A temporal perspective on stress hormones and memory

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CHAPTER V

A delayed action of glucocorticoids degrades the enhanced formation of emotionally-arousing memories over neutral ones

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In rodents, glucocorticoids can regulate neural mechanisms underlying learning and memory in a time-dependent way. This was evidenced in *in vitro* studies where synaptic plasticity as well its efficacy mediated by noradrenergic activity was bidirectionally regulated by glucocorticoids in ways that represented either a rapid facilitatory or a delayed inhibitory action. This time-dependent effect has been proposed to result from an instantaneous non-genomic and a late-onset gene-mediated mechanism respectively. In the present study, we attempted to extend this insight to discovery at the human-level research. With a randomised double-blind, placebo-controlled, within-subject design, we tested whether the memory encoding for either neutral or negatively emotional information (in a picture learning task) differs significantly among three pharmacological conditions: application of 20 mg hydrocortisone 3 hours before encoding (i.e. "early" application, allowing the development of a *delayed* cortisol effect), application of 20 mg hydrocortisone just prior to encoding (i.e. "late" application, concentrating on *rapid* cortisol effects), and the placebo control. It was shown that the drug treatments did not result in apparent changes in the overall numbers of pictures remembered; however, the ratio of successfully remembered negative versus neutral pictures was significantly decreased through "early" drug application, thereby reflecting a diminished weight of emotional information in overall memory formation. fMRI data further corroborated this type of regulation by demonstrating a reduced activity of the left hippocampus in the early hydrocortisone treatment group. In sum, these findings indicate that emotional information can lose its relative weight (in association with memory enhancement) in encoding, when modulated by a proposed, delayed gene-mediated glucocorticoid mechanism; by so, the significance of emotional items in memory formation is hampered.

Introduction

An abundance of evidence supports the susceptibility of memory faculties to the influence of stress and its associated hormones (e.g. glucocorticoids) (McEwen and Sapolsky, 1995; Roozendaal et al., 1997; Lupien and Lepage, 2001; Kim and Diamond, 2002; Shors, 2006). On the one hand, stress is considered a "bad guy" due to its deleterious effects on memory functions under specific circumstances (de Kloet et al., 1999; Sapolsky, 2000) – such as during memory retrieval (de Quervain et al., 1998; Roozendaal et al., 2008; Wolf, 2008) or after chronic stress (McEwen, 2004; Pittenger and Duman, 2008; Wolf, 2008); on the other hand, it is also observed that an acute stress event, particularly of significant affective relevance, is better retained in memory than any routine incidence entailing trivial challenge (Cahill and McGaugh, 1998; Joels et al., 2006).

Negatively emotionally-arousing information is often inherent in a stressful condition. Emotional prevalence is demonstrated by the superiority of emotional arousing information in the process of memory encoding and retention (Cahill and McGaugh, 1995; Kensinger and Corkin, 2003). In animal studies, negative information often takes the form of aversive stimuli, which invariably lead to enhanced memory for the aversive scenario (LeDoux et al., 1990; LaLumiere et al., 2004). It has been proposed that activation of the amygdala is crucial in assigning incoming information an "emo-

tional tag" (Richter-Levin and Akirav, 2003), thereby differentiating the significance of the information and facilitating the remembering of aversive conditions (Cahill et al., 1996; Canli et al., 2000; van Stegeren et al., 2005). As a stressful experience is often collateral to emotional arousal, such a condition entails even greater complexity; in this regard, if stress arises in association with negative emotion provocation, its impact on memory is established upon a synthesis of amygdala stimulation and the regulation of stress hormones. Perceived stress reliably induces stimulation of the hypothalamic-pituitary-adrenal axis and the autonomic nervous system, resulting in the releases of glucocorticoids and catecholamines (adrenaline and noradrenaline) respectively (Tsigos and Chrousos, 2002; Smith and Vale, 2006). Ample evidence from human studies demonstrates that memory formation of emotionally arousing events is subject to regulation by stress or glucocorticoid treatments (Buchanan and Lovallo, 2001; Cahill et al., 2003; Abercrombie et al., 2006; Schwabe et al., 2008). This culminated in the "amygdala modulation" theory, which proposes that glucocorticoid-mediated regulation of memory requires noradrenergic activity within the basolateral amygdala, and that the basolateral amygdala can effectively modulate various memory processes in other brain regions including, among others, the hippocampus (Roozendaal, 2003; McGaugh, 2004; Richter-Levin, 2004; Vuilleumier et al., 2004; Roozendaal et al., 2006).

As such, there is a growing understanding of and an increasing interest in the regulatory functions of stress hormones in memory. Focusing on the cellular and molecular bases of these neuromodulators, a theoretical model has been proposed that attempts to divide the actions of the hormones into two types of temporally-linked regulations, in relation to a fast-acting mechanism and a slow-onset action respectively. There is indication that the time-dependency of the regulations can virtually lead to opposing ends of memory functionality (Joels et al., 2006). This is best exemplified by glucocorticoids. While their gene-mediated signalling pathways – recruiting the nuclear receptors (i.e. MRs: mineralecorticoid receptors and GRs: glucocorticoid receptors) and requiring an adequate length of time to channel a complex of intracellular events to transcriptional activity – have been well studied (Vreugdenhil et al., 2001; Kellendonk et al., 2002; Zhou and Cidlowski, 2005), their instant, nongenomic effects attract additional attention (Chen and Qiu, 1999; Borski, 2000; Makara and Haller, 2001). A nongenomic effect was recently identified at the cellular level where a brief administration of stress-levels of corticosterone rapidly and reversibly enhanced glutamate transmission of hippocampal CA1 pyramidal neurons, in which case a specific mechanism of the membrane-linked MRs was implicated (Karst et al., 2005).

At the network level, we have previously examined the time-dependent effects of corticosteroids employing a "synaptic plasticity" model of learning and memory – long-term potentiation (LTP), which represents the most widely-acknowledged neurobiological model for memory to date (Martin and Morris, 2002; Morris, 2003). It was shown that in hippocampal CA1 neurons, an identical stimulation paradigm variably modifies synaptic strength, with enhancement achieved by acute corticosteroids application and suppression arising from brief pre-treatment of the hormone hours in advance (Wiegert et al., 2005; Wiegert et al., 2006). In a following study, we have further demonstrated a time-dependent hormonal regulation of LTP in the hippocampal dentate gyrus (DG); the bidirectional pattern was notably found for the interactions between the glucocorticoid and

noradrenergic systems (Pu et al., 2007). Appreciating the significant role of the amygdala in emotional memory, an additional study was performed within the basolateral amygdala; although the functional bidirectionality was not readily identified, we nevertheless showed a suppressive effect of corticosteroids on the amygdala LTP mediated though β-adrenergic activation (Pu et al., 2009).

Until now, our insight into the time-dependent glucocorticoid regulation was built entirely upon *in vitro* animal models. Any conclusion drawn from such is far from being complete. Therefore, we were particularly interested in extending our findings to human-level observation. Here, we approached this issue by tapping a human behavioural study, in which the respective memory for emotional-arousing and neutral information was tested, and, more significantly, the glucocorticoid regulatory effects on these memories were examined. To increase the explanatory power of the study, functional magnetic resonance imaging (fMRI) was applied in monitoring real-time brain activitation during encoding, and this was done in conjunction with a subsequent memory paradigm. The latter discloses the underpinning of memory formation by contrasting the brain activity between successful and unsuccessful memories (i.e. the Dm effect – difference due to memory) (Fernandez and Tendolkar, 2001; Paller and Wagner, 2002), and implementation of such a paradigm was based on a previous study that illustrated Dm effects at the amygdala and medial temporal lobe (MTL) in relation to emotional memory (Dolcos et al., 2004).

In the current study, we tested the hypothesis – based upon previous animal studies (Wiegert et al., 2006; Pu et al., 2007, 2009) – that delayed effects of glucocorticoids in the human brain, developing gradually over time prior to learning, *impair* subsequent memory, whereas rapid hormone effects can *promote* it. The design required that human volunteers memorise a series of pictures of either negative or neutral valence and have their brain activity monitored by fMRI during encoding (i.e. picture viewing). Experiments were performed in a randomised double-blind, placebo controlled fashion, in which 20 mg hydrocortisone or placebo was orally administered to ensure that a rapid glucocorticoid effect, a delayed one, and the control could be differentiated across sessions. We specifically focused on the following questions: 1) whether there is an emotional effect on memory; 2) whether memory can be regulated time-dependently by glucocorticoids; and 3) whether the emotional effect on memory is also subject to glucocorticoid regulation. In this text, only a set of preliminary results are reported, which will constitute the major output of a large-scale human research project that will be published in a more extensive form elsewhere.

Materials and Methods

Participants

The study was performed in accordance with the institutional guidelines of the local ethics committee (CMO Region Arnhem-Nijmegen, the Netherlands) and the Declaration of Helsinki. All participants had given informed consent to their participation prior to the commencement of the study.

At the current stage, 12 young male volunteers (aged 21 – 29, median 23) were included in the study, all right-handed. The inclusion was based on assessing their backgrounds to examine whether they were free from any of the following criteria: history of head injury, historical or current treatment of psychiatric, neurological, or endocrine disorders, regular use of corticosteroids, psychotropic or recreative substances, frequent and heavy smoker or drinker, recent illnesses (within 3 weeks), history of autonomic failure, current periodontitis, claustrophobia, acute inflammatory diseases, acute peptic or duodenal ulcers, intensive physical exercises, irregular day-night cycle, presence of metal objects against MRI safety. Furthermore, participants had been screened with Beck Depression Inventory (Beck, 2002), Spielberg Trait Anxiety Inventory (T-anxiety) (van der Ploeg, 1980, 1981), and NEO FFI Personality Inventory (Costa, 1992), which would have provided any indication of a pathological level of depression, anxiety and neuroticism. The participants, in addition, were not experiencing ongoing stressful events or major life events during the experiment periods and had no prior exposure to pictures used in the study.

As an introductory interview had been made for individual participants, during which they had undergone a T1-weighted anatomical scan; therefore, all participants were experienced in MRI environment before the study sessions began.

Procedure

A single session: Participants were invited to totally 3 counterbalanced experimental sessions. A single session consisted of two consecutive afternoons, with the first one comprising drug administration and the picture encoding task and the second the memory recall tests (Figure 1). Selection of the afternoon as an optimal testing period reflects the need of diminishing the impact of diurnal variations in cortisol levels. Participants were instructed to refrain from using any recreational drug since 3 days prior to each session and from having alcohol, tobacco and exercise 24 hrs in advance. They were also prohibited from brushing teeth, flossing, or having any drink (but water) within 2 hours beforehand. They were asked to take a light lunch and do so no later than ½ hours before arrival; their lunch could not contain any citrus products, coffee, tea, milk and sweets (e.g. hot chocolate) (Maheu et al., 2005). Throughout the entire study period, they had no further food intake and had merely water to drink.

On the first afternoon, the participant arrived at the laboratory at 12:15 ± ¾ hrs; he was instructed by the investigator about the procedures to follow, so that a comparable familiarity with the task was either established or reinstated. The participant was also aware that an unknown amount of monetary award would be given in proportion to his performance in recall tests, thus his commitment and effort could be encouraged. After ½ – ¾ hr from arrival, two salivary samples measuring his baseline level of cortisol were collected.

During the entire period (~ 3 ¾ hrs) prior to the encoding task, the participant had been waiting in a quiet, isolated room where he was free to conduct most personal activities but anything potentially stressful (e.g. video games). One capsule (containing hydrocortisone or placebo) at once was provided to him at two time-points: 3 hrs and ½ hrs preceding the encoding task. Salivary samples

were regularly collected at various time-points that might reveal crucial changes in cortisol levels before and after drug administration and before and after the encoding (as later explained).

During the encoding task (~ 1 hr), the participant lay supine in the scanner and viewed the screen via a mirror positioned onto the head coil. It was emphasised that he lay still, with eyes open fixing the centre of the screen where study materials (i.e. pictures) were displayed. The participant was required to watch the pictures for the entire presentation time (6 sec) and the fixation cross during the inter-picture intervals (4 – 8 sec). As described below, two categories of pictures were used – neutral or negative; accordingly, he needed to rate the valence of each picture with a right-hand button press as an orientation task. Pictures were displayed in a pseudorandom order – no more than two pictures of the same valence were shown consecutively, and the first slides were always neutral, so that the ceiling effect resulting from a combined effect of arousal and primacy could be prevented (Cahill et al., 2003).

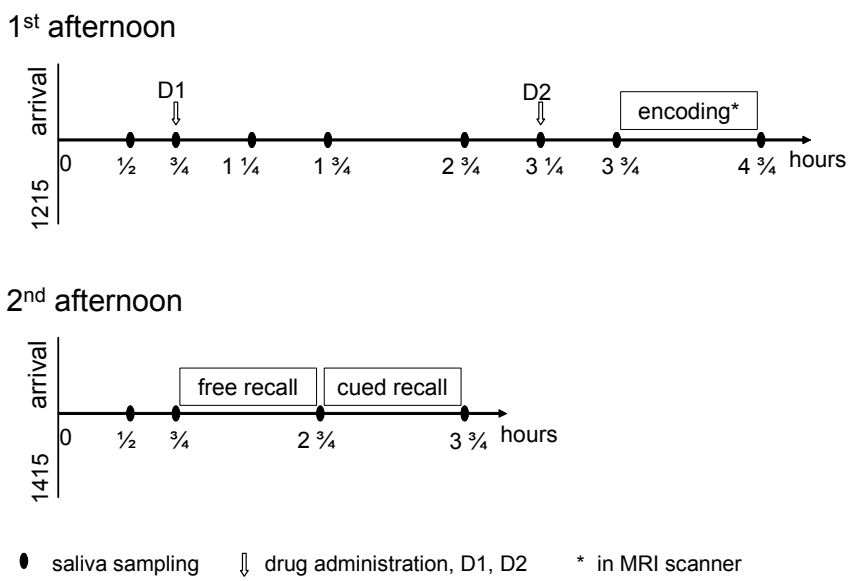

Figure 1 A scheme of an individual experimental session (totally 3 sessions of this for the complete within-subject study), in which the drugs were applied at two different time-points prior to the encoding tasks (on the 1st day afternoon) and saliva samples were collected at various time-points over the entire session.

On the second day, the participant arrived at 14:15 ± ¾ hrs. After an acclimatization period of ½ – ¾ hr, baseline measurement was made by collecting two salivary samples. Salivary samples were

further collected before and after individual memory tests. The participant stayed in a quiet, isolated room and committed himself to, first, a free recall memory test (~ 1 hr), and then a cued recall test (~ 1 hr). In both tests, the participant was required to write to the utmost detail all the characteristics of the pictures he could remember, so that an outsider would, with the information provided, identify the pictures as distinctively recognisable (Dolcos et al., 2004). The cued recall test differed from the free recall in that the participant had received one- or two-word written cues (of similar valence to that of the picture) that may facilitate his recall. All results were later scored by two raters; a "hit" (defining a remembered picture) was only considered when a consensus between the two raters was reached.

Drug administration and counterbalancing: The participant needed to undergo 3 counterbalanced experimental sessions, with an approximate inter-session interval of one month. The whole procedure for individual sessions remained identical except that the drug administration schemes differed from session to session. All drug capsules, containing either 20 mg hydrocortisone (to elevate circulating cortisol levels) or placebo, were administered orally.

In order to ensure a double-blind, placebo-controlled paradigm and to monitor the time-dependent effect of cortisol, the following schedules were used: 1) 1st capsule containing hydrocortisone, 2nd placebo – to reveal the *delayed* drug effect of cortisol; 2) 1st placebo, 2nd hydrocortisone – to disclose the *rapid* cortisol effect; and 3) 1st placebo, 2nd placebo – the control. For a particular participant, a specific schedule was followed during one session only – that is, that he had to follow 3 different schedules for all 3 sessions. The orders of drug administration were fully counterbalanced among participants.

Stimulus materials

An individual stimulus set presented during the encoding task consisted of 80 negative and 80 neutral pictures, supplemented by 41 null events (fixation cross). Three different sets were used for all 3 study sessions (counterbalanced across participants), which were free from inter-set differences in terms of arousal and valence.

Pictures used were selected from both a standard set of affective pictures (IAPS: International Affective Picture System) (Lang, 1999) and an additional collection of new pictures downloaded from the Internet and included based on their similarity in valence and content to the IAPS set. To confirm the homogeneity of new pictures and the IAPS ones, 20 male volunteers were invited to rate the pictures on a scale from 1 to 9 for arousal and valence according to the Self-Assessment Manikin (SAM) (Bradley and Lang, 1994), during which the new pictures were mingled with standard IAPS pictures. The selection was based on valuation of arousal and valence ratings. Negative pictures were chosen due to their moderate-to-high arousal quality (average score 5.5, S.E.M. = 0.7) and negative valence (average score 3.1, S.E.M. = 0.7), – as measured by SAM (Bradley and Lang, 1994). Neutral ones were selected for relatively low arousal degrees (average score 2.5, S.E.M. = 0.7) and neutral valence (average score 5.3, S.E.M. = 0.3). Individual sets contained around 50 %

newly rated neutral and 15 % newly rated negative pictures; and chromatic features and complexity were matched within the sets whilst content overlaps were minimized.

Endocrine, psychological and physical measures
Cortisol: Cortisol levels were monitored by collecting saliva samples at various time-points during both days of study (Figure 1), following a pattern that was reflective of the elevations after drug administration and the magnitudes around memory tasks.

Typically, during a single session, on the first day (t_0 = 0 hr, as arrival), the following time-points (approximately) were scheduled for saliva collection: t_1 = ½ hr (baseline), t_2 = ¾ hr (baseline right before drug 1), t_3 = 1 ¼ hrs, t_4 = 1 ¾ hrs, t_5 = 2 ¾ hrs, t_6 = 3 ¼ hrs (right before drug 2), t_7 = 3 ¾ hrs (right before encoding), t_8 = 4 ¾ hrs (right after encoding). On the second day (t_0 = 0 hr, as arrival), the following time-points (approximately) were chosen: t_1 = ½ hr (baseline), t_2 = ¾ hr (baseline right before free recall), t_3 = 1 ¾ hrs (right after free recall and before cued recall), t_4 = 2 ¾ hrs (right after cued recall).

Saliva was collected using a commercially available collection device (Salivette®, Sarstedt, Germany). To collect a sample, the participant was required to place the cotton swab supplied by the salivette inside his mouth and chewed it gently for no less than 1 min. The swab was then returned to the salivette, which was subsequently stored in the freezer at -25 °C until assay. Biochemical analysis was performed at a collaborator's site (Department of Biopsychology, TU Dresden, Germany). After thawing, salivettes were centrifuged at 3,000 rpm for 5 minutes, which resulted in a clear supernatant of low viscosity. Salivary-free cortisol concentrations were determined employing chemiluminescence immunoassay (CLIA) with a high sensitivity of 0.16 ng / ml (IBL; Hamburg, Germany).

Positive and negative affect: Both affective states were assessed using the PANAS scales (Watson et al., 1988; Peeters, 1996) at the following time-points during the first day of each session: ½ hr after arrival, before encoding when inside the scanner, and after encoding when inside the scanner.

Heart rates: The cardiac rhythm of the participant was measured during scanning by using a pulse oximeter connected to his left index finger. He was required to keep his left hand still during the entire scanning period.

MRI Acquisition
Participants were scanned by a Siemens (Erlangen, Germany) MAGNETOM Avanto 1.5 Tesla MRI scanner equipped with an 8-channel head coil. Three series of blood oxygenation level dependent (BOLD) T2*-weighted gradient echo EPI images were acquired with the following parameters: TR = 2340 ms, TE = 35 ms, FA = 90 °, 32 axial slices approximately aligned with AC-PC plane, slice matrix size = 64 x 64, slice thickness = 3.5 mm, slice gap = 0.35 mm, FOV = 212 x 212 mm^2. Owing to its relatively short TE, this sequence yields optimal contrast-to-noise ratio in the medial temporal lobe, hippocampus and amygdala. The whole period of functional imaging spanned approximately 1

hour. High resolution anatomical images were acquired for individuals by a T1-weighted 3D Magnetization-Prepared RApid Gradient Echo (MP-RAGE) sequence, which employed the following parameters: TR = 2250 ms, TE = 2.95 ms, FA = 15 °, orientation: sagittal, FOV = 256 x 256 mm^2, voxel size = 1.0 mm isotropic.

Functional MRI Data Analysis

All data acquired were processed and analyzed by using Statistical Parametric Mapping software (SPM 5; UCL, London) and in-house software. The first five EPI-volumes were discarded to allow for T1 equilibration. Prior to analysis, all images linking to the encoding task were motion-corrected by rigid body transformation and sum of squared differences minimization. They were further adjusted for temporal differences in sampling across slices. All functional images were co-registered with the participant-specific high-resolution T1-weighted anatomical images through normalized mutual information maximization. The anatomical image was subsequently used to normalize all scans to the MNI T1-152 (Montreal Neurological Institute) space. The functional images were resampled with a voxel size of 2 mm isotropic. Finally, all images were smoothed with an isotropic 8-mm full-width half maximum Gaussian kernel in order to accommodate residual functional/anatomical variances between participants.

Data were analyzed by applying a general linear model whereby individual events were modelled on the basis of subsequent remembering, emotional valence and session (i.e. drug conditions). The six covariates corresponding to the movement parameters obtained from the realignment procedure were altogether included. Regressors were temporally convolved with the synthetic hemodynamic response function of SPM 5. To reduce the differences between scan sessions, the average signal per scan was estimated with global normalization by using proportional scaling. The single subject parameter estimates from each session and condition resulting from the first-level analysis were included in subsequent random effects analysis. For the second-level random-effects analysis, a factorial ANOVA was performed whereby drug conditions (control, delayed, rapid), emotional valence (negative vs. neutral), and subsequent memory (remembered vs. forgotten) were defined as within-subject factors. Statistical tests were family-wise error (FWE) corrected for multiple comparisons using Gaussian random field theory across the whole brain. Based on our *a priori* hypothesis, data concerning the regions of interest: MTL structures (e.g. the hippocampus, the amygdala) were corrected for reduced search regions (based on size) and small volumes through several anatomical masks (i.e. automated anatomical labelling derived masking images of the bilateral hippocampus, bilateral amygdala, unilateral hippocampus, and unilateral amygdala). A conjunction analysis had only been used for identifying functional overlaps between the subsequent memory effect and emotional valence. For all analyses, statistical thresholds were set at $P < 0.05$.

Behavioural and physiological analyses

Behavioural and physiological data were exported to SPSS and analysed by repeated measures ANOVA. *P* values < 0.05 were accepted as significantly different. If main effects or interactions involving the order factor were noticed, the drug order was also included as a between-subjects factor. In this text, unless otherwise mentioned, all data are presented as value ± S.E.M..

Results

Endocrine, psychological and physiological measures

Cortisol: 20 mg hydrocortisone (CORT) was effective to elevate circulating cortisol levels, as reflected in saliva samples. The elevation was evident from 30 minutes after oral drug administration, and continued beyond a period of one hour (Figure 2A). For early CORT application (the active substance was applied at the 1st drug point, allowing the development of a *delayed* cortisol effect), significant increases of salivary cortisol levels in comparison with the immediate before-drug level (t_2: mean ± S.E.M = 5.88 ± 0.71 nmol/L) were found at the time-points from t_3 until t_8 (Figure 2A). For late CORT application (the active substance was applied at 2nd drug point, mediating a *rapid* cortisol effect), when compared with the immediate before-drug level (t_6: mean ± S.E.M. = 4.38 ± 0.48 nmol/L), significant increases were found at t_7 and t_8. For placebo control, there was no main effect of time over the entire first-day period ($F_{(3,35)} = 1.660$, $P > 0.05$).

It was important to identify the difference among all 3 drug conditions in salivary cortisol levels before or after the encoding task. For the time-point right before the encoding (t_7), there was an overall difference among all drug conditions ($F_{(1,12)} = 6.162$, $P < 0.05$); and this was attributed to a significant difference between placebo control and early CORT application (delayed effect) ($P < 0.01$), and between placebo control and late CORT application (rapid effect) ($P < 0.05$), in the absence of any difference between the early and late drug conditions ($P > 0.05$). For the time-point after the encoding (t_8), an overall difference was also found ($F_{(1,15)} = 24.904$, $P < 0.001$), which was attributable to a difference between the placebo control and early CORT application ($P < 0.05$), and between the control and late CORT application ($P < 0.001$), and between early and late CORT applications ($P < 0.01$).

For the second day of memory recall tests, there was no main effect of time over the entire period of the test for individual drug conditions (early CORT application: $F_{(1,15)} = 1.358$, $P > 0.05$; late CORT application: $F_{(2,22)} = 0.892$, $P > 0.05$; placebo control: $F_{(1,14)} = 3.366$, $P > 0.05$) (data not shown).

PANAS: For all drug conditions, a consistent reduction in the degrees of positive affect was seen over the study period; a main effect of time was identified for each drug condition (early CORT application: $F_{(2,22)} = 9.102$, $P < 0.01$; late CORT application: $F_{(2,22)} = 15.322$, $P < 0.001$; placebo control: $F_{(2,22)} = 14.668$, $P < 0.001$) (Figure 2B). The differences were mainly found between the degrees of positive affect after the encoding and that after arrival (all $P < 0.01$) or that just before the encoding task (all $P < 0.01$). No overall differences in positive affect were found between various drug conditions: neither before encoding ($F_{(2,22)} = 0.691$, $P > 0.05$) nor afterwards ($F_{(2,22)} = 1.591$, $P > 0.05$).

As regards the negative affect, there were not shown any apparent changes over time for all drug conditions (early CORT application: $F_{(2,22)} = 0.892$, $P > 0.05$; late CORT application: $F_{(2,22)} = 1.844$, $P > 0.05$; placebo control: $F_{(2,22)} = 2.593$, $P > 0.05$). Neither was there any overall difference among

drug conditions found at the time before or after encoding ($F_{(2,22)}$ = 1.166, P > 0.05 and $F_{(2,22)}$ = 3.274, P > 0.05 respectively).

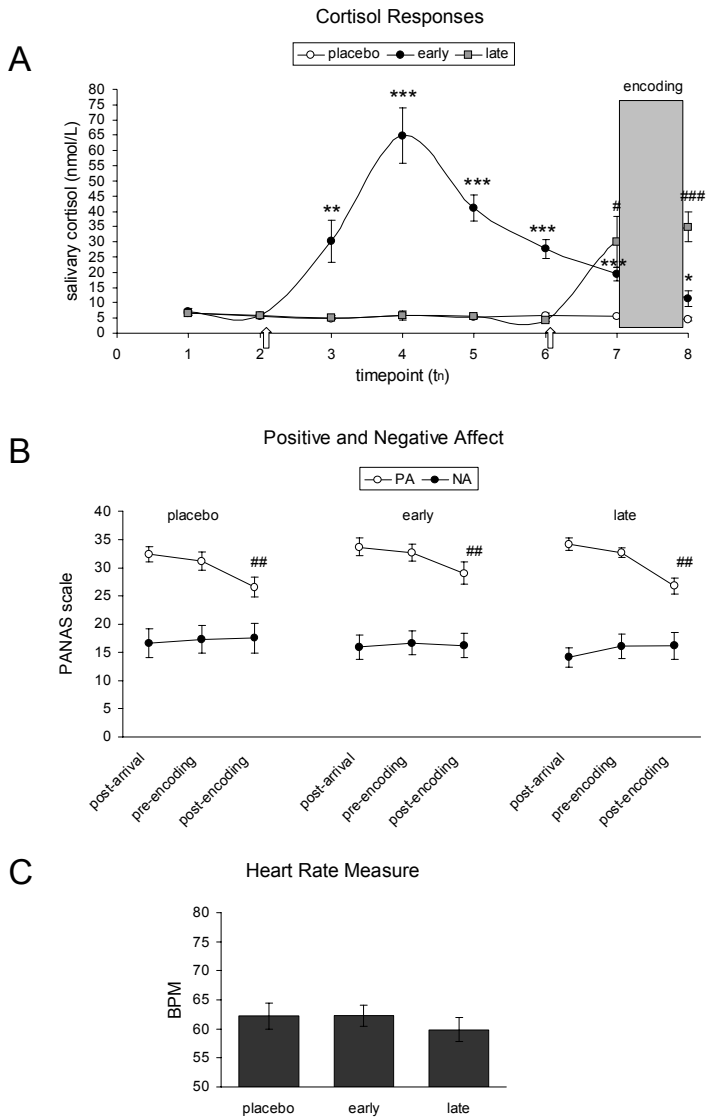

Figure 2 Endocrine, psychological and physiological measures during the first-day session. (A) cortisol levels were substantially elevated after 30 minutes from oral drug administration, and such an elevation lasted for

hours. *, **, ***: $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively, for the cortisol level at the individual time-point as compared to the immediate before-drug level (t_2) in the early CORT condition. #, ###: $P < 0.05$, $P < 0.001$ respectively, for the cortisol level at the individual time-point as compared with the immediate before-drug level (t_6) in the late CORT condition. Arrows indicate the time when the drug was administered. (B) Across all 3 session, participants' subjectively-felt positive affect was decreased after encoding, though negative affect was not significantly altered. ##: $P < 0.01$, compared with both the post-arrival level and the pre-encoding level. (C) Mean heart rates during encoding across all 3 sessions. Little difference across drug conditions was found. Placebo: the placebo control; early: early CORT application (delayed cortisol effect); late: late CORT application (rapid cortisol effect).

It can be concluded that watching emotional pictures inside the MRI environment, regardless of the treatment, may significantly diminish the levels of positive affect; however, such experience does not necessarily lead to a heightened sense of negative affect.

Heart rate: Mean heart rates for the entire encoding period during individual drug conditions were: 62.31 ± 1.80 (mean ± S.E.M.) for early CORT application, 59.87 ± 2.05 for late CORT application, and 62.22 ± 2.28 for placebo control (Figure 2C). There was no overall difference among the 3 drug conditions ($F_{(2,22)} = 1.243$, $P > 0.05$). Heart rates were to a large extent indicative of sympathetic activity during encoding, thus such activities were not found to have altered across different conditions. In the current study, heart rates were consistently measured after a resting (waiting) period of up to 3 ¾ hrs, thus any changes in value responding to the encoding task would have been noticed from a stable, physiologically basal level.

In view of the endocrine, psychological and physiological results, we arrived at a conclusion that endogenous physiological and psychological responses to the presentation of emotional pictures and the fMRI procedures were mostly homogeneous across all 3 within-subject sessions (i.e. 3 drug conditions), apart from a major difference in endogenous cortisol levels arising from external pharmacological manipulation. It is worth noting that peripheral autonomic responses are not the indicator of noradrenergic responses occurring centrally in the brain in relation to emotional arousal (Strange and Dolan, 2004).

Memory performance

The memory of the pictures viewed on the previous day was tested in two separate tests: a first free recall test followed by a cued recall test. In a primary level of analysis, memory performance was measured on the basis of the absolute number of pictures remembered (Table 1). For both tests, there was a main effect of emotional valence for all 3 study sessions (free recall: $F_{(1,11)} = 70.019$, $P < 0.001$; cued recall: $F_{(1,11)} = 8.947$, $P < 0.05$). Significantly more negative pictures were remembered than neutral ones. However, in either of the tests, there was no indication of a main drug condition effect across the sessions (free recall: $F_{(2,22)} = 0.427$, $P > 0.05$; cued recall: $F_{(2,22)} = 0.427$, $P > 0.05$), nor was an interaction effect between drug conditions and valence available (free recall: $F_{(2,22)} = 0.399$, $P > 0.05$; cued recall: $F_{(2,22)} = 2.397$, $P > 0.05$).

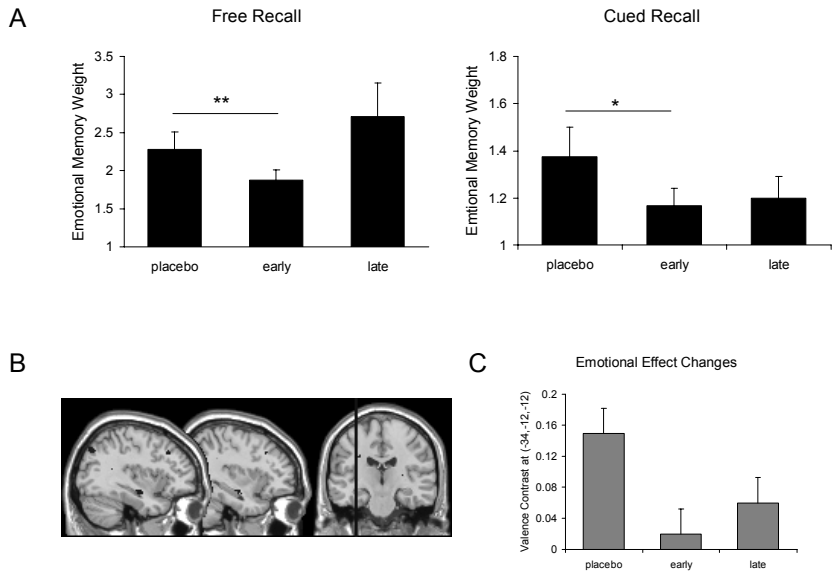

Figure 3 Behavioral and fMRI data for memory performance. (A) Memory results shown for the free recall test and the cued recall test. Consistently, early CORT application (*delayed* effect) resulted in a reduction of the "emotional memory weight" (expressed as negative pictures remembered devided by neutral pictures remembered) as compared with the placebo control in both tests. *, **: $P < 0.05$, $P < 0.01$ respectively, compared with the control. It should be noted that there was also a difference between the early condition and the late condition in the free recall test ($P < 0.05$), which is here not marked explicitly. (B) fMRI results show a significant drug condition X emotional valence interaction, as identified in the left anterior hippocampus. $T = 3.44$, $P < 0.05$. (C) At the signal peak within the left hippocampus, it appeared that the signal contrast derived from the main valence effect was significantly reduced, in a way mimicking the result shown by cued recall test. Value estimates with 90 % confidence interval are shown.

Subsequently, we employed a special index for analysis that represents the extent of "emotional enhancement", computed as the number of remembered negative pictures divided by the number of remembered neutral pictures (emotion weight rate = negative pictures remembered / neutral pictures remembered). Hence, for each session (and each drug condition), a single emotional weight (EW)-rate was derived (Figure 3A). By comparing these rates, we observed that there was a significant main effect of drug conditions across all 3 session for the free recall test ($F_{(1,15)} = 4.190$, $P < 0.05$), and similarly, a trend of significance ($F_{(2,22)} = 3.349$, $P = 0.054$) in the cued recall. Pair-wise comparisons demonstrated that, for free recall, there was a significant difference between the effects of early CORT application (i.e. delayed effect) and the placebo control ($P < 0.01$), and between early CORT application and later CORT application (i.e. rapid effect) ($P < 0.05$); likewise, in cued recall, a significant difference between early CORT application and the control was found (P

< 0.05). Thus, both tests (Figure 3A) conjointly indicate that early drug application – by allowing the development of a delayed (presumably gene-mediated) cortisol effect – can diminish the "emotional memory weight" attributed to negative information, which can be crucially relied upon for emotional memory enhancement.

Neuroimaging

For the purpose of fMRI analysis, we set up a statistical model by utilising the behavioural data from the cued recall test integrated with those from the free recall test. Such an approach was taken with the consideration of: a) the number of the memory hits counted in the free call test alone was relatively small, this seriously undermines the efficacy and reliability of contrasting any effect between the remembered and forgotten items; b) the cued recall test facilitates memory recall via externally provided cues, its results being more indicative of the outcomes of previous successful encoding with limited reference to the capability of retrieval; in our design, the neural activity was monitored *during* the encoding phase, thus a focus on the cued recall results is most relevant; c) occasionally, there were memory hits found in the free recall test, but not by the cued recall test; undoubtedly, such hits represents the outcomes of successful encoding, but due to the occasional performance variations they were not detected by another test. In this regard, both tests had produced complementary results to be used in the following analyses, in which all memory hits as identified by either of the tests would be aggregated into a single category of "remembered", and only those pictures that were unmentioned in both of them were considered "forgotten".

		placebo *(control)*	**early drug** *(delayed CORT effect)*	**late drug** *(rapid CORT effect)*
Free recall	Neutral pictures remembered	16 (2.90)	18 (2.22)	18 (3.64)
	Negative pictures remembered	31 (3.04)	32 (2.32)	34 (2.61)
Cued recall	Neutral pictures remembered	36 (4.05)	38 (3.47)	40 (4.16)
	Negative pictures remembered	45 (3.17)	43 (3.00)	44 (2.58)

Table 1 Memory Performance. Figures are shown for picture numbers with S.E.M. in brackets.

First of all, there was a main Dm effect (i.e. successful memory formation) found across sessions. Increased neural activity was identified in regions of the inferior frontal gyrus, inferior temporal gyrus, inferior parietal gyrus and the hippocampus. All appear to be lateralized to the left hemisphere. A main effect of emotional valence was found in several frontal and temporal regions, the fusiform gyrus, cerebellum, and particularly in the hippocampus and amygdala, which corroborated earlier findings that pinpointed the engagement of the amygdala and MTL in memory processing of emotional stimuli (Canli et al., 2000; Dolcos et al., 2004). Interestingly, it seemed that the Dm main effect and the emotion main effect were actually correlated in specific MTL regions – the left hippocampus and the left amygdala, as this was demonstrated by a conjunction analysis that had combined those contrasts displaying the main Dm effect and the main emotion effect. A main drug condition effect was only noticed between the placebo control and early CORT application, and localised to the left middle cingulum and right middle frontal cortex. (For an overview of the *T* values and statistical significances for individual effects, see Table 2 at the end of the chapter; note that only those effects qualified for a significance of $P < 0.05$ were reported).

Furthermore, we examined the interaction effects among all main factors. No significant interaction effect was observed on neural activity as a consequence of the drug condition-by-Dm effect interaction or of the drug condition-by-Dm effect-by-emotion valence interaction. However, a significant effect was identified for the Dm-by-emotion interaction in the superior medial frontal region. Above all, we were interested in knowing whether a drug condition-by-emotion interaction effect indeed existed, as this would correspond to our behavioural results, implying that "emotional memory weight" was subject to drug manipulation. For this test, we defined our *a priori* regions of interest to be the amygdala and the hippocampus, and the bilateral structures were examined in each separate hemisphere, as it seemed that the memory effect was predominant in the left hemisphere. This paralleled a view of the lateralised functionality of the amygdala or the hippocampus in associating emotional effects with subsequent memory performance, as indicated by several studies (Canli et al., 2000; Dolcos et al., 2004). With such an approach, we identified a significant interaction between the emotional valence and the drug condition (placebo control vs. early CORT application only), localised to the left hippocampus (Figure 3B). At the site (the anterior hippocampus) where such an effect was maximal, it appeared that the signal contrast generated by emotional valence was substantially reduced in the condition of early drug application, in comparison with the control condition. The difference of the contrasts was less observable between late CORT application and the control, and between early CORT application and late CORT application (Figure 3C). This entirely mirrored the pattern of differences as recognised in the behavioural results, and displayed a good alignment between the neuroimaging data and behavioural results, in both functional and structural terms.

Discussion

In the current study, we tested in humans the hypothesis – as established upon earlier findings at the animal level (Wiegert et al., 2006; Pu et al., 2007, 2009) – that the delayed effect of glucocorticoids can impair subsequent memory formation whereas the rapid hormone effect may facilitate it.

To this end, we deliberately manipulated drug application of hydrocortisone, attempting to achieve a time-dependent pattern of the drug efficacy. Exogenous hydrocortisone administration was for this reason preferable over stress exposure, since it allowed the precise control of "timed" elevations of hormone levels and the isolation of the glucocorticoids function as the main determining factor. Consistently, we observed that the drug treatment merely introduced differences in the timing and amount of active cortisol, without impacting on the subjective affective ratings and sympathetic activity. Early administration of hydrocortisone unequivocally resulted in marked increases of salivary cortisol over several hours prior to encoding, virtually allowing a sufficient length of time to elapse before the onset of encoding, which permitted the development of gene-mediated actions. For the late drug administration group, salivary cortisol levels were elevated during the entire encoding session, a condition that purports to facilitate memory formation. It should be noted that, although the early treatment group had significantly lower cortisol levels at the end of the encoding period than did the late drug group, a comparably high cortsiol level was discernible at the outset. We can therefore not fully exclude that the proposed delayed hormonal effect was herein confounded by a putative rapid effect. If so, the rapid effect would not have escaped from being detected at the behavioural level in the intended testing, which seems to not have achieved; thus, the influence of this potential confounder may be limited. However, such a view merits any further investigation that may allow an even longer delay between the hydrocorticone administration and picture encoding.

Behavioural observations

The major behavioural finding of this study is that negative information gains a preferential retention in memory over neutral information, and this bias is subject to regulation by glucocorticoids, contingent upon the timing of the hormone application. This extends previous views on the stress hormone's regulatory influence on emotional memory (Cahill and McGaugh, 1998; Buchanan and Lovallo, 2001; Cahill et al., 2003; Abercrombie et al., 2006; Roozendaal et al., 2008). In those studies, elevations in corticosteroid level occurred shortly before or after the encoding and lead to enhanced memory of emotional information. In our hands, introducing a lengthy delay between the elevation in corticosteroid levels and the encoding, thus allowing a full development of gene-mediated actions, unambiguously resulted in a *suppression* of the distinction power between the memories of negative and neutral information. It should be realised that glucocorticoids- or stress-induced impairment of emotional memory is not unprecedented (Rimmele et al., 2003); as was once shown in the case of increased hormone levels shortly before memory retrieval (Kuhlmann et al., 2005; Kuhlmann and Wolf, 2006). Furthermore, impaired declarative memory may arise from an extended application of glucocorticoids over several days – unlike through a brief single-dose administration, possibly reflecting certain of gene-mediated hormone mechanisms instead of any rapid action (Newcomer et al., 1999).

It needs to be recognised that in our study, cortisol did not reduce the total number of negative pictures remembered. In general, the numbers of remembered pictures (in both categories) were comparable among variable drug conditions. Thus, the overall amount of information encoded and retained did not alter significantly, but rather the proportion of the respective category of encoded information. It thus reflects a shifted balance between the weights of two types of information – i.e.

neutral and negative – in memory, without modification of the global level of information encoding. Of note, though, our present observations are based on a relatively small cohort, which clearly needs to be extended before definite conclusions can be drawn.

If the present observations hold in an even larger cohort, one implication is then that within a defined mental resource pool, certain forms of competition may take place, and the strengths of "competitor" information can be uplifted or undermined through modulatory efforts. Such a "competition view" was taken by Diamond and associates (Diamond et al., 2005) in account of potentially opposite memory behaviour resulting from stress. Indeed, a recent human study reported that pre-learning psychosocial stress could impair long-term declarative neutral memories, meanwhile enhancing emotional ones (Payne et al., 2007). Also, pre-treatment of cortisol a couple of hours in advance resulted in impaired memory for neutral verbal stimuli, concurrent to an increase in negative stimuli remembered, as displayed in a recognition task right after learning (though, not quite identical to our results) (Tops et al., 2003). In addition, another study has illustrated a developed enhancement of emotional memory recall resulting from cortisol treatment, which was clearly linked to a parallel decrease in neutral memory (Kuhlmann and Wolf, 2006). This lends support to the notion that one type of information gains weight at the other's loss.

A readily identifiable functional relevance is that stress-induced rises in cortisol in conjunction with emotionally distressing situations favours the encoding of emotionally "tagged" over neutral aspects (Richter-Levin and Akirav, 2003; Richter-Levin, 2004). Notably, information is being tagged or emotionally-weighted in alignment with its significance relative to other, and gaining a predominant representation that is required for achieving an essential advantage in dealing with challenges in which it is inherent. A lowering of the emotional weight would invariably signal an impaired adaptive memory function in this sense; this can at least, as shown by this study, be achieved through a *prior* glucocorticoid action, which allows the full development of gene-mediated mechanisms. This probably occurs in a condition that preceding stress induction inhibits the distinction – thus the enhanced encoding – of later-occurring stressful incidences.

Neural substrate

We located the effect of glucocorticoid-mediated impairment of emotional memory weight to the brain region that underlies the effectiveness of this regulation – the left anterior hippocampus. It is undoubted that this is an area centrally targeted by the stress hormones in influencing memory function (Dolcos et al., 2004; Richardson et al., 2004). However, it was still surprising to notice that: first, this region did not exhibit a subsequent memory-by-emotion-by-drug condition (control vs. early drug application) effect; second, emotional effect being one of the key variables, amygdala involvement was not seen for the emotion-by-drug condition interaction effect. It is tempting to consider that as Dm represents a direct substrate of informational processing of memory, the emotional weight actually falls into a second-level processing that codes the relativity of the primary information representing a derivative of emotional stimulation embedded in an information acquisition context; thus its susceptibility to hormonal regulation is not reflected at the basic level encoding, as the Dm would have indicated. On the other hand, if emotional stimulation initially engages the

amygdala and initiates information differentiation (Cahill et al., 1996; McGaugh, 2004; Costafreda et al., 2008), its effect can well be translated into an encoding effort that is integrated into the overall hippocampal activity in memory function; thus amygdala activity could be an upstream event that is least modifiable by the hormonal regulation aiming at shifting its already assigned weight (to individual information representations). Still, the Dm effect and the emotional effect are unlikely separable in the current context, as a conjunction of both effects at the left hippocampus was clearly present. This may be relevant to certain mechanisms recruiting both emotional and information inputs, which are still elusive. Less significantly, there seemed to be a tendency of left lateralisation for several identified effects; if not arising from the handness, this may likely indicate a certain lateralised functional relevance. In several earlier studies, the specificity of effects being localised to the left MTL structures was documented (Canli et al., 2000; Dolcos et al., 2004; Matsuoka et al., 2007); however, this mostly was restricted to females instead of males (Cahill et al., 2004).

In conclusion, we have identified a delayed, presumably gene-mediated action of glucocortoids on emotional memory, represented primarily by a suppression of the relative weight of emotional information over neutral one, which takes its effect by regulating left hippocampal functions. Such a finding can yield valuable insights for the development of novel therapeutic approaches in quelling excessively strengthened emotional memories in diseases like posttraumatic stress disorder (Yehuda, 2002; de Quervain, 2008), emphasising readjustment of their balance with neutral, routine information through “timed” pharmacological treatment.

Region	Coordinates			Peak T-score
	x	y	z	
Main effect of Dm				
remembered > forgotten				
Inferior Frontal, L	-48	8	28	6.01**
	-46	40	8	5.28*
Inferior Temporal, L	-52	-60	-12	5.44**
Inferior Parietal, L	-48	-40	50	5.28*
Hippocampus, L	-16	-6	-12	4.22##
	-26	-4	-22	3.90#
Main effect of emotion valence				
negative > neutral				
Mid Temporal, L	-52	-64	8	13.05***
Cerebellum, L	-16	-72	-46	7.70***
Fusiform, R	44	-46	-18	13.40***
Inferior Frontal, R	54	36	0	8.38***
Precuneus, R	4	-56	30	7.78***
Superior Medial Frontal, R	4	50	32	7.50***
	4	30	54	4.91*
Rectus, R	6	56	-16	6.10***
Hippocampus, L	-18	-6	-14	6.48###
Hippocampus, R	18	-4	-12	8.77###
	22	-24	-6	3.71#
Amygdala, L	-24	-4	-18	6.60†††
Amygdala, R	22	-4	-16	7.83†††
Main effect of drug conditions				
placebo control > early CORT application				

Mid Cingulum, L	-6	-42	42	5.19*
Mid Frontal, R	28	42	34	4.92*
Dm x Emotion				
Positive interaction				
Superior Medial Frontal, L	-2	66	20	5.24*
Dm ∩ Emotion				
remembered > forgotten & negative > neutral				
Hippocampus, L	-16	-6	-12	4.22##
	-26	-4	-22	3.90#
Amygdala, L	-26	-2	-22	3.76++
	-20	-4	-12	3.51+
Drug condition x Emotion				
placebo control > early CORT application				
Hippocampus, L	-34	-12	-12	3.44&

Table 2 FMRI results. *, **, ***: $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, whole brain corrected. #, ##, ###: $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, small volume corrected with the bilateral hippocampus AAL mask. †††: $P < 0.001$, small volume corrected with the bilateral amygdala mask. +,++: $P < 0.05$, $P < 0.01$, respectively, small volume corrected with the left amygdala mask. &: $P < 0.05$, small volume corrected with the left hippocampus mask.