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Exploring common changes after acute mental stress and acute tryptophan depletion: Resting-state fMRI studies

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ABSTRACT

Stress and low serotonin levels are important biological factors in depression and anxiety etiologies. Although studies indicate that low serotonin levels, stress, and other factors may interact in depression/anxiety psychopathology, few studies have investigated the potentially shared neural substrates. We conducted resting-state fMRI scans pre- and post-stress task, and under control and tryptophan depletion condition, to explore the common changes induced by acute mental stress (AMS) and acute tryptophan depletion (ATD). The present study targeted regions within core brain networks – default mode network, salience network, executive control network, and emotion network – reported altered in AMS and ATD, and used regional homogeneity (ReHo) and functional connectivity (FC) analyses to explore their overlapped effects. We additionally examined the relationships among core neural networks – operationalized as an index of resource allocation bias that quantifies the shift from internal to external modes of processing. We found both manipulations induced increased ReHo of the amygdala and decreased ReHo of the posterior cingulate cortex (PCC). The PCC-amygdala FC was negatively correlated with the change of negative affect, whereas the right dorsolateral prefrontal cortex and right anterior insula FC was positively associated with anxiety level. In addition, we found that a greater shift to an external mode was correlated with higher anxiety level under both conditions. Common changes induced by acute mental stress and acute tryptophan depletion confirmed our hypothesis that AMS and ATD induce changes in common neural pathways, which in turn might mark vulnerability to depression and anxiety.

1. Introduction

Mental stress and low serotonin (5-hydroxytryptamine; 5-HT) levels are among the most well-recognized etiological factors for mood disorders such as major depression disorder (MDD) and anxiety disorders (Bogdan and Pizzagalli, 2006; Gotlib et al., 2008). MDD and anxiety patients often have deficient serotonergic function and greater cortisol release than healthy individuals under stress (GR, 1995; Heninger et al., 1984). Temporarily inducing mental stress using distressful tasks or temporally reducing central serotonin levels through restricting serotonin precursor, tryptophan, intake (an experimental method called acute tryptophan depletion, ATD (Young et al., 1985)) can effectively evoke depressive mood or anxiety in vulnerable individuals (van Donkelaar et al., 2011). Studies have indicated potential neurobiological interactions between mental stress and low serotonin in the development of depression (Contesse et al., 2000; Drevets, 1998; Heisler et al., 2007). Serotonin can govern the activity of the hypothalamic-pituitary-adrenal (HPA) axis (the central stress system) by controlling the activity of corticotropin-releasing factor (CRF) neurons (Thomas et al., 1999). Meanwhile, adrenocorticotropic hormone releasing factor, the neurohormone related to stress, can inhibit the activity of 5-HT neurons, and consequently inhibits the synthesis of serotonin (Kirby et al., 2008). Considering the shared molecular substrates and similar behavioral manifestations induced by acute mental stress (AMS) and serotonin deficiency, we hypothesize that they share neural substrates vital to the vulnerability of depression/anxiety – a conjecture still lacking direct tests in the literature.

fMRI is a powerful imaging tool for measuring brain activity

Abbreviations: AMS, acute mental stress; ATD, acute tryptophan depletion; ReHo, regional homogeneity; FC, functional connectivity; DMN, default mode network; SN, salience network; ECN, executive control network; EN, emotion network; PCC, posterior cingulate cortex; RAI, right anterior insula; rdlPFC, right dorsolateral prefrontal cortex; LAMY, left amygdala; IRAB, index of resource allocation bias

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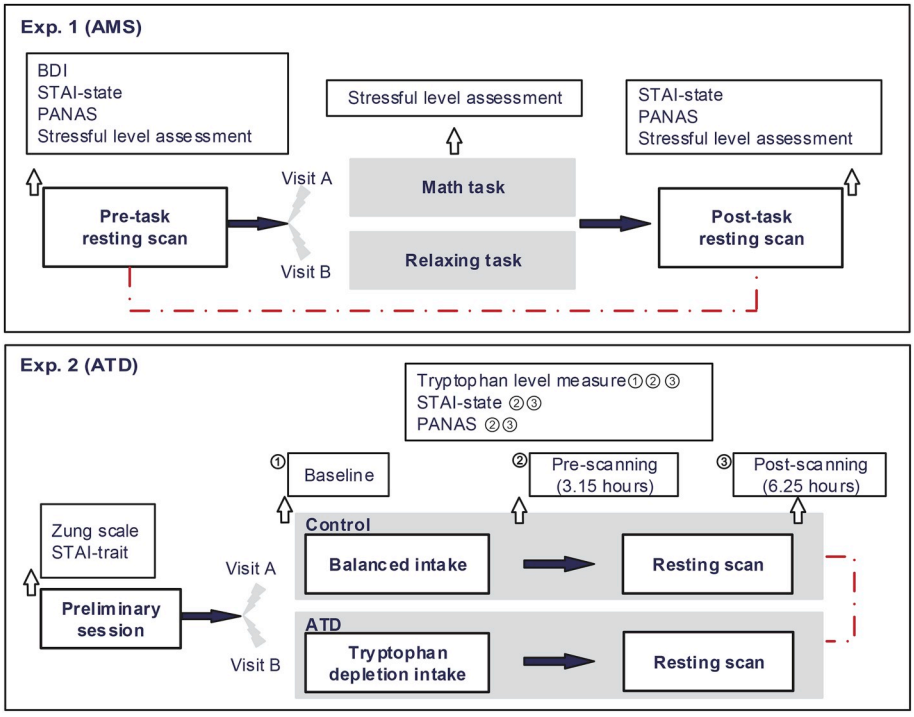

Fig. 1. Schematic diagram of the two separate experiments (red dotted line indicates the conditions for comparison in two experiments). Resting-state scans were conducted in pre- and post-tasks (math calculation and breath counting) in Exp. 1, and in control and acute tryptophan depletion (ATD) state in Exp. 2. Behavior measurements and the tryptophan level were collected with certain intervals. Exp. 1: acute mental stress; Exp. 2: acute tryptophan depletion. BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; PANAS, Positive and Negative Affect Scale.

changes induced by diseases or manipulated by internal/external stimulations. fMRI studies have shown that the neural networks most commonly linked to depression/anxiety include the default mode and memory network (DMN/MN) which is related to internally directed self-referential thoughts, the salience network (SN) which detects bottom-up salient events and reallocates the attention resources, executive control network (ECN) which is mainly involved in external executive function, and emotion network (EN) which processes emotional information (Drevets, 1998; Greicius, 2008; Greicius et al., 2007; Kaiser et al., 2015; Maletic et al., 2007; Manoliu et al., 2014; Mayberg et al., 1999; Ottowitz et al., 2002; Sheline et al., 2009; Whitfield-Gabrieli and Ford, 2012). Atypical functioning in these networks has been found in AMS studies as well as ATD studies. Post- versus pre-stress resting-state fMRI in healthy adults revealed increased amygdala-ventromedial prefrontal cortex (vmPFC) connectivity (Quaedflieg et al., 2015), and within dorsal attention network connectivity (Soares et al., 2013), as well as decreased amygdala-posterior cingulate cortex (PCC)/precuneus connectivity (Maron-Katz et al., 2016) and within DMN connectivity (Soares et al., 2013). Resting-state fMRI study under ATD also revealed the decreased activity of DMN regions (precuneus/PCC and medial prefrontal cortex) and increased dorsolateral prefrontal cortex (dlPFC)-DMN connectivity (Kunisato et al., 2011), as well as decreased within DMN connectivity (Helmbold et al., 2016). In summary, the core brain regions in DMN (PCC/precuneus, vmPFC, orbital frontal cortex-OFC and hippocampus), ECN (dlPFC, inferior parietal cortex), SN (insula, anterior cingulate cortex-ACC), and EN (amygdala, OFC, and vmPFC) have been frequently reported to be manipulated by both AMS and ATD, indicating the possible common neural substrates underpinning AMS and ATD, and the common changes induced by these two factors may predict individuals' vulnerability to depression/anxiety and advance our understanding of depression/anxiety psychopathology.

Understanding how and why DMN, ECN, SN, and EN interact in depression/anxiety could provide important clues for eventual remediation of these disorders. An interesting theory has posited that SN, in particular right anterior insula (RAI), can mediate the switch of attention between internal mode (governed by DMN) and external mode (dominated by ECN) (Menon, 2011; Menon and Uddin, 2010; van Tol et al., 2014). The subtraction of SN-ECN and SN-DMN functional connectivity (FC), which was referred to as a resource allocation index, was found associated with smoking urge scores in healthy smoking subjects under abstinence (Lerman et al., 2014). A study in depressed patients also found an alternation in the balance between the internal and external mode in those with higher depressive rumination levels, increasing the former while diminishing the latter (Hamilton et al., 2011). These studies highlight the importance of studying the relationships among the three networks in understanding attention reallocation for mental disorders. We hypothesize that the triple network interactions may reflect a similar process – changing between internal and external focus – in response to AMS and ATD.

1.1. Aims of the study

To our knowledge, there is no direct comparison of AMS and ATD induced brain changes except for meta-analysis. However, meta-analysis may bias the conclusion due to different preprocessing and analysis pipelines across studies. Therefore, in the present study, we conducted an AMS study at Tsinghua University, and exploratorily analyzed an ATD study dataset collected at Duke University, using the same analysis pipeline. Regional homogeneity (ReHo) which evaluates local activity, FC which measures connections among regions/networks and the triple network interactions which examine attention resources allocation were compared in both samples – providing additional evidence for the generality of our conclusions across different data collection procedures. We hypothesized that common changes in core networks would be observed under AMS and ATD, and those changes would be associated with the individual's depression/anxiety vulnerability trait.

2. Materials and methods

The two datasets were collected from two independent experiments (experiment 1 on AMS; experiment 2 on ATD), scanning pipelines are shown in Fig. 1. Here we describe the two experiments successively and separately. Demographic information and psychometric characteristics for the two experiments are summarized in Table 1.

Table 1
Subject's demographics, depression severity, trait anxiety and mood measures in AMS (Exp. 1) and ATD (Exp. 2) experiment. BDI, Beck Depression Inventory; Zung score, Zung Self-Rating Depression Scale.

	Exp. 1		Exp. 2	
	Pre-math	Post-math	Control	ATD
N	17		20	
Age	22 ± 0.68		23 ± 0.73	
Gender (F/M)	5/12		10/10	
Depression Severity	4.31 ± 0.94 (BDI)		28.60 ± 1.37 (Zung score)	
Trait Anxiety	\		46.90 ± 0.54	
State Anxiety	42.70 ± 1.40	43.81 ± 1.40	33.65 ± 2.00	34.65 ± 2.20
Negative Affect	16.38 ± 0.90	16.81 ± 0.90	12.75 ± 0.60	12.35 ± 0.70
Positive Affect	33.15 ± 1.31	31.59 ± 1.48	24.57 ± 1.99	24.19 ± 2.05

2.1. Experiment 1 (acute mental stress)

2.1.1. Participants

Seventeen healthy college students (mean age = 22y, right-handed, 12 males) were recruited in this study; subjects who had any history of head injury, neurological disorders, or other major medical conditions were excluded. The Beck Depression Inventory (BDI) was used to screen for depression (scores < 13). The BDI score was also collected one year later after the scanning. This study was approved by the local Medical Ethics Committee at Tsinghua University School of Medicine, and informed written consent was obtained from all subjects. Math calculation and breath counting tasks (Supplementary Materials) were used to induce stressful and relaxing state, respectively; these manipulations have been validated in our earlier studies (Paul et al., 2013; Wang et al., 2013). Subjects came twice (one for stressful and one for relaxing task), apart from 5 to 10 days, to complete the study in a counterbalanced order. Two six-minute and fifteen-second resting-state fMRI scans were acquired with one before and one after each task (stressful or relaxing).

See Supplementary Materials for details on mood evaluation and data acquisition.

2.2. Experiment 2 (acute tryptophan depletion)

2.2.1. Participants

Twenty healthy participants (mean age = 23y, right-handed, 10 males) were recruited into this study, with no history of head injury, neurological disorders, or other major medical conditions. Participants were screened using the Zung Self-Rating Depression Scale (scores < 36). All participants provided written informed consent under a protocol approved by the Institutional Review Board at Duke University School of Medicine. The ATD experiment was double-blind, placebo-controlled, and counterbalanced, our previous study has shown successfully reduced tryptophan level through the same ATD manipulation (Wang et al., 2009). All participants visited thrice, including a preliminary session, a control session and an ATD session (Supplementary Materials), each separated by 7–10 days. Subjects were asked to maintain a low-protein diet on the day before and fast on the mornings of each control/ATD session. A five-minute resting-state fMRI scan was conducted in each of the control and ATD sessions. Given that it takes about 4–5 h for plasma tryptophan to decrease to the lowest level, we only acquired resting fMRI 4 h after the intake of tryptophan-depletion (or full protein) compounds.

See Supplementary Materials for details on mood evaluation and data acquisition.

2.3. Data analysis

All preprocessing steps (Supplementary Materials) and analysis procedures for experiment 1 and experiment 2 were the same, the overview of the analysis pipeline is shown in Fig. 2. Our interest contrast was resting-state measurement post- versus pre-stress (or counting) task in Exp. 1 and post ATD versus control conditions in Exp. 2 and the intersection effect between the two experiments. We are interested in how the brain activity and connectivity changes in networks reported associated with depression and anxiety (EN, DMN, SN, and ECN) under stress and low serotonin levels, so we conducted the analysis and reported results focusing on core networks. Resting-state ReHo and FC analysis were conducted in DPABI V1.3 (http://rfmri.org/DPABI), and group analyses were conducted in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/).

2.3.1. Resting-state regional homogeneity (ReHo) measurement

To assess changes in regional neural activity synchronization after stressful and ATD conditions, resting-state ReHo for each subject was calculated. The Kendall's correlations coefficient (KCC) of the time series of a given voxel with its nearest neighboring voxels was used to estimate the extent of regional synchronization for ReHo (Zang et al., 2004). The default 26 neighboring voxels were included in KCC calculation. ReHo and ALFF (amplitude of low-frequency fluctuation) are two approaches to reveal regional functional abnormality; both are reliably correlated with cerebral blood flow in most of the brain cortex (Li et al., 2012), while ReHo is more sensitive to depict regional dysfunction because it accounts for the dynamic features of the time-courses of neighboring voxels (An et al., 2013).

2.3.2. ROI-based functional connectivity

ROI selection and FC calculation: Considering that template ROIs might not be specific for our dataset and using ROIs which showed changed activity might increase the sensitivity to detect FC change, we extracted representative clusters within core networks that showed

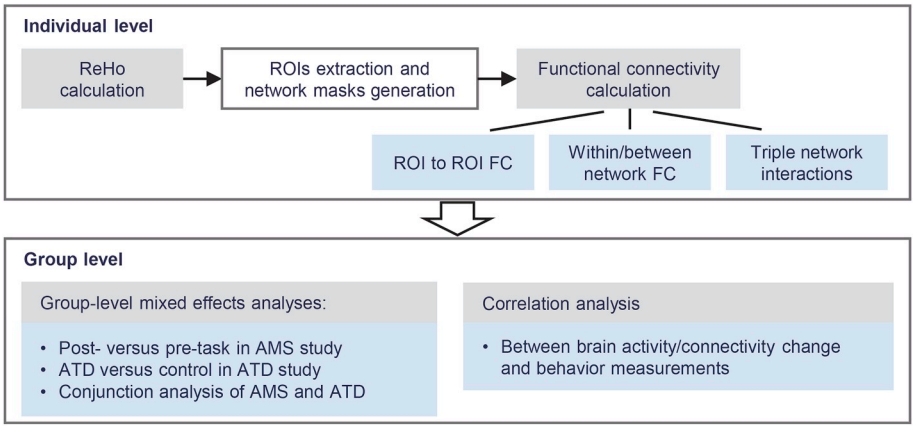

Fig. 2. Analysis pipeline for both acute mental stress (AMS) and acute tryptophan depletion (ATD) experiment. The regional homogeneity (ReHo) was calculated voxel-wise and clusters which showed changed ReHo among core networks were selected as regions of interest (ROIs) to evaluate functional connectivity and triple network interactions (SN, DMN, and ECN). Group analyses were applied to identify common changes shared by AMS and ATD, and correlation analysis was conducted to examine the association of brain changes and behavior output.

significant changes in ReHo under stressful vs. baseline state or ATD vs. control state as seeds, to compute seed-to-seed FC maps for each subject. In this study, ROIs within EN, DMN, SN, and ECN that showed ReHo changes in either Exp. 1 or Exp. 2 were chosen for FC analysis. The seeds were the left amygdala, the overlapping cluster of increased ReHo in the two experiments, the PCC, the overlapping cluster of decreased ReHo in the two experiments, and the RAI and right dorsolateral prefrontal cortex (rdlPFC), which showed increased ReHo post-relative to pre-stress.

Network masks generation and within/between network FC evaluation: To assess the within network connectivity, we first generated network templates based on seed-based connectivity maps of RAI, rdlPFC and PCC, then we applied the meta-analysis atlas of these three networks (http://neurosynth.org/) as masks, and the overlapped areas between our network templates from the two experiments and the masks were referred to as our final SN, ECN, and DMN. The connectivity between the PCC and the DMN, the rdlPFC and the ECN, and the RAI and the SN were measured to represent the within network connectivity. The connectivity between ROIs of DMN, ECN, SN, and EN was calculated as between network connectivity.

2.3.3. Network interactions

We were also interested in how the attention allocation changes between internal (DMN) and external (ECN) mode post-AMS and post-ATD. Therefore, we examined the interactions among the triple networks by focusing on three key nodes: PCC, RAI, and rdlPFC, all of which showed alterations in ReHo after AMS or ATD. The previous study assumed that the SN controls the interaction between the external (ECN) and internal (DMN) modes, and used the subtraction of SN-DMN FC and SN-ECN FC as a resource allocation index (Lerman et al., 2014). However, since the control node allocates attention resources across internal and external modes, we believe the connectivity strength between the control node and the two networks should change in opposite direction during resource competition, i.e., while the RAI-rdlPFC FC strength increases, RAI-PCC FC strength decreases, that is, the RAI-PCC FC increases given PCC is typically negatively connected to RAI in resting-state. Therefore, we used RAI-dlPFC plus (rather than minus) RAI-PCC FC to define an index of resource allocation bias (IRAB, Eq. (1)). The IRAB will increase when the attention shifts from internal mode to external mode, and vice versa.

$$IRAB = FC_{RAI-rdlPFC} + FC_{RAI-PCC} \quad (1)$$

2.3.4. Group analysis

Voxel-wise mixed effects analyses were applied for the whole brain group analyses. For the AMS study, our interesting contrast was post- versus pre-task condition, i.e., between post- and pre-breath counting task. Considering the fact that the information processing differences between the breath counting task and the stress tasks not only includes the degree of stressfulness but also different cognitive processing, we focused on post-math vs. pre-math comparison in AMS. However, given the practical issue, we did not collect pre-ATD or control baseline resting-state data for the ATD study, our interesting contrast was post-ATD vs. post-control conditions. To examine the common ReHo changes across the two experiments, a conjunction analysis between the post- versus pre-stress and ATD versus control condition was conducted using SPM8. Independently, a paired *t*-test was conducted for the post- vs. pre-stress condition in Exp. 1 and ATD versus control condition in Exp. 2. To make the two studies more comparable and also better control baseline differences, we also conducted an exploratory analysis (paired *t*-test) on stress-induced changes (post-stress – pre-stress) vs. relaxation-induced changes (post-relaxation – pre-relaxation) contrast. The results can be found in the Supplementary Materials. In order to ensure the reliability of our measures between studies, and within subjects, we conducted a two-sample *t*-test for ReHo between the pre-math condition in AMS study and the control condition in ATD study using data collection site as a covariate to measure the baseline difference between two cohorts, and a paired *t*-test for ReHo between the pre-math condition and pre-relaxation condition to measure within subject stability. The significance level for voxel-wise statistical tests was set at a threshold of $p < 0.05$ with cluster-wise false discovery rate (cFDR) correction for multiple comparisons. All t statistical maps were overlaid on a single anatomical image provided in the MRIcroGL software (http://www.mccauslandcenter.sc.edu/mricrogl/).

2.3.5. Post-hoc correlation analysis

In order to better elucidate the psychological mechanism underpinning the alteration of the brain activity and connectivity, we calculated the Pearson correlation coefficient between the brain activity/connectivity changes (ReHo, FC, and IRAB) and behavior measures (self-rated stressful level, anxiety level, and BDI) with a threshold of $p < 0.05$.

2.3.6. ROI identifications from group independent component analysis (gICA)

To ensure that the FC related findings were not biased by our ROI selection method, we also tested all the significant findings using ROIs extracted from two gICA analyses (N of components = 20 and 150 respectively), the gICA was conducted in GIFTv3.0b (http://mialab.mrn.org/software/gift/). To make sure we are comparing the same components/regions between conditions of the two studies, we conducted gICA on all resting-state data from the two studies (including pre- and post-stressful/relaxing tasks from Exp. 1 and resting-state data under ATD and control conditions from Exp. 2). By specifying the number of components of 20, we identified the DMN, SN, and ECN through visual inspection (SFig. 3). By increasing the number of components to 150 (the maximum number constrained by the total time-points of the fMRI data and the PCA procedure before ICA), the networks were decomposed into separate nodes (one or two regions in a component), ROIs of the PCC, RAI, rdlPFC and left amygdala were extracted in accordance with the ReHo change-based ROIs (SFig. 4). To be noted, we used the overlapping regions between the nodes identified from the gICA of 150 components and the corresponding networks identified from the gICA of 20 components as ROIs. More details and results are described in the Supplementary Materials.

3. Results

3.1. Behavior and tryptophan measures

Experiment 1: As shown in Fig. 3, engaging in a math task significantly increased the self-assessed stressful level by 40% (paired *t*-test, $p < 0.01$), whereas subjects showed no significant changes in the stress level during the relaxing task (paired *t*-test, $p = 0.45$) (Fig. 3.1). Consistently, the respiration rate was also increased after the math task (paired *t*-test, $p < 0.05$) but not after the relaxing task (paired *t*-test, $p = 0.29$) (Fig. 3.2).

Experiment 2: The ATD manipulation successfully reduced plasma tryptophan levels on average by 75%. A significant reduction in the plasma tryptophan level at 11:40am before the fMRI session relative to that at the baseline (measured at 8:30am, paired *t*-test, $p < 0.001$) was observed, and the lower level was maintained until the end of the scan with an average decrease of 81% (STable 1). There was no significant difference (paired *t*-test, $p = 0.57$) in baseline tryptophan level between the ATD and Control visit.

3.2. fMRI results

Reports of the conjunction analysis and paired *t*-test for each experiment are presented in Stable2 and STable 3 respectively. Only common results (from the conjunction analysis) or results within core networks in which we are interested are shown and discussed. In order

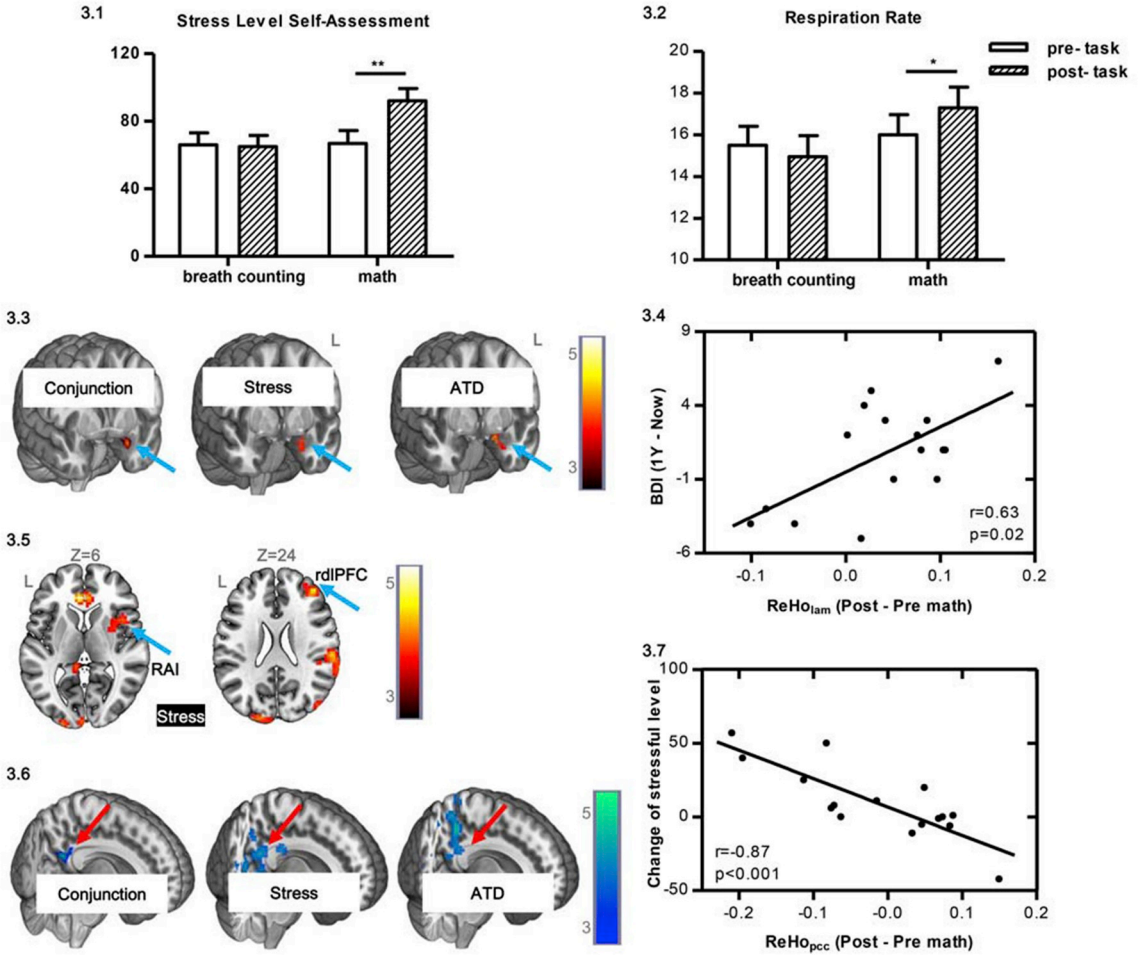

Fig. 3. 1 Self-assessed stressful level increased after the math but not after the relaxing task (**: $p < 0.01$); 2 Respiration rate increased after the math but not after the relaxing task (*: $p < 0.05$); 3 Conjunction analysis (left) revealed increased regional homogeneity (ReHo) of the left amygdala after the math task (middle) and the acute tryptophan depletion (ATD, right) condition; 4 In the math task, the increment of ReHo (post- versus pre-math task) in the left amygdala was correlated with the change of BDI (Beck Depression Inventory) after one year; 5 ReHo of the right anterior insula (RAI) and right dorsolateral prefrontal cortex (rdlPFC) increased after math task; 6 Conjunction analysis (left) revealed decreased ReHo of the posterior cingulate cortex (PCC) in post- versus pre-math task (middle) and in the ATD state relative to the control state (right); 7 The ReHo change of the PCC in the math task was negatively correlated with the change of stressful level.

to compare the fMRI results of the two independent experiments directly, we describe the results from the two studies together in sections below. The fMRI results of the relaxing task were not shown since it did not induce any significant change post- vs. pre-the task.

3.2.1. ReHo results

No significant baseline difference was observed between AMS and ATD conditions, nor between math and relaxing tasks which suggested the stability of between studies and within subjects.

ReHo Increase: Conjunction analysis revealed increased ReHo in the left amygdala after both AMS (post- vs. pre-stress) and ATD (ATD vs. control) condition (Fig. 3.3). In the AMS study, the resting-state ReHo change (post- vs. pre-stress) was positively correlated with the subject's depression severity ($r = 0.61$, $p = 0.02$) (SFig. 1), and it was also associated with depression severity increase one-year later ($r = 0.63$, $p = 0.02$) (Fig. 3.4). After the AMS task, there was also a significantly increased ReHo of the RAI and rdlPFC (Fig. 3.5).

ReHo Decrease: Conjunction analysis revealed decreased ReHo in the PCC under both AMS and ATD conditions (Fig. 3.6). In addition, the ReHo decrement of the PCC after AMS was correlated with the increase of self-rated stressful level ($r = -0.87$, $p < 0.001$) (Fig. 3.7).

3.2.2. ROI-based functional connectivity

Changes within networks: We have found significantly decreased within-DMN connectivity (pre-math mean $FC_{PCC\text{-}DMN} = 0.89$, $SE = 0.002$; post-math mean $FC_{PCC\text{-}DMN} = 0.82$, $SE = 0.004$) and increased within-ECN connectivity (pre-math mean $FC_{rdlPFC\text{-}ECN} = 0.62$, $SE = 0.006$; post-math mean $FC_{rdlPFC\text{-}ECN} = 0.70$, $SE = 0.005$) post- vs. pre-stressful task but not under ATD vs. control condition. The BDI score was significantly correlated with decreased DMN FC post-stress task ($r = -0.62$, $p = 0.01$) (SFig. 2).

Changes between networks: There was a significantly increased FC between RAI and rdlPFC post- relative to pre-stress task (pre-math mean $FC_{RAI\text{-}rdlPFC} = 0.39$, $SE = 0.01$; post-math mean $FC_{RAI\text{-}rdlPFC} = 0.52$, $SE = 0.02$), and the change was correlated with the state anxiety level pre-stress task ($r = 0.67$, $p = 0.005$) (Fig. 4.1 middle). There was no significant change in $FC_{RAI\text{-}rdlPFC}$ between ATD and control conditions, however, the increased $FC_{RAI\text{-}rdlPFC}$ was correlated with the increased anxiety level in ATD relative to control conditions ($r = 0.59$, $p = 0.007$) (Fig. 4.1 right). Although $FC_{PCC\text{-}LAMY}$ (LAMY = left amygdala) did not show significant change after stress and ATD, it was negatively correlated with the change of negative affect ($r = -0.56$, $p = 0.03$ in the AMS; $r = -0.61$, $p = 0.004$ in the ATD study) (Fig. 4.2 middle: AMS; Fig. 4.2 right: ATD).

Triple-Network interactions: Regarding IRAB of interactions among DMN, SN, and ECN, those who had higher baseline anxiety level showed increased IRAB post- relative to pre-stress task ($r = 0.70$, $p = 0.003$) (Fig. 5 left). We did not find similar results in the relaxing

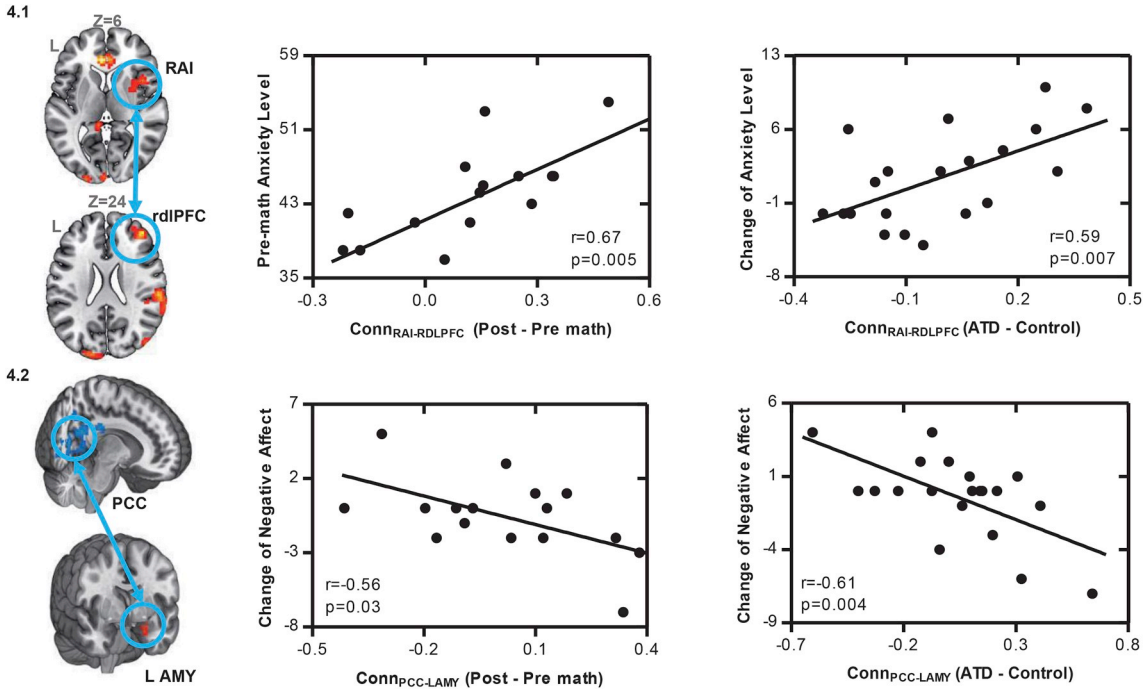

Fig. 4. 1 $FC_{RAI\text{-}rdlPFC}$ change was positively correlated with the baseline anxiety level under post- versus pre-math condition (middle) and positively correlated with the change of anxiety level under ATD (acute tryptophan depletion) versus control condition (right); 2 $FC_{PCC\text{-}LAMY}$ change was negatively correlated with the change of negative affect under: post- versus pre-math condition (middle), ATD versus control condition (right). RAI, right anterior insula; rdlPFC, right dorsolateral prefrontal cortex; PCC, posterior cingulate cortex; LAMY, left amygdala.

task. Similarly, In the ATD experiment, the trait anxiety level was also correlated with greater IRAB (r = 0.59, p = 0.006) (Fig. 5 right).

Replication of FC findings using gICA identified ROIs: In general, the majority of the FC findings stand using gICA-based ROIs, but the statistical power is lower than the results of ReHo-based ROIs (SFig. 5, SFig. 6). There is only one finding that has not been replicated, the decreased within DMN FC post-stress task was not correlated with BDI scores using the gICA-based ROIs. Detailed results can be found in the Supplementary Materials.

4. Discussion

Previous studies have shown that both AMS and ATD increase vulnerability to depression/anxiety. Here, we compared changes in brain resting state activity (using ReHo) and connectivity after AMS and during ATD using data collected from two independent samples. Consistent to our hypotheses, we found common changes under AMS and ATD in ReHo of the PCC, amygdala, and correlation of PCC-amygdala as well as rdlPFC-RAI FC with behavioral changes. By further studying the relationships among the three core neural networks, DMN, ECN and SN, using the index of resource allocation bias, we found that the index was correlated with anxiety level under both AMS and ATD conditions. These results confirmed our hypothesis that AMS and ATD induce changes in common neural pathways, and further suggest that these neural pathways may be the basis of neural pathology for vulnerability to anxiety/depression. Overall, four key changes were found which will be discussed in turn.

(1) **PCC, a key node of the DMN, showed decreased resting-state ReHo under both AMS and ATD.** ReHo reduction in the PCC under

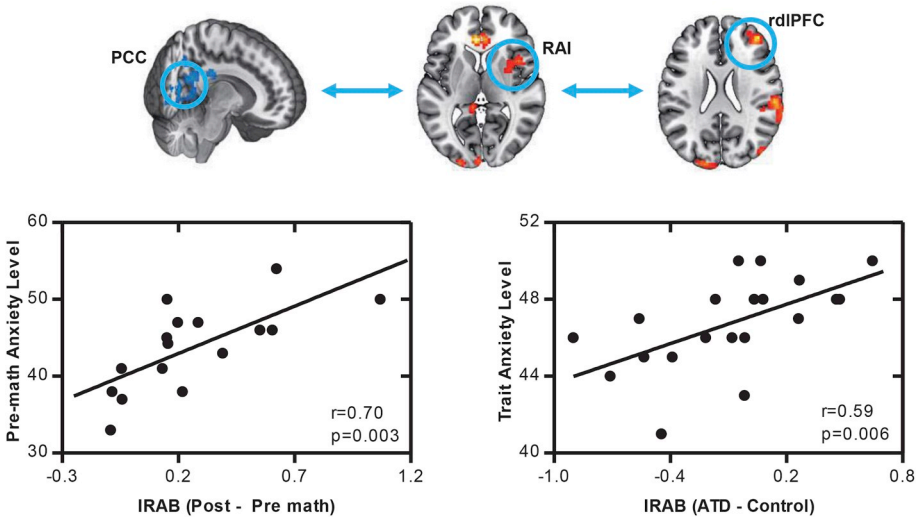

Fig. 5. Left: Post- versus pre-math, the index of resource allocation bias (IRAB) was positively correlated with baseline anxiety level; Right: Acute tryptophan depletion (ATD) versus control, the IRAB was positively correlated with the trait anxiety level. PCC, posterior cingulate cortex; RAI, right anterior insula; rdlPFC, right dorsolateral prefrontal cortex.

AMS was correlated with increased stressful level, while within-DMN connectivity reduction under AMS was associated with BDI scores. These findings are consistent with previous studies on AMS (Soares et al., 2013) and on ATD (Helmbold et al., 2016; Kunisato et al., 2011; Soares et al., 2013). The PCC, together with other nodes of DMN is involved in self-consciousness and self-related mental processing (Cavanna and Trimble, 2006). Reduced PCC ReHo could reflect reduced self-related mental processing after AMS and ATD. It is understandable that within-DMN connectivity was suppressed immediately following the cognitively demanding stressful task relative to before the task. During ATD, there was no cognitive task before the resting-state run, and there was no reduction/inhibition of the whole DMN ReHo. Of note that hyperactivation of PCC and hyperconnectivity within DMN in individuals with depression and anxiety are frequently reported (Greicius et al., 2007), which is opposite to our healthy subjects under acute stress and ATD. This is not surprising given that our study is focused on AMS and ATD, whereas depression/anxiety should be an outcome of chronic stress and sustained low serotonin level, and adaptive hyperactivity/hyperconnectivity of DMN could have developed to increased attention to the self or rumination. Nevertheless, both depression/anxiety patients and healthy subjects under AMS or ATD exhibited alterations in PCC resting activity and within DMN connectivity, which suggests that the DMN, in particular, the PCC, is a vulnerable region to AMS and ATD.

(2) **The RAI and rdlPFC showed increased regional homogeneity and connectivity after acute stress.** We suggest that when facing stress, emotional salience increases along with increased RAI activity, which subsequently alerts prefrontal cortex for emotion regulation. Therefore, an increase in dlPFC activity is necessary to enhance cognitive processing to adapt to the stressful state as cortisol stress hormones and glucocorticoid increase (Yuen et al., 2009). Supportively, we indeed found increased RAI and dlPFC ReHo post- relative to pre-stress task. Again, our findings in AMS are opposite to previous studies that lower dlPFC activity has been reported in subjects who may have experienced chronic stress, i.e. patients with depression (Siegel, 2007) and subjects with high risk of depression (An et al., 2013). Similar findings were not found in the ATD study. The PFC is richly supplied with serotonergic neurons, and ATD might not be powerful enough to dampen PFC function. Alternatively, the changes in the dlPFC might only be revealed under a task which demands recruitment of executive function as opposed to during resting state (Robinson and Sahakian, 2009). However, the majority of prior ATD studies investigating effects on executive functions have reported non-specific or negative findings (Mendelsohn et al., 2009). Notably, there was an increase in the correlation between RAI-dlPFC connectivity and anxiety level under stress and ATD, suggesting in those with higher anxiety level, greater RAI and dlPFC FC might be found post-ATD. Greater coupling between RAI and dlPFC post-stress and post-ATD in those with higher anxiety level could be related to enhanced emotional salience for executing adaptation.

(3) **The interaction of triple networks was correlated with the anxiety level.** Our study showed that the IRAB was correlated with baseline anxiety level in the stressful experiment, and associated with trait anxiety level in the ATD condition. The correlation between the anxiety level and ATD has also been reported previously (Klemenhagen et al., 2006; Robinson et al., 2012). Extending previous findings, we found that both AMS and ATD induced greater synchronization between the RAI and dlPFC (ECN for external mode) in those with higher anxiety levels as well as suppressed the homogeneity of PCC (DMN for internal mode), which indicates a possible attention shift from internal network to the external network when facing adverse environments.

(4) **The regional homogeneity of amygdala and connectivity between PCC and amygdala may be another two biomarkers for depression vulnerability.** Both experiments induced increased ReHo of the amygdala. As a key region related to emotional processing, in particular negative emotion, increased amygdala activation has been frequently reported in trait anxiety (Fakra et al., 2009) and depression (Sergerie et al., 2008; Sheline et al., 2001). Numerous studies have evidenced increased amygdala activation during aversive emotional processing, therefore, it is not surprising that we have found increased amygdala ReHo under both AMS and ATD. Unlike reports in the literature which often emphasize the importance of the amygdala-MPFC connectivity in emotion regulation/anxiety/depression, we found lower amygdala-PCC connectivity associated with stronger negative affect under AMS and ATD conditions. Decreased amygdala-PCC FC in those with higher negative affect could reflect a tendency of disruption of self-preventative thought and empathy/mind process (Chase et al., 2014). Others also implied that amygdala-PCC connectivity could be related to effortful emotion regulation (Maron-Katz et al., 2016). Nevertheless, the association of decreased amygdala-PCC FC with negative affect suggests that amygdala-PCC FC could also be a marker of depression/anxiety vulnerability.

Collectively, these results indicate that the neural pathways underpinning the common changes that occur during AMS and ATD might cover a wide range of neural networks from emotional salience network (the amygdala, insula) to executive network (i.e, the dlPFC). In order to adapt to the adverse environment, the attention resources may need to be shifted from internal mode (subserved by DMN) to external mode (subserved by ECN) for effortful self-regulation and adjustment. Our findings of the positive correlation between the IRAB and anxiety level support this concept in healthy individuals. Higher baseline/trait anxiety level is linked to greater attention resource reallocation from internal mode to external mode. Given that the IRAB represents a summation of interactions among the three core networks, we believe it is a promising measure in predicting depression/anxiety vulnerability.

Although we used clusters that showed significant changes in ReHo in the AMS and ATD studies as ROIs for the ROI-to-ROI FC analysis which could possibly be circular in the analysis, none of the FC changes were correlated with the ReHo changes of either ROI. Therefore, there is little risk of circularity. The results from our additional gICA-based ROI analysis were consistent with the findings from ReHo-based ROIs. Therefore, we believe our reported results were not biased by our ROI selection method.

While this study is the first to compare shared neural pathways in response to AMS and ATD, small sample size of each experiment, relatively short resting-state scans and lenient threshold in the study limit the generalization of our conclusions. Larger samples with longer scan length in future studies are warranted. We currently examined the shared neural substrates related to stress and tryptophan depletion through two independent studies. Future studies with a within-subject design and combining ATD and AMS in the same experiment can better address this question. Although we regressed out the impact of age and gender in our data analyses, future studies with larger samples are needed to confirm our results and to determine whether age or gender influence the findings. Another caveat of our study is that we only used subjective self-rating as a measure of stress level and cortisol measures were not obtained from all participants for analyses. It would also be important to collect measures of trait anxiety in future work. Our AMS study was mainly focused on post- vs. pre-stress contrast, and the ATD study was focused on the post-ATD vs. post-control contrast, which seems not so compatible. We then exploratorily used the (post – pre) stress vs. (post – pre) relaxation contrast other than the post-stress vs. post-relaxation contrast given the intra-class correlation coefficients (ICC) values from the pre-relaxation and pre-stress baseline scans were not high (SFig. 7), and the findings were similar to that from post- vs. pre-stress contrast (SFig. 8). We did not have a baseline resting fMRI scan before subjects taking the beverages in the ATD study because it

was not practical to let subjects wait for 3–5 h (for the peak of low tryptophan) in the scanner to get a second scan, we thus cannot rule out the effects of the baseline difference from the ATD vs. control contrast.

In summary, the present study found that AMS and ATD share similar alterations in resting state activity (reduced ReHo in the PCC and increased ReHo in the amygdala) and network interactions (increased IRAB for those have higher anxiety level) during resting state. Although some changes in these regions and in network interactions were opposite to previous findings in depression/anxiety, our results indicate that these regions and networks are key to depression/anxiety vulnerability. Future studies in confirming the usefulness of these biomarkers for depression/anxiety vulnerability would help advance our understanding of the etiology of depression/anxiety and may help prevent the depression/anxiety at an early stage of these disorders.

Conflicts of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.03.025.

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