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Alternations in functional connectivity of amygdalar subregions under acute social stress

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ABSTRACT

The amygdala has long been considered a vital region involved in acute and chronic stress responses. Extensive evidences from animal and human studies suggest that the functional connectivity of amygdalar subnuclei (basolateral amygdala (BLA), centromedial amygdala (CMA) and superficial amygdala (SFA)) undergo specific alterations in stress-related psychopathology. However, whether and how intrinsic functional connectivity within the amygdalar subcomponents is differently altered in the aftermath of an acute stressor remains unknown. In the present study, using a within-subject design, we examined the impact of acute psychological social stress on the functional connectivity of amygdalar subregions at rest. Results showed that stress mainly affected the connectivity pattern of CMA. In particular, in the stress condition compared with the control, the connectivity of CMA to left posterior cingulate cortex and right thalamus was decreased under stress, while the connectivity of CMA to left caudate connectivity was increased at rest post-stressor. The findings suggest that healthy individuals may adapt to threatening surroundings by reducing threatening information input, and shifting to well-learned procedural behaviors.

1. Introduction

Acute stress has been shown to have both immediate and chronic effects on brain functions (Hermans et al., 2014; Maron-Katz et al., 2016; Pruessner et al., 2008; Quaedflieg et al., 2015). Amygdala plays a critical role in manifesting autonomic responses to acute stress, being involved in both swift activation of autonomic nervous system (ANS) and slower activation of hypothalamic-pituitary-adrenal (HPA) axis (Ulrich-Lai and Herman, 2009; Veer et al., 2011). Studies in humans and rats revealed changes in the gray matter volume and spine density of amygdala when under chronic stress (Drevets et al., 2008; Mitra et al., 2005). Previous studies have detected exaggerated activation of amygdala under acute stress and in stress-related psychopathologies such as post-traumatic stress disorder (PTSD), major depressive disorder (MDD), and generalized anxiety disorder (GAD) (Dougherty et al., 2004; Shin and Liberzon, 2010; Stein et al., 2007).

Emotion dysregulation has been linked to stress-related psychiatric disorders. Patients with PTSD have been found to show hypervigilance to potential threat, adopting a less adaptive emotion regulation strategy, such as emotion suppression and rumination (Ehring and Ehlers, 2014; Messman-Moore and Bhuptani, 2017; Yehuda et al., 2015). Amygdala is also a core brain region involved in experiencing emotions. Altered amygdala activation under stress is documented to play an important part in stress-related emotion dysregulation (LeDoux, 2007; Nicholson et al., 2017). For example, a previous study found that the coupling between amygdala and medial PFC was negatively correlated with the severity of hyperarousal to unpleasant words in PTSD patients (Sadeh et al., 2014).

The amygdala is a complex structure composed of multiple structurally and functionally distinct subregions, including the basolateral amygdala (BLA), centromedial amygdala (CMA) and superficial amygdala (SFA) (Amunts et al., 2005; LeDoux, 2003). The BLA, as an input area, plays an important role in integrating multisensory information and associating these information with emotional values via interactions with multiple cortical and subcortical brain structures, including sensorimotor area, thalamus, prefrontal cortex, hippocampus and insula (Janak and Tye, 2015b; Phelps and LeDoux, 2005). The CMA is identified as an output area and is involved in the orchestration of autonomic and somatic reactions through projections to the hypothalamus, dorsal striatum, paraventricular nucleus and periaqueductal gray (LeDoux, 2007; Moreno and González, 2007; Amy Krain Roy et al., 2009). The SFA lies adjacent to the olfactory cortex, and is found to be

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more sensitive to socially relevant information than BLA and CMA (Bzdok et al., 2013; Goossens et al., 2009).

Extensive evidence suggests that the functional connectivity of these amygdalar subnuclei show specific alterations in stress-related psychopathology (Brown et al., 2014; Amy K Roy et al., 2013). For example, Brown et al. demonstrated that the PTSD group and trauma-exposed control group had different resting-state connectivity pattern of BLA complex, while the CMA showed no difference in connectivity between the groups (Brown et al., 2014). In a GAD study, the functional connectivity between the CMA and widespread brain areas, including ventromedial and ventrolateral prefrontal cortex (PFC), caudate nucleus, insula and superior temporal gyrus, changed in the patient group in comparison to the control group. In the same study the SFA and BLA both showed altered connectivity with cerebellum and brainstem (Amy K Roy et al., 2013). However, whether and how the intrinsic functional connectivity of the amygdalar complex (BLA, CMA, and SFA) are differently altered in healthy individuals following an acute stressor remains unknown.

In the current study, using a within-subject design, we examined whether and how acute psychological social stress orchestrated resting-state connectivity of amygdala subregions. Our within-subject design could better control for any influence of individual variations on the dependent variable compared to the between-subject design adopted by previous studies (Kruse et al., 2017; Maier et al., 2015). We focused on the change in connectivity between amygdalar subregions and (i) subcortical structures (i.e. putamen, caudate, thalamus) which have been documented to be structurally and functionally connected with the amygdala and involved in emotion perception and processing, as well as (ii) the prefrontal cortex (including left and right inferior frontal gyrus, middle frontal gyrus and superior frontal cortex), which contribute to cognitive control and emotion modulation (McEwen and Morrison, 2013; Amy K Roy et al., 2013; Amy Krain Roy et al., 2009; Vogel et al., 2015; Y. Yin et al., 2011). Moreover, previous studies found that the connectivity between the amygdala and the posterior cingulate cortex (PCC) was associated with psychiatric disorders such as PTSD and social anxiety disorder (Bluhm et al., 2009; Liao et al., 2010). Thus, we are also focusing on how the relation between the amygdala and the PCC changed following an acute stressor.

2. Methods

2.1. Participants

Thirty volunteers (age: 20.6 ± 2.0 years (18–25 years); 15 females) participated in this study. All participants were healthy and without a history of major medical, psychiatric, or neurological diseases. None of them reported any history of smoking, use of illicit substances or current use of any psychoactive medications. No participant had previously enrolled in stress-related research. Participants were instructed to refrain from heavy exercise and caffeine at least for 12 h before the experiments. All participants provided written informed consent according to protocols approved by the South China Normal University Institutional Review Board.

2.2. Experimental design and procedure

All experiments were conducted between 1300 h and 1800 h to control for diurnal variations of cortisol secretion. Participants were exposed to acute stress and control conditions in two separate sessions spaced at least 30 days apart, with the order of exposures counter-balanced across subjects. As showed in Fig. 1A, after an acclimation period of 20 min following arrival (T1), baseline saliva samples and affect ratings were collected. Then participants were informed of the stress or control task (see *stress induction*) and were given 5 min of preparation time. After preparation (T2), affect ratings were recorded again. Then participants went through the formal stress or control task, with saliva samples and affect ratings collected upon task completion (T3). Next, 8 min of resting-state fMRI data were collected. After completion of resting state fMRI scan, participants were engaged in 3 sessions of stop signal task (SST) (Hu et al., 2016), the results of which are reported elsewhere. After each session of SST (T4, T5, and T6), saliva samples and affect ratings were collected.

2.3. Stress induction

In the stress condition, participants completed the Trier Social Stress Test (TSST), a well-validated stressor consisting of an impromptu speech and a mental arithmetic task (Kirschbaum et al., 1993). TSST included a preparation period (5 min), and the TSST (a public speaking task - a mock job interview: 5 min; and a mental arithmetic task - serial subtraction: 5 min). Participants were instructed to prepare an application for a job of their choice and introduce themselves to the search committee with a free speech of 5 min duration. They were to convince the committee that they were the most suitable candidate for the position. To increase task engagement, participants were asked to write down their dream job before the preparation period. They delivered the 5-min speech (without notes) in front of a video camera and the committee (one woman and one man) who were trained to remain emotionally neutral. Without prior knowledge, in front of the same committee, they were then asked to subtract the number 13 serially from 1022 in English as fast and accurately as possible for 5 min. On any error the committee asked the participant to start again at 1022. In the control condition, to ensure a comparable cognitive load, participants went through the same tasks without the committee and video camera, in other words, there was no social evaluative stress in control condition.

2.4. Physiological and psychological measures

Salivary cortisol was assessed at multiple time points throughout the experiment (Fig. 1A). Saliva samples were collected with the Salivettes (Sarstedt, Germany) and were stored at −15 °C until assayed. Cortisol concentrations in saliva (in ng/mL) were measured by performing ELISA (catalog No. SLV 4635; DRG, Germany). Participants rated their positive emotions (calm, relaxed, peaceful, confident, and energetic) and negative emotions (nervous, anxious, scared, tired, and upset) on a four-point scale from 1 (“not at all”) to 4 (“extremely”) at multiple time points throughout the procedure (Fig. 1A).

2.5. Imaging protocol

Images were obtained with a 3-Tesla MRI scanner (Siemens) at the Brain Imaging Center at South China Normal University. The scanner was equipped with a 12-channel head coil. Structural images were collected using a T1-weighted gradient-echo scan (TR = 1900 ms, TE = 2.52 ms, flip angle = 9°, field of view = 256 × 256 mm, matrix = 256 × 256, 1 mm^3 isotropic voxel, and 176 slices). Functional blood oxygenation level dependent (BOLD) signals were then acquired with a single-shot gradient echo echo-planar imaging (EPI) sequence. Thirty-two axial slices parallel to the AC-PC line covering the whole brain were acquired with TR = 2000 ms, TE = 25 ms, flip angle = 90°, field of view = 220 × 220 mm, matrix = 64 × 64, 32 slices with slice thickness = 4 mm and no gap. Slice scanning order was ascending interleaved. Two hundred and forty images were acquired for the resting state scan.

2.6. Imaging data processing

The fMRI data were preprocessed and analyzed using Statistical Parametric Mapping version 8 (SPM8, Wellcome Department of Imaging Neuroscience, University College London, U.K.), and Data Processing & Analysis for (Resting-State) Brain Imaging (DPABI; http://

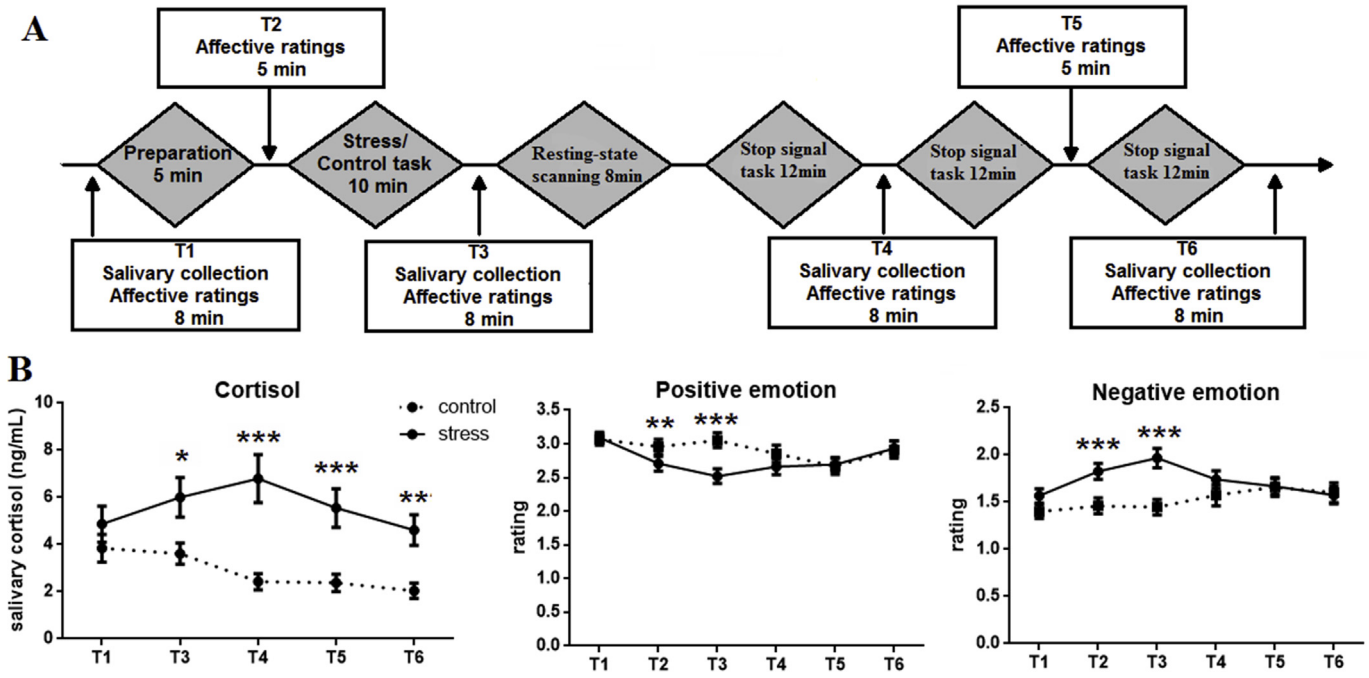

Fig. 1. Experimental procedure and manipulation check. (A) The timeline of the experiment. After an acclimation period of 20 min following arrival, participants were required to go through the Trier Social Stress Test (TSST) with (the stress condition) or without (the control condition) social evaluative processes. After the formal tasks, resting state data were collected. Saliva samples were collected at T1, T3, T4, T5, and T6. Affective ratings were collected at T1, T2, T3, T4, T5, and T6. (B) Cortisol and positive/negative emotional responses under control and stress condition. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

rfmri.org/DPABI) (Yan et al., 2016). After discarding the first 10 vol, the remaining 230 fMRI volumes were first slice-time corrected and later motion corrected using a least squares approach and a 24-parameter autoregressive model (Friston 24-parameter model) (Friston et al., 1996). Next, motion-corrected functional data were co-registered to the subject's own structural T1-weighted images, and segmented into grey matter, white matter, and cerebrospinal fluid. Then, the DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lieal-gebra) technique was performed to create an average structural brain template from all subject's T1 images (Ashburner, 2007). The segmented BOLD volumes were normalized into a standardized MNI space using the DARTEL template, re-sampled to 3 mm × 3 mm × 4 mm isotropic voxel, spatially smoothed with a 6 mm FWHM Gaussian filter, and temporally band-pass filtered to 0.01–0.1 Hz to reduce the effect of very low frequency drift and high frequency physiological noise. Nuisance signal corrections were performed on the 24 head-motion parameters, cerebrospinal fluid (CSF), white matter, and global signals.

To address head motion concerns in resting-state fMRI analyses, we calculated the average voxel-specific framewise displacement (FD) at group-level (Jonathan D Power et al., 2012; J. D. Power et al., 2014). FD measure indexes the movement of the head from one volume to the next and is calculated as the sum of the absolute values of the differentiated realignment estimates (by backward differences) at every time point (Jonathan D Power et al., 2012). The mean FD in stress and control groups were 0.11 ± 0.03 and 0.12 ± 0.03, respectively. One-sample *t*-test showed that they were significantly less than 0.2 mm (both $p < 0.001$). Furthermore, paired *t*-test showed no significant differences in FD between the two conditions ($p = 0.53$). Then, for functional connectivity analysis, we removed frames with FD > 0.5 mm ("scrubbing"). One time point before "bad" time points and two time points after "bad" time points were deleted. There was no significant difference in the percentage of time points removed between the stress and the control conditions (0.017 ± 0.036% vs. 0.010 ± 0.023%, $t(29) = 1.079$, $p = 0.290$, Cohen's $d = 0.316$).

We used the maximum probabilistic map of amygdala subregions derived by Amunts et al. (2005), using SPM Anatomy Toolbox v2.2. Maps exist for the amygdalar basolateralsubregion (BLA), centromedial subregion (CMA), and the superficial subregions (SFA) for each hemisphere (see voxel parcellation map in Amunts et al., 2005). Only voxels with at least a 50% probability of belonging to one of these subregions were included in an ROI, and each voxel was assigned to only one subregion. Bilateral amygdala ROIs were created by combining the bilateral whole amygdala, BLA, CMA, and SFA maps, respectively. The BOLD time courses were averaged spatially over each of the 4 seeds (bilateral whole amygdala and BLA, CMA, and SFA). We computed the correlation coefficient between the averaged time course of each seed region and the time courses of all other brain voxels for individual subjects in the stress and control condition, respectively. Then, we converted these image maps to z score maps by Fisher's z transform (Jenkins and Watts, 1968): $z = 0.5log_e\left[\frac{1+r}{1-r}\right]$.

To test for group differences in the bilateral whole amygdala ROI, a second-level analysis was performed in which bilateral amygdala seeded z-score maps were submitted to a paired *t*-test (stress vs. control). Then, a group-level flexible factorial analysis was applied with *subject, group* (stress/control), and *subregion* (bilateral BLA/CMA/SFA) as factors. Within this ANOVA we calculated both main effects (*group* and *subregion*) as well as the *group X subregion* interaction. To further visualize significant effects in interaction, the average connectivity data (βweights) were extracted and submitted to repeated-measures ANOVA. For all reported analyses, a family-wise error (FWE) corrected threshold of $p < 0.05$ using small volume correction (svc) was set. The ROIs for svc included subcortical structures (i.e. putamen, caudate, thalamus) which have been documented to be structurally and functionally connected with the amygdala and are involved in emotion perception and processing, as well as the prefrontal cortex (including left and right inferior frontal gyrus, middle frontal gyrus and superior frontal cortex), which contribute to cognitive control and emotion modulation (McEwen and Morrison, 2013; Amy K Roy et al., 2013; Amy Krain Roy et al., 2009; Vogel et al., 2015; Y. Yin et al., 2011). Moreover, previous studies found that the connectivity between the amygdala and the PCC was associated with psychiatric disorders such as PTSD and social anxiety disorder (Bluhm et al., 2009; Liao et al., 2010). Thus, we

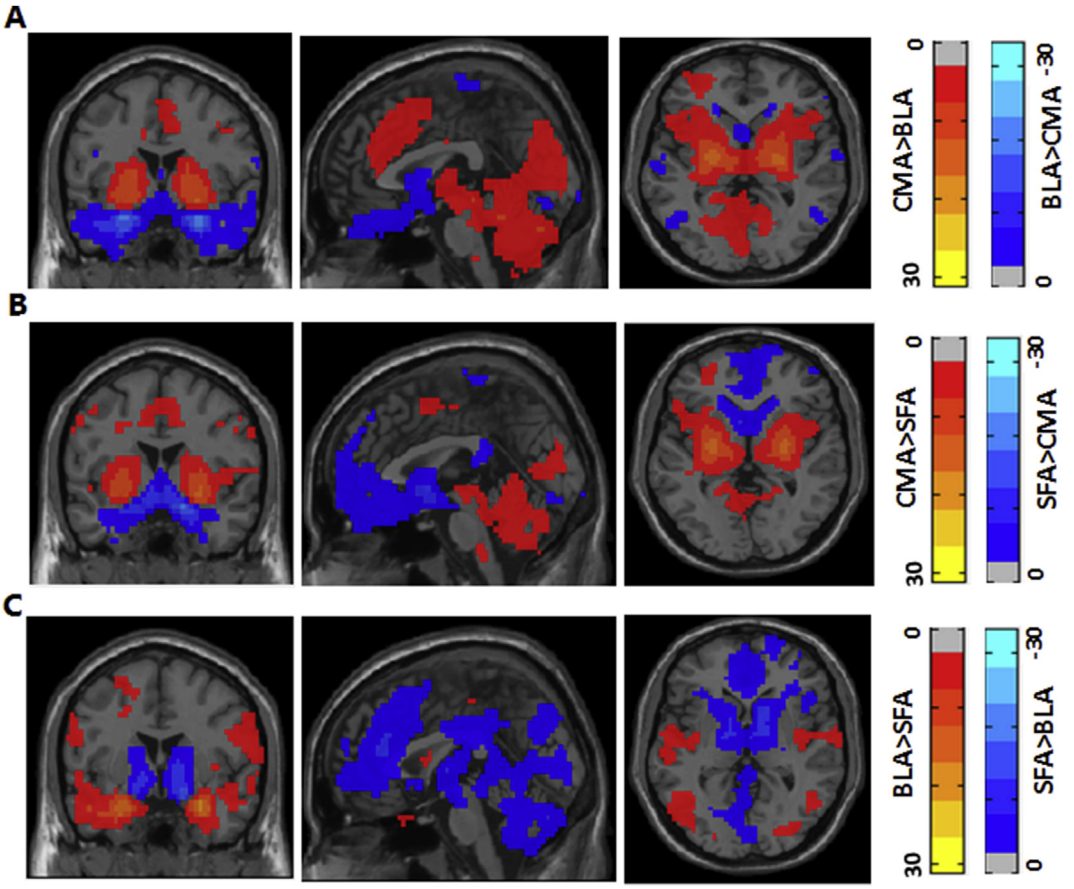

Fig. 2. Brain areas showing differential connectivity to the centromedial (CMA), the basolateral amygdala (BLA) or superficial amygdala (SFA) during resting-state functional magnetic resonance imaging. (A) difference between CMA and BLA; (B) difference between CMA and SFA; (C) difference between BLA and SFA.

also focused on how the relation between the amygdala and the PCC changed following exposure to an acute stressor. Other activated brain areas were reported at a liberal threshold, $p < 0.001$ uncorrected, for 10 continuous voxels. For display purposes, all images are depicted at $p < 0.005$.

3. Results

3.1. Physiological responses to acute stress

For salivary cortisol (Fig. 1B), a repeated-measures ANOVA was applied, with *Treatment* (Control vs. Stress) and Time point (T1, T3, T4, T5, and T6) as two within-subject factors. Three participants cortisol could not be assayed at few time points due to insufficient saliva. The remaining 27 participants were included in the ANOVA of salivary cortisol. For all reported analysis, Greenhouse-Geisser correction was applied when sphericity was violated. Results showed that the main effect of treatment ($F(1, 26) = 16.258$, $p < 0.001$, $\eta^2 = 0.385$), and time point ($F(4, 104) = 4.159$, $p = 0.010$, $\eta^2 = 0.138$), and the interaction between treatment and time point were significant ($F(4, 104) = 4.453$, $p = 0.006$, $\eta^2 = 0.146$). Post-hoc *t*-tests showed that, at T1, participants did not differ between stress and control sessions in salivary cortisol level ($t(29) = -0.967$, $p = 0.342$). However, at T3 ($t(26) = 2.677$, $p = 0.013$), T4 ($t(26) = 4.503$, $p < 0.001$), and T5 ($t(26) = 4.112$, $p < 0.001$) and T6 ($t(26) = 3.947$, $p = 0.001$), salivary cortisol levels were higher in the stress condition than the control condition.

For positive and negative emotion ratings, we also carried out Treatment (Control vs. Stress) by Time point (T1, T2, T3, T4, T5, and T6) repeated-measures ANOVAs (Fig. 1B). For positive emotions ratings, the main effect of treatment ($F(1, 29) = 4.355$, $p = 0.046$, $\eta^2 = 0.131$) and time point ($F(5, 145) = 6.556$, $p < 0.001$, $\eta^2 = 0.184$) were significant; the interaction between treatment and time point was also significant ($F(5, 145) = 7.505$, $p < 0.001$, $\eta^2 = 0.206$). Post-hoc *t*-tests showed that positive affect was lower in the stress condition than control condition at T2 ($t(29) = -2.801$, $p = 0.009$), and T3 ($t(29) = -4.817$, $p < 0.001$). For negative emotion ratings, results revealed a significant main effect of treatment ($F(1, 29) = 8.553$, $p = 0.007$, $\eta^2 = 0.228$), and a significant main effect of time ($F(5, 145) = 3.354$, $p = 0.015$, $\eta^2 = 0.104$). The interaction between Treatment and Time was significant ($F(5, 145) = 7.495$, $p < 0.001$, $\eta^2 = 0.205$). Post-hoc *t*-tests revealed significantly higher negative emotion ratings in stress condition than in control condition at T2 ($t(29) = 4.413$, $p < 0.001$) and T3 ($t(29) = 4.763$, $p < 0.001$). These findings suggested that the manipulation of acute stress was successful.

3.2. Resting state functional connectivity results

For bilateral whole amygdala, a paired *t*-test revealed no significant difference in the brain regions of interest in the stress condition - control condition. Compared with the stress condition, increased connectivity in the left putamen ([-24 -6 4], voxel = 13, $p = 0.036$, svc) was found in the control condition.

Consistent with the reported results of bilateral whole amygdala, the flexible factorial analysis revealed a significant main effect of Group on the left putamen, showing a decreased connectivity in the stress minus control comparison ([-24 -6 4], voxel = 15, $p = 0.05$, svc). There was also a main effect of subregion within broad brain regions, including the bilateral anterior cingulate, orbital cortex, temporal lobe, prefrontal cortex and the postcentral gyrus. Consistent with previous

studies (Amy Krain Roy et al., 2009; Vogel et al., 2015), direct comparison between the BLA and CMA subdivisions found that CMA showed stronger connectivity than BLA with bilateral dorsal striatum, anterior cingulate, orbital frontal cortex, dorso-medial frontal cortex, occipital lobe and cerebellum. BLA had stronger connectivity than CMA with ventro-medial and ventro-lateral PFC, temporal lobe, precentral and postcentral gyrus. Regions that showed stronger connectivity to the CMA than SFA included bilateral dorsal striatum, insula, sensorimotor cortex, and cerebellum. Regions showing stronger connectivity with SFA than CMA included ventro-medial and ventro-lateral PFC, and hippocampal cortex. Moreover, compared with BLA, SFA had stronger connectivity with dorso-medial frontal cortex, parahippocampal cortex, thalamus, occipital lobe, PCC, precuneus, and cerebellum. BLA had stronger connectivity than SFA with large parts of the temporal lobe, hippocampus, precentral and postcentral gyrus (see Fig. 2).

The interaction between *group* and *subregion* revealed significant or marginally significant clusters in the right thalamus ([15–33 4], voxel = 12, $p = 0.037$, svc), left PCC ([-15 -51 20], voxel = 19, $p = 0.032$, svc), right inferior frontal gyrus (IFG, [45 18 -8], voxel = 32, $p = 0.052$, svc), and left caudate ([-18 0 24], voxel = 9, $p = 0.059$, svc) (see Fig. 3). The average connectivity values (βweights) from these significant clusters were extracted and submitted to repeated-measures ANOVA, with *group* (stress and control) and *subregion* (bilater BLA, CMA and SFA) as two factors. The results showed that the *group* X *subregion* interaction was significant in right thalamus ($F(2, 58) = 6.690$, $p = 0.002$, $\eta^2 = 0.187$), left PCC ($F(2, 58) = 10.935$, $p < 0.001$, $\eta^2 = 0.274$), right IFG ($F(2, 58) = 12.036$, $p < 0.001$, $\eta^2 = 0.241$), and left caudate ($F(2, 58) = 10.183$, $p < 0.001$, $\eta^2 = 0.260$). As shown in Fig. 3, as per the post hoc test results, the CMA connectivity to left caudate showed a significant increase in the stress condition than in the control condition ($t = 2.250$, $p = 0.032$), whereas the connectivity of CMA with left PCC and right thalamus were significantly decreased in the stress condition ($t = -2.644$, $p = 0.013$; $t = -2.969$, $p = 0.006$). There were no such effects in the SFA and BLA. These results suggest that the CMA was the core subregion affected by the acute stressor in the current study.

4. Discussion

In the present study, we explored whether and how acute social stress affects the functional connectivity of amygdalar subregions (BLA, CMA and SFA) in resting-state. Our results showed that stress mainly affected the connectivity pattern of CMA. In particular, the CMA-left PCC and CMA-right thalamus connectivity significantly decreased in the stress condition compared with the control condition, while the connectivity of CMA to left caudate significantly increased in the stress condition.

Compared with the control group, the connectivity between CMA and left caudate was increased after an acute stressor. This result is consistent with previous studies showing that CMA has an intense connectivity with the dorsal striatum (Amy Krain Roy et al., 2009; Vogel et al., 2015). The dorsal striatum (i.e. caudate and putamen) has been documented to be associated with habitual behavior (Corbit et al., 2012; Everitt and Robbins, 2013; L. Schwabe and Wolf, 2012). Further, it has been proposed that the connectivity of the amygdala with brain regions involved in habitual and automatic behavioral responses increase after stress induction (Hermans et al., 2014; Lars Schwabe and Wolf, 2013). For instance, a previous study found that within approximately 9 min after a socially evaluated cold-pressure task (SECPT), social stress increased centromedial amygdala connectivity with the caudate, and this effect was positively correlated with the stress-induced cortisol response (Vogel et al., 2015). A similar shift from flexible and controlled response to habitual and procedural behavior under stress was found in memory related studies (Lars Schwabe and Bolam, 2017; L. Schwabe and Wolf, 2012; Lars Schwabe and Wolf, 2013). In an fMRI study using a probabilistic classification-learning task, stress increased the connectivity between amygdala and dorsal striatum, and induced a shift from hippocampus-based declarative memory to a dorsal striatum dependent procedural memory (Lars Schwabe et al., 2013). Our results are consistent with previous findings and extended them by showing enhanced CMA-caudate functional connectivity after TSST induced stress in resting state.

The connectivity between the CMA and left PCC and right thalamus decreased after an acute stressor. The PCC is a core region of the default mode network and has been shown to be involved in self-relevant

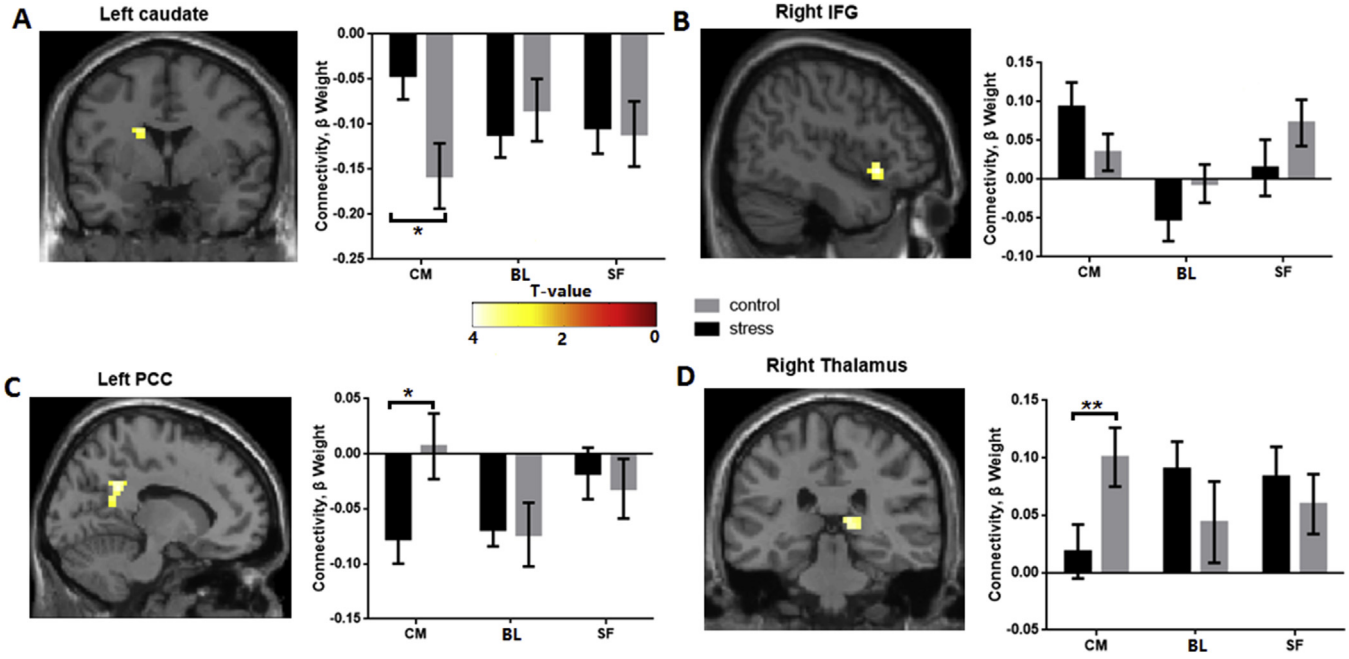

Fig. 3. Brain clusters significant or marginally significant for group X subregion interaction. The average connectivity (βweights) were extracted from (A) the left caudate, (B) the right IFG, (C) the left PCC, and (D) the right thalamus. CM, centromedial; BL, basolateral; SF, superficial; IFG, inferior frontal gyrus; PCC, posterior cingulate cortex. *$p < 0.05$, **$p < 0.01$.

thoughts, mind wandering and emotion evaluation (Mars et al., 2012; Mason et al., 2007; Vogt and Laureys, 2005; Vogt et al., 2006). Although there is plenty of literature suggesting a positive coupling between the amygdala and PCC in stress-related diseases (Gentili et al., 2009; Liao et al., 2010), the evidence showing the opposite patterns are accumulating (Bluhm et al., 2009; Chase et al., 2013). For instance, a previous study using resting state fMRI found that postpartum depressed women had less PCC-amygdala connectivity compared to a control group, which might be associated with maladaptation to the responsibilities of motherhood in postpartum depressed mothers (Chase et al., 2013). In a PTSD study, the connectivity between the PCC and the amygdala, as well as the hippocampus and insula were decreased in patients with PTSD in comparison to a control group, which could be related to abnormal emotional reactions to environmental stimuli (Bluhm et al., 2009). In the present study, it is plausible that the decreased connectivity between CMA and PCC was related to abnormal self-referential processing and emotion regulation after acute stress. It is worth noting that Veer et al. also used TSST to induce acute stress, and contradictory to our result, found increased connectivity between the amygdala and PCC (Veer et al., 2011). However, the study tested resting state fMRI an hour after stress induction, while we focused on the time period about 12 min after the TSST (see Fig. 1). Further, Veer et al. reported the connectivity of the whole amygdala, whereas we focused on amygdalar subregions.

Thalamus is a core brain region involved in fear circuitry and believed to be a gateway for primary sensory output to amygdala to induce defensive responses (Duval et al., 2015; Yan Yin et al., 2011). In accordance with its function of transferring information, previous studies found that the activation of thalamus increased in response to threatening stimulus in stress-relevant disorders, for example, SAD and PTSD (Giménez et al., 2012; Patel et al., 2012). Unlike these studies, our study participants were healthy adults. As such we speculated that the inhibited pathway of amygdala-thalamus had reduced the incoming sensory information of threat after acute psychological social stress, thus attenuating the influence of stressor on healthy individuals in order to maintain homeostatic balance.

We found that acute stress mainly affected the connectivity pattern of CMA. The CMA is the output center of amygdala, playing an important part in automatic behavioral and emotional responses to fear and threat, while the BLA inhibits these unconditional processes (Ciocchi et al., 2010; LeDoux, 2007; Tye et al., 2011). When the BLA was lesioned and thus the inhibition from BLA to intact CMA was impaired, a BLA-damaged patient showed hyper-vigilance to fearful and threatening stimuli (Janak and Tye, 2015a; Terburg et al., 2012; Tye et al., 2011; Wang et al., 2017). Such increased vigilance was supposed to be associated with acute panic and anxiety. In addition to these lesion studies, a previous study found that the central nucleus of amygdala (CeA) played an prominent role in early life stress (ELS) group, showing robust predictive paths from CeA to BLA and SFA (Grant et al., 2015). Our results are consistent with these studies, showing that the CMA is susceptible to acute stressors and its connectivity with the emotion-related brain regions is altered.

There are several concerns about the feasibility of examining amygdalar subfields using conventional functional imaging methods. However, previous studies that employed the same methods found distinct functional connectivity patterns between amygdalar subregions (Eckstein et al., 2017; Amy Krain Roy et al., 2009). Further, we used DARTEL to create an average structural brain template from all the subject's T1 images, and registered BOLD volumes to MNI template using the DARTEL template, thus substantially increasing the accuracy of our results. In addition, as shown in Fig. 2, the functional connectivity of amygdalar subregions with the left PCC and left caudate in both stress and control conditions were mainly negative (except the CMA-left PCC connectivity in control). Global signal regression is considered as a causative factor in the induction of such negative correlations, leading the sum correlation of the seed region to be a negative value (Murphy et al., 2009). However, the global signal contains many physiological noises, for example, vasomotion, blood pressure, and cardiac cycles. Thus it is recommended is that the global signal should be removed in resting-state functional connectivity analysis (Chang and Glover, 2009; Fox et al., 2009). The global signal is composed of both neural and non-neural signals. It is argued that removing global signal regression in the processing of resting state functional connectivity data is not inherently right or wrong and different approaches may likely reveal complementary insights about the brain's functional organisation (Murphy and Fox, 2017). Finally, the use of multiple independent ROIs in our study increases the number of independent comparisons and therefore the FWE rate. As such, our findings and conclusions should be treated as preliminary and with caution, warranting further replication and expansion with a larger sample size.

To conclude, in the present study, we found that acute social stress mainly affected the connectivity pattern of CMA with other brain structures, but not those of BLA and SFA. In particular, compared with the control condition, stronger CMA-left caudate connectivity was found in the stress condition, while the connectivity of CMA to left PCC and right thalamus were significantly decreased after stress exposure. It is tempting to speculate that the changed connectivity of CMA with these brain regions observed in the present study indicates that healthy individuals in acute stress tend to reduce incoming information and shift to well-learned procedural behaviors to adapt to the threatening surroundings.

Declaration of conflicting interests

The authors declared that they had no conflicts of interest with respect to their research, authorship or the publication of this article.

Author contributions

R. Yu developed the study concept. J. Chang analyzed and interpreted the data under the supervision of R. Yu. J. Chang drafted the manuscript, and R. Yu provided critical revisions. All authors approved the final version of the manuscript for submission. We thank J. Hu for collecting the data.

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Appendix A. Supplementary data

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