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Endogenous cortisol level interacts with noradrenergic activation in the human amygdala

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Abstract

Animal studies show that high cortisol levels exert their effect on stressful task performance via modulation of the amygdala. Availability of noradrenaline in this brain region appears to be a critical prerequisite for this effect. This relationship between noradrenaline and cortisol is explained by an animal model where the amygdala constitutes a crucial region for this interaction. In humans this model has not been extensively tested so far. In a previously reported study human subjects (aged 20.93 ± 2.38) were scanned using fMRI when watching sets of emotional and neutral pictures after taking the β-adrenergic antagonist propranolol or placebo. Stimulus sets consisted of 92 pictures, divided in four emotional categories that ranged from neutral scenes of domestic objects (CAT1) to extremely negative scenes of mutilation or accidents (CAT4). Confrontation with arousing emotional pictures, accompanied by increased noradrenaline levels, evoked increased amygdala activation under placebo but not under betablocker condition. This new and additional analysis of this data set was carried out to determine the effect of differential endogenous cortisol levels on amygdala activation. Cortisol levels during scanning were determined using salivary samples and subjects were post hoc divided in a High ($n = 14$) and Low cortisol group ($n = 14$). When subjects were watching emotional stimuli, presumably associated with enhanced noradrenaline (NA) levels, amygdala activation was contrasted between the two cortisol groups. We hypothesized that emotional stimuli would elicit more amygdala activation in the High than in the Low cortisol group. Here we demonstrate indeed a significant interaction effect of the endogenous cortisol level with increasing activation in the amygdala under placebo but not under betablocker condition, thereby extending the rodent based model of a synergistic effect of the two stress hormones to the human.

Keywords: Amygdala; fMRI; Noradrenaline; Cortisol; Human

1. Introduction

Catecholamines such as noradrenaline (NA)[1] and adrenaline, in interaction with glucocorticoids, play a key role in both normal homeostasis and in sympathetically mediated responses to stress. They also act as neurotransmitters in the central nervous system where they take care of the fast first reactions in the 'fight or flight' response. The stress

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[1] *Abbreviations:* NA, noradrenaline; CORT, cortisol; NTS, nucleus of the solitary tract; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; HPA, hypothalamic–pituitary–adrenal axis.

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response becomes evident as a rapid activation of the sympathetic nervous system, leading to the release of NA from a widely distributed network of synapses and adrenaline from the adrenal medulla (de Kloet, Joels, & Holsboer, 2005). Systemic adrenaline can activate β-adrenoceptors on vagal afferents terminating in the nucleus of the solitary tract (NTS). In turn, noradrenergic cell groups in the NTS project directly to the amygdala or indirectly via the locus coeruleus, a brain nucleus located in the brainstem (Roozendaal, 2002). This brain area is regarded as the most important source of brain norepinephrine. It projects to the basolateral amygdala where it is critically involved in emotional arousal induced memory facilitation.

A second and relatively slower response to stress is executed by the secretion of cortisol (CORT). Psychological stressors that activate limbic structures such as amygdala and hippocampus also lead to activation of the paraventricular nucleus of the hypothalamus. Corticotropin or adrenocorticotropic hormone (ACTH) is secreted from the anterior pituitary in response to corticotropin-releasing hormone (CRH) from the hypothalamus. In this way the hypothalamic–pituitary–adrenal (HPA) axis is involved in the second wave of the stress response where ACTH stimulates the adrenal cortex to secrete glucocorticoids such as cortisol. Cortisol, that freely passes the blood brain barrier, affects all bodily and brain tissues, which response is normally leading to a renewed homeostasis via a negative feedback loop (Lovallo & Thomas, 2000). Although cortisol receptors can be found in almost all brain areas, a relatively high density of receptors is found in the prefrontal cortex, the NTS, amygdala and hippocampus (Gold, Drevets, & Charney, 2002).

Finally, the relationship between noradrenaline release and the control of glucocorticoid release is embodied by a limbic system—HPA axis interaction via projections of hippocampal and amygdalar efferents that relay with neurons in the bed NTS, hypothalamus and brainstem to access corticotropin releasing hormone neurons (Herman, Ostrander, Mueller, & Figueiredo, 2005).

A wide array of studies focused on the effect of these stress hormones (nor) adrenaline and cortisol on memory performance in animals and humans (Cahill & McGaugh, 1996; Lupien et al., 2005a; McGaugh, 2004; van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998) and on the mechanisms and structures involved in this process in animals (McGaugh, 2000, 2002; Roozendaal et al., 2006a) and humans (Bremner, 2003; McIntyre, Power, Roozendaal, & McGaugh, 2003; Pare, 2003; Phan, Wager, Taylor, & Liberzon, 2002; Vermetten & Bremner, 2002a, 2002b).

It became clear from studies on patients with brain damage that the amygdalae are essential nuclei in the brain in the labeling, perception, encoding and other processes related to emotional memory (Adolphs, Tranel, & Buchanan, 2005; Anderson & Phelps, 2001). Reviewing the relevant studies on this subject, the role of the amygdala as pivotal structure in emotional information processing has been established also in humans (Labar & Cabeza, 2006; Phan et al., 2002; Zald, 2003). In addition, it was essential to show that the amygdala is the site of action for noradrenergic activation in emotional information processing in humans. Some firm evidence now exists that—also in humans—the emotional information processing in the amygdala is mediated by noradrenaline (Hurlemann et al., 2005; Strange, Hurlemann, & Dolan, 2003; van Stegeren et al., 2005).

The role of cortisol in relation to amygdala activity and cognitive performance has delivered a more complex image (de Kloet, Oitzl, & Joels, 1999; Lupien & McEwen, 1997). These studies indicate that glucocorticoids (GCs) have diverse and often conflicting effects on memory function. The evidence suggests that the consequences of glucocorticoid activation on cognition depend largely on the different memory phases and functions investigated (working memory; encoding, consolidation or retrieval) (Lupien, Maheu, & Weeks, 2005b; Roozendaal, 2002; Roozendaal, Okuda, de Quervain, & McGaugh, 2005). Moreover the role of both mineralocorticoid and glucocorticoid receptors in the various memory stages should be taken into account as well as the context in which this corticoid-receptor activation takes place (de Kloet et al., 1999). Very similar to the effects of catecholamines, pre- and post-training activation of glucocorticoid-sensitive pathways involving glucocorticoid (GR) receptors enhances memory consolidation in a dose-dependent inverted-U fashion. In humans it was shown that elevated cortisol levels during memory encoding enhances the long-term recall performance of emotionally arousing pictures relative to neutral pictures (Buchanan & Lovallo, 2001; Kuhlmann & Wolf, 2006) although other studies found mixed results (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003; Putman, Van Honk, Kessels, Mulder, & Koppeschaar, 2004). On the other hand evidence for a detrimental effect on delayed memory retrieval by high cortisol levels has been found in rodents (de Quervain, Roozendaal, & McGaugh, 1998) as well as in humans (de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Het, Ramlow, & Wolf, 2005; Wolf, Kuhlmann, Buss, Hellhammer, & Kirschbaum, 2004). In short, while some progress has been made in relating the effects of cortisol to specific memory phases, the neural correlates underlying these effects have not been elucidated sufficiently in the human.

Based on recent findings on the amygdala's role in mediating acute effects of glucocorticoids on memory consolidation in rats, the relation between noradrenaline and corticosterone (in animals) could be summarized by a model proposed by Roozendaal and McGaugh (Roozendaal, 2000, 2002; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006b), which reflects the interaction of noradrenaline and cortisol. The core of the model is that high cortisol levels can exert its effect only *under the condition* that noradrenaline is present in the amygdala. NA in the amygdala is a necessary prerequisite for CORT to have an effect on amygdala activation as well as on other in memory involved brain areas. This led to the hypothesis for the pres-

ent study in which we wanted to test if this animal model on the interaction of NA and CORT could be applicable to humans as well.

Only a few human studies to date have looked at the interaction of these two stress hormone systems on memory, but all without imaging. Cahill and colleagues used a cold pressor stress procedure and succeeded to induce elevated cortisol levels after subjects viewed slides of varying emotional content. Elevated cortisol levels enhanced memory for emotionally arousing slides but not for relatively neutral slides. They suggest that in humans cortisol may interact with the degree of arousal at initial encoding of information to modulate memory consolidation (Cahill, Gorski, & Le, 2003). Another study assessed whether the effects of cortisol (induced by a psychosocial stress task) on working memory depend on the level of adrenergic activity (as measured by sympathetic activation) during memory performance. It was found that adrenergic activation is essential for the impairing effects of stress-induced cortisol on working memory (Elzinga & Roelofs, 2005).

Here we present a new and additional analysis of a recent imaging study (van Stegeren et al., 2005) to investigate endogenous cortisol effects on amygdala activation in humans. In that study amygdala activation, monitored with functional magnetic resonance imaging (fMRI), increased with emotional intensity of the pictures under placebo condition, as has been also found by others (Cahill et al., 1996; Canli, Zhao, Brewer, Gabrieli, & Cahill, 2000; Zald, 2003). Betablockade with propranolol, a non selective β-adrenergic antagonist selectively decreased amygdala activation for emotional pictures but not for neutral pictures. It was concluded that the neurotransmitter noradrenaline also mediates amygdala activity in humans when processing emotional stimuli. Hence, emotional stimuli lead to increased activation in the amygdala and this amygdala activation is noradrenergic dependent (Hurlemann et al., 2005; Strange et al., 2003; van Stegeren et al., 2005).

In this experiment cortisol levels of all subjects were measured before and immediately after the scanning procedure. This provided us with a marker of the subject's (endogenous) cortisol level during the experiment when watching sets of pictures—varying from neutral to extremely negative emotional—in the scanner. If a high cortisol level interacts with noradrenergic activation in the amygdala, then viewing emotional compared to neutral pictures should result in increased amygdala activation in subjects with a high versus low cortisol level. This hypothesis was tested here.

2. Materials and methods

2.1. Subjects

Twenty-eight right-handed subjects out of 30 participants (14 male, 14 females; mean age 20.93 ± 2.38, ranging from 18 to 28 years) without medical or psychiatric history successfully completed the experiment that was approved by the Medical Ethical Committee of the VU University Medical Centre (VUMC) and informed consent was obtained from all subjects. Two subjects dropped out, because one female subject withdrew from the experiment before it was finished and the other (male) subject fell asleep in the scanner.

2.2. Saliva sampling and cortisol measurement

Cortisol levels were assessed out of salivary samples obtained using Salivette sampling devices (Sarstedt, Rommelsdorf, Germany). Free cortisol levels are measured using a commercially available immunoassay (IBL, Hamburg, Germany). Inter- and intra-assay variations are below 15%. After an acclimatization period of 15 min cortisol levels (CORT) of all participants were measured at baseline (t_0), immediately before entering the scanner (t_1) and immediately after the scanning procedure (t_2) (Fig. 1a). Because we were not able to measure salivary cortisol levels directly during scanning, we tried to obtain a good estimation of the cortisol level during the scanning procedure. We defined a mean cortisol level (ScanCort) by calculating the mean of the cortisol levels just before they went into the scanner and immediately after they left the scanner. So the mean cortisol level was calculated between t_2 and t_1 with $[\mathrm{CORT}(t_2) + \mathrm{CORT}(t_1)]/2$. We then used a median split to obtain a High_Cort versus Low_Cort group, both consisting of 14 subjects that were—just by coincidence—completely evenly divided over the sexes.

2.3. Procedure

On two consecutive days subjects came to the fMRI department of the VUMC and received a betablocker, a non-selective noradrenergic antago-

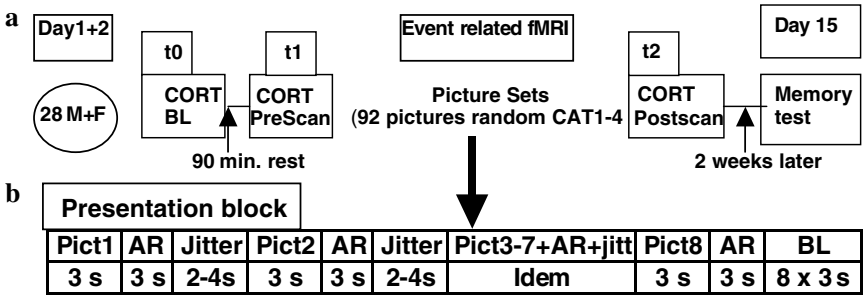

Fig. 1. Experimental design and procedure. (a) Study design: The first session took place on two consecutive days where 14 male (M) and 14 female (F) subjects came to the scanning department for this fMRI study. Saliva samples for cortisol measurements were obtained at t_0, t_1 and t_2. (b) Structure of one 'presentation block'. Lying in the scanner subjects were presented with blocks of 8 pictures (Pict) containing random assortments of pictures across all four emotional categories. After each picture subjects were asked on screen for an affective rating (AR) of the previous picture with 1 being 'not emotional at all' to 4 being 'extreme emotional'. These individual emotional ratings were used to classify the pictures for further event-related fMRI analysis. BL, baseline, gray screens; Jitt, jitter.

nist, or placebo, double blind and crossed over. The outcome of this drug manipulation on amygdala activation and memory has already been reported (van Stegeren et al., 2005). Because in an earlier study (Maheu, Joober, Beaulieu, & Lupien, 2004) the application of propranolol (80 mg) significantly enhanced free cortisol levels approximately 1.5 h after its administration, we chose to first look at the data in this design of the placebo (PL) scans only, in order to avoid contamination of the results.

Stimulus sets consisted of 92 pictures, divided in four emotional categories that ranged from neutral scenes of domestic objects (CAT1) to extremely negative scenes of mutilation or accidents (CAT4) and are extensively described elsewhere (van Stegeren et al., 2005). The sets were presented in an event-related jittered design (Donaldson & Buckner, 2001) and analyzed with a general linear model (GLM). After pre-scan cortisol measurements subjects were positioned in the MRI scanner, where a structural scan was made first. Then the experimental (event-related) functional imaging started. Subjects were presented with blocks of 8 pictures containing random assortments of pictures across all four emotional categories. After each picture (presentation time 3 s), subjects were asked on screen (within 3 s) to indicate the emotional intensity of the previous picture by pressing one of four buttons with their right hand, with 1 being 'not emotional at all' to 4 being 'extreme emotional'. These individual emotional ratings were used to classify the pictures for further event-related fMRI analysis. After a block of 8 pictures with 8 emotional ratings by the subject, 8 gray screens served as a resting (baseline) period, also presented as events and jittered (Donaldson & Buckner, 2001) (Fig. 1b).

2.4. fMRI acquisition

Imaging was carried out on a 1.5 T Sonata MR scanner (Siemens, Erlangen, Germany), using a polarized head coil with foam padding to restrict head movement. Twenty-three slices positioned perpendicular to the long axis of the hippocampus and completely covering the amygdala and hippocampus were collected using a gradient-echo echo-planar imaging pulse-sequence (in-plane voxel size 3×3 mm; 2.5 mm thick, 0.5 mm gap; repetition time, 2130 ms; echo time, 50 ms; total number of scans in each subject ~620). A T1-weighted structural MRI-scan was also acquired (MPRAGE; inversion time: 300 ms, TR = 15 ms; TE = 7 ms; flip angle = 8°; 160 coronal slices, $1 \times 1 \times 1.5$ mm voxels). Each subject's functional images in the current experiment were first inspected to ensure adequate signal in both amygdalae.

2.5. Gender

Subjects' sex (14 men and 14 women) has been entered as a separate variable in both the fMRI data analysis as well as the memory data analysis. No significant interaction effects with cortisol levels were noticeable. Splitting the groups by sex and cortisol levels left us with an $n = 7$ per cell. We therefore decided to leave further specifications about these non-significant effects out and report the results with respect to the Cortisol effects only, which is the main focus of this study.

2.6. fMRI data analysis

All MRI analyses were carried out using FEAT (FMRI Expert Analysis Tool) Version 5.4, part of FSL 3.2 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl; (Smith et al., 2004)). Pre-statistics processing included slice-timing correction using Fourier-space time-series phase-shifting; motion correction (Jenkinson, Bannister, Brady, & Smith, 2002); non-brain removal (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 8 mm; mean-based intensity normalization of all volumes by the same factor; high-pass temporal filtering (Gaussian-weighted LSF straight line fitting, with sigma = 100.0 s). Time-series statistical analysis was carried out using local autocorrelation correction (Woolrich, Ripley, Brady, & Smith, 2001), modeling in each subject the events using a double γ-hemodynamic response function and its temporal derivative. Functional images were co-registered to high-resolution scans and subsequently to standard space images.

At first level comparisons of interest were implemented as linear contrasts (Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004). Amygdala activation was analyzed contrasting each emotional category to baseline and with contrasts comparing increasing emotional categories (CAT2, 3 and 4) with the neutral CAT1 (CAT2 > CAT1; CAT3 > CAT1; CAT4 > CAT1).

At higher-level an ANCOVA model was used with the mean cortisol level during scanning (ScanCort) as regressor and with scan session as additional covariate to account for possible differences between scan 1 and 2. The effect of ScanCort was pictured for all first level contrasts (e.g. CAT3 > 1, CAT4 > 1). Additionally another higher-level analysis was carried out using an ANCOVA model with the two cortisol groups (High and Low Cortisol). For all first level contrasts, the average activation was determined for the High and Low_Cort groups, as well as the difference between the groups (Cort_High > Cort_Low; and the inverse contrast Cort_Low > Cort_High). For group averages, Z (Gaussian T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold of $p = 0.05$ (Friston, Worsley, Frakowiak, Mazziotta, & Evans, 1994). The analyses testing for group differences were restricted to the amygdalar regions that were activated in either group average, because we had very specific hypotheses about this region beforehand. Given the small sizes of the amygdalae as regions of interest (ROIs), these statistic images were thresholded using $Z > 2.3$, uncorrected.

3. Results

3.1. Cortisol

No significant difference in cortisol level was found between t_1 and t_2 ($F(1,27) = 1.13$; $p = .30$), meaning that cortisol levels were not affected by the scanning procedure itself. Mean values of the High_Cort versus Low_Cort group pointed at significantly different cortisol levels between the groups as they were created (Mean ± SD: High_Cort = 8.78 ± 1.97; Low_Cort = 5.40 ± 1.11, $p < .001$). Subjects were tested throughout the day from 9 am until 6 pm. As can be expected, based on the circadian rhythm of cortisol, a (moderate) correlation was found between test time and CORT (Spearman's $\rho = -.38$, $p < .05$). Differences between the High and Low_Cort group remained however, when time of day was controlled for in an ANCOVA. Men and women did not differ in cortisol level at t_0, t_1 or t_2 and were (coincidentally) equally distributed over the High and Low_Cort groups (7 in each).

3.2. fMRI data: Amygdala activation

The analyses under PL condition showed a significant main effect of cortisol level (ScanCort) for the contrast of CAT3 > 1 pictures ($Z = 3.2$, cluster-corrected $p = .05$), but not for CAT4 > 1 pictures. Since the variance of cortisol levels is relatively high, we chose to repeat the analyses with the group divided in a High and Low Cort group.

The analyses under PL condition showed significant effects for the High_Cort > Low_Cort groups, when subsequent emotional categories were compared. Preliminary evidence to support the main hypothesis of this study was provided by the contrasts where amygdala activation in the High_Cort condition was contrasted with the activation in the Low_Cort condition. Increased amygdala activation for

the emotional pictures, compared to the neutral CAT1 pictures, was significantly higher in the High_Cort compared to the Low_Cort group, pointing at an interaction of emotional intensity, which we hypothesized to be related to noradrenergic activation, with endogenous cortisol level.

Comparing activation for the contrast of CAT2 > 1 and the group High in cortisol with the Low_Cort group led to a trend in amygdala activation (Fig. 2a). But comparing the High_Cort > Low_Cort group for the CAT3-1 and CAT4-1 contrast led to significant clusters located in the right amygdala complex (Fig. 2b and c). *Z*- and *p*-values and local maximum coordinates of these analyses are presented in Table 1. No effect was found for the inverse contrasts of Low > High cortisol groups and session effects were not significant.

Table 1
Placebo condition: comparison of activation in High_Cort > Low_Cort

Placebo condition: High_Cort > Low_Cort					
First level contrast	Cluster corr. *P*	Max *Z*	*x* (mm)	*y* (mm)	*z* (mm)
Cat 2 > 1	0.07	2.31	−14	−6	−12
Cat 3 > 1	0.04*	3.36	10	−4	−14
Cat 4 > 1	0.04*	3.36	24	0	−14

* Significant with cluster corrected threshold $Z = 2.3$, $p < .05$.

3.3. Amygdala activation: Interaction with CORT under PL and BB condition

Although there were some arguments to specifically look at the PL condition only, in order not to be disturbed by possible interaction effects of the BB on cortisol levels, the data of activation under BB condition were also available. It would be interesting indeed to explore whether cortisol levels under BB condition would differentially affect amygdala activation, or even not affect this activation at all. If the model, tested here, would be applicable to human interaction of NA and CORT, then the substantial decrease or absence of NA activation under betablockade should blunt cortisol effects (hence would result in no or decreased High > Low differences in the BB condition). This was tested in additional analyses.

Analogously to the first (PL only) analysis, two groups with respect to CORT levels during scanning were created within the BB condition by a median split. This led to a High_Cort versus Low_Cort group with significantly different cortisol levels (for BB condition: Mean ± SD: High_Cort = 13.05 ± 3.79; Low_Cort = 6.06 ± 1.55, $p < .001$). Comparing mean cortisol levels between the drug conditions revealed that during scanning CORT levels were higher

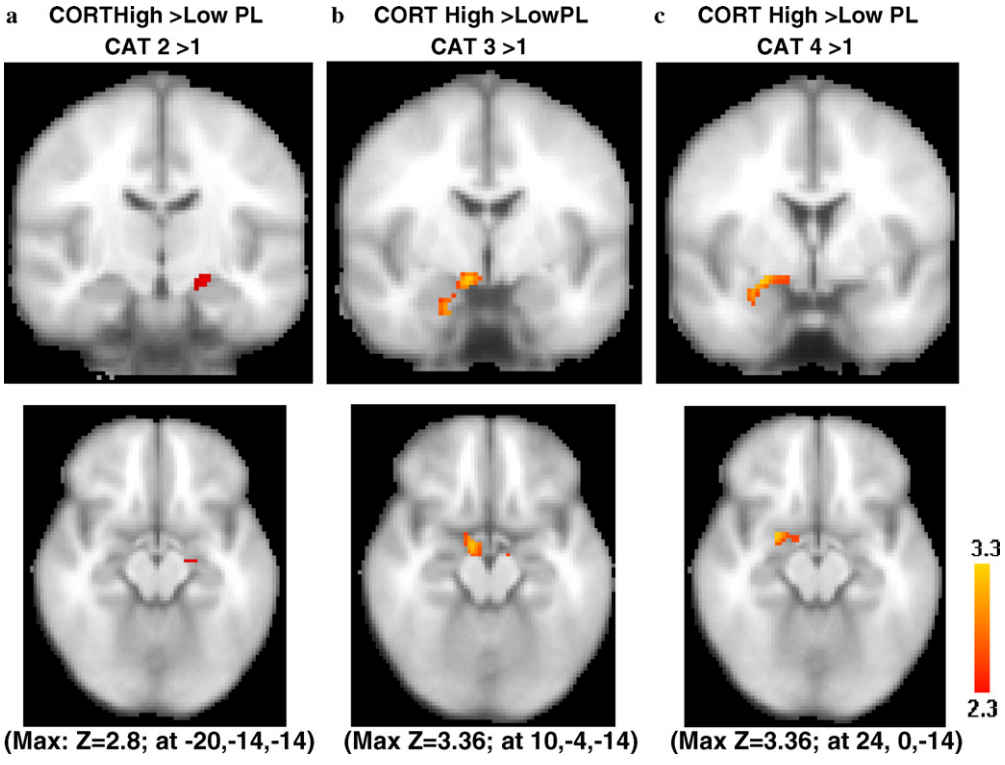

Fig. 2. Interaction of groups with high (Cort_High) and low (Cort_Low) cortisol levels with noradrenergic response in amygdala under placebo (PL) condition. Coronal (top panels) and transversal (bottom panels) sections at the level of the amygdala as region of interest (ROI) for contrasts of CAT2, 3 and 4 with the neutral CAT1 pictures. Interaction of Cort_High > Low groups with increasing emotional intensity of the pictures (hypothesized by us to be related to increasing noradrenergic levels) is portrayed. Right in pictures is left in brain. The cluster corrected threshold is $Z = 2.3$. (a) CAT2 > 1: Contrast of activation during CAT2–CAT1 pictures comparing Cort_High > Cort_Low; max. cluster in Left amygdala at (−20, −14, −14), $Z = 2.8$; $p < .08$; (b) CAT3 > 1: contrast of activation during CAT3–CAT1 pictures comparing Cort_High > Cort_Low groups; max. cluster in R amygdala at (10, −4, −14), $Z = 3.4$; $p < .05$; (c) CAT4 > 1: contrast of activation during CAT4–CAT1 pictures comparing Cort_High > Cort_Low groups; max. cluster in R amygdala at (24, 0, −14), $Z = 3.4$; $p < .05$.

under BB condition than under placebo condition ($p < .05$). Comparing cortisol levels of the Low_Cort groups of both drug conditions with an ANOVA did not show a significant difference. There was a significant difference between the High_Cort groups of BB versus PL, however ($p < .001$).

Analyses and thresholding comparable to the PL condition were carried out for the group under BB condition separately. No significant activation passed the threshold for the High > Low_Cort comparisons of any of the category contrasts (Cat2 > 1, Cat3 > 1 or Cat4 > 1). This supports the idea that decreasing noradrenaline levels in the brain by betablockade erases the differential positive effect of a high compared to low endogenous cortisol level on amygdala activation.

We then carried out an analysis, exploring the interaction effect between drug application (PL > BB) with endogenous cortisol level on amygdala activation for emotional versus neutral pictures. We directly compared brain activation under PL and BB condition with contrasts for High > Low Cort groups in the subsequent picture categories. Note that interpreting the results should be done with caution, since the High and Low_Cort groups refer to different absolute levels within each drug group. Calculating the difference in activation of PL > BB, interacting with Cort_High > Low levels, a small cluster was found in the amygdala for CAT3 > 1 ($Z = 2.35$; max. voxel at $x, y, z = 20, -8, -24$) and a significant cluster in the right amygdala for the CAT4 > 1 contrast ($Z = 3.01$; $p = 0.05$; max. voxel at $x, y, z = 14, 0, -12$) (Fig. 3). This cannot be attributed to the High cortisol levels as such, because these are even higher in the BB group. It supports the idea, that it is indeed the noradrenergic activation in the amygdala, evoked by emotional pictures of CAT3 and CAT4, that enables higher cortisol levels to exert an additional effect on amygdala activation. No significant effect of the reverse comparison of BB > PL or of CORT_Low > High was found.

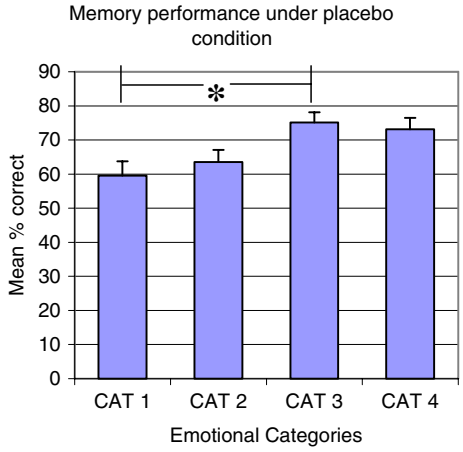

Fig. 4. Memory performance ranging from emotional neutral (CAT1) to extremely negative emotional (CAT4) pictures under placebo condition. (*$p < .05$.)

3.4. Memory

Memory performance was assessed two weeks after scanning. As has been reported earlier (van Stegeren et al., 2005) memory performance for the emotional CAT3 and CAT4 stimuli was better than that of the neutral CAT1 pictures under PL condition (Fig. 4). A MANOVA GLM was carried out using SPSS12.0.1 on the memory scores of the PL group with emotional intensity (EMO) as within repeated measures (Cat1, Cat2, etc.) and with CORT (High versus Low) as between variable. No main or interaction effect of the endogenous cortisol level during scanning on memory performance two weeks later was found.

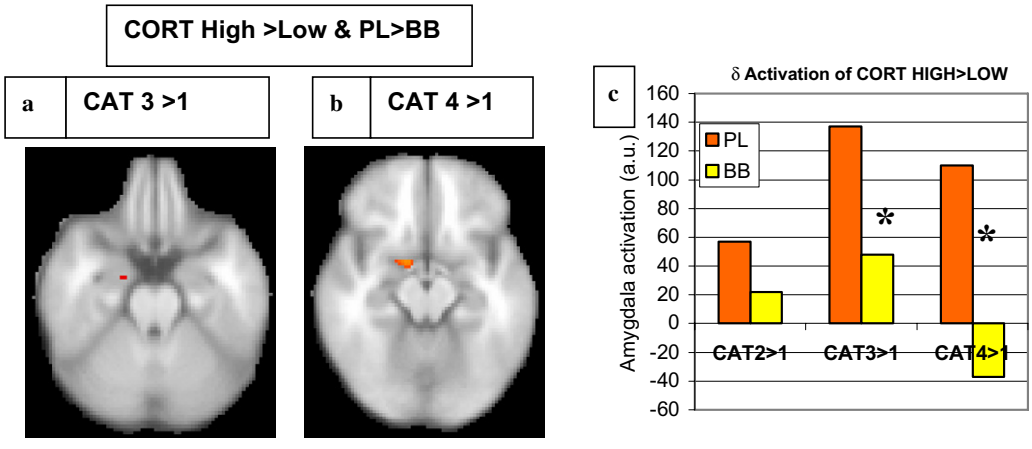

Fig. 3. Interaction of PLACEBO (PL) > Betablocker (BB) with High > Low CORT. Amygdala activation under betablocker (BB) and placebo (PL) condition was contrasted both ways and delivered significant activation for the PL > BB comparison. This contrast was then compared for the High versus Low cortisol groups. A small cluster in the amygdala is visible for this comparison (PL > BB, CORT High > Low) when watching emotional CAT3 compared to CAT1 pictures ($Z = 2.35$; $x, y, z = 20, -8, -24$) (a). An even bigger cluster remained when comparing the most emotional CAT4 > CAT1 pictures ($Z = 3.01$; $x, y, z = 14, 0, -12$) (b). The difference in activation for the High > Low_Cort groups was calculated and applied in each analysis of PL and BB group, for the successive contrasts of the emotional categories. Then PL and BB groups were compared and the contrast of PL > BB in interaction with the High > Low_Cort group resulted in significant activation for the CAT3 > 1 and CAT4 > 1 contrast (*$p < .05$) (c).

4. Discussion

These analyses support our hypothesis that endogenous cortisol levels at the time of encoding interact with the level of noradrenergic activation in the amygdala in man. Our findings agree with the model of Roozendaal and McGaugh obtained in rats (McGaugh, 2000, 2004; Roozendaal, 2000, 2002), recently underlined in two studies that focused on the synergistic actions of glucocorticoids and emotional arousal-induced noradrenergic activation of the BLA (Roozendaal et al., 2006a, 2006b). They state that this interaction constitutes a neural mechanism by which glucocorticoids may selectively enhance memory consolidation for emotionally arousing experiences (Roozendaal et al., 2006a) and plays a role in processes such as auditory fear conditioning as well (Roozendaal et al., 2006a). This model assumes that the glucocorticoid effects on memory consolidation are critically depending on the noradrenergic level in the BLA and the interaction with other brain areas. We here found support for a role of endogenous cortisol levels in human amygdala activation. The endogenous cortisol level of our subjects interacted with noradrenergic activation within the amygdala: subjects that had high cortisol levels showed significantly more amygdala activation during emotional pictures than the group low in cortisol. This additional effect of the High > Low cortisol level was visible as a small cluster in the left amygdala when comparing lightly emotional CAT2 pictures with neutral CAT1 pictures. But confrontation with CAT3 and extreme emotional CAT4 pictures led to a significantly activated cluster (even when thresholded with a cluster corrected p) in the right amygdala, when High > Low cortisol groups were contrasted.

The analyses with the BB group do give additional indications that this model is applicable in humans too. Of course the question arises if propranolol might influence brain hemodynamics in general. In that case the propranolol-induced decrease in amygdala activation as we found, might reflect changes in brain hemodynamics rather than blockade of noradrenergic signaling. Two aspects of our findings however argue against this option. Firstly, no main effect of propranolol on brain activation is found when compared with placebo. If our findings would be the result of a general decrease in hemodynamic perfusion of the brain, then similar decreases in activation would be found when watching pictures of all four emotional categories. This is however not the case. Increased amygdala activation for the emotional CAT3 pictures (hypothesized by us to be related to increased noradrenergic activation) was selectively reduced by this dosage of propranolol, when compared to the neutral CAT1 pictures. Secondly, this effect of propranolol during the confrontation with emotionally arousing pictures is even more supportive for a selective and not a general hemodynamic effect if one realizes that the emotional and neutral pictures were randomly presented.

When NA levels are decreased with this dosage of a betablocker (80 mg propranolol), the differential effect of the High > Low CORT level that affected amygdala activation in the placebo group disappeared. It is clear that these results should be investigated in a new experimental study where drug application and control groups are designed in such a way as to rule out some of the contamination that is apparent in this study. Hence, although this study is supportive for this solidly tested model in animals, the ultimate support for a comparable model in humans should be furnished by well-designed glucocorticoid challenge studies.

In line with earlier studies (Kizildere, Gluck, Zietz, Scholmerich, & Straub, 2003; Maheu et al., 2004) cortisol levels were higher in response to the betablocker condition, although not significantly in this study. In a clinical study (Kizildere et al., 2003) propranolol was supplied 2 h before a CRH-test that provoked an increase in ACTH as well as cortisol levels. Propranolol was shown to decrease the CRH-stimulated cortisol response in healthy human subjects. Not many studies specifically looked into the question as to why cortisol levels rise in reaction to the betablocker propranolol. Some studies noted a propranolol-induced increase in plasma ACTH and/or serum cortisol level after CRH-stimulation during hypoglycemia (Jezova-Repcekova, Klimes, Jurcovicova, & Vigas, 1979; Lager, Jagenburg, von Schenck, & Smith, 1980), but this increase in cortisol was also occurring independently from the preceding (hormonal) CRH stimulation or hypoglycemic situation, so just by providing propranolol (Maheu et al., 2004). It appears that none of these effects—on ACTH and cortisol level responses—may be directly on the adrenocortical level but may be more systemic, since β-adrenergic receptors were not found on normal human adrenocortical cells (Kizildere et al., 2003).

Several studies (Adolphs et al., 2005; Cahill et al., 1996, 2000) have shown that amygdala activity is enhanced when subjects view or encode emotional compared to neutral pictures. Recently it was established that this amygdala activation is mediated by noradrenaline (Hurlemann et al., 2005; Strange et al., 2003; van Stegeren et al., 2005). The present study is the first to show that endogenous cortisol levels amplify this noradrenergic effect in humans. Subjects with a relatively high cortisol level showed more amygdala activation when watching emotional pictures compared to neutral pictures than subjects with a low cortisol level. We showed that the endogenous cortisol level at least exerts its effect during the encoding phase of emotional material on amygdala activation. The current data do not allow speculating about the effects of cortisol during the other phases of the memory process.

It might seem surprising that cortisol levels were not affected by the scanning procedure, a possible stressor in its own right, but also not by the stimulus material during scanning. It consisted of a set of horrible emotional scenes (with mutilations and accidents), that indeed was capable of triggering the noradrenergic system. This was shown in an earlier pilot, where heart rate in reaction to the

emotional stimuli was significantly different from the response to the neutral pictures (van Stegeren et al., 2005). Moreover, salivary α-amylase that has been shown to be a good marker for noradrenergic activation in several studies now (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004), did increase in our subjects under placebo condition during scanning, but not under betablockade (van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Also in the scanner amygdala activation was higher for the CAT3 and CAT4 pictures compared to the neutral CAT1 pictures. But cortisol levels were not different just before compared to immediately after scanning, pointing at a difference in responsiveness of these stress hormone systems. Recently, some studies also found that cortisol levels did not change in subjects from pre-task to post-task in stress conditioning paradigms (Jackson, Payne, Nadel, & Jacobs, 2006; Zorawski, Cook, Kuhn, & LaBar, 2005). In a large meta-analysis reviewing lab studies to see which procedures do and which ones do not lead to cortisol responses, Dickerson and Kemeny (2004) conclude that tasks containing both uncontrollable and social-evaluative elements were associated with the largest cortisol and adrenocorticotropin hormone changes and the longest times to recovery. But the average effect size for tasks consisting of 'emotion induction' were not significantly different from zero, indicating that on average, emotion induction tasks per se did not elicit a significant cortisol response. This led us to conclude that the median split in a High versus Low_Cort group should not be regarded as a difference in task-related cortisol response, but more as a difference in endogenous 'state' of the subjects when watching the pictures during the fMRI scanning.

There is some evidence that corticosteroid hormones play an important role in the control of vascular smooth muscle tone by the permissive effects in potentiating vasoactive responses to norepinephrine (Ullian, 1999; Yang & Zhang, 2004). So, not only it is true that animal studies show that NA is a necessary prerequisite to allow an effect of cortisol, mainly via the basolateral nucleus of the amygdala, conversely a high level of glucocorticoids affect the impact of raised norepinephrine or noradrenaline levels on the vascular smooth muscle tone, leading to vasoconstriction. A mechanism like this is difficult to match with our findings. These studies (Ullian, 1999; Yang & Zhang, 2004) were however mainly related to supra-physiological CORT levels that were present either by application of cortisol agonists or in hypercortisolaemia. Secondly, these studies describe a dose-response relationship between cortisol and the effect of NA on vascular tone. We conclude that the intense interaction of the two stress systems occurs at multiple levels (e.g. also for the vascular response), but that the natural order of the stress response pleads for an HPA response to stress that is facilitated when the SNS system is already active.

The memory data were not in line with the imaging data. Cortisol has been found to both enhance memory consolidation and reduce memory retrieval, leading to mixed predictions about its total effect on long-term memory (Lupien et al., 2005a; Pitman & Delahanty, 2005). The fine-tuning of the hormonal interplay between the noradrenergic and cortisol system at encoding and its effect on long-term memory performance is apparently affected by many factors in time. So a significant synergistic effect of cortisol in the noradrenergic system of the amygdala at the time of encoding does not translate directly into improved long-term memory performance. In a recent article Wilkinson and Halligan (2004) are discussing the relevance of behavioral corroboration of imaging data in the field of cognition. They argue that the demand for behavioral corroboration in functional imaging experiments is heavily depending on our knowledge about which parts of the cognitive process in question produce the observed behavioral responses, and which parts produce the observed neural activations.

It is not easy to compare the outcome of this study with the few human studies mentioned that were looking at interaction effects between the stress hormone systems (Cahill et al., 2003; Elzinga & Roelofs, 2005). The design is not comparable because in the first study the cortisol level was manipulated endogenously by a stress procedure (Cahill et al., 2003) and elevated cortisol levels were evoked only during the consolidation phase. In the second study the memory phase studied relates to working memory (Elzinga & Roelofs, 2005) as compared to the long term recognition memory performance in this study.

We emphasize that in this study High versus Low_Cort levels are referring to the physiological status of the subjects during scanning and were not manipulated experimentally. Maybe higher or exogenously manipulated cortisol levels would have translated into positive memory effects two weeks later. This hypothesis is supported by two pharmacological studies which observed that treating subjects with 20 or 30 mg of hydrocortisone (which induces cortisol levels in the upper physiological range) before the presentation of IAPS pictures lead to enhanced emotional memory facilitation in memory tests conducted one day (Kuhlmann & Wolf, 2006) or one week (Buchanan & Lovallo, 2001) later. Thus while the differences in endogenous cortisol levels observed in the present study between the Low_Cort and the High_Cort groups appears to have influenced amygdala activity during encoding, more substantial cortisol elevations might be needed in order to reliably induce behavioral effects on a memory test 1 or 2 weeks later.

Of course it is unclear in our study how long the High versus Low_cort groups remained in this status of significantly different cortisol levels after the experiment was over. So it is unknown how long cortisol levels remained different during the consolidation phase of the processing of the pictures presented. It might be true that for an additional effect on memory performance cortisol levels should remain high for a considerable amount of time. Also, it could be argued that the effect on the ultimate memory performance could be depending on whether or not the cortisol response is related to the 'to be remembered material' itself. Okuda et al. investigated whether the influence of glucocorticoid hormones on memory depends on the level of emotional

arousal induced by the training experience itself. When rats were extensively habituated to the training apparatus (in the absence of any objects) they were less aroused by object recognition training than rats not given prior habituation training. Their findings suggest that training-induced emotional arousal may be essential for glucocorticoid effects on object recognition memory (Okuda, Roozendaal, & McGaugh, 2004; Roozendaal et al., 2006b). Several of these issues should be faced in future studies and pleads again for a well-controlled glucocorticoid challenge study.

Recently reviewing the literature on psychological and neural mechanisms underlying emotional memory processes Labar and Cabeza (2006) conclude that the memory enhancing effects of emotional arousal involve interactions between subcortical and cortical structures and engagement of central and peripheral neurohormonal systems that are coordinated by the amygdala (Labar & Cabeza, 2006). This lab study supports this role of the amygdala in the interactive play between noradrenergic and cortisol effects in humans when confronted with emotional stimuli.

In sum, we demonstrated a significant interaction effect of the endogenous cortisol level with noradrenergic activation in the amygdala during the perception of emotional material. This can initiate additional studies with planned differences in glucocorticoid levels to elucidate the relationship between these two stress hormone systems, memory performance and amygdala activation.

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