E.R. de Kloet, M.S. Oitzl & E. Vermetten (Eds.)
Progress in Brain Research, Vol. 167
ISSN 0079-6123

CHAPTER 20

Interaction of endogenous cortisol and noradrenaline in the human amygdala

Anda H. van Stegeren[1,*], Oliver T. Wolf[2], Walter Everaerd[1] and Serge A.R.B. Rombouts[3]

[1]*Department of Clinical Psychology and Cognitive Science Center, University of Amsterdam, Roetersstraat 15, 1018 WB Amsterdam, The Netherlands*
[2]*Universität Bielefeld, Abteilung für Psychologie,T5-221, Universitätsstrasse. 25, 33615, Bielefeld, Germany*
[3]*Department of Radiology, Leiden University Medical Center; Department of Psychology, Leiden University and Leiden Institute for Brain and Cognition (LIBC), Leiden, The Netherlands*

Abstract: Animal studies show that glucocorticoid effects on memory depend on noradrenergic activation within an intact amygdala. Testing this model in humans is the subject of the present fMRI study. Healthy subjects watched emotional and neutral stimuli after having received a betablocker or placebo. Cortisol levels of all subjects were determined and served as a marker of the subject's (endogenous) cortisol level during the experiment. Viewing emotional pictures resulted in increased amygdala activation compared to neutral pictures and this effect was enhanced in subjects with a high versus low cortisol level under placebo condition. Betablockade with propranolol, lowering the noradrenergic level in the amygdala, disrupted this effect and apparently the interaction with cortisol. These data support the hypothesis that high endogenous cortisol levels at the time of encoding interact with noradrenergic activation in the amygdala in man.

Keywords: amygdala; fMRI; noradrenaline; cortisol; human

Introduction

Memories of emotional or traumatic events tend to be better remembered than are daily returning neutral or non-emotional events. Why are incidents like the dramatic school shooting on the Virginia Tech campus in Blacksburg or the terrorist suicide attacks on September 11, 2001 so well remembered? One of the explanations for this memory difference is the extent to which the body is physiologically aroused. Studying the role of stress hormones on emotional memory is the focus of our recent research.

What was already known in human emotional information processing can be summarized as follows: first, several imaging studies show that confronting humans with emotionally valenced information (visual, auditory, or aversive tastes) activates the amygdala (Phan et al., 2002). The amygdala appears to be a core structure in all forms of emotional information and memory processing.

Second, the search for neurotransmitters and hormones involved in memory for stressful events provide clear evidence for a role of noradrenaline (NA). Studies with rats show that noradrenergic

*Corresponding author. Tel.: +31205256799;
Fax: +31206391369; E-mail: A.H.vanStegeren@uva.nl

DOI: 10.1016/S0079-6123(07)67020-4

agonists when injected systemically or locally in the basolateral nucleus of the amygdala (BLA) lead to better memory performance on stress tasks. Doing the opposite, so blocking the NA receptors with antagonists like betablockers (BBs), leads to decreased memory performance. This last aspect is also shown in humans (Cahill et al., 1994; van Stegeren et al., 1998).

Finally, it is shown in fMRI studies that NA is playing a role in this process by its effect on the noradrenergic receptors within the amygdala. Blocking the NA receptors with the BB propranolol when watching emotional and neutral pictures, not only blocks long term emotional memory performance, but also reduces amygdala activation when watching emotional pictures (Hurlemann et al., 2005; van Stegeren et al., 2005). Herewith NA is regarded as an essential neurotransmitter within the human amygdala.

The role of cortisol (CORT), as a second(ary) stress hormone involved in traumatic events or situations, has also been extensively studied. The effects of CORT or corticosteroids on memory can best be described as 'confusing': facilitating effects of CORT as well as impairing effects on cognitive performance have been described (Buchanan and Lovallo, 2001; Abercrombie et al., 2003; Lupien et al., 2005). Studying the effect of stress on memory, individual differences such as personal history, gender and age are important whereas context and memory phase during which stress is experienced, are also playing a role (de Kloet et al., 1999; Joels et al., 2006).

The relation between NA and corticosterone (in animals) can be described by a model (Roozendaal, 2000) that implies that the role of the amygdala, with its noradrenergic receptors in the BLA, is essential for any glucocorticoid-mediated effect on memory. This is determined by selective lesions of the BLA or infusions of beta-adrenoreceptor antagonists into the BLA that blocks the memory-modulating effects of systemic injections of glucocorticoids. So, glucocorticoid effects on memory depend on noradrenergic activation within an intact BLA that can interact with other brain regions.

Testing this model in humans is the subject of the present study. We present a new and additional analysis of a recent imaging study (van Stegeren et al., 2005) to investigate endogenous CORT effects on amygdala activation in humans. Healthy subjects watched emotional and neutral stimuli after having received a BB or placebo (PL). CORT levels of all subjects are measured before and immediately after the scanning procedure providing us with a marker of the subject's (endogenous) CORT level during the experiment. If CORT interacts with NA also in the human amygdala, then viewing emotional pictures should result in increased amygdala activation in subjects with a High_CORT versus Low_CORT level under PL condition. Betablockade, lowering the noradrenergic level in the amygdala, should then disrupt this effect. This hypothesis is tested here.

Methods and procedure

The first two sessions took place on two consecutive days where 14 male (M) and 14 female (F) subjects (age 20.93 ± 2.38) without medical or psychiatric history came to the scanning department of the Free University Medical Centre (VUMC). Informed consent was obtained from all subjects. Double blind, they received either a BB (propranolol, 80 mg) or PL in random order over the two days. Salivary samples for CORT measurements were obtained using Salivettes sampling devices (Sarstedt, Rommelsdorf, Germany) at baseline (t0), immediately before entering the scanner (t1) and 30 min later, immediately after the scanning procedure (t2). Trying to obtain a good estimation of the CORT level during the scanning procedure, salivary CORT levels just before and immediately after the scanning were averaged with $[\mathrm{CORT}(t1) + \mathrm{CORT}(t2)]/2$. Two groups (High_CORT versus Low_CORT) were then obtained by a median split.

In the scanner subjects were presented with a set of 92 pictures containing random assortments across 4 emotional categories. After each picture (presentation time 3 s) subjects were asked on screen for an affective rating of the previous picture with 1 being 'not emotional at all' (CAT1) (e.g. domestic subjects) to 4 being 'extremely

emotional' (CAT4) (e.g. mutilation). These individual emotional ratings were used to classify the pictures for further event-related fMRI analysis. Two weeks later recognition memory was tested by presenting subjects with the original stimulus sets combined with filler sets that had comparable emotional valence. Memory was expressed as the percentage correctly recognized pictures.

fMRI acquisition and analysis

Imaging was carried out on a 1.5 T Sonata MR scanner (Siemens, Erlangen, Germany). A T1-weighted structural MRI-scan was acquired before functional imaging began [see (van Stegeren et al., 2005, 2007) for more extensively described methods and fMRI specifications]. All fMRI analyses were carried out using FEAT 5.4, part of FSL 3.2; www.fmrib.ox.ac.uk/fsl.

Amygdala activation was analyzed with contrasts comparing increasing emotional categories (CAT2, 3 and 4) with the neutral CAT1 (CAT2 > CAT1; CAT3 > CAT1; CAT4 > CAT1). At higher level the effect of CORT level on all contrasts was calculated, as well as the difference between the groups (CORT_High > CORT_Low; and the inverse contrast CORT_Low > CORT_High). Statistic images were first cluster corrected ($Z > 2.3$; $p = 0.05$). Given the small sizes of the amygdalae as regions of interest (ROI), these statistic images were thresholded using $Z > 2.3$, uncorrected.

Results

Cortisol

No significant difference in CORT level is found between t_1 and t_2 ($F(1, 27) = 1.13$; $p = 0.30$), meaning that CORT levels are not affected by the scanning procedure itself. As can be expected based on a median split, CORT level during scanning in the High_CORT versus Low_CORT group is significantly different (mean ± SD: High_CORT = 8.78 ± 1.97 nmol/l; Low_CORT = 5.40 ± 1.11 nmol/l, $p < 0.001$).

fMRI data: placebo only

Starting with the analysis of only the PL condition, a significant effect for the High_CORT > Low_CORT groups on amygdala activation is found, when subsequent emotional categories are compared (CAT2 > 1: trend $p = 0.07$; CAT3 > 1: $p < 0.05$; CAT4 > 1: $p < 0.05$). So increased amygdala activation for the emotional pictures, compared to the neutral CAT1 pictures, is significantly higher in the High_CORT compared to the Low_CORT group. This points at an interaction between NA levels, which we hypothesize to be related to emotional intensity, and endogenous CORT levels.

PL > BB interaction with CORT_High > CORT_Low

Support for the model would be even stronger if this interaction effect between NA and endogenous CORT would be disturbed under BB condition, in which NA levels are substantially lowered. This was exactly what we found: amygdala activation did not pass the threshold under BB condition for the High_CORT > Low_CORT groups with emotional pictures. Note that interpreting the results should be done with caution, since the High_CORT and Low_CORT groups refer to different absolute levels within each drug group. However, when calculating the difference in activation of PL > BB, interacting with CORT_High > CORT_Low concentrations, a cluster was found in the right amygdala for CAT3 > 1 ($Z = 2.35$; max. voxel at x, y, $z = 20, -8, -24$) and for the CAT4 > 1 contrast ($Z = 3.01$; $p = 0.05$; max. voxel at x, y, $z = 14, 0, -12$) (see Figs. 1 and 2).

Memory

Memory performance was assessed 2 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. Betablockade with propranolol

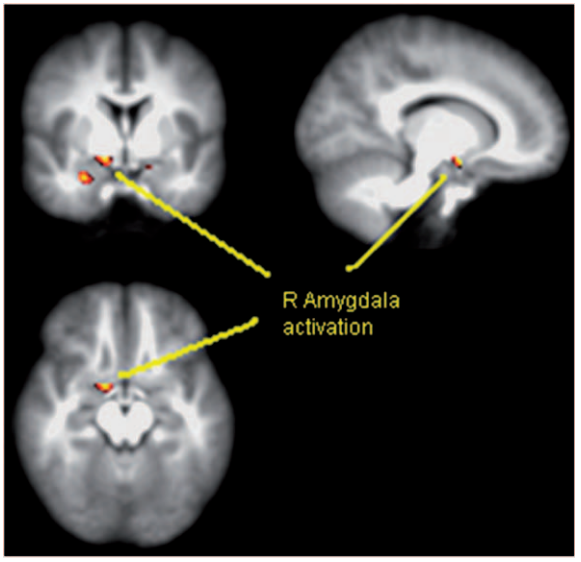

Fig. 1. PL > BB interacting with CORT_High > CRT_Low: a significant cluster in the R amygdala remains when comparing the most emotional CAT4 with CAT1 pictures ($Z = 3.01$; x, y, $z = 14, 0, -12$). R in picture = L in brain.

significantly obliterated this effect for the CAT3 > 1 difference. But no main or interaction effect of the endogenous CORT level during scanning on memory performance 2 weeks later was found.

Discussion

These data support our hypothesis that high endogenous CORT levels at the time of encoding interact with noradrenergic activation in the amygdala in man. They also agree with the model obtained in rats (Roozendaal, 2000, 2002), recently underlined in a study that focused on the synergistic actions of glucocorticoids and emotional arousal-induced noradrenergic activation of the BLA. This interaction constitutes a neural mechanism by which glucocorticoids may selectively enhance memory consolidation for emotionally arousing experiences (Roozendaal et al., 2006).

Although we were able to show that NA levels rose during the scanning, by measuring salivary alpha amylase levels as an indicator

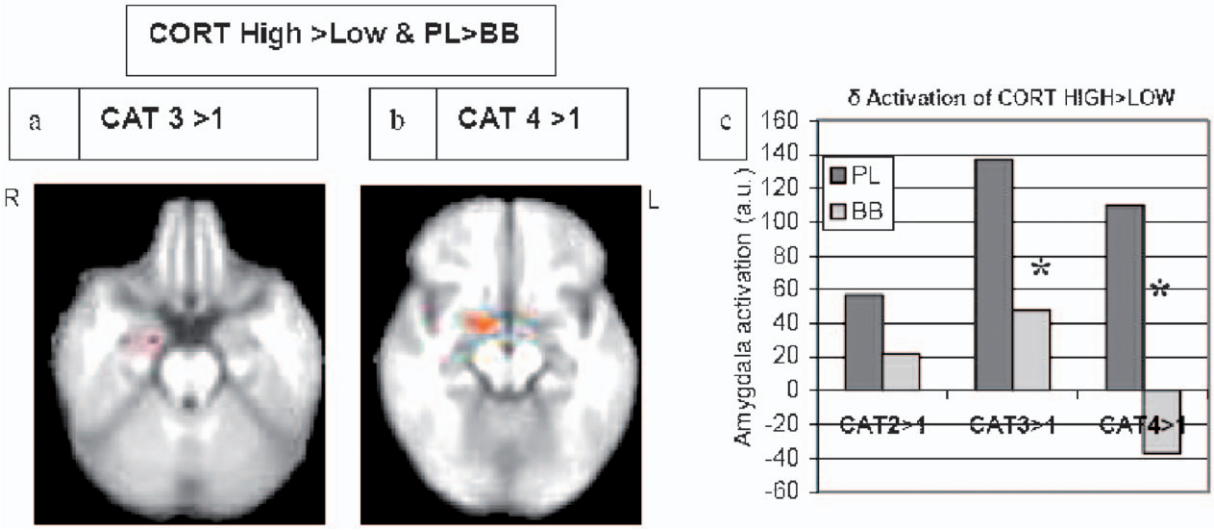

Fig. 2. Interaction of placebo (PL) > betablocker (BB) condition with High_CORT > Low_CORT groups. Significant activation for the PL > BB comparison remains in the R amygdala when High_CORT–Low_CORT group activation is contrasted. A small cluster in the amygdala is visible comparing CAT3 > 1 activation (a) and a bigger cluster for CAT4 > 1 activation (b). This means that amygdala activation that is present in subjects with High_CORT levels under PL condition during emotional CAT4 pictures is decreased when under BB condition. Delta scores (differences) in activation for High_CORT versus Low_CORT groups are plotted for emotional picture categories contrasted with neutral (CAT1) picture activation by drug groups (c). Reprinted from van Stegeren et al. (2007) Neurobiol. Learn. Mem. Copyright (2007), with permission from Elsevier.

for sympathetic-adreno-medullar system (SAM-system) activity (van Stegeren et al., 2006), it might seem surprising that CORT levels were not affected by the scanning procedure itself. A large meta-analysis reviewing laboratory studies to see which procedures do and which ones do not lead to CORT responses (Dickerson and Kemeny, 2004) shows that tasks containing both uncontrollable and social-evaluative elements are associated with the largest CORT and adrenocorticotropin hormone changes and the longest times to recovery, but that emotion induction tasks per se do not elicit a significant CORT response. High_CORT and Low_ CORT levels in this study should then be more regarded as a difference in endogenous 'state' than as a difference in a task-related CORT response.

The BB-induced decrease in amygdala activation cannot be explained by changes in brain hemodynamics in general, since no main effect of propranolol on brain activation is found during all four emotional categories. It is the selective reduction of increased amygdala activation during the emotional CAT3 pictures (hypothesized by us to be related to increased noradrenergic activation) that is shown here. Decreasing NA levels with this dosage of propranolol wipes out the differential effect of the High_CORT > Low_CORT concentrations that leads to increased amygdala activation in the PL group.

Focussing solely on the interaction of NA and CORT is of course a simplification of what happens during stress in reality. First, the stress system has two modes of operation, a fast and a slower mode, with a variety of stress hormones and agents [such as corticotropin releasing hormone (CRH), vasopressin and urocortins] playing a role in CORT release. Second, corticosteroids operate in both modes by means of mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in different ratios and with distinctive as well as interacting functions (de Kloet et al., 2005). Finally, in a recent overview the facilitating and impairing effects of stress on memory are unified in a theory that explains both functions of corticosteroids (Joels et al., 2006). The authors propose that if convergence in time and space takes place, stress hormones help to store the information attached to the event for future use. They make a distinction in rapid, non-genomic effects of stress hormones such as NA, CRH and CORT that can facilitate the encoding of information when (a) they act in the same areas that are involved in processing of the information to be remembered and (b) do so around the time that synaptic strengthening in these areas takes place. Additionally, CORT or corticosterone initiates a slower genomic signal that will suppress unrelated information reaching these circuits some time after the stressful event. This dual effect of corticosterone serves to enhance the signal-to-noise ratio of important information (Joels et al., 2006).

It is clear that the presented results on the interaction between NA and CORT in humans should be investigated in a new experimental study where drug application and control groups are designed in a way to rule out some of the drawbacks of this study — such as the post-hoc nature of the CORT grouping. 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.

Abbreviations

BB	betablocker
BLA	basolateral nucleus of amygdala
CORT	cortisol
CRH	corticotropin releasing hormone
GR	glucocorticoid receptor
NA	noradrenaline
MR	mineralocorticoid receptor
PL	placebo
ROI	regions of interest
SAM-system	sympathetic-adreno-medullar system

References

Abercrombie, H.C., Kalin, N.H., Thurow, M.E., Rosenkranz, M.A. and Davidson, R.J. (2003) Cortisol variation in humans affects memory for emotionally laden and neutral information. Behav. Neurosci., 117: 505–516.

Buchanan, T.W. and Lovallo, W.R. (2001) Enhanced memory for emotional material following stress-level cortisol treatment in humans. Psychoneuroendocrinology, 26: 307–317.

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

Dickerson, S.S. and Kemeny, M.E. (2004) Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull., 130: 355–391.

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

Joels, M., Pu, Z., Wiegert, O., Oitzl, M.S. and Krugers, H.J. (2006) Learning under stress: how does it work? Trends Cogn. Sci., 10: 152–158.

de Kloet, E.R., Joels, M. and Holsboer, F. (2005) Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci., 6: 463–475.

de Kloet, E.R., Oitzl, M.S. and Joels, M. (1999) Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci., 22: 422–426.

Lupien, S.J., Fiocco, A., Wan, N., Maheu, F., Lord, C., Schramek, T. and Tu, M.T. (2005) Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology, 30: 225–242.

Phan, K.L., Wager, T., Taylor, S.F. and Liberzon, I. (2002) Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage, 16: 331–348.

Roozendaal, B. (2000) 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology, 25: 213–238.

Roozendaal, B. (2002) Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiol. Learn. Mem., 78: 578–595.

Roozendaal, B., Okuda, S., Van der Zee, E.A. and McGaugh, J.L. (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. Proc. Natl. Acad. Sci. U.S.A., 103: 6741–6746.

van Stegeren, A.H., Everaerd, W., Cahill, L., McGaugh, J.L. and Gooren, L.J. (1998) Memory for emotional events: differential effects of centrally versus peripherally acting beta-blocking agents. Psychopharmacology (Berl.), 138: 305–310.

van Stegeren, A.H., Goekoop, R., Everaerd, W., Scheltens, P., Barkhof, F., Kuijer, J.P. and Rombouts, S.A. (2005) Noradrenaline mediates amygdala activation in men and women during encoding of emotional material. Neuroimage, 24: 898–909.

van Stegeren, A., Rohleder, N., Everaerd, W. and Wolf, O.T. (2006) Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. Psychoneuroendocrinology, 31: 137–141.

van Stegeren, A.H., Wolf, O.T., Everaerd, W., Scheltens, P., Barkhof, F. and Rombouts, S.A. (2007) Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. Neurobiol. Learn. Mem., 87: 57–66.