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**Abstract**

Cortisol’s effects on memory follow an inverted U-shaped function such that memory retrieval is impaired with very low concentrations, presumably due to insufficient activation of high-affine mineralocorticoid receptors (MR), or with very high concentrations, due to predominant low-affine glucocorticoid receptor (GR) activation. Through corresponding changes in re-encoding, the retrieval effect of cortisol might translate into a persistent change of the retrieved memory. We tested whether partial suppression of morning cortisol synthesis by metyrapone, leading to intermediate, circadian nadir-like levels with presumed predominant MR activation, improves retrieval, particularly of emotional memory, and consistently changes the memory. In a randomized, placebo-controlled, double-blind, within-subject cross-over design, 18 men were orally administered metyrapone (1 g) vs. placebo at 4:00 AM to suppress the morning cortisol rise. Retrieval of emotional and neutral texts and pictures (learned 3 days earlier) was assessed 4 h after substance administration and a second time one week later. Metyrapone suppressed endogenous cortisol release to circadian nadir-equivalent levels at the time of retrieval testing. Contrary to our expectations, metyrapone significantly impaired free recall of emotional texts (p < .05), whereas retrieval of neutral texts or pictures remained unaffected. One week later, participants still showed lower memory for emotional texts in the metyrapone than placebo condition (p < .05). Our finding that suppressing morning cortisol to nadir-like concentrations not only impairs acute retrieval, but also persistently weakens emotional memories corroborates the concept that retrieval effects of cortisol produce persistent memory changes, possibly by affecting re-encoding.

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1. Introduction

Cortisol is a potent modulator of memory, which differentially affects processes of encoding, consolidation, and retrieval (de Quervain, Aerni, Schelling, & Roozendaal, 2009; Kelemen, Bahrendt, Born, & Inostroza, 2014; Schwabe, Joels, Roozendaal, de Quervain, Aerni, Schelling, & Roozendaal, 2009; Kelemen, Rothfischer, Reichwald, & Hautzinger, 2005; Kuhlmann, Piel, & Wolf, 2005). Generally, it enhances encoding of information, but impairs memory retrieval, especially of negative material. Of note, memory retrieval is not only impaired at strongly elevated cortisol levels (de Quervain, Roozendaal, & McGaugh, 1998; de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Domes, Rothfischer, Reichwald, & Hautzinger, 2005; Kuhlmann, Piel, & Wolf, 2005), but also at minimum levels after suppression of cortisol synthesis by metyrapone (Marin, Hupbach, Maheu, Nader, & Lupien, 2011; Rimmele, Meier, Lange, & Born, 2010), suggesting an inverted U-shaped function that describes the relationship between memory retrieval and cortisol concentrations (Schilling, Kolsch, Larra, Zech, Blumenthal, Frings, & Schachinger, 2013). The inverted U-shaped response function has been linked to an imbalance in mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) activation with both enhanced GR activation at high cortisol levels and reduced MR activation at very low cortisol levels mediating impairing effects on retrieval. In line with this notion, administration of metyrapone at a dose of 3 g almost completely suppressed endogenous cortisol release, and this was accompanied by a significantly impaired free recall of texts and pictures, in particular when emotional (Rimmele et al., 2010). Thus, optimal memory retrieval is expected when MRs are occupied to a great extent, but not GRs, i.e. conditions presumably

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achieved during the circadian nadir of cortisol release (de Kloet, Oitzl, & Joels, 1999; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007).

Besides an acutely impairing effect on retrieval, there is first evidence that the impairing effect of metyrapone on memory retrieval persists beyond the acute period of cortisol suppression and is still present at a second retrieval test 4 days later (Marin et al., 2011).

That study showed a persisting decrease of emotional, but not neutral memories for pictures with 1.5 g of metyrapone, but not with 0.75 g of metyrapone given before the first retrieval of the test materials. Salivary cortisol measures indicated that a significant suppression of cortisol was achieved only after the 1.5 g dosis.

Here, we tested the effect of metyrapone-induced cortisol inhibition during the morning hours on acute retrieval and the persistence of this effect over an even longer 1-week interval. Adopting the framework of an MR/GR activation balance that determines the direction of glucocorticoid effects, we chose a dose of 1 g metyrapone. Based on pilot studies and our previous work (Rimmele et al., 2010), this dose was expected to only partially block cortisol release and to induce cortisol levels comparable with those during the circadian nadir of pituitary-adrenal activity where MRs are estimated to be occupied by 50–70%, in the absence of substantial GR occupation (Kalman & Spencer, 2002; Reul & de Kloet, 1985; Spencer, Miller, Moday, Stein, & McEwen, 1993). We expected that such predominance of MR over GR occupation would acutely enhance memory retrieval. Assuming that the effect on retrieval goes along with a parallel effect on re-encoding, we further expected that the acute enhancement in retrieval after metyrapone would persist during a second retrieval test 1 week later.

2. Methods

2.1. Participants

Eighteen healthy native German-speaking men (mean age 22.17 ± 2.50 years; mean body mass index 22.92 ± 1.65 kg/m²) participated in the double-blind, within-subject cross-over study, which was approved by the local ethics committee. Subjects provided informed consent and were paid for participation.

2.2. Procedure and memory tasks

Each participant was tested in two conditions (metyrapone vs. placebo), separated by an interval of at least 12 days, with the order of conditions balanced across subjects. Each condition included a learning session and two retrieval sessions (Fig. 1A). In the learning session (9:00–11:00 AM), participants memorized emotional and neutral texts (Wagner, Degirmenci, Drosopoulos, Perras, & Born, 2005) and pictures (Lang, 1999). Substance administration took place at 4:00 AM before the first retrieval session (between 7:45 AM and 9:00 AM, 3 days after the learning session). To this end, subjects slept in the laboratory (lights off at 11:00 PM) and were shortly awakened for oral administration of either metyrapone (1 g, Novartis Pharma, Switzerland, half life in plasma 20–120 min) or placebo. Retrieval of the texts took place between 7:45 AM and 8:30 AM, and retrieval of the pictures between 8:30 AM and 9:00 AM. Cortisol, ACTH, epinephrine and norepinephrine levels were assessed repeatedly in blood sampled at 2:00 AM and 3:30 AM, and following substance administration every 30 min from 4:30 AM until 10:00 AM. The second retrieval session took place one week later in the afternoon (2:00–4:00 PM).

For assessment of text memories in each of the two learning sessions, participants were instructed to read one emotional and one neutral text, which were printed on a sheet of paper, thoroughly within 4 min (abundant time to complete the readings) and to memorize as many details as possible for later recall. The order of the experimental texts within a session and the order of parallel versions on the subject’s two test occasions were balanced across subjects. Immediately after learning, participants wrote down the previously read text as exactly as possible in order to obtain a measure of the original encoding level. Free recall was assessed in the same way in the first retrieval session 3 days (after pill administration) as well as in the second retrieval session 10 days (without any pill administration) after learning. Assessment of memory performance was based on the number of correctly recalled content words. Validity of this measure has been confirmed in previous experiments (Schuerer-Necker, 1994).

For assessment of picture memory in the learning session, participants were instructed to memorize 50 negative and 50 neutral pictures. Following the presentation of each picture for 4 s, using the Self Assessment Manikin (1, highly positive; 9, highly negative; 1, very much arousing; 9, not at all arousing) (Bradley, Greenwald, Petry, & Lang, 1992; Lang, 1999), emotional and neutral pictures were rated significantly different on valence (average rating – emotional 5.92 ± .26; neutral 4.27 ± .18; f(14) = 6.24, p < .001) and arousal (emotional 5.10 ± .46; neutral 6.79 ± .34; f(14) = 5.89, p < .001). Arousal and valence ratings did not differ between the parallel versions (all p > .19). The two sets were counterbalanced across the metyrapone and placebo condition. Immediately after encoding, during the first retrieval session 3 days (after receiving placebo or metyrapone) and the second retrieval session 10 days after learning (without pill administration), participants’ free recall was assessed by asking them to list, for each picture recalled, as many details as they could remember with no time constraint.

2.3. Psychological control variables

At the beginning of the retrieval sessions, attention, mood, calmness, wakefulness, and working memory were assessed using the d2 letter cancellation test (Brickenkamp & Zilm, 1998), the Positive and Negative Affect Scale (PANAS) (Watson, Clark, & Tellegen, 1988), the Multidimensional Mood Questionnaire (Steyer, Schwenkmezger, Notz, & Eid, 1997), and the Digit Span subtest (forward, backward) of the Wechsler Adult Intelligence Scale (Wechsler, 1981). Additionally at the end of the retrieval sessions, working memory was assessed with the Sternberg task as previously described (Lupien, Gillin, & Hauger, 1999).

2.4. Hormonal measures

Blood samples were immediately centrifuged and stored at −80 °C until assay. Cortisol and ACTH concentrations were assessed using Immulite (Siemens Medical Solutions Diagnostics, Los Angeles, CA; sensitivity 2 μg/dl for cortisol, 9 pg/ml for ACTH). Plasma epinephrine (E) and norepinephrine (NE) were assessed with standard high-performance liquid chromatography (ChromSystems, Munich, Germany; sensitivity 15 pg/ml for E and NE). Interassay coefficients of variation for all assays were <10%. E levels were mostly below detection threshold and are not reported.

2.5. Data analyses

Two independent raters, blind to treatment, quantified written free recall. Statistical analysis was based on analyses of variance (ANOVA) with repeated-measures factors for ‘treatment’ (metyrapone vs. placebo) and ‘session’ (‘learning session vs. 1st retrieval session, and 1st vs. 2nd retrieval session, respectively) and for the memory variables, the additional factor ‘emotionality’ (neutral vs. emotional). For hormone levels, analyses included repeated-measures factors ‘treatment’ and ‘time of measurement’. Where appropriate, Greenhouse-Geisser corrections of degrees of freedom were used. Significant ANOVA effects were specified by
pairwise contrasts using $t$ tests. The significance level was set to $\alpha < .05$. Four participants did not come to the last session.

### 3. Results

#### 3.1. Memory for texts and pictures

Immediate free recall of texts at the end of the learning session, and before any treatment, was closely comparable between conditions ($all \ p > .11$). However, text memory decreased differentially in the metyrapone vs. placebo condition from the learning to the first retrieval session ($session \times treatment$ interaction: $F(1,17) = 4.80; \ p < .05$; main effect of session: $F(1, 17) = 29.29, \ p < .001$). Metyrapone acutely impaired free recall of content words of the emotional texts ($34.36 \pm 3.13$ vs. placebo: $41.97 \pm 2.82$ words; $t(17) = 2.78, \ p < .05$), but not of the neutral texts ($p > .32$ for $t$ test; $F(1,17) = 6.39, \ p < .05$ for main effect of treatment, Fig. 2A). At the second retrieval which took place one week later, when the substance had been cleared from the body, participants in the metyrapone condition still showed the memory retrieval impairment for emotional texts ($32.93 \pm 2.89$ recalled words in the metyrapone condition vs. $40.43 \pm 3.25$ in the placebo condition; $t_{(13)} = 2.19, \ p < .05$), but not for neutral texts ($19.00 \pm 2.71$ words after metyrapone and $19.46 \pm 2.41$ words after placebo; $treatment \times emotionality \ F(1,13) = 6.96, \ p < .05$, Fig. 2A). Memory did not change from the 1st to the 2nd retrieval ($all \ p > .58$). More content words were recalled for emotional than neutral texts ($all \ p < .01$).

Similar to memory for texts, the number of recalled pictures and memory for picture details was comparable between treatment conditions during learning ($all \ p > .75$) and decreased from the learning to the first retrieval session (main effect of session: $F(1,17) = 34.06, \ p < .001$ for number of recalled pictures, $F(1,17) = 42.16, \ p < .001$ for details), but were not affected by treatment (for respective session $\times$ treatment interactions $p > .53$, Fig. 2B). Recall of pictures and picture details was comparable between the metyrapone and the placebo condition in the two retrieval sessions for both emotional and neutral pictures (for
3.2. Hormonal measures

At the time of retrieval testing (7:30–9:00 AM), plasma cortisol levels were strongly increased after placebo (15.82 ± 8.1 µg/dl) but suppressed after administration of metyrapone (3.37 ± 0.37 µg/dl; t(16) = 16.47, p < .001; Fig. 1B). At this time, levels of ACTH were distinctly higher in the metyrapone (178.22 ± 21.58 pg/ml) than in the placebo condition (31.21 ± 2.58 pg/ml; t(16) = 6.97; p < .001). Plasma norepinephrine did not differ between treatments (p > .62), but increased similarly in the placebo and metyrapone condition from 2:00 AM to 10:00 AM (F(1,95, 33.07) = 57.76; p < .001 for main effect of time).

Because the differential effect of metyrapone on retrieval of texts vs. pictures was unexpected, we more carefully compared cortisol levels during retrieval of texts (7:45–8:30 AM) and retrieval of pictures (8:30–9:00 AM). Indeed, cortisol levels were more strongly suppressed by metyrapone at the time of text recall (3.08 ± 0.34 µg/dl) than during picture recall (4.30 ± 0.44 µg/dl, t(17) = 9.97, p < .001; Fig. 1B), while in the placebo condition cortisol levels were higher during text recall (16.98 ± 0.86 µg/dl) than picture recall (13.14 ± 7.66 µg/dl; F(1,16) = 112.5; t(16) = 8.31, p < .001, p < .001 for treatment x time; Fig. 1B).

3.3. Control variables

Measures of sleepiness after awakening (Stanford Sleepiness Scale) (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973), attention, working memory performance, and reported mood and wakefulness did not differ between treatment conditions (all p > .15). Subjects were not able to correctly identify whether they had received metyrapone or placebo (χ² test; p > .48). No side effects of metyrapone were observed.

4. Discussion

We found that suppression of the morning cortisol rise by 1 g metyrapone impaired free recall of emotional texts, while leaving recall of neutral texts or of emotional and neutral pictures unaffected. This impairment was still evident at a second retrieval test, taking place one week after substance administration.

The present study originated from the assumption that the balance between MR and GR activation determines glucocorticoid effects on memory retrieval (Harris, Holmes, de Kloet, Chapman, & Seckl, 2013; Rimmele, Besedovsky, Lange, & Born, 2013). MRs show distinctly higher affinity to cortisol than GRs and thus are predominantly occupied at times of low endogenous cortisol release, as during the early night when the 24-h cortisol nadir is reached in humans (Reul & de Kloet, 1985; Spencer et al., 1993). In previous studies blocking endogenous cortisol synthesis almost completely with a threefold higher dose (3 g) of metyrapone or directly blocking mineralocorticoid receptors with spironolactone robustly impaired emotional memory retrieval, a finding that we ascribed to insufficient MR activation (Rimmele et al., 2010, 2013). By contrast, GR activation is predominant during times of high endogenous cortisol like during stress. At such high cortisol levels, memory retrieval has been found to be impaired rather than improved. Four reasons might explain this outcome. First, MR might not be involved in upholding memory retrieval. However, although a suppression of cortisol within the normal physiological range similar to the circadian nadir (i.e., to 3–5 µg/dl) was successfully achieved by this 1-g dose, contrary to our prediction, emotional memory retrieval was impaired rather than improved. Four reasons might explain this outcome. Second, MR expression might be up-regulated during the
morning hours compared with expression levels during the early night. In this case, even graded reductions in cortisol during the morning would lead also to a substantial reduction in MR activity with the effect determined by insufficient MR occupation per se rather than by relative predominance of MR over GR as we had hypothesized. Indeed, MR expression has been found to be up-regulated in the beginning of the active phase in rodents although overall knowledge about the circadian MR regulation is scarce (Reul, van den Bosch, & de Kloet, 1987). Against this backdrop, it remains a speculation that predominant MR activation in conjunction with improved emotional memory retrieval had occurred with even more fine-tuned, slighter suppression of cortisol synthesis. Third, while MR occupation contributes to upholding memory retrieval, predominance of MR might not necessarily lead to an enhancement of this function above normal baseline performance. This view is indeed consistent with another study showing diminished retrieval of emotional memory after 1.5 g metyrapone where as 0.75 g remained ineffective (Marin et al., 2011), and it would also fit with the present failure of metyrapone to affect retrieval of pictures as this took place at times, during which cortisol suppression was less effective. Also direct MR agonists (like fluorohydrocortisone) compared to a combined activation of MRs and GRs failed to improve retrieval function in a previous study (Tytherleigh, Vedhara, & Lightman, 2004). Fourth, metyrapone could have influenced memory performance indirectly, for example by increasing the secretion of progesterone and deoxycorticosterone (Krügers, Maslam, Korf, Joels, & Holsboer, 2000). However, actions of these hormones are expected to be in opposite direction on the MR (Funder, 2005; Souque, Fagart, Couette, Davioud, Sobrio, Marquet, & Rafestin-Oblin, 1995) and thus would not explain the current effects.

The second main finding of this study is that the acute impairment in the retrieval of emotional text memories after metyrapone was still present at a second retrieval one week later, i.e., long after the substance had cleared from the system, and any suppression of cortisol synthesis was presumably absent. This finding confirms and extends a previous study showing persistence of retrieval impairment for emotional memories over 4 days (Marin et al., 2011) and can be well explained by metyrapone disrupting re-encoding. Explicit retrieval of a memory is assumed to go along with the formation of a new encoding of the retrieved information, thus effectively enhancing the memory trace (Nadel & Moscovitch, 1997; Roediger & Butler, 2011). Given that cortisol supports new encoding (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003; Buchanan & Lovatto, 2001; Rimmele, Domes, Mathiak, & Hautzinger, 2003), a similar effect on re-encoding does not come as a surprise and is more likely than an effect on reconsolidation following re-encoding, which should happen at a slower time scale after memory reactivation (Nader & Hardt, 2009; Schiller & Phelps, 2011). Yet, we cannot rule out the alternative explanation that the persistence of the memory impairment may be due to the lack of cortisol required for modulation of the reconsolidation process, which would likewise become evident in a subsequent retrieval. Indeed recent studies suggest that cortisol (corticosterone in rodents) via MRs or GRs may play a role not only in consolidation, but also in reconsolidation processes (Akirav & Maroun, 2013; Kruk, Haller, Meelis, & de Kloet, 2013; Taubenfeld, Riceberg, New, & Alberini, 2009).

Considering the importance of re-encoding processes for the long-term retention of memory, the observation of a persistently reduced emotional memory retrieval after suppressing cortisol during retrieval bears important clinical implications. Retrieval in conditions of suppressed cortisol release might be a method to permanently weaken unwanted memories, for example, in patients with posttraumatic stress disorder.

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