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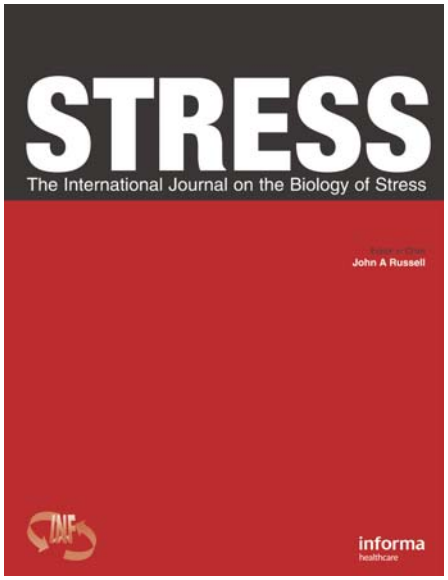

Delayed memory effects after intense stress in Special Forces candidates: Exploring path processes between cortisol secretion and memory recall

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Abstract

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Delayed memory effects after intense stress in Special Forces candidates: Exploring path processes between cortisol secretion and memory recall

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Keywords: *delayed recall, glucocorticoids, immediate recall, multiple mediation, real-life stress, Rey-Osterrieth complex figure*

Testing protocols were submitted to and approved by the standing ethics committee of the Open University, the Netherlands.

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Introduction

Ample studies show that increased cortisol secretion can have various immediate effects on human cognition. Tollenaar et al. (2009), however, demonstrated that a single session of exogenously administered cortisol not only has immediate negative effects, but may also result in delayed memory retrieval deficits (see also De Quervain et al. 1998). With little, if any, information about the persistence of cortisol effects on cognitive performance when evoked by endogenous reactions to naturalistic stressors, the present study investigated delayed cortisol effects, non-intrusively measured in saliva, on memory retrieval after cortisol secretion was triggered by stress exposure to a strenuous Special Forces (SF) selection exercise. Empirical work combining these research features is limited and little is known about the neuroendocrinological path processes between stress exposure and memory retrieval. Yet, the identification of such processes is important because it may lead to the refinement of existing theoretical paradigms (MacKinnon et al. 2008; Spencer et al. 2005). Alternatively, as Rosenberg (1968, p. 63) neatly stated: "In the absence of such mediating or intervening mechanisms, one ends up with facts, but with incomplete understanding".

Cortisol is the principal glucocorticoid in humans and is secreted by the stress-responsive hypothalamic-pituitary-adrenal (HPA) axis, essentially a complex system of direct and indirect feedback mechanisms (Kudielka et al. 2009). Due to specific characteristics, such as its lipophilic structure and molecular size, cortisol readily passes the semi-permeable blood-brain-barrier that functions as a brain-protective interface and possesses various carrier-mediated transport systems for small molecules (Ohtsuki and Terasaki 2007). Subsequently, and in interaction with other transmitter systems, cortisol can modulate memory in various ways (Joëls et al. 2006), which may depend upon the envisaged memory phase. For instance, while elevated cortisol concentrations usually facilitate memory when cortisol is released during the consolidation phase (Andreano and Cahill 2006; Smeets et al. 2008), but see Rimmele et al. (2003), it may impair memory retrieval regardless of the time of the day (Buchanan and Tranel 2008; De Quervain et al. 1998; 2000; Oei et al. 2007; Smeets 2011). The memory impairments seem to be more pronounced under higher levels of acute stress, when the activity level of the sympathetic nervous system is high (De Quervain et al. 2007; Kuhlmann and Wolf 2006). This is consistent with models that emphasize an important role

for noradrenergic activity in the basolateral part of the amygdala (Roozendaal and McGaugh 2011).

Furthermore, there are indications of an inverted U-shaped relationship between cognitive performance and cortisol secretion (Andreano and Cahill 2006; Joëls et al. 2006; Salehi et al. 2010). While moderate stress levels tend to facilitate cognitive performance, research on military populations has provided consistent evidence that intense psychological distress causes robust endocrinological alterations (Morgan et al. 2000; 2001; 2002) and reduced performance (Morgan et al. 2006; Taverniers et al. 2010; Taylor et al. 2009). McEwen and Sapolsky (1995) attributed the U-shaped relationship between cognitive performance and corticoid secretion to divergent affinities of two nuclear receptors; mineralocorticoid receptors (MRs; high affinity for cortisol) and glucocorticoid receptors (GRs; significantly lower affinity for cortisol). Memory facilitation seems to occur in the situation where MRs are fully and GRs are only partially saturated with glucocorticoids. It is only when GRs are fully occupied that a decline in memory performance is observed (Abercrombie et al. 2003). While there is some evidence of the primary role of GRs or even the MR/GR ratio (Roozendaal and McGauch 2011), both animal and human research confirm the presence of dense concentrations of GRs in specific areas of the brain (Patel et al. 2008; Perlman et al. 2007). Hitherto, however, no studies provided statistical support for the above pictured processes that represent the path processes between stress exposure and memory retrieval, respectively via cortisol reactivity and/or subjective stress.

Tollenaar et al. (2009) investigated immediate and prolonged effects of a single dose of 35 mg cortisol on memory retrieval of emotional and neutral information. They found that exogenously administered cortisol causes significant memory impairments shortly after cortisol administration and demonstrated that this effect was not abolished after a recovery or (passive) wash-out period of 1 week. Similarly, Tollenaar et al. (2008a) found impairing effects of cortisol on long-term (6 months) memory retrieval after acute psycho-social stress (Tollenaar et al. 2008b). Tollenaar at al. (2008a) proposed that long-term memory effects could be related to diminished rehearsal and re-encoding under the influence of cortisol, thereby weakening the non-retrieved memory traces. Research in rodents, however, suggested reconsolidation – the renewed consolidation after memory traces pass a labile period during which they are prone to changes – as a possible mechanism behind delayed memory effects (e.g. Debiec et al. 2006) and that the glucocorticoid system can affect the reconsolidation

mechanism of (avoidance) memory (Tronel and Alberini 2007). In human research, Hupbach and colleagues (2007) demonstrated reconsolidation mechanisms and labile declarative episodic memories after subtle reminders triggered integration of new information. To our knowledge, there is currently no information about the persistence of these effects on visuo-spatial declarative memory after exposure to extreme stress, except for the study by Morgan et al. (2006), which registered impaired delayed visuo-spatial memory retrieval 6 h after extreme military training. The authors introduced the Rey-Osterrieth complex figure (ROCF), but they neither focused on delayed effects nor included psychoneuroendocrinological correlates.

Extending the combined work of Morgan and Tollenaar and their respective colleagues, the current field experiment examined immediate and delayed effects of cortisol reactivity under intense real-life conditions. Ongoing SF selection programs provide ideal opportunities to ethically conduct this type of research in healthy men. Given that the SF stressor neatly matched Lupien's (2009) four situational characteristics that trigger cortisol secretion (i.e, Novelty, Unpredictability, Threat to the ego, and loSs of control; NUTS), excessive cortisol increases were expected for the stress group. Subsequently, the elevated cortisol concentrations were assumed to negatively affect immediate and delayed visuo-spatial recall capacities (after a 24 h recovery period; Morgan et al. 2006; Taverniers et al. 2010; Tollenaar et al. 2008a; 2008b; 2009).

The study further hypothesized that the effects of intense naturalistic stress on memory would be mediated through cortisol secretion. To investigate this, the path processes between stress exposure and memory retrieval (direct or indirect via cortisol reactivity and/or subjective stress) were examined with a distribution-free multiple mediation (MM) procedure. A brief explanation of the applied procedure and its rationale is considered appropriate to interpret the findings (Preacher and Hayes 2008). Henceforth, it is important to note that, where 'direct' of 'indirect' effects are discussed, a distinction has to be made between a methodological/statistical emphasis (see further) and a neuropsychological one. Importantly, the statistical emphasis reflects by no means the idea of a direct effect that cortisol would have on cognitive performance, as these are influenced by a complex interplay between diverse transmitter systems.

With regard to statistical analyses, MacKinnon (2008) argues that strictly significant two-variable relationships (e.g. bivariate correlations, *t*-tests, and ANOVAs) are a methodologically necessary, though insufficient condition to demonstrate causality.

Moreover, such statistics are unable to explain how, or via which path(s), effects occur. Path identification implies the idea of mediation analysis and MM analyses are straightforward extensions of single mediator models (MacKinnon et al. 2007). MM analyses, however, contribute important additional advantages such as: (1) the reduced risk of parameter bias due to omitted variables; (2) the exploration of the significance of overall indirect effects; (3) the determination of the impact of *specific* mediators – under condition of the presence of other mediators; and (4) the possibility to compare competing theories within a single research model. Given the multidimensional characteristics of stress effects, these are important advantages because, while a MM procedure arithmetically computes all variables separately, the procedure inherently acknowledges their theoretical and/or practical relatedness and this in the presence of other potentially mediating variables. Evidently, the risk of omitted variables can never be excluded and the model can only perform analyses according to the introduced data.

While earlier techniques for mediation analysis were often "somewhat arcane", Preacher and Hayes (2008, p. 881) proposed a computer-intensive asymptotic and nonparametric resampling strategy (i.e., the product approach and bootstrapping) that (1) is straightforward, (2) does not require a normal data distribution and accepts skewness, and (3) admits bivariate independent variables. These characteristics render it ideal for experimental research with often small sample sizes. Figure 1, Panel A represents a two-variable relationship between the independent variable (X) and the dependent variable (Y) via a single path (c). Panel B shows the MM model for indirect effects between stress (X) and ROCF outcomes (Y), via the potentially mediating variables cortisol (M_1) and subjective stress (M_2), and the respective path indices (c', a_i, b_i).

[Insert Figure 1 about here]

Method

The experiment was embedded within the standard annual Belgian SF selection procedure and stress was evoked in a mock prisoner of war exercise identical to that in Taverniers et al. (2010). The precise context is restricted by confidentiality and only scientifically relevant information is provided. Testing protocols were submitted to and approved by the standing ethics committee of the Open University of the Netherlands. All procedures were carried out according to the Helsinki Declaration's requirements and in full understanding, with both written and oral consent, of the participants.

Participants

Participants were 24 healthy, physically fit males with normal body mass index (Mujica-Parodi et al., 2008). Ages ranged from 21 to 35 years (M = 27.04 years, SD = 4.09). All were active duty Belgian Armed Forces members and recruitment followed as they volunteered as SF candidates. It was explained to participants that participation was voluntary and that accepting or rejecting the request to participate would by no means, positively or negatively, affect the selection result. Before the selection week, participants were medically tested, and on location assessed for endocrine disorders and the use of medication. Underscoring the strenuousness of the selection application, of the original 40 SF volunteers 16 dropped-out of the selection process before the delayed visuo-spatial recall test started. Data from these candidates were not used for analyses.

Measures and materials

Saliva sampling and cortisol analyses. Salivary cortisol is a valid, reliable, and non-invasive index of unbound fractions of cortisol in the blood (Kirschbaum and Hellhammer, 1989; 1994; Nicolson, 2008). Salivary samples were collected with pre-numbered cotton roll devices (Salivette®; Sarstedt, Etten-Leur, the Netherlands) and stored at -20°C immediately after collection. Subsequently, the samples were thawed and centrifuged at 21.1g 4°C for 5 min at the Dresdner Technical University LabServices. Salivary free cortisol concentrations were analyzed using a commercial chemiluminescence immunoassay (IBL, Hamburg, Germany). Mean intra- and inter-assay coefficients of variation were typically less than 8% and 12%, respectively, and the lower and upper detection limits were 0.015 µg/dl (0.41 nmol/L) and 4.0 µg/dl (110.4 nmol/L), respectively.

Subjective stress. Subjective stress was assessed by the National Aeronautics and Space Administration (USA) (NASA) Task Load Index (TLX; Hart and Staveland 1988), a multi-dimensional rating scale that combines information about the magnitude of six independent task load-related subscales (mental demands, physical demands, time demands, own performance, effort, and frustration). The TLX is considered a highly sensitive assessment technique and has often been used in military research (Rubio et al. 2004). Total scores were obtained by summing raw scores of the six subscales that ranged from 0 to 20.

Rey-Osterrieth complex figure. The Rey-Osterrieth complex figure (ROCF) is a standardized neuropsychological test for the evaluation of non-verbal abilities, memory recall, attention, planning, and working memory (Knight and Kaplan 2003). The complexity of the ROCF stems from 36 different elements that are difficult to memorize verbally. While children usually apply a piecemeal approach to copy and recall, most adults use a more holistic, configurational approach. Whereas the traditional starting point of the test, the copy phase, essentially assesses visuo-constructive ability, the immediate recall paradigm permits the assessment of visuo-spatial abilities within declarative memory (Shin et al. 2006). The delayed recall paradigm, by contrast, allows for the assessment of delayed effects and the computation of visuo-spatial memory decline (Lezak et al. 2004). A computerized version of the ROCF was presented in black-on-white for 45 seconds and with a size-on-screen of 12x8 cm. Given the risk of a ceiling-effect with healthy and highly motivated SF candidates – essentially due to the known low variability of the ROCF copy scores in healthy subjects (Shin et al. 2006), the copy phase was omitted and participants were only offered 45 min on-screen visual access to the ROCF. Subsequently, they had three minutes for immediate recall. After a recovery period of 24 h, the ROCF test for delayed effects was delivered in group (in a classroom), according to the unintentional, single trial, delayed recall protocol (Shin et al. 2006). For both the immediate and the delayed memory effects, the ROCFs were scored double-blind, applying the Denman scoring system (DnSS; Knight 2003). Compared to other quantitative ROCF scoring systems, the DnSS provides a larger scoring range that extends from 0 (theoretical minimum) to 72 (maximal score; 2 points per element). Higher scores represent better memory recall performance.

Control measures. The degree to which the HPA axis is activated during stressful events can show considerable individual variation depending upon character issues and life-history events (Kudielka et al. 2009). Three control measures for individual differences were

considered to be of interest in the current situation: [1] The 22-item impact of event scale-revised (IES-R; Weiss and Marmar 1997; translated and back-translated), probably the most widely used self-report measure in the field of traumatic stress impact that assesses the potential risk of developing post traumatic stress disorder (PTSD). The IES-R was chosen for its good psychometric qualities as well as for its sensitivity to detect lower symptom levels (Creamer et al. 2003). Answering possibilities ranged from *not at all* (0) to *extremely* (4). Cronbach alpha was 0.85 (Intrusions: 0.80, Hyper-arousal: 0.43, and Avoidance: 0.74). [2] The 15-item dispositional resilience scale (DRS15-R; translated and back-translated) for personality hardiness (Bartone 2007), a personality aspect that provides a natural advantage in stressful circumstances and that is associated with increased outcome performance in stress research with, amongst others, SF candidates (Bartone et al. 2008; Eid and Morgan 2006). Answering possibilities ranged from *not at all true* (0) to *completely true* (4). Cronbach alpha was 0.60 (Commitment: 0.60, Control: 0.62, and Challenge: 0.55). [3] The Generalized Cognitive Test Battery (GCTB), a standard issued Belgian Armed Forces cognitive ability test (Irvine 2006). The GCTB assesses cognitive performances in five domains, collated to one general factor for cognitive ability that ranges from 0 to 20. No internal consistency measures are available.

Procedure. Prior to the experiment, all participants were physically and psychologically screened according to procedures identical to those described in Taverniers et al. (2010). After arrival at the training centre candidates signed a written informed consent form, were instructed to remove all external identification marks, and received a chest number to increase anonymity. In the course of day one, they completed the IES-R and DRS15-R scales. Participants were deprived of food, drinks, smoking, and heavy physical exercise at least 90 min prior to the cortisol measurements. They were not deprived of sleep the night before.

After a group-wise salivary cortisol baseline measurement (T_0, at 18.00 h), participants were randomly assigned to a control (n = 12) or stress (n = 12) condition, and exposed to a no-stress filler task or to SF stress treatment. The SF stress treatment consisted of a strenuous, genuinely unexpected, and uncontrollable mock prisoner of war exercise that lasted 60 ± 5 min. More specifically, participants in the stress group were abruptly and forcefully captured, physically constrained, and subsequently interrogated. To promote similar levels of cognitive load, the control group completed administration tasks and ran non-stressful weapons manipulation tasks during the same timeframe.

After stress exposure, two more saliva samples were taken. Apart from the test for delayed effects, all participants were tested individually according to identical procedures. The time point for the first cortisol measurement (T_1) coincided with the start of the ROCF test (19.35 h for the first participant). The moment for the second cortisol measurement (T_2), at T + 15 min, was based on previous findings that indicated that a period of 15 min coincides with the highest cortisol increases (Joëls et al. 2006; Morgan et al. 2000; 2001; 2002). After running the entire exercise – more practical tests were ran after the ROCF, participants were instructed to complete the TLX, while reflecting on their respective stress exposures. Delayed cortisol effects on visuo-spatial memory were tested in group, in a class room, after a 24 h recovery period, counting from the cortisol baseline measurement. Figure 2 depicts the experimental time-line and provides clock times.

[Insert Figure 2 about here]

Statistical analyses. When appropriate, bivariate correlations analyses, independent samples *t*-tests, and mixed model analyses of variance (ANOVAs) were used to analyze the data (SPSS 16). Peak cortisol responses at different points in time (ΔCORT) were computed as: $\Delta CORT = T_{Peak} - T_{0.}$ If required (in case of excessive skewness), cortisol data (nmol/L) were log-transformed and Greenhouse-Geisser corrected *p*-values are reported when appropriate. Analyses were two-tailed and alpha was set at 0.05. Memory decline was computed as: Memory decline = (Immediate recall – Delayed recall)/Immediate recall*100 (Lezak et al. 2004). In line with procedures described by Preacher and Hayes (2008), MM analyses (for immediate and delayed ROCF outcomes) were performed according to the distribution of the product approach to determine the importance of the direct effect (vs. the total effect), and by bootstrapping resampling procedures [untransformed ΔCORT data; 5,000 iterations; 95% bias corrected and accelerated (BCa) confidence intervals (CI); after MacKinnon et al. (2004)] to assess both the outcome invariance of the specific mediators and the unique most significant mediator.

Results

Group equivalence. Table 1 shows the outcomes of all control measures and that group equivalence was achieved.

[Insert Table 1 about here]

Stress and salivary cortisol responses. Baseline salivary cortisol measurements (all subsequent cortisol measures are expressed in nmol/L) revealed no differences between the groups (two-sided independent samples *t*-test [$t(22) = 0.27$; $p = 0.79$]). The 2 (Group; Control, Stress) x 3 (Time; T_0, T_1, T_2) mixed model ANOVA yielded a significant between subjects main effect of Group [$F(1,22) = 46.12$; $p < 0.001$, partial $\eta^2 = 0.69$], a significant effect of Time [Wilks' $\lambda = 0.47$, $F(2,21) = 12.03$; $p < 0.001$, partial $\eta^2 = 0.53$], and a significant Group x Time interaction effect [Wilks' $\lambda = 0.27$, $F(2,21) = 27.76$; $p < 0.001$, partial $\eta^2 = 0.73$]. The course of cortisol concentrations for both groups, with non-transformed data, is shown in Figure 3.

[Insert Figure 3 about here]

For subjective stress, an independent samples *t*-test on TLX scores after stress exposure yielded a significant difference between the control ($M = 9.17$, $SE = 1.39$) and the stress ($M = 58.92$, $SE = 2.80$) group [$t(16.1) = -15.94$; $p < 0.001$], which qualified the circumstances as being stressful.

Immediate and delayed memory effects. A two-sided independent samples *t*-test revealed significant group differences in mean ROCF scores for the control and the stress group [$t(22) = 3.00$; $p < 0.01$], indicating that immediate ROCF recall performance had significantly deteriorated after intense stress and robust cortisol secretion. Figure 4 further shows that there were delayed stress effects as ROCF recall differences transferred over time. First, the 2

(Group; Control, Stress) x 2 (Time; $ROCF_1$, $ROCF_2$) mixed model ANOVA yielded a significant between subjects effect of Group [$F(1,22) = 9.91$; $p < 0.005$, partial $\eta^2 = 0.31$], a significant effect of Time [Wilks' $\lambda = 0.59$, $F(1,22) = 15.62$; $p < 0.001$, partial $\eta^2 = 0.42$], but no interaction ($p = 0.55$). In sum, the above findings indicate significant delayed effects of endogenous cortisol concentrations on memory performance after a recovery period of 24 h. Computation of memory decline determined that participants belonging to the control and the stress groups forgot at comparable rates of, respectively, 4.51% and 3.51%.

[Insert Figure 4 about here]

Path processes. Prior to the MM analyses, bivariate correlations were calculated among all studied variables, for both groups separately. For the control group, except for the two ROCF measurements [$r(12) = -0.95$, $p < 0.01$], no other significant relationships were found. Table 2 shows the correlations for the intense stress group. The significant correlations in the stress group provide support for the hypothesized relationship between increases in cortisol concentrations and the deterioration of both cognitive outcomes.

[Insert Table 2 about here]

For the immediate ROCF recall, the MM analyses revealed that the total (*c path*) and the direct effects (*c' path*) from X to Y were -8.83 ($p < 0.01$) and 1.58 (n.s.), respectively, while the directions of all *a* and *b* paths were as expected. Thus, the total indirect effect was different from zero (i.e., the combined mediators were the significant contributors to the overall effect). Examination of the specific indirect effects indicated that ΔCORT was the unique significant mediator with a BCa CI ranging from -14.06 to -2.79 (does not contain a zero; Table 3). Similar results were found for the delayed ROCF as the differences between total [*c path*: -8.17, $p < 0.005$] and direct effects [*c' path*: -1.20 (n.s.)] from X on Y differed significantly. Again, all *a* and *b* path-results were directed as expected. Examination of the

specific indirect effects confirmed that ΔCORT was once more the unique significant mediator with a BCa CI ranging from -12.02 to -2.46 (does not contain a zero; see Table 3).

[Insert Table 3 about here]

Discussion

The current study investigated immediate and prolonged effects of cortisol secretion that was evoked by intense naturalistic stress. First, the study confirmed that exposure to stringent military stressors triggers robust cortisol secretion, which significantly impairs immediate visuo-spatial declarative memory recall. These findings, under identical conditions, have been discussed in Taverniers et al. (2010) and accord with work under comparable stress conditions of both Morgan et al. (2000, 2001; 2002) and Taylor et al. (2007). However, by extending the study of Taverniers et al. (2010), the present study demonstrated lasting effects of high endogenously evoked cortisol concentrations on visuo-spatial declarative memory.

These findings are also in line with recent studies by Tollenaar et al. (2009), who administered exogenous cortisol and employed a wash-out period of one week, and with Tollenaar et al. (2008a), who looked at memory performance after an acute laboratory stressor. The latter authors used a 6 months delay. The cognitive findings also accord with those of Morgan et al. (2006) who measured ROCF effects after a 6 h delay, but did not focus on psychoneuroendocrine correlates of visuo-spatial memory effects. Although neuropsychological work has shown more complexity (i.e. the effect of cortisol is influenced by other transmitter systems), the current study introduced a sophisticated method for MM analyses (Preacher and Hayes 2008) and was, to our knowledge, the first to mathematically demonstrate the importance of cortisol reactivity as the single most significant mediator, relative to the other variables under consideration, leading to cognitive performance decline (see MacKinnon et al. 2007).

The current results also indicate that the impairing effects of cortisol on memory retrieval originate at an early stage of memory formation; as memory decay seems to remain relatively stable with similar decline rates for those who were and those who were not exposed to stress.

This supports the proposed mechanism of diminished rehearsal and, more specifically, the hampered encoding and re-encoding under the influence of high cortisol concentrations. In the course of that process, non-retrieved memory traces are weakened or lost (Tollenaar at al. 2008a).

While two-variable relationships are, strictly, a necessary though methodologically insufficient condition to determine causal relationships (MacKinnon 2008; Preacher and Hayes 2004; 2008), the current study introduced MM analyses and provided mathematical support for the generally accepted idea in psychoneuroendocrinology that cortisol reactivity *in itself,* rather than the direct intense stress treatment and indirect subjective stress experiences, affects cognitive performance. The statistical revelation of a path process between stress and cognitive processing via cortisol secretion (and not via subjectively experienced stress) is novel, albeit consistent with ample findings from psychoneuroendocrinological research. As far as it concerns the studied variables, the current research replenishes related work by providing statistical support that there was a unique significant indirect effect via cortisol reactivity that caused the decline in memory performance; potentially provoked by divergent corticosteroid affinities of MRs and GRs in the brain (McEwen and Sapolsky 1995) and instigated though saturated GRs in the associated brain areas (Abercrombie et al. 2003).

Limitations

This study evidently has some limitations that need consideration to interpret its findings. First, due to the practical feasibility of inducing severe stress, the number of participants that could be recruited and tested was not high. Nevertheless, there was a significant impairing effect on memory retrieval in the stress group. Second, the study did not measure salivary cortisol concentrations at the time of the delayed ROCF test, 24 h after the stress exposure. Therefore, it cannot be determined for certain whether cortisol levels were back to baseline at that time. Given the participants' activity spectrum (identical for all), hours prior to the delayed ROCF test, one can reasonably assume that cortisol levels were significantly reduced and that the memory impairment was not due to a renewed acute stress effect. In addition, to enable generalizations across sexes, populations that include female participants are desirable in the future. Accordingly, future work should envisage replications on larger and more heterogeneous population samples. Given that stress is a multifaceted phenomenon and an aggregate of a complex interplay of both subjective and objective correlates, it would be desirable to include more psychobiological measurements of stress correlates. Furthermore, it

is important to note that the current MM analyses only involve the variables that were assessed within the study. Omitted variables could play an important practical role in the complex interplay between stress and cognitive performance. From a practical stance, it would be interesting in future research to examine the effects of diverse types of reactivation of memory traces (Hupbach et al. 2007), but also to relate memory dysfunctions to performance tasks that could range from elementary to more complex memory functions. Finally, while the current study mathematically identified cortisol secretion as the principal mediator between stress and memory retrieval in two separate analyses, the latter were strongly correlated (Table 2). Accordingly, further research on genuinely independent databases and, preferably, with more than two potentially mediating variables (e.g. testosterone and/or autonomic stress markers such as alpha-amylase) would be highly recommended to fully identify the path processes between stress and memory recall. Evidently, the MM procedure should be seen as an analytic tool and the study of such analyses would only make sense if the *a priori* defined path model conceptually makes sense.

Future directions

Despite the aforementioned limitations, this study investigated an important, though largely overlooked phenomenon, namely delayed effects of intense endogenously evoked cortisol concentrations on memory, in a well-controlled real-world setting. Moreover, the study performed MM analyses and, hitherto, no such work has been reported in the general field of psychoneuroendocrinology (MacKinnon et al. 2007). In effect, a closer look at the presently applied methodology might offer additional research opportunities. First, the current mediators were significantly correlated. MM effects, however, are often attenuated to the degree to which the mediators are correlated (comparable to the phenomenon of multicolinearity; Preacher and Hayes 2008). Given that psychobiological and subjective strain measures usually do not correlate well (Dickerson and Kemeny 2004), the applied strategy for mediation analyses might open additional possibilities for future work. Secondly, in (multiple) mediation analyses a significant effect could appear even if the *a priori* relationship between the independent and the dependent variable is not significant. This situation could occur when examining opposing mediation processes and when the test of mediated effects has more statistical power than the test of the overall relation between the independent and the dependent variable (MacKinnon 2008; Shrout and Bolger 2002). Finally, Spencer et al. (2005) claim that statistical sophistication and process identification are essential for a psychological

field to fully mature. From a scientific point of view and, given sufficient follow-up research, it is proposed that the demonstrated method could contribute to the refinement of existing paradigms in psychobiological stress research.

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Declaration of interest

The authors declare not to have any conflict of interest that could bias this work.

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Figures

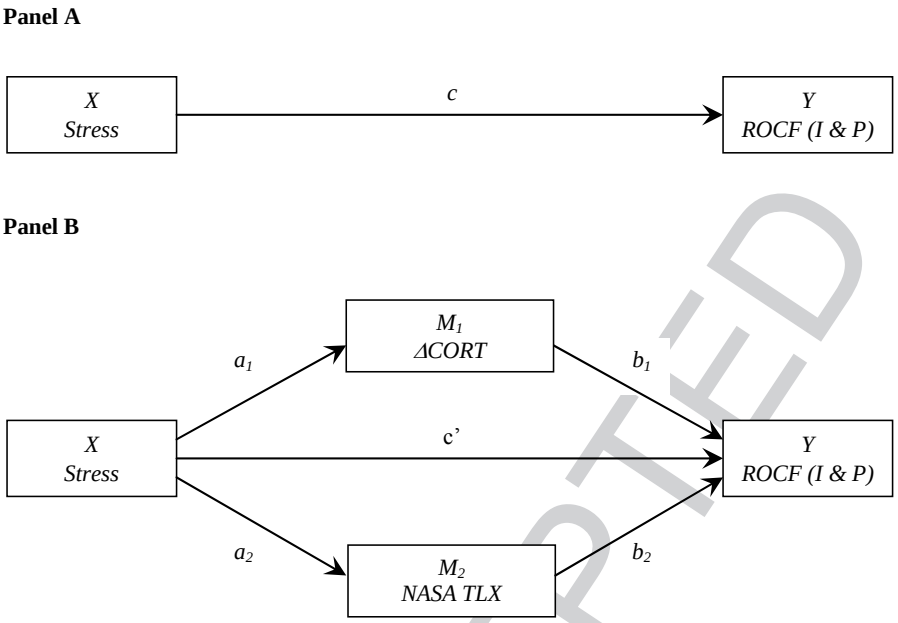

Figure 1. Panel A represents a direct effect of the independent (*X*; stress treatment) on the dependent variables [*Y*; immediate (*I*) and delayed (*P*) ROCF (Rey-Osterrieth complex figure) performance] with a single path index (*c*). Panel B represents a multiple mediation design with similar *X* and *Y*, two mediators (M_1 and M_2), and path indices (a_1, a_2, b_1, b_2, and *c'*). ΔCORT: change in salivary cortisol concentration; NASA TLX: National Aeronautics and Space Administration (USA) Task Load Index.

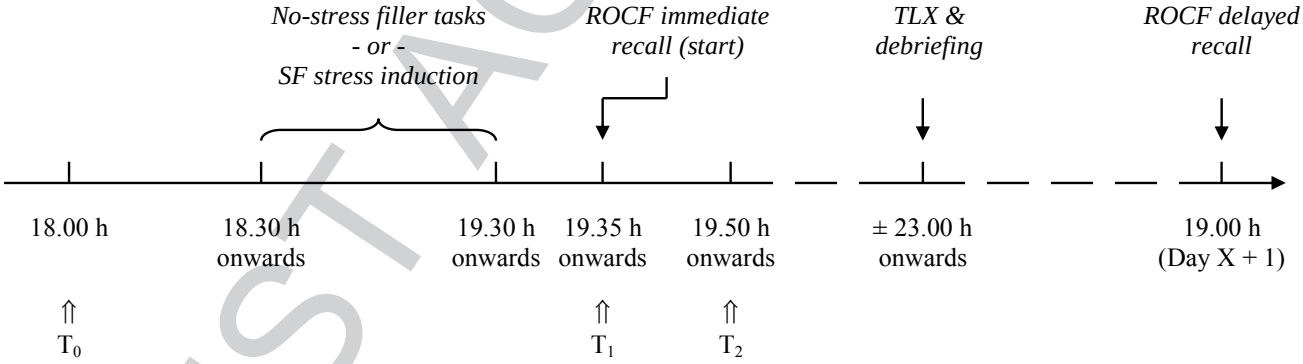

Figure 2. Experimental time-line (clock times; the annotation 'onwards' indicates a sequence of individual assessments with an interval of approximately 10 min) for the control vs. the SF (Special Forces) stress group; baseline saliva sampling (T_0); test instructions, cortisol saliva sampling (T_1), and subsequently Rey-Osterrieth complex figure (ROCF) immediate recall; second cortisol saliva sampling (T_2) at T+15; NASA (National Aeronautics and Space Administration (USA)) Task Load Index (TLX) scoring and debriefing; measurement of delayed effects after a 24 h recovery period. X: stress.

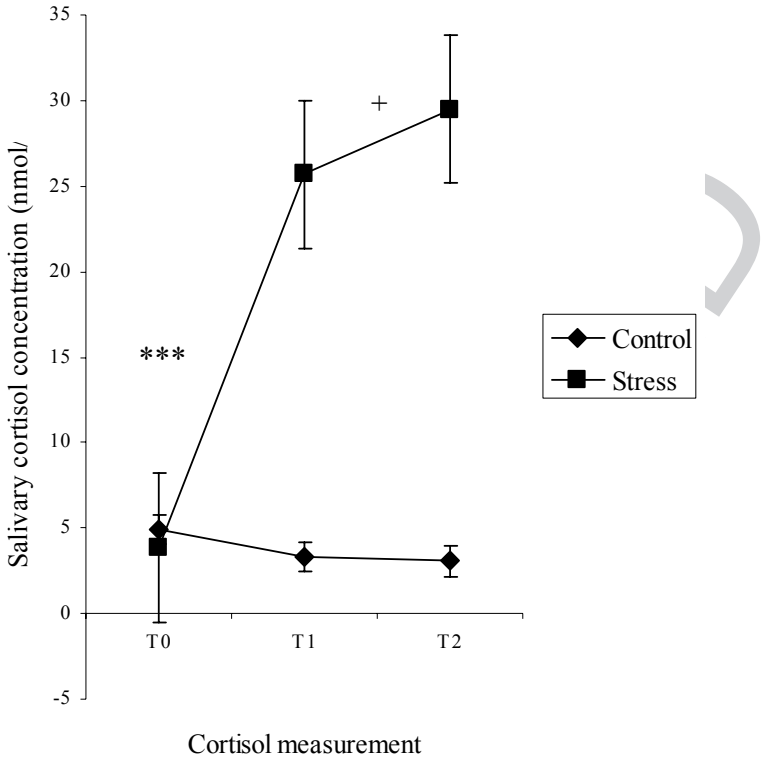

Figure 3. Result of mixed model ANOVA with untransformed salivary cortisol measures ($M \pm 1$ *SE*) for the control ($n = 12$) vs. the SF (Special Forces) stress group ($n = 12$) (Times: T_0 at T-75 min, T_1 immediately post-stress, and T_2 at T+15 min). *** $p < 0.001$; + $p = 0.07$; other differences were not significant.

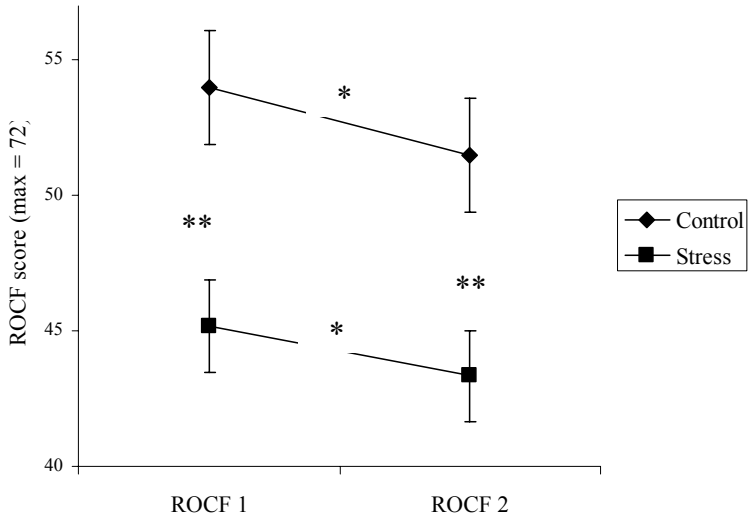

Figure 4. Result of mixed model ANOVA with pairwise comparisons, depicting mean performance scores (± 1 *SE*) for the control ($n = 12$) vs. the SF (Special Forces) stress group ($n = 12$) on the Rey-

Osterrieth complex figure; immediate recall (ROCF 1) and delayed recall (ROCF 2). ** $p < 0.01$; ** $p < 0.05$

Tables

Table 1. Means (*M*), standard errors of measurement (*SE*), and *t*-values for the control measures [impact of event scale-revised (IES-R), the dispositional resilience scale-revised (DRS15-R), and the generalized cognitive test battery (GCTB)] for the control ($n = 12$) and the stress ($n = 12$) group.

	Control group		Stress group		*t**	Significance
	M	*SE*	*M*	*SE*		
Age (year)	27.08	(1.25)	27.00	(1.16)	0.05	n.s.
IES-R	10.42	(2.87)	12.25	(1.86)	- 0.54	n.s.
DRS15-R	36.67	(0.85)	35.33	(0.79)	1.15	n.s.
GCTB	13.73	(0.72)	12.85	(1.11)	0.66	n.s.

* df = 22; n.s.= not significant

Table 2. Results from bivariate correlation analyses for the stress group ($n = 12$) concerning subjective stress (TLX), peak salivary cortisol response (ΔCORT), and both immediate and delayed Rey-Osterrieth complex figure (ROCF) scores.

	Variable	1	2	3	4
1	TLX score	-			
2	ΔCORT	0.19	-		
3	Immediate ROCF recall	-0.29	-0.77**	-	
4	Delayed ROCF recall	-0.19	-0.81**	0.91**	-

** $p < 0.01$

Table 3. Bootstrapping results for outcome invariance, revealing that peak cortisol response (ΔCORT), relative to the direct (total) effect and subjective stress (TLX), is the unique significant mediator between stress treatment and ROCF memory retrieval (N = 24). The table's midsection contains the results from both the immediate and the delayed recall (Immediate | Delayed).

Effect	Bias corrected and accelerated CI		Result
	Lower	Upper	
Total	-26.87 | -21.44	13.89 | 16.18	n.s.
ΔCORT	-14.06 | -12.02	-2.79 | -2.46	*
TLX	-20.36 | -16.22	19.55 | 20.46	n.s.

n.s.: not significant, * Significant [95% bias corrected and accelerated confidence intervals (CI); 5,000 iterations]