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Eyeblink conditional discrimination learning in healthy young men is impaired after stress exposure

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Abstract

Stress is known to influence the hippocampus. Eyeblink conditional discrimination learning is dependent on the hippocampus, but the effects of stress on the task are unknown. Male participants were allocated to a psychosocial stress condition (Trier Social Stress Test) or a control condition. Afterwards, a conditional discrimination task was performed. A tone (the CS) predicted an airpuff (the US) only when preceded by a specific visual stimulus (a red or a green colored square, the S+ and S−). Stressed participants showed a rise in cortisol and an increase in negative affect. Stressed participants also failed to acquire the conditional discrimination. They responded to all of the presented CS irrespective of the preceding occasion setter (S+ or S−). Controls, in contrast, acquired the discrimination rapidly. The present study provides further evidence for an impairing effect of acute stress on tasks relying on the hippocampal formation.

Descriptors: Stress, Cortisol, Conditional discrimination learning, Humans, Hippocampus

Stress leads to a rapid activation of the sympathetic nervous system (SNS), causing the release of (nor)adrenalin from the adrenal medulla. Simultaneously, the hypothalamus-pituitary adrenal (HPA) axis is activated. Corticotrophin-releasing hormone (CRH) and, consecutively, adrenocorticotrophin (ACTH) stimulate the release of glucocorticoids (GCs, in humans mostly cortisol) from the adrenal cortex (Joels & Baram, 2009). These stress messengers can have rapid as well as delayed effects on neural excitability. Influences of stress on the hippocampus have been especially well characterized. Initially, stress causes a rapid excitation (mediated via noradrenalin, CRH, and nongenomic GC effects) of hippocampal neurons (Diamond, Campbell, Park, Halonen, & Zoladz, 2007; Joels, Karst, DeRijk, & De Kloet, 2008; Joels, Pu, Wiegert, Oitzl, & Krugers, 2006). With a short delay, the genomic GC effects then unfold and cause a reduction in neuronal excitability and plasticity (Diamond et al., 2007; Joels et al., 2006, 2008).

A wealth of studies conducted during the past decades has established that these stress mediators can influence learning and memory (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Sandi & Pinelo-Nava, 2007; Wolf, 2008, 2009). Studies in rodents have demonstrated that the effects of stress can be investigated using eyeblink conditioning. The advantage of using such a basic learning paradigm is that the neuronal circuits involved are well understood (see Christian & Thompson, 2003). Numerous studies from Shors's lab have demonstrated that stress influences hippocampal-independent delay conditioning as well as hippocampal-dependent trace conditioning (Shors, 2004). Interestingly, effects on both paradigms rely on an intact hippocampus and amygdala, since damaging one of these structures abolishes the effects of stress (Bangasser & Shors, 2007; Waddell, Bangasser, & Shors, 2008).

In humans, we have recently demonstrated that acute stress induced by the Trier Social Stress Test impairs the acquisition of delay eyeblink conditioning (Wolf, Minnebusch, & Daum, 2009). Other authors have reported on the modulation of trace conditioning (a form of learning in which a short temporal gap occurs between the conditioned stimulus [CS; the tone] and the unconditioned stimulus [US; the airpuff]) by stress and stress hormones. Effects of mild stress and pharmacological manipulations have been reported (Duncko, Cornwell, Cui, Merikangas, & Grillon, 2007; Nees, Richter, Lass-Hennemann, Blumenthal, & Schachinger, 2008; Vythilingam et al., 2006). Moreover, impaired trace conditioning has been observed in patients with chronic endogenous hypercortisolemia (Grillon, Smith, Haynos, & Nieman, 2004).

In contrast to delay and trace eyeblink conditioning, no previous study has investigated the impact of stress on eyeblink conditional discrimination learning (also referred to as occasion setting; Green & Woodruff-Pak, 2000) in the human. This is a complex form of associative learning during which participants have to learn that a CS (e.g., a tone) only predicts an US (the puff of air) when preceded by a specific discriminative stimulus (e.g., a light in a specific color). In contrast, the same CS does not predict the US when preceded by a light in a different color. Previous research has established the crucial role of the hippocampus in this paradigm (Green & Woodruff-Pak, 2000). For example, temporal lobe lesions in humans cause a deficit in conditional discrimination learning (Daum, Channon, Polkey, & Gray, 1991; Fortier et al., 2003). Patients learn the conditioned response, but fail to acquire

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the discrimination. They respond to all of the CSs irrespective of the preceding discriminator. Similar alterations occur during human aging (Bellebaum & Daum, 2004). These findings are in line with the idea that the hippocampus is important for the association of discontinuous items (Wallenstein, Eichenbaum, & Hasselmo, 1998).

The goal of the present study was to investigate the effects of acute stress on eyeblink conditional discrimination learning. Based on the effects of acute stress on the hippocampus reviewed above and based on the crucial role of the hippocampus in eyeblink conditional discrimination learning, we expected that stress would impair conditional discrimination. Moreover, we predicted that the stress-induced cortisol increase would be associated with the behavioral impairment. In addition, we investigated the effects of stress on extinction, which immediately followed the acquisition trials, in an exploratory fashion.

Method

Participants and Design

Thirty-seven young, healthy male university students who were recruited by advertisements participated in this study. We decided to study males only in order to avoid the potential modulatory influence of female sex steroids on HPA reactivity and/or on the effects of stress on the brain. Menstrual cycle-associated changes in gonadal steroids have been shown to modulate HPA activity (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). More importantly, oral contraceptive (OC) usage is associated with a blunted free cortisol response to the public speech stressor used in this study, most likely reflecting the OC-induced increase in cortisol-binding globulin (Kirschbaum, Pirke, & Hellhammer, 1995; Kirschbaum et al., 1999). Psychoneuroendocrine stress studies are thus faced with the problem of having to study freely cycling women only, who are very difficult to recruit at German universities (e.g., Preuß & Wolf, 2009; Schoofs, Preuss, & Wolf, 2008), or to study OC users, knowing in advance that they will show a blunted cortisol response (Cornelisse, van Stegeren, & Joels, 2011). Finally, some memory studies suggest that the sensitivity to glucocorticoids changes over the course of the menstrual cycle (Andreano, Arjomandi, & Cahill, 2008; Schoofs & Wolf, 2009) or with OC usage (Kuhlmann & Wolf, 2005). In the current study, we therefore decided to focus exclusively on males, since funding limitations and time constraints did not allow us to study a sufficiently large sample size in order to address the complicated issue of sex differences with sufficient power.

None of the participants had a history of neurological, psychiatric, or general medical problems, color-vision or auditory deficits, or any prior experience with conditioning studies. A further exclusion criterion was the intake of medications.

Subjects were randomly assigned to the STRESS ($n = 18$) or the CONTROL ($n = 19$) group. Mean age was 25.2 years ($SD = 3.5$) in the STRESS and 25.3 years ($SD = 3.4$) in the CONTROL group; the average body mass index (BMI) was 24.5 kg/m^2 ($SD = 1.6$) in the STRESS and 24.4 kg/m^2 ($SD = 3.2$) in the CONTROL group. The two groups did not differ significantly in those variables. To control for effects of the time of day, the experiment was always conducted between 10 and 12 am. The study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki and was approved by the Ethics Committee of the Medical Faculty of the Ruhr University, Bochum, Germany. Written informed consent was obtained from all participants.

Stress and Control Conditions

Psychosocial stress was induced in the STRESS group by means of the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993), an established procedure that reliably leads to elevated cortisol levels via activation of HPA axis. In the first part of the TSST, the subject is asked to try to convince a "selection committee" (a man and a woman) of his assets for a fictitious job in a 5-min free speech which follows a 2-min preparation period. The "job interview" is videotaped and followed by a 5-min task requiring mental arithmetic (counting backward from 2043 in steps of 17).

The control task completed by the subjects of the CONTROL group entails a 5-min speech about a recently read book or a recently seen movie, a committee is not present, and the performance is not taped. The free speech is also followed by a 5-min mental arithmetic task (counting forward in steps of 15). Unlike the stress condition, the control condition (called the Placebo TSST) lacks the stress-inducing components of the TSST (social evaluative threat and uncontrollability) and does not lead to a significant cortisol increase (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009; Kuhlmann, Piel, & Wolf, 2005).

Conditional Discrimination

After completion of the stress or control condition, subjects underwent an eyeblink conditional discrimination learning task, which has previously been shown to be sensitive to temporal lobe/hippocampal lesions in human patients (Daum et al., 1991) and to aging effects in healthy subjects (Bellebaum & Daum, 2004). In this task, the color of a light starting each trial predicted whether or not the following tone would be accompanied by a corneal puff of air. The task entailed 72 acquisition (divided into 6 blocks of 12 trials) and 12 extinction trials.

Each acquisition block consisted of 8 S+ and 4 S− trials. In an S+ trial, a colored square was shown for 2 s on a computer screen at eye level, followed by a 3-s delay and an 800-ms CS tone (1000 Hz, 70 db) as well as an 80-ms airpuff constituting the US (200 mmHg). The US was administered to the cornea of the right eye from a distance of 1 cm via a nozzle mounted on goggles. US onset was 720 ms after CS onset, both stimuli coterminated. In an S− trial, a different-colored square presented for 2 s was followed by a 3-s interval and a tone identical to the CS tone on an S+ trial, but without an airpuff. For half of the subjects in each group, a red square served as the S+ and a green square as the S-, the other half was presented with the opposite pattern. Both versions lead to comparable conditioning performance (see Bellebaum & Daum, 2004; Daum et al., 1991). The order of trial types was randomized in each block; mean intertrial interval duration was 12 s. After 72 acquisition trials, the 12 extinction trials (8 S+ and 4 S− trials) ensued without interruption or warning. The technical details of stimulus presentation and recording of eyelid movement by means of a photocell system are described in detail elsewhere (Bellebaum & Daum, 2004).

Since insight into the stimulus contingencies influences discrimination performance, a postexperimental interview was conducted after completion of the conditioning task. After initial free recall of the structure of the experiment, the questionnaire assessed knowledge of stimulus relations in a set of structured questions (i.e., "If a green square was shown, was the tone followed by a puff of air?" For details, see Bellebaum & Daum, 2004).

Conditioning Data Analysis

Eyeblink data were analyzed offline using the EEG Analyst software (Daum et al., 1993; Wolf et al., 2009). Latencies and amplitudes of eyeblinks with a minimum amplitude of 0.8 mm were scored (for analysis details, see Bellebaum & Daum, 2004). A CR was defined as an eyelid-closing movement that occurred no earlier than 450 ms after CS onset and no later than US onset. Eyelid closures with latencies between US onset and 160 ms after US onset were scored as URs. UR amplitudes were analyzed for the first block of trials due to a blend of CRs and URs later on during the course of conditioning.

In order to obtain a single parameter for the ability to discriminate between S− trials and S+ trials, a discrimination index was calculated as described in detail elsewhere (Daum et al., 1991). In short, the following formula was used: S+ trial responses / S+ trial responses + 2 × S− trial responses. Reponses in S− trials were multiplied × 2 in order to account for the fact that eight S+ but only four S− trials were presented in each block.

Saliva Sampling and Cortisol Assessment

Saliva was collected using Salivette collection devices (Sarstedt, Germany) before (baseline) and immediately after (+1 min) stress induction/the control condition, immediately before the conditioning procedure was started (+10 min), and after completion of the conditioning session (+40 min). In order to obtain a single parameter integrating the four cortisol measures, an area under the curve (AUC) index was calculated for each participant using the trapezoid formula as described in the literature (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Free cortisol levels were measured using a commercially available chemiluminescence-immuno-assay (CLIA; Immuno-Biological Laboratories [IBL], Hamburg, Germany). Inter- and intra-assay variations were below 10%. Hormone analysis was conducted at the laboratory of Prof. Clemens Kirschbaum at the University of Dresden, Germany.

Affect Measurement

A checklist containing adjectives (Positive and Negative Affect Schedule, PANAS; Watson, Clark, & Tellegen, 1988) was used for affect measurement. The PANAS entails 10 items assessing positive affect (e.g., interested and enthusiastic) and 10 items assessing negative affect (e.g., upset and ashamed). Each item is rated on a 5-point scale. The PANAS was completed upon arrival at the laboratory and again after cessation of the stress or control task.

Statistical Analysis

The conditioning data of the two groups were compared by means of repeated measures analysis of variance (ANOVA), with Greenhouse-Geisser adjustment or orthogonal polynomial decomposition. In addition, planned *t* tests as well as Pearson correlations were conducted.

Results

Cortisol

The cortisol responses are illustrated in Figure 1. An ANOVA with the factors GROUP (STRESS vs. CONTROL) and TIME (baseline, +1, +10, +40) yielded a significant interaction

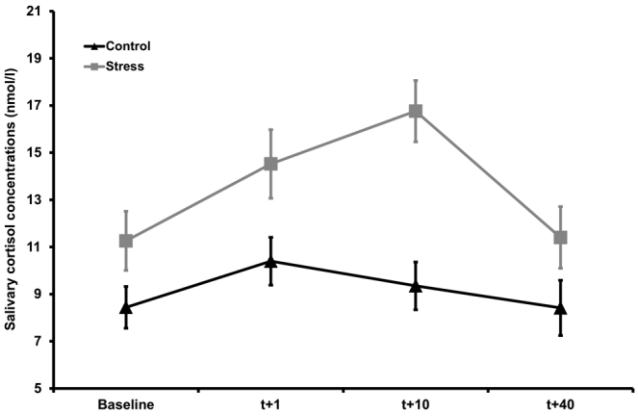

Figure 1. Salivary cortisol concentrations in participants exposed to the Trier Social Stress Test (TSST) or a control condition (Placebo TSST). The baseline sample was collected directly prior to and the t + 1 sample immediately after the stress or control condition (which lasted 15 min). The t + 10 and t + 40 samples were taken 10 and 40 min after cessation of the TSST or Placebo TSST. The conditional discrimination learning took place between the +10 and +40 sample. Stressed participants had significantly elevated cortisol levels compared to baseline (as well as compared to controls) at t + 1 and t + 10.

($F(3,105) = 3.46$, $p = .032$). In the STRESS group, the cortisol levels were significantly higher 1 min and 10 min after stress induction (both $p < .038$) compared to baseline, but had returned to baseline levels after completion of the conditioning. In the CONTROL group, the cortisol levels did not differ significantly from baseline at any of the subsequent points of time (all $p > .38$). As a consequence, the STRESS group had higher concentrations compared to the CONTROL group at +1 ($p < .05$) and +10 ($p < .01$) min after the TSST and Placebo TSST, respectively. No significant differences were observed at baseline and +40.

Affect

The PANAS affect ratings were analyzed for negative and positive items separately by ANOVAs with the factors GROUP (STRESS vs. CONTROL) and TIME (before and after TSST or Placebo TSST). Analysis of negative affect yielded a significant GROUP × TIME interaction ($F(1,35) = 8.63$, $p = .006$), with stress leading to an increase in negative affect (baseline $M = 1.39 \pm .48$ (*SD*), poststress $M = 1.64 \pm .50$), while there was no change in the control group (baseline $M = 1.32 \pm .46$, postcontrol $M = 1.24 \pm .38$). Analysis of positive affect did not yield any significant stress effects.

UR Amplitude

Mean UR amplitude was 2085.5 (*SD* = 1062) in the STRESS and 2725.4 (*SD* = 1638) in the CONTROL group; this difference was not significant ($p = .17$). Mean UR amplitude did not correlate with cortisol levels (neither with the four points of time, at which cortisol levels were assessed (baseline, +1, +10, +40), nor with the AUC). It did not correlate with the behavioral performance either (first CR, CR frequency on S+ and S− trials).

Acquisition of Conditional Discrimination Learning

On average, the first CR occurred after 11.2 trials (*SD* = 14.3) in the CONTROL and after 9.6 trials (*SD* = 12.1) in the STRESS group;

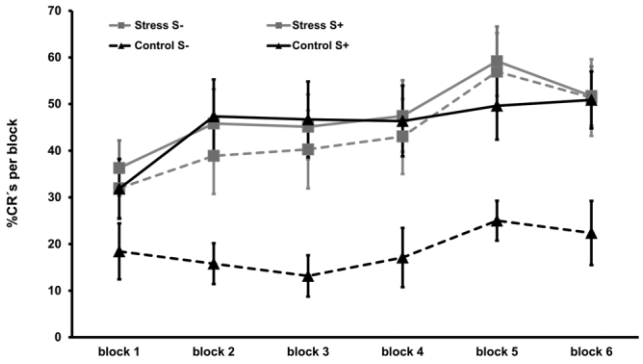

Figure 2. Conditional discrimination learning in participants exposed to the stress or the control condition. Each of the six blocks consisted of 12 trials. Learning performance is expressed in percentages. Controls learned quickly to respond to only those CS that were preceded by an S+. In contrast, stressed participants failed to differentiate between S+ and S− trials. They responded to both stimuli with an increasing amount of CRs.

this difference was not significant ($p = .72$). The course of CR frequency on S+ and S− trials across acquisition is illustrated in Figure 2.

An ANOVA with the factors GROUP, TRIAL TYPE (S+ vs. S−), and BLOCK (1–6) yielded significant main effects of TRIAL TYPE ($F(1,35) = 15.0$, $p < .001$), with higher CR rates on S+ compared to S− trials, and BLOCK (linear trend) ($F(1,35) = 24.3$, $p < .001$), reflecting significant CR rate increases and thereby significant learning across blocks. The GROUP × TRIAL TYPE interaction was also significant ($F(1,35) = 8.6$, $p = .006$). The interaction emerged due to significantly higher CR rates in the STRESS group compared to the CONTROL group on S− trials ($F(1,35) = 11.8$, $p = .002$), whereas there was no significant group difference on S+ trials ($p = .81$). Analyses in the separate groups showed that the CONTROL group acquired significant discrimination in terms of higher CR rates on S+ compared to S− trials ($p = .001$), while this was not the case in the STRESS group ($p = .295$). None of the other interactions reached significance (all $p > .2$).

Extinction

Extinction effects were evaluated by comparing CR rates on S+ and S− trials during the last acquisition block and during the extinction block. In addition to a significant BLOCK effect ($F(1,35) = 37.9.0$, $p < .001$), which reflected significant extinction, an ANOVA yielded a significant TRIAL TYPE × BLOCK interaction ($F(1,35) = 4.63$, $p = .038$), which reflected a stronger reduction of CR rate on S+ compared to S− trials. There was also a significant GROUP × TRIAL TYPE × BLOCK interaction ($F(1,35) = 9.73$, $p = .004$). This effect was due to significantly stronger reductions of CR rate in the CONTROL group compared to the STRESS group on S+ trials (GROUP × BLOCK, $F(1,35) = 4.91$, $p = .033$) and marginally stronger reductions of CR rate in the STRESS group compared to the CONTROL group on S− trials ($F(1,35) = 4.09$, $p = .051$). Further analyses showed that in the CONTROL group there were significant extinction effects on S+ trials ($p < .001$), but not on S− trials ($p = .527$; see Figure 3). In the STRESS group, there were significant extinction effects on both S+ trials ($p = .008$) and S− trials ($p = .004$).

Awareness

A subject was classified as aware if he indicated the correct stimulus contingencies in the postexperimental interview, that is, if he correctly reported the association between the color of the S+/S− square and tone-puff of air/tone-alone combinations in the initial free recall part or answered the questions related to the contingencies correctly. Ten of the 18 subjects in the STRESS group (56%) and 15 of the 19 subjects in the CONTROL group (79%) were rated as aware based on this criterion. This difference in awareness frequency was not significant (Chi-square test = 2.3, $p = .129$).

Associations Between Cortisol and Discrimination Learning

In order to further explore the relationship between individual differences in cortisol responsivity and conditional discrimination, two sets of analyses were performed. As in our previous study on stress and delay conditioning (Wolf et al., 2009), we separated the stress group into (cortisol) responders and nonresponders. A cortisol response was defined as an increase of 2.5 nmol/l or larger between the baseline level and the +10 measure (see Schoofs & Wolf, 2009, for details). Using this rather conservative criterion, the stress group was divided into 10 responders and 8 nonresponders. An ANOVA with the factors GROUP (responder, nonresponder, control), TRIAL TYPE (S+ vs. S−) and BLOCK (1–6) revealed significant main effects of TRIAL TYPE ($F(1,35) = 7.9$, $p = .008$), with higher CR rates for S+ compared to S− trials, and BLOCK (linear trend) ($F(1,35) = 24.5$, $p < .001$), showing increased CR rates over the course of time. Furthermore, there was a significant GROUP × TRIAL TYPE interaction ($F(1,35) = 4.8$, $p = .015$). Subsequent paired comparisons showed significant discrimination performances for the CONTROL group ($F(1,35) = 15.1$, $p = .001$), a trend towards significant discrimination performances for the NONRESPONDER group ($F(1,35) = 4.5$, $p = .071$), and no significant differences between S+ and S− trials for the RESPONDER group ($p = .78$). Results are displayed in Figure 4.

As an additional approach, correlational analyses were conducted. The AUC index was correlated with the discrimination index (see Method for description of both indices). Results revealed a negative correlation for the entire group ($n = 37$) ($r = -.44$, $p < .01$). In order to assure that this association was not just secondary to the between-group differences in cortisol and discrimination performance, a partial correlation was computed

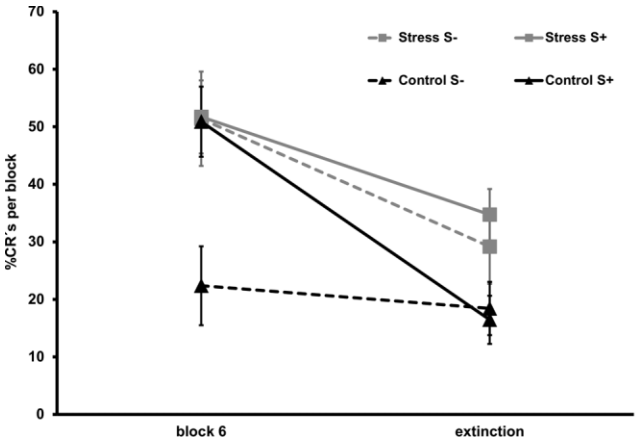

Figure 3. Extinction of conditional discrimination learning in participants exposed to the stress or the control condition. The figure contrasts performance in the last acquisition block (#6) with the average percentage of CRs displayed during the extinction block (which consisted of 12 trials). Stressed participants extinguished their responses to S+ as well as S− stimuli, but exhibited a slower extinction rate than controls.

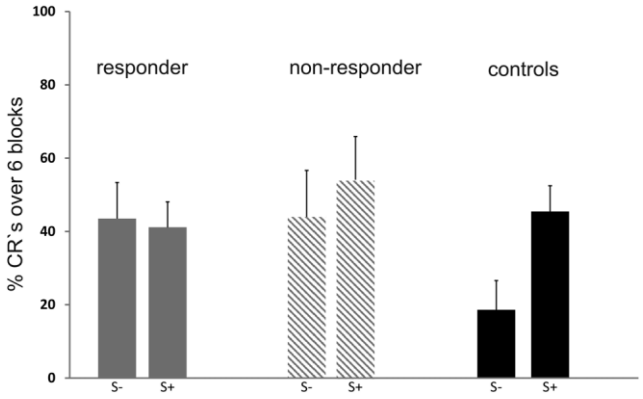

Figure 4. Acquisition of conditional discrimination learning (displayed as percentage of CRs over the entire six acquisition blocks) in participants showing a stress-induced cortisol response (responder, $n = 10$), participants exposed to stress but failing to show a robust cortisol response (nonresponder, $n = 8$), and controls not exposed to the stressor ($n = 19$). Controls showed significant conditional discrimination as evident by a significantly ($p < .01$) larger proportion of responses to S+ compared to S− trials. Cortisol nonresponders showed a trend towards successful discrimination ($p = .07$), while responders showed no evidence for conditional discrimination.

controlling for the influence of group membership. The correlation remained sizeable ($r = -.31$), but fell short of significance ($p = .06$).

Discussion

The present study examined the impact of acute psychosocial stress on conditional discrimination learning. As to be expected, results revealed that the stressor leads to increased salivary cortisol levels as well as increased negative affect. The conditional discrimination task was acquired quite rapidly by the control group. A clear difference between responses to the CS that was preceded by the S+ (the occasion setter), compared to the CS that was preceded by the S-, was already apparent after the first block (consisting of 12 trials). This is in line with previous studies using this paradigm in young, healthy participants (Bellebaum & Daum, 2004). A single acute stress exposure had clear effects on performance in this task. Stressed participants failed to acquire the discrimination. Irrespective of the occasion setter used (S+ or S−), they responded to each CS with an eyeblink (an US). Thus, their responses to the S+ and the S− did not differ from each other. Both responses increased over the course of the six acquisition blocks and were highly similar to the S+ responses in the control group. The control group showed rapid extinction. In contrast, extinction of the stress group was less pronounced, with a parallel decrease of response to the S+ and S− stimuli. Thus, stress impaired the acquisition of conditional discrimination and had (possibly as a consequence thereof) a negative influence on extinction.

It is important to emphasize that these findings do not reflect unspecific effects of the acute stress exposure. Both groups showed comparable US amplitudes. In addition, both groups showed a similar learning curve for the responses to the S+ trials. The results are quite similar to observations made in patients with temporal lobe lesions (Daum et al., 1991). In contrast, age-associated changes appear to impact discrimination as well, but to additionally impact overall CR frequency (Bellebaum & Daum, 2004). The latter finding is thought to reflect age-associated cerebellar dysfunction.

This study is the first to report the effects of stress on conditional discrimination learning in the human. Previous human eyeblink conditioning studies have focused on hippocampus-dependent trace conditioning. Duncko and colleagues reported that a milder stressor (cold pressor task) enhanced trace eyeblink conditioning (Duncko et al., 2007). In contrast, patients with Cushing's syndrome, who display chronic endogenous hypercortisolemia, were impaired in a trace conditioning task (Grillon et al., 2004). Similarly, a single administration of cortisol impaired trace conditioning in patients with post-traumatic stress disorder (PTSD; Vythilingam et al., 2006). These findings, together with the current study, might reflect a nonlinear (inverted U-shaped) dose response relationship between glucocorticoids and hippocampus-mediated conditioning tasks. This interpretation would be in line with electrophysiological as well as behavioral studies in rodents (Diamond et al., 2007; Sandi & Pinelo-Nava, 2007).

Having said this, our data failed to find evidence for an inverted U-shaped relationship. The responder analysis as well as the correlational analysis suggest a linear negative association between cortisol and conditional discrimination. This is in line with our previous study on delay eyeblink conditioning (Wolf et al., 2009) as well as with previous studies in the area of declarative memory (Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001). Future pharmacological dose response studies are needed to address this issue more appropriately.

The finding that stressed participants acquired the response to the S+ stimuli at a similar speed as the participants from the control group might at first glance contradict our previous finding of an impaired delay eyeblink conditioning after stress exposure (Wolf et al., 2009). However, a comparison of the two paradigms is problematic since, in conditional discrimination, the US is preceded by two stimuli (S and CS). The acquisition of the motor response itself is therefore conceivably easier.

Studies with neurological patients have established that the hippocampus is crucial for successful acquisition in conditional discrimination learning (Daum et al., 1991; Fortier et al., 2003). Importantly, this is not secondary to the fact that conditional discrimination tasks typically use trace (instead of delay) conditioning (Fortier et al., 2003). In line with findings in rodents with hippocampal lesions (Ross, Orr, Holland, & Berger, 1984), patients with hippocampal damage show increased CRs to the CS in S+ and S− trials. It has been suggested that this response pattern supports the notion that the hippocampus is important for acquiring "if–then" rules (Daum et al., 1991). Other authors have suggested that the hippocampus plays a role in the association of discontinuous items (Wallenstein et al., 1998) or in the establishment of relational memories (Konkel & Cohen, 2009).

The stress-induced alterations observed in the current study could reflect acute and transient inhibitory effects of glucocorticoids on hippocampal neurons (Diamond et al., 2007). This hypothesis is support by several recent human neuroimaging studies reporting decreased hippocampal activity after stress (Henckens, Hermans, Pu, Joels, & Fernandez, 2009; Pruessner et al., 2008; Weerda, Muehlhan, Wolf, & Thiel, 2010) or glucocorticoid (de Quervain et al., 2003; Oei et al., 2007) treatment.

The current findings share some features with recent results obtained from an appetitive instrumental learning task. Stressed participants or participants treated with cortisol in combination with yohimbine exhibited rigid habitual behavior, which was not influenced by the value of the reward used (Schwabe & Wolf, 2009; Schwabe, Tegenthoff, Hoffken, & Wolf, 2010; Schwabe & Wolf, in

press). In contrast, participants from the control groups showed goal-directed behavior and responded only to those stimuli that were associated with a valuable reward. In the present study, stress also led to an inflexible response behavior characterized by CRs, which occurred irrespective of the presence or absence of the discriminative stimulus (the S+). Taken together, these recent findings support the notion that stress impairs flexible cognitive response styles and induces a rigid, stimulus-driven response style (Schwabe, Wolf, & Oitzl, 2010).

So far, the discussion of our findings has focused on the potential role of the stress-induced activation of the HPA axis. Of course, cognitive and affective mediators need to be considered as well. Stressed participants might have been distracted by rumination. The fact that rumination or cognitive interference plays a critical role in mediating the impact of stress on other cognitive processes (e.g., working memory and long-term memory) has been established in previous field studies (Stawski, Sliwinski, & Smyth, 2006, 2009). Future laboratory studies might benefit from assessing these potential cognitive mediators in more detail.

Extinction in the stress group was less efficient compared to controls. The interpretation of the findings is hampered by the fact that the two groups showed different learning successes. Since stressed participants failed to acquire the discriminatory potency of the S+, they experienced the acquisition as only partially reinforced. It is well established that partial reinforcement causes slower extinction compared to fully reinforced designs (Fester & Skinner, 1957). In order to investigate selective effects of stress on extinction, future studies should induce stress before extinction, but after acquisition (Quirk & Mueller, 2008; Rodrigues, LeDoux, & Sapolsky, 2009). In these studies, a longer delay between acquisition and extinction should be chosen, since it is known that extinction is influenced by the time interval between acquisition and extinction (Chang & Maren, 2009; Maren & Chang, 2006).

In the current study, we investigated males only. The conclusions to be drawn are thus restricted to this sex, which is a limitation of the study. It remains to be established whether similar results occur in women. In our previous delay eyelid conditioning studies, we observed that men and women showed a highly comparable effect (impairment) in delay conditioning after being exposed to the TSST (Wolf et al., 2009). Human research on classical conditioning appears to suggest that fear conditioning (Jackson, Payne, Nadel, & Jacobs, 2006; Merz et al., 2010; Stark et al., 2006; Zorawski, Blanding, Kuhn, & Labar, 2006), but not eyeblink conditioning (Wolf et al., 2009), is modulated by stress in a sex-dependent fashion. However, given the strong evidence for sex differences observed in rodent eyeblink stress studies (Dalla & Shors, 2009; Shors, 2004), additional research on this topic is certainly warranted.

In the current study, we tested the effects of an acute moderate psychosocial laboratory stressor on learning performance in young healthy males. The current findings' relevance for psychiatric conditions characterized by HPA alterations is unknown. Patients with major depressive disorder, for example, show elevated cortisol levels and signs of hippocampal atrophy. Patients with PTSD often show reduced basal cortisol levels but have hippocampal atrophy as well (for reviews, see Schlosser, Wolf, & Wingenfeld, 2011; Wingenfeld & Wolf, in press). It would be interesting to investigate conditional discrimination under resting as well as under stressful conditions in these patient populations.

In sum, the present experiments demonstrate that a single exposure to a brief psychosocial laboratory stressor impairs conditional discrimination learning. We suggest that this effect might be mediated by inhibitory effects of the stress hormone cortisol on neuronal plasticity of the hippocampus. This hypothesis needs to be tested using pharmacological approaches. Moreover, the neural underpinning of the observed effect of stress should be further elucidated using functional imaging techniques.

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