Psychoneuroendocrinology 71 (2016) 64–72

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Stress in the zoo: Tracking the impact of stress on memory formation over time

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ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 28 April 2016
Accepted 28 April 2016

Keywords:
Stress
Memory formation
Memory encoding
Glucocorticoids
Noradrenaline
Autonomic nervous system

ABSTRACT

Although stress is well known to modulate human memory, precisely how memory formation is altered by a stressful encounter remains unclear. Stress effects on cognition are mainly mediated by the rapidly acting sympathetic nervous system, resulting in the release of catecholamines, and the slower acting hypothalamus-pituitary-adrenal axis secreting cortisol, which induces its effects on cognition through fast, non-genomic actions and delayed, genomic actions. Importantly, these different waves of the physiological stress response are thought to dynamically alter neural processing in brain regions important for memory such as the amygdala and the hippocampus. However, the precise time course of stress effects on memory formation is still unclear. To track the development of stress effects on memory over time, we tested individuals who underwent a stressful experience or a control procedure before a 2-h walk through a zoo, while an automatic camera continuously photographed the events they encoded. In a recognition memory test one week later, participants were presented with target photographs of their own zoo tour and lure photographs from an alternate tour. Stressed participants showed better memory for the experimental treatment than control participants, and this memory enhancement for the stressful encounter itself was directly linked to the sympathetic stress response. Moreover, stress enhanced memory for events encoded 41–65 min after stressor onset, which was associated with the cortisol stress response, most likely arising from non-genomic cortisol actions. However, memory for events encoded long after the stressor, when genomic cortisol actions had most likely developed, remained unchanged. Our findings provide novel insights into how stress effects on memory formation develop over time, depending on the activity of major physiological stress response systems.

1. Introduction

Our everyday life is full of challenges, deadlines and demands. For many of us, stress is so common that it is accepted as part of life. Stress, however, can change the way we feel, think, and behave (Diamond et al., 2007; Lupien et al., 2009; Sandi and Haller, 2015). In particular, stress is known to impact learning and memory processes (Diamond et al., 2007; Joëls et al., 2006; Schwabe and Wolf, 2013). Although stress-induced changes in memory formation have major implications for stress-related mental disorders, such as depression or post-traumatic stress disorder (PTSD; Pitman et al., 2012) as well as educational or occupational settings, the literature on the influence of stress on memory formation is heterogeneous, with some studies reporting enhanced and others impaired memory after stress (e.g. Diamond et al., 2007; Payne et al., 2006; Sandi et al., 1997; Schwabe et al., 2008; Zoladz et al., 2011). Thus, exactly how stress affects memory formation is not well understood.

It is commonly assumed that the impact of stress on memory formation depends on the temporal proximity of the stressful encounter and the event that is encoded (Joëls et al., 2011; Schwabe et al., 2012). Stress experienced during or shortly before learning is thought to enhance memory formation whereas stress long before learning is assumed to suppress new encoding to protect the memories formed in the stressful situation from interference (Joëls et al., 2011; Schwabe et al., 2012). These time-dependent effects of stress on memory have been linked to time-dependent physiological and endocrine changes that occur in response to stressful encounters (Joëls and Baram, 2009; Joëls et al., 2011). Within seconds after stressor onset, the autonomic nervous system (ANS) is activated, resulting in the release of catecholamines, such as adrenaline or noradrenaline, which prepare the organism for 'fight-or-flight'. A second, slower system activated under stress is the hypothalamus-pituitary-adrenal (HPA) axis which

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http://dx.doi.org/10.1016/j.psyneuen.2016.04.027
0306-4530/

leads to the release of corticosteroids (in humans mainly cortisol), reaching peak levels approximately thirty minutes after stressor onset. Upon reaching the brain, corticosteroid hormones operate via two modes of action: a rapid, non-genomic mode mediated by membrane-bound receptors and a slower, genomic mode of action mediated by intracellular receptors that is assumed to set in 60–90 min after a stressful event (Joëls et al., 2012). Thus, there are (at least) three waves of the physiological stress response: rapidly acting ANS activity, early non-genomic cortisol actions, and slow genomic cortisol actions. Critically, it is assumed that these different stress response waves have a distinct, perhaps even opposite impact on memory processes (Joëls et al., 2011). More specifically, rapidly released catecholamines are thought to facilitate memory formation by enhancing neural excitability and glutamatergic transmission in relevant brain structures such as the basolateral amygdala and the hippocampus (Hu et al., 2007; Joëls et al., 2011; Onur et al., 2009). These effects of noradrenaline are thought to be further promoted by non-genomic cortisol effects (Joëls et al., 2011; Karst et al., 2010; Karst et al., 2005). Non-genomic cortisol effects appear to enhance neural activity and noradrenergic input in the amygdala (Roozendaal et al., 2006b; van Stegeren et al., 2007), again leading to enhanced memory performance. In contrast, later genomic effects of cortisol are assumed to suppress new encoding by decreasing and normalizing neural excitability in these structures to allow for enhanced prefrontal cognitive control long after the stressful encounter (Henckens et al., 2010; Karst et al., 2010; Lovallo et al., 2010). Although this time-dependency of stress effects on memory formation appears to be widely accepted in the field, studies that directly assess the dynamics of memory formation after a stressful event are lacking. Rather, this widely held hypothesis is based on a literature primarily comprising studies that investigated memory formation at defined time points after a stressor, e.g. 0, 10, or 30 min after a stressful event (Domes et al., 2002; Payne et al., 2006; Schwabe et al., 2008; Smeets et al., 2009; Zoladz et al., 2011). However, in order to understand when stress effects on memory formation arise, how long they last, and to what extent they are linked to the activity of major stress response systems, memory processes need to be assessed continuously during and after a stressful event.

Moreover, to date virtually all studies investigating stress effects on memory were performed in strictly controlled laboratory settings, using by and large arbitrary learning material (Domes et al., 2002; Schwabe et al., 2008; Smeets et al., 2009). But can these findings actually be translated to real-life settings? Given that stress effects on memory have major implications, for example, for educational or clinical settings, the translation of findings to natural environments is essential. Thus, the aims of this experiment were twofold. First and foremost, we aimed to unravel the dynamics of stress effects on memory formation by testing how stress affects the formation of memories during the first hours after a stressful event. Second, we aimed to assess the impact of stress on memory formation in a natural environment. To this end, we used a unique paradigm in which participants first underwent a stress or control manipulation before they encoded experiences on a 2-h tour through a zoo. Both during the stress/control manipulation and during the zoo tour participants were carrying a camera that automatically took pictures from their first-person perspective. This paradigm allowed us to exert control over the encoding of real-world events and to determine when exactly after stressor onset a specific event was encoded. Memory performance was tested one week later in a recognition test that included photographs of the participant's own stress/control experience and zoo tour, as well as lures from an alternative tour through the zoo. In order to track activity of the ANS and the HPA axis, we assessed participants pulse and blood pressure in the context of the stress (or control) manipulation and measured salivary cortisol every 6 min across the entire encoding session. We predicted that stress would enhance memory for the stressful event itself, when autonomic arousal is high and catecholamines facilitate neural activity in the amygdala and hippocampus, and for events encoded during the rapid mode of cortisol action, about 30 min post-stress, when non-genomic cortisol effects support the effects of catecholamines. However, memory was expected to be impaired for events that were encoded about 90 min after stress, when the genomic effects of cortisol decrease activity of amygdala and hippocampus.

2. Material and methods

2.1. Participants and experimental design

Sixty-six healthy individuals with normal or corrected-to-normal vision who had not visited the Hamburg zoo for at least ten years participated in this experiment (35 men, 31 women; mean age = 25.7 years, SD = 3.6 years). We excluded individuals with current medication intake or any medical condition including allergic reactions to animals, or lifetime history of any neurological or psychiatric disorders. We further did not include smokers and women taking hormonal contraceptives as smoking and hormonal contraceptives affect the stress response (Kirschbaum et al., 1999; Rohleder and Kirschbaum, 2006). Moreover, women were not tested during their menses. The study protocol was approved by the review board of the German Psychological Society (LS022015), all participants provided written informed consent and received a moderate monetary compensation (35 €, approximately 40 USD) for participation.

A mixed design with the between-subjects factor treatment (stress vs. control manipulation) and the within-subject factor time of encoding relative to treatment onset was used to investigate time-dependent effects of stress on memory formation. Participants were randomly assigned to the stress or control group. Data from one participant was not available due to technical failure of the camera and another participant did not return for day 2 (both male, stress group). Furthermore, one participant (female, stress group) was identified as an outlier based on canonical statistical criteria (Tabachnick and Fidell, 2005; hit rate more than 2.5 SD below the overall mean) and thus excluded from the analyses of recognition data. Therefore, the number of participants included in the analyses of recognition memory was 63 (stress: 16 men, 15 women, control: 17 men, 15 women). Finally, four participants had data on only approximately 100 instead of 120 min of walking due to weather conditions (heavy rain) or technical problems, but were still included in the analyses.

2.2. Procedure

All testing for both experimental sessions took place in the afternoon (12:15-19:30) and all participants were tested individually.

Day 1. Upon their arrival at the zoo (Tierpark Hagenbeck, Hamburg, Germany), participants provided a baseline saliva sample (see Section 2.3) and their vital signs (heart rate, blood pressure) were measured using an automatic blood pressure monitor with arm cuff (Omron, the Netherlands). Furthermore, participants completed the Spielberger State-Trait Anxiety Inventory (German version; Laux et al., 1981) and a German mood questionnaire (MDBF; Steyer et al., 1994) that assesses subjective mood on three scales: depressed vs. elevated mood, restlessness vs. calmness, and sleepiness vs. wakefulness. Sum scores per scale range from 8 to 40 and high scores represent elevated mood, calmness, and wakefulness, respectively. From then on, participants carried a camera (Autographer, OMG plc., UK) around their neck, facing forward at the height of their sternum. The camera automatically took approx-

imately three full-color pictures (136° wide angle) per minute for the remainder of the experimental session on day 1 (including stress/control manipulation and zoo tour). Pictures were recorded using Autographer's algorithm based on changes in brightness, acceleration, direction, location, and motion of the motif. The participants were instructed not to cover the camera, and to try to memorize as much as possible from their zoo visit.

Next, participants were brought to a separate room where they underwent either the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) or a non-stressful control procedure. The TSST is a standard stress-induction protocol for humans, known to reliably activate both the ANS and the HPA axis (Kirschbaum et al., 1993). Briefly, the TSST simulates a 15-min job interview comprising a public speech about why the participant would be a good candidate for the job and a rather difficult mental-arithmetic task (counting backwards from 2043 in steps of 17). Throughout the TSST, participants were videotaped and evaluated by two serious, non-reinforcing committee members. In the control condition, participants were alone in the room and they could chose to speak about a holiday, movie, or book followed by a simple mental arithmetic task (counting forwards from zero in steps of 15); there was no evaluative committee and the participants were not videotaped.

To assess the effective stress induction by the TSST, participant's vital signs and subjective mood were assessed again immediately after the TSST/control procedure. In addition, a saliva sample was taken and participants rated how difficult, unpleasant, and stressful the procedure was on three scales ranging from 0 ('not at all') to 100 ('very much'). Thereafter, participants started a guided walk through the zoo along a predefined route (see Fig. 1). There were two possible non-overlapping routes (A and B) that were counterbalanced across groups, as was the direction of the walk (clockwise or counter-clockwise). To avoid being on the photographs, the experimenter stayed behind the participant all the time and did not interact with the participant except for taking saliva samples every six minutes, indicating the route, and making sure the participant is in time on the indicated route. After exactly two hours of walking, all participants were guided back to the start of the route, the camera was switched off, and they provided a last saliva sample, vital signs, and mood assessment. Participants of the stress group were debriefed about the stress induction procedure (but not about the aims of the experiment as a whole), and left the zoo immediately.

Day 2. Seven days after day 1, participants came to the laboratory and again provided a saliva sample, vital signs, and subjective mood assessment. Thereafter, a recognition test assessed participants' memory for events from day 1 (Fig. 1). From all photographs taken for a participant (during the experimental manipulation and the zoo tour), we selected one photograph per minute for the recognition test. We based the selection on image sharpness, absence of details directly identifying the participant or the experimenter (e.g. clothes), and as few motif repetitions as possible. In the recognition test, participants were presented with photographs taken during their own visit to the zoo (targets, one target per minute of 2-h session) and photographs taken during the walk of another participant (lures). This other participant was tested on the same day at approximately the same time – to control for weather and lighting conditions – but on the other route (i.e. when a participant walked route A, the lures were photographs from a participant that walked route B, and vice versa). In addition to the photographs from the zoo tour, the recognition test also included photographs from the participants' own stress/control experience and photographs which were taken independently with slight variations to the standard room setup (e.g. window blind open vs. closed or video camera removed vs. present). In total, participants were presented with 264 photographs (12 photographs depicting the stress/control manipulation of the participant, 120 photographs showing events encountered on the zoo tour at a specific time point after the stress/control manipulation, 12 lures for the stress/control manipulation, and 120 lures from the walk of another participant). Participants saw one picture at a time on a computer screen, in random order, and were asked to indicate whether they had seen this scene during day 1 by pressing one of two arrow keys (yes, no). The correspondence between arrow keys (left, right) and answers (yes, no) varied randomly over trials. If participants responded with 'yes', they were further requested to indicate their confidence on a scale from 1 ('guess') to 4 ('very sure'). Finally, participants completed Beck's Depression Inventory (German version; Hautzinger et al., 1994) to control for depressive mood and were subsequently debriefed about the aims of the study. For one participant (stress condition), the interval between testing days was 10 days due to scheduling problems, but the data from this participant did not differ from the others. Therefore, we kept her data in the analyses.

2.3. Saliva sampling

In order to measure concentrations of the stress hormone cortisol, 24 saliva samples were taken per participant using Salivette® collection devices (Sarstedt, Germany). At the end of each test day, all samples were stored at −18 °C (−0.4 °F). At the end of the study, the samples were thawed for biochemical analysis, and the fraction of free cortisol was assessed using a commercially available chemiluminescence immunoassay (IBL, Tecan Group, Switzerland). This kit has a high sensitivity with its lower limit of cortisol detection being 0.3312 nmol/l. All inter- and intra-assay coefficients of variance were <10%.

2.4. Data analysis

To test whether the stress induction was successful, data on subjective mood, vital signs, and salivary cortisol were subjected to mixed-design analyses of variance (ANOVA) with the between-subjects factor treatment and the within-subject factor time. To investigate post-hoc group differences in the ratings of the TSST/control procedure, and to test whether stress effects on mood, vital signs, or cortisol levels persisted on day 2, independent-samples *t*-tests were used.

To investigate time-dependent effects of stress on memory formation, we calculated the hit rate in percent for each six-minute interval (in line with the 6 min interval of saliva sampling). To exclude correct guesses, we removed correct responses that were followed by a confidence rating of 1 from the analysis ('guess', Takashima et al., 2006; we did not analyze confidence ratings further). Next, we subtracted the individual false alarm rate for the TSST/control procedure from the hits for this procedure. Similarly, the participant's overall false alarm rate for pictures from the zoo tour was subtracted from the hit rate for each interval during the walk. The resulting memory accuracy scores were subjected to mixed-design ANOVAs with the between-subjects factor treatment and the within-subject factor time of encoding after stress/control manipulation onset.

In order to align the memory data with the time course of the stress response, we then grouped the memory data into six larger time intervals (Fig. 2): autonomic activation (averaging the two intervals of the stress/control manipulation), and five blocks of four six minute intervals each following the time course of cortisol secretion (cortisol rise and plateau, early decline, late decline, approaching baseline, steady levels). We again calculated mixed-design ANOVAs, followed by independent-samples *t*-tests. To test for associations between memory performance and the individual physiological stress responses using Pearson's *r*, we calculated the area under the curve with respect to the increase from before to 20 min after (fifth saliva sample) the offset of the TSST/control procedure (AUCi; Pruessner et al., 2003) as an integrated mea-

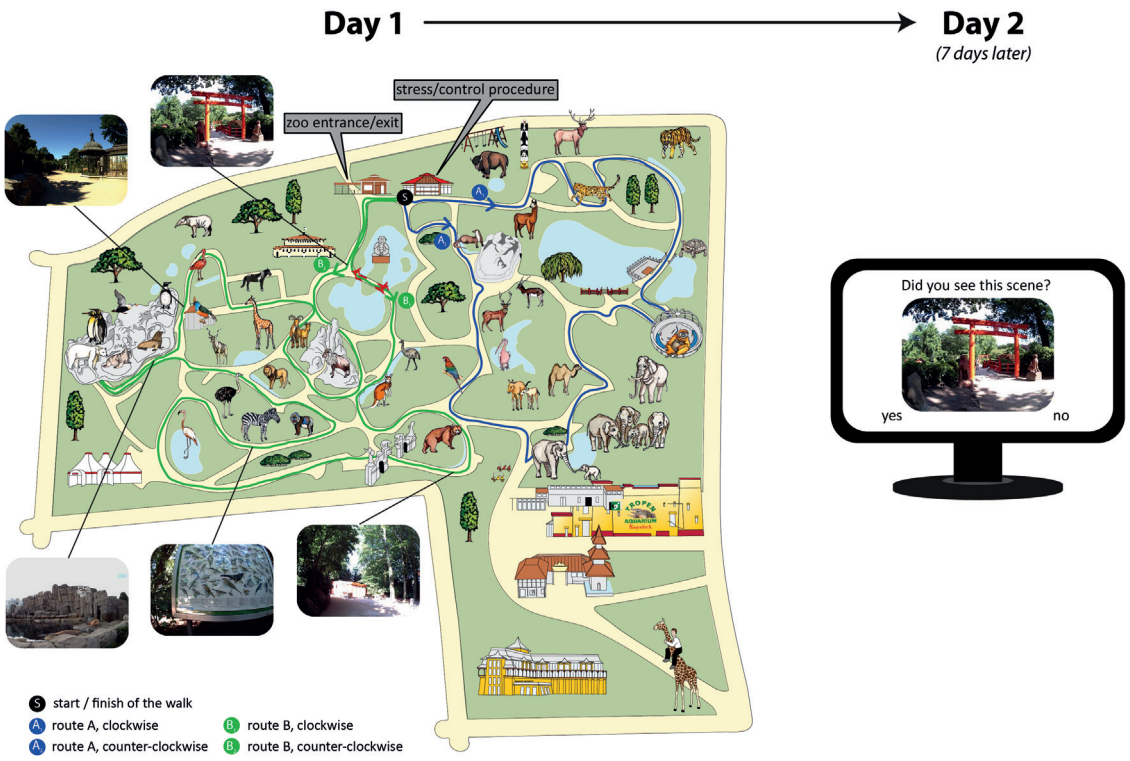

Fig. 1. Map of the zoo (left) and study procedure. Participants came to the Hamburg zoo on day 1, underwent a stress or control procedure and then walked through the zoo along a predefined route (A or B, indicated in blue and green, respectively). The routes and directions (clockwise or counter-clockwise indicated by '1' or '2') were balanced across groups. A camera around the participants' neck took approximately three photographs per minute, example pictures are displayed. Seven days later (day 2, right), participants came to the laboratory for a recognition test. Map of the zoo modified, with permission, from Tierpark Hagenbeck, Hamburg, Germany. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sure of the cortisol response to the stressor/control manipulation. To approximate sympathetic activation, we used the increase in blood pressure from before to immediately after the stress/control manipulation. All analyses were conducted using SPSS 22 (IBM, NY). The alpha level was set to 0.05 for all analyses (two-tailed), and Greenhouse-Geisser correction was used to correct for violations of sphericity.

3. Results

3.1. Subjective and physiological parameters confirm successful stress induction

As expected, subjective and physiological responses to the TSST verified the successful stress-induction. The TSST was rated as significantly more difficult, unpleasant, and stressful than the control procedure (all $t(62) > 50$, all $p < 0.001$, all $d > 1.2$, Table 1). Moreover, the TSST led to increases in depressed mood (time × treatment: $F(1.6,97.5) = 7.14$, $p = 0.003$, $\eta_p^2 = 0.20$, Table 1) and restlessness (time × treatment: $F(1.6,98.4) = 5.86$, $p = 0.007$, $\eta_p^2 = 0.09$) resulting in group differences directly after the stress/control procedure (restlessness: $t(62) = -2.66$, $p = 0.010$, depressed mood [trend]: $t(62) = -1.86$, $p = 0.067$), whereas groups did not differ before the experimental manipulation (mood: $p = 0.146$, restlessness: $p = 0.560$) or at the end of day 1 (mood: $p = 0.547$, restlessness: $p = 0.913$). The exposure to the TSST also increased systolic (time × treatment: $F(1.9,120.6) = 3.41$, $p = 0.037$, $\eta_p^2 = 0.05$, Table 1) and diastolic blood pressure (time × treatment: $F(1.9,117.1) = 4.23$, $p = 0.019$, $\eta_p^2 = 0.06$, main effect of treatment: $F(1,62) = 4.51$, $p = 0.038$, $\eta_p^2 = 0.07$, Table 1), indicating an activation of the ANS after the TSST in the stress group despite the absence of an effect on heart rate (time × treatment $p = 0.256$, main effect of treatment: $p = 0.728$). Finally, the TSST led to a pronounced increase in salivary cortisol levels (time × treatment: $F(1.7,99.4) = 8.72$, $p = 0.001$, $\eta_p^2 = 0.13$, main effect of treatment: $F(1,57) = 8.76$, $p = 0.004$, $\eta_p^2 = 0.13$, Fig. 2a). Whereas there was no difference between the groups in cortisol levels before the TSST ($t(62) = -1.73$, $p = 0.089$), cortisol levels increased after the stress manipulation, reaching peak levels approximately 23 min after TSST onset, and starting to decline 41 min after the stressor, reapproaching baseline after 95 min. This pattern resulted in group differences from 23 min after the onset of the TSST/control manipulation onwards until the end of day 1 ($0.001 \leq ps \leq 0.048$, the only exceptions being 101 and 125 min post stressor, $p = 0.131$ and $p = 0.060$).

Importantly, before memory testing on day 2, groups were comparable in their subjective ratings and physiological parameters (wakefulness $p = 0.175$, mood $p = 0.558$, restlessness $p = 0.997$, systolic blood pressure $p = 0.203$, diastolic blood pressure $p = 0.972$, heart rate $p = 0.691$, cortisol $p = 0.488$). Therefore, group differences in memory accuracy on day 2 cannot be attributed to differences in stress system activity at test. Moreover, groups did not differ in the control variables trait anxiety (stress group: M = 33, SD = 5.6, control group: M = 36, SD = 9.4; $p = 0.181$) or depressive symptoms (stress group: M = 5, SD = 4.6, control group: M = 6, SD = 6.4; $p = 0.370$).

3.2. Stress boosts memory formation depending on stress system activity

Stress before encoding significantly enhanced memory formation in a time-dependent manner (treatment × time of encoding: $F(14.3,788.5) = 1.84$, $p = 0.028$, $\eta_p^2 = 0.03$). In order to better understand how the different waves of the stress response affected memory formation, we analyzed memory accuracy over six

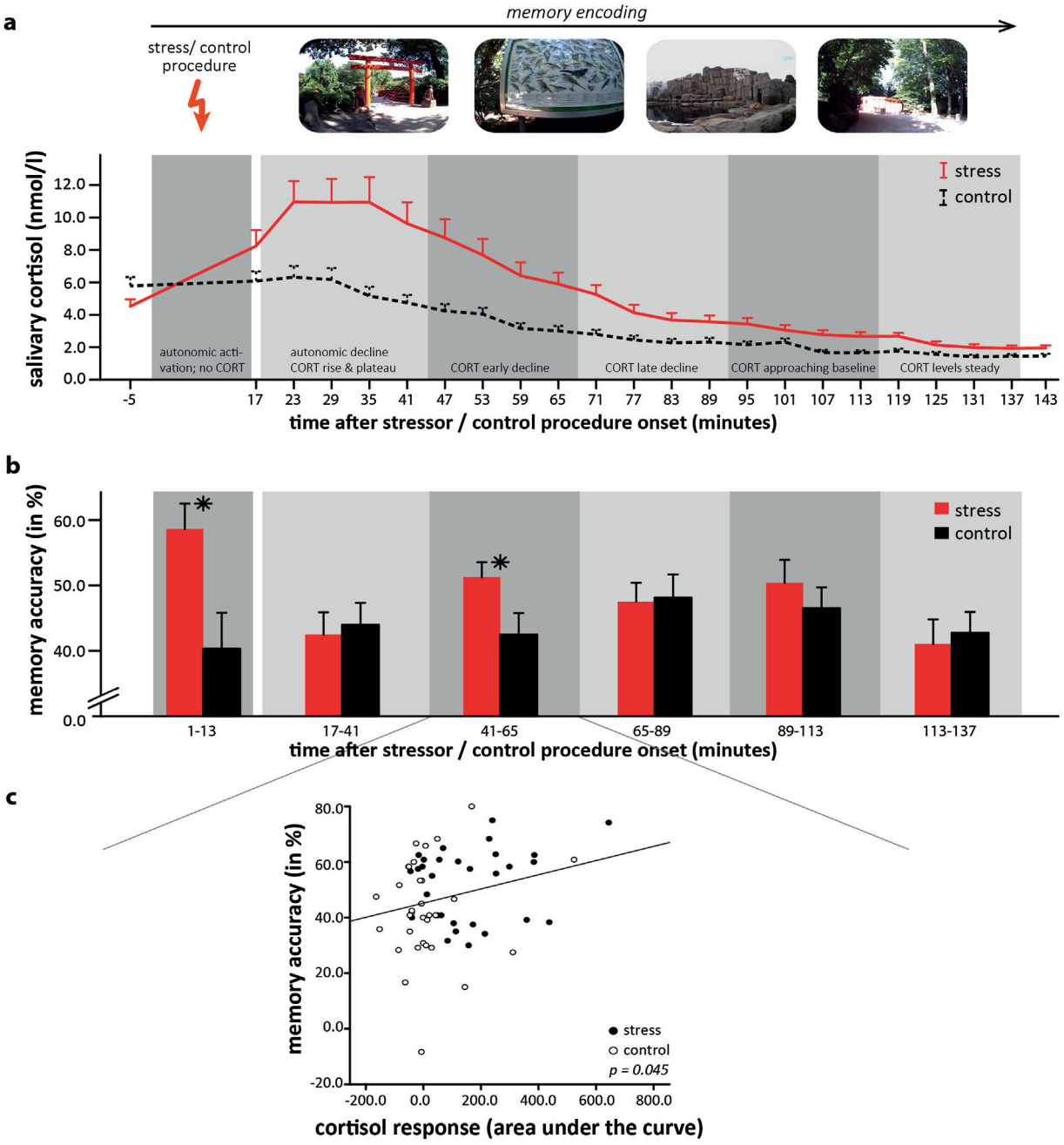

Fig. 2. Experimental procedure, cortisol response, and memory performance. (a) Participants' mean salivary cortisol levels over the course of day 1. A timeline of the experimental procedure is shown on top. Whereas cortisol levels were still rising during encoding of the stressful situation, they were elevated during the walk through the zoo. (b) Memory accuracy (hits – false alarms) on day 2 for photographs taken during the stress (or control) manipulation and the zoo tour on day 1, displayed according to the different periods in which learning took place after stressor/control onset. Stress increased memory formation both during the phase of autonomic activation (i.e. under stress) and the early decline of cortisol levels. (c) The cortisol response to the stress/control manipulation was directly associated with the memory accuracy for memories encoded during the early decline of cortisol levels (41–65 min after stressor onset, $r = 0.255$, $p = 0.045$). CORT = salivary cortisol; error bars represent standard errors of the mean, $^{*}p < 0.05$ compared to the control group.

larger time intervals covering different phases of the stress response (Fig. 2b). Again, we found a time-dependent enhancement of memory due to stress (treatment × stress response interval: $F(3.1,180.4) = 4.44$, $p = 0.004$, $\eta_p^2 = 0.07$). Specifically, during the stressful encounter, when autonomic arousal was high but cortisol levels were not yet elevated, stress increased memory formation ($t(61) = 2.03$, $p = 0.047$). When the autonomic effects wore off and cortisol levels rose to peak level, we did not find a group difference ($p = 0.782$). However, for events encoded about 41–65 min after the stressor onset, when cortisol levels had reached a plateau and already started to decline, the stress group again showed better memory performance compared to the control group ($t(61) = 2.34$, $p = 0.023$). Stress did not affect memory formation for events encoded more than 65 min after the stressor (65–89 min after stress onset: $p = 0.989$, 91–113 min: $p = 0.325$, 113–137 min: $p = 0.728$). Given that some previous studies reported differences between men and women in stress effects on memory processes (Andreano and Cahill, 2006; Guenzel et al., 2014), we investigated in an additional analysis whether the observed time-dependent effects of stress on memory differed in men and women. However, this analysis did not yield a gender × treatment or gender × time of

Table 1
Subjective mood, vital signs, and subjective ratings in both experimental groups across the experiment.

Variable	Control group				Stress group			
	Before control procedure	After control procedure	End of day 1	Day 2	Before stress induction	After stress induction	End of day 1	Day 2
Subjective mood								
Restlessness vs. calmness	34 (0.8)	31 (1.0)	36 (0.6)	32 (1.1)	33 (0.7)	27** (1.1)	36 (0.6)	32 (1.0)
Depressed mood vs. elevated mood	35 (0.6)	34 (0.9)	36 (0.6)	34 (0.8)	36 (0.6)	31# (1.1)	36 (0.7)	33 (0.9)
Sleepiness vs. Wakefulness	31 (0.9)	31 (0.9)	29 (1.1)	31 (0.9)	30 (1.2)	29 (1.2)	27 (1.2)	29 (1.2)
Vital signs								
Heart rate (bpm)	82 (2.4)	81 (2.7)	76 (2.4)	85 (2.3)	83 (2.4)	84 (2.4)	75 (2.3)	86 (2.5)
Diastolic blood pressure (mmHg)	79 (1.8)	82 (1.5)	79 (1.3)	81 (1.3)	81 (1.3)	90** (1.9)	82 (1.5)	81 (1.4)
Systolic blood pressure (mmHg)	117 (2.1)	120 (2.7)	117 (2.4)	116 (1.9)	121 (2.5)	129* (3.2)	118 (2.0)	120 (2.5)
Rating of stressor/control procedure								
Difficult	–	35 (4.5)	–	–	–	70*** (3.0)	–	–
Unpleasant	–	27 (4.7)	–	–	–	61*** (4.0)	–	–
Stressful	–	37 (4.5)	–	–	–	69*** (4.1)	–	–

Note: Data represent mean (standard error).
# $p < 0.10$ compared to control group.
* $p < 0.05$ compared to control group.
** $p < 0.01$ compared to control group.
*** $p < 0.001$ compared to control group.

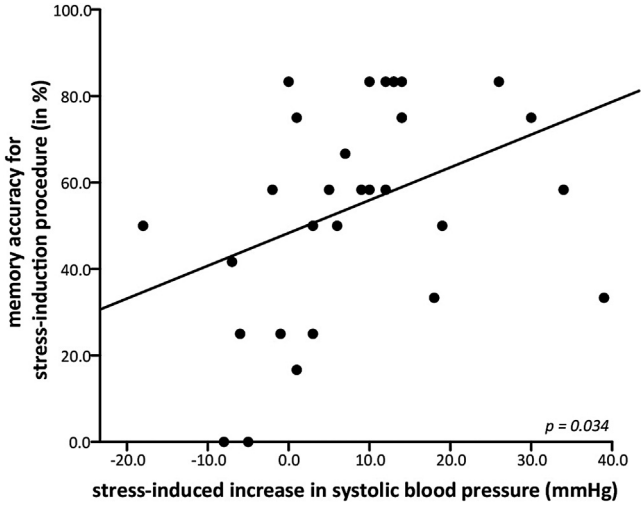

Fig. 3. The stress-induced increase in systolic blood pressure was associated with memory accuracy for the stress induction procedure. Participants with a stronger systolic blood pressure response to the stressor showed increased memory accuracy for items encoded during the stressful procedure.

encoding × treatment interaction (all $p > 0.20$), suggesting that the observed effects were comparable for both males and females.

In a next step, we analyzed the role of the different physiological stress response systems in the observed memory enhancement. According to the known temporal profiles of action for the ANS and HPA axis (Joëls and Baram, 2009), we hypothesized that the stress-induced increase in memory formation during the stressful situation itself would be associated with the ANS, whereas the memory increase for events encoded 41–65 min after stressor onset relative to the control procedure would be linked to the cortisol response to the stress/control manipulation. Indeed, we found a positive correlation between the stress-induced increase in systolic blood pressure as a marker of ANS activity with memory accuracy for the stress procedure ($r = 0.382$, $p = 0.034$, see Fig. 3). This correlation was stronger than the association in the control group (correlation: $r = -0.138$, $p = 0.451$; correlation difference between stress and control groups: $z = 2.04$, $p = 0.021$; correlation over both groups: $r = 0.165$, $p = 0.196$). For events encoded 41–65 min after stress onset, blood pressure was not associated with the observed memory enhancement but even tended to correlate negatively with memory performance for those events ($r = -0.302$, $p = 0.098$). Conversely, cortisol concentrations were not associated with memory for items encoded during the stress/control procedure itself ($r = -0.014$, $p = 0.912$) but correlated significantly with memory accuracy for items encoded 41–65 min after the onset of the stress/control manipulation ($r = 0.255$, $p = 0.045$, Fig. 2c; stress group only: $r = 0.167$, control group only: $r = 0.139$, both $p > 0.2$). Note that the pattern of results remained when removing the person with the lowest memory accuracy ($r = 0.250$, $p = 0.052$).

4. Discussion

Stress is commonly assumed to exert time-dependent effects on memory formation that develop in relation to the temporal profile of action of major stress response systems, in particular the ANS and HPA axis (Joëls et al., 2011; Schwabe et al., 2012). However, to date studies have only focused on memory formation at specific time points after the stressful event, thus precluding an analysis of how stress effects on memory develop with an increasing temporal distance between stressor and encoded event. Here, we set out to continuously measure the effects of stress on memory formation in a natural environment, tracking both the stress response and memory performance precisely over more than two hours.

Overall, we found that stress enhanced memory formation time-dependently which was related to the activity of both major stress response systems. Immediate activation of the ANS, reflected in the stress-induced increase in blood pressure, boosted memory formation for the stressful encounter itself. In contrast, there was no increase in blood pressure in the control group, suggesting a lack of autonomic activation, and thus no enhancement of memory. At a neural level, there is convincing evidence that catecholamines in the basolateral amygdala, specifically norepinephrine (NE), mediate these immediate stress effects on episodic memory formation in the hippocampus (McGaugh et al., 1996; Rasch et al., 2009). For instance, the enhancing effect of emotions or cortisol administration on amygdala and hippocampal activation and later memory performance can be abolished by the administration of the beta-blocker propranolol, thus supporting the necessity of catecholamine release for an emotional memory enhancement (Roozendaal et al., 2006b; Strange and Dolan, 2004).

In addition, activation of the HPA axis, which results in a relatively slow release of cortisol, enhanced memory formation for events encoded about 41–65 min after the stressor onset. This

enhancement was associated with the cortisol response to the experimental manipulation. Cortisol acts on a slower time-scale than NE but appears to involve the same pathway, i.e. its effects are mediated by NE in the amygdala (Roozendaal et al., 2006a). For instance, another study demonstrated that amygdala activation in response to emotional stimuli, which could be blocked by the beta blocker propranolol, was particularly strong in participants with high cortisol levels during encoding (van Stegeren et al., 2007). Although our findings suggest that catecholamines and cortisol relate to stress effects that are dissociable in time, this does not preclude the possibility that these stress effects are synergistic actions of both NE and cortisol acting on the same neurons. For instance, the effects of cortisol may be contingent on prior exposure to catecholamines as predicted by previous studies in rodents arguing for interacting effects of cortisol and NE in the basolateral amygdala (Quirarte et al., 1997; Roozendaal et al., 2006b). Future studies may employ pharmacological manipulations, such as beta-adrenergic blockade by propranolol, in order to assess whether the memory enhancing effects of non-genomic cortisol action depend on the prior action of catecholamines.

Our results are generally in line with previous laboratory studies demonstrating enhancing effects of stress on memory formation during and after stressful situations (Domes et al., 2002; Henckens et al., 2009; Sandi et al., 1997; Schwabe et al., 2008). The present findings, however, go significantly beyond previous reports in several ways. For instance, we found that stress boosted memory formation when either noradrenergic activation was high or cortisol levels were clearly elevated and started already to decline. However, we found no effect when ANS activation was fading and cortisol levels were just reaching peak levels, perhaps because it takes some time for cortisol to induce its effects on the brain. Moreover, it is assumed that genomic corticosteroid effects arising sometime after stress would impair memory formation by reducing and normalizing amygdala and hippocampal activity to allow for increased prefrontal cognitive control (Henckens et al., 2010; Joëls et al., 2011; Lovallo et al., 2010; Morsink et al., 2006; Pavlides et al., 1996; Schwabe et al., 2012). For instance, firing activity in hippocampal neurons is decreased some hours after stress (Joëls and de Kloet, 1989) and the induction of long-term potentiation, a crucial mechanism for learning, is hampered several hours after stress exposure (Pavlides et al., 1996). Although we assessed memory formation for events up to 2 h after stress offset, we did not find evidence for delayed, genomic effects hindering memory formation. One possible explanation may be that genomic effects did not appear until even more than 2.5 h after stressor onset. However, this explanation seems unlikely given that other genomic effects have been reported in this time window before (e.g. Joëls and de Kloet, 1989), although differences between species are possible and rodent findings may not easily translate to humans. Another possible explanation may be that genomic effects are somewhat weaker and thus harder to detect with our sample size than rapid, non-genomic effects. Furthermore, genomic effects may not necessarily impair memory formation but aim at reinstalling homeostasis and normalizing brain function (Joëls et al., 2012). Thus, the reversal of enhancing stress effects back to control levels after 1 h may be due to both the decreasing cortisol levels and early genomic effects. Finally, in our study the stress exposure and memory encoding took place within the same larger context, a visit to the zoo. It may well be that genomic cortisol effects only suppress the encoding of new information that belong to a new or sufficiently distinct context (Schwabe et al., 2012). In line with this reasoning, previous studies found enhanced memory formation for stressor-related information (Smeets et al., 2007) even when encoding took place two hours after stress onset, i.e. when genomic effects were considered to be at play (Smeets et al., 2009). Thus, it may be that the learning material has to be clearly unrelated to the stressful situation and/or acquired in a distinctly new context in order to find impairing (genomic) effects on memory formation. This context-dependency of stress effects on memory formation might also explain contradictory findings of decreased memory formation after stress (Elzinga et al., 2005; Kirschbaum et al., 1996; Quaedflieg et al., 2013). With respect to the effects of context, it should be kept in mind that, in contrast to many laboratory studies, the encoding and retrieval sessions in the current study took place in different contexts (the zoo vs. a university laboratory). While this context shift was unavoidable for practical reasons, it may have affected memory retrieval (Schwabe and Wolf, 2009). Although it appears reasonable to assume that this context shift should have affected the stress and control group to a similar extent, this may not be necessarily be the case.

We focused here on stress effects on memory formation which incorporates encoding as well as consolidation processes. Given that we induced a long-lasting stress response prior to encoding that may have also affected early consolidation, we cannot distinguish between stress effects on encoding and consolidation. Although this distinction is theoretically highly relevant and stress is known to exert facilitating effects on consolidation (Cahill et al., 2003; Roozendaal et al., 2006c), our study resembles the real-life situation in which stress effects on encoding and consolidation cannot be separated.

Furthermore, it is well known that the effects of stress on memory can differ depending on the emotionality of the study material (Payne et al., 2006; Schwabe et al., 2008; Smeets et al., 2009; Zoladz et al., 2011). Here, we investigated time-dependent effects of stress on memory for slightly negative to slightly positive material, events encoded during a stressful procedure and a walk through the zoo. An intriguing issue for future studies with high relevance to the clinic will be to investigate how our findings relate to more arousing material such as longer lasting emotional experiences.

Additionally, it should be noted that the participants were explicitly instructed to memorize the events on day 1. Such intentional encoding strategies were often associated with better memory performance and rely on partially different neural systems compared to incidental encoding (Bernstein et al., 2002; Grady et al., 1998). Although it is unclear whether acute stress differentially affects memories encoded incidentally and intentionally, it would be interesting to test in future experiments whether our findings hold for incidental encoding. Furthermore, we employed an interval of one week between encoding and retrieval and found positive associations between ANS and HPA axis activity and memory performance. It should be kept in mind, however, that a previous study found a positive correlation between cortisol levels at encoding and later memory only in participants who were allowed to sleep between encoding and retrieval and not in participants who stayed awake (Bennion et al., 2014). Thus, it may well be that the enhanced memory formation after stress relies at least in part on the interaction between stress modulators and consolidation processes during sleep.

In sum, we set out to assess precisely how memory formation develops in the aftermath of a stressful event in a natural environment. Using a unique approach that allowed us to track the impact of stress on memories for events encoded at specific time points continuously over 2.5 h after stressor onset, we demonstrate that stress may enhance memory formation for events in specific time windows and that these facilitating effects develop in parallel to the action of major stress response systems. Considering that stress is so common in our everyday lives, affecting cognition at work, and in educational and clinical settings, our study takes the important step of demonstrating the existence and time-dependency of stress effects on human memory formation in a natural environment.

Role of funding source

This study was funded by the University of Hamburg, which had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Author contributions

L.S. conceived the study, both authors contributed to the study design. Testing and data acquisition was performed by S.V. Both authors analyzed and interpreted the data. S.V. drafted the manuscript, L.S. provided critical revisions. Both authors approved the final version of the manuscript for submission.

Conflict of interest

None of the authors has any conflicts of interests.

Acknowledgements

We gratefully acknowledge the support of the Tierpark Hagenbeck, Hamburg, Germany. Furthermore, we thank Freba Hamid, Mewes Muhs, Rebecca Nixdorf, Verônica Leal-Nunes, and Sonja Timmermann for their help during data collection.

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