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Memories in context: On the role of cortisol

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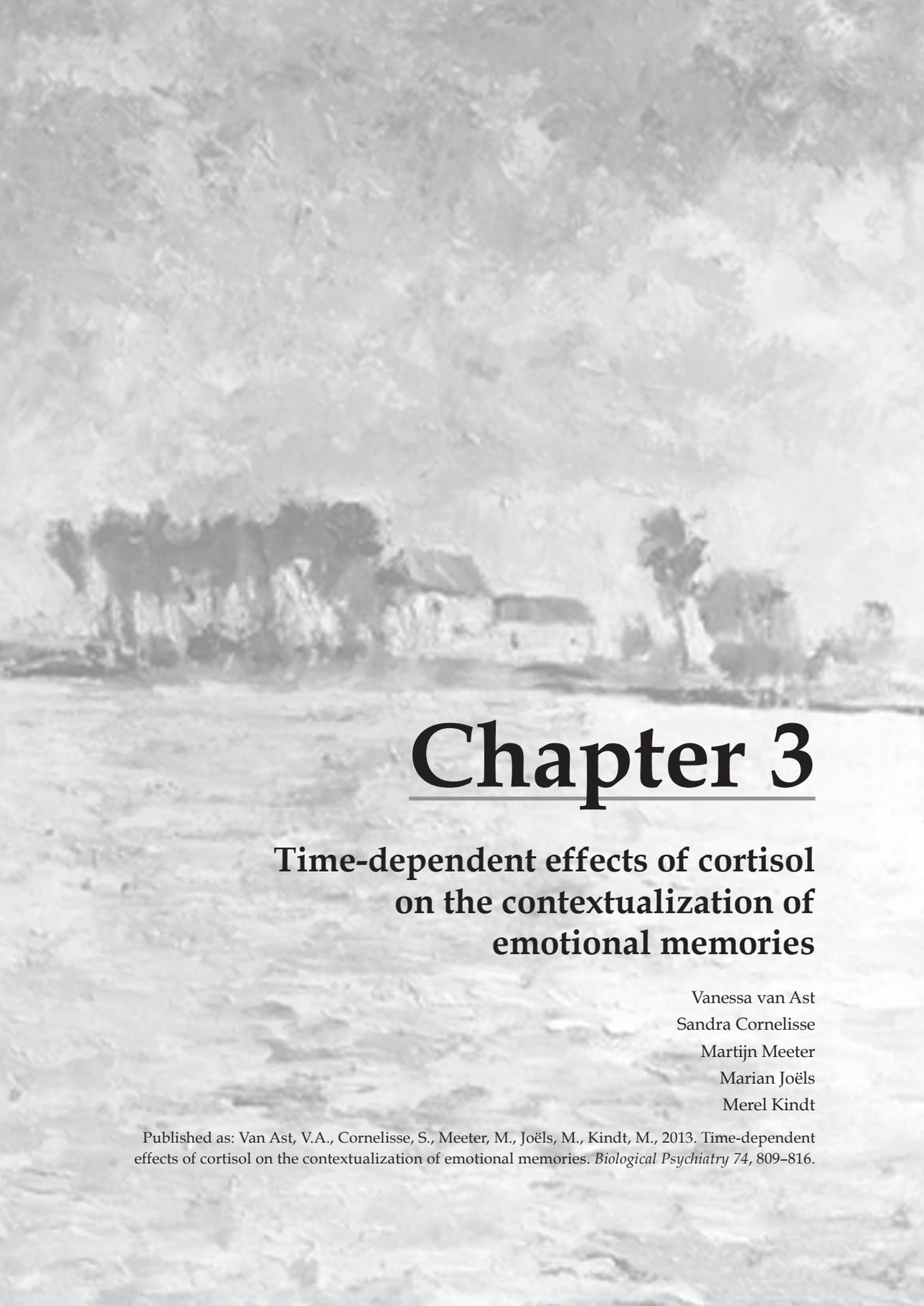
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Chapter 3

Time-dependent effects of cortisol on the contextualization of emotional memories

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SUMMARY

The inability to store fearful memories into their original encoding context is considered to be an important vulnerability factor for the development of anxiety disorders like posttraumatic stress disorder (PTSD). Altered memory contextualization most likely involves effects of the stress hormone cortisol, acting via receptors located in the memory neurocircuitry. Cortisol via these receptors induces rapid non-genomic effects followed by slower genomic effects, which are thought to modulate cognitive function in opposite, complementary ways. Here we targeted these time-dependent effects of cortisol during memory encoding, and tested subsequent contextualization of emotional and neutral memories. In a double blind, placebo controlled design, 64 men were randomly assigned to one of three groups, 1) receiving 10mg hydrocortisone either 30 minutes (rapid cort effects) or 2) 210 minutes (slow cort) before a memory encoding task, or 3) receiving placebo at both times. During encoding, participants were presented with neutral and emotional words in unique background pictures. Approximately 24 hours later, context dependency of their memories was assessed. Recognition data revealed that cortisol's rapid effects impair emotional memory contextualization, while cortisol's slow effects enhance it. Neutral memory contextualization remained unaltered by cortisol, irrespective of the timing of the drug. This study shows distinct time-dependent effects of cortisol on the contextualization of specifically emotional memories. The results suggest that rapid effects of cortisol may lead to impaired emotional memory contextualization, while slow effects of cortisol may confer protection against emotional memory generalization.

1. INTRODUCTION

Memories are more likely to be remembered when the retrieval context resembles the encoding context (Godden and Baddeley, 1975; Smith and Vela, 2001; Tulving and Thomson, 1973). Such contextual dependency of memories is highly adaptive as it can help to retrieve memories that are likely to be appropriate in a specific context. Consequently, the ability to store memories into their original encoding context (i.e., memory *contextualization*) may protect against memory generalization. Since patients suffering from posttraumatic stress disorder (PTSD) display augmented memory generalization (Elzinga and Bremner, 2002), contextualization of emotional memories seems to be compromised in PTSD (Acheson et al., 2012; Brewin and Holmes, 2003; Ehlers and Clark, 2000; Liberzon and Sripada, 2008). The hippocampus, which is supposed to subserve context effects on memory (Chun and Phelps, 1999; Davachi, 2006; Kalisch et al., 2006; Rasch et al., 2007), is a main target of the stress hormone cortisol (Joëls and Baram, 2009). Through its effects on the hippocampus, cortisol may impair the contextual dependency of memories, but whether this is indeed the case in healthy humans is presently unknown. In general, the literature on the potential role of cortisol in (traumatic) memory formation is equivocal. For instance, one recent animal study showed that corticosteroids, injected after fear-conditioning, reduce context-specific fear responses the next day, causing PTSD-like symptoms (Kaouane et al., 2012). Conversely, another study showed that corticosteroid administration prior to acute stress could *dampen* subsequent behavioral characteristics of PTSD (Rao et al., 2012).

One possible explanation for such paradoxical effects of corticosteroids is that these hormones exert time-dependent effects on neurobiological processes, with disparate net effects on behavior (de Kloet et al., 2005; Joëls et al., 2011; 2012; Schwabe et al., 2010c; 2012). After a stressful event, non-genomic corticosteroid actions quickly enhance neural activity mediated by glutamate in mice, particularly in the amygdala (Karst et al., 2010; 2005). In humans, acutely elevated cortisol levels generally suppress hippocampal activity (Henckens et al., 2009; Lovallo et al., 2010; Pruessner et al., 2008). In interaction with noradrenergic activation, corticosteroids (via rapid actions) enhance human amygdala activity (van Marle et al., 2010; 2009; van Stegeren et al., 2010; 2007), facilitate instinctive, habitual behavior (Schwabe et al., 2010b) and negative response biases (Enkel et al., 2010; Kukolja et al., 2008), while impairing higher order controlled executive processes (Elzinga and Roelofs, 2005). Together, these behaviors may promote survival at the short-term, helping the organism e.g. to select the most appropriate strategy immediately after stress, though at the cost of remembering contextual details. Some hours after stress, slower long-lasting ge-

nomic corticosteroid actions develop (de Kloet et al., 2005; Wiegert et al., 2005). The available data suggest that these slow actions serve to restore homeostasis following stressful periods (Diamond et al., 2007; Joëls et al., 2012; McEwen, 2007). In agreement, slow corticosteroid effects have been shown to enhance cognitive self-control (Oitzl et al., 2001), enhance working memory processing involving the dorsolateral PFC (Henckens et al., 2011), promote sustained attentional processing (Henckens et al., 2012b), and reduce amygdala activity (Henckens et al., 2012b; 2010). As such, slower genomic corticosteroid effects may aid in remembering a certain event in a more cognitively controlled, contextualized, manner.

Given these findings, we here probed these two time-domains and tested the hypothesis that rapid cortisol effects (presumably through non-genomic pathways and in interaction with arousal-evoked central adrenergic release caused by the emotional words, Kensinger and Corkin, 2004; Roozendaal et al., 2006b) impair the contextual dependency specifically of emotional memories; and that delayed (presumably gene-mediated) effects of cortisol enhance contextual dependency of subsequent emotional memories. To test this, we randomly assigned healthy young men to one of three groups: 1) a group receiving placebo at 210 minutes and 10mg hydrocortisone at 30 minutes before encoding (rapid cort); 2) a group receiving cortisol 210 minutes and placebo 30 minutes before encoding (slow cort); and 3) a group receiving placebo at both times. During encoding, participants were presented with neutral and emotional (i.e., of negative valence and high arousal) words in unique background pictures. Approximately 24 hours later, memory contextualization was assessed: half of the emotional and neutral words were tested in intact contexts, while the other half of the words were tested in rearranged context combinations (Talamini et al., 2010; Tambini et al., 2010). In addition to objective alterations in memory performance, we conducted exploratory analyses to test for changes in the subjective quality of memories (Yonelinas, 2002).

2. METHODS AND MATERIALS

2.1. Participants

In total 64 male subjects gave written informed consent. Participant characteristics are given in the **supplementary results**. The local ethical committee of the University of Amsterdam approved the study. Inclusion criteria as assessed by self-report were: no past or present psychiatric or neurological condition, no diagnosis of dyslexia, and age between 18 and 35 years. Men having any somatic or endocrine disease (e.g., acute asthma), or taking any medication known to influence central nervous system or endocrine systems were excluded from participation. A final exclusion

criterion constituted non-adherence to the encoding instructions on day 1. Further, participants were asked to refrain from taking any drugs three days prior to participation, and to get a night of proper sleep, refrain from heavy exercise, alcohol and caffeine intake 12 hours prior to participation, and not to eat, drink, smoke or brush teeth two hours before participation. Subjects were rewarded for their participation with course credits or paid € 65,-.

2.2. Drug administration and assessment

Hydrocortisone and placebo (albochin) treatments were administered through identically appearing pills. A single dose of 10 mg of hydrocortisone was employed to elevate endogenous cortisol to a level equivalent to moderate acute stress (Abercrombie et al., 2003). Salivary free cortisol concentrations were assessed with Salivette collection devices (Sarstedt, Nümbrecht, Germany). Cortisol samples were taken at 10 time points spread throughout the experiment (**Figure 1**) and subsequently stored at -25 °C. Upon completion of the study, samples were sent out to Dresden (Technische Universität, Germany) where salivary free cortisol concentrations were measured using a commercially available chemiluminescence immuno-assay with high sensitivity of 0.16 ng/ml (IBL, Hamburg, Germany).

2.3. Memory Measurements

2.3.1. Encoding

The encoding task was modeled freely after (Talamini et al., 2010; Tambini et al., 2010). To induce emotional versus neutral declarative memories, participants were shown 30 neutral, low arousing words, and 30 negative, high arousing words on a small gray rectangle presented against color pictures of natural scenes or city landscapes (see **Figure 2**). We utilized words because these are easier than pictures to match on a range of dimensions (e.g., frequency or familiarity) that affect memory performance (Kensinger, 2004). Furthermore, memory-enhancing effects for arousing words are likely mediated by rapid arousal-evoked central adrenergic release from the locus coeruleus (Sara, 2009) mediated by the amygdala (Kensinger and Corkin, 2004; Roozendaal et al., 2006b). Subjects were instructed to vividly imagine the meaning and content of each word in the background to promote 1) deep encoding (Craik and Lockhart, 1972), 2) to create an association of the word with its unique context, and 3) to create a complete and rich episodic memory. Each individual word was presented for 5 sec together with a unique context, in random order. Next, participants evaluated their mental image on arousal and valence dimensions using self-assessment manikins (SAM; (Bradley and Lang, 1994)) in a fixed time window

of 4 seconds for each rating. In between trials, a fixation cross was presented for 1 second. Words were concrete nouns drawn from a validated database (Hermans and de Houwer, 1994), consisted of 5 to 10 letters and contained no more than 3 syllables. All words were balanced, so that the emotional and neutral words did not differ in terms of familiarity ratings, length, and/or amount of syllables, but did significantly differ in terms of valence and arousal. Background pictures contained no distinguishing objects, thus distinctiveness of the contexts likely relied on their unique spatial configuration. The task began with three practice trials that at the same time functioned as a buffer for possible primacy effects on subsequent memory. All memory tasks were presented using E-prime (version 2.0; Psychology Software Tools, Inc.; Pittsburgh, PA).

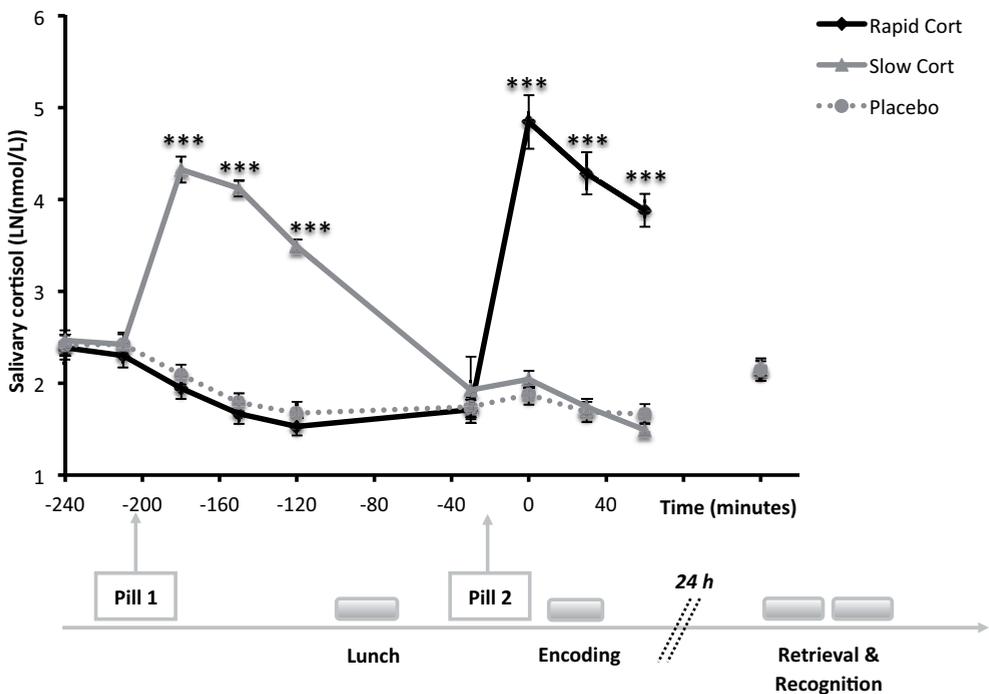


Figure 1. Overview of the experimental design and salivary cortisol curves. Participants received a pill 210 minutes (pill 1) and 30 minutes (pill 2) prior to memory encoding ($t = 0$) at day 1 that could contain 10 mg hydrocortisone or placebo (albochin). Hydrocortisone administration in both groups significantly elevated salivary cortisol as compared to placebo, but did not differ immediately before each pill intake or during baseline at day 2. Throughout the experiment, saliva samples were taken at 240, 210, 180, 150, 120, 30 and 0 minutes before encoding, 30 and 60 minutes after encoding, and before the surprise recall and recognition test 24 h later. Error bars represent standard error of the mean (s.e.m.). Significant Bonferroni corrected differences with placebo are depicted by *** = $p < 0.005$.



Figure 2. Experimental paradigm. To induce neutral versus emotional declarative memories, subjects were shown 30 emotional and 30 neutral words on a small gray rectangle (image not to scale), each in combination with unique color pictures of natural scenes or city landscapes. During recognition testing the following day, participants were presented with the 60 old words that were presented during encoding on day 1, intermixed with 60 foil words (30 emotional and 30 neutral) that were not presented before. Crucially, to assess context dependency of memories, half of the old words were presented in the same word-context combination (intact) as at the first session while the other half of the word-context pairs were rearranged (rearranged) to form new pairs. The foil items were presented against backgrounds viewed during the first session. Subjects indicated whether they recognized the word as having seen during the imagination task by responding “old” or “new.”

2.3.2. Surprise memory tests

See **supplementary methods** for a description of the retrieval test. During the recognition test, participants were presented with the 60 old words that were presented during memory encoding on day 1, intermixed with 60 foil words (30 emotional and 30 neutral) that were not presented before. Crucially, half of the old word-context pairs was presented in the same combination as at the first session, while the other half of the word-context pairs was rearranged to form new pairs. All of the foil words were combined with one of the old contexts. Consequently, each context was presented twice in total. The test was self-paced. Subjects were instructed to indicate whether or not they recognized the word as having seen during the encoding task, by responding “old” or “new” respectively. In addition they were told that the background image may or may not be the same, but that they should focus on the words. Reaction times of the responses were recorded. See **supplementary methods** for further description of the recognition task.

2.4. Design and general procedure

In a between-subjects, placebo-controlled, double blind study design, participants were randomly assigned to either the rapid cort, (hydrocortisone 30 min prior to testing) or slow cort (hydrocortisone 210 min prior to testing) or placebo group (see experimental outline in **Figure 1**). Testing took place in between 12 pm and 8 pm, when endogenous cortisol levels are stable and relatively low (Pruessner et al., 1997). Upon arrival, participants read the information brochure, were screened by means of an interview to assess eligibility for participation, signed the informed consent and filled out the trait scale of the State-Trait Anxiety Inventory (STAI-T; (Spielberger et al., 1970) to probe potential individual differences in processing emotional material). Baseline self-reported mood states were assessed with the STAI state scale (STAI-S; Spielberger et al., 1970)) and the Positive Affect and Negative Affect Schedule (PANAS; Watson et al., 1988)). A first baseline saliva sample was given as well. Then, we used the “Cube and Paper Test”, a test of allocentric manipulation (i.e., the ability to process configural cues among distal environmental relationships; Gilbertson et al., 2007), to control for possible individual differences in memory contextualization processes. Directly following a second baseline sample, participants received their first pill (cortisol or placebo). To ascertain that 1) in the slow cort group cortisol levels would return to baseline and 2) non-genomic effects would be absent during encoding, a three-hour waiting period was inserted during which participants either read or studied. Four more saliva samples were obtained and participants ate lunch. Waiting took place in the same room as testing in order to prevent any (unwanted) additional context effects on memory. The second pill (cortisol or placebo) was given three hours after the first. In order to reach peak plasma levels (Czock et al., 2005) and activate non-genomic effects in the brain for the rapid cort condition, a second resting period of 30 min was inserted. Participants gave another saliva sample and again filled out the mood questionnaires (PANAS, STAI-S). After waiting, an eighth saliva sample was taken, followed by the encoding task and two more samples. Time in between the two testing sessions was kept at 24 hours as much as possible. The session the following day commenced with the mood questionnaires (STAI-S, PANAS), a saliva sample and surprise retrieval and recognition tests. A final post-experimental questionnaire assessed 1) motivation and concentration to complete the encoding task, 2) whether participants expected a memory test, and 3) insight into which substance was received at what time.

2.5. Data analysis

Statistical analysis was performed using the SPSS statistical software package (SPSS Inc., Chicago, Illinois). The effect of hydrocortisone administration on salivary corti-

sol during the first testing day was assessed by means of a mixed ANOVA with the within-subjects factor Sample (S1-S9) and between-subjects factor Group. To assess participants' recognition memory performance as a function of context and emotion, hit rate (i.e., correct classification of previously presented words as "old") was calculated per factor (i.e., neutral intact, neutral rearranged, emotional intact, emotional rearranged). False alarm rates (i.e., misclassification of new words as "old") were calculated as a function of emotion. Using the hit and false alarm rates d-prime sensitivity index was calculated, according to signal detection theory. For this goal hit rates were truncated at 0.975, and false alarm rates at 0.025 (Stanislaw and Todorov, 1999). Recognition data were initially analyzed by means of an omnibus ANOVA with the repeated measures factors Emotion and Context (intact, rearranged), while Group was the between-subjects factor. See **supplementary methods** for a description of analysis of the retrieval data, and additional analyses on the recognition data. A Greenhouse-Geisser procedure was used in case of violation of the sphericity assumption in ANOVAs. Alpha level was set at .05 for all statistical analyses.

3. RESULTS

3.1. Cortisol levels

Figure 1 displays salivary cortisol levels for the three experimental groups during the experiment. As expected, the ANOVA for salivary cortisol levels showed a significant Group×Time interaction ($F_{8,472}=86.10, p<0.001, \eta_p^2=0.745$). Planned comparisons with placebo showed that in the slow cort group cortisol levels were increased from 30 min after *first* pill intake until at least 90 minutes later (S3-S5; all $t_{59}>13.22, ps<0.001$). Right before *second* pill intake, salivary cortisol levels of the slow cort group did not differ anymore from placebo (S6; $t_{59}=0.68, ns$). Further planned comparisons showed that in the rapid cort group, cortisol levels were increased 30 min after *second* pill intake until the end of the first session (S7-S9; all $t_{59}>10.86, ps<0.001$). On day 2, there were no differences in cortisol levels between the three groups (S10; $F_{2,59}=0.05, ns$). The exit interview showed that participants were unable to identify the substance received ($X^2(1)=0.180, ns$).

3.2. Memory performance

Subjective ratings during encoding confirmed that the induction of emotional versus neutral mental images was successful (see **supplementary results** and **Figure S1**). The cued retrieval data revealed that memory retrieval was enhanced with intact contexts and that emotional memories were less context-dependent, but the timing

of cortisol did not alter these processes. Overall the rapid cort group retrieved less memories (for elaboration, see **supplementary results** and **Figure S2**).

The omnibus ANOVA on recognition d-prime data (**Figure 3A**) showed that Context exerted a strong influence on memory recognition: Memory was better for words that were presented in their original context vs. a rearranged context ($F_{1,59}=178.44$, $p<0.001$, $\eta_p^2=0.752$). Recognition of emotional versus neutral words depended on context (Context \times Emotion interaction; $F_{1,59}=8.54$, $p=0.005$, $\eta_p^2=0.126$) as well. Furthermore, overall recognition performance was sensitive to the timing of cortisol ($F_{2,59}=3.92$, $p=0.025$, $\eta_p^2=0.117$), with the rapid group performing worse than both the placebo ($F_{1,59}=6.98$, $p=0.011$, $\eta_p^2=0.106$) and slow cort ($F_{1,59}=4.76$, $p=0.033$, $\eta_p^2=0.075$) groups. This effect was caused by an enhanced false alarm rate in the rapid cort group (see **supplementary results**). Crucial to the hypothesis at hand, contextual dependency of emotional and neutral memories varied as a function of Group (Context \times Emotion \times Group interaction; $F_{2,59}=3.26$, $p=0.045$, $\eta_p^2=0.100$).

To decompose this three-way interaction we separately analyzed neutral and emotional memories. Neutral memories were strongly context-dependent ($F_{1,59}=153.80$, $p<0.001$, $\eta_p^2=0.723$), but context-dependency was not modulated by cortisol ($F_{2,59}=0.06$, $p=ns$). Emotional memories were context-dependent as well ($F_{1,59}=41.48$, $p<0.001$, $\eta_p^2=0.413$). Importantly, this context-dependency was modulated by timing of cortisol (Context \times Group; $F_{2,59}=6.88$, $p=0.002$, $\eta_p^2=0.189$). To further decompose this interaction, we entered difference scores of the rearranged condition minus the intact condition (i.e., contextualization; a larger contextualization index reflects greater contextual dependency of memories) in an ANOVA with the between-subjects factor Group and within-subjects factor Emotion (see **Figure 3B**). Note that the difference scores within this analysis reflect the factor 'Context' from the omnibus analysis. In order to investigate how exactly the timing of cortisol altered the contextual dependency of emotional memories, we used planned contrasts to directly compare contextualization (i.e., the difference score) across groups. These revealed that compared with placebo, cortisol administration several hours *prior to* encoding (slow cort) resulted in significant enhancement of the contextualization of emotional memories ($t_{59}=2.01$, $p=0.049$, $d=0.621$), while elevated cortisol levels *during* encoding (rapid cort) resulted in a marginally significant impairment of the contextualization of emotional memories ($t_{59}=1.85$, $p=0.069$, $d=0.561$). Contrasting neutral and negative memory contextualization within each group confirmed that negative memories tended to be decontextualized in the placebo group ($F_{1,59}=3.51$, $p=0.066$, $\eta_p^2=0.056$), an effect that was even stronger present in the rapid cortisol group ($F_{1,59}=6.88$, $p=0.001$, $\eta_p^2=0.161$). By contrast, neutral and negative memories were equally contextualized in the slow cortisol group ($F_{1,59}=0.41$, ns).

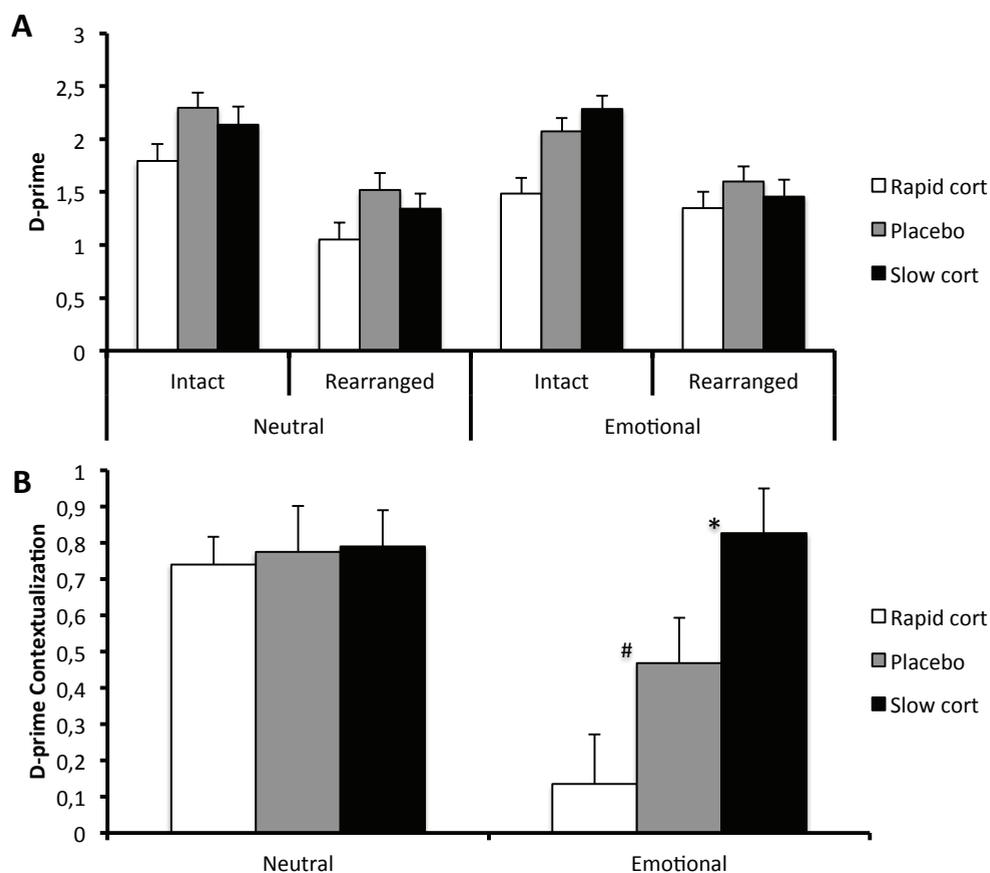


Figure 3. Recognition performance. **(A)** Graphs depict memory performance indexed by D-prime as a function of group (rapid cort, slow cort or placebo), context (intact or rearranged) and emotion (neutral or emotional). Error bars represent standard error of the mean (s.e.m.). **(B)** Contextual dependency of neutral and emotional memories. Difference scores between D-prime values for the rearranged conditions subtracted from the intact conditions are depicted. A higher difference score reflects greater contextual dependency of memories. As can be seen cortisol did not in any way affect the contextualization of neutral memories, but pronoucedly affected the contextualization of *emotional* memories. The significant planned contrast between the slow cort and placebo group is depicted with $*=p<0.05$ and the marginally significant planned contrast between the rapid cort group and placebo is depicted with $\#=p<0.07$.

The above relationships continued to exist when rerunning the same analyses with subjective mood ratings from the first or second day as covariates, showing that the timing of cortisol did not interact with mood to alter memory contextualization. Further, analysis of hit rates revealed generally the same pattern of results as revealed by the d-prime analysis, although the effects were only marginally significant (**supplementary results** and **Figure S3**). Finally, exploratory analyses

of the subjective quality of memories (Remember-Know judgments) indicated that recollection was unaffected by the timing of cortisol, but slow cortisol enhanced familiarity sensitivity to context for emotional memories (**supplementary results** and **Figure S4**).

4. DISCUSSION

A key question is under what circumstances cortisol may exert protecting as opposed to detrimental effects on memory contextualization. We tested the hypothesis that *rapid* cortisol effects impair the contextual dependency specifically of emotional memories and that *delayed* effects of cortisol enhance the contextual dependency of subsequent emotional memories. To probe these two time-domains, cortisol elevations were induced directly, or some hours prior to memory encoding. In agreement with our hypothesis, cortisol's rapid effects impaired emotional memory contextualization, while cortisol's slow effects enhanced the contextualization of emotional memory. In contrast, the contextualization of neutral memory remained unaltered by cortisol irrespective of the timing of the drug.

The rapid -presumably non-genomic- effects of cortisol show that acute cortisol during encoding decreased contextualization of emotional memories, though this was only marginally significant. Also, we found that acute cortisol during encoding enhanced the false alarm rate for the emotional words (see **supplementary results**), which explains why there was a general memory impairment in the rapid group as measured by d-prime. The enhanced false alarm rate can however not explain the decreased contextualization in this group, since contextualization is the difference between memory performance in intact and rearranged context conditions, both calculated employing the same false alarm rate (i.e., it is a constant). Together, the enhanced false alarm rate for emotional memories and reduced contextualization by rapid cortisol corroborate previous studies, all suggesting that stress may enhance consolidation of the gist of an experience at the cost of detailed information (Mather, 2007; Payne et al., 2006; 2002).

On the other hand, slow -presumably genomic- effects of cortisol during encoding, enhanced contextualization of emotional memories. Theories of delayed effects of cortisol on the brain suggest that these serve an adaptive function by promoting a variety of higher order cognitive functions (Henckens et al., 2012b; 2011; 2010; Joëls et al., 2012; Oitzl et al., 2001). Our results are in line with this notion and demonstrate how slow corticosteroid effects can drastically alter the way experiences are processed, stored and subsequently remembered. Overall, this suggests that delayed effects of cortisol not merely restore baseline cognitive functioning (McEwen, 2007),

but rather lead to a redistribution of resources towards superior executive functioning.

Taken together, the rapid and slow cortisol effects of cortisol appear to affect contextual processing in opposite directions. As the hippocampus is believed to subservise context effects on memory (Chun and Phelps, 1999; Davachi, 2006; Kalisch et al., 2006; Rasch et al., 2007) and is a main target of cortisol (Joëls and Baram, 2009) it is likely that this structure mediated the present time-dependent effects of cortisol. However, cortisol did not affect *general* contextualization processes, because neutral information was not affected. Thus, apart from the hippocampus, our cortisol manipulations probably affected other brain areas as well. One likely area is the basolateral amygdala (BLA). The BLA is believed to be a site of storage for memories of fearful or stressful experiences (Schafe et al., 2001). In rodents, a single acute dose of corticosterone was sufficient to enhance glutamatergic transmission through MRs (Karst et al., 2010) and facilitate noradrenergic actions on synaptic plasticity (Liebmann et al., 2009). Conversely, >1 hr after corticosterone administration noradrenergic actions were gradually suppressed (Liebmann et al., 2009; Pu et al., 2009). In agreement, corticosterone administered prior to acute stress prevented subsequent increases in anxiety; this was associated with reduced spinogenesis in the BLA (Rao et al., 2012). Perhaps, our cortisol manipulations affected the amygdala in a similar manner. This is possible, since the encoding of emotional arousing words was shown to depend on an amygdala-hippocampal network (Kensinger and Corkin, 2004) that can be amplified by stress (Qin et al., 2012). Remembrance of non-arousing words is supported by more controlled processes mediated by a prefrontal cortex-hippocampal network (Kensinger and Corkin, 2004). Taken together, we propose that interacting rapid cortisol effects -presumably in the hippocampal-amygdalar network- may have caused emotional memories to be consolidated in an isolated, context-independent and generalized manner. Slower corticosteroid effects on the other hand may have caused a shift in the brain towards more cognitively controlled processes, supported by a prefrontal cortex-hippocampal network, dealing with the emotional, arousing memories in a comparable manner as the neutral ones.

Time-dependent effects of cortisol on contextualization became apparent in the d-prime and hit rate recognition data, but not in the retrieval data. Further exploratory analyses revealed that recollection remained unaffected by cortisol, but slow cortisol enhanced familiarity sensitivity to context for emotional memories (though the effect was only marginally significant). This latter finding suggests that the enhanced contextualization observed for the slow cortisol group could be explained by an enhanced familiarity in intact contexts, rather than by altered recollection (see **supplementary results**). At first sight, such a pattern of results may be consistent

with the view that retrieval and recollection on the one hand and familiarity on the other are subserved by separate, dissociable brain systems (e.g., Brown and Aggleton, 2001). However, the fact that we found strong context and cortisol effects -both supposedly mediated by the hippocampus- on familiarity (or recognition) may be difficult to reconcile with a view in which the hippocampus is not involved in familiarity. Perhaps then, the current cortisol manipulation, and most pronouncedly the 'slow' effect, may have primarily affected weak memories, strengthening their hippocampal components. This would be in line with suggestions that weak memories are most likely to fit a familiarity profile (e.g., Kirwan et al., 2010; Squire et al., 2007). Clearly, such ideas require more in-depth investigations.

Another unresolved, but related, question is whether the process of contextualization is associative in nature (i.e., comprising several separate constituents), or unitizing in nature (i.e., representing separate stimulus components as a single compound; Staresina and Davachi, 2010). Neuroimaging studies are particularly well suited to investigate which brain regions mediate cortisol effects on these memory processes.

From a clinical perspective, our current findings may also shed light on the question how cortisol can sometimes contribute to traumatic memory formation (Cohen et al., 2009; Kaouane et al., 2012) but in other cases can exert protective effects (Rao et al., 2012). Here we show that one and the same hormone can induce disparate effects on behavior: not only the absolute levels of cortisol may play an important role in modulating certain memory processes, but also the *delay* between elevation in cortisol levels and encoding, (and perhaps consolidation, reconsolidation and retrieval too) of traumatic events. This is in line with the temporal dynamics model (Diamond et al., 2007), the emotional tagging hypothesis (Richter-Levin and Akirav, 2003) and theories by Joëls and colleagues (Joëls et al., 2012), which all predict differential effects by corticosteroids in the time-domain shortly after stress as opposed to several hours later (though directionality and region specificity of effects differ among these theories). This insight not only bears important implications for basic experimental research into the mechanisms of emotional memory modulation, it also suggests that corticosteroids might protect against traumatic memory formation when administered hours ahead of exposure to possible traumatic experiences (for instance in soldiers sent out to a war scene). Interestingly, cortisol has been suggested as a facilitating agent in exposure therapy (de Quervain et al., 2011; de Quervain and Margraf, 2008; Soravia et al., 2006). If genomic effects of cortisol indeed enhance the contextual dependency of memories, clinicians should take care to administer cortisol in close proximity of exposure/extinction as these are thought to be highly

context dependent processes (Bouton, 2004). In such a way, maximum generalizability of extinction learning may be realized.

The present study has several limitations. First, we used exogenous administration of hydrocortisone to probe the two time-domains of hormonal action. While this has the great advantage that it allows investigation of corticosteroid actions in isolation, it clearly differs from the situation after real-life stress. In line with the present findings of rapid cortisol effects, one study in humans showed that social stress impaired context-dependent memory of an object-location task, but did not explicitly relate this effect to stress-induced cortisol levels (Schwabe et al., 2009a). Thus it remains to be seen if disparate effects on contextualization of emotional memory are also seen with short versus long delays between encoding and *stress exposure*, and whether these effects indeed relate to stress-induced cortisol. Further, our design does not allow to distinguish between possible effects of cortisol on attention allocation (Putman and Roelofs, 2011) during encoding and effects on how information is subsequently maintained. Finally, besides timing, many other factors may moderate the complex relationship between cortisol and memory contextualization such as hippocampal volume (Gilbertson et al., 2007; 2002), the experience of previous trauma (Resnick et al., 1995), chronic stress (McEwen, 2003), amygdala reactivity (Admon et al., 2009), or genetic make-up (de Kloet et al., 2005). Further experimental research into these modulatory mechanisms in healthy and clinical populations is warranted.

In conclusion, this study shows distinct time-dependent effects of cortisol on the contextualization of emotional memories. The results suggest that rapid effects of cortisol may lead to impaired emotional memory contextualization, while slow effects of cortisol may confer protection against emotional memory generalization.

SUPPLEMENTARY MATERIAL

1. SUPPLEMENTARY METHODS

1.1. Retrieval (word stem completion)

On the second day of the experiment, participants returned to the lab to complete two surprise memory tasks. During the first test (word stem completion), participants were presented with the first two letters of all the words presented during the memory encoding task of the first session. The word stems were shown in a gray rectangle presented against a color picture. Word stems were unique, and subjects were asked to use the stem as a memory cue and to complete the word by typing in the remaining letters. In addition, they were told that the background picture could be the same as the previous day, but not necessarily so. Only those items that could be recalled were to be completed. In case the participants could not remember the word, they were asked to respond with 'x'. The task was self-paced. Reaction times of the responses were recorded. Crucially, half of the words (i.e., 15 emotional and 15 neutral) were presented in the same context as the previous day ('intact context' condition), while the other half were presented in a context that was previously seen, but not in this specific word-context combination ('rearranged context' condition).

1.2. Recognition

Besides possible *objective* alterations in context dependent memory performance, it could be the case that the timing of cortisol administration modulates the *subjective* quality of these memories. That is, recognition of some items may be based on a detailed vivid feeling of re-experience (recollection), whereas other items may be recognized on the basis of a sense that the item has been previously encountered (a sense of familiarity) (Yonelinas, 2002). Thus, if participants indicated that they recognized the word as old, participants underwent a typical 'Remember/Know' procedure. Prior to commencement on the recognition test, the experimenter orally explained the exact distinction between remember and know responses, making use of validated instructions (Geraci et al., 2009; Rajaram, 1993). In short, subjects were asked to give a "remember" response when the word brought back to mind a specific detail from the episode in which the word had been experienced, such as a sensory detail, a thought, or a feeling. They were asked to give a "know" response when they knew having encountered the word, but did not consciously recollect anything about its actual occurrence or what happened or what was experienced at the time of its occurrence. When subjects judged the presented word to be new, they were asked to indicate how confident they were of their response on a scale from 1

through 5 to equalize the number of questions that had to be answered after each alternative answer. We did so to balance the number of questions after a new/old decision. Otherwise, participants would perhaps have been more biased towards indicating 'new', as it would have meant fewer actions to continue to the next trial. Thus, since the confidence rating was merely a filler task, we have not analyzed these ratings.

1.3. Data analysis

Group differences in sample characteristics were assessed by univariate analyses of variance (ANOVAs) with the between-subject factor Group (rapid cort, slow cort, placebo). Arousal and valence ratings of the words obtained during the encoding task from day 1 were analyzed by means of a repeated measures ANOVA with the repeated measure Emotion as within-subject factor and Group as the between-subjects factor. Proportions of correct answers were calculated for the retrieval data, as a function of context and emotion. To assess the subjective quality of memories we calculated recollection as $(\text{remember}_{\text{hit}} - \text{remember}_{\text{fa}}) / (1 - \text{remember}_{\text{fa}})$ and as familiarity d' -prime (using $(\text{know}_{\text{hit}} / (1 - \text{remember}_{\text{hit}}))$ vs. $\text{know}_{\text{fa}} / (1 - \text{remember}_{\text{fa}})$), in line with recommendations by Yonelinas (1998). These latter scores take into account the fact that the probability of making a know response to a presented word is constrained by the number of remember responses made to presented words, because participants were instructed to respond 'know' to items that were familiar and not recollected (Yonelinas et al., 1998). Retrieval, recollection and familiarity data were analyzed by means of ANOVAs with the repeated measures factors Emotion and Context, and Group as the between-subjects factor. Significant effects were followed up by relevant planned comparisons.

2. SUPPLEMENTARY RESULTS

2.1. Participant characteristics

Two participants (one from the rapid cort group and one from the slow cort group) indicated that they did not adhere to the encoding instructions on day 1. They were excluded from analysis, based on the *a priori* exclusion criteria. The final sample consisted of 62 subjects with a mean age of 21.6 years (SD = 2.6 years) and a mean body mass index (BMI) of 22.9 (SD = 3). The rapid cort, slow cort and placebo groups did not differ in terms of age, BMI, trait anxiety, or score on the cube and paper task (all $F_s < 2.4$, not significant (ns)). In addition, the three groups did not differ on subjective mood (state anxiety, positive affect and negative affect) before memory encoding (all $F_s < 1.92$, ns), or before memory retrieval (all $F_s < 0.97$, ns). Participants

from all groups had been awake for a comparable amount of time prior to testing ($F_{2,59} = 0.55$, ns), and were all as motivated and concentrated to complete the memory task (all F s < 1.80, ns). None of the participants had expected a surprise memory task.

2.2. Subjective ratings during memory encoding

To assess whether the induction of neutral versus emotional mental images was successful, we analyzed the neutral and emotional words on ratings of valence and arousal obtained during the encoding task. Ratings are depicted in **Figure S1**. As expected, the emotional words imagined in their respective contexts were rated significantly more negative ($F_{1,59} = 334.58$, $p < 0.001$, $\eta_p^2 = 0.850$) and arousing ($F_{1,59} = 175.57$, $p < 0.001$, $\eta_p^2 = 0.748$) than the neutral words. Importantly, there were no effects of Group on valence ($F_{2,59} = 0.26$, ns) or arousal ($F_{2,59} = 0.10$, ns) ratings, and these ratings did not correlate with overall memory performance or contextualization. Thus, the experienced intensity of the stimuli during encoding did not mediate possible effects of cortisol timing on subsequent memory (Baron and Kenny, 1986).

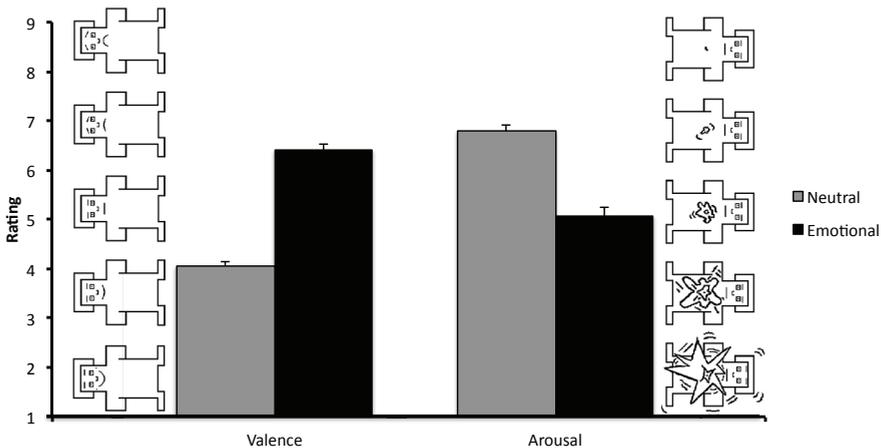


Figure S1. Arousal and valence ratings. The graph depicts arousal and valence ratings of the mental images of the words in contexts that participants formed during encoding at the first day of the experiment. Error bars represent standard error of the mean.

2.3. Cued retrieval

As can be seen in **Figure S2**, analysis of the cued retrieval data showed that Context exerted a strong influence on whether a memory was retrieved or not ($F_{1,59} = 98.31$, $p < 0.001$, $\eta_p^2 = 0.625$): With intact contexts, words were easier remembered than with rear-

ranged contexts. Furthermore, emotional memories were generally easier retrieved than neutral memories ($F_{1,59} = 13.06, p = 0.001, \eta_p^2 = 0.181$), though successful retrieval of emotional versus neutral memories marginally depended on context (Context \times Emotion interaction; $F_{1,59} = 3.95, p = 0.051, \eta_p^2 = 0.063$). Specific planned comparisons indicated that this interaction was mainly caused by higher retrieval of emotional memories as compared to neutral memories within rearranged contexts ($F_{1,59} = 25.61, p < 0.001, \eta_p^2 = 0.303$). Within intact contexts, retrieval of emotional and neutral memories did not significantly differ ($F_{1,59} = 0.36, ns$). Most pertinent to the hypothesis at hand, there were no interactions of Context and/or Emotion with Group (all $F_s < 1.59, ns$). There was, however, a main effect of Group ($F_{2,59} = 3.95, p = 0.025, \eta_p^2 = 0.118$), indicating that independent of Context or Emotion, retrieval varied across the groups. Follow up comparisons indicated that overall retrieval of the rapid cort group was relatively impaired as compared with both the placebo ($F_{1,59} = 5.66, p < 0.021, \eta_p^2 = 0.088$) and slow

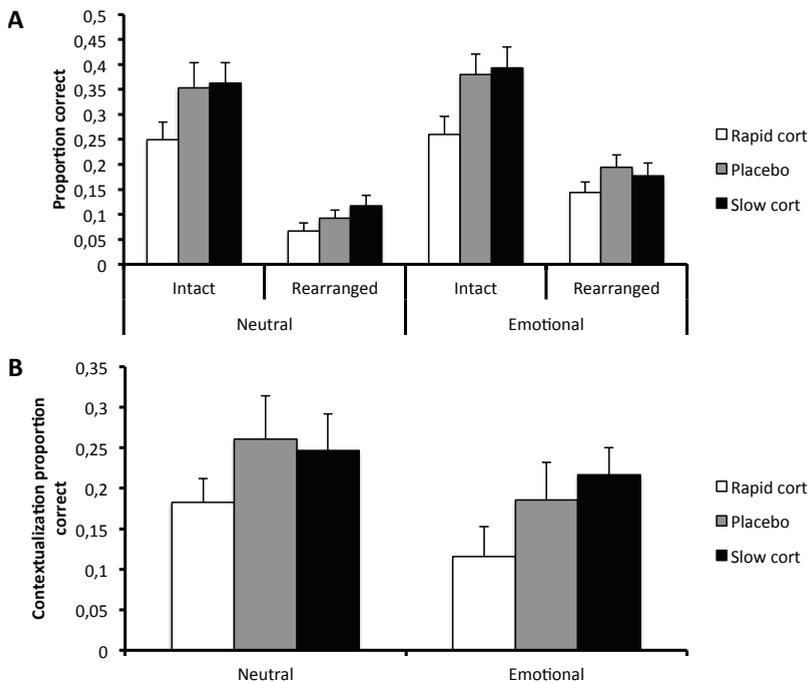


Figure S2. Cued retrieval data. **(A)** Mean proportion correct responses during the cued recall memory task at day 2, as a function of group, context and emotion. **(B)** Contextual dependency of neutral and emotional memories, as a function of group. Differences scores are depicted between proportions correct for the rearranged conditions subtracted from the intact conditions. A larger difference score reflects greater contextual dependency of memories. Error bars represent standard error of the mean.

cort groups ($F_{1,59} = 6.4, p < 0.021, \eta_p^2 = 0.098$), while the slow cort and placebo groups did not differ from each other ($F_{1,59} = 0.06, ns$).

Analysis of reaction times showed that responses were generally quicker in the intact contexts ($F_{1,59} = 37.04, p < 0.001, \eta_p^2 = 0.386$). No other effects were significant on reaction times (all F s $< 1.42, ns$).

2.4. Recognition: Hit and false alarm rate

An analysis comparable to the analysis of d-prime was repeated with hit rate as the dependent variable. As can be seen in **Figure S3A** the pattern of results was comparable with those found with d-prime: Context exerted a strong influence on memories ($F_{1,59} = 73.580, p < 0.001, \eta_p^2 = 0.555$). Negative memories were overall better remembered ($F_{2,59} = 157.119, p < 0.001, \eta_p^2 = 0.727$). Recognition of emotional versus neutral memories again depended on context (Context \times Emotion interaction; $F_{1,59} = 15.352, p < 0.001, \eta_p^2 = 0.206$). Unlike the d-prime analysis, there was no effect of cortisol timing on overall hit rates (main effect of Group; $F_{2,59} = 1.03, ns$). Most pertinent to the hypothesis at hand, contextual dependency of emotional and neutral memories tended to vary as a function of cortisol timing (Context \times Emotion \times Group interaction; $F_{2,59} = 2.888, p = 0.064, \eta_p^2 = 0.089$; **Figure S3B**), though the effect was slightly weaker than the effect observed in the d-prime analysis.

To decompose this three-way interaction we separately analyzed hit rates for neutral and emotional memories, as was done for d-prime. Neutral memories were strongly context-dependent ($F_{1,59} = 116.96, p < 0.001, \eta_p^2 = 0.665$), but context-dependency was not modulated by cortisol (Context \times Group; $F_{2,59} = 0.08, p = ns$). Emotional memories were strongly context-dependent as well ($F_{1,59} = 40.63, p < 0.001, \eta_p^2 = 0.408$). This context-dependency was modulated by timing of cortisol (Context \times Group; $F_{2,59} = 6.45, p = 0.003, \eta_p^2 = 0.179$). To further decompose this interaction, we entered contextualization scores for these emotional memories (i.e., difference scores of the rearranged condition minus the intact condition; a larger contextualization index reflects greater contextual dependency of memories) in an ANOVA with the between-subjects factor Group (see **Figure S3B**). Note that the difference scores within this analysis reflect the factor 'Context' from the previous analysis. In order to investigate how exactly the timing of cortisol altered the contextual dependency of emotional memories, we used planned contrasts to directly compare contextualization across groups. These revealed that compared with placebo, cortisol administration several hours *prior to* encoding (slow cort) resulted in a marginally significant enhancement of the contextualization of emotional memories ($t_{59} = 1.86, p = 0.065$), while elevated cortisol levels *during* encoding (rapid cort) resulted in a marginally significant impairment of the contextualization of emotional memories ($t_{59} = 1.88, p = 0.065$).

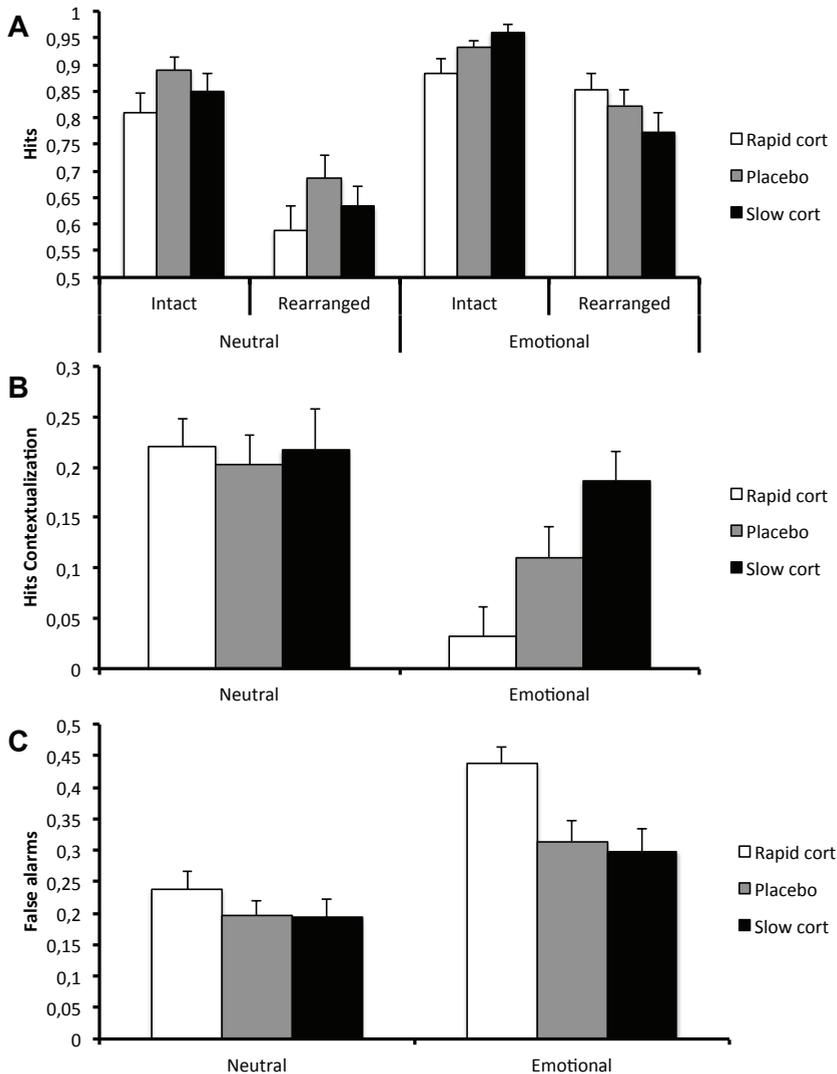


Figure S3. Hit and false alarm rates during recognition testing. **(A)** Graph depicts memory performance indexed by mean hits, as a function of group (rapid cort, slow cort or placebo), context (intact or rearranged) and emotion (neutral or emotional). **(B)** Contextual dependency of neutral and emotional memories. Differences scores are depicted between hit rate values for the rearranged conditions subtracted from the intact conditions. A larger difference score reflects greater contextual dependency of memories. As can be seen in the figure, cortisol did not in any way affect the contextualization of neutral memories, but pronouncedly affected the contextualization of emotional memories. **(C)** False alarm rate, as a function of condition (rapid cort, slow cort or placebo), context (intact or rearranged) and emotion (emotional or neutral). The rapid cort group displayed significantly more false alarms on the emotional words as compared to both other groups.

Next, false alarm rates for neutral and emotional words were analyzed (note that the foils do not have a study context, and there are therefore no separate false alarm rates for intact and rearranged contexts). Emotional words elicited more false memories than neutral words ($F_{1,59} = 72.85, p < 0.001, \eta_p^2 = 0.553$). Depending on Emotion, the timing of cortisol exerted a marginally significant effect on false alarm rate (Emotion \times Group $F_{2,59} = 3.06, p < 0.055, \eta_p^2 = 0.094$). As can be seen in **Figure S3C**, this effect was mainly caused by inflated false memories by the rapid cort group on emotional words ($t_{59} = 2.66, p = 0.01$).

Finally, accuracy effects of cortisol were not caused by a change in speed-accuracy trade-off due to cortisol, as there were not any effects of Group on reaction times (all $F_s < 2.9$, ns), though the responses were generally faster with intact contexts ($F_{1,59} = 21.76, p < 0.001, \eta_p^2 = 0.269$).

2.5. Recognition: Subjective quality of memory

It could be the case that our cortisol manipulations modulated the *subjective* quality of memories. That is, recognition of some items may be based on a detailed vivid feeling of re-experience of a specific past episode (recollection), whereas other items may be recognized on the basis of a sense that the item has been previously encountered, but is noncontextual in nature (a sense of familiarity) (Aggleton and Brown, 1999; Mandler, 1980; Yonelinas, 2002) (see **Figure S4**). From these notions it can be predicted that context congruency should enhance recollection, but leave familiarity unaffected. Since we did not have strong predictions concerning the effects of cortisol, we analyzed these in an explorative fashion. Analyses showed that recollection was strongly enhanced when the testing context was congruent with the encoding context ($F_{1,59} = 99.456, p < 0.001, \eta_p^2 = 0.628$). None of the other effects reached significance (all $F_s > 2.976$, ns).

Analysis of the familiarity responses revealed that, contrary to expectation, context congruency did enhance the sense of familiarity ($F_{1,59} = 22.895, p < 0.001, \eta_p^2 = 0.280$). Interestingly, this effect was marginally modulated by Emotion and Group (Context \times Emotion \times Group; $F_{1,59} = 2.71, p = 0.075, \eta_p^2 = 0.085$). ANOVAS for neutral and emotional memories separately revealed that for neutral memories the intact context condition enhanced the feeling of familiarity ($F_{1,59} = 19.066, p < 0.001, \eta_p^2 = 0.244$) but this effect was not modulated by Group ($F_{2,59} = 0.760, p = \text{ns}$). For the negative memories, there was only a trend Context effect ($F_{2,59} = 2.968, p = 0.090, \eta_p^2 = 0.048$) that was modulated by Group (Context \times Group; $F_{1,59} = 3.908, p = 0.025, \eta_p^2 = 0.117$). Comparisons as done for recognition scores indicated that the intact and rearranged context conditions did not significantly differ from each other for the placebo and rapid groups (all $F_s < 0.229$, ns), but did differ for the slow group ($F_{2,59} = 10.204, p = 0.002, \eta_p^2 = 0.147$). Direct comparison of contextualization difference

scores confirmed that contextualization of familiarity responses were enhanced for the slow group as compared with the placebo ($t_{59} = 2.66, p = 0.010$) and rapid ($t_{59} = -2.95, p = 0.041$) groups. In the slow condition, an intact context thus seemed to enhance the subjective feeling of familiarity for negative memories. Though these analyses were only explorative and warrant further investigation, it may suggest that the enhanced contextualization effect in the slow group may be explained by increased strength of the memory trace (Squire et al., 2007).

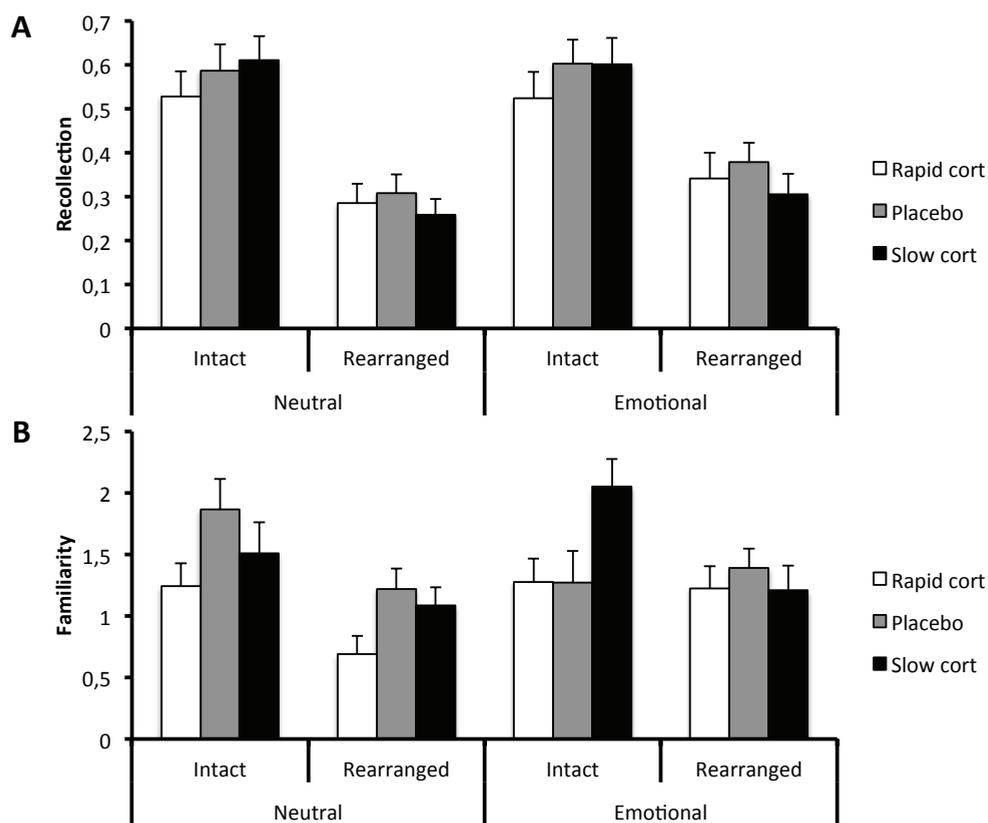


Figure S4. Subjective quality of memories. **(A)** Mean recollection scores. Cortisol did not affect recollection. **(B)** Mean familiarity responses (d'). Participants in the slow cortisol condition generally gave more familiar responses in the intact context.

2.6. Recognition: Additional analyses

It is possible that being able to retrieve a word in the cued recall test would make it easier to also recognize it. However, since words that are recallable are those that are better represented in memory, it is likely that they would be recognized anyway,

that is, also without a preceding recall test. Although the procedure was the same for all experimental groups, we checked whether recall score is a confounder in our design by testing whether inclusion of general memory performance during retrieval altered the general pattern of results. To do so, we re-ran the main analyses (i.e., the Context \times Emotion \times Group mixed ANOVA) of the current paper, but included general retrieval performance as a covariate. Results showed that the three-way interaction with d-prime as dependent variable became even slightly stronger ($F_{2,59} = 3.44, p = 0.039, \eta_p^2 = 0.106$), while this same interaction effect was again not present in the recollection data ($F_{2,59} = 0.014, p = \text{ns}$). Also, the familiarity analysis was still marginally significant ($F_{2,59} = 2.821, p = 0.068, \eta_p^2 = 0.089$). These results suggest that performance during retrieval did not affect the major findings from the present study.

The foil words during recognition were presented along with old contexts. Consequently, contexts were all presented twice. Though trials were presented randomly we wanted to exclude the possibility that old or new word-context combinations were presented more often as first trial (or second) than the other. Context repetitions took place as often for old as new words and these repetitions did not differ between the groups ($F_{2,62} = 0.48, p = \text{ns}$). Finally, there were no effects of context repetitions (i.e., first presentation versus second presentation) on memory performance ($F_{1,59} = 0.62, p = \text{ns}$), and memory performance on the first or the second context presentation was not altered by cortisol timing ($F_{2,59} = 0.69, p = \text{ns}$).