individuals in our healthy control group. This is a common effect in the literature [1, 81], but leaves us unable to determine the extent to which the observed effects with amygdala connectivity were due to current depression, higher levels of perceived stress or both. Second, our post-stress resting data was collected about 40–60 min after the onset of the acute stress manipulation. This may have limited our ability to detect some effects in the immediate aftermath of the stressor. Moreover, the post-stress resting state scan was collected after the tasks including a reinforcement learning task. It is possible that this task had effects on the post-stress resting state scan. This might introduce a potential confound. Third, we have used Harvard-Oxford atlas, a widely used atlas in prior papers in MDD and stress, to define the corticolimbic network nodes. An important criterion in choosing this atlas was to strike a balance between minimizing the number of nodes and edges while covering the brain areas of interest. However, it should be noted that the parcellation of the regions in Harvard-Oxford atlas is based on structural data and is not data-driven. Lastly, our sample sizes for each group were only moderate, and we may have been under-powered to detect smaller effects, such as associations with the multivariate signatures and sex effects.

CONCLUSION

Taken together, these findings highlight attenuated connectivity between basolateral amygdala and prefrontal cortex as a specific response to acute stress in healthy controls that may be chronically present in patients with current depression and high levels of perceived stress. Moreover, this connectivity profile was selectively associated with exaggerated responses to negatively valanced feedback during a reinforcement learning task. This circuit may be an important target for stress-linked psychopathology, such as disorders of mood and anxiety.

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ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Mental Health R00MH102355 and R01MH108605 to MTT and K01MH126308 to JAC. Data management through REDCap was supported by UL1 TR000424. We also gratefully acknowledge support from Andrew Teer, Sandra Goulding, Amanda Shamblaw, Amanda Arulpragasam, Nicolas Rohleder, and Laurie Scott.

AUTHOR CONTRIBUTIONS

SH planned and performed all primary analyses and drafted the manuscript; JAC aided with data collection and analysis, interpretation of results, and edited the manuscript; BAMD, MRN and ECH aided in data collection, data analysis, and edited the manuscript; PAK aided in multivariate data analysis, interpretation of results, and edited the manuscript; MTT conceived the study, oversaw analysis and helped draft the manuscript.

COMPETING INTERESTS

The authors report no conflicts of interest, financial or otherwise. In the past three years, MTT has served as a paid consultant to Blackthorn Therapeutics and Boehringer Ingelheim. None of these entities supported the current work, and all views expressed herein are solely those of the authors.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41380-023-02056-5.

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