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Dissociating effects of acute photic stress on spatial, episodic-like and working memory in the rat

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Highlights

- We compare acute photic stress effects on three memory dependent tasks.
- Focus on the normalization phase of the stress response.
- Spatial memory performance was detrimentally affected in a spatial water maze task.
- Object-location deficits but no temporal-object impairments were observed in an episodic-like memory task.
- Rats' performance was unaffected in an operant delayed matching-to-sample task.

Abstract

Adaptively responding to acute stress has been of great importance for human and animal survival. However, for our species, stress-related disorders are putting an ever-increasing burden on healthcare systems. It is thus crucial to understand the basic processes and cognitive changes associated with acute stress. Here, we examined the effects of acute stress exposure on spatial (water maze) and memory (delayed match to sample and episodic-memory-like tasks) performance. We found striking performance deficits in stressed animals navigating in the water maze. We also found, in an episodic-like memory task, striking object-location deficits, but not in temporal-object association learning in stressed animals. Finally, no differences were apparent for any delay periods (up to 30 s) in a delayed match to sample task. Taken together, these results show a strong differential effect of acute stress on differing memory processes.

Keywords

Acute stress; spatial memory; episodic memory; DMTS; Water maze; Reference memory

1. Introduction

The physiological response to acute stressors – the acute stress response – serves a variety of adaptive responses. However, a sustained stress response may cause a variety of deleterious effects. Estimates for the US alone suggest work-related stress costs relate to $150 billion p.a. in lost productivity, absenteeism, poor decision-making, stress-related mental illness, and substance abuse [34]. Many studies have shown deleterious effects of stress, especially of an acute nature on cognition in humans and rodents alike (for review see [1], [2], [3] and [4]). Here we examine the effects of acute stress on a variety of cognitive processes, in order to better understand how stress may differentially affect differing functions. We focus in particular on the normalization phase of the acute stress response, where the brain is thought to recover from the acute stress exposure (for review, see [1]). Our aim was to characterize in detail the behavioral changes associated with this phase by introducing a 30 min break after the stress exposure, and before testing animals in the various memory tasks.

Stressors applied before learning impair spatial memory performance (24 h post-training) in the water maze (MWM) and the radial arm water maze (RAWM) [5], [6] and [7]. In all three studies the duration of the stressor was between 30 min and 1 h but for both Diamond et al. [5] and Park et al. [7] training trials started immediately after the stress ceased, whereas in

Kims' study there was a delay of 4–5.5 h. Similarly, a mild elevated platform stress (30 min), in very young rats (3–4 weeks old), showed retrieval impairments 30 min after training [8]. Interestingly, the same study reported no effects with a short (15 s) foot-shock protocol. A 2 min predator exposure did not show memory deficits when learning started 30 min after the stress resulted however in a large memory enhancement when immediately introduced prior to the learning trials [9].

Testing episodic-like memory in rodents is more challenging than spatial memory [10]. Based on results presented in [11] we adapted their protocol to test episodic-like memory. The protocol is based on a combination of an object-recognition task and a temporal memory task to introduce episodic-like events where the animal is tested for object recency and object–place associations. The concept behind this protocol is that the rats' spontaneous exploration behavior favors novelty [11], [12] and [13]. Kart-Teke et al. [11] have shown that rats demonstrate "what, where, and when" memory based on object preference exploration patterns. In their experiments rats recognized previously explored objects and remembered their order of appearance. For example rats spent more time exploring an "old familiar" object relative to a "recent familiar" object. This led to the conclusion that the animals recognized previously explored objects and remembered their order of first appearance. Further, rats preferred a displaced "recent familiar" object, compared to a stationary "recent familiar" object, while the exact opposite was observed for the displaced and stationary "old familiar" objects suggesting an integrated memory for objects, place and time. In an additional set of experiments they applied a mild injection stress 30 min before training during the sample trials, which abolished any differences in object exploration. Thus this task provides us with the opportunity to extend our understanding of higher-order memory processes with a strong spatial component.

Delayed matching (or non-matching)-to-sample tests have been extensively used to assess working memory in rodents [14], [15], [16] and [17]. Animals are generally presented with a sample stimulus during the sample phase and after a certain retention interval have to remember the matching (DMTS) or the non-matching (DNMTS) stimulus of the sample trial. Although, several publications have reported prominent working memory performances in humans under high stress conditions (e.g. [18] and [19]) there is to the best of our knowledge no report of acute extrinsic stress effects on rodents involving working memory tasks such as the DMTS.

Here we systematically compare performance on these spatial, episodic-like and short-term memory tasks. We predict that there will be differential patterns of sensitivity to the recovery phase after exposure to an acute stressor.

2. Materials and methods

2.1. Animals

A total of 76 male Hanover Wistar rats (B&K, United Kingdom), weighing between 420 and 465 g at the start of the experiment, were used for the study. Rats were housed in pairs in a temperature-controlled laminar airflow unit and maintained on a 12:12 light–dark cycle (08:00–20:00 h). All tests were carried out between 12:00 and 16:00 o'clock. Rats received food and water ad libitum. Experiments were carried out in accordance with regulations laid out by LAST Ireland and were compliant with the European Union directives on animal experimentation (European Community Council Directive 86/609/EEC).

2.2. Stress protocol

For all behavioral experiments rats were randomly assigned to either the stress group(s) or the control group. Rats assigned to the stress group(s) underwent a 30 min light stress exposure. This mild stress consisted of 30 min of exposure to bright light (~120 CD) in a small round bucket. After 30 min of bright light exposure, rats were allowed another 30 min of rest before training or testing in the respective experiments was started. The use of photic stress was based on earlier work in the lab [20] which demonstrated that this technique reliably induced a stress response. This mild-to-moderate stressor was chosen over more extreme stressors as being more naturalistic (as compared to electric shock for example). A settling period of 30 min was introduced so the normalization phase in respect of the stress response timeline would have been initiated [21].

2.3. Water maze task

Before training in the water maze commenced, rats (n = 16) were habituated for three days to the experimenter for 10 min per animal per day. The black tank for the water maze, which was 1.5 m in diameter, 40 cm in height, was filled with water (30 cm, ~22 °C). A black curtain (with two big white cues) around the water tank was used to separate the recording environment from the rest of the room and room lights were dimmed to allow continuous and noise-free tracking of the animals. Rat movements were tracked with EthoVision 3.0 software (Noldus, NL) via a camera mounted above the tank.

The main training protocol consisted of five days of learning trials with semi-random starting position and a fixed hidden platform submerged about 1.5 cm below the water surface in the southwest quadrant of the tank. A trial consisted of 60 s where the animal was allowed to search for the platform (if the animal did not find the platform it was guided to the target platform), a 15 s period where the animal was allowed to remain on the platform and finally 30 s in a holding box near the tank before the next trial started. Rats were introduced into the tank always facing the wall and allowed to slide into the water. After four trials per day, the rats were dried with a towel and kept in a heated environment for a short time before being placed back into their home cages.

The number and location of the start positions were chosen to ensure near equal length to the platform and that the animal was not memorizing specific routes but was orienting itself via the distal cues provided [22]. For the probe trial, the platform was removed from the tank and the animal was introduced once for 60 s, in the northeast quadrant, into the tank to search for the supposed location of the platform. To ensure any differences in performance were not due to visual deficits caused by the light stress in the stress group, a separate visual cue session was undertaken on the next day. For this session, the platform was reintroduced in the previous location (SW) in the tank with a white flag attached (~30 cm × 30 cm) and the animals were started four times in each of the four starting positions. After a one-day break, a three-day reversal training scheme was started. In short, the platform location was placed in the opposite quadrant (NE) and the starting positions were reversed accordingly (see Fig. 1B).

FIGURE 1 here

2.4. Episodic-like memory task

The open-field environment (90 cm × 90 cm × 35 cm, black painted wooden walls) was indirectly illuminated by four 60 W bulbs. A black curtain surrounding the experimental set-up prevented other visual cues present in the room being seen by the animals. Two distal visual cues were attached to the black curtain to allow reliable and consistent spatial orientation for the animals. A video camera mounted above the center of the environment was used for recording behavior; the samples were stored to allow offline analysis. Between each of the trials the objects, the arena as well as the floor, were cleaned thoroughly with an alcohol-based cleaning solution to extinguish any odors present from previous trials. Two distinct sets of objects were used for the task. One set of objects was identical white non-transparent bottles with a smooth surface and a plastic top. They were 20 cm in height with a base diameter of 10 cm. The other set was a stack of identical Duplo® pieces with a rectangular base (7 cm × 7 cm) and of similar height as the bottles (~20 cm). Duplo® pieces assembling the stack had different colors (red, green, blue) and extended twice further than the actual size of the base. To ensure that objects could not be knocked over by the animals during exploration, they were stuck with Blutack® to the floor. The objects had no known ethological significance for the rats and had never been paired with a reinforcer. For habituation purposes the rats (n = 32) were handled for three days and were allowed to explore the empty arena for 5 min each. Generally the behavioral task paradigm closely adhered to Kart-Teke et al. [11]. Each rat received two sample trials and a test trial. Rats were always introduced into the center of the arena and allowed to explore the environment freely for 5 min. In the first trial four objects of the same type (A) were placed in the arena at set locations (see Fig. 1C). After the rats had finished their 5 min of exploration they were given a 60 min break before the second trial. The second trial was identical to the first one, except that four novel objects (B) were present. Two of these objects were placed in two other locations, which did not contain objects during sample trial one. The particular objects presented on trials 1 and 2 were randomly determined for each rat. After 60 min rats received the last trial (test trial) for 5 min. This probe trial was identical to the sample trials, except that two objects from trial 1 ("old familiar" objects, A1) and two objects encountered from trial 2 ("recent familiar" objects) were presented. One of each object was presented in a new position, whereas the other objects remained at their previous position.

In order to assess the effects of stress on contrasting memory and learning phases during the task we designed a protocol stressing animals at different time points throughout the procedure. The first stress group was exposed to light 60 min before entering trial 1 (PreT1 group) supposedly interfering already with the acquisition of object and place memory. The second stress group (PreT2) was exposed immediately after trial 1 to photic stress interfering with consolidation of trial 1 learning as well as with later stages of the protocol from that moment on. The third group (PostT2) was stressed immediately after Trial 2 thus interfering with the consolidation of Trial 2 learning and the behavior during the Test Trial (see Fig. 1C).

The time spent by animals exploring the object was scored offline using the recorded video files for each rat. Exploration time was scored, when the rat actively approached the objects and had further contact with the objects. As the objects were distant enough from the corners and the walls it allowed the rat to fully circle the arena and around the objects, this circling behavior was not counted as active exploration of the objects even if unintended contact might have been established between the objects and the rats. If rats were rearing upon the objects it was counted as active exploration as it was interpreted as an interest in the upper parts of the object rather than climbing efforts by the rat (as due to the texture and height of the object this was unlikely).

2.5. Delayed match to sample task

In total 12 rats were trained in a commercially available three-lever operant system from Med Associates (VT, US). Two retractable levers were mounted on the front wall, and one retractable lever was mounted on the back wall. Between the two front levers a food hoper was fitted where sucrose pellets (TestDiet, USA) were delivered when the task criteria were correctly fulfilled (see Fig. 1A). The boxes were individually contained within a sound attenuated box (Med Associates) to reduce distraction during the task and a small lamp within the box provided enough light during the task. Operant boxes were connected to an interface system (Med Associates) which was in turn connected to the PC to allow data storage and analysis via Med Associates Med-PC® program.

Before behavioral training started, rats were habituated to the experimenter for 5 days – 10 min each. During this time animals were food-restricted to reduce their weight to 85% of their original ad libitum weight. After rats reached the weight criterion, the delayed-matching to sample (DMTS) training protocol started. First, rats were trained for two days to lever press for food reward on a continuous reinforcement schedule, i.e. pressing any lever would result in the delivery of a sucrose pellet to the hopper. On the subsequent two days the levers were retracted once pressed, delivering a pellet and then extending again to allow animals to habituate to the sound of the retracting levers. This protocol was also run on a continuous reinforcement schedule. To extinguish lever biases, levers which were pressed three consecutive times would, after the 5th day, not give any more pellets forcing the animal to press a different lever. The next phase of training involved a randomized presentation of the left or right lever. Once one of those levers was pressed, it retracted and the back lever was presented. A pellet was only delivered if this back lever was pressed. In addition, a 10-s inter-trial interval was initiated before the next trial. This procedure was repeated for two days. Once rats finished the training program successfully, rats started with the basic DMTS task. At the start of each session the house light was turned on with the levers in the retracted position. Animals were initially trained on the task contingencies with no enforced delay between the sample and the choice component (0-delay condition). At the start of each trial, one response lever was randomly selected and inserted into the chamber. As soon as the lever press response was registered, the lever was retracted and the rear lever on the opposite wall was extended. Once the response on the back lever was registered the two front levers were inserted into the chamber together. A response on the matching lever to the sample lever was designated correct, the levers were retracted, a pellet was delivered to the hopper, the house light remained on and an inter-trial interval of 10 s was initiated before the next trial began. A response on the non-matching sample lever resulted in an incorrect response, the levers were retracted, no pellet was delivered, the house light was extinguished and the 10-s interval was initiated before the next trial started. Rats were required to meet a criterion of 75% for three consecutive days on this program before the delay category was introduced. In the next stage of training a randomized 1–5 s delay was introduced between the response on the sample lever and the extension of the rear lever. In the final stage of training the random delay was extended to a maximum of 30 s, requiring the rat to wait for the extension of the rear lever before moving to the choice phase Three criteria had to be fulfilled before animals were allowed to undergo the stress protocol: (i) the overall performance had to be above 75% for three consecutive days; (ii) the correct response for all delay categories (1–5 s, 6–10 s, 11–15 s, 16–20 s, 21–25 s, 26–30 s) had to be above 65% for three consecutive days; (iii) at least 90% of all required trials had to be completed for three consecutive days.

2.6. Statistical analysis

In the water maze task overall performance over the training protocol between the two groups (control vs. stress) was analyzed via a two-way repeated measure ANOVA. Comparison of days between groups was performed with the Bonferroni post hoc test. For the cue trial and the probe trial, Lillieferos normality testing returned a non-normal distribution of the data. Those data were tested with a non-parametric Mann–Whitney *U* test. In the episodic memory task statistical analysis followed the original paper of [11]. Within-group differences between objects pairs were analyzed with the Wilcoxon signed rank test. In the DMTS test a two-way repeated measure ANOVA was used to compare performances between groups. Thereafter, a pairwise Student–Newman–Keuls post hoc test was run to assess the performance difference for each interval between the two regimes (control vs. stress) for each delay category. All statistics were calculated using the Statistical Package for the Social Sciences (SPSS) 16 software and SigmaPlot v.11.

3. Results

3.1. Water maze task

The stress group performed significantly worse than the control group for latency and distance traveled to reach the target platform over the training period. A two-way repeated measures ANOVA with training condition (control vs. stress) as the between-group measure and acquisition session as the within-group measure revealed a highly significant difference for the latency ($F_{[316]} = 17.7$, $p < 0.001$) and distance to platform ($F_{[316]} = 16.5$, $p < 0.001$). There was no significant interaction for distance to platform ($F_{[316]} = 1.77$, $p = 0.147$) or latency ($F_{[316]} = 1.35$, $p = 0.135$). In addition, no overall difference for the velocity was found between the two groups ($F_{[316]} = 3.629$, $p = 0.057$). With regard to individual performance days, the stress group showed a significant performance drop in the latency to reach the platform on days two ($t_{[63]} = 2.23$, $p = 0.029$) and five ($t_{[63]} = 3.23$, $p = 0.002$) (see Fig. 2A).

FIGURE 2 here

During the probe trial (no platform present) stressed rats spent less time in the target area ($Z_{[15]} = -2.31$, $p = 0.02$). Further, analysis of the mean number of crossings into a target field (three times the diameter of the platform) revealed that stressed animals crossed the target area less than their control counterparts ($Z_{[15]} = -2.12$, $p = 0.035$). Interestingly, a one-way ANOVA analysis revealed a significant difference in the four quadrant analysis for the control animals ($F_{[28]} = 17.4$, $p < 0.001$) but not for stressed animals ($F_{[28]} = 5.3$, $p > 0.05$). Controls spent a significantly greater amount of time in the goal quadrant over the NW ($p < 0.001$), the SE ($p = 0.005$) and the NE ($p = 0.002$) quadrants. In contrast, no difference between the goal quadrant and any other quadrant was found for the stress group (SW–SE: $p = 0.69$; SW–NE: $p = 0.10$; SW–NW: $p = 0.44$) (see Fig. 2B).

The cue trial showed no significant differences between the stress and control groups ($Z_{[15]} = -1.55$, $p = 0.16$). Similarly, in trial two, performance did not significantly differ ($Z_{[15]} = -1.17$, $p = 0.278$) between groups either. The three-day reversal protocol revealed no differences in performance measured in distance toward the platform or latency to reach the platform between the groups (two-way repeated measure ANOVA) or between groups when compared against each other on each day (Student–Newman–Keuls post hoc test).

3.2. Episodic-like memory task

15 s of total object exploration was set as the threshold value for each trial; otherwise, the rat was excluded from analysis. Less than 15 s of total object exploration was recorded for one subject in the control group, two rats in the PreT1, none in the PreT2 and 4 in the PostT2 group. This resulted in an $n = 11$ in the control group, 10 in the PreT1 group, 12 for PreT2 and 8 for the PostT2 group. Importantly, following the original findings of Kart-Teke et al. [11], control rats showed the expected exploration pattern (see Fig. 3A and B). First, rats displayed a significantly higher preference of object A1 (old stationary) over object B1 (recent stationary) ($Z_{[10]} = -2.22$, $p = 0.026$) arguing for a temporal-object memory. Further, a significant difference ($Z_{[10]} = -2.48$, $p = 0.0128$) was found for object exploration between the two recent objects (B2 > B1). Thus, animals showed an increased preference for the recently displaced object versus the recent stationary object, indicating a memory for object–place association. In contrast to Kart-Teke et al. [11], we did not find significant exploration differences between A1 and A2, indicating the older memory no longer discriminates between displaced and stationary objects. PreT1 stressed animals showed a preference for A1 over B1 ($Z_{[9]} = -2.19$, $p = 0.028$), similar to controls, in contrast to controls over B2 ($Z_{[9]} = -2.09$, $p = 0.037$) (see Fig. 3C). Hence, there was no difference between exploration of two recent objects in the PreT1 group ($Z_{[9]} = -0.86$, $p = 0.386$). This loss of B2 > B1 exploration argues for a loss of object–place memory. Furthermore, there was a difference between A2 and B2 ($Z_{[9]} = -2.8$, $p = 0.005$).

FIGURE 3 here

In the PreT2 group, preference for A1 over B1 was preserved ($Z_{[11]} = -2.67$, $p = 0.007$) but, no preference of B2 over B1 was found ($Z_{[11]} = -0.76$, $p = 0.44$), arguing for a deficit in object–place memory, but an unaffected temporal-object memory (see Fig. 3D). Similarly, the PostT2 group showed significant exploration for A1 > B1 ($Z_{[7]} = -2.24$, $p = 0.025$) and B2 ($Z_{[7]} = -2.24$, $p = 0.025$), but not B2 over B1, revealing a deficit for object–place memory, but intact temporal-object memory (see Fig. 3E). Stress after T2 did not affect the processing and consolidation for object B, otherwise, we would expect a much higher exploration for both B objects.

For both sample trials one (one-way ANOVA: A1: $F_{[140]} = 1.46$, $p = 0.24$; A2: $F_{[140]} = 0.75$, $p = 0.55$, A3: $F_{[140]} = 0.4$, $p = 0.75$, A4: $F_{[140]} = 0.53$, $p = 0.66$) and two (B1: $F_{[140]} = 0.49$, $p = 0.69$; B2: $F_{[140]} = 0.15$, $p = 0.923$, B3: $F_{[140]} = 0.24$, $p = 0.86$, B4: $F_{[140]} = 0.32$, $p = 0.81$) no difference between explorations, for each object between the groups, was found indicating that merely object position was not a factor for increased or decreased object exploration.

3.3. Delayed match to sample task

10 of 12 animals reached the performance criterion 75% correct trials and were allowed in the study. Animals continued to perform the task without a significant change in performance for any of the delays, after stress (see Fig. 4). Repeated measures ANOVA revealed no significant overall difference between treatments ($q = 1.01$, $p = 0.5$); the Student–Newman–Keuls post hoc test did not reveal any significant differences for any delays. Interestingly, however, the average time needed for a trial increased significantly after stress exposure ($t = 4.26$, $p = 0.003$).

FIGURE 4 here

4. Discussion

We find striking performance decrements on the water maze and the episodic-like memory task but not the working memory task after exposure to acute stress.

4.1. Water maze task

Stressed animals displayed a striking performance decrease compared to controls during training. When retention was tested 24 h later in a probe trial, stressed animals showed marked deficits in performance, assessed by numbers of crossings of the target area, the duration spent in it and the latency to reach the target area. Moreover, quadrant analysis revealed that the controls discriminated the goal quadrant from the other three quadrants. Importantly, both groups did not show differences when performing in the cued water maze task. Hence, it is a safe assumption that the rats did not experience deficits in vision because of light exposure. Stressed rats did not show a significant decrease during the reversal trials. One likely explanation is that animals acclimatized to the bright light exposure and its stressful nature for the rats was decreased. However, we do see a similar difference in the learning curve slopes (R1–R2) as during the original training days (D1–D2) between the two groups. Yet performance levels do not differ significantly between groups. This raises the question about how much stress interferes with reference memory (understanding the principle of the task) which may contribute to the original deficit observed.

Nevertheless, these results fill an important gap in literature as the only two studies which investigated spatial working memory in the normalization period of the stress response (e.g. after a 30 min delay between stressor and training) had crucial differences in their experimental variables [9] and [23]. In [23] retention was not tested after 24 h preventing direct comparisons between the studies. Further, in [9] only a very short predator stress was applied, which may produce opposing effects compared to longer exposures to the same stressor (see [5] and [9]). Yet, both studies reported near normal memory performance. Furthermore, in both studies the strain of the animal and the valence of the stressor were different, though we mainly accredit the discrepancies across studies to the timeline of stress duration and testing.

4.2. Episodic-like memory task

Importantly, control animals obeyed two main original predictions. First, the results show an increased preference of the recently displaced object versus the recent stationary object (see [11]). In contrast, stressed animals failed to differentiate between both objects, which is most apparent in the PostT2 and PreT1 groups. Hence, all three stressed groups showed a remarkable deficit in object–place association. Interestingly, contradicting earlier results [11] the temporal object association was intact in stressed animals. This temporal-object element is presented by the increased exploration of A1 over B1 as seen in our control animals. Whereas, in their study, a single injection stress of saline 30 min prior to sample trial one (comparable to PreT1) has abolished any differences, our results do present a preserved temporal-object association after 30 min of photic stress. Thus the divergent effect of acute stress only shows an impairment of the place–object association while leaving the temporal-object association unaffected. The valence of the stressor might explain the differences in stress results with regard to the study by Kart-Teke et al. [11].

4.3. Delayed match to sample task

During the DMTS operant task neither did we observe a between treatment effect nor a performance change for any of the delay category observed. This is an intriguing finding as a functioning hippocampus has been shown to be important in the long delays during working memory tasks [24]. One explanation could be that animals might employ strategies or postural mediations to solve the task without fully exploiting their working memory capabilities. The possibility of body alignment toward the future correct response was first raised by Gutnikov et al. [25]. However, as one of our operant boxes was fitted with a camera and allowed visual inspection of the animals' behavior during the delay period we observed postural mediation very infrequently. The failure to induce any effect on the DMTS sample test is striking. Stressed animals did require significantly more time on average to solve a trial compared to their regular performance under control conditions. One possibility would be that animals tend to start more slowly than normally increasing the total time needed for the trials.

4.4. Overall discussion

Our results demonstrate a clear segregation of the effects of acute stress during the normalization phase between spatial and working memory and temporal-object memory. Rats showed a striking impairment in the spatial memory components of both the water maze and the episodic-like memory task. They depicted normal behavioral performance during the working memory task and the temporal-object association of the episodic-like memory task. Although we did not directly test the involvement of certain brain regions it appears that, based on the observed results, memory processes which require a high amount of hippocampal activation are highly susceptible during the normalization phase to acute stress. Spatial reference memory as tested in the water maze and object–place associations as tested in the episodic-like memory task have been extensively linked to the hippocampus [26], [27], [28], [29] and [30]. Temporal order memory and working memory appear to depend mainly on an intact prefrontal cortex [31] and [32]. This network appears to be mostly spared during our behavioral tasks by the effects of the stress episode during this normalization phase. There is some ambiguity to what extent the hippocampus is involved in the DMTS task as recent publications only attributes major hippocampal contribution to long-delay performances. Presumably, other brain areas may be able to absorb some processes and functions shadowing effects within behavioral tasks [33] in cases were milder stressors are experienced and hippocampal activation may not be substantial (e.g. the DMTS task).

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FIGURE 1

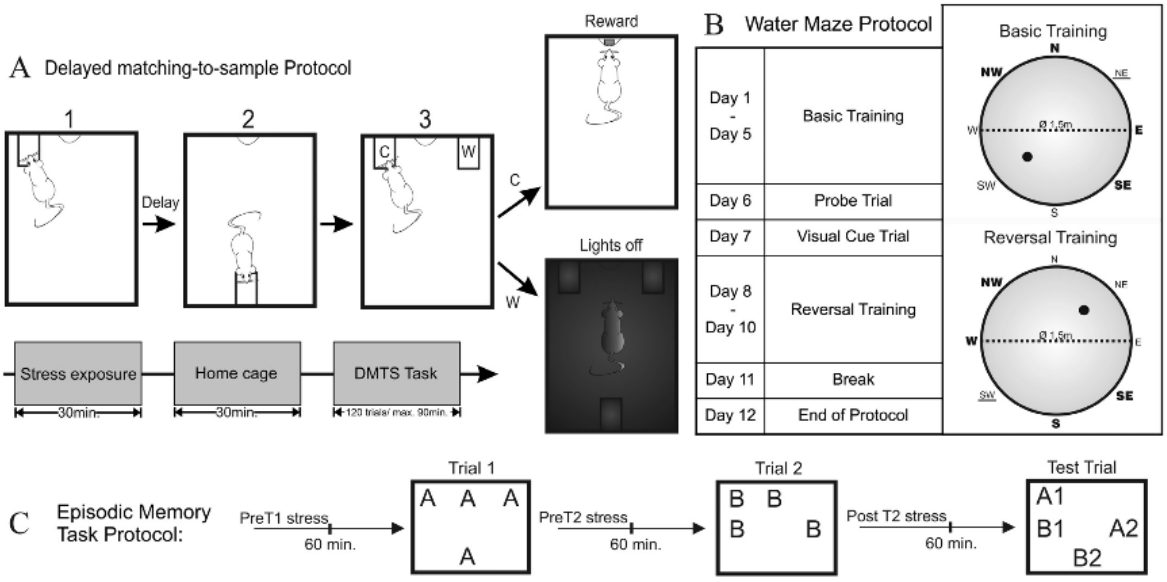

Fig. 1.

(A) Schematic drawing of the DMTS task in the operant boxes. In this example the rat has to press the left presented lever (1) and after a random delay between 1 and 30 s it has to press the back lever (2). In the next step the rat is presented with both front levers. If the rat presses the correct left lever (c) it will receive a sugar pellet as reward but if the animals fails to remember its previous press and chooses the right lever (w) the lights go off and no sugar pellet will be delivered. Below is a timeline of the operant stress protocol presented. Control trials (DMTS task only) were never performed on the same day as a stress trial. (B) The left table shows the timeline of the water maze protocol. Animals were stressed before each training day for 30 min via the photic stress procedure and were allowed another 30 min of rest before the trials started. The graphs on the right depict the protocol and set-up of the water maze. Bold letters indicate starting positions; the black circle indicates the position of the platform. The underlined NE area depicts the entry point of the probe trial. (C) Schematic drawing of the episodic-like memory protocol. A refers to the “old” objects familiar from trial 1, B refers to the “recent” objects from trial 2. The numbers indicate if the object was displaced (2) or presented in the familiar location (1). Hence, A1 is referred to as “old familiar stationary”, A2 is “old familiar displaced, B1 is “recent familiar stationary” and B2 is “recent familiar displaced”. Trial duration is 5 min each and inter trial interval is 60 min.

FIGURE 2

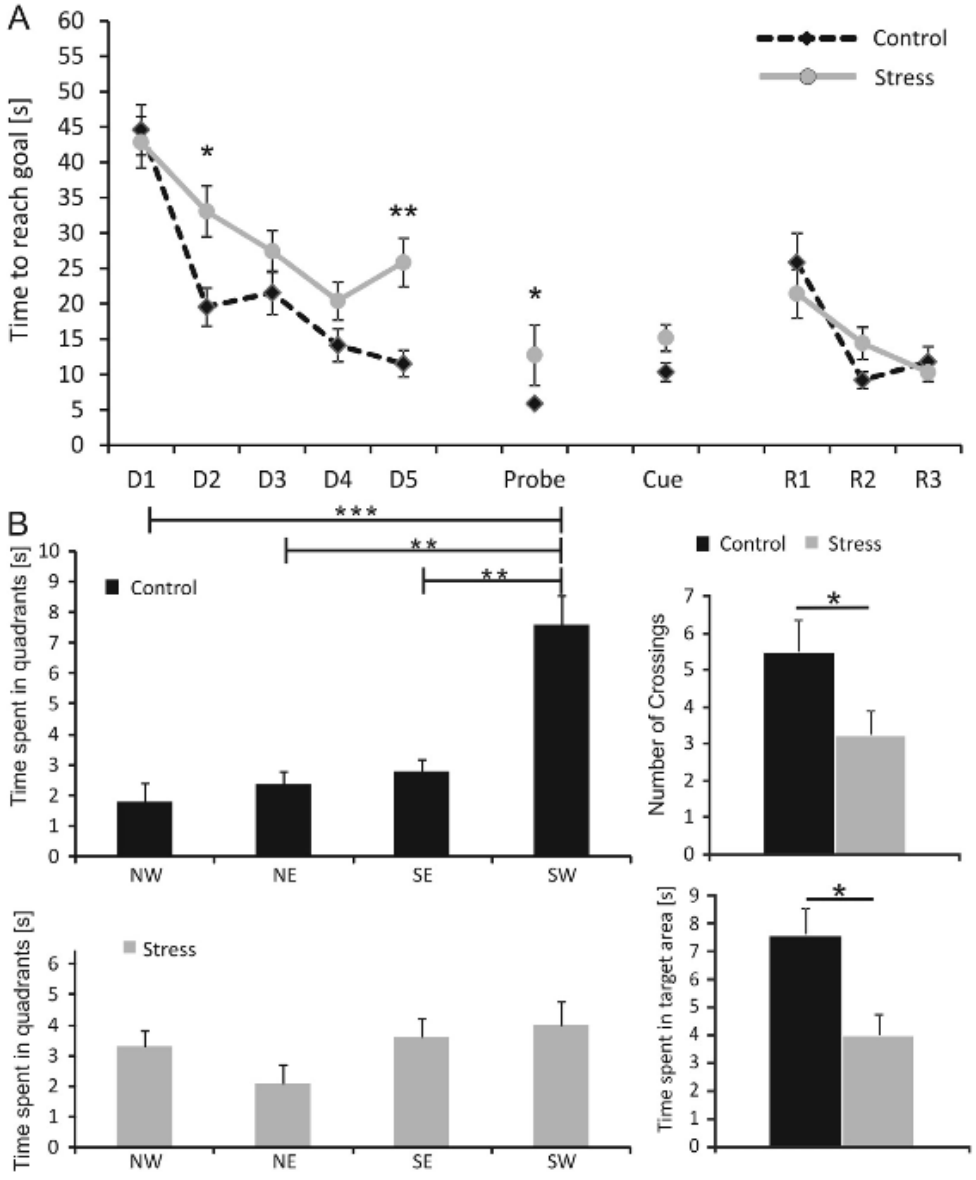

Fig. 2.

(A) Latency to reach the platform over all experimental days; the black dotted line shows the performance expressed in latency to reach the platform site over the whole protocol for the control group. Gray continuous lines represent stress group. For the probe trial, latency to reach the area covering twice the platform size was used as a measure of performance. Basic training: D1–D5; reversal training R1–R3; data presented as mean ± SEM; (B) detailed probe trial results; the left two diagrams show the time spent in the respective quadrants during the 60 s probe trial. SW represents the target quadrant and NE the opposite (=starting) quadrant. Control animals (black bars) show clear distinction of the target quadrant over any other quadrant whereas stressed animals (gray bars) fail to do so. The two right diagrams present other forms

of visualization of the probe trial results. Stressed animals (gray bars) show less crossings through the target area (three times platform size) and spend significantly less amount time within this target area. Data presented as mean + SEM; $n = 8$; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

FIGURE 3

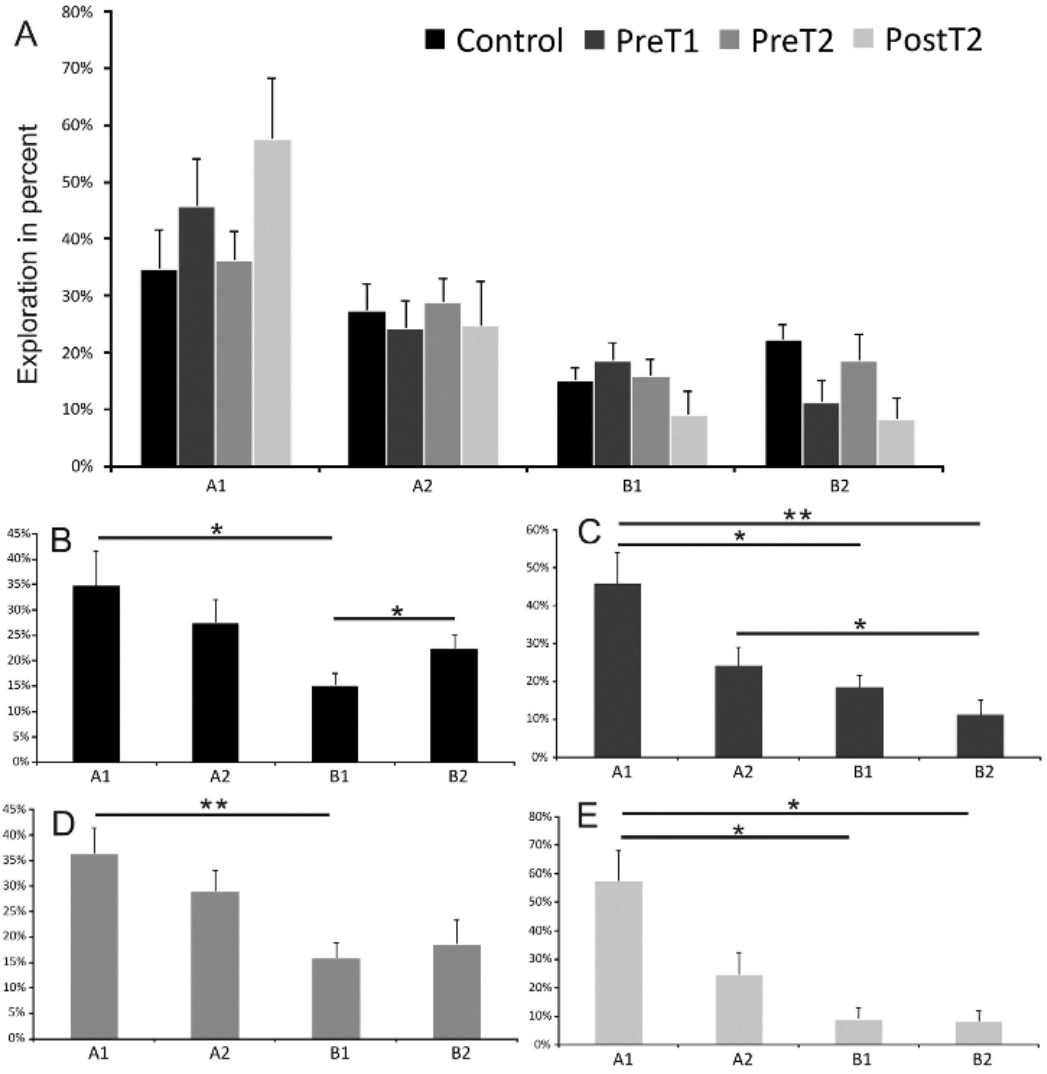

Fig. 3.

Episodic memory task results: the chart displays the mean exploration in percent of all objects between the groups in the test trial (A). For ease of reading, no significant differences are shown in the top graph. Black bars denote the control group; A1 = “old familiar stationary” object, A2 = “old familiar displaced” object, B1 = “recent familiar stationary” object and B2 = “recent familiar displaced” object. The different shades of gray depict the results of the stress group. Below (B–E) the data is separated into each group with the respective significance. Control animals show an increased interest of B2 over B1 indicating the expected object–place memory. However this memory trace disappears in any of the stress groups; the temporal-object element of the task is presented by the increased exploration of A1 over B1 which appears to be independent of stress exposure. Data presented as mean + SEM, $n = 11$ for control group, $n = 10$ for PrT1, $n = 12$ for PreT2, $n = 8$ for PostT2, $*p < 0.05$, $**p < 0.01$.

FIGURE 4

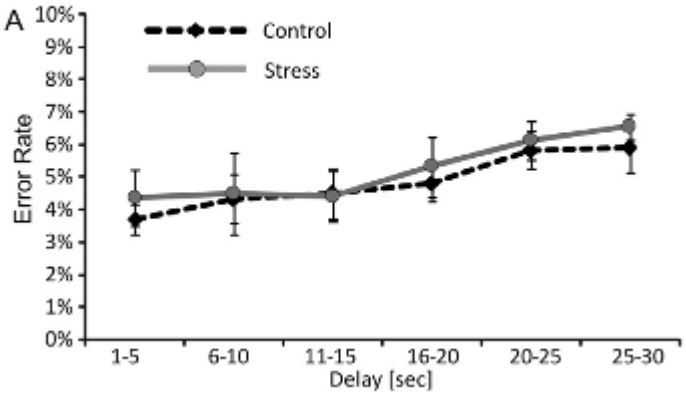

Fig. 4.

DMTS performance: error rate (no. of errors/total number of lever presentations) over the different grouped time delays during the probe trial. Control animals (black dotted line) perform nearly identical to the stressed animal group (gray line). Data presented as mean ± SEM, n = 10 per group.