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ORIGINAL ARTICLE

Noradrenergic-glucocorticoid modulation of emotional memory encoding in the human hippocampus

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Background. Current rodent models emphasize the joint action of the stress mediators noradrenaline (NE) and cortisol (CORT) in conferring a memory advantage of emotional over neutral stimuli.

Method. Using a pharmacological strategy of tackling this stress-related mechanism to enhance human episodic (autobiographical) memory, we measured amygdala-hippocampal responses during encoding of emotional and neutral stimuli with functional magnetic resonance imaging in 51 healthy subjects under four pharmacological conditions in a double-blind parallel group design: (i) placebo; (ii) the NE-reuptake inhibitor reboxetine (4 mg); (iii) hydrocortisone (synthetic CORT) (30 mg); or (iv) both agents in combination.

Results. Differential drug effects were found in the left hippocampus, whereas hydrocortisone alone selectively decreased hippocampal responses to emotional relative to neutral stimuli, reboxetine potentiated hippocampal responses to these stimuli. Importantly, the inhibitory influence of hydrocortisone was reversed by co-administration of reboxetine.

Conclusions. Our results imply that stress levels of CORT alone attenuate hippocampal responses to emotional stimuli, an effect possibly related to a regulatory negative feedback loop. However, when simultaneously elevated to stress levels, NE and CORT act together to synergistically enhance hippocampal activity during encoding of emotional stimuli, a mechanism that may turn maladaptive under circumstances of traumatic stress.

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Key words: Cortisol, emotion, fMRI, hippocampus, memory, noradrenaline, stress.

Introduction

More than any other species, humans are beneficiaries and victims of their emotional memories. While the adaptive value of these memories for survival and reproductive success is obvious, excessive stress often turns them into a source of anxiety and depression (Roozendaal *et al*. 2009). This is best evidenced in post-traumatic stress disorder (PTSD), which is characterized by the clinical triad hypermnesia/re-experiencing symptoms, avoidance behaviour and hyperarousal in response to an inescapable life-threatening stressor. By focusing on the link between stress-related amygdala hyper-responsiveness (Rosenkranz *et al*. 2010) and overexpression of conditioned fear and avoidance behaviour (Yamamoto *et al*. 2009), the prevailing rodent models of PTSD emphasize fear memory dysregulation in the aetiology of the illness. However, these models fail to account for the hypermnesia/re-experiencing symptoms, which indicate emotional episodic (autobiographical) memory dysfunction (Brewin, 2008) and sometimes dominate the clinical phenotype. To treat or perhaps even prevent these symptoms, a valid neurocircuitry model of how stress influences, and interferes with, emotional episodic memory function is essential.

Current perspectives from functional magnetic resonance imaging (fMRI) studies in healthy human subjects suggest the interaction of two endogenous modulators, noradrenaline (NE) and cortisol (CORT), in mediating the impact of stress on amygdala and hippocampus (van Stegeren *et al*. 2007, 2010), which together orchestrate the efficient encoding of emotional episodic memories (Dolcos *et al*. 2004; Richardson *et al*. 2004). Consequently, a combination of stress-related NE–CORT interactions and exaggerated amygdala-hippocampal responses triggered by

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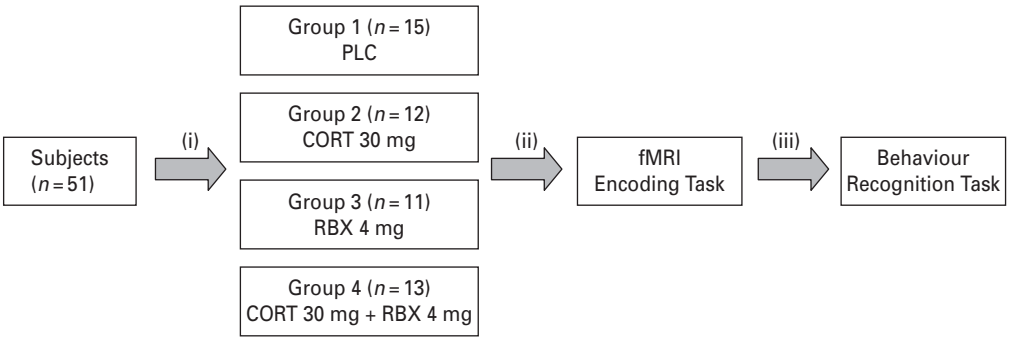

Fig. 1. The randomized double-blind placebo-controlled parallel-group design and the timeline of the study are illustrated. (i) Random allocation to one of four treatment groups; (ii) functional magnetic resonance imaging (fMRI) scan of encoding 2 h 25 min after treatment; (iii) behavioural recognition test 1 week later. PLC, placebo; CORT, cortisol; RBX, reboxetine.

these interactions is thought to underlie overactive emotional episodic encoding in PTSD (Hurlemann, 2008). Using fMRI in healthy volunteers, we recently showed that combined pharmacological elevation of NE and CORT triggered an amygdala response bias to fearful faces (Kukolja *et al*. 2008*a*), which experimentally mimics amygdala hyper-responsiveness to fearful faces in PTSD patients (Rauch *et al*. 2000; Shin *et al*. 2005). A crucial question is whether elevations of NE and CORT also affect hippocampal function. The rationale of the present fMRI experiment was thus to measure – within the established experimental framework of amygdala hyper-responsiveness to fearful faces (Kukolja *et al*. 2008*a*) – hippocampal activation during encoding of emotional *versus* neutral material. We hypothesized that NE and CORT – when simultaneously elevated to moderate stress levels – would act together by synergistically enhancing hippocampal function during emotional episodic encoding, a mechanism that may turn maladaptive under circumstances of excessive stress.

Method

Subjects

Altogether, 62 healthy adults (students at the University of Aachen and trainees at the Research Center Jülich, recruited by local advertisement) participated in the present study after providing written, informed consent. The sample characteristics are detailed online in Supplementary Method. The study had full ethical approval. One subject was excluded from further analysis due to excessive head movements during the fMRI session, three subjects were excluded due to a computer failure to record responses at recognition and seven subjects were excluded due to salivary CORT measurement failure. Thus, the data from 51 subjects were included in the analysis.

Encoding session

The experiment consisted of an encoding and a recognition session 1 week apart (Fig. 1). FMRI was measured while subjects were engaged in an episodic encoding task (Fig. 1) based on emotional and neutral stimuli selected from the International Affective Picture System (IAPS; Lang *et al*. 2008). Stimulus delivery and response recording during encoding and recognition was carried out using Presentation11 (Neurobehavioral Systems, Inc., USA). Specifically, 72 neutral pictures [defined by low arousal ratings ranging from 2.32 to 4.19 and valence ratings ranging from 4.14 to 7.97, according to the IAPS manual (Lang *et al*. 2008)] and 72 emotional pictures [defined by high arousal ratings ranging from 5.35 to 7.34 and valence ratings ranging from 1.31 to 4.79, according to the IAPS manual (Lang *et al*. 2008)] were randomly displayed in the centre of an LCD screen behind the scanner, visible to the subjects via a mirror system (viewing distance 254 cm). Each stimulus was presented for 4000 ms, followed by an inter-stimulus interval lasting 1710 ms. In total, 40 null-events in which a white fixation cross was displayed on a black background were interspersed. This resulted in variable stimulus-onset asynchronies and allowed a comparison of the blood-oxygen-level-dependent signal of the event types of interest with a no-stimulus baseline. In order to ensure proper stimulus processing, subjects were asked to indicate via button presses whether or not a given stimulus was emotional. The encoding session lasted 22 min.

Drug administration

In a between-subjects, double-blind study design, participants were randomly allocated to one of four treatment groups. Importantly, these groups did not differ in demographic and cognitive characteristics (i.e. age, education, IQ, general memory functioning, mood and valence and arousal ratings, see Supplementary

Material). The first group (PLC) received four tablets of saccharose placebo, the second (CORT-only) received three tablets of hydrocortisone (10 mg each) and one tablet of saccharose placebo, the third (RBX-only) received one tablet of reboxetine (4 mg) and three tablets of saccharose placebo and the last (RBX-CORT) received one tablet of reboxetine (4 mg) and three tablets of hydrocortisone (10 mg each) (Fig. 1; for randomization methods, see Supplementary Material). Reboxetine is a highly selective inhibitor of presynaptic NE reuptake (Kent, 2000; Scates & Doraiswamy, 2000). A 4-mg single oral dose of reboxetine was administered in analogy to our previous pharmacological fMRI studies of amygdala response changes following noradrenergic stimulation (Kukolja *et al*. 2008*a*; Onur *et al*. 2009). A 30-mg single oral dose of hydrocortisone was administered to elevate CORT activity to moderate levels ranging between low (20 mg) and high (40 mg) stress levels (Abercrombie *et al*. 2003). Drugs were administered 2.5 h prior to scanning. Further details are available online in Supplementary Material. For an assessment of CORT levels, saliva samples were collected immediately before drug administration (CORT1) as well as before (CORT2) and after (CORT3) scanning from each subject using Salivette collection devices (Sarstedt, Germany). After scanning, a venous blood sample was taken for the measurement of reboxetine serum levels.

Recognition session

The recognition session was performed 1 week after encoding to exclude confounding effects of drug action (Fig. 1). Subjects were placed in a quiet test room equipped with a computer set-up and presented with all 144 pictures previously shown during encoding. A total of 72 new pictures were intermixed (36 emotional and 36 neutral), resulting in an old/new ratio of 2:1. Subjects underwent a 45-min remember-know recognition test, requiring button presses to indicate whether: (i) they had seen the presented picture (remember); (ii) the picture looked familiar (know); or (iii) they had never seen the picture before. Prior to further data analysis, χ^2 tests were calculated, ensuring that subjects had not been guessing when performing the recognition test. To analyse recognition performance, the detection sensitivity index *d*-prime (*d′*) was calculated in accordance with the signal detection analysis (Green & Swets, 1966; Macmillan, 1993; Stanislaw & Todorov, 1999), relating the number of correctly recognized items with the sum of all positive responses, including false positives. This enabled us to estimate a performance score that was unaffected by any individual response bias. *d′* scores were analysed using repeated-measures three-way analysis of variance (ANOVA) including the between-subjects factors RBX (RBX, no RBX) and CORT (CORT, no CORT) and the within-subjects factor emotion (emotional, neutral). Gender (male, female) was added as a covariate to ensure that effects were generalizable, irrespective of gender. Adding gender as a fourth factor would not have added valuable information to this analysis because sample sizes of male and female subjects were too small ($n=5$–8) within each treatment group. Significant main effects and interactions were further investigated using *post-hoc t* tests, with the significance level p being Bonferroni-adjusted for multiple comparisons. To assess potential dose–response relationships, *d′* scores for emotional and neutral pictures were correlated separately with salivary CORT and serum RBX levels. For the closest estimate of CORT levels during scanning, we used the mean of pre- and post-scan CORT levels [mCORT level = (CORT2 + CORT3)/2]. CORT levels were determined in all subjects, such that data from all subjects (irrespective of treatment group, reflecting either endogenous or endogenous plus exogenous CORT) were included in the correlation analysis. Due to large inter-individual variability, mCORT levels [which originally were not normally distributed (Kolmogorov–Smirnov test $n=51$, $Z=1.802$, $p<0.005$)] were log-transformed ($\mathrm{mCORT_{log}}$) to obtain normal distribution (Kolmogorov–Smirnov test $n=51$, $Z=1.194$, $p=0.116$), meeting requirements for a correlation analysis. An analogous analysis was performed when correlating serum RBX-levels with *d′* scores. In this case, only subjects receiving reboxetine ($n=24$) were included in the analysis, because inclusion of non-medicated subjects would have distorted the correlation analysis. After the recognition session, subjects were asked to rate the pictures with regard to arousal and pleasure on a scale ranging from 1–9 (not arousing – very arousing; very unpleasant – very pleasant, respectively). Arousal and valence ratings were analysed using repeated-measures ANOVA, with the between-subjects factor treatment group (PLC, CORT-only, RBX-only, RBX-CORT) and the within-subjects factor emotion (emotional, neutral). Valence and arousal ratings are reported online in Supplementary Material. Significant results of *post-hoc* tests are reported after Bonferroni-correction for multiple comparisons.

fMRI hardware and image processing

Functional magnetic resonance images were acquired using a TRIO 3-T whole-body scanner (Siemens, Germany) equipped with a standard head coil for radio frequency transmission and signal reception. Sequence parameters are documented online in Supplementary Material. The image pre-processing was performed using Matlab7 (The MathWorks Inc., USA) and

SPM5 (http://www.fil.ion.ucl.ac.uk/spm) (for details, see online Supplementary Material). The data were analysed using a general linear model as implemented in SPM5 (Kiebel & Holmes, 2003) in an event-related fashion. In accord with previous studies (van Stegeren *et al*. 2005), all trials containing subsequently recognized items (i.e. pictures categorized as 'remembered' or 'known') were collapsed in the fMRI analysis in order to capture all neural activity related to successful encoding of these items. For each subject, simple main effects for each condition were computed using baseline contrasts. These first-level individual contrasts were then entered into a second-level group analysis using an ANOVA with the between-subjects factors RBX (RBX, no RBX) and CORT (CORT, no CORT) and the within-subjects factors emotion (emotional, neutral) and memory (recognized, forgotten) employing a random effects model (Penny & Holmes, 2003). In analogy to the behavioural data analysis, gender was added as a covariate to the ANOVA. Violations of sphericity were accounted for by modelling non-independence across parameter estimates from the same subject and by allowing unequal variances both between conditions and subjects. Due to our a priori anatomical focus on amygdala and hippocampus, we restricted the analyses to these structures by defining regions of interest (ROI) based on cytoarchitectonic maximum probability maps (Amunts *et al*. 2005) as available in the SPM Anatomy toolbox (Eickhoff *et al*. 2005; see also Goossens *et al*. 2009; Hurlemann *et al*. 2010; Onur *et al*. 2010). To identify regions differentially active as a function of emotion, subsequent memory and treatment, we used F-contrasts to test for main effects and interactions. Activations yielded by the resulting SPM(F) maps are reported at $p<0.05$, corrected for family-wise error (FWE) by reference to the Gaussian random field theory (Worsley *et al*. 1996). To disentangle these effects, we performed subsequent tests for simpler interactions or simple main effects, whenever applicable, within the clusters of voxels found active in the primary analysis. For these *post-hoc* tests, FWE correction was applied only for the respective cluster of voxels at which the analysis was performed because, on the one hand, *post-hoc* tests are only meaningful in voxels where higher-order effects are found and, on the other, *post-hoc* tests to voxels are not justified where no higher-order effect is present.

Results

Behavioural data

Recognition memory

Repeated measures ANOVA on d' detection sensitivity (Greenhouse–Geisser corrected for non-sphericity) revealed a main effect of emotion (mean $\pm$ S.D.) [d': $F(1,46)=45.22$, $p<0.0001$]. This influence of emotion was extremely robust and not further modulated by treatment, i.e. there were neither main effects of RBX or CORT nor interaction effects on d' (Table 1). *Post-hoc* paired t tests including the four treatment groups revealed that the main effect of emotion was due to enhanced detection sensitivity [d': $t(50)=-8.13$, $p<0.0001$] for emotional relative to neutral items (Table 1).

Table 1. *Behavioural results*

		PLC	CORT-only	RBX-only	RBX-CORT
d'	N	1.0 (0.4)	1.3 (0.4)	1.0 (0.7)	1.2 (0.4)
	E	1.6 (0.4)	1.8 (0.5)	1.5 (0.6)	1.6 (0.7)

E, emotional; N, neutral; CORT, cortisol; RBX, reboxetine; PLC, placebo.

d-prime (d') scores were gathered 1 week after pharmacological intervention.

Values are shown as mean (S.D.).

Correlation analyses

There was a positive correlation between d' detection sensitivity and $mCORT_{log}$ for neutral items, i.e. the detection sensitivity improved as a function of increasing CORT levels ($n=51$, $r=0.293$, $p<0.05$). For emotional items, no such relationship was found ($n=51$, $r=0.085$, $p=0.55$) (Fig. 2*d*). There were no significant correlations between RBX and d' for either neutral or emotional items (neutral: $n=24$, $r=0.012$, $p>0.05$; emotional: $n=24$, $r=-0.061$, $p>0.05$).

Imaging data

Main effects

The ROI analysis showed a main effect of emotion, irrespective of the factors subsequent memory or treatment, which encompassed both amygdala and hippocampus bilaterally (Table 2, Fig. 2*a*). Subsequent paired t tests revealed that activations of amygdala and adjacent anterior hippocampus were greater for emotional than for neutral items. In contrast, more distant posterior parts of the hippocampus (especially the cornu ammonis subregion) showed the reverse pattern, with greater responses to neutral than to emotional items ($p<0.05$, FWE-corrected) (Table 2). Moreover, amygdala and hippocampus bilaterally showed a main effect of subsequent memory. *Post-hoc* paired t tests confirmed that, in both regions, activation was greater for subsequently remembered relative to subsequently forgotten items, irrespective

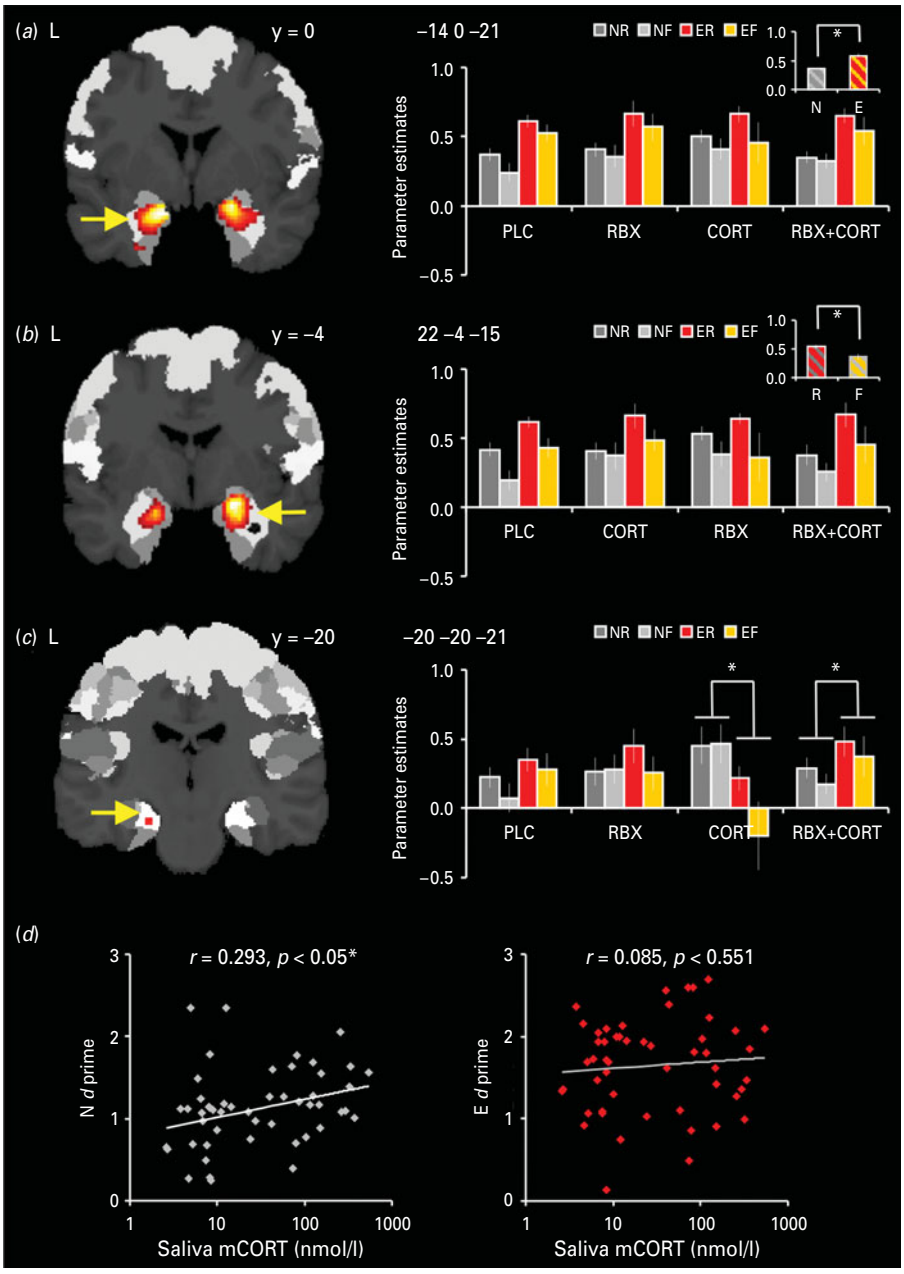

Fig. 2. Brain activation clusters projected upon maximum probability maps provided by the Anatomy toolbox (see text). (*a*) Main effect of emotion; (*b*) main effect of memory; (*c*) three-way interaction between reboxetine (RBX), cortisol (CORT) and emotion. For illustration purposes only, activations are shown at a threshold of $p < 0.001$, uncorrected. Signal plots show parameter estimates for each condition. Small plots in (*a*) and (*b*) show mean parameter estimates comprising the main effects of emotion and memory, respectively. (*d*) Correlation between log-transformed mean CORT levels (mCORT) and *d*-prime (*d'*) scores for neutral (N) and for emotional (E) pictures. PLC, placebo; ER, emotional recognized; EF, emotional forgotten; NR, neutral recognized; NF, neutral forgotten; R, recognized; F, forgotten; L, left; R, right.

of the factors emotion or treatment ($p < 0.05$, FWE-corrected) (Table 2, Fig. 2*b*). As collinearity between the observed main effects is likely, we additionally performed separate analyses of subsequent memory effects for emotional and neutral items. These analyses document a main effect of subsequent memory for emotional items in the amygdala bilaterally and a main effect of subsequent memory for neutral items in both the right amygdala and hippocampus (Table 2).

Interaction effects

Crucially, the ANOVA showed a two-way interaction between RBX and emotion in the left hippocampus.

Table 2. *Functional magnetic resonance imaging data*

		x	y	z	Z	p	vx	Effect
Main effect of emotion								
Amygdala	L	−16	0	−21	6.78	<0.0005	340	E>N
	R	22	−2	−17	6.65	<0.0005	295	E>N
	R	32	0	−39	3.19	<0.05	1	E>N
Hippocampal formation	R	22	0	−23	5.55	<0.0005	43	E>N
	R	32	−2	−41	3.80	<0.05	4	E>N
	L	−12	−2	−23	6.00	<0.0005	85	E>N
	R	28	−24	−11	4.62	<0.001	104	E>N
	L	−20	−26	−9	5.35	<0.0005	199	E>N
	R	20	−36	11	3.95	<0.05	3	N>E
	R	26	−40	5	4.58	<0.001	8	N>E
	L	−32	−40	−3	3.58	<0.05	1	N>E
Main effect of memory								
Amygdala	R	24	−2	−19	5.34	<0.0005	241	r>f
	L	−18	−4	−19	4.54	<0.0005	130	r>f
Hippocampal formation	R	22	0	−23	4.63	<0.05	24	r>f
	L	−16	0	−25	3.69	<0.05	2	r>f
	L	−20	−10	−21	3.94	<0.05	14	r>f
	R	24	−12	−19	4.63	<0.0005	151	r>f
	L	−32	−20	−15	3.59	<0.05	2	r>f
	L	−30	−28	−19	3.69	<0.05	4	r>f
	L	−34	−28	−19	3.69	<0.05	1	r>f
	L	−24	−30	−17	3.78	<0.05	1	r>f
Main effect of memory (neutral only)								
Amygdala	R	24	−4	−15	4.17	<0.005	93	N(r)>N(f)
Hippocampal formation	R	36	−20	−15	4.53	<0.001	49	N(r)>N(f)
	L	−26	−34	−13	3.78	<0.05	1	N(r)>N(f)
	L	−24	−36	−11	3.59	<0.05	1	N(r)>N(f)
Main effect of memory (emotional only)								
Amygdala	R	24	−2	−19	4.07	<0.005	87	E(r)>E(f)
	L	−18	−4	−19	3.45	<0.05	15	E(r)>E(f)
Hippocampal formation	R	22	0	−23	3.68	<0.05	1	E(r)>E(f)
Interaction RBX × emotion								
Hippocampal formation	L	−16	−20	−21	3.59	<0.05	2	see Table 3
Interaction RBX × CORT × emotion								
Hippocampal formation	L	−20	−20	−21	3.57	<0.05	1	see Table 3

E, Emotional; N, neutral; r, recognized; f, forgotten; L, left; R, right; RBX, reboxetine; CORT, cortisol.
Brain activations at $p<0.05$ family-wise error-corrected.
Listed are the effects that yielded significant results and *post-hoc* tests in the respective clusters.

Post-hoc t tests revealed that this interaction effect was based upon opposite main effects of emotion in the RBX groups (RBX-only, RBX-CORT) and in the non-RBX groups (PLC, CORT-only). In the RBX groups, greater hippocampal activation was elicited by emotional items (Table 3*a*); whereas in the non-RBX groups, neutral items elicited greater hippocampal activation (Table 3*a*). In a neighbouring cluster, a three-way interaction between RBX, CORT and emotion was found. This interaction effect appeared to be driven by a stronger two-way interaction between RBX and emotion in the CORT groups (CORT-only, RBX-CORT) than in the non-CORT groups (PLC, RBX-only) (Fig. 2*c*). Indeed, the presence of a two-way interaction between RBX and emotion in the CORT groups (CORT-only, RBX-CORT) contrasted with the absence of such interaction in the non-CORT groups (PLC, RBX-only) ($p>0.05$) (Table 3*b*). *Post-hoc t* tests revealed that, in the RBX-CORT group, greater hippocampal responses were elicited by emotional items, whereas neutral items elicited greater hippocampal responses in the CORT-only group (Table 3*b*, Fig. 2*c*).

Table 3. *Functional magnetic resonance imaging data : interaction RBX × emotion (left hippocampus) – post-hoc tests*

			Z	*p*	Effect
(*a*) Interaction RBX × emotion at x = −16, y = −20, z = −21			3.57	< 0.05	RBX groups (E > N) > non-RBX groups (E > N)
RBX groups[a]	Main effect of emotion		1.77	< 0.05*	RBX groups (E > N)
non-RBX groups[a]	Main effect of emotion		3.24	< 0.005*	non-RBX groups (N > E)
emotional[a]	Main effect of RBX		2.08	< 0.05*	RBX groups (E) > non-RBX groups (E)
neutral[a]	Main effect of RBX		–	N.S.	–
(*b*) Interaction CORT × RBX × emotion at x = −20, y = −20, z = −21			2.73	< 0.005	
Non-CORT groups[a]	Interaction RBX × emotion		–	N.S.	–
CORT groups[a]	Interaction RBX × emotion		4.31	< 0.0005*	RBX-CORT (E > N) > CORT-only (E > N)
	RBX-CORT[b]	Main effect of emotion	2.14	< 0.05*	RBX-CORT(E > N)
	CORT-only[b]	Main effect of emotion	3.66	< 0.001*	CORT-only (N > E)
	Emotional[b]	Main effect of RBX	2.32	< 0.05*	RBX-CORT(E) > CORT-only (E)
	Neutral[b]	Main effect of RBX	–	N.S.	

E, Emotional ; N, neutral ; N.S., not significant.

(*a*) Sequential *post-hoc* tests decomposing the reboxetine (RBX) × emotion interaction in the left hippocampal formation (x = −16, y = −20, z = −21).

(*b*) Sequential *post-hoc* tests decomposing the RBX × cortisol (CORT) emotion interaction in the left hippocampal formation (x = −20, y = −20, z = −21).

[a] and [b] Indicate second-order and third-order *post-hoc* tests, respectively.

* Indicates the significance levels of the *post-hoc* tests adjusted for the size of the respective cluster where the main interaction effect was found.

RBX groups comprised the RBX-only group and the RBX-CORT group. Non-RBX groups comprised the placebo group (PLC) and the CORT-only group. Conversely, CORT groups comprised the CORT-only group and the RBX-CORT group and non-CORT groups comprised the PLC group and the RBX-only group.

There were no further two-, three- or four-way interactions between the factors RBX, CORT, emotion and subsequent memory (all p values > 0.05). Taken together, these results suggest that treatment with hydrocortisone selectively decreased hippocampal responses to emotional relative to neutral items, whereas treatment with reboxetine potentiated hippocampal responses to these stimuli. Importantly, the inhibitory influence of hydrocortisone was reversed by co-administration of reboxetine.

Discussion

Imaging results

The present pharmacological fMRI study in 51 healthy subjects showed that experimental elevation of NE and CORT differentially modulated hippocampal responses in an emotional episodic encoding task, depending on whether NE and CORT signalling were challenged separately *versus* in combination. While elevated NE levels enhanced hippocampal responses to emotional items, elevated CORT levels required the presence of elevated NE levels to promote processing of emotional items, suggesting that CORT alone decreased hippocampal function, whereas it increased the effects of noradrenergic stimulation. In a related experiment, we have shown that elevation of NE and CORT to moderate stress levels induced an amygdala response bias to fear (Kukolja *et al*. 2008*a*), which is equivalent to the amygdala hyper-responsiveness to fear in patients with PTSD (Rauch *et al*. 2000 ; Shin *et al*. 2005). When switching the task to emotional episodic encoding, we observed a similar response bias in the hippocampus, which is known to orchestrate, together with the amygdala, the efficient episodic encoding of emotional memories (Burgess *et al*. 2002 ; Phelps, 2004 ; LaBar & Cabeza, 2006).

In the present study, both amygdala and hippocampus showed robust subsequent memory effects, i.e. their activation during encoding of items predicted later memory for these items, irrespective of whether these items were emotional or neutral. While this result is suggestive of a collinearity between emotion and subsequent memory effects, being the most likely reason for the lack of an interaction between these factors, it is important to note that subsequent memory effects were preserved even when emotional and neutral items were analysed separately, confirming the central role of amygdala and hippocampus, and their interplay, in episodic encoding (Burgess *et al*. 2002 ; Phelps, 2004 ; LaBar & Cabeza, 2006). To some

degree, our results imply a functional architecture related to the emotionality of items, with a preferential response bias of the amygdala to the encoding of emotional items and a preferential response bias of the hippocampus to the encoding of neutral items. However, the absence of an interaction between emotion and subsequent memory effects is reflective of largely overlapping amygdala and hippocampal activations, suggesting that episodic encoding engages, to a large extent, the same subset of amygdala-hippocampal neurons.

The observed two-way interaction between the factors emotion and reboxetine treatment suggests that noradrenergic stimulation facilitates hippocampal processing of emotional items at the cost of neutral items. Presuming that enhanced hippocampal responses are reflective of increased encoding efficiency, the present results – on a neural level – support the hypothesis that privileged encoding of emotional information is mediated by elevations of NE and CORT signalling. Originally, Cahill *et al*. (1994) have shown, within the experimental context of an emotional story paradigm, that β-noradrenergic blockade with propranolol specifically eliminated the consolidation advantage of emotional episodic memories. Consistent with this landmark finding, accumulating evidence from pharmacological fMRI studies suggests that propranolol, when administered prior to episodic encoding, blocks the amygdala response to subsequently remembered emotional information (Strange & Dolan, 2004) and to emotional information, irrespective of subsequent memory (van Stegeren *et al*. 2005). Here, we demonstrate a reciprocal neural effect by using a strategy of noradrenergic stimulation instead of inhibition.

In the present study, elevation of CORT shifted encoding-related hippocampal activity towards increased responses to neutral information and decreased responses to emotional information. This result replicates our previous findings (Kukolja *et al*. 2008*a*) and is consistent with observations that elevations of CORT levels reduce fear responses in humans (Soravia *et al*. 2006; Putman *et al*. 2007), an inhibitory effect implying a protective negative feedback regulation (Soravia *et al*. 2006). However, when NE levels were simultaneously elevated, the inhibitory effect of CORT was reversed, such that CORT synergistically interacted with noradrenergic stimulation in jointly amplifying hippocampal activity. Thus, from a functional perspective, the presence or absence of elevated NE signalling quintessentially determines whether CORT activates or deactivates the hippocampus, suggesting a molecular 'switch' by which hippocampal function is physiologically controlled. This hypothesis is in line with rodent studies documenting a noradrenergic dependency of CORT effects on emotional memory (Quirarte *et al*. 1997; McGaugh & Roozendaal, 2002; Roozendaal *et al*. 2006, 2009).

Behavioural results

As expected, emotional items were much better recognized than neutral items. Interestingly, however, stress levels of CORT predicted better encoding of neutral items. This result does not only corroborate evidence of the memory-enhancing potential of CORT (Buchanan & Lovallo, 2001; Abercrombie *et al*. 2003; Het *et al*. 2005; Joels *et al*. 2006; Diamond *et al*. 2007; Kukolja *et al*. 2008*b*), but also illustrates that neutral items, when paired with (pharmacologically) elevated CORT levels, which usually tag emotional items, can achieve the privileged memory status of these items. This finding underlines that physiological mechanisms underlying the memory advantage of emotional events can, in principle, be mimicked by pharmacological means. In contrast, no additional effect of CORT on the encoding of emotional items was observed, suggesting that their emotional salience was sufficient for driving amygdala and hippocampus to optimal performance, without additional benefit from elevated CORT signalling.

The neural effects of reboxetine are not reflected in the behavioural data, which is consistent with the absence of memory-modulating effects following noradrenergic stimulation with RBX (Papps *et al*. 2002; Chamberlain *et al*. 2006) or the α_2-receptor antagonist yohimbine in other fMRI studies (van Stegeren *et al*. 2010). While reboxetine has been shown to modulate emotional episodic encoding in the short term (Hurlemann *et al*. 2005, 2007), long-term effects may require much more intense and/or prolonged noradrenergic stimulation to evolve. Thus, the present study was successful in modelling the adaptive neural mechanisms by which moderate stress may shape amygdala and hippocampal function but could, of course, not model the effects of excessive NE release associated with traumatic stress.

Conclusions

The present pharmacological fMRI study translates evidence of stress-related NE–CORT interactions in the modulation of amygdala and hippocampal function from experimental animals to the human. Together with our previous study, the present findings illustrate the adaptive influence of NE and CORT on amygdala-hippocampal responses. These responses may turn maladaptive under circumstances of traumatic stress, leading to pathological hyperencoding and hyperconsolidation of episodic memories of trauma.

Note

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/psm).

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Declaration of Interest

None.

References

Abercrombie HC, Kalin NH, Thurow ME, Rosenkranz MA, Davidson RJ (2003). Cortisol variation in humans affects memory for emotionally laden and neutral information. *Behavioral Neuroscience* **117**, 505–516.

Amunts K, Kedo O, Kindler M, Pieperhoff P, Mohlberg H, Shah NJ, Habel U, Schneider F, Zilles K (2005). Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. *Anatomy and Embryology* (*Berl*) **210**, 343–352.

Brewin CR (2008). What is it that a neurobiological model of PTSD must explain? *Progress in Brain Research* **167**, 217–228.

Buchanan TW, Lovallo WR (2001). Enhanced memory for emotional material following stress-level cortisol treatment in humans. *Psychoneuroendocrinology* **26**, 307–317.

Burgess N, Maguire EA, O'Keefe J (2002). The human hippocampus and spatial and episodic memory. *Neuron* **35**, 625–641.

Cahill L, Prins B, Weber M, McGaugh JL (1994). Beta-adrenergic activation and memory for emotional events. *Nature* **371**, 702–704.

Chamberlain SR, Muller U, Blackwell AD, Clark L, Robbins TW, Sahakian BJ (2006). Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science* **311**, 861–863.

Diamond DM, Campbell AM, Park CR, Halonen J, Zoladz PR (2007). The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. *Neural Plasticity* **60803**, 1–33.

Dolcos F, LaBar KS, Cabeza R (2004). Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. *Neuron* **42**, 855–863.

Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K, Zilles K (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* **25**, 1325–1335.

Goossens L, Kukolja J, Onur OA, Fink GR, Maier W, Griez E, Schruers K, Hurlemann R (2009). Selective processing of social stimuli in the superficial amygdala. *Human Brain Mapping* **30**, 3332–3338.

Green DM, Swets JA (1966). *Signal Detection Theory and Biophysics*. Wiley: New York.

Het S, Ramlow G, Wolf OT (2005). A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinology* **30**, 771–784.

Hurlemann R (2008). Noradrenergic-glucocorticoid mechanisms in emotion-induced amnesia: from adaptation to disease. *Psychopharmacology* (*Berlin*) **197**, 13–23.

Hurlemann R, Hawellek B, Matusch A, Kolsch H, Wollersen H, Madea B, Vogeley K, Maier W, Dolan RJ (2005). Noradrenergic modulation of emotion-induced forgetting and remembering. *Journal of Neuroscience* **25**, 6343–6349.

Hurlemann R, Matusch A, Hawellek B, Klingmuller D, Kolsch H, Maier W, Dolan RJ (2007). Emotion-induced retrograde amnesia varies as a function of noradrenergic-glucocorticoid activity. *Psychopharmacology* (*Berlin*) **194**, 261–269.

Hurlemann R, Walter H, Rehme AK, Kukolja J, Santoro SC, Schmidt C, Schnell K, Musshoff F, Keysers C, Maier W, Kendrick KM, Onur OA (2010). Human amygdala reactivity is diminished by the beta-noradrenergic antagonist propranolol. *Psychological Medicine* **40**, 1839–1848.

Joels M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ (2006). Learning under stress: how does it work? *Trends in Cognitive Sciences* **10**, 152–158.

Kent JM (2000). SNaRIs, NaSSAs, and NaRIs: new agents for the treatment of depression. *Lancet* **355**, 911–918.

Kiebel S, Holmes AP (2003). The General Linear Model. In *Human Brain Function* (ed. R. S. J. Frackowiak, K. Friston, C. D. Frith, R. J. Dolan, C. J. Price, J. Ashburner and W. D. Penny), pp. 725–760. Academic Press: San Diego.

Kukolja J, Schläpfer TE, Keysers C, Klingmüller D, Maier W, Fink GR, Hurlemann R (2008*a*). Modeling a negative response bias in the human amygdala by noradrenergic-glucocorticoid interactions. *Journal of Neuroscience* **28**, 12868–12876.

Kukolja J, Thiel CM, Wolf OT, Fink GR (2008*b*). Increased cortisol levels in cognitively challenging situations are beneficial in young but not older subjects. *Psychopharmacology* (*Berlin*) **201**, 293–304.

LaBar KS, Cabeza R (2006). Cognitive neuroscience of emotional memory. *Nature Reviews Neuroscience* **7**, 54–64.

Lang PJ, Bradley MM, Cuthbert BN (2008). International affective picture system (IAPS): affective ratings of pictures and instruction manual. Technical Report A-8. University of Florida: Gainesville, FL.

McGaugh JL, Roozendaal B (2002). Role of adrenal stress hormones in forming lasting memories in the brain. *Current Opinion in Neurobiology* **12**, 205–210.

Macmillan NA (1993). Signal detection theory as data analysis method and psychological decision model. In *A Handbook for Data Analysis in the Behavioral Sciences: Methodological Issues* (ed. G. Keren and C. Lewis), pp. 21–57. Erlbaum: Hillsdale, NJ.

Onur OA, Walter H, Schlaepfer TE, Rehme AK, Schmidt C, Keysers C, Maier W, Hurlemann R (2009). Noradrenergic enhancement of amygdala responses to fear. *Social Cognitive and Affective Neuroscience* **4**, 119–126.

Onur OA, Schlaepfer TE, Kukolja J, Bauer A, Jeung H, Patin A, Otte DM, Shah NJ, Maier W, Kendrick KM, Fink GR, Hurlemann R (2010). The N-methyl-D-aspartate receptor co-agonist D-cycloserine facilitates declarative learning and hippocampal activity in humans. *Biological Psychiatry* **67**, 1205–1211.

Papps BP, Shajahan PM, Ebmeier KP, O'Carroll RE (2002). The effects of noradrenergic re-uptake inhibition on memory encoding in man. *Psychopharmacology* (*Berlin*) **159**, 311–318.

Penny WD, Holmes AP (2003). Random effects analysis. In *Human Brain Function* (ed. R. S. J. Frackowiak, K. J. Friston, C. D. Frith, R. J. Dolan, C. J. Price, J. Ashburner and W. D. Penny), pp. 843–850. Academic Press: San Diego.

Phelps EA (2004). Human emotion and memory: interactions of the amygdala and hippocampal complex. *Current Opinion in Neurobiology* **14**, 198–202.

Putman P, Hermans EJ, Koppeschaar H, van Schijndel A, van Honk J (2007). A single administration of cortisol acutely reduces preconscious attention for fear in anxious young men. *Psychoneuroendocrinology* **32**, 793–802.

Quirarte GL, Roozendaal B, McGaugh JL (1997). Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences USA* **94**, 14048–14053.

Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, Orr SP, Pitman RK (2000). Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biological Psychiatry* **47**, 769–776.

Richardson MP, Strange BA, Dolan RJ (2004). Encoding of emotional memories depends on amygdala and hippocampus and their interactions. *Nature Neuroscience* **7**, 278–285.

Roozendaal B, McEwen BS, Chattarji S (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience* **10**, 423–433.

Roozendaal B, Okuda S, van der Zee EA, McGaugh JL (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences USA* **103**, 6741–6746.

Rosenkranz JA, Venheim ER, Padival M (2010). Chronic stress causes amygdala hyperexcitability in rodents. *Biological Psychiatry* **67**, 1128–1136.

Scates AC, Doraiswamy PM (2000). Reboxetine: a selective norepinephrine reuptake inhibitor for the treatment of depression. *Annals of Pharmacotherapy* **34**, 1302–1312.

Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, Macklin ML, Lasko NB, Cavanagh SR, Krangel TS, Orr SP, Pitman RK, Whalen PJ, Rauch SL (2005). A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Archives of General Psychiatry* **62**, 273–281.

Soravia LM, Heinrichs M, Aerni A, Maroni C, Schelling G, Ehlert U, Roozendaal B, de Quervain DJ (2006). Glucocorticoids reduce phobic fear in humans. *Proceedings of the National Academy of Sciences USA* **103**, 5585–5590.

Stanislaw H, Todorov N (1999). Calculation of signal detection theory measures. *Behavior Research Methods, Instruments and Computers* **31**, 137–149.

Strange BA, Dolan RJ (2004). Beta-adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. *Proceedings of the National Academy of Sciences USA* **101**, 11454–11458.

van Stegeren AH, Goekoop R, Everaerd W, Scheltens P, Barkhof F, Kuijer JP, Rombouts SA (2005). Noradrenaline mediates amygdala activation in men and women during encoding of emotional material. *Neuroimage* **24**, 898–909.

van Stegeren AH, Roozendaal B, Kindt M, Wolf OT, Joels M (2010). Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. *Neurobiology of Learning and Memory* **93**, 56–65.

van Stegeren AH, Wolf OT, Everaerd W, Scheltens P, Barkhof F, Rombouts SA (2007). Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. *Neurobiology of Learning and Memory* **87**, 57–66.

Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC (1996). A unified statistical approach for determining significant signals in images of cerebral activation. *Human Brain Mapping* **4**, 58–74.

Yamamoto S, Morinobu S, Takei S, Fuchikami M, Matsuki A, Yamawaki S, Liberzon I (2009). Single prolonged stress: toward an animal model of posttraumatic stress disorder. *Depression and Anxiety* **26**, 1110–1117.