Corticotosteroids are key modulators of human cognition. They are released in response to stress as the end product of the hypothalamic–adrenal–pituitary (HPA) axis, and are known to readily cross the blood–brain barrier to affect brain processing (1). Corticotosteroids ensure sufficient energy supply to challenged tissues and control the excitability of neuronal networks, and are thereby thought to support and regulate the stress response (2). The hormones exert their actions upon binding of the mineralocorticoid (MR) and glucocorticoid receptor (GR), abundantly expressed in the brain (3–5). Recent animal research has indicated that receptor-binding causes both immediate nongenomic effects (6) and slow, genomic effects that manifest themselves several hours after stress exposure (7, 8). By these distinct mechanisms, corticotosteroids seem to influence neural plasticity in a time-dependent manner (9).

So far, most research on modulation of cognition has focused on medial temporal lobe structures, where corticotosteroids have been shown to affect neuronal excitability, synaptic plasticity, and processes of memory retrieval and consolidation (10, 11). However, moderate to high levels of receptor expression in the prefrontal cortex (PFC) (5) make this structure susceptible to corticotosteroid modulation as well. A current working hypothesis states that corticotosteroids’ rapid nongenomic effects work in concert with the effects of catecholamines during the early phase of the stress response (9, 12), and thereby optimize rapid adaptive behavior by reallocating neural resources away from higher-order cognitive processing regions in the PFC to promote vigilance, instinctive behavior, and the encoding of the stressful experience into memory (13). Meanwhile, the corticotosteroid-induced genomic cascade is initiated, which is hypothesized to restore PFC function in the aftermath of stress (13). Although findings from both animal (14, 15) and human literature (16, 17) provide initial evidence for corticotosteroid modulation of PFC signaling, both the neural and functional consequences on higher-cognitive function and their time-dependency remain to be tested.

Here, we targeted both the rapid (putatively nongenomic) and slow (putatively genomic) effects of corticosteroids on PFC processing using a working memory (WM) paradigm during functional MRI (fMRI) in humans. WM refers to a system maintaining relevant information in a temporary buffer that is constantly updated to guide behavior (18). It is typically associated with the activation of a frontoparietal executive function network, including the dorsolateral prefrontal cortex (DLPFC) (18). Implementing a randomized, double-blind, placebo-controlled design, 72 young, healthy men received 10 mg hydrocortisone—known to mimic corticosterone levels observed during moderate to severe stress—either 30 min (to target corticotosterone rapid effects) or 240 min (to assess corticotesteroid slow effects) before a numerical n-back task. To investigate whether corticosteroid effects depend on task difficulty, we manipulated WM load using a 0-, 1-, 2-, and 3-back condition.

Results

Physiological and Psychological Measures. As expected, oral administration of 10 mg hydrocortisone increased salivary cortisol levels to those observed during moderate to severe stress (19) (Fig. 1), which was evidenced by a significant main effect of group \(F(2,65) = 43.30, P < 0.001\) and a time × group interaction \(F(18,116) = 26.17, P < 0.001\). Increased levels were observed from 30 min postadministration onward in both hydrocortisone administration conditions, and the levels remained elevated for at least 90 min. As intended, treatment resulted in elevated cortisol levels during fMRI scanning in the rapid hydrocortisone condition, whereas the levels in the slow condition had already returned to baseline.

Postexperiment debriefing showed that participants were unable to identify the substance received. As expected, hydrocortisone administration did not affect autonomic measures of heart rate [main effect of drug: \(F(2,64) < 1\)] and heart rate variability [\(F(2,64) < 1, \text{NS}\)] (Table 1). Although significant reductions in levels of depression scores [Friedman’s ANOVA; \(\chi^2(2) = 8.99, P = 0.011\)], anger scores [\(\chi^2(2) = 7.43, P = 0.024\)], vigor scores [\(\chi^2(2) = 79.05, P < 0.001\)], and tension scores [\(\chi^2(2) = 18.38, P < 0.001\)] were observed over the course of the experiment, levels of fatigue [\(\chi^2(2) = 52.40, P < 0.001\)] increased, none of these factors was affected by drug administration. Hence, differences in brain activity found between drug conditions cannot readily be explained by any physiological or psychological side effects of drug administration.
Working Memory Performance. Separate ANOVAs for both performance measures of accuracy and reaction times (RTs) were conducted with WM load as within-subject factor, and drug condition as between-subject factor. There were robust effects of WM load on both accuracy [F(3, 63) = 107.72, P < 0.001] and RTs [F(3, 63) = 97.96, P < 0.001] (Fig. 24). These analyses revealed no significant main effect of drug [accuracy: F(2, 65) = 1.59, P = 0.212; RTs: F(2, 65) = 1.98, P = 0.146] or a WM-load × drug interaction (accuracy and RTs: all F < 1) on both performance measures, although a tendency toward shorter RTs and improved accuracy could be observed for the slow hydrocortisone (CORT) group (Fig. 24). Because performance on the n-back task can be regarded as a combined measure of both accuracy and RTs of responding (both assessing voluntary attention (23) and efficacy of information processing (24)), the two measures were combined to create one overall WM performance measure (25) (Materials and Methods). Analysis of this combined performance measure revealed that CORT administration indeed affected WM performance (F(2, 42) = 3.25, P = 0.045). This main effect of drug was driven by an improved performance of the slow CORT group compared with the rapid CORT group [T(42) = 6.58, P = 0.014, Fig. 2D] and close to significant improvement compared with the placebo group [T(43) = 3.59, P = 0.065]. The rapid CORT and placebo groups did not differ on WM-performance [T(42) = 0.233, NS]. The observed effects seemed to be driven by drug effects at high WM load [2- and 3-back conditions; F(2, 64) = 3.34, P = 0.042], as there was no significant difference between drug conditions at low WM load [0- and 1-back; F(2, 64) = 1.68, P = 0.195]. However, the drug × load interaction failed to reach significance [F(2, 64) < 1].

Brain Activation. We first identified brain regions activated by performing the numerical n-back task by contrasting 3- 2- 1-back with 0-back conditions (collapsing across groups). As expected, the WM task activated an extended set of brain regions in the bilateral prefrontal cortex (including the DLPCF), bilateral inferior parietal cortex, inferior occipital lobe, cerebellum and other related regions (Table 2 and Fig. S1A). The opposite contrast, regions deactivated by WM processing, revealed the default mode network including the posterior cingulate cortex, the ventral medial PFC extending into the orbitofrontal cortex and the medial temporal lobe (Table 2 and Fig. S1B).

To examine how corticosteroids affect working memory processing over time, we first identified those brain regions the activity of which was modulated by any of the drug conditions. This analysis showed that the only significant effect of hydrocortisone was observed in the left DLPCF [t( = -40, y = 42, z = 32) F(2, 194) = 11.52, P_corrected = 0.050] (Fig. 3). We next extracted the data from this cluster to analyze whether the effects of hydrocortisone were moderated by WM load using orthogonal contrasts. First of all, the main effect of WM load was significant [F(2, 63) = 4.83, P = 0.011] and showed that the DLPCF displayed greater activity with increasing load. More importantly, the effect of WM load on DLPCF activation was mediated by hydrocortisone administration [drug × WM-load interaction; F(4,128) = 2.56, P = 0.042]. Further analyses showed that this interaction was driven by more prominent drug effects at high compared with low WM loads [drug × WM-load (1- vs. 2-back); F(2, 64) = 5.17, P = 0.008, drug × WM-load (one- vs. three-back); F(2, 64) = 4.57, P = 0.014], whereas the drug effect between the high WM-load conditions (two- and three-back) did not differ significantly [F(2, 64) < 1].

To determine which of the drug conditions induced these DLPCF effects, we continued with pairwise follow-up tests among the three drug conditions. These analyses revealed that the observed drug × WM-load interaction effect in the DLPCF was caused by the slow effects of corticosteroids under high WM-load. A history of corticosteroid elevation apparently induced increased high WM-load processing in the DLPCF compared with both placebo [F(1, 43) = 6.31, P = 0.016] and the rapid corticosteroid conditions [F(1, 42) = 12.82, P = 0.001]. Current elevation

Table 1. Physiological and psychological measures

<table>
<thead>
<tr>
<th>Mood state</th>
<th>Placebo</th>
<th>Rapid CORT</th>
<th>Slow CORT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression 1 (t = 30 min)</td>
<td>0.26 (0.13)</td>
<td>0.82 (0.37)</td>
<td>0.65 (0.32)</td>
</tr>
<tr>
<td>2 (t = 255 min)</td>
<td>0.09 (0.06)</td>
<td>0.64 (0.35)</td>
<td>0.13 (0.07)</td>
</tr>
<tr>
<td>3 (t = 375 min)</td>
<td>0.04 (0.04)</td>
<td>0.59 (0.24)</td>
<td>0.13 (0.10)</td>
</tr>
<tr>
<td>Anger 1 (t = 30 min)</td>
<td>0.61 (0.23)</td>
<td>1.18 (0.40)</td>
<td>1.00 (0.43)</td>
</tr>
<tr>
<td>2 (t = 255 min)</td>
<td>0.30 (0.19)</td>
<td>0.45 (0.23)</td>
<td>0.48 (0.20)</td>
</tr>
<tr>
<td>3 (t = 375 min)</td>
<td>0.22 (0.18)</td>
<td>0.73 (0.29)</td>
<td>0.87 (0.32)</td>
</tr>
<tr>
<td>Fatigue 1 (t = 30 min)</td>
<td>1.17 (0.30)</td>
<td>1.68 (0.50)</td>
<td>2.70 (0.61)</td>
</tr>
<tr>
<td>2 (t = 255 min)</td>
<td>1.35 (0.44)</td>
<td>1.55 (0.52)</td>
<td>2.43 (0.56)</td>
</tr>
<tr>
<td>3 (t = 375 min)</td>
<td>3.52 (0.67)</td>
<td>5.23 (0.69)</td>
<td>4.22 (0.71)</td>
</tr>
<tr>
<td>Vigor 1 (t = 30 min)</td>
<td>12.65 (0.79)</td>
<td>10.50 (0.77)</td>
<td>11.70 (0.90)</td>
</tr>
<tr>
<td>2 (t = 255 min)</td>
<td>10.43 (0.68)</td>
<td>8.73 (0.75)</td>
<td>10.26 (0.96)</td>
</tr>
<tr>
<td>3 (t = 375 min)</td>
<td>7.57 (0.88)</td>
<td>4.86 (0.82)</td>
<td>7.13 (0.91)</td>
</tr>
<tr>
<td>Tension 1 (t = 30 min)</td>
<td>1.00 (0.27)</td>
<td>1.36 (0.29)</td>
<td>1.30 (0.46)</td>
</tr>
<tr>
<td>2 (t = 255 min)</td>
<td>0.35 (0.13)</td>
<td>1.09 (0.35)</td>
<td>0.96 (0.30)</td>
</tr>
<tr>
<td>3 (t = 375 min)</td>
<td>0.26 (0.16)</td>
<td>0.64 (0.20)</td>
<td>0.17 (0.10)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>65.60 (1.96)</td>
<td>67.04 (2.57)</td>
<td>68.30 (2.41)</td>
</tr>
<tr>
<td>Heart rate variability (ms²)</td>
<td>70.76 (4.95)</td>
<td>62.71 (4.99)</td>
<td>67.02 (6.60)</td>
</tr>
</tbody>
</table>

Data are mean (SEM). CORT, hydrocortisone.

Fig. 1. Experimental design and salivary cortisol curves. Participants received two capsules (drug1 and drug2) containing either 10 mg hydrocortisone (CORT) or placebo at different time points before the numerical n-back task (0/1/2/3-back). Hydrocortisone intake significantly elevated salivary cortisol levels in both hydrocortisone administration conditions to levels observed during moderate to severe stress. Mood, Mood based on POMS questionnaire (20-22). Error bars represent SEM.

Fig. 2. Behavioral performance in n-back task. (A) Mean error rates and RTs of the 0-, 1-, 2-, and 3-back conditions for the three drug conditions did not reveal any effects of hydrocortisone (CORT). (B) Combination of error rates and RTs into one overall WM performance measure revealed that the slow CORT group outperformed both other groups. Error bars represent SEM.
in corticosteroid level had no such effect on DLPFC activation [rapid CORT vs. placebo: $F(1,42) = 1.24$, NS].

**Discussion**

Here we targeted both the rapid (presumably nongenomic) and slow (presumably genomic) effects of corticosteroids on prefrontal working memory processing. Results revealed time-differential effects for corticosteroids' actions, with their slow effects increasing WM-related activation of the DLPFC and thereby improving WM performance, whereas corticosteroids' rapid effects did not induce any observable effect.

Previous work in animals has provided initial evidence that corticosteroids, in addition to their well-established slow genomic effects, also exert rapid nongenomic effects (9). The hormones have been shown to rapidly affect neuronal plasticity by binding to membrane mineralocorticoid receptors (MR), leading to a change in glutamate release (6). At the same time, a corticosteroid-induced genomic cascade is initiated by the binding of primarily intracellular glucocorticoid receptors (GRs) that, upon binding, translocate to the nucleus, where they function as transcription factors to modulate the expression of more than 200 genes (26).

In the medial temporal lobe, these rapid and slow actions of corticosteroids were shown to have fundamentally distinct consequences in that they either enhanced or inhibited neuronal plasticity, respectively (6–8). Here, we aimed to dissociate these two effects on the human PFC by first establishing 10 mg of hydrocortisone at either 30 or 240 min before the WM task. The timing of the rapid corticosteroid condition was based on a previous study in our laboratory revealing an elevation in human salivary cortisol levels from 30 min onward (27), and most prominent rapid, quickly reversible effects with corticosteroids administered directly to hippocampal slices in rodents (6). The genomic effects of corticosteroids, on the other hand, generally do not start earlier than 3 h after exposure to high corticosteroid levels (28, 29), and these effects last for hours (28, 30). Thus, administration of hydrocortisone at 30 min before scanning probably caused sufficient high levels of the hormone in the brain to evoke rapid nongenomic effects, whereas this delay was too short to allow development of gene-mediated events. Conversely, when hydrocortisone was applied at 240 min before testing, hormone levels were so low (similar to baseline) during the behavioral task that nongenomic actions are not likely to happen, yet allowed enough time for the gene-mediated actions to occur.

Under conditions of acute stress, working memory is generally impaired (31–33), whereas neuronal firing and long-term potentiation in the PFC are known to be decreased (34–36). These effects are at least partly caused by the stress-related hormones norepinephrine and dopamine, which are known to impair prefrontal cortex function in higher doses (37). They subserve the initial fight-or-flight response by prioritizing rapid instinctive behavior (as mediated by, e.g., the amygdala) and emotional memory encoding (38, 39) over complex, higher-order cognitive functions as performed by the prefrontal cortex (37, 40). Because previous studies have shown that the rapid effects of corticosteroids act in concert with (and to amplify) the effects of catecholamines on long term memory (9, 12), we hypothesized impaired WM performance in the rapid CORT condition. However, we did not observe any rapid, nongenomic effects of corticosteroids on either WM performance or DLPFC activation. Previous studies of corticosteroid modulation of working memory performance show rather conflicting results on this topic. Studies have reported no effects on WM performance (41, 42), corticosteroid-induced improvements in both humans (43) and animals (44), as well as impairments (45) depending on concurrent sympathetic activation (31) or WM
load (46). The latter findings suggest that fast actions of corticosteroids indeed have effects additive to those of noradrenergic activation in WM impairment. Work in rodents has shown that this concurrent noradrenergic activity of the amygdala actually is essential for corticosteroid-induced impaired WM to occur (47). In line with this, a recent human study of the effects of norepinephrine and corticosteroids on the neural correlates of memory formation showed that, specifically, the administration of both hormones caused a strong deactivation in the prefrontal cortex, whereas no such effects were observed when only corticosteroids were administered (17). Here we used different levels of difficulty (WM load), which presumably triggered different levels of arousal, but did not observe any rapid modulatory effects of hydrocortisone on WM performance or DLPFC processing. However, the levels of emotional arousal reached due to this manipulation most likely did not reach arousal levels observed under conditions of stress. Therefore, this issue of potentially interacting rapid corticosteroid and noradrenergic effects on PFC functioning remains open for future research. Regardless, our results show that corticosteroids by themselves do not modulate WM performance or WM-related DLPFC activity in a rapid nongenomic manner.

Corticosteroids’ slow, genomic effects on the other hand have often been seen as essential for adaptation and restoration of homeostasis following situations of acute stress (48). Here we provide a unique demonstration that exactly these delayed effects of corticosteroids boost WM processing. This effect was strongest at high WM load when cognitive demand is highest. Our findings of enhanced WM by corticosteroids are supported by two recent rodent studies in which the administration of corticosterone in the prefrontal cortex was shown to enhance glutamatergic transmission in PFC pyramidal neurons by increasing surface levels of NMDA- and AMPA-receptor subunits (44, 49). Moreover, one of these studies (44) showed that stress improved performance on a WM task 4 h later, but not immediately. Both this increase in glutamatergic transmission and improved behavioral performance were abolished by the administration of a selective GR antagonist, pointing towards the involvement of this receptor. Because the rapid stimulatory nongenomic effects of corticosteroids are thought to be mediated by corticosteroid binding of membrane MRs (6), this observed corticosteroid-induced WM improvement most likely involves a genomic mechanism. These findings in animals, together with the time-delay implemented for assessing the slow corticosteroid effects in this study, suggest that the observed improvement in WM-processing is mediated via a GR-dependent genomic mechanism. However, the administration of a GR antagonist would be necessary to explicitly test this hypothesis in humans. Although extremely interesting and necessary for future understanding of corticosteroid effects, the realization of such an experiment is currently prohibited for practical reasons, as no selective GR antagonist has yet been registered for human use. Mifepristone (RU-486) is the only compound commercially available (50); however, it is known to cross the blood–brain barrier only at very high concentrations (51) and, more importantly, to also act as a very potent progesterone receptor antagonist (52), which might cause many unwanted side effects. Future studies are therefore necessary to elucidate the exact underlying mechanism of the observed potentiation of WM processing. Nevertheless, we here show that, specifically, corticosteroids’ slow actions boost WM processing in the DLPFC, which are likely mediated via a GR-dependent genomic mechanism.

Other limitations of this study should be mentioned. First of all, the behavioral effects observed in this study were not very strong. Although trends were seen in absolute measures of reaction time and error rate, these trends failed to reach significance. Only the combination of both measures revealed an indication for enhanced performance in the slow corticosteroid group. However, because both measures contribute to behavioral performance in their own distinct ways (23, 24), we think that this combination is actually warranted. The combination of error rates and reaction times is often used to determine the speed–accuracy tradeoff displayed by participants. This speed–accuracy tradeoff refers to the fact that there is usually a tradeoff between these two measures, with either short reaction times causing many errors, or longer reaction times reducing the number of errors (53). Here however, we observed both faster and more accurate responses by participants in the slow CORT group compared with the other groups, so instead of a shift in tradeoff, we found additive effects both pointing towards improved performance.

It cannot be excluded that the lack of a strong behavioral effect is partly caused by the relatively low number of subjects in our fMRI study; this number is obviously lower than for less laborious psychopharmacological studies. Behavioral output is dependent on a multitude of factors (e.g., intelligence or motivation), and the variation in WM performance within each group is therefore quite substantial. For this reason, effects with rather small effect sizes, such as observed here, are not easily detected in humans, certainly with the between-subjects clear evidence was used. Regardless, we found significant brain effects that were in line with the behavioral effect, providing corroborative evidence. A second explanation for the rather weak behavioral effect might be that the dose of hydrocortisone administered was too low to induce stronger effects. We used 10 mg hydrocortisone in this study, because this dose is known to increase salivary cortisol levels to physiological levels observed under conditions of moderate to severe stress (17, 56, 57), and use of such a higher dose might possibly have induced stronger behavioral effects.

Another limitation of this study is that we investigated men only, which limits the generalization of the obtained results to women. Women are known to display HPA axis reactivity different from that of men and to exhibit smaller and more variable responses to stress (58), which appear to depend on the phase of the menstrual cycle and use of hormonal contraceptives (59). Although sex differences are important to consider, this issue was beyond the scope of this initial study, which is why we opted to recruit the population with the most stable response to corticosteroids, and excluded women from participation.

Finally, this pharmacological study obviously is not an exact copy of naturally occurring circumstances. Real-life cortisol release in response to stress is accompanied by the release of many other neuromodulators, such as norepinephrine, corticotropin-releasing hormone, dopamine, and serotonin (60). Mere administration of hydrocortisone lacks the interaction with these modulators, but does reveal a cleaner mechanistic account for the pure corticosteroid effect, which was the aim of this study.

Regardless of these potential limitations, the present results reveal a major finding of this study: slow corticosteroid effects for the existence of time-dependent effects of corticosteroids on human brain processing. The importance of this timing factor, although widely acknowledged in animal literature (61), has so far been neglected in human studies on corticosteroid effects. The majority of previous studies tested for corticosteroid effects ~1 h after hydrocortisone administration (62–64), most probably resulting in a mix of corticosteroids’ rapid, nongenomic and slow, genomic effects. Our data suggest that future research on corticosteroids, along with the understanding of their effects, would greatly benefit from the incorporation of this crucial timing factor in experimental designs. Second, corticosteroids’ slow effects were shown to augment DLPFC processing and to facilitate WM performance. Because previous research has indicated that working memory and prefrontal processing are impaired under conditions of acute stress by the rapid actions of catecholamines (37), we speculate that these slow corticosteroid effects may counteract these changes and help the brain to recover in the aftermath of stress. Thereby, they may serve a highly adaptive function in normalizing brain processing when stress has subsided.

Materials and Methods

A more detailed description of the methods applied can be found in SI Materials and Methods.
Participants. Seventy-two young (age range, 18–29 years; median, 21 years), right-handed, healthy male volunteers gave written informed consent to participate in the study. Individuals with any history of or current psychiatric, neurological, or endocrine disorders, or receiving any medication that affects central nervous system or endocrine systems, were excluded from participation. In addition, four participants were excluded from analyses because they displayed either abnormal basal salivary cortisol levels (>3 SDs above mean; one participant), or showed no elevation in salivary cortisol level in response to hydrocortisone intake, resulting in 23 men in the placebo group, 23 in the slow CORT group, and 22 in the rapid CORT group. The study was executed in accordance with the Declaration of Helsinki and approved by the local ethics committee (CMO region Arnhem-Nijmegen, The Netherlands).

Procedure. Experiment. To reduce the impact of diurnal variation in cortisol levels, all testing was performed in the afternoon, between 1200 hours (±30 min) and 1800 hours (±30 min), when hormone levels are relatively stable. Upon arrival, participants received an information brochure about the procedure, gave informed consent, and completed an intake questionnaire to ensure that inclusion and exclusion criteria were met. Next, 30 min after arrival, a first saliva sample was taken, followed by another sample 15 min later, to measure a reliable baseline level. Participants were asked to complete a first Profile of Mood States (POMS) questionnaire (20–22), after which they were briefly trained in the WM task to ensure proper understanding during scanning. Immediately after the second saliva sample (at $t = 240$ min) participants received the first capsule. During the entire period (~3.5 h) before scanning, participants waited in a quiet room where they were free to conduct any activities except for anything potentially arousing (e.g., video games). At 30 min before scanning, participants were asked to complete another POMS questionnaire, and received the second capsule. Both drug capsules, containing either 10 mg CORT or placebo (cellulose), were administered orally. This dose is known to elevate salivary cortisol levels to moderate to high stress levels (19, 54, 55), and has been shown to be successful in the induction of corticosteroid effects on declarative memory (54, 55). Depending on the group to which the participant was (randomly) assigned he received either the first capsule containing placebo, the second containing placebo (placebo group); the first capsule hydrocortisone, the second capsule placebo (slow CORT group); or the first capsule placebo, the second capsule hydrocortisone (rapid CORT group).

n-back task. At about 4.5 h after arrival participants were taken to the scanner room where they were asked to conduct an n-back task. Using a blocked design, participants completed eight cycles of alternating 0-, 1-, 2-, and 3-back conditions, interleaved by a short fixation period (2.4 s) (Fig. 2). Within each block, a pseudorandom digit sequence (no more than two repetitions) consisting of 12 single digits was shown to participants. Each digit was presented for 400 ms, followed by an interstimulus interval of 1400 ms. Each block started with a 2-s cue presentation indicating the 0-, 1-, 2-, or 3-back condition, resulting in an interblock interval of 26 s. Blocks were presented in a mirrored design avoiding covariation with linear drift. During the 0-back condition, participants were asked to decide whether or not the current item had appeared one position back in the sequence. Similarly, in the 2- and 3-back condition, participants were instructed to detect whether the current item had appeared two or three positions back, respectively. Each sequence contained either two or three targets, and participants were asked to make a button press with their right index finger as fast as possible when detecting a target. To ensure proper understanding and sufficient performance, participants practiced each condition twice earlier that day outside the MRI scanner (at $t = 240$ min) and twice inside immediately before the actual task ($t = 0$ min).

Physiological and Psychological Measures. Salivary cortisol measure. Cortisol levels were measured from saliva at 10 time points (Fig. 1): baseline measurements twice at the beginning of the experiment ($t = −255$ and $−240$ min), and eight samples ($t = −210, −180, −150, −30, 0, 30, 60, and 90$ min) to assess cortisol changes throughout the experiment. Saliva was collected using a commercially available collection device (Salivette, Sarstedt).

Heart rate. Cardiac rhythm of the participants was measured during scanning using a pulse oximeter placed on their left index finger. Participants were instructed to keep their hands as still as possible during the measurement. Heart rate frequency was calculated using in-house software.

Mood state. Mood state was assessed using the POMS questionnaire (20–22) at three time points: at the beginning of the experimental day ($t = −255$ min), just before entering the fMRI scanner ($t = −30$ min), and just before departure ($t = 90$ min).

Physiological and Psychological Statistical Analysis. Behavioral and physiological data were analyzed in SPSS 15.0 (SPSS, Inc) using mixed-model ANOVAs with WM load (3 vs. 2 vs. 1 vs. 0-back) as within-subject factor and CORT manipulation (placebo vs. slow CORT vs. rapid CORT) as between-subjects factor. Participant age was included as covariate. Due to the high levels of skewness and kurtosis of the POMS questionnaire (20–22), mood scores were analyzed using nonparametric tests (Friedman test). The two measures of working memory performance, accuracy and reaction times, were analyzed both separately and combined as one overall WM performance measure using Stouffer’s z-score method (65). This method first applies a z-transformation to both independent variables and subsequently combines them (here by subtraction) into one overall z-score. Alpha was set at 0.05 throughout.

fMRI Data Analysis. Data were analyzed using Statistical Parametric Mapping software (SPM; University College London), following standard preprocessed procedures (SI Materials and Methods), data were analyzed using a general linear model, in which individual events were modeled based on drug condition and working memory load (1-, 2- or 3-back contrasted vs. 0-back (baseline)). Regressors were temporally convolved with the canonical hemodynamic response function of SPM. The six covariates corresponding to the movement parameters obtained from the realignment procedure were also included in the model. To reduce unspecific differences between scan sessions, global normalization using proportional scaling was applied. The single-subject parameter estimates from each session and condition obtained from the first-level analysis were included in subsequent random effects analyses. For the second-level analysis, a factorial ANOVA was used, with working memory load (1-, 2-, 3-back) as within-subject factor, drug condition (placebo vs. slow CORT vs. rapid CORT) as between-subjects factor, and participant age as covariate.

Given strong neurophysiological evidence for the locus of corticosteroid receptors (5) and its involvement in WM processing (18), the DLPCF was a region of interest. Data concerning this region was corrected for reduced search volume through an anatomical mask as defined by the WFU PickAtlas Tool, version 2.4 (bilateral middle frontal gyrus). A threshold of $P < 0.05$, familywise error whole-brain corrected, was applied to all other regions.

ACKNOWLEDGMENTS. This work was supported by Grant 021.002.053 from The Netherlands Organization for Scientific Research.

Supporting Information

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SI Materials and Methods

Participants. To ensure stable effects of hydrocortisone over all participants, women were excluded from participation. Women are known to display HPA axis reactivity different from that in men, exhibiting smaller and more variable cortisol responses to stress (1), depending on menstrual cycle phase and use of hormonal contraceptives (2, 3). Furthermore, individuals who met any of the following criteria were excluded from participation: history of head injury; autonomic failure; history of or current psychiatric, neurological, or endocrine disorders; current periodontitis; acute inflammatory disease, acute peptic or duodenal ulcers; regular use of corticosteroids; treatment with psychotropic medications, narcotics, β-blockers, steroids, or any other medication that affects central nervous system or endocrine systems; medical illness within the 3 weeks before testing; self-reported mental or substance use disorder; daily tobacco or alcohol use; regular night shift work; or current stressful episode or major life event. Moreover, volunteers with high scores on depression [score >8 on the Beck Depression Inventory (4)] were excluded from participation.

Procedure. Before arrival. Before inclusion all eligible participants received an extensive information brochure, listing all inclusion and exclusion criteria and roughly explaining the setup of the experiment. If criteria were met (according to the participant’s own insights), an appointment was made. To minimize differences in baseline cortisol levels, participants were instructed not to use any recreational drugs for 3 days, and to refrain from drinking alcohol, exercising, and smoking for 24 h, before the appointment. Furthermore, participants were requested not to brush their teeth, floss, or eat and drink anything but water for 1 h before the session, enabling adequate saliva sampling for cortisol assessment. They were asked to take a light lunch and do so no later than 1 h before arrival; their lunch could not contain any citrus products, coffee, tea, milk, or sweets (5). Throughout the entire study period, participants were given only water to drink, except for a scheduled lunch at 150 min before scanning. Scanning. At ~4.5 h after arrival, participants were taken to the scanner room and the procedures were explained. Participants lay supine in the scanner and viewed the screen through a mirror positioned on the head coil. They were asked to lie as still as possible, to keep their eyes open, and to look directly and continuously at the center of the screen in front of them.

fMRI Data Preprocessing. The first five EPI volumes were discarded to allow for T1 equilibration. Before fMRI analysis, the images were motion corrected using rigid body transformations and least sum of squares minimization. Subsequently, the images were temporally adjusted to account for differences in sampling times across different slices. All functional images were then coregistered with the high-resolution T1-weighted structural image using normalized mutual information maximization. The anatomical image was subsequently used to normalize all scans into MNI152 (Montreal Neurological Institute) space. All functional images were resampled with a voxel size of 2 mm isotropic. Finally, all images were smoothed with an isotropic 8-mm, full-width-at-half-maximum Gaussian kernel to accommodate residual functional/anatomical variance among subjects.

Salivary Cortisol Measure. Saliva was collected using a commercially available collection device (Salivette, Sarstedt). For each sample, the participant first placed the cotton swab provided in each Salivette tube in his mouth and chewed gently on it for 1 min to produce saliva. The swab was then placed back in the Salivette tube, and the samples were stored in a freezer at ~25 °C until assayed. Laboratory analyses were performed at the Department of Biopsychology, Technical University, Dresden, Germany. After thawing, salivettes were centrifuged at 3,000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Salivary free cortisol concentrations were subsequently measured using a commercially available chemiluminescence immunoassay with high sensitivity of 0.16 ng/mL (IBL).

Brain Activation Maps. Visualizations of activations were created using MRIcron (http://www.sph.sc.edu/comd/rorden/mricron/) by superimposing statistical parametric maps thresholded at $P < 0.001$ uncorrected (unless specified otherwise) onto a canonical T1-weighted image in standard MNI152 space.

Fig. S1. Brain activation related to WM processing. (A) The n-back task activated a widespread set of brain regions in the bilateral prefrontal cortex (including the DLPFC), bilateral inferior parietal cortex, inferior occipital lobe, and cerebellum. (B) Regions deactivated by WM processing were the default mode network, including the posterior cingulate cortex, ventral medial PFC extending into the orbitofrontal cortex, and medial temporal lobe. Statistical parametric maps are familywise error corrected. Table 2 provides exact coordinates and values.

Fig. S2. n-Back task containing a 0-, 1-, 2-, and 3-back condition. Participants were instructed to press a button when the currently viewed number was identical to the one that they had seen n numbers before. For the 0-back condition, they were asked to press whenever they saw a numeral 1. All participants completed eight blocks of each condition, with every sequence consisting of 12 digits with either two or three targets.