The stress connection: Neuroimaging studies of emotion circuits in social stress, personality, and stress-related psychopathology

Veer, I.M.

CHAPTER 3

Beyond acute social stress: Increased functional connectivity between amygdala and cortical midline structures

Veer, I. M., Oei, N. Y. L., Spinhoven, P., van Buchem, M.A., Elzinga, B. M., & Rombouts, S. A. R. B. (2011). *Neuroimage*, 57(4), 1534-1541.

ABSTRACT

Whereas we know a fair amount on the role of the amygdala in the acute stress response, virtually nothing is known about its role during the recovery period after the stress has waned. Functional connectivity analysis of the amygdala during this period might be useful in revealing brain circuits promoting adaptive recovery from a stressful event, as well as consolidation of emotionally relevant information in preparing for future challenges. Healthy participants were randomly assigned to either a psychosocial stress task (n = 18; stress group) or a comparable non-stressful control procedure (n = 20; controls). To study the prolonged effects of stress on amygdala functional connectivity, resting-state fMRI scans were acquired an hour after the stress task. Amygdala functional connectivity with other brain regions was assessed using seed-based correlations. The stress group exhibited a strong physiological and behavioral reaction to psychosocial stress exposure. Compared with controls the stress group showed increased amygdala functional connectivity with three cortical midline structures: the posterior cingulate cortex and precuneus (p < .05, corrected), and the medial prefrontal cortex (p < .05, small volume corrected). An hour after psychosocial stress, changes in amygdala functional connectivity were detected with cortical midline structures involved in the processing and regulation of emotions, as well as autobiographical memory. It is hypothesized that these effects could relate to top-down control of the amygdala and consolidation of self-relevant information after a stressful event. These results on functional connectivity in the recovery phase after stress might provide an important new vantage point in studying both sensitivity and resilience to stress.

INTRODUCTION

When we face a stressful situation, our brain initiates a stress response. The amygdala plays a key role in evoking this response, as it signals danger and, more generally, emotional salience of incoming sensory information to the rest of the brain to prepare ourselves for appropriate action (LeDoux, 2000; Phillips, Drevets, Rauch, & Lane, 2003a). Through its neuronal projections to several brainstem nuclei and the hypothalamus, the amygdala excites both the autonomic nervous system (ANS) and hypothalamic–pituitary–adrenal (HPA) axis. The ANS promotes a swift physical and behavioral response through the release of catecholamines, such as adrenaline and noradrenaline. In contrast, slower acting stress agents such as cortisol are secreted through activation of the HPA-axis to warrant homeostasis after the stressful event (Ulrich-Lai & Herman, 2009). A balanced integration of both pathways enables an adaptive modulation of both the physical and the behavioral stress response (Joëls & Baram, 2009).

To date, effects of stress on the amygdala have mostly been described during or directly after stress. For example, during psychosocial stress deactivation of limbic regions, including the amygdala, was found (Pruessner et al., 2008), whereas after psychosocial stress our group demonstrated increased amygdala responsivity towards negative stimuli during an emotional working memory task (Oei et al., 2012). Similar results were obtained by van Marle et al. (2009) after letting participants watch negatively arousing movie clips as a stressor. Using that same stress induction paradigm, these researchers also found increased functional connectivity (FC) between the amygdala and brain regions mediating autonomic activity, such as the dorsal anterior cingulate cortex (ACC) and brainstem. Thus, the effects found immediately following a stressor might possibly relate to activation of the acute autonomic stress response by the amygdala (van Marle, Hermans, Qin, & Fernández, 2010). In contrast, studying the recovery period after a stressful event is equally important, as prolonged activation during this period has been related to the development of psychopathology and somatic disease (Brosschot, Gerin, & Thayer, 2006). Nonetheless, relatively little is known about the role of the amygdala during this period when homeostasis rather

than immediate survival is being promoted, relating to processes such as the inhibition of autonomic responses evoked by the stressor, as well as emotion regulation and memory consolidation.

The amygdala receives modulatory input from cortical brain regions, which dampen its responsivity in the aftermath of negatively arousing events (LeDoux, 2000). Particularly regions in the medial prefrontal cortex (mPFC) have been found to be involved in modulating amygdala activity during emotional conflict and regulation of autonomic and affective responses, most notably the perigenual division of the anterior cingulate cortex (Egner, Etkin, Gale, & Hirsch, 2008; Etkin, Egner, Peraza, Kandel, & Hirsch, 2006; Gianaros et al., 2008; Pezawas et al., 2005; Wager et al., 2009), but also the ventro- and dorsomedial (vm/dm) portions of the PFC (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Urry et al., 2006). Interestingly, cortisol was found to strengthen FC between the amygdala and dmPFC more than four hours following its administration (Henckens et al., 2010). In addition, these same regions showed an increased inverse relation in glucose metabolism after psychosocial stress: Higher metabolism in the dmPFC was associated with lower metabolism in the amygdala (Kern et al., 2008). Moreover, steeper (i.e., more normative) decreases in diurnal cortisol were related to a stronger inverse coupling between the amygdala and the vmPFC during regulation of negative affect (Urry et al., 2006). These findings suggest an important role for an interaction between the mPFC and amygdala in achieving adaptive emotion regulation in the period following stress, potentially mediated by cortisol or stress in general.

Besides initiating the acute stress response, the amygdala is a key structure in promoting memory consolidation of emotionally salient information through its interactions with the hippocampus (McGaugh, 2004; McGaugh, Cahill, & Roozendaal, 1996). The amygdala seems to be essential in mediating the effects of stress hormones on learning and memory consolidation (Roozendaal et al., 2009). Therefore, increased interactions between the amygdala and hippocampus may underlie the enhancing effects of stress and/or cortisol and noradrenalin on emotional memory found in human studies (Buchanan & Lovallo, 2001; Cahill et al., 2003; Kuhlmann & Wolf, 2006; Strange & Dolan, 2004). The improved memory consolidation for emotionally rele-

vant and arousing information after a stressful experience is hypothesized to represent a mechanism that enables us to prepare for and adaptively face similar challenging situations in the future.

Resting-state (RS-)fMRI has become an important tool to study functional interactions in the human brain in the absence of overt behavior (Fox & Raichle, 2007). This makes the technique especially useful for studying diffuse states of the brain, such as stress, and may therefore provide valuable insights on how stress affects the neural circuitry underlying emotion regulation and memory consolidation when the acute phase of the stress has waned. Moreover, RS-fMRI has been found to provide reliable measures of amygdala FC that corroborate results of white matter tracing studies in non-human primates (Amaral & Price, 1984; Ghashghaei & Barbas, 2002): amygdala FC has been observed with several brain regions supporting the processing, regulation and consolidation of emotionally salient events, such as the mPFC, including the anterior cingulate cortex (ACC), dm/vmPFC and orbitofrontal cortex (OFC), as well as the insula, hippocampus and brainstem (Robinson, Laird, Glahn, Lovallo, & Fox, 2010; Roy et al., 2009; Stein et al., 2007a).

In the current study we investigated the long-term influence of psychosocial stress on resting-state FC (RSFC) of the amygdala stretching beyond the acute stress response, during the recovery phase. Healthy male participants were exposed to either social stress or a comparable non-stressful control condition before entering the MRI scanner. Amygdala RSFC was assessed one hour after stress exposure, when the acute stress response had already waned. We expected that stress would lead to increased RSFC between the amygdala and the mPFC, potentially pointing to top-down modulatory control over the amygdala. Secondly, we expected the amygdala to show increased interactions with brain areas involved in (emotional) memory formation and consolidation, such as (peri)hippocampal regions.

METHODS

PARTICIPANTS

Forty-seven male volunteers from the general population were recruited by means of advertisements. All participants were screened before inclusion. Eligibility criteria were: no history of disease or chronic disease requiring medical attention, no dyslexia, no color blindness, no current use of prescribed medication and/or use of remedies containing corticosteroids, no use of psychotropic drugs, no current or past psychiatric problems, as was determined by the Amsterdam Biographical interview (ABV) (de Wilde, 1963), the total score on the Dutch version of the Symptom checklist (SCL-90) (Arrindell & Ettema, 1986), the Dutch version of the Beck Depression Inventory (BDI) (Bouman et al., 1985), and the State-Trait Anxiety Inventory (STAI) (Spielberger, 1983). Furthermore, participants were required to have a Body Mass Index (BMI, in kg/m^2) between 19 and 26, to be between 18 and 30 years old, and to be right-handed. Forty participants were deemed eligible and included in the study. Participants were randomly assigned to either the experimental or control group in a randomized two-group design. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center, and written informed consent was given by all participants.

MATERIALS

Stress manipulation

To induce stress, the Trier Social Stress Test (TSST) was employed (Kirschbaum et al., 1993). The TSST protocol has consistently proven to raise cortisol levels (Kirschbaum & Hellhammer, 1994). This laboratory stressor consists of a ten-minute anticipation period, followed by a five-minute free speech that had to include one's positive and negative characteristics. After the anticipation period, the speech was given in front of a selection committee of three psychologists. Subsequently, participants had to perform a five-minute arithmetic task (counting backwards from 1033 to zero, in steps of 13) in front of the same committee. One of its members responded to

incorrect answers by saying out loud "incorrect, please start over", while keeping up the participant's performance by means of a clearly visible scoreboard. In the control condition, participants used the same anticipation period of ten minutes to think of a movie to their liking, about which they had to answer open questions on paper for five minutes in the same laboratory room, though without any audience. Thereafter, they were instructed to count backwards from 50 to zero at a slow pace, which lasted for another five minutes.

Physiological assessments

Salivary cortisol was assessed at multiple time points through- out the procedure (see procedure) using Salivettes (Sarstedt, Germany). Saliva sampling is a stress-free method to assess unbound cortisol (Kirschbaum & Hellhammer, 1994). Saliva samples were stored at –20 °C until assayed at Prof. Kirschbaum's laboratory (http://biopsychologie.tu-dresden.de). Cortisol concentrations in saliva (in nmol/L) were measured using a commercially available chemiluminescence-immuno-assay kit with high sensitivity (IBL, Hamburg, Germany). Inter- and intra-assay coefficients of variation were below 10 %. Systolic blood pressure (SBP, mm Hg), diastolic blood pressure (DBP, mm Hg), and heart rate (HR, bpm) were furthermore recorded outside the scanner room at multiple time points using an automatic wrist blood pressure monitor (OMRON, R5-I) to assess autonomic nervous system responsivity to the stressor. Furthermore, heart rate was monitored during RS acquisition using a pulse oximeter attached to the middle finger of the left hand. The average heart rate was logged every minute. In addition, the total number of respiratory peaks was counted, as was recorded by means of a respiratory belt around the chest. Repeated measures ANOVAs and post-hoc independent sample *t*-tests were carried out on the physiological data and VAS scale for each time point using SPSS Version 16.0 (SPSS Inc.).

FMRI data acquisition

Imaging data were acquired on a Philips 3T Achieva MRI scanner using an eight-channel SENSE head coil for radiofrequency reception (Philips Healthcare, Best, The Netherlands). Whole-brain RS-fMRI data were acquired using T_2^*-weight-

ed gradient-echo echo-planar imaging (EPI) with the following scan parameters: 160 volumes; 38 axial slices scanned in ascending order; repetition time (TR) = 2200 ms; echo time (TE) = 30 ms; flip angle = 80°; FOV = 220 × 220 mm; 2.75 mm isotropic voxels with a 0.25 mm slice gap. A high-resolution anatomical image (T_1-weighted ultra-fast gradient-echo acquisition; TR = 9.75 ms; TE = 4.59 ms; flip angle = 8°; 140 axial slices; FOV = 224 × 224 mm; in-plane resolution 0.875 × 0.875 mm; slice thickness = 1.2 mm), and a high-resolution T_2^*- weighted gradient echo EPI scan (TR = 2.2 s; TE = 30 ms; flip angle = 80°; 84 axial slices; FOV = 220 × 220 mm; in-plane resolution 1.96 × 1.96 mm, slice thickness = 2 mm) were acquired for registration to standard space.

FMRI data preprocessing

Prior to analysis, all resting-state fMRI data sets were submitted to a visual quality control check to ensure that no gross artifacts were present in the data. Next, data were analyzed using FSL Version 4.1.3 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). The following preprocessing steps were applied to the EPI data sets: motion correction, removal of non-brain tissue, spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM), grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and a high pass temporal filter of 100 s (i.e., ≥.01 Hz). The RS dataset was registered to the high resolution EPI image, the high resolution EPI image to the T_1-weighted image, and the T_1-weighted image to the 2 mm isotropic MNI-152 standard space image (T_1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada). The resulting transformation matrices were then combined to obtain a native to MNI space transformation matrix and its inverse (MNI to native space).

FMRI time course extraction and statistical analysis

For the current study, a seed based correlation approach (Fox & Raichle, 2007) was employed to reveal brain regions that are functionally connected to the amygdala during rest (e.g., Roy et al., 2009). To this end, binary masks of the bilateral amygdala

were created using the Harvard–Oxford Subcortical Atlas, as provided in MNI standard space within FSL: the center voxel was determined for the left and right amygdala, and spherical regions of interest (ROIs) were subsequently created around these voxels using a radius of 4 mm. Next, using the inverse transformation matrix, the amygdala masks were registered to each participant's RS-fMRI preprocessed dataset. The mean time course was subsequently extracted from the voxels falling within each amygdala mask in native space. These time courses were entered as a regressor in a general linear model (GLM), together with nine nuisance regressors, comprising the white matter signal, CSF signal, six motion parameters (rigid body: three translations and three rotations), and the global signal. The latter regressor was included to further reduce the influence of artifacts caused by physiological signal sources (i.e., cardiac and respiratory) on the results (Fox & Raichle, 2007). Each individual model was tested using FEAT version 5.98, part of FSL. The resulting individual parameter estimate (PE) maps, together with their corresponding within-subject variance maps, were then resliced into 2 mm isotropic MNI space and fed into a higher level between-groups mixed effects analysis (two-sample *t*-test). First, whole-brain *z*-statistic images were thresholded using clusters determined by an initial cluster-forming threshold of $z > 2.3$ and a (corrected) cluster significance threshold of $p < .05$ (Worsley, 2001). A small volume correction was applied for regions known to have functional and/or anatomical connections to the amygdala (Amaral & Price, 1984; Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a), and which were a priori hypothesized to be affected by stress in this study: the mPFC, including the pgACC, vm/dmPFC and OFC, as well as the hippocampus. Masks of these regions of interest were defined based on the Harvard–Oxford (sub)cortical probability atlases, as provided in FSL, and were then used to mask the raw statistical images. Subsequently, correction for multiple comparisons was carried out for only those voxels present in the ROI masks, using cluster based thresholding with the same parameter settings as for the whole-brain analysis ($z > 2.3$, $p < .05$).

Procedure

On the day of scanning participants arrived at either 8:30 or 10:30 a.m. The arrival time of the participants was balanced both between and within groups to keep morning cortisol levels as comparable as possible. Participants were asked to refrain from caffeine or sugar containing drinks, and not to eat two hours before arrival time to minimize unwanted effects on cortisol levels. After arrival, participants were seated in a quiet waiting room, where instructions were given about the protocol. Exactly 30 minutes after arrival, participants were given instructions belonging to either the control or stress condition. Both protocols started outside the scanner, where participants were either told to prepare a presentation, or to think about a movie to their liking. After preparation, they were brought to a quiet room in which the committee was seated (stress) or the movie questionnaire was handed out (control), and both protocols were continued. Each took 20 minutes to complete. Afterwards, the participant was brought to the scanner. The scanning protocol consisted of an emotional working memory task (Oei et al., 2012), several anatomical scans, and the RS scan which was acquired at the end of the scan protocol, 60 minutes after completion of the TSST. For the RS scan, participants were instructed to lie still with their eyes closed during the entire scan in the darkened scanner room. Saliva was sampled at five time points throughout the procedure: before ('baseline') and after the anticipation phase of the TSST or control condition ('pre TSST'), at the end of the TSST or control condition just before entering the scanner ('post TSST'), immediately after finishing the task scan ('post task') and immediately after the RS scan outside the scanner ('post RS'). At the exact same moments, a 10-point Likert scale was used to inquire about the subjectively perceived stress levels. Blood pressure and heart rate were sampled at the same time points, except when the participant was inside the scanner room due to MR-incompatibility of the equipment. An exit-interview, and, if applicable, a debriefing regarding the TSST followed at the end of the procedure. Subsequently, participants were thanked and paid for their participation in the study.

RESULTS

Two participants from the stress group were discarded in the analysis: one participant exhibited an extreme cortisol level at baseline (120 nmol/L), probably reflecting saliva sample contamination, while data from one participant could not be acquired due to scanner failure. The resulting analyses were therefore carried out on 20 control participants (mean age 23.95 ± 2.52 years) and 18 participants who were exposed to psychosocial stress (mean age 23.94 ± 3.12 years). The stress and control group did not differ in terms of age, BMI, STAI trait or state scores, and baseline heart rate, blood pressure or cortisol (all: $p > .1$).

Physiological and behavioral results

The stress group showed a strong physiological reaction to the stressor as measured by the salivary cortisol levels (see **Figure 3.1a**), which was confirmed by a Group-by-Time interaction, $F(1.69, 60.96) = 9.9$, $p < .001$. Post-hoc t-tests showed higher cortisol values in the stress group before ($p < .001$) and after ($p < .01$) the working memory task, and directly after the RS scan ($p < .05$) compared with controls. This effect was also reflected by the concurrent increase of the subjective stress ratings (see

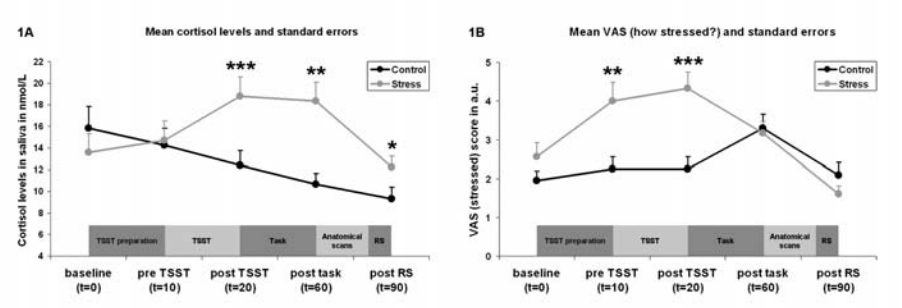

Figure 3.1 (**A**) Mean salivary cortisol levels and standard errors for both the stress and control group at the five time points of sampling (t = time in minutes from baseline). Note: *** $p < .001$, ** $p < .01$, * $p < .05$. (**B**) Mean subjective stress scores and standard errors for both the stress and control group at the five time points of sampling (t = time in minutes from baseline). Note: *** $p < .001$, ** $p < .01$, * $p < .05$.

Figure 3.1b), as was confirmed by the Group-by-Time interaction, $F(3.36, 120.98) = 19.21$, $p < .001$. Here, post-hoc tests showed higher ratings for the stress group before ($p < .01$) and after ($p < .001$) the TSST, but not after the RS scan. Lastly, both systolic and diastolic blood pressure (SBP/DPB) showed a Group-by-Time interaction, $F(3, 108) = 18.24$, $p < .001$ and $F(3, 108) = 6$, $p = .001$, respectively. While SBP showed a trend ($p = .088$) before the TSST, DBP was already increased in the stress group ($p < .014$). Both SBP and DBP were increased in the stress group after the TSST ($p < .001$) compared with the control group. We did not find a difference in heart rate and frequency of respiration during the RS scan, and in blood pressure directly after the RS scan between the two groups (all $p > .1$).

Functional connectivity results

Figure 3.2a shows the joint amygdala resting-state functional connectivity patterns for the two groups separately, as well as their overlap and differences. Within both groups the connectivity pattern largely overlapped with areas described to have functional and anatomical connections with the amygdala in previous studies (Amaral & Price, 1984; Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a). Areas involved included: brainstem, hippocampus, hypothalamus, subgenual cingulate cortex, dorsal cingulate cortex, posterior lateral orbitofrontal cortex, insula, temporal poles, and the primary visual cortex. The majority of these regions together form "the emotional brain", dedicated to the processing and regulation of emotion (Pessoa, 2008). A detailed description of the areas involved is provided in **Table 3.1**.

Compared to the control group, the stress group showed increased amygdala RSFC with the posterior cingulate cortex (PCC) and the adjacent precuneus ($p < .05$, corrected; see **Figure 3.2b**). In addition, when applying a small volume correction for our regions of interest, increased amygdala RSFC was demonstrated within the vmPFC in the stress group compared to the control group. However, changes in amygdala functional connectivity with the hippocampus were not found, which is contrary to our expectations. Post-hoc tests revealed that the effects were not driven by either the left or right amygdala alone. Lastly, no differences were observed for the opposite contrast control > stress.

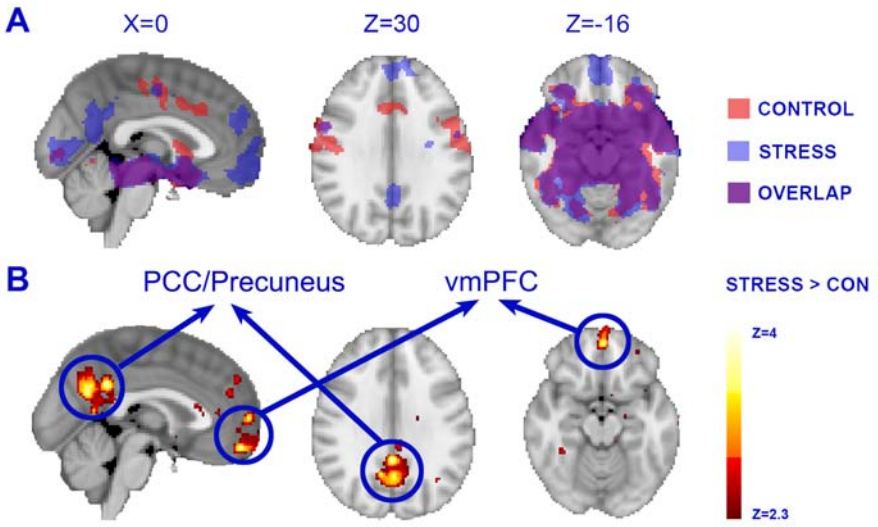

Figure 3.2 Group main (**A**) and between groups (**B**) effects of joint amygdala resting-state functional connectivity overlaid on the 2 mm MNI standard space template. Group main effects are cluster corrected at $p < .05$. Between group effects are shown uncorrected at $z > 2.3$ for illustration purposes. The left side of the brain corresponds to the right hemisphere and vice versa.

DISCUSSION

In the current study we investigated whether psychosocial stress modulates RSFC of the amygdala with other brain regions important for the processing, regulation and consolidation of emotionally salient events in healthy participants during the recovery phase, when the acute stress response has waned. It was expected that stress would increase amygdala RSFC with the mPFC, supporting regulatory feedback on the amygdala during recovery from the stressful event. In addition, increased amygdala RSFC was expected with regions facilitating (emotional) memory formation and consolidation, such as the hippocampus and its adjacent structures, indicating an increased propensity to store emotionally salient information in memory after stress.

The seed based correlation approach employed in this study generated whole brain RSFC patterns of the amygdala similar to those reported in previous studies (Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a). The comparison between the stress and control group yielded two major findings. Firstly, increased RSFC was found with the posterior cingulate cortex (PCC) and the adjacent precuneus. The

Table 3.1 Amygdala resting-state functional connectivity results

Region	Hemisphere	Cluster size 2mm voxels	Peak voxel coordinates (MNI)			*z*-value
			x	y	z	
Control						
Positive						
lateral orbitofrontal	R	35890	30	34	-18	5.09
cortex	L		-30	34	-16	5.29
hippocampus	R		28	-22	-16	6.03
	L		-26	-20	-16	6.19
putamen	R		30	-14	-4	6.39
	L		-30	-16	0	6.14
globus pallidus	R		24	-4	0	6.2
	L		-20	0	2	5.62
insula	R		42	-2	-8	5.55
	L		-40	-6	-8	4.81
hypothalamus	R		6	-4	-12	4.04
	L		-6	-2	-26	4.95
subcallosal cortex	R		8	10	-14	4.99
	L		-6	16	-14	4.54
temporal pole	R		46	10	-16	5.35
	L		-52	10	-16	5.36
superior temporal	R		54	-34	4	3.76
gyrus	R		48	-24	-4	3.57
	L		-54	-14	-8	4.54
	L		-52	-34	2	4.04
middle temporal	R		56	-12	-14	5
gyrus	L		-56	-14	-10	4.34
occipital cortex	R		14	-86	4	3.6
	L		-6	-92	4	4.52
brainstem	R		-2	-34	-16	6.13
dorsal anterior	R	7318	8	-8	40	4.27
cingulate cortex	L		-8	-8	44	4.23
postcentral gyrus	R		62	-16	38	4.82
	L		-46	-16	36	4.76
precentral gyrus	R		60	4	32	4.67
Negative						
posterior cingulate	R	12325	4	-36	26	4.41
cortex	L		-4	-36	26	4.3
precuneus	R		6	-66	30	3.13
	L		-8	-70	32	3.67

Table 3.1 Continued.

Region	Hemisphere	Cluster size 2mm voxels	Peak voxel coordinates (MNI)			*z*-value
			x	y	z	
Control						
Negative						
lateral frontal pole	R	4027	26	58	10	4.08
	L		-34	58	6	3.75
perigenual anterior cingulate cortex	R	1300	4	36	10	2.94
medial superior frontal gyrus			-2	26	50	3
Stress						
Positive						
lateral orbitofrontal cortex	R	41463	36	36	-12	4.25
	L		-38	32	-16	3.88
hippocampus	R		32	-14	-20	6.07
	L		-24	-30	-10	6.35
putamen	R		32	-16	0	5.47
	L		-30	-20	2	4.73
globus pallidus	R		24	-4	2	4.57
	L		-20	-4	0	4.98
insula	R		40	-10	-8	4.38
	L		-42	-4	-2	4.47
hypothalamus	R		4	-2	-14	5.1
	L		-6	-2	-14	5.39
subcallosal cortex	R		10	12	-18	5.81
	L		-6	14	-16	6.26
temporal pole	R		48	8	-24	5.23
	L		-42	10	-24	5.16
superior temporal gyrus	R		60	-12	-2	4
	R		52	-22	-4	4.04
	L		-62	-12	-8	4.86
	L		-52	-22	0	4.76
middle temporal gyrus	R		58	-12	-16	5.19
	L		-50	2	-22	4.82
occipital cortex	R		24	-94	0	3.81
	L		-8	-88	0	4.22
brainstem	R		-4	-34	-14	6.36
posterior cingulate cortex			0	-48	32	3.39
precuneus	R		2	-58	12	4.25
	L		-4	-58	8	4.31
dorsal anterior cingulate cortex	R	1556	8	-6	42	2.97
	L		-6	0	42	3.7
	L		-16	-40	56	3.2
precentral gyrus	R		40	-12	42	3.96
	L		-34	-16	44	4.4

Table 3.1 Continued.

Region	Hemisphere	Cluster size 2mm voxels	Peak voxel coordinates (MNI) x	y	z	*z*-value
Stress						
Positive						
ventromedial prefrontal cortex		2301	4	52	-14	5.31
dorsomedial prefrontal cortex			0	46	26	4.45
Negative						
lateral frontal pole	R	12371	30	60	4	3.64
	L		-28	60	18	3.76
medial superior frontal gyrus		84	0	22	50	3.35
Stress>Control						
posterior cingulate cortex	R	1260	2	-46	32	3.68
precuneus			0	-62	26	3.63
ventromedial prefrontal cortex		270	0	54	-16	3.68*
frontal pole			2	60	6	3.69*

Note: all *z*-values are corrected for multiple comparisons ($p < .05$), except for z-values with a * ($p < .05$, small volume corrected)

PCC/precuneus area is implicated in autobiographical memory processes (Buckner & Carroll, 2007; Cavanna & Trimble, 2006; Vann, Aggleton, & Maguire, 2009). Recently, evidence for a direct ascending anatomical connection between the basolateral nucleus and retrosplenial cortex, the most caudal part of the PCC, was found in the macaque brain (Buckwalter, Schumann, & Van Hoesen, 2007). The existence of such a connection seems to be supported by studies showing RSFC between the two regions in humans (Robinson et al., 2010; Stein et al., 2007a). In addition, a recent study found white matter pathways between these regions and the hippocampus (Greicius, Supekar, Menon, & Dougherty, 2009), a pivotal brain structure for storing and retrieving episodic information (Squire & Zola-Morgan, 1991). Further, the amygdala is richly and reciprocally connected to the hippocampus in primates (Amaral, 1986). We thus speculate that the current finding might reveal the cortico–limbic circuit through which stress enhances memory formation of emotionally salient events

(McGaugh, 2004; Roozendaal et al., 2009). While this could reflect a beneficial mechanism, served to adaptively face similar situations in the future, increased connectivity in this circuit may also turn maladaptive, thereby promoting disproportionate memory consolidation of negative experiences. This, in turn, may eventually form a basis for unwanted intrusive memories, a key symptom in posttraumatic stress disorder (Brohawn, Offringa, Pfaff, Hughes, & Shin, 2010), but also common to depression and anxiety. Nonetheless, in this study we did not explicitly test for memory of emotionally salient information. Therefore, future studies are warranted to investigate whether stress actually modulates emotional memory through increased FC between the amygdala and precuneus.

The second major finding in our study was that psychosocial stress, in line with our expectations, increased amygdala RSFC with the medial prefrontal cortex (mPFC). Especially the ventral part of the mPFC (vmPFC) has dense and reciprocal anatomical connections to the amygdala (Ghashghaei & Barbas, 2002; Ghashghaei, Hilgetag, & Barbas, 2007), which might drive the connectivity observed between these regions (Robinson et al., 2010; Roy et al., 2009; Stein et al., 2007a). Although the pgACC, acknowledged as part of the mPFC, has been described most extensively as a target region for top-down inhibitory control over the amygdala (Pessoa, 2008; Pezawas et al., 2005; Phillips, Drevets, Rauch, & Lane, 2003a), other studies report on yet another part of the mPFC, similar to the location we found in our study, that is implicated in regulating amygdala responses (Heinz et al., 2005; Johnstone et al., 2007; Urry et al., 2006). In addition, glucose metabolism in this region was shown to decrease with higher levels of cortisol, resulting from a comparable psychosocial stressor, and was inversely related to the metabolism of the hippocampus/amygdala (Kern et al., 2008). Further, a more normative diurnal cortisol pattern was found to relate to stronger functional coupling between the vmPFC and amygdala during downregulation of negative affect (Urry et al., 2006). Lastly, cortisol administration was shown to increase FC between the amygdala and mPFC (Henckens et al., 2010). Therefore, the current result might be in line with the notion that the amygdala receives modulatory control from the mPFC to regulate expression of emotions, or more specifically, to regulate the brain's response to stress. An overload in stress

may impact exactly this feedback circuit and thereby contribute to the pathogenesis of stress-related psychiatric disorders, such as depression, anxiety and posttraumatic stress disorder, as decoupling of these regions has been well-documented in relation to disturbed emotion regulation (Heinz et al., 2005; Johnstone et al., 2007; Phillips, Drevets, Rauch, & Lane, 2003b; Shin et al., 2006; Veer et al., 2010).

The midline brain regions, PCC/precuneus and mPFC, found in the current study are the core constituents of the default mode network (DMN) (Raichle et al., 2001). This network is proposed to be related to mind wandering (Mason et al., 2007), autobiographical memory processes (Buckner & Carroll, 2007), and self-referential thought (Gusnard, Akbudak, Shulman, & Raichle, 2001; Northoff & Bermpohl, 2004; Northoff et al., 2006; Raichle et al., 2001). Furthermore, in line with these functional accounts, the DMN is hypothesized to provide the infrastructure for integrating past, present and future events related to the self (Buckner & Carroll, 2007). This would enable us to reflect on and learn from past experiences, which is essential for adaptively coping with future challenges. Therefore, increased amygdala connectivity with these DMN regions could reflect stress-induced facilitation of self-evaluative processes under or after emotionally salient experiences. This might be particularly strong in our paradigm, because of the social evaluative component in the stressor we applied. Some support for this hypothesis, although taken tentatively, can be found in studies of social phobia showing increased activity in the precuneus/PCC and vmPFC when viewing emotional facial expressions (Gentili et al., 2009) and increased vmPFC within the DMN at rest (Liao et al., 2010). In addition, abnormally increased RSFC within the DMN has been described in other stress-related psychiatric disorders, such as major depression (Greicius et al., 2007), and posttraumatic stress disorder (Lanius et al., 2010). It is important to note that self-referential activity, as might be reflected by the enhanced connectivity with DMN regions, is compatible with both our previous accounts, being improved memory for emotionally salient events and downregulation of emotional states, as both processes are dependent on evaluation of the situation one encountered. Lastly, from a dynamical network perspective, it is highly plausible that separate resting-state connectivity networks engage or disengage in different configurations, depending on the circumstances to be dealt

with. Consequently, we might actually observe that the amygdala-centered connectivity network under scrutiny connects to the DMN to meet the demands set by a stressful situation.

We did not observe increased RSFC of the amygdala with the hippocampus itself and/or its adjacent areas after stress. However, the amygdala borders the hippocampus, which makes it hard to segment the two structures from one another, especially when dealing with the coarse resolution of functional MRI scans. When also taking into account the spatial smoothing applied during preprocessing, the time series derived from our amygdala seeds might have been 'contaminated' by signal from the hippocampus. Effects on our results could be twofold: subtle differences in connectivity between the amygdala and hippocampus may have been swamped through partial overlap in signal, as might be suggested by the very high correlation with the hippocampus in both groups. Secondly, the increased PCC/precuneus connectivity might actually be mediated by the hippocampus, which is supported by the strong white matter pathways between these regions (Greicius et al., 2009). Nonetheless, such a scenario would furthermore underscore that our results could relate to increased emotional memory formation after a stressful event.

Using the TSST, a real life psychosocial stress situation, we were successful in raising both physiological and subjective stress levels of participants in the stress group, as was reflected by substantial increases in the salivary cortisol response, blood pressure, and subjective stress ratings. The stress group demonstrated a cortisol response almost twice as high as their baseline levels, whereas the control group showed a steady decrease in their cortisol levels over the course of the experiment. Notably, the stress group still demonstrated higher cortisol levels than controls when the RS-fMRI scan was acquired, an hour after the TSST was completed. The group that was already stressed by the TSST rated their subjective experience of stress as higher before entering the scanner than the control group. However, stress-free controls showed an increase of subjective stress while inside the scanner, probably due to lying inside an MRI scanner, as all participants in the current study were scanner-naïve. Nonetheless, both groups were close to baseline directly after the RS scan. Therefore, it is likely that we were not able to show connectivity related to the immediate stress

response as shown by Van Marle et al. (2010). However, we do find robust differences that can only be attributed to the stressful experience our experimental group encountered. In our opinion, these differences could be interpreted to reflect processes promoting recovery and adaptation in the post-stressor period, either conscious or unconscious, to warrant homeostasis.

A limitation of the current study is the possible influence of physiological differences between the stress and control group on the functional connectivity effects we observed. Firstly, we have tried to minimize this by adding the global signal as a confound regressor, which has previously been shown to reduce effects of physiological fluctuations on the data (Fox & Raichle, 2007). Secondly, although heart rate and respiration were not measured comprehensively during resting-state data acquisition, our crude sampling method did not reveal any differences during RS data acquisition. Lastly, it is important to note that in a previous study a significant decrease in both heart rate and blood pressure was found within 10 min following the TSST, with heart rate already being returned to baseline levels (Oei et al., 2006). Therefore, we think it is unlikely that the differences in functional connectivity found one hour after stress exposure could be attributed to differences in physiological fluctuations between the two groups.

A second limitation of our study pertains to the possible influence that the emotional working memory task might have had on the amygdala functional connectivity patterns, although the RS scan was acquired 20 minutes post-task. Analysis of the task showed increased amygdala responsivity towards negative emotional stimuli after stress. Therefore, the differences in functional connectivity we observed might also be caused by a more thorough perception and processing of such stimuli under or after a stressful condition. This does, however, fit our hypothesis that the stress-induced increase in amygdala RSFC with the PCC/precuneus could reflect enhanced emotional memory. However, there was no association between amygdala responsivity on the task and the strength of the amygdala RSFC with the PCC/precuneus and the vmPFC. Though taken tentatively, this might speak against influence of the task on the current results.

On a final note, we should be cautious in relating our results to adaptive recovery from stress. Although we have measured amygdala RSFC in the recovery phase after stress, we cannot directly compare our effects to RSFC under acute stress or to a measure of recovery encompassing the hour after stress, which could have strengthened our hypothesis. However, we can compare our results to those obtained by Van Marle et al. (2010), albeit different stressors were used. The authors showed RSFC patterns pointing to autonomic activation in the direct aftermath of stress, whereas our results in absence of such activation better fit recovery processes such as regulatory feedback and preparation for future hardships. Nevertheless, we do recommend including a measure of recovery (rate) in future studies, so allowing a better characterization of amygdala RSFC in the post-stressor period.

In sum, here we show for the first time that psychosocial stress increases amygdala resting-state functional connectivity with the precuneus/PCC and vmPFC, areas known to be involved in memory, emotion regulation and social cognition. This result might be attributable to behavioral homeostasis after stress, which stretches beyond the initial stress response. Although our results are likely to reflect a healthy and adaptive response to a stressful situation, these may also provide a link to the pathogenesis of stress-related psychopathology and provide an important new vantage point in studying both sensitivity and resilience to stress in general.