



## Enhanced resting-state connectivity of amygdala in the immediate aftermath of acute psychological stress<sup>☆</sup>

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### ABSTRACT

Recent neuroimaging studies investigating responses to stressful stimuli may importantly further our understanding of psychological trauma etiology. However, theory posits that sustained activation of these stress circuits after the stressful event may play an equally important role in the development of stress-related psychopathology. Importantly, such post-stress network changes remain poorly characterized. The amygdala with its connections is crucially positioned in the central stress circuitry that mediates the initial stress response. Hence, we investigated post-stress amygdala-centered connectivity patterns in order to characterize the aftermath of acute, experimentally-induced stress in healthy humans. We recorded resting-state functional MRI in 26 female participants following a period of moderate psychological stress induced by means of aversive (vs. emotionally neutral) movie watching with a self-referencing instruction. Next, we implemented a seedregion analysis calculating the voxel-wise correlation with the anatomically extracted time-series of the amygdala. Various stress measures confirmed successful stress induction. Moreover, we demonstrated enhanced functional coupling of the amygdala with dorsal anterior cingulate cortex, anterior insula, and a dorso-rostral pontine region, which appears to overlap with the anatomical location of the locus coeruleus (LC), when contrasting the stress with the control group. Thus, we show that the aftermath of acute stress is qualified by prolonged activation in an amygdala-connectivity network. This pattern of co-activation may indicate an extended state of hypervigilance that promotes sustained salience and mnemonic processing after stress. Characterization of the post-stress brain state may provide initial insight into the early phases of psychological trauma formation.

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### Introduction

A set of recent neuroimaging studies have begun to unravel the neural dynamics underlying the acute stress response in healthy humans (Gianaros et al., 2008; Pruessner et al., 2008; Wang et al., 2005). These findings are of great value in our understanding of psychological trauma etiology. Yet, theoretical notions suggest that the prolonged activation of noradrenergically-driven stress circuits specifically after the stressful event may be equally relevant for the initial phase of traumatic illness (Krystal and Neumeister, 2009; Morgan et al., 2003). Importantly, post-stress activation patterns of such stress-related brain networks remain largely uninvestigated.

The amygdala is critically positioned in the central stress circuitry and mediates an initial surge in vigilance that optimizes the detection and assessment of threats to homeostasis (de Kloet et al., 2005; van

Marle et al., 2009). For this, the amygdala depends largely on ascending, excitatory catecholaminergic pathways like the dense noradrenergic innervation from the locus coeruleus (LC), an autonomic pontine nucleus that is the main source of norepinephrine (NE) in the forebrain (Sara, 2009). The amygdala and LC are richly and reciprocally connected (Valentino and Van Bockstaele, 2008; Van Bockstaele et al., 2001) and their interaction is important for the upregulation of arousal in response to salient or stressful events (Aston-Jones et al., 1991; Joëls and Baram, 2009; Quirarte et al., 1998; Valentino and Van Bockstaele, 2008). In mediating the autonomic arousal that accompanies vigilant states, the amygdala is additionally coupled to the dorsal anterior cingulate cortex (dACC) and anterior insula (AI), key regions in autonomic-interoceptive processing (Craig, 2009; Critchley, 2005). Their joint activation with amygdala and various brainstem structures during rest has recently been described to constitute an intrinsic connectivity network that is particularly involved in continuous salience processing within the homeostatic, emotional, and cognitive domain (Seeley et al., 2007).

A surge in vigilance as part of a normal stress response serves clear adaptive function. However, sustained activation of these stress-related brain circuits after the stressful event may result in allostatic load (McEwen, 2007) and in case of excessive stress or phenotypically

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vulnerable individuals be at the basis of trauma etiology (Krystal and Neumeister, 2009; Morgan et al., 2003; Yehuda and LeDoux, 2007). To date, this highly relevant time window qualified as the immediate aftermath of stress stays uncharacterized.

To investigate post-stress activation of the amygdala-centered stress network, we probed sustained functional coupling of the amygdala with LC, dACC, and AI directly after experimentally-induced, psychological stress in a group of healthy women. We recorded resting-state Blood Oxygenation Level Dependent (BOLD)-fMRI after moderate stress was induced by means of aversive (vs. emotionally neutral) movie watching with a self-referencing instruction. Using a seedregion analysis that calculated the voxel-wise correlation with the extracted time course of the anatomically defined amygdala, we then tested the prediction that the direct aftermath of acute stress is characterized by enhanced functional connectivity between amygdala and LC (as main source of NE innervation), and between amygdala and dACC and AI (as main constituents of the above-described salience network).

## Materials and Methods

### Participants

Twenty-nine healthy women participated in this study. Participants had normal or corrected vision. They reported no history of psychiatric, neurological, or endocrine disease and no current use of psychoactive or corticosteroid drugs. Nor did they report a habit of watching violent movies or playing violent videogames, or a history of being victim or eye-witness of severe physical/emotional trauma. Avoiding confounds related to gender differences (Wang et al., 2007) and menstrual cycle-dependent variance in stress responsiveness (Ossewaarde et al., 2010) only women taking oral contraceptives were included. Scanning took place in the final two weeks of the cycle ensuring relatively stable gonadal hormone levels. Written informed consent was obtained before the experiment and the study was approved by the local ethical review board (CMO Region Arnhem-Nijmegen, The Netherlands) in accordance with the declaration of Helsinki.

Participants were randomly assigned to either the stress ( $n = 13$ ; age:  $21 \pm 2.2$ , range: 18–25) or control group ( $n = 13$ ; mean age:  $20 \pm 1.8$ , range: 18–24). Data of 3 additional participants were excluded due to technical failure or failure to complete the procedure.

### General procedure

To ensure low and relatively stable levels of endogenous cortisol the experiment took place in the afternoon. An acclimatization period of 1.5 hours following arrival was used to collect baseline saliva samples and affect ratings. Additionally, participants completed various personality questionnaires, including the Spielberger Trait Anxiety Inventory (STAI, 20 item Dutch translation, Van der Ploeg, 1981; van der Ploeg et al., 1980; Spielberger et al., 1970) and the NEO Five Factor Inventory (NEO FFI, Costa and McCrae, 1992). To avoid further anticipatory stress in the control group, participants were then told which experimental group they were assigned to before being escorted to the MR scanner. The full fMRI session consisted of three tasks that were each preceded and followed by different movie clips and thus embedded in either a continuously stressful or neutral context (Fig. 1). These included a task involving passive viewing of dynamic emotional facial expressions (van Marle et al., 2009), a working memory task (Qin et al., 2009) and a reward expectancy task. Critically, these tasks were identical between the two conditions of interest (stress induction vs. control condition). After the fourth and final movie clip, approximately 60 minutes after the start of the experiment, a resting-state run was recorded that is reported here (details below). This period of resting-state thus

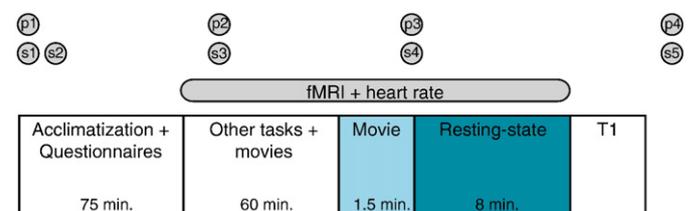
characterized the immediate aftermath of a continuously stressful (vs. emotionally neutral) experience. The experiment ended with a structural scan. A debriefing procedure followed after participants left the scanner.

### Stress induction

Moderate psychological stress was induced in the stress condition by showing short movie clips containing scenes with strongly aversive content (extreme violence), selected from a commercially available movie (Irréversible, 2002 by Gaspar Noé), inside the MRI scanner. In contrast, the control condition consisted of watching equally long movie clips from another movie (Comment j'ai tué mon père, 2001 by Anne Fontaine), which were equal in luminance and similar in language and human/face presence, but contained only non-arousing scenes. Participants were asked to constantly and attentively view the movie clips after short introductory texts put them in the scene from an eye-witness perspective thereby attempting to involve them maximally in the experience. This method of stress induction closely corresponds to the determinants of the human stress response as described by Mason (Mason, 1968), i.e., unpredictability, novelty, and uncontrollability. Furthermore, it meets the criteria described by Joëls and colleagues (Joëls et al., 2006) for stress enhanced memory to occur, i.e., close spatio-temporal proximity of stressor and task (task immediately followed by stressor within fMRI environment). Finally, previous studies by our laboratory and others have shown that similar methods elicit measurable physiological stress responses (Cousijn et al., 2010; Henckens et al., 2009; Nejtek, 2002; Wittling and Pfluger, 1990). The movie clip preceding the resting-state run was 1.30 minutes in duration. The resting-state run consisted of 8 minutes of task-free, undirected wakefulness. Participants were instructed to relax and think of nothing in particular. They were told to keep their eyes open to rule out sleeping, which was checked with a MRI compatible eye-tracking device using infrared light from SensoMotoric Instruments (MEyeTrack-LR). The scanner room was almost completely dark.

### Physiological and subjective measurements of stress

Heart rate was continuously recorded throughout scanning (Fig. 1) to assess the autonomic response to the stress manipulation. For this, we used an infrared pulse oximeter (accompanying the MRI scanner, Siemens, Erlangen, Germany) placed on the left index finger. Offline artifact correction and analysis of heart rate signal, calculating heart rate frequency (HRF) and heart rate variability (HRV) was done using in-house software. HRF was calculated as 60/



**Fig. 1.** Experimental design. Eight minutes of resting-state BOLD fMRI recording (dark blue box) followed a series of experimental paradigms, embedded in either an acutely stressful (stress group) or neutral (control group) context by means of intermittent, adjoining short movie clips with strongly aversive or emotionally neutral content, respectively. A fourth and final movie clip directly preceded the resting-state run (light blue box). Resting-state consisted of task-free, undirected wakefulness with eyes open. To monitor the effects of stress induction salivary cortisol samples (S1–5) and subjective affect ratings (as measured with PANAS, P1–4) were collected at baseline and at different time points before, immediately preceding and after the resting-state run. Heart rate was recorded continuously. min, minutes. T1, structural scan.

mean interbeat interval and HRV as the root mean squares of successive differences between successive interbeat intervals. HRV shows a decrease as a function of stress (Berntson et al., 1997; Porges, 1995). HRF and HRV were analyzed for each of the four movie clips as main autonomic markers of stress induction over the course of the experiment. Baseline-correction of heart rate data was done by subtracting the corresponding values derived from the resting-state run as by that time HRF and HRV normalized and showed no group difference (both HRF and HRV:  $T(24) < 1$ ). Data of four participants (three in the stress group) were discarded because of excessive signal artifacts.

To assess the hypothalamic-pituitary-adrenal (HPA) axis response, saliva was sampled using salivette collection devices (Sarstedt, Rommelsdorf, Germany) to determine the level of free cortisol. We sampled two baseline measurements prior to the fMRI session and three additional measurements: 1.) following an earlier task, 2.) following the fourth and final movie clip, just prior to the resting-state run, and 3.) 30 minutes after the fourth movie clip (22 minutes after the resting-state run and 15 minutes after leaving the scanner). All measurements were baseline-corrected. All samples were stored at  $-20^{\circ}\text{C}$  until analysis. Samples were prepared for biochemical analysis by centrifuging at 3,000 rpm for 5 minutes, which resulted in a clear supernatant of low viscosity. Salivary-free cortisol concentrations were determined by the Department of Biopsychology, TU Dresden, Germany, employing a chemi-luminescence-assay (CLIA) with high sensitivity of 0.16 ng/ml (IBL; Hamburg, Germany).

Subjective state was assessed by obtaining the positive and negative affect schedule (PANAS; (Watson et al., 1988)) once at baseline and at three additional time-points coinciding with saliva sampling. Ten items for positive and ten for negative affect were rated on a five-point scale ranging from '1 - not at all' to '5 - extremely'. Separate scores for positive and negative affect were baseline-corrected.

For all stress measures statistical analyses were performed using repeated measures ANOVAs over all time points of measurement with stress induction (stress versus control) as between subjects factor. Whenever necessary, further testing was done using t-tests. Alpha was set at .05 throughout.

### Image acquisition

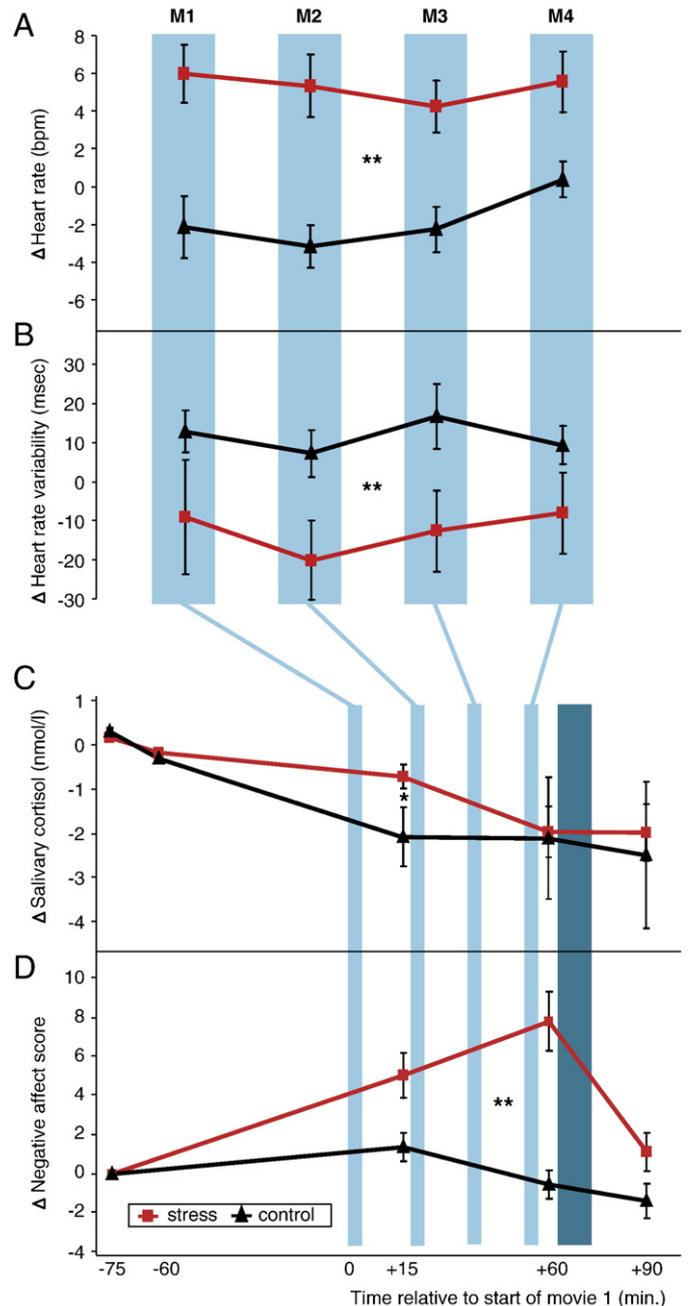
Whole brain  $T2^*$  weighted gradient echo EPI BOLD-fMRI images were acquired with a Siemens (Erlangen, Germany) TIM Trio 3.0 Tesla MR-scanner equipped with an eight channel phased-array head coil, using an ascending slice acquisition (30 axial-slices, TE/TR: 25/1530 ms, flip angle  $71^{\circ}$ , FOV:  $212 \times 212$  mm, matrix  $64 \times 64$ , 3.8 mm slice thickness, .4 mm slice gap). 319 images were acquired during the resting-state run. In order to reduce artifacts caused by inhomogeneity around air-tissue interfaces, we used a relatively short TE, an oblique axial angulation, and reduced echo-train length (de Zwart et al., 2006) by means of Factor 2 accelerated GRAPPA (Griswold et al., 2002). High-resolution structural images ( $1 \times 1 \times 1$  mm) were obtained using a  $t1$ -weighted MP-RAGE sequence (TE/TR: 2.96/2300 ms, flip angle:  $8^{\circ}$ , FOV:  $256 \times 256 \times 192$  mm, GRAPPA acceleration factor 2).

### Image analysis

Image processing and statistical analyses were performed using SPM5 ([www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)). The first five EPI volumes were discarded to allow for  $T1$  equilibration, and the remaining images were realigned using rigid body transformations. The mean image was then coregistered to the structural MR-image. Subsequently, images were transformed into common stereotactic space (MNI152  $T1$ -template), and resampled into 2 mm isotropic voxels. Spatial

smoothing was performed using a Gaussian kernel of 8 mm full-width at half-maximum.

Our goal was to examine functional connectivity patterns of the amygdala in a period following acute stress. To this end, we implemented a seed region analysis, calculating the correlation of the amygdala's time course with the rest of the brain. First, the amygdala's time course was extracted using an anatomical mask that was created from an independent sample in standard (MNI152) space by thresholding ( $P > .35$ ) a probability map obtained through manual anatomical segmentation of the amygdala in 21 individuals'



**Fig. 2.** Autonomic, hypothalamic-pituitary-adrenal axis, and subjective responses to stress induction and control condition. Averaged, baseline-corrected heart rate frequency (A) and heart rate variability (B) during all four movie clips of the experiment (light blue boxes) for the stress and the control group. Baseline-corrected salivary cortisol levels (C) and subjective negative affect ratings (D) assessed at baseline and various time points throughout the experiment. Error bars represent SEM, bpm, beats per min. \*  $p < 0.05$ ; \*\*  $p < 0.005$ .

T1 images (Palmen et al., 2006) (see upper most part of Fig. 3). The mask consisted of  $234 \times 2 \times 2 \text{ mm}^3$  voxels. Second, the first eigenvariate of the set of time courses from voxels comprising the amygdala was calculated for each subject. The resulting time series was used as a covariate of interest in a whole-brain, linear regression, statistical parametric analysis. The realignment parameters, consisting of six parameter rigid body transformations (3 translations and 3 rotations) used for motion correction, were additionally included to model potential movement artifacts. Contrast parameter images for the seed region covariate generated at the single subject level were then submitted to 2nd level random effects analysis. Statistical parametric maps were created within SPM5 using a two sample t-test contrasting the stress induction group versus the control condition group.

Our statistical threshold was set at  $p < 0.05$ , family-wise-error (FWE) rate corrected for multiple comparisons across the whole brain or the region of interest (ROI) using a small volume correction. Given our primary hypothesis of enhanced amygdala coupling to the LC, dACC and AI (Seeley et al., 2007), these regions were targeted as ROI's. Specifically, in close accordance with previous studies reporting on the LC, we implemented a reduced spherical search volume (10 mm radius) around a previously reported (functionally defined) center coordinate for LC (Schmidt et al., 2009). For the dACC and the AI, the search volumes were anatomically defined using the WFU Pickatlas (Maldjian et al., 2003).

## Results

### Stress measures

At baseline the two experimental groups did not differ in cortisol level, or subjective negative or positive affect ratings, nor in STAI score or any of the NEO FFI subscale scores (all  $T_{(24)} < 1$ ).

For each of the four movie clips in the extended experiment, averaged and baseline-corrected HRF and HRV are presented in Figs. 2A and B, respectively. A 4 (time) by 2 (stress induction) ANOVA revealed a main effect of stress induction for both HRF ( $F_{(1,21)} = 16.8$ ,  $p < 0.005$ ) and HRV ( $F_{(1,20)} = 5.0$ ,  $p < 0.005$ ). For HRF, but not HRV, individual t-tests revealed significant group differences for each of the four separate time points (all  $p < 0.01$ ). Together, these findings indicate increased sympathetic and decreased parasympathetic tonus throughout the experiment as a result of stress induction.

Baseline-corrected salivary cortisol levels are presented in Fig. 2C. A 3 (time) by 2 (stress induction) ANOVA revealed an interaction between the factors time and stress induction ( $F_{(2,23)} = 6.5$ ,  $p < 0.05$ ), which was carried by a difference in salivary cortisol levels between experimental groups at time point 3 (15 min after the first movie clip, stress > control,  $T_{(16,2)} = 1.8$ ,  $p < 0.05$ , one-sided). No difference between groups was found at time points 4 (directly following the final movie clip/preceding the resting-state run) and 5 (30 minutes after the final movie clip/15 minutes out of the MRI scanner) Additionally, we found a drop in cortisol levels below baseline, most likely due to high (stress) anticipation and diurnal fluctuation.

Fig. 2D shows baseline-corrected subjective negative affect ratings as measured by the PANAS. An ANOVA revealed a main effect of stress induction (stress > control,  $F_{(1,24)} = 18.7$ ,  $p < 0.001$ ) and an interaction between time and stress induction ( $F_{(3,22)} = 8.4$ ,  $p < 0.001$ ). Subsequent separate independent t-tests revealed significantly higher negative ratings for the stress group than the control group at time point 3/second rating ( $T_{(24)} = 2.6$ ;  $p < 0.05$ ) and time point 4/third rating ( $T_{(24)} = 5.1$ ;  $p < 0.001$ ). No effects of stress induction or interaction effects were found for positive affect ratings.

Together, these results point towards successful induction of moderate levels of psychological and physiological stress and negative emotional state in the stress group preceding the resting-state run.

### fMRI results

In an effort to investigate amygdala connectivity in the immediate aftermath of acute stress, we implemented an ROI seedregion analysis on eight minutes of resting-state data following stress induction.

First, brain regions were identified that were functionally coupled to amygdala across both experimental groups (taking stress induction and control groups together). This analysis yielded multiple activation clusters showing temporal co-activation with amygdala, including hippocampus, ACC, precuneus, parahippocampal gyrus, inferior, middle and superior frontal gyrus, middle orbital gyrus, insula, and brainstem ( $p < 0.05$ , FWE corrected for whole brain volume, see Table 1).

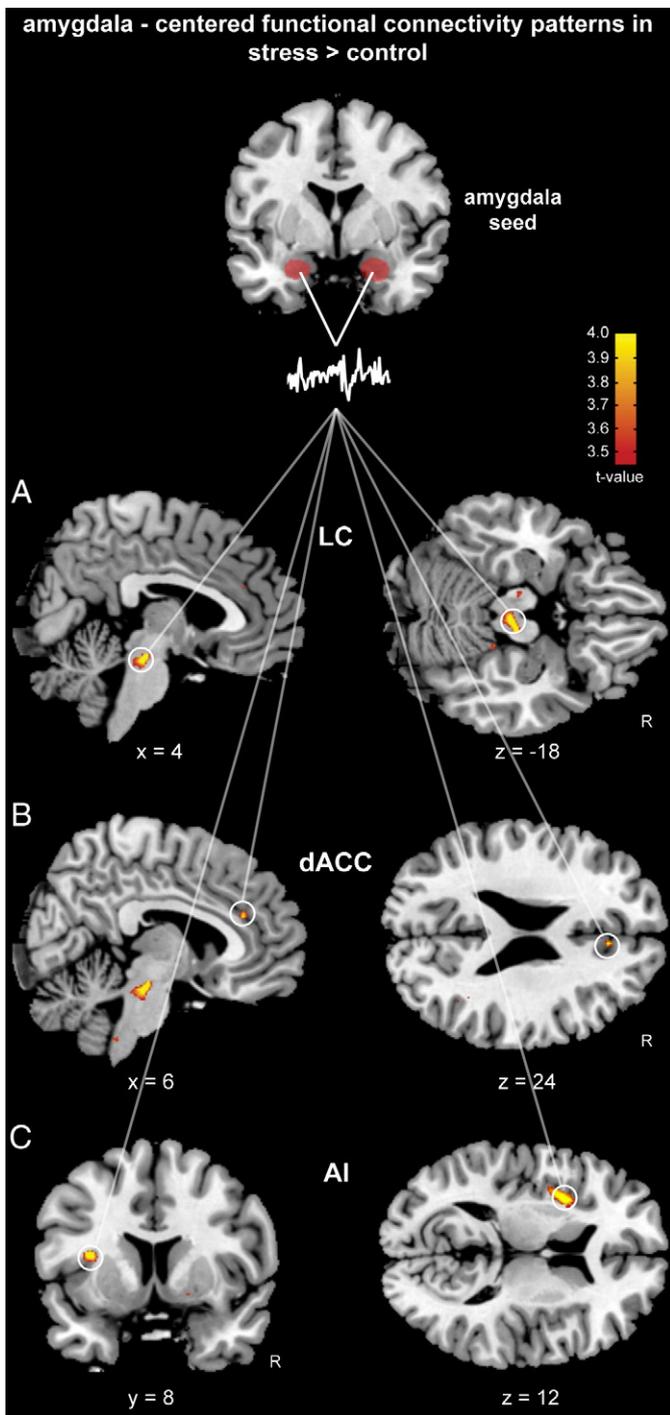
Second, when contrasting the stress induction and the control group, we found enhanced functional coupling of the amygdala with a dorso-rostral pontine region that appears to overlap with the anatomical location of the LC (local maximum: [MNI coordinates (x, y, z)]: 4, -24, -18;  $T_{(1,24)} = 4.49$ ,  $p = 0.012$ , svc-FWE) (Fig. 3A, Table 1). Additionally, we found enhanced temporal co-activation of amygdala with the dACC (local maximum: 6, 34, 24;  $T_{(1,24)} = 4.12$ ,  $p = 0.043$ , svc-FWE) (Fig. 3B) and the AI (local maximum: -32, 8, 12;  $T_{(1,24)} = 4.41$ ,  $p = 0.034$ , svc-FWE) (Fig. 3C). For clarity reasons and to emphasize the selectivity of the effects we also report in Table 1 the few additional activation clusters in this contrast that reached a statistical threshold of  $p < 0.001$ , uncorrected and had a cluster size of at least 10 voxels. These results however are not considered significant (lack an a priori hypothesis) and will therefore not be discussed.

**Table 1**

Peak voxels and corresponding T values of regions that show functional coupling with bilateral amygdala for both groups combined and for main effect of stress induction.

	hemi	MNI coordinates			t-value
		x	y	z	
<i>Both groups</i>					
Amygdala	R	26	-6	-14	12.35***
	L	-24	-6	-14	11.54***
Hippocampus	L	-22	-34	4	10.43***
	R	28	-10	-26	10.09***
Thalamus	R	18	-32	6	9.92***
	L	-4	-16	8	10.26***
Insula	L	-32	18	-14	9.99***
	R	42	22	-4	8.26***
Anterior cingulate cortex	L	-32	-6	16	7.46***
	R	4	46	8	9.98***
Superior temporal gyrus	L	-60	0	0	9.59***
	R	54	10	-12	8.67***
Precuneus	L	-4	-54	64	9.52***
	R	8	-78	50	8.27***
Cerebellum	L	-40	-54	-32	9.20***
	L	-32	-36	-24	8.84***
Fusiform gyrus	L	-36	22	-8	8.40***
	R	58	16	-2	8.17***
Parahippocampal gyrus	R	28	-48	-6	8.31***
	R	20	-16	-20	7.72***
Middle frontal gyrus	L	-16	-26	-16	6.94***
	R	36	34	46	8.30***
Superior frontal gyrus	L	-36	56	6	7.97***
	L	-20	-2	62	8.28***
Middle orbital gyrus	R	2	40	-14	8.08***
	L	-8	48	-10	7.29***
Brainstem	R	10	-32	-12	6.94***
<i>Main effect of stress induction</i>					
Brainstem	R	4	-24	-18	4.49**
Insula	L	-32	8	12	4.41**
	L	-42	-20	20	4.30*
Anterior cingulate cortex	R	34	-16	18	3.96*
	R	6	34	24	4.12**
Superior temporal gyrus	R	46	-28	18	3.93*

\* $p < 0.001$  (uncorrected), with a cluster size of at least 10 voxels; \*\* $p < 0.05$  (small-volume corrected); \*\*\* $p < 0.05$  (FWE corrected for whole brain volume); R: right; L: left.



**Fig. 3.** Seedregion connectivity analysis showing enhanced functional coupling between amygdala (seedregion) and (A) a dorso-rostral pontine region spatially overlapping with the anatomical location of the locus coeruleus (LC), (B) the dorsal anterior cingulate cortex (dACC) and (C) the anterior insula (AI) in a period of task-free, undirected wakefulness directly following acute stress (contrasted with an emotionally neutral control condition). Sagittal and axial (A and B) and coronal and axial (C) t-maps are thresholded at  $p < 0.001$ , uncorrected for display purposes and overlaid onto a canonical T-1 weighted image in standard MNI152 space. R, right.

Finally, stress induction did not result in reduced amygdala coupling (no significant clusters in opposite contrast: control > stress).

## Discussion

The present study aimed at investigating the brain state that characterizes the immediate aftermath of acute stress. Using an

amygdala-centered connectivity analysis, we found enhanced functional coupling between amygdala and dACC, AI, and brainstem in a resting-state period directly following experimentally-induced, moderate psychological stress. The functional relevance of sustained coupling within this amygdala-centered stress network is discussed below.

The amygdala exhibited enhanced coupling with dACC and AI. These regions are strongly implicated in mediating the autonomic bodily arousal that normally accompanies vigilant states. Both dACC and AI are densely and reciprocally connected with amygdala (Augustine, 1996; Ongur and Price, 2000) with dACC mostly sending projections to amygdala (Ghashghaei et al., 2007). Their joint activation pattern in many neuroimaging studies on emotion (Kober et al., 2008) points towards functionally specialized modules for autonomic efference (dACC) and interoceptive feedback (AI) that integrate respectively the motivation and subjective feeling that constitute any emotion (Craig, 2009; Critchley, 2005).

Furthermore, recent work by Seeley and colleagues on resting-state networks suggests that together with amygdala and related brainstem regions the dACC and AI form an intrinsic connectivity network that is particularly geared towards 'personal' salience processing; that is: the continuous integration of the affective, autonomic, and visceral representations of biologically salient events to guide adaptive behavior (Seeley et al., 2007). Our data now implies that this background scan for homeostatic salience is persistently augmented in the immediate aftermath of acute stress.

The amygdala additionally demonstrated increased post-stress co-activation with the brainstem, more specifically, a dorso-rostral subdivision of the pons that appears to overlap with the anatomical location of the LC. It needs to be noted that fMRI lacks the spatial resolution to pinpoint signal activation or co-activation to anatomically minute structures such as distinct brainstem nuclei. Thus, a definite localization of the observed effect to the LC is methodologically not feasible. However, the overlap with the known anatomical location of the LC (Schmidt et al., 2009; Sterpenich et al., 2006) and the prior hypothesis concerning amygdala-LC connectivity and prolonged stress effects make such a localization plausible. The amygdala and the LC/NE system are tightly coupled in the context of acute stress (de Kloet et al., 2005; Joëls and Baram, 2009). In mediating the surge in vigilance in response to stressful or salient events, the amygdala depends mostly on dense noradrenergic innervation from LC. Exposure to various stressors activates the LC (Abercrombie and Jacobs, 1987; Valentino and Van Bockstaele, 2008) and leads to increased NE levels in the amygdala (Galvez et al., 1996; Quirarte et al., 1998). Moreover, it has been recently shown that LC directly mediates stress-induced NE-transmission in the amygdala (Buffalari and Grace, 2007, 2009). Since our method does not provide information on the directionality of the correlated activity, we can not exclude the possibility that the described network originates from LC rather than amygdala. However, given the strong feedback projections from amygdala to LC, their joint action in the context of stress can be best understood as part of a reciprocal feedback loop (Valentino and Van Bockstaele, 2008; Van Bockstaele et al., 2001).

These findings, together with previous reports indicating that this type of stress induction is linked to increased noradrenergic action (Cousijn et al., 2010; Henckens et al., 2009), suggest that the observed protracted amygdala-centered connectivity patterns after stress may be in part driven by elevated central NE-signaling. We realize that this claim remains speculative, since directly measuring elevated central NE-signaling is not possible in this type of research. A future study aimed at pharmacologically blocking NE-signaling after stress (e.g., with propranolol) and showing attenuated coupling within the illustrated network could more conclusively demonstrate a potential relationship with augmented noradrenergic signaling. Alternatively, the observed effects could be related to other stress-related neuromodulators like dopamine, whose signaling is tightly coupled to the LC/NE system (Sara, 2009).

We have previously shown that controlled stress induction resulted in the hypervigilant processing of emotional stimuli by amygdala and related sensory areas (van Marle et al., 2009). Here, we extend these findings by showing that even in the absence of an emotional processing task or any direct perceptual input, an amygdala-centered network exhibits prolonged functional coupling in the direct aftermath of acute stress. This can be functionally interpreted as an extended state of hypervigilance: A prolonged period of possibly LC-originating arousal that promotes a preparatory state of readiness in a stress/salience network consisting of amygdala, dACC, and AI. This interpretation is tentative since our resting-state design did not allow for a direct behavioral measure of vigilance. Nevertheless, the highly selective post-stress coupling of amygdala with brain regions that support vigilance processing, as outlined above, supports this conclusion.

An extended state of central arousal may additionally facilitate memory consolidation. An essential aspect of the stress response is to equip the organism with a potent memory trace of the stressful event for future reference (McGaugh, 2004; Roozendaal et al., 2009). There is now abundant evidence from both animal and human neuroimaging studies that increased  $\beta$ -adrenergic action in the basolateral amygdala underlies the enhancing effect of stress and stresshormones on memory (Roozendaal et al., 2006; Strange and Dolan, 2004; van Stegeren et al., 2005). Our finding of enhanced brainstem-amygdala coupling after stress may be indicative of the immediate, prioritized memory consolidation of the stressful/aversive movie content driven by LC-originating NE-innervation of the amygdala. Alternatively, strengthened brainstem-amygdala coupling after stress could be related to increased (ruminative) recollection of the stressful/aversive movie material during rest, as a recent study showed enhanced LC-amygdala coupling at retrieval of emotional context (Sterpenich et al., 2006). A future study could directly test this by associating post-stress activation of this circuit with a behavioral measure of memory.

The present results most likely represent normal, adaptive changes to moderate, experimentally induced stress and therefore its direct implications for trauma formation after real-world stress are limited. Still, on a purely speculative basis, our results of sustained activation of the amygdala-connectivity network after stress may be informative for the process of psychological trauma etiology. Under pathological conditions of severe stress similar mechanisms may lead to the (NE-dependent) over-consolidation of stress or fear memories (Pitman, 1989; Rauch et al., 2006) and/or a chronic state of hypervigilance. Additionally, the results may be meaningful in relation to known individual differences in stress responsivity determined by genetic and epigenetic factors (Cousijn et al., 2010; de Kloet et al., 2005; de Quervain et al., 2007). Phenotypically vulnerable individuals that show a failure to adequately contain and timely constrain an initialized stress response may be specifically at risk to develop traumatic illness through pathologically prolonged activation of similar stress circuits after trauma (Yehuda and LeDoux, 2007). Alternatively, the development of trauma after real-world stress may be associated with a pattern of brain activation and physiological changes that differs qualitatively, rather than quantitatively, from this relatively mildly stressful state as it can be induced experimentally in healthy volunteers.

A possible limitation of this study is that the resting-state run is (the concluding) part of a larger fMRI session with multiple tasks (see methods). These tasks however, were identical between the two conditions of interest (stress induction vs. control condition). Theoretically, we can not exclude the possibility of an interaction between the stress effects on these tasks and the resting-state connectivity. Importantly though, the only task with potential spatial overlap in activation patterns (an emotional facial processing task) was maximally separated from the resting-state run in time. Simultaneously, one could consider these different cognitive and

affective operations during the acutely stressful context as necessary to model any true aftermath of acute stress in real world situations. A second possible limitation is that because of our ROI analysis (choosing amygdala as a seedregion and dACC, AI, and LC as ROI's) we can not exclude that stress also induced changes in additional brain regions that are not covered by our spatially restricted analysis. However, seeding from amygdala seems justified in the context of stress (Joëls and Baram, 2009) and the very limited amount of additional activation clusters that survived the lenient threshold of  $p < 0.001$ , uncorrected, in the stress vs. control contrast (Table 1), emphasizes the selectivity of the results.

In sum, our data show that the direct aftermath of experimentally induced, moderate stress is characterized by the sustained activation of amygdala-centered stress circuits. Prolonged functional coupling within this network suggests an extended state of hypervigilance that may facilitate protracted salience and mnemonic processing after stress. Characterization of post-stress network changes in humans may represent a first step towards understanding the early phase of psychological trauma etiology.

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