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Phasic deactivation of the medial temporal lobe enables working memory processing under stress

Helena Cousijn [a,b], Mark Rijpkema [a], Shaozheng Qin [a,c], Guido A. van Wingen [a,d], Guillén Fernández [a,e,*]

[a] Radboud University Nijmegen, Donders Institute for Brain, Cognition and Behavior, 6500 HB Nijmegen, The Netherlands
[b] Department of Psychiatry, Warneford Hospital, University of Oxford, Oxford, UK
[c] Department of Psychiatry and Behavioral Science, Stanford University School of Medicine, Palo Alto, CA 94303, USA
[d] Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[e] Radboud University Nijmegen Medical Centre, Department of Cognitive Neuroscience, 6500 HB Nijmegen, The Netherlands

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ABSTRACT

Demanding cognitive tasks are sometimes carried out under stressful conditions. Several studies indicate that whereas severe stress impairs performance, moderate stress can enhance cognitive performance. In this study, we investigated how moderate stress influences the neural systems supporting working memory. We embedded an N-back working memory task in a moderately stressful context, as indicated by our physiological stress measures, and probed phasic and tonic human brain activity using two fMRI-techniques: conventional blood oxygen level dependent fMRI and arterial spin labeling (ASL). The results showed that the stress induction, as compared to the neutral control condition, led to slightly faster reaction times without changes in accuracy. In general, working memory processing was associated with increased activity in a frontoparietal network and reduced activity in the medial temporal lobe (MTL). The stress induction led to enhanced reduction of phasic MTL responses, specifically the hippocampus and amygdala. In addition, ASL showed that stress increased tonic amygdala activity, while tonic hippocampal activity was unaffected. These findings suggest that the influence of stress on MTL deactivation during working memory processing is task-related rather than a general consequence of the stressful state. The temporal suspension of hippocampal processing in favor of more task relevant processes may allow subjects to maintain normal performance levels under moderate stress.

Introduction

Demanding cognitive tasks are sometimes carried out under stressful conditions. Several studies indicate that whereas severe stress impairs performance (Aston-Jones et al., 1999), moderate stress can be beneficial (Lewis et al., 2008; Duncko et al., 2009; Weerda et al., 2010). While the effects of supraoptimal levels of catecholamines on the prefrontal cortex appear to mediate impaired cognitive performance (Arnsten and Li, 2005), little is known about the neural mechanisms that enable normal or even better performance under stressful conditions.

For cognitively demanding tasks, working memory (WM) processes are needed to maintain and manipulate relevant information in a temporary buffer that is constantly updated to guide behavior (Baddeley, 2003). Usually, WM processes are accompanied by increased fronto-parietal activity (Fletcher and Henson, 2001) and by deactivations of the so-called default-mode network (DMN), including the medial temporal lobe (MTL) (Callicott et al., 2000; Egan et al., 2003; Meyer-Lindenberg et al., 2001). This shift in balance between the DMN and fronto-parietal network is assumed to support reallocation of available neuronal resources to regions required for task-specific processing (Khalili-Mahani et al., 2010).

The brain's response to stress is orchestrated by the action of neuromodulators, mainly the fast-acting catecholamines norepinephrine and dopamine and the more slowly acting hormone cortisol (McEwen, 2007). Catecholamine receptors are distributed throughout the brain including the MTL (Sara, 2009) and cortisol receptors are abundant in the amygdala and hippocampus, which makes these structures particularly sensitive to the effects of stress (De Kloet et al., 1998). It has been shown that when stress is induced with a challenging mental arithmetic task, MTL activity decreases (Pruessner et al., 2008).

Previous studies that specifically investigated the effects of stress on WM-processing observed mixed results (Duncko et al., 2009; Oei et al., 2006; Porcelli et al., 2008; Qin et al., 2009), which might in part be caused by differences in stress levels due to variability in subject population and the stress induction procedures used. However, the results of some studies suggest that maintaining performance during stress might depend on the ability to suppress MTL activity. DMN deactivation predicts better working memory performance (Anticevic et al., 2010b) and stress induction during cognitive tasks leads to further hippocampal deactivation

* Corresponding author at: Radboud University Nijmegen, Donders Institute for Brain, Cognition and Behavior, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Fax: +31 24 36 10989.
E-mail address: g.fernandez@donders.ru.nl (G. Fernández).

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(Khalili-Mahani et al., 2010; Weerda et al., 2010). The amygdala appears to regulate the effect of stress on WM (Roozendaal et al., 2004) and lower amygdala responses during a WM-task predict better WM performance (Anticevic et al., 2010a).

Here, we investigated the influence of stress on the neural mechanisms supporting WM. To this end, we induced moderate stress by showing strongly aversive (vs. neutral) movie clips, and assessed the effect of our stress manipulation by measuring heart rate as well as salivary cortisol and α-amylase levels. We used conventional BOLD fMRI to measure how stress modulates phasic neural responses during an N-back WM task. Furthermore, as stress has been shown to change tonic activity in both the amygdala and the hippocampus (Cousijn et al., 2010; Peres et al., 2007; Tillfors et al., 2001), we also measured changes at a slightly slower time-scale by studying brain perfusion using continuous arterial spin labeling (CASL). The use of these two MRI techniques allowed us to disentangle task-related and state-related changes in brain activity and investigate whether stress effects on WM are the result of the stressful brain state or are directly related to altered, task-related WM processing.

Materials and methods

Participants

Forty-one healthy young men (aged 18–35 years) with normal or corrected-to-normal vision participated in this study. Only men were included because hormonal fluctuations across the menstrual cycle and hormonal contraceptives may influence the stress response (Kirschbaum et al., 1999; Ossewaarde et al., 2010). Participants reported no history of psychiatric, neurological, or endocrine diseases and no current use of psychoactive drugs or corticosteroids. All had participated in previous MRI experiments to ensure that no stress response would be evoked by unfamiliarity with the environment and procedures. Written informed consent was obtained before the experiment. The study was carried out in accordance with the guidelines of the local ethical review board (CMO Region Arnhem-Nijmegen, The Netherlands) and in accordance with the declaration of Helsinki. Data of one participant were excluded due to technical failure and MRI data of another participant were excluded due to excessive head movement during scanning.

General procedure

On the day of the experiment, all subjects were scanned while completing the N-back task in a state of stress and in a neutral condition. The order of the stress and neutral conditions was counterbalanced across subjects. In the week preceding the experiment, participants were contacted by phone and answered a set of questions that formed the exclusion criteria for this experiment. After the phone call, participants were sent a salivette collection device (Sarstedt, Rommelsdorf, Germany) and were asked to take a baseline saliva sample in the late afternoon on the day before the experiment. The experiment itself took place in the afternoon or early evening to ensure low and relatively stable levels of endogenous cortisol.

Upon arrival, participants had the experiment explained to them, completed the trait version of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970), and practiced the N-back task. In the scanner, they completed both the stress and neutral condition. Each of these conditions consisted of the N-back task, which was presented in between stressful or neutral movies (Fig. 1). Immediately after the last movie, perfusion was measured with CASL (Wang et al., 2005b). Before and after the stress and control condition, saliva samples and subjective affect ratings were collected and heart rate was measured throughout the experiment. The two conditions were separated by approximately 20 min in which a structural MRI scan was made for anatomical normalization purposes.

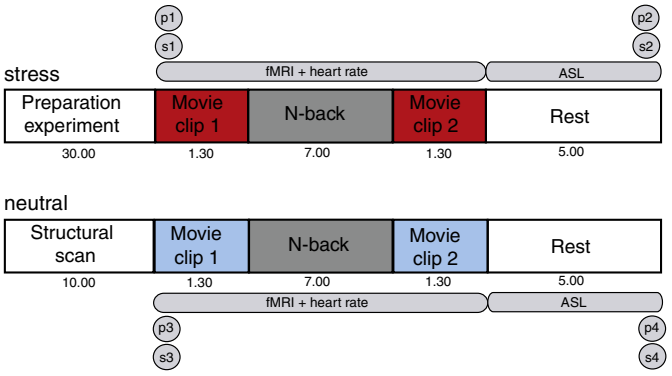

Fig. 1. Overview and timeline of the experimental design. The upper part of the figure shows the stress condition with the red blocks representing stress movies, while the lower part shows the neutral condition with the blue blocks representing the neutral movie clips. However, in the study these orders were counterbalanced so that for half the participants the neutral condition was the first condition. Preparation took place outside the scanner. Before the first movie clip, participants completed an amygdala activation task, as described in Cousijn et al., 2010. Therefore, the N-back task took place approximately 8 min after the first stress induction. The numbers below the movies and task indicate the time in minutes, P = PANAS, S = saliva sampling. Acquisition of each PANAS and each saliva sample took 5 min, thereby creating a 20 minute gap between the conditions.

Stress induction

Psychological stress was induced by showing short movie clips within the MRI scanner containing scenes with strongly aversive content (extreme violence) selected from a commercially available movie (Irréversible, 2002, by Gaspar Noé). The selected clips appeared in the movie at time points 22.30, 39.40, 42.20, and 53.30 min. During the control condition, participants watched 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 but contained only emotionally neutral and non-arousing scenes. After short introductory texts, participants were asked to watch the movies attentively and take an eye-witness perspective as to involve them maximally in the action taking place in the movie clips. The present stress induction procedure closely corresponds to the determinants of the human stress response as described by Mason (1968), i.e., unpredictability, novelty, and uncontrollability. Moreover, previous studies have shown that this method elicits a measurable physiological and psychological stress response (Cousijn et al., 2010; Qin et al., 2009; van Marle et al., 2009).

N-back task

Using a blocked design, participants completed 6 cycles of alternating 0- and 2-back conditions interleaved with a jittered resting-fixation baseline ranging from 8 to 12 s (average 10 s). Within each block, a random digit sequence consisting of 15 single digits was shown to participants. Each digit was presented for 400 ms, followed by an interstimulus-interval of 1400 ms. Each block lasted 27 s and started with a 2 s cue presentation indicating the 0- or 2-back condition. During the 0-back condition, participants were asked to detect whether the item currently presented on the screen was a "1". During the 2-back condition, participants were asked to detect whether the current item had appeared two positions back in the sequence. Participants were instructed to make a button press as quickly and accurately as possible when detecting a target. Before fMRI scanning, participants were trained (with 6 cycles of alternating 0- and 2-back) to minimize interindividual variability and reduce practice effects. N-back accuracy was calculated by the proportion of hits to misses and false alarms.

Psychological and physiological measurements

Subjective mood was assessed by obtaining scores on the Positive And Negative Affect Scale (PANAS) (Watson et al., 1988) before and after the stress and the control condition (four in total). Ten items for positive and ten for negative affect had to be rated on a five-point scale ranging from 1—not at all to 5—extremely. Individual mean scores were calculated for subjective negative affect.

To assess the autonomic response and the HPA-axis response to the context manipulation, saliva was sampled with salivette collection devices to determine the levels of α-amylase and cortisol. Samples were taken on the day before the experiment and before and after both conditions (five in total) and were stored at −20 °C until analysis. The analysis was carried out at the Biopsychology department in Dresden, Germany, as described by Rohleder et al. (2006). For one subject no data were acquired and for one subject the analysis did not succeed, while data of a third subject were not taken into account because he consumed caffeine shortly before the experiment.

To assess autonomic activity throughout the experiment, we continuously recorded heart rate with an infrared pulse oximeter (accompanying the MRI scanner, Siemens, Erlangen, Germany) placed on a finger of the left hand. Offline artifact correction and analysis of the heart rate frequency was done with in-house software written in Matlab 7.6. The heart rate was averaged for the duration of each movie clip and the task. For one subject, data were not available.

fMRI data acquisition

During both the stress and the control condition, whole brain T2*-weighted blood oxygenation level-dependent (BOLD) fMRI data were acquired using echo-planar imaging (EPI) with a Siemens TIM Trio 3.0 Tesla MR-scanner using an ascending slice acquisition sequence (37 axial slices, volume repetition time (TR) = 2.18 s, echo time (TE) = 25 ms, flip angle = 90°, slice matrix size = 64 × 64, slice-thickness = 3.0 mm, slice gap = .3 mm, field of view (FOV) = 212 mm). Two hundred and six images were acquired during each N-back task. These parameters were chosen to optimize detection of MTL activity and therefore the repetition time was not synchronized to the trial length.

At the end of both conditions, resting-state CASL data were recorded with an ascending slice acquisition sequence (labeling time = 2 s, post label delay time = 1 s, label offset = 8.0 cm, TR = 3.69 s, TE = 11 ms, flip angle = 90°, matrix size = 64 × 64, slice thickness = 5 mm, slice gap = 1.5 mm, FOV = 224 mm, bandwidth = 2694 Hz per pixel). Eighty images were acquired for each participant in each condition. For three participants, no CASL data were acquired due to technical failure.

High-resolution structural images were acquired using a T1-weighted three dimensional magnetization-prepared rapid gradient echo (MP-RAGE) sequence (TR = 2.3 s, TE = 3.03 s, flip angle = 8°, 192 contiguous sagittal slices, slice matrix size = 256 × 256, FOV = 256 mm).

fMRI data analysis

Image preprocessing and statistical analysis of the BOLD fMRI data was performed using SPM5 (Wellcome Department of Imaging Neuroscience, London, UK). The first five EPI volumes were discarded to allow for T1 equilibration. Remaining functional images were realigned with rigid body transformation and coregistered to the anatomical T1-weighted MR-image. Subsequently, images were transformed into a common stereotactic space (MNI152 T1-template) and resampled into $2 \times 2 \times 2$ mm^3 isotropic voxels. Spatial smoothing was performed with an isotropic 3D Gaussian kernel of 8 mm full-width at half-maximum.

Statistical analysis was performed within the framework of the general linear model. The two N-back conditions were modeled separately as boxcar regressors and convolved with the canonical hemodynamic response function of SPM5. Additionally, realignment parameters were included to model potential movement artifacts. Contrast parameter images generated at the single subject level for both conditions relative to baseline were submitted to second level group analysis. This group analysis was a 2 (WM-load: 0-back vs. 2-back) × 2 (stress induction: stress vs. neutral) within-subject analysis of variance. We chose to look at direct comparisons of the 0-back and 2-back tasks because comparing demanding cognitive processing to a low level cognitive task that is matched for all visual and motor aspects, rather than rest, allows you to observe more specific patterns of brain activity. Statistical tests were corrected for multiple comparisons across the entire brain by using the family-wise error (FWE) correction as implemented in SPM based on random field theory at a threshold of $p = .05$. Given our a priori interest in the MTL, we specifically investigated the amygdala and hippocampus using a small volume correction (SVC) (Worsley et al., 1996). These regions were anatomically defined using the WFU Pickatlas (Maldjian et al., 2003).

Preprocessing of the CASL-data was carried out with the SPM-based ASL perfusion fMRI data processing toolbox using standard settings (Wang et al., 2008). Images were realigned and spatial smoothing was applied with a 3D isotropic kernel with 9 mm full-width at half-maximum, followed by image coregistration between the raw EPI and structural images. Perfusion difference images were calculated by pair-wise subtraction of the label/control image pairs. The amygdala and hippocampus activation clusters that were observed in the stress by WM load-interaction of the conventional BOLD fMRI analysis were extracted at a threshold of $p < .001$ uncorrected. These clusters were defined as regions of interest (ROIs) and were then transformed back into the anatomical space for each individual subject, thereby creating ROIs for all individual participants. Mean perfusion data were extracted for these ROIs and subjected to paired-samples t-tests to determine differences in perfusion between the stress condition and neutral condition.

Results

Psychological and physiological stress measures

To establish whether the stress induction was successful, heart rate was recorded throughout the stress and control conditions and measures of salivary cortisol, salivary α-amylase, and subjective negative affect ratings were taken before and after each experimental condition. Heart rate data were analyzed with a 3 (time points) × 2 (stress vs. neutral) repeated measures ANOVA across averaged measures during the N-back task and its two surrounding movie clips. We found a main effect of stress ($F(1,76) = 46.62$, $p < 0.001$), and further t-tests confirmed that heart rate was consistently higher during all three phases of the experiment in the stress condition (all $t(38) > 2.29$, $p < .03$). Stress by time interactions were found for salivary cortisol ($F(1,36) = 4.46$, $p = .042$), salivary α-amylase ($F(1,36) = 7.97$, $p = .008$), and negative affect ($F(1,39) = 20.43$, $p < .001$). Increases were observed during the stress condition (mean cortisol (nmol/l): from 7.68 to 8.32; mean α-amylase (U/l): from 47.77 to 55.30; mean negative affect: from 12.85 to 16.29), while decreases were observed during the neutral condition (mean cortisol: from 7.97 to 6.39; mean α-amylase: from 58.06 to 49.36; mean negative affect: from 13.23 to 12.55). All these measures indicate that our stress induction procedure led to a significant but, when compared to real-life stress situations (Chatterton et al., 1997; Morgan et al., 2000), relatively moderate increase in stress levels. Therefore, we assume the stress induced in our experiment to be moderate rather than severe.

N-back performance

Repeated-measures ANOVAs were carried out on both accuracy data and reaction times with WM load (0 vs. 2) and stress (stress vs. neutral) as within-subject factors. A main effect of stress on reaction times was observed ($F(1,39) = 5.16$, $p = .029$), indicating that

participants were faster to respond to the stimulus when they were stressed (stress 898 ms; neutral 916 ms). The ANOVA on reaction time data did not reveal a stress by load interaction ($p = .59$), indicating that stress improved reaction times in both the 0-back and 2-back condition. There was a robust main effect of WM load on reaction times ($F(1,39) = 77.50$, $p < .001$), with participants responding faster in the 0-back condition (0-back 847 ms; 2-back 967 ms).

The ANOVA on the accuracy data revealed no interaction between stress and WM load ($p = .77$), but did show a robust main effect of WM load ($F(1,39) = 34.4$, $p < .001$), indicating that in both the stress and neutral condition participants were more accurate in the 0-back condition (0-back 0.97; 2-back 0.85). There was no main effect of stress ($p = .39$: stress 0.91; neutral 0.90). The faster responses under conditions of stress, without a loss of accuracy, form an indication that the moderately stressful conditions created here could enable performance. However, the actual effect of stress on processing speed was medium (Cohen's $d = .404$), so other mediating factors might also be responsible for the reduction in reaction times.

BOLD fMRI results

First, by contrasting 2- with 0-back conditions, we replicated robust activations in the frontoparietal network (Figs. 2a, b) consisting of the bilateral dorsolateral prefrontal cortex (DLPFC) (local maxima at [36, 40, 28] and [−32, 52, 16], $p < .05$, FWE-corrected) extending into the middle and inferior PFC, the bilateral parietal inferior cortex (local maxima at [−38, −42, 46] and [44, −40, 46], $p < .05$ FWE-corrected), and cerebellum (local maxima at [−32, −58, −30] and [28, −60, −28], $p < .05$, FWE-corrected).

Furthermore, WM tasks, and in particular N-back tasks, have been shown to lead to deactivation of a set of regions called the DMN (Hampson et al., 2006). To assess these deactivations we contrasted the 0-back with the 2-back condition. This analysis revealed WM related deactivation in a set of regions (Fig. 2c) including the hippocampus extending into the amygdala (local maxima at [−28, −36, −14] and [28, −34, −14], $p < .05$, FWE-corrected), the ventral medial PFC (local maxima at [−2, 62, 12] and [−8, 64, 20], $p < .05$, FWE-corrected), insula (local maxima [−38, −6, −8] and [40, 12, 4], $p < .05$, FWE-corrected) and posterior cingulate cortex (local maximum[−2, 50, −6]), $p < .05$, FWE-corrected). These deactivations were specific for the 2-back condition, because contrasting 2-back with fixation revealed a similar pattern, while contrasting 0-back with fixation did not show deactivations in any of these areas.

More important for the question under investigation, the results revealed a stress by WM load-interaction in both the left hippocampus (MNI coordinates: −26, −38, −4], $Z = 3.73$, $p = .002$ (SVC)) and the left amygdala (MNI coordinates: [−26, 2, −18], $Z = 2.81$, $p = .033$ (SVC)). In both structures, the WM related deactivation was modulated by stress, so that the deactivation was relatively stronger in the stress than in the neutral condition (Fig. 3). This pattern of results suggests that maintaining normal or even optimizing WM performance under stressful conditions is associated with increased deactivation of the MTL system.

To explore individual differences, we looked at possible correlations of the observed interactions with trait anxiety as measured with the STAI, but did not observe any significant correlations (amygdala $r = -.187$, $p = .351$; hippocampus $r = .085$, $p = .608$). One potential explanation for these negative findings is that the amount of stress-induced deactivation of the MTL is not linearly related to this state anxiety measure.

CASL fMRI results

Conventional BOLD fMRI is a measure of phasic neural responses, but stress also leads to more slowly modulated state changes and might therefore, next to phasic responses, affect tonic activity in the MTL (Tillfors et al., 2001; Wang et al., 2005a). Thus, we probed such stress-induced changes in tonic MTL activity by measuring regional brain perfusion using another fMRI technique, continuous ASL, as a correlate of tonic activity. We extracted perfusion data, calculated by pairwise subtraction of the label/control image pairs, from the clusters of hippocampus and amygdala activation found with conventional fMRI by creating ROIs for each individual participant. The results showed an increase in tonic amygdala perfusion during the stress condition as compared to the neutral condition ($t(35) = 2.39$, $p = .02$), but no significant difference in tonic activity in the hippocampus ($t(35) = -1.2$, $p = .24$). Thus, tonic amygdala activity was affected by stressful environmental conditions, while tonic hippocampal activity appeared unaffected (Fig. 4). If anything, hippocampal activity was slightly higher in the neutral condition than in the stress condition.

Together with our conventional fMRI findings, these results form an indication that different mechanisms underlie the stress-enhanced WM-related deactivation in the hippocampus and amygdala. Whereas stress enhances phasic WM-related decreases in the hippocampus but not baseline perfusion, the WM-related deactivation in the amygdala should be interpreted in light of a tonically increased baseline. To assess whether phasic deactivation from the increased baseline only occurs under conditions of high load or already during more basic task processing, we analyzed our BOLD fMRI data further. This analysis revealed that amygdala activity for the contrast between rest and 0-back was not significantly different between the stress and neutral condition ($p > .05$, SVC), while the 2-back vs. rest contrast revealed a significant difference in activity between the stress and neutral condition (MNI coordinates: [−26, 2, −18], $Z = 3.05$, $p = .017$ (SVC)). This

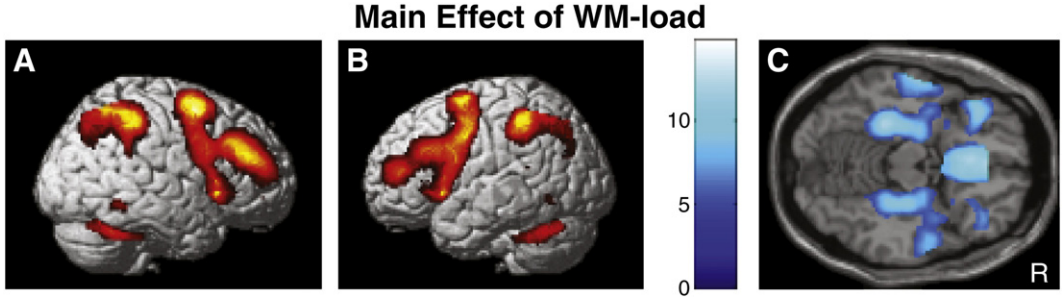

Fig. 2. Brain activations observed in the N-back WM task, thresholded at $p < .05$ whole-brain family-wise error corrected. A) Render image displaying brain regions on the right side of the brain that showed more activation during the 2-back than the 0-back task. B) Render image displaying brain regions on the left side of the brain that showed more activation during the 2-back than 0-back task. C) Transversal view of the brain ($z = 14$) showing brain areas that were deactivated with increased WM load. The image has been superimposed on a single-subject T1-weighted image.

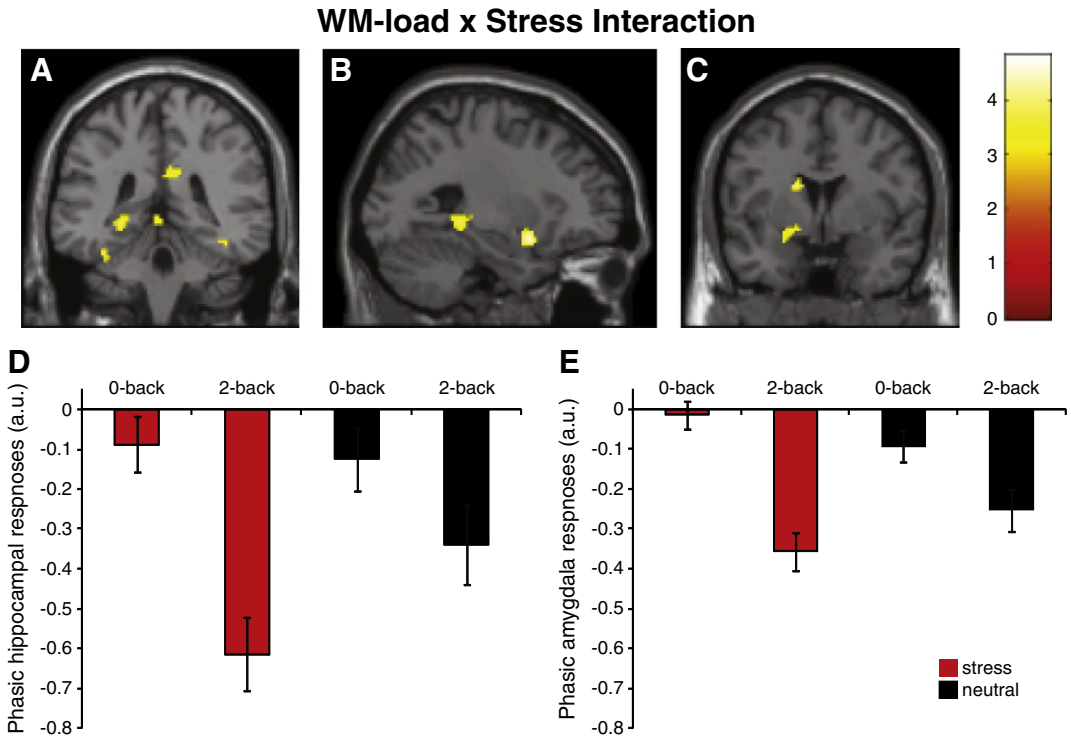

Fig. 3. The deactivation of the hippocampus and amygdala is modulated by stress. A) Coronal view of the left hippocampus showing the stress by WM-load interaction ($y = -38$). B) Axial view of both the hippocampus and amygdala showing the stress by WM-load interaction ($x = -26$). C) Coronal view of the left amygdala showing the stress by WM-load interaction ($y = 2$). The bar graphs demonstrate how both the hippocampus (left) and amygdala (right) become more deactivated during a state of stress. The figures show the statistical comparisons ($p < .001$ uncorrected for visualization purposes) superimposed on a single-subject T1-weighted image.

indicates that the proposed deactivation of the amygdala to compensate for an increase in baseline might only be required under conditions of high cognitive demand.

Discussion

The results of this study show that faster WM performance under moderate stress is accompanied by an augmented reduction of phasic activity in the hippocampus and amygdala. This suggests that reduced MTL activity enables working memory performance under conditions where coping with stress is possible. We speculate that the phasic deactivation might reduce interfering influences relayed by the MTL and thereby allow subjects to maintain or even optimize performance on cognitively demanding tasks. Furthermore, our combination of imaging techniques allows us to demonstrate that there is no detectable change in tonic hippocampal activity, suggesting that these results are related to WM-processing and not to general stress-induced changes in baseline brain activity. In contrast to the hippocampus, perfusion measurements showed that stress did increase tonic activity in the amygdala. This observed increase is in line with previous studies addressing stress-induced changes in tonic amygdala activity (Tillfors et al., 2001) and indicates that the phasic WM-related deactivations in the amygdala might be related to suppression of high baseline tonic activity (Aston-Jones et al., 1999).

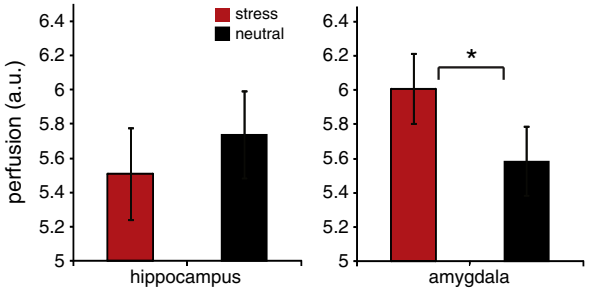

Fig. 4. The effects of stress on perfusion in the amygdala and hippocampus. While there is no significant difference between perfusion in the stress condition and neutral condition in the hippocampus (left), there is a significant increase in perfusion in the stress condition in the amygdala (right) (a.u., arbitrary units).

The reasons for the phasic deactivations might be found in theories concerning DMN activity. It has been proposed that the brain functions in a default mode that can be changed by task demands (Raichle et al., 2001). In this default mode state, there is sustained ongoing information processing in a network of brain structures (Gusnard et al., 2001). When cognitive demands are placed on the organism, specific brain functions unique to the baseline state are temporarily suspended (Raichle et al., 2001). This has specifically been shown to occur under conditions of increased WM-load (Esposito et al., 2006).

The redistribution of resources from task-irrelevant brain regions toward task-relevant brain regions has been demonstrated to be important for maintaining WM-performance (Pomarol-Clotet et al., 2008). Under conditions of stress or emotional distraction, this redistribution becomes even more crucial. Dolcos and McCarthy (2006) showed in their study how limbic processing of task-irrelevant emotional stimuli interferes with performance on a WM-task. Anticevic et al. (2010a) extended these findings by showing that negative interference is associated with decreased prefrontal and increased limbic activity. These studies differ from the present study in that emotional distracters were presented during the task rather than stress induced before the task. However, they form an illustration of how dual processing of cognitively demanding as well as negative information might require a reallocation of brain resources. Therefore, under conditions of stress that are still present during the cognitive task, as indicated by our physiological measures, the limbic system might require deactivation. In our study, this deactivation was accompanied by a slight decrease in reaction times without a loss of accuracy, indicating that suppression

of MTL activity might prevent interference with performance. This is in line with previous studies indicating that the deactivation of task-irrelevant regions leads to better WM-performance (Anticevic et al., 2010b).

The slight, stress-induced facilitation of WM-processing might be due to the fact that our movie clips induced only a moderate stress response, as well as our choice of N-back task. In a study with a similar set-up (Gray et al., 2002) in which a 3-back task was used, it was found that the induction of a negative emotional state enhances spatial working memory and impairs verbal working memory. In our experiment, with a numerical N-back task, we did no replicate their findings of clear enhancement and impairment. Furthermore, all participants in our experiment had experience with MRI-experiments, thereby avoiding the induction of additional stress related to the novelty of the experimental procedure (Tessner et al., 2006). Additionally, all participants in this experiment were male, who have been shown to respond differently to stressors than females (Bale, 2006; Kudielka and Kirschbaum, 2005). These factors might also explain why we did not observe a change in DLPFC activity which has been reported previously (Qin et al., 2009). However, that study indicated that more severe stress is correlated with an increase in reaction times as well as a reduction of DLPFC activity. In another study (Weerda et al., 2010), it was found that increased DLPFC activity is associated with better performance. Taken together, these studies suggest that a stress-related maintenance or improvement of WM performance is associated with a reduction of task-irrelevant activity in the MTL, whereas a stress-related impairment of WM performance is associated with a reduction of task-relevant activity in the DLPFC. However, as there was no manipulation of stress severity in this study, additional studies in which moderate as well as more severe stress is induced are required to test this hypothesis.

Two neurochemical mechanisms might contribute to the pattern of activation seen in this study. First of all, WM processing has been shown to depend on catecholaminergic signaling (Chamberlain et al., 2006), with stimulation of specific classes of receptors being crucial for working-memory performance (Wang et al., 2007). However, only at moderate levels do catecholamines have beneficial effects on prefrontal functioning, whereas high concentrations are detrimental (Arnsten and Li, 2005). It has been proposed that increased norepinephrine facilitates reorganization of functional networks in the brain, which might be a dynamic mechanism coordinating prefrontal and MTL functioning under conditions of stress (Bouret and Sara, 2005). Additionally, the effects of stress on the brain are mediated by cortisol (Kloet de et al., 2005). While catecholamines optimize rapid adaptive behavior, cortisol has both rapid effects via membrane-located mineralocorticoid receptors (Karst et al., 2005) and induces a slower cascade that is thought to prevent activation (Joëls and de Kloet, 1989) and impair functioning (Pavlides et al., 1995) of MTL structures. Studies administering cortisol in humans have also shown that cortisol can lead to a rapid deactivation of the amygdala and hippocampus. (Henckens et al., 2010; Lovallo et al., 2010). This way, catecholamines and cortisol work in concert to optimize the use of resources during acute stress (Roozendaal et al., 2006).

Previous studies suggested that different mechanisms may underlie stress-induced deactivations of the hippocampus and amygdala (Pruessner et al., 2008). The use of two imaging techniques that measure phasic and tonic activity independently allowed us to provide support for this suggestion. In the hippocampus, phasic activity was suppressed independent of detectable changes in tonic activity. Although our experiment might not have been powerful enough to detect a change in baseline activity, the slight trend toward a decrease in baseline activity in the hippocampus in the stress condition indicates that the mechanism is unlikely to be the same as in the amygdala, where the phasic responses might at least be partly due to suppression of the enhanced tonic activity (Aston-Jones et al., 1999). One could speculate that the deactivations observed in the hippocampus temporarily suppress memory retrieval, while deactivations of the amygdala might suppress stress-induced fear processing. When these suppressions are effective, subjects are able to perform.

In conclusion, our findings show that WM processing during moderate stress is associated with enhanced deactivation of the MTL, presumably to suppress task-irrelevant activity. In the hippocampus, this deactivation appears directly related to the task, because no state-related changes could be detected with our measure of tonic activity. In the amygdala, tonic activity increased rather than decreased, so the reduction in phasic responses observed there could be related to an increase in tonic baseline activity. Our study underlines the importance of addressing both tonic and phasic changes when employing a stress manipulation for a comprehensive interpretation. Most importantly, together with results from other studies, our results provide an indication that with moderate levels of stress an adaptive reallocation of resources away from the MTL enables performance on cognitively demanding tasks.

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