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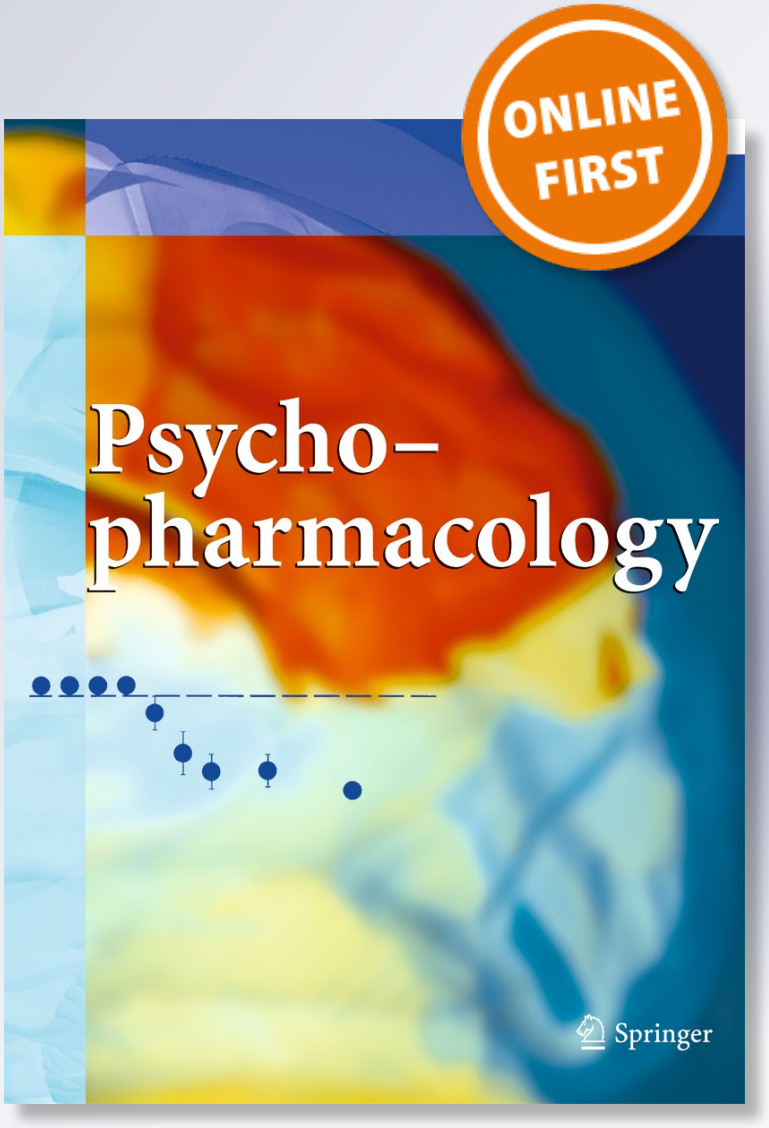

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ORIGINAL INVESTIGATION

ADRA2B genotype differentially modulates stress-induced neural activity in the amygdala and hippocampus during emotional memory retrieval

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Abstract
Rationale Noradrenaline interacts with stress hormones in the amygdala and hippocampus to enhance emotional memory consolidation, but the noradrenergic-glucocorticoid interaction at retrieval, where stress impairs memory, is less understood.
Objectives We used a genetic neuroimaging approach to investigate whether a genetic variation of the noradrenergic system impacts stress-induced neural activity in amygdala and hippocampus during recognition of emotional memory.
Methods This study is based on genotype-dependent reanalysis of data from our previous publication (Li et al. Brain Imaging Behav 2014). Twenty-two healthy male volunteers were genotyped for the *ADRA2B* gene encoding the α2B-adrenergic receptor. Ten deletion carriers and 12 noncarriers performed an emotional face recognition task, while their brain activity was measured with fMRI. During encoding, 50 fearful and 50 neutral faces were presented. One hour later, they underwent either an acute stress (Trier Social Stress Test) or a control procedure which was followed immediately by the retrieval session, where participants had to discriminate between 100 old and 50 new faces.
Results A genotype-dependent modulation of neural activity at retrieval was found in the bilateral amygdala and right hippocampus. Deletion carriers showed decreased neural activity in the amygdala when recognizing emotional faces in control condition and increased amygdala activity under stress. Noncarriers showed no differences in emotional modulated amygdala activation under stress or control. Instead, stress-induced increases during recognition of emotional faces were present in the right hippocampus.
Conclusion The genotype-dependent effects of acute stress on neural activity in amygdala and hippocampus provide evidence for noradrenergic-glucocorticoid interaction in emotional memory retrieval.

Keywords Acute stress · Memory retrieval · Emotional memory · fMRI · *ADRA2B* · Amygdala · Hippocampus

Abbreviations

fMRI	Functional magnetic resonance imaging
BOLD	Blood oxygenation level dependent
BMI	Body mass index
TSST	Trier Social Stress Test
ISI	Inter-stimulus-interval
MDBF	Multidimensional Mood State Questionnaire
EPI	Echo planar imaging
TR	Time to repeat
TE	Time to echo
MPRAGE	Magnetization prepared rapid acquisition gradient echo
FWHM	Full-width-half-maximum

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HRF	Hemodynamic response function
FWE	Family-wise error
ROIs	Regions of interest
IPC	Inferior parietal cortex
NA/A	Noradrenaline and adrenaline
PTSD	Posttraumatic stress disorder
LTL	Left temporal lobectomy
RTL	Right temporal lobectomy

Introduction

Genetic variations linked to noradrenergic neurotransmission have been shown to contribute to individual differences in emotional memory and responsiveness to acute stress. A common deletion of the *ADRA2B* gene which codes for the presynaptic noradrenergic α2B receptor has been found to act as a loss-of-function polymorphism that prevents receptor adaptation to agonist stimulation and impacts emotional memory (de Quervain et al. 2007b; Small et al. 2001). Behavioral studies provide evidence that the functional deletion is linked to enhanced memory for emotional items (de Quervain et al. 2007b). It has been suggested that enhancements in emotional memory in deletion carriers may be related to higher arousal and better visual processing of negative emotional items at encoding (Todd et al. 2013a, b). One neural mechanism that may contribute to enhanced emotional arousal in deletion carriers is increased amygdala responsiveness. Functional magnetic resonance imaging (fMRI) data suggest that carriers of the deletion variant exhibit a higher blood oxygenation level dependent (BOLD) response in the amygdala and increased amygdala–insula coupling during encoding of pictures with negative emotional valence (Rasch et al. 2009). There is also evidence that the *ADRA2B* deletion modulates amygdala processing under acute stress. Cousijn et al. (2010) induced stress by showing short movie clips with highly aversive content and were able to demonstrate that further increases in amygdala responses to dynamically morphing emotional faces were only observed in deletion carriers, which suggests that stress modulates the processing of emotional stimuli in a genotype-specific way.

The latter finding is in line with a large body of evidence that indicates that the effects of stress on emotional memory depend on an interaction of glucocorticoids with the noradrenergic system in the basolateral amygdala which subsequently impacts neural activity in other brain regions, including the prefrontal cortex and hippocampus (McGaugh et al. 1996; Roozendaal et al. 2009b). Although most studies have focused on noradrenergic-glucocorticoid mechanisms in memory consolidation, several lines of evidence indicate that such interactions are also crucial in mediating stress effects at retrieval. For example, administration of the β-adrenoceptor antagonist propranolol prevents stress-induced effects on memory retrieval (de Quervain et al. 2007a; Schwabe et al. 2009). Further, intra-amygdala infusions of propranolol blocked the impairing effect of a glucocorticoid receptor agonist infused into the hippocampus prior to memory retrieval (Roozendaal et al. 2004). In other words, noradrenergic activity in the basolateral amygdala modulates the effects of hippocampal glucocorticoids on memory retrieval. Along these lines, we have shown on behavioral level, that stress exposure prior to retrieval of emotional face memory impairs performance and that the *ADRA2B* deletion preserved emotional faces from this stress-induced memory impairment (Li et al. 2013).

The present study aimed to investigate whether the *ADRA2B* deletion impacts stress-induced neural activity in amygdala and hippocampus during the retrieval of emotional faces. Using a genetic neuroimaging approach, we reanalyzed previously published fMRI data of participants who performed an emotional face recognition memory task and were exposed to acute psychosocial stress prior to memory retrieval (Li et al. 2014). Genotype was included as between-subject factor to the original analysis to focus on individual differences in emotional memory retrieval under stress. Additionally, we also analyzed encoding-related neural activity prior to stress application to gauge genotype-dependent differences during encoding of emotional items. Participants were genotyped for the *ADRA2B* deletion, and data was grouped into deletion carriers and noncarriers. We hypothesized that elevated cortisol levels caused by acute psychosocial stress will change neural activity in amygdala and hippocampus in a genotype-specific way.

Methods

Participants

Participants were young, healthy men between 18 and 31 (24.25±0.75)years of age and a body mass index (BMI) between 18 and 25. None of them suffered from any acute or chronic disease, or took medication. The study was approved by the ethics committee of the University of Oldenburg, and participants provided written informed consent. The data of 27 participants were included into the behavioral analysis, and the data of 22 participants were included into the fMRI data analysis. Reasons for exclusion of MRI data were excessive movement (>4 mm) or incomplete coverage of the most ventral parts of the amygdala. We chose only men as participants mainly due to greater stability of hormone levels; also, a recent paper suggested that psychosocial stress has opposite effect on women and men in fear learning (Merz et al. 2013).

Design and procedure

We combined a standardized psychosocial stress protocol with a face recognition memory task which consisted of an encoding and retrieval phase, separated by 100 min (60-min break, 15-min psychosocial stress test, and additional time for structural scan, genetic probe and cortisol collection, mood self-ratings, and preparation for scanning). Scanning was performed during training (to familiarize participants with the task in the fMRI environment), encoding, and retrieval. A short delay between encoding and retrieval was used to decrease task difficulty. Participants underwent a stress or a respective control procedure in a within-subject design outside the scanner prior to the retrieval phase. Stress and control sessions were separated by 1 week (±1 day). The order of the stress and control condition was randomized across subjects. Testing took place between 9:00 a.m. and 3:00 p.m., and most of the participants were tested starting at 9:00 a.m. (four participants were tested at 12:00 a.m.). Physiological and mood self-rating data were collected at four time points. Each participant was tested at the same time of the day in the stress and control session to account for circadian rhythms in stress hormone levels. Different face stimuli were used in each session.

Psychosocial stress

We used the Trier Social Stress Test (TSST) (Kirschbaum et al. 1993) to induce psychosocial stress in a laboratory setting. After an anticipatory preparation period, participants had to perform a free speech in front of a committee (consisting of a man and a woman dressed in white coats) as a fictitious job interview, followed by a mental arithmetic task (counting backwards from 2,043 in steps of 17). Each of the three periods lasted 5 min, while participants were video- and voice-recorded. This protocol is a combination of social-evaluative threat and an uncontrollable situation, which is consistently associated with a significant cortisol increase in saliva and blood (Dickerson and Kemeny 2004). In the control condition, the participants were instructed to perform a free speech about a recently experienced journey and counting forwards from zero in steps of 15 in an empty room without committee or recording (Het et al. 2009). Participants were informed prior to their participation about their participation in both a stress and control procedure. They were told only prior to the preparation period of stress test whether they were in the stress or control procedure.

Face recognition memory task

We used a face recognition memory task since fearful face stimuli have been shown to strongly activate the amygdala and hippocampus (Dolcos et al. 2005). Fearful and neutral faces were selected from several databases, adjusted in luminance, and presented on a gray background. We created two sets of face stimuli. Whether set I or II was presented in the control or stress condition was counterbalanced across subjects and across sessions, and the pictures in each set were counterbalanced with previously rated emotionality, arousal, and picture quality, as well as the gender of the faces. For further description of the stimuli, see Li et al. (2013) and Li et al. (2014). During encoding, participants were presented randomly with 100 faces with either fearful or neutral facial expressions (fearful/neutral=1:1). Each face was displayed for 2.5 s, with an inter-stimulus-interval (ISI) ranging from 2.5 to 12.5 s where a baseline stimulus consisting of a scrambled face with a central fixation cross was presented. Participants used their index and middle fingers of the right hand to press one of two buttons to indicate the gender of the displayed face. They were told to encode the faces for later retrieval. We used the gender classification task to make sure that all participants paid a similar amount of attention to the displayed faces, without explicit judgment of emotion. Seventy minutes after encoding, participants were exposed to either the TSST or the control condition followed immediately by the recognition task. In the retrieval phase, participants were presented randomly with 150 fearful and neutral faces: 100 faces from the encoding session (old) and 50 new (fearful/neutral=1:1). Each face was displayed for 2.5 s, with an ISI ranging from 2.5 to 12.5 s during which the baseline stimulus was shown. Participants were instructed to discern old from new face by pressing the corresponding button (index or middle finger of the right hand). Prior to the encoding session, participants underwent a training phase for 15 min on both days inside the scanner. The training session consisted of a short encoding followed by a retrieval phase using a set of faces, which were not used during the real task. Accuracy and reaction time during encoding were analyzed for potential genotype effects (deletion/noncarriers) by means of independent *t* tests. Accuracy and reaction times at retrieval were analyzed with mixed-design ANOVAs with the within-subject factors stress condition (control/stress), emotion (fearful/neutral), and the between-subject factor genotype (deletion/noncarriers). Significant effects were followed by post hoc *t* tests. All statistical analyses were performed using SPSS 18.0 (SPSS GmbH, Munich, Germany).

Data acquisition and analysis

Physiological and subjective measures

Salivary concentrations of free cortisol, blood pressure, pulse, and subjective mood ratings were collected at four time points: (i) prior to the encoding phase, (ii) prior to the

TSST/control condition, (iii) directly afterward, and (iv) after completion of the retrieval session (i.e., approx. 40 min after TSST). Saliva was collected using Salivette collection devices (Sarstedt AG & Co., Nümbrecht, Germany), which were stored afterward at −20 °C until biochemical analysis. Biochemical analysis was performed by the lab of Prof. Dr. C. Kirschbaum, Dresden, Germany: salivary levels of free cortisol were measured using a luminescence immunoassay (IBL GmbH, Hamburg, Germany). Inter- and intra-assay variations were below 10 %. In two participants, cortisol values could not be determined due to technical problems in one of the sessions; hence, cortisol analyses rely on the data of 25 participants.

Affective responses were assessed with the German version of the Multidimensional Mood State Questionnaire (MDBF) (Steyer et al. 1997) after collection of saliva samples. The questionnaire consists of 24 items with a five-point rating scale each. These 24 items rely on three underlying dimensions: good mood–bad mood, alertness–tiredness, and calmness–nervousness.

Physiological and mood effects were analyzed with mixed-design ANOVAs with the factors time point (before TSST, after TSST, after scanning), stress condition (control/stress), and genotype. Significant effects were followed by post hoc *t* tests.

Genotyping

DNA was extracted from oral epithelium cells according to Walsh et al. (1991). Genotyping was performed according to Rasch et al. (2009) by the Institute for Polymorphism and Mutation Analysis, Homburg, Germany. One participant was a homozygous carrier of the *ADRA2B* deletion, 12 participants were heterozygous, and 14 were noncarriers. In the reduced fMRI dataset, one participant was a homozygous carrier of the *ADRA2B* deletion, nine were heterozygous, and 12 were noncarriers. As in previous studies (Cousijn et al. 2010; de Quervain et al. 2007b; Li et al. 2013; Rasch et al. 2009), we treated homozygote and heterozygote carriers of the deletion variant as one group (deletion carriers).

fMRI data acquisition and analysis

A 1.5 T Siemens MAGNETOM Sonata MRI system with an eight-channel head coil was used to obtain T_2* weighted gradient echo planar imaging (EPI) volumes with BOLD contrast (time to repeat (TR)=2 s; time to echo (TE)=50 ms; flip angle α=90°). The volumes consisted of 25 axial slices with an in-between gap of 0.75 mm recorded in ascending order. Each slice had a matrix size of 64×64 voxels with a voxel size of 3×3×3 mm. After completion of encoding phase, a T_1-weighted scan (176 contiguous slices, each slice 448×512 voxels, voxel size=1×1×1 mm^3) was conducted to collect a high-resolution structural reference volume of each participant. A magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence was employed with TR=1.97 s, TE=3.93 ms, and α=15°.

The functional data was analyzed with standard preprocessing in SPM8 (FIL, Welcome Trust Centre for Neuroimaging, UCL, London, UK) for both the encoding and retrieval phase. To correct for head motion, functional time series were spatially realigned and unwarped. The structural T_1-weighted volume was registered to a mean functional image. For spatial normalization of the functional images to the MNI reference brain (Montreal Neurological Institute, Montreal, Canada), we first ran segmentation on the mean EPI image. Subsequently, we ran segmentation on the resulting bias-corrected image in native space to achieve normalization. Finally, normalized functional volumes, which were resampled to 2×2×2-mm-sized voxels were spatially smoothed with a three-dimensional Gaussian kernel of 8-mm full-width-half-maximum (FWHM).

First, we analyzed potential genotype-dependent effects during encoding of fearful and neutral faces. Single subject data during encoding were modeled with four regressors of interest modeling the effects of neutral and fearful faces prior to the stress and control condition. At retrieval, single data were modeled with 16 regressors of interest modeling the effects of neutral and fearful faces in the stress and control condition as a function of novelty and correctness (e.g., control condition: fearful old face which was correctly recognized; control condition: fearful old face which yielded an incorrect answer; control condition: fearful new face which was correctly recognized; etc.).

In both sessions (encoding and retrieval), we additionally included six rigid-body motion parameters as nuisance regressors to account for movement artifacts. Each regressor was convolved with a canonical hemodynamic response function (HRF), and voxel time series were high-pass filtered at 1/128 Hz to account for nonphysiological slow drifts in the measured signal and modeled for temporal autocorrelation across scans with an autoregressive AR (1) model. The first five volumes of the echo planar imaging series were discarded prior to pre-processing to account for T_1-equilibrium effects.

To investigate genotype effects on encoding of emotional memory, contrasts coding for BOLD signal increases to all fearful as compared to all neutral faces were entered into a two-sample *t* test comparing deletion carriers with noncarriers. To gauge the genotype-dependent effects on stress-induced modulation of emotional memory retrieval, contrasts coding for BOLD signal increases to fearful as compared to neutral faces under stress as compared to control (i.e., [stress fearful–stress neutral]–[control fearful–control neutral]) were entered into the two-sample *t* test comparing *ADRA2B* deletion carriers with noncarriers. For completeness, interactions are also shown for each genotype separately. For both data acquired

during encoding and retrieval, we performed first a whole brain analysis and subsequently a region of interest (ROI) analysis on the two prior ROIs, the amygdala and the hippocampus. ROIs were derived from the Wake Forest University Pickatlas toolbox (TD Brodmann areas+, Maldjian et al. (2003)). All brain activations are reported at $p \leq 0.05$ using family-wise error (FWE) correction at cluster level for the whole brain analysis and at peak level for the two ROIs. To illustrate significant interactions, mean beta values were extracted from significant clusters using the REX toolbox (The Gabrieli Lab at MIT). Mean beta values were plotted as a function of stress condition, emotion, and genotype.

Results

Physiological and behavioral results

We found strong cortisol, physiological, and subjective responses to acute psychosocial stress as implemented by the TSST. The ANOVA revealed a time by stress condition interaction for the cortisol concentration ($F(2, 46)=8.85$, $p<0.01$), the systolic blood pressure ($F(2, 50)=3.57$, $p<0.05$), the pulse ($F(2, 50)=4.79$, $p<0.05$), and subjective ratings of mood (good–bad mood scale MDBF, $F(2, 50)=32.96$, $p<0.001$) and calmness (calmness–concern scale MDBF, $F(2, 50)=37.44$, $p<0.001$). No genotype by stress condition, genotype by time, or genotype by stress condition by time interactions was found. Note that the first time point (arrival) was not included into the analysis since it was only collected to control for potential pre-encoding differences between genotypes. There were no pre-encoding differences in cortisol, pulse, systolic blood pressure, or any of the subjective ratings (all $p>0.1$). Diastolic blood pressure was however higher in control at arrival ($t(26)=-2.49$, $p=0.02$). Hence, *ADRA2B* genotype did not impact stress-induced changes of cortisol level, blood pressure, pulse, or subjective mood ratings.

Response accuracy during the gender classification task during the encoding phase was high in both groups but significantly higher in noncarriers (deletion carriers, 81.92± 0.75 %; noncarriers, 85.29±0.97 %; $t(25)=-2.71$; $p<0.05$). RTs did not differ between deletion carriers and noncarriers ($t(25)=0.52$, $p=0.61$). There were no differences between the two sessions (control/stress) with respect to accuracy and RT in the gender classification task (RT: $F(1, 26)=2.02$, $p=0.17$; accuracy: $F(1, 26)=0.67$, $p=0.42$).

Response accuracy and RTs at retrieval are shown in Fig. 1. We found a tendency for a stress condition by emotion by genotype interaction effect with respect to memory accuracy ($F(1, 25)=4.11$, $p=0.054$, see Fig. 1a). None of the other possible main effects or interactions was significant. Post hoc paired *t* tests indicate that deletion carriers showed a tendency for lower recognition rates for fearful faces as compared to neutral faces in the control condition ($t(12)=-2.131$, $p=0.054$). In contrast, noncarriers showed a significantly lower memory accuracy for fearful faces as compared to neutral faces in the stress condition ($t(13)=-3.428$, $p<0.01$).

RTs analysis revealed a significant stress condition by emotion by genotype interaction ($F(1, 25)=6.997$, $p<0.05$, see Fig. 1b). Post hoc paired *t* tests revealed that deletion carriers showed significantly slower RTs to

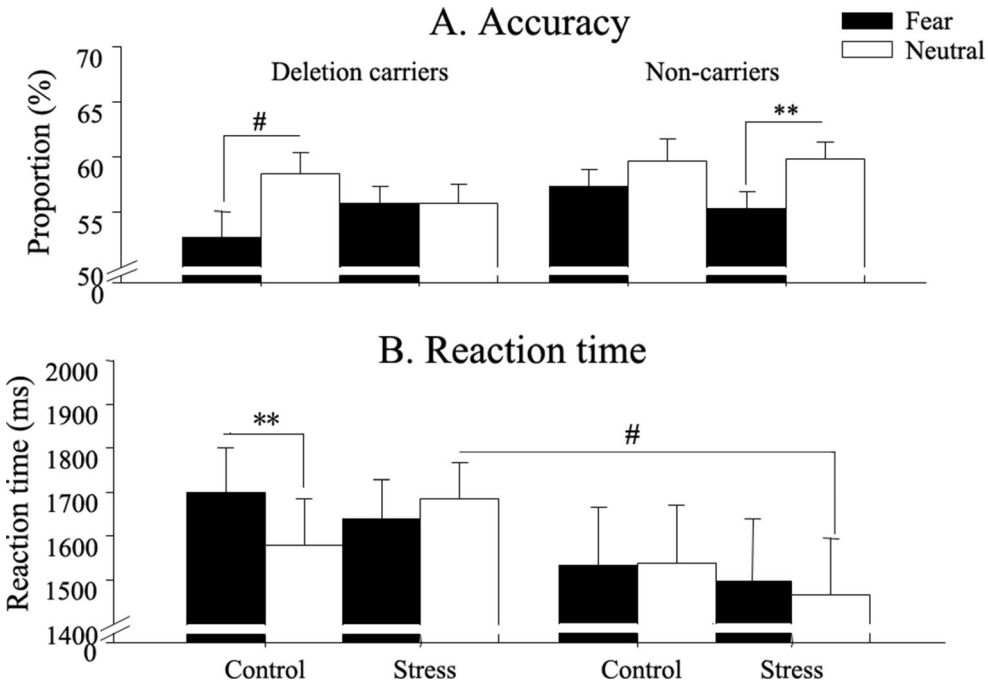

Fig. 1 Performance in the face recognition task in deletion carriers and noncarriers illustrating the interaction of the stress condition and emotion with respect to accuracy (**a**) and RT (**b**). (*p* values of post hoc *t* tests: $^{**}p \leq 0.01$; $\#p \leq 0.1$)

fearful as compared to neutral faces in the control condition ($t(12)=3.115$, $p<0.01$).

To investigate the effects of order (stress first/control first) on the behavioral level, we included order as a second between-group factor (stress condition first or control first) into the analysis resulting in a four way ANOVA with the factors order, genotype, stress condition, and emotion. The results revealed no significant influence of order on the genotype effects.

Neural activity at encoding

We performed a genotype by emotion interaction analysis on the fMRI data at encoding to investigate whether *ADRA2B* genotype differentially modulates processing of neutral and fearful faces prior to exposing participants to the stress or control procedure. The whole brain analysis revealed that noncarriers showed higher neural activity in the left inferior parietal cortex (IPC; −56, −52, 46, $Z=4.24$; $p<0.05$, cluster-level FWE corrected, cluster size 112 voxels, Fig. 2c) when encoding fearful faces as compared to neutral faces, while the reverse pattern was seen in deletion carriers (see Fig. 2d). There was no brain region showing the opposite interaction. There were no genotype-dependent differences in the amygdala or hippocampus.

Neural activity at retrieval

We performed a genotype by stress condition by emotion interaction analysis on the fMRI data at retrieval to test whether stress differentially modulates neural activity during emotional recognition memory in carriers and noncarriers of the *ADRA2B* deletion. The whole brain analysis yielded no significant difference between carriers and noncarriers of the deletion. We found however a significant three-way interaction in our two prior regions of interest, the left and right amygdala (left: −20, −8, −16; $Z=3.87$; $p<0.01$, FWE corrected; cluster size 35 voxels; right: 24, −4, −14; $Z=3.28$; $p<0.05$, FWE corrected; cluster size 47 voxels; Fig. 3c) and the right hippocampus (32, −28, −12; $Z=3.23$; $p<0.05$, FWE corrected; cluster size 16 voxels; Fig. 4c). Figure 3a, b illustrates the stress condition by emotion interaction separately for both genotypes in the amygdala. Note that the interaction in the amygdala was only present in deletion carriers and due to lower neural activity during recognition of fearful as compared to neutral faces in the control condition and higher neural activity in response to emotional as compared to neutral faces under stress (Fig. 3d, e). Neural activity in the right amygdala during recognition of neutral and fearful faces was not significantly different in noncarriers, but a significant effect of stress in noncarriers was found in the left amygdala. Figure 4a, b illustrates the stress condition by emotion interaction for both genotypes in the right hippocampus. In contrast to the findings in the amygdala, the genotype by stress condition by emotion interaction was only significant in noncarriers and can be explained by an increase in neural activity during fearful versus neutral faces under stress.

To investigate the effects of order on the neural level, we performed the same four-way ANOVA as above on mean beta estimates of amygdala and hippocampus and found no

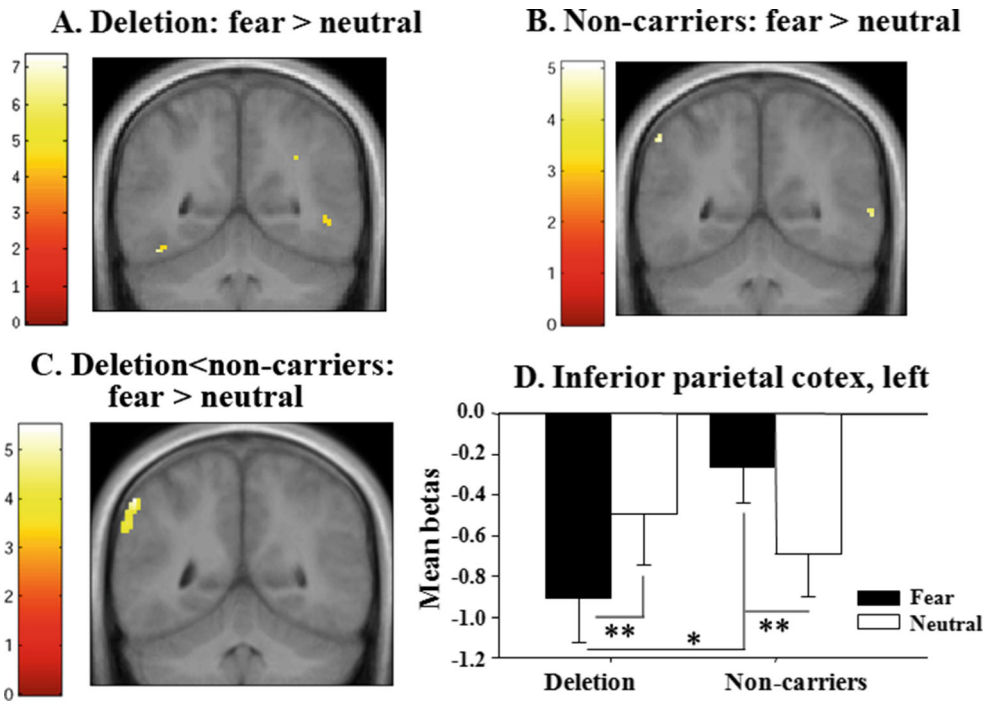

Fig. 2 Neural activity [fearful–neutral] at encoding. **a** Neural activity in deletion carriers. **b** Neural activity in noncarriers. **c** Genotype by emotion interaction [noncarriers (fearful–neutral)–deletion carriers (fearful–neutral)] in the left inferior parietal cortex (IPC). **d** *Bar charts* depict mean betas estimates of the activated cluster (IPC: −56, −52, 46) as a function of genotype (deletion carriers/noncarriers) and emotion (fearful/neutral). For display purposes, activation clusters are thresholded at $p\leq0.001$ (uncorrected) and are rendered on a mean T1 image of all participants. (post hoc *t* tests on mean betas: *$p\leq0.05$; **$p\leq0.01$)

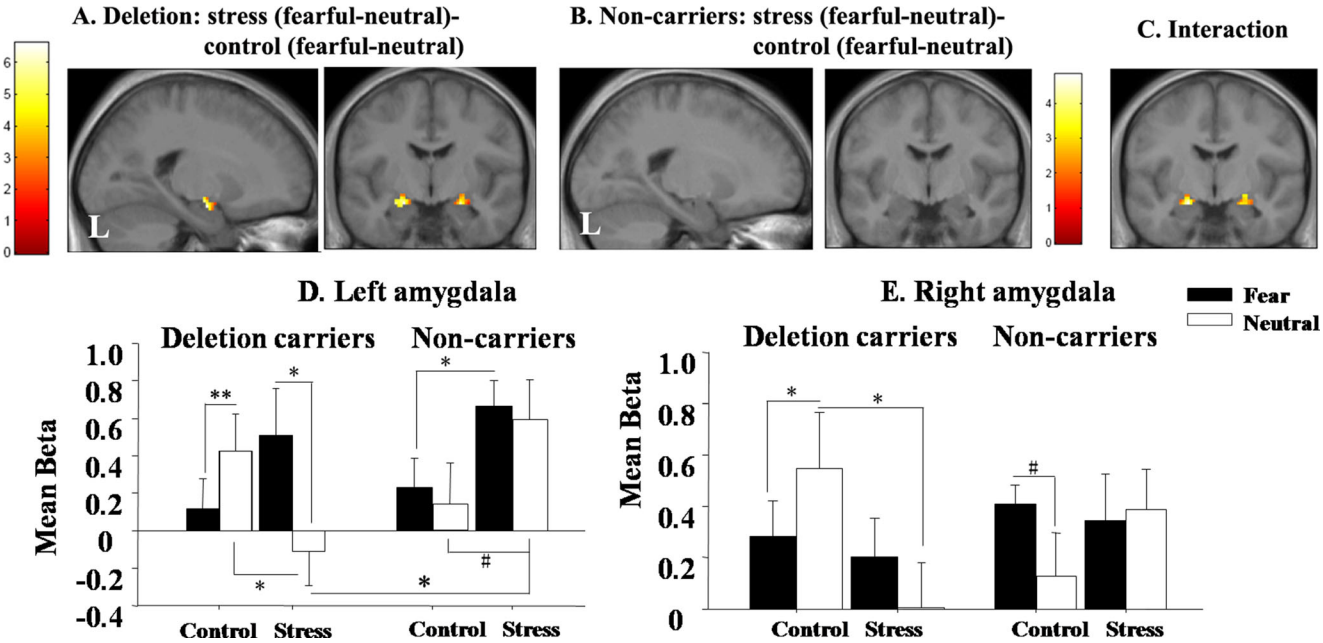

Fig. 3 Neural activity [stress (fearful–neutral)–control (fearful–neutral)] in the left and right amygdala at recognition. **a** Neural activity in deletion carriers. **b** Neural activity in noncarriers. **c** Genotype by stress condition by emotion interaction. **d**, **e** *Bar charts* depict mean betas estimates of the activated clusters (left amygdala: −20, −8, −16; right amygdala: 24, −4, −14) as a function of genotype, stress condition, and emotion. For display purposes, activation clusters are thresholded at $p \leq 0.001$ (uncorrected) and are rendered on a mean T1 image of all participants. (post hoc *t* tests on mean betas: $*p \leq 0.05$; $**p \leq 0.01$; $\#p \leq 0.1$)

significant order effect nor interactions with order. There was also no significant influence of testing time on our results.

In summary, recognition-related neural activity provides evidence for a stress-induced modulation of emotional

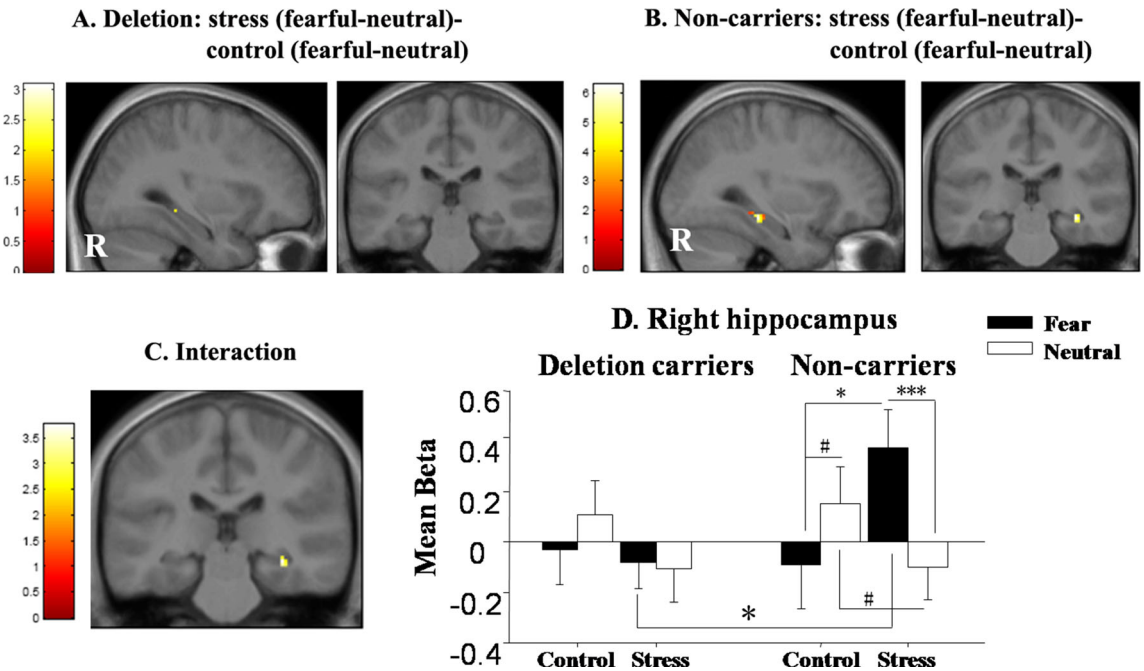

Fig. 4 Neural activity [stress (fearful–neutral)–control (fearful–neutral)] in the right hippocampus at recognition. **a** Neural activity in deletion carriers. **b** Neural activity in noncarriers. **c** Genotype by stress condition by emotion interaction. **d** *Bar charts* depict mean betas estimates of the activated cluster (hippocampus: 32, −28, −12) as a function of genotype, stress condition, and emotion. For display purposes, activation clusters are thresholded at $p \leq 0.001$ (uncorrected) and are rendered on a mean T1 image of all participants. (post hoc *t* tests on mean betas: $*p \leq 0.05$; $**p \leq 0.01$; $\#p \leq 0.1$)

memory in the left and right amygdala in carriers of the *ADRA2B* deletion, while noncarriers exhibited a stress-induced modulation in the right hippocampus.

Discussion

Relying on a small number of volunteers, our data demonstrate that a genetic variation that influences noradrenergic neurotransmission modulates neural activity in the amygdala and hippocampus after stress exposure in an emotional face memory paradigm. Our genetic neuroimaging study is in line with prior studies showing that the *ADRA2B* deletion modulates emotional memory (de Quervain et al. 2007b; Li et al. 2013) and amygdala activity when processing emotional faces in stress-free and stressful situations (Cousijn et al. 2010; Rasch et al. 2009).

Molecular studies provide evidence that glucocorticoid receptors are highly enriched in hippocampus, amygdala, and frontal lobe. Further, noradrenergic receptors are abundant in the amygdala and therefore essential for emotional arousal (reviewed in Lupien and Lepage (2001); Roozendaal et al. (2009a)). The release of noradrenaline in the amygdala is crucial for enabling memory consolidation (reviewed in McGaugh (2000)). It has been suggested that the amygdala and hippocampus are core regions for interactions between stress and emotional memory. Psychopharmacological studies in animals point toward a role of post-encoding noradrenergic activity in the amygdala in modulating emotional memory consolidation in the hippocampus and other regions, where it may interact with glucocorticoid-related process (see review by Todd et al. (2011)). Noradrenaline interacts with stress hormones in the amygdala and hippocampus to enhance emotional memory consolidation (reviewed in McGaugh and Roozendaal (2009)); the synchronization of the two brain areas is also necessary for the retrieval of emotional memories (reviewed in (Buchanan 2007)). In humans, it has been shown by means of fMRI that conjoint pharmacological increases of noradrenaline and cortisol levels increase amygdala activation to negative emotional stimuli (Kukolja et al. 2008) and that the effects of noradrenergic blockade on amygdala responses to emotional stimuli depend on the level of endogenous cortisol (van Stegeren et al. 2008).

The retrieval of recently learned memories needs the coordinated action of noradrenergic neurons in the locus coeruleus and amygdala which activate fronto-hippocampal networks (reviewed in Sara (2009)). Animal studies found that lesions of the amygdala or blockade of β-adrenergic receptors prevent the spatial memory retrieval impairment induced by intrahippocampal infusions of a glucocorticoid receptor agonist (Roozendaal et al. 2003, 2004). These findings indicate that glucocorticoids impair memory retrieval by facilitating noradrenergic mechanisms in the amygdala, which mediate and enable hippocampal glucocorticoid effects. By using knockout mice that lack noradrenaline and adrenaline (NA/A), Murchison et al. (2004) found that noradrenaline is critical for hippocampus-dependent fear conditioning memory retrieval. Human studies have shown that blocking noradrenergic activity with the β-adrenergic receptor antagonist propranolol abolished the declarative memory enhancement for emotional items at retrieval, which suggests that the emotional memory enhancement is highly dependent on noradrenaline (Kroes et al. 2010; Strange and Dolan 2004). Further, blockade of noradrenergic activity prior to stress or cortisone treatment impacts memory retrieval of emotional items, independent on whether cortisone enhanced (Schwabe et al. 2009) or impaired (de Quervain et al. 2007a) emotional memory. Hence, noradrenergic activity is a critical modulator of stress effects on emotional memory retrieval.

Using the same paradigm in a behavioral study with a larger number of volunteers, we previously found that deletion carriers showed slower reaction times to emotional faces in the control condition, which was not observed under stress or in noncarriers (Li et al. 2013). We speculated that the slower reaction times in recognizing emotional faces are primarily driven by a shift of attention toward a potential threat in the environment, suggesting an increase in emotional reactivity in deletion carriers. The behavioral data showed here replicate our prior behavioral findings (Li et al. 2013) even though in a relatively small sample. Additionally, the data showed a tendency for decreased recognition accuracy for emotional faces in deletion carriers in the control condition. The lower emotional memory performance in deletion carriers is in contrast to the findings of de Quervain et al. (2007b) who reported increased memory for negatively and positively valenced emotional scenes in deletion carriers. Differences in stimulus material and the length of the delay between encoding and retrieval may have contributed to these differences (see also Li et al. (2013) for a further discussion).

Our fMRI data at retrieval revealed a three-way interaction of *ADRA2B* genotype, stress condition, and emotional memory which was evident in the amygdala and hippocampus. Using a ROI analysis in a relatively small sample size, we show a pattern of amygdala activity that parallels to some extent the behavioral finding since BOLD responses to emotional and neutral items differed under stress and control only in deletion carriers but not in noncarriers.

A variety of studies provide evidence that *ADRA2B* genotype modulates neural circuits related to cognitive functions (e.g., episodic memory, emotional memory) and mood disorders (e.g., posttraumatic stress disorder (PTSD), depression, reviewed in Frodl et al. 2008; Rasch et al. 2010; Todd et al. 2011, also see a recent paper by Koutroumani et al. 2013). Prior fMRI data acquired at encoding, further suggest increased activation of the amygdala (Rasch et al. 2009) and

inferior frontal gyrus (Urner et al. 2011) in deletion carriers which contributed to successful emotional memory formation. Our data yielded neither any evidence for genotype-dependent differences in amygdala activity for neutral and fearful faces at encoding nor did we find any evidence of increased neural activity in visual cortices which would indicate better visual processing of negative emotional items as proposed by Todd et al. (2013b). Instead, we provide evidence for a genotype-dependent modulation of neural activity in the left inferior parietal cortex when encoding fearful faces. The left inferior parietal cortex has previously been shown to contribute to processing fearful faces in several fMRI studies (reviewed in Fusar-Poli et al. (2009)). The lack of effects on amygdala activity may have been due to our implicit emotion processing task at encoding (Fusar-Poli et al. 2009) or the small number of volunteers.

Exposure to acute psychosocial stress prior to memory retrieval was however found to impact retrieval-related amygdala activity in a genotype-dependent manner. While deletion carriers showed decreased neural activity in the amygdala when retrieving emotional as compared to neutral faces in the control condition and increased amygdala activity under stress, noncarriers showed no differences in amygdala activation for neutral and emotional faces, neither under stress nor in the control condition. Note that during memory retrieval, neural activity in response to emotional items per se was not different between genotypes nor were there any differences in stress-induced changes in amygdala activity to emotional items as previously reported for encoding (Cousijn et al. 2010; Rasch et al. 2009) or after hydrocortisone in an emotional interference task (Henckens et al. 2012). Human fMRI data suggest that a stress-related noradrenergic activation is needed for a large-scale brain-state shift that occurs after stressors and promotes rapid defense mechanisms, including autonomic-neuroendocrine control and vigilant attentional reorienting (Hermans et al. 2011). Therefore, different *ADRA2B* genotypes might be affected differently in their performance under stress. A study in war refugees suggests that deletion carriers showed a significantly higher score for reexperiencing symptoms of traumatic events than did noncarriers, suggesting that deletion carriers have higher risks for PTSD (de Quervain et al. 2007b), which might be associated with higher activation of the right amygdala to negative emotional pictures in deletion carriers ((Rasch et al. 2009), also reviewed in Wilker et al. (2013)). Here, we add to this evidence by showing stronger amygdala activation at retrieval for fearful as compared to neutral faces in deletion carriers.

The second brain area which showed a significant genotype by stress condition by emotion interaction was the right hippocampus. Here, noncarriers showed higher neural activity to emotional faces under stress. Patients with right temporal lobectomy (RTL) produced significantly fewer autobiographic memories of unpleasant events than the left temporal lobectomy (LTL) and control group, indicating that the right (but not the left temporal lobe) is involved in the retrieval of negatively valenced, high-intensity memories (Buchanan et al. 2006). A previous fMRI study reported increased left hippocampal activation during retrieval of emotional nouns and showed that this increase was abolished by blocking noradrenergic activity with propranolol (Strange and Dolan 2004). Further evidence for a noradrenergic-glucocorticoid interaction during memory encoding comes from a pharmacological fMRI study by Kukolja et al. (2011). The authors were able to show that cortisol increased neural responses in hippocampus to emotional items only in the presence of noradrenergic stimulation.

In summary, although with a small sample size, our data indicate that a loss-of-function polymorphism which increases the availability of noradrenaline modulates the effects of acute psychosocial stress on emotional memory recognition and related neural activity in amygdala and hippocampus.

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Conflict of interest The authors have no conflict of interest to declare.

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