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Short communication

Acute stress negatively affects object recognition early memory consolidation and memory retrieval unrelated to state-dependency

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ABSTRACT

It is well known that stress affects memory performance. However, there still appears to be inconstancy in literature about how acute stress affects the different stages of memory: acquisition, consolidation and retrieval. In this study, we exposed rats to acute stress and measured the effect on memory performance in the object recognition task as a measure for episodic memory. Stress was induced 30 min prior to the learning phase to affect acquisition, directly after the learning phase to affect consolidation, or 30 min before the retrieval phase to affect retrieval. Additionally, we induced stress both 30 min prior to the learning phase and 30 min prior to the retrieval phase to test whether the effects were related to state-dependency. As expected, we found that acute stress did not affect acquisition but had a negative impact on retrieval. To our knowledge, we are the first to show that early consolidation was negatively affected by acute stress. We also show that stress does not have a state-dependent effect on memory.

Acute stress exposure results in the activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of catecholamines and glucocorticoids respectively [1–3]. After release, glucocorticoids (GCs), cortisol in humans and corticosterone in rodents, can bind to two types of GC receptors, which are differentially distributed in the central nervous system. The mineralocorticoid receptor (MR) has a high affinity for GCs and is mostly located to limbic regions, whereas the glucocorticoid receptor (GR) has a low affinity for GCs and is mostly located to sub-cortical and cortical regions. Both of the receptor types are highly expressed in the hippocampus [4]. Obviously, the effects of GC release on the central nervous system are dependent on the level of the GCs and the proportion of MRs and GRs saturated.

Acute activation of the MR facilitates acquisition and extinction processes, whereas the GR is essential for consolidation and detrimental for retrieval of declarative/episodic memory [5]. Additionally, studies have shown involvement of players other than GCs in different memory processes, including vasopressin and its vasopressin 1b receptor [6], cannabinoid receptors [7], and the endogenous opioid system [8]. The understanding of the effect of acute stress on the different memory processes, i.e. acquisition, consolidation and retrieval, has grown over the recent years [9,10]. However, there is still a lot of inconsistency in literature, which has a lot to do with the differential effects that acute stress can have. In fact, a lot the effects of acute stress on different memory processes depends on the type of stress, the intensity of the stress, and the context of the stressor [9,11]. Furthermore, it is unknown whether the effects of acute stress on memory are the result of a state-dependent effect, which has been shown for other treatment conditions (e.g. [12]).

A study by Li et al. already elaborated on the effect of acute restraint stress on the different stages of memory [13]. However, it remains unclear whether there is an effect on early consolidation, and whether the effects found can be related to state-dependency. In order to further explore the effects of acute stress on episodic memory, and whether these effects are related to state-dependency, we used the object recognition task (ORT) in rats with acute stress induced at different time points to affect either acquisition, consolidation or retrieval. A state-dependent effect was assessed by inducing stress before the first as well as before the second trial. Furthermore, we established a forgetting curve to determine the optimal inter-trial interval for measuring the effects of acute stress on the different stages of memory, and in particular early consolidation.

The object recognition task was performed as described previously [14,15]. In brief, in a first learning trial (T1), the rat is allowed to explore a circular arena with two identical objects for 3 min. After an interval of 3 h, the rat is placed back into the arena, with one of the objects replaced by a novel object (T2), and is again allowed to explore for 3 min. Exploration time for each object is recorded, and the main

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outcome measure is the discrimination index (d2), which is the difference in exploration time between the novel and familiar object, divided by the total exploration time. All animals were first habituated to handling, the arena, and the objects, and all animals were trained until a stable forgetting curve could be obtained ranging from 1 h to 24 h intervals (see [16] for full details). We applied a 3 h inter-trial interval based on the forgetting curve, since this was the longest interval where a reliable memory performance could be observed in order to assess possible negative impact of stress. Approximately 100 µl of blood was taken to measure corticosterone levels at baseline, 30 min after acute stress exposure, and 45 min after stress exposure. Blood samples were collected via saphenous vein puncture using a heparin-coated tubes and were kept on ice and subsequently centrifuged at 5000 rpm for 10 min at 4 °C, after which the plasma was frozen down to 80 °C for subsequent determination. All samples were run in duplicate using a commercially available RIA kit for rat corticosterone from MP Biomedicals (Corticosterone I^{125} for rodents, MP Biomedicals). The stress conditions were compared with a virtual group that shows no discrimination (d2 = 0) and a standard error mean (SEM) that corresponds to the average SEM (0.065) of independent samples in previous studies [17], in order to determine whether the stress impaired memory. A one-way ANOVA with a Dunnett post-hoc test was used to compare the conditions with the virtual group. Comparisons between corticosterone time points were done using a one-way repeated measures ANOVA with a Dunnett post-hoc test. Comparisons between exploration times were done using a one-way ANOVA with Bonferroni post-hoc test.

The study was approved by the local Animal Ethical Committee according to governmental guidelines. Acute stress was induced in twenty-four three-month-old male Wistar rats (310–390 grams) by placing the animals in a standard type 3 Makrolon cage filled with 2 cm of water (20 °C) for 5 min. To affect acquisition processes, we administered the stress 30 min before T1. Stress was administered immediately after T1 to affect the consolidation processes. To affect retrieval processes we administered the stress 30 min before T2. Finally, we examined the state-dependency of these effects by administering the stress 30 min before T1 and before T2. For unstressed conditions, again twenty-four three-month-old male Wistar rats weighing 310–390 grams were used. The animals were housed individually in standard type 3 Makrolon cages with sawdust bedding in an air-conditioned room, with a reversed light/dark cycle of 12/12 h (lights off from 5:00 to 17:00). Exploration times were scored manually by an experimenter who was blinded to the experimental conditions.

A forgetting curve was established, which showed that the length of the inter-trial interval had a significant effect on memory ($F(4,115) = 4.629$; $P < 0.01$). Animals were able to remember the familiar object after a 1 h ($P < 0.001$) and 3 h ($P < 0.001$) inter-trial interval, but not after 5 h or 24 h (Fig. 1A). This indicates that the animals were properly habituated to the arena and the objects, and the 3-h inter-trial interval was the maximal interval to examine possible detrimental effects of stress in the different stages of memory. Corticosterone levels were increased three-fold ($F(1.609,16.09) = 18.22$; $P < 0.001$) compared to baseline at 30 min ($P < 0.001$) and 45 min ($P < 0.001$) following acute stress exposure (Fig. 1B), suggesting that the stress induction was successful. Total exploration times for T1 (e1) and T2 (e2) were significantly different (e1 $F(4,115) = 21.61$; $P < 0.001$; e2 $F(4,115) = 40.38$; $P < 0.001$). For the unstressed condition, e1 = 27.9 ± 2.5 s ($P < 0.05$ compared to all stressed conditions) and e2 = 24.9 ± 1.6 ($P < 0.05$ compared to all stressed conditions). When exposed to acute stress 30 min before T1, e1 = 11.0 ± 1.0 and e2 = 10.8 ± 1.1; when exposed to acute stress directly after T1, e1 = 11.9 ± 1.0 and e2 = 11.3 ± 0.9; for acute stress 30 min before T2, e1 = 12.1 ± 1.1 and e2 = 12.9 ± 1.0; and when exposed to stress 30 min before T1 and before T2, e1 = 39.4 ± 5.3 ($P < 0.001$ compared to the other stress conditions) and e2 = 31.6 ± 2.3 ($P < 0.001$ compared to the other stress conditions). Under unstressed conditions, animals were able to remember the familiar object ($P < 0.001$), and similar results were found when animals were exposed to acute stress 30 min before T1 ($P < 0.05$), suggesting that acquisition processes were not affected upon acute stress (Fig. 2A). A one-way ANOVA showed that stress had a negative effect on memory ($F(4,115) = 4.494$; $P < 0.01$). When animals were stressed immediately after T1, or 30 min before T2, animals were unable to remember the familiar object ($P = 0.233$ and $P = 0.834$ respectively, compared to the virtual group), suggesting that both memory consolidation and retrieval were negatively affected by acute stress exposure (Fig. 2A). The effects of acute stress were not caused by a state-dependent effect, since stress 30 min before both T1 and T2 also resulted in animals forgetting the familiar object (Fig. 2B; $P = 0.696$ compared to the virtual group).

The current study showed that 5-min, acute stress exposure increased corticosterone levels three-fold, and negatively affected memory consolidation and retrieval. No effects were found for acquisition, which is consistent with previous literature [13]. The effects of acute stress on retrieval are consistent with many other studies (for an in-depth review, see [18]; for a meta-analysis, see [9]), and the first study already dates back to 1998, when de Quervain and colleagues found that stress prior to long-term memory retrieval negatively affected spatial memory [19]. Based on our forgetting curve, we used a 3-h inter-trial interval to measure the effects of stress on acquisition, consolidation, and retrieval. Early consolidation is known to last until 3 h after the learning phase, whereas late consolidation only starts after 3 h. Given the length of our inter-trial interval, we are most likely measuring the effects of acute stress on early consolidation. Therefore, the results indicate that acute stress negatively affects early consolidation, and to our knowledge we are the first to show this. Additionally, given the length of the inter-trial interval, it cannot be excluded that the effects found on retrieval when animals were exposed to acute stress 30 min before T2 may also be the result of an interference with the late phase of early consolidation.

Total exploration times for T1 and T2 were significantly higher for the group receiving stress before T1 and T2 compared to unstressed conditions, whereas e1 and e2 were lower for all other groups compared to the unstressed group. A difference in exploration times is unexpected and may be the result of environmental factors. However, a

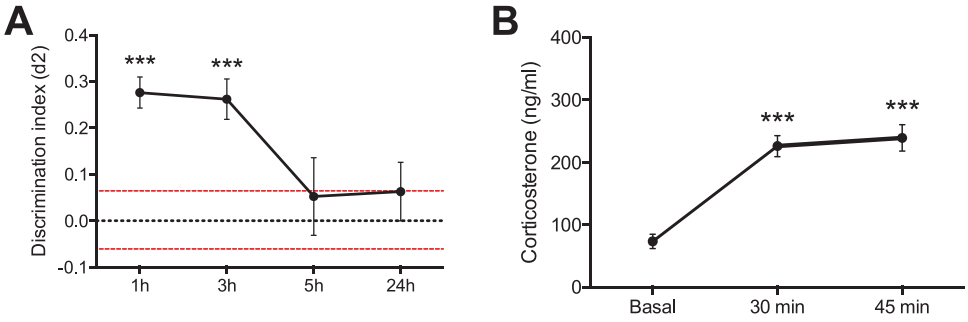

Fig. 1. (A) Forgetting curve depicting memory performance of the animals in the object recognition task (ORT) after several inter-trial intervals under unstressed conditions. Area between red dashed lines indicates the SEM range of the virtual group showing no discrimination (mean: 0, SEM: 0.065). Asterisks represent statistical significance from the virtual group (one-way ANOVA using a Dunnett's post-hoc test). ***$P < 0.001$, n = 24. (B) The effects of acute stress on corticosterone levels measured 30 min and 45 min after stress exposure. Asterisks represent statistical significance (one-way repeated measures ANOVA, Dunnett's correction for multiple comparisons) compared to basal corticosterone levels. *** $P < 0.001$; n = 12. All data are represented as mean ± SEM.