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Research report

Post-training self administration of sugar facilitates cognitive performance of male C57BL/6J mice in two spatial learning tasks

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ABSTRACT

Spatial memory can be strengthened by adverse stimuli that activate the stress system, and administration of the stress hormone corticosterone in close-context with the learning task. Less is known about modulation of spatial memory by post-training positive reinforcers (reward). Cognitive performance was assessed in male C57BL/6J mice using two learning tasks: the water maze (WM) and circular hole board (CHB). Sugar was chosen as a post-training reinforcer. We expected that the free access to sugar immediately (0 h) after training would facilitate spatial memory; delayed access to sugar (4 h after training) or no sugar served as controls. In both tasks, 0 h sugar mice showed superior performance, indicated by shorter latencies and distances to the trained spatial location. The memory facilitating effect of sugar became visible at distinct times during training: on the CHB from the first trial onwards, in the WM on training days 4 and 5. Sugar-rewarded mice kept their superior performance during the free exploration/swim trial, expressed by more persistent search strategies for the exit hole or platform. Post-training sugar reward in close-context with performance strengthens memory via modulation of consolidation. This finding supports the integrative theory of reinforcement and memory. We suggest that our experimental set-up will allow to differentiate between direct effects on memory and alterations in reward processes in animal models of stress-related diseases.

1. Introduction

Memory formation is modulated by task-inherent appetitive and aversive characteristics. Other stimuli occurring in close context with the task either impair or enhance memory [8,19]. Decades ago, Huston and colleagues presented a memory processing theory of reinforcement, proposing that the reinforcer acts on a memory of the response or of the stimulus-response contiguity [13,14]. It has provided a framework for studies that have demonstrated a close correspondence between memory promoting and reinforcing effects of natural reinforcers like food, but also of electrical and chemical stimulation of the brain [15].

Here we address the effect of a post-training natural reinforcer on cognitive performance in two spatial learning paradigms in mice: the well known and commonly used water maze (WM) [23] and the circular hole board (CHB) [4]. Both tasks have been originally designed for rats. Mice prefer dry-land over wet mazes [26,27,29]. For mice, the degree of the task-inherent aversive characteristics differs largely [29], in parallel with the activation of the stress system and secretion of glucocorticoid hormones [9,16]. For example, increasing the aversiveness of the task, like lowering the water temperature in the water maze, increases the secretion of the stress-hormone corticosterone and results in memory improvement in rats [2,25]. Injections of corticosterone have comparable effects on memory (for review [16]). The WM is regarded as life-threatening while the CHB is considered to be less (or not) aversive, as the animal walks to locate a hole leading to its home cage. Thus, modulation of the adverse components of a task facilitates learning and memory processes (e.g., lowering water temperature, increasing strength of electric shock in fear conditioning paradigms [24,25]). In contrast, memory facilitating effects of positive rewarding stimuli are less well studied. Using plain food as reinforcer, requires prior food deprivation of the subjects which is a stressor itself, known to change circadian corticosterone secretion and glucose levels [17,18]. Mice like sweets, so we decided to give the mice free access to glucose (sugar corns) as reinforcer.

It is well known that glucose facilitates cognitive performance and that peripheral glucose administration improves memory in

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aversive and appetitive tasks. In mice, glucose has always been administered via invasive techniques like intraperitoneal injections [22]. An intraperitoneal injection is an acute stressor, resulting in increased heart rate, body temperature and elevated concentrations of corticosterone [6,21]. As described above, stressors given in close context with a task have memory facilitating effects [16].

To dissect the rewarding properties of the post-training stimulus from interference with the stressful method of application, male C57BL/6J mice got free access to sugar in their home cage, either immediately post-training (0 h) or 4 h after the last training trial of the day. Separation of the rewarding stimulus in-time from the training event controls for general metabolic effects [8,19]. We expect that (1) post-training self administration of glucose will reinforce memory resulting in superior cognitive performance and (2) the pattern of memory facilitation will be task-dependent.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (3 months; $n = 44$) were purchased from Charles-River laboratories. Upon arrival at the animal facilities (LACDR, University of Leiden, The Netherlands), mice were single housed and transported to the experimental room to acclimatize for 2 weeks before the start of the experiment, in a temperature ($21 \pm 1\,^{\circ}C$) and humidity ($55 \pm 5\%$) controlled room; food and water *ad libitum;* 12:12 h light-dark cycle (lights on at 07:00 h). All experiments were performed between 09:00 and 14:00 h. Experiments were approved by the Local Committee for Animal Health, Ethics and Research of the University of Leiden. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

2.2. Experimental design

Separate groups of mice were used in the two spatial learning tasks. Water maze (WM; $n = 8$/group): (1) post-training self administration of sugar in close context (0 h-sugar), i.e., immediately upon return to their home cage; (2) post-training self administration of sugar out of context (4 h-sugar), i.e., 4 h after the last daily training trial in home cage as control for possible metabolic effects of sugar and (3) controls, i.e., no-sugar. The WM program started with a free swim trial, followed by 4 days of spatial training and finished with another free swim trial 3 days later. Circular hole board (CHB; $n = 10$/group): (1) post-training self administration of sugar in close context (0 h-sugar); i.e., immediately upon return to their home cage and (2) controls, i.e., no-sugar. The CHB program started with a free exploration trial, followed by 4 days of training and finished with another free exploration trial three days later.

Behaviour was recorded on videotape and analyzed with Ethovision 1.97 (Noldus Information & Technology BV, Wageningen, The Netherlands). The software sampled the position of the mouse 5 times per second. To calculate the distance walked on the CHB, the minimal distance between samples was set at 3 cm.

2.3. Self administration of sugar

Mice were familiarized with the sugar corns before WM and CHB training started. A feeding cup (2.5 cm × 2.3 cm) was glued to the bottom of the home cage in the corner opposite to the nest. During the week before training started, mice got free access to sugar 3 times (30 mg sugar corns; every other day). At 09:00 h, the grid of the cage was lifted, the sawdust was removed from the feeding cup, and sugar was placed in the cup. Mice consumed all the sugar within 10 min. Mice remained in their home cage and were not handled during the administration procedure. Following the last training trial of the day, mice had free access to sugar in their home cage either immediately (0 h-sugar) or delayed (4 h-sugar), after having located the platform in the WM or exit tunnel in the CHB, or when the maximum trial duration had expired.

2.4. Water maze

Three days before spatial training started, the white pool (140 cm diameter, side walls 50 cm high) was filled with 2 cm of warm water ($26 \pm 1\,^{\circ}C$). This was the mouse's first contact with water and it was allowed to walk around for 120 s.

2.4.1. Training trials

The pool was filled with warm water ($26 \pm 1\,^{\circ}C$; ±25 cm deep) and made opaque by the addition of chalk. A platform (8 cm diameter) was situated 0.5 cm below the surface of the water, invisible for the mouse. The ratio between the surface area of the pool and the platform was 270:1. The mouse was placed in the water at one of four possible equally spaced release points. A maximum of 60 s was allowed, during which the mouse had to find the platform and climb onto it. If the mouse did not find the platform itself it was guided there using a grid (20 cm × 6 cm). Mice remained on the platform for 15 s. Animals were run sequentially with an inter-trial interval of approximately 10 min. After each trial, mice were placed under a red-light warming lamp for 3 min. A free swim trial preceded and followed the spatial training trials (platform was absent: FST-before: 120 s; FST-after: 60 s).

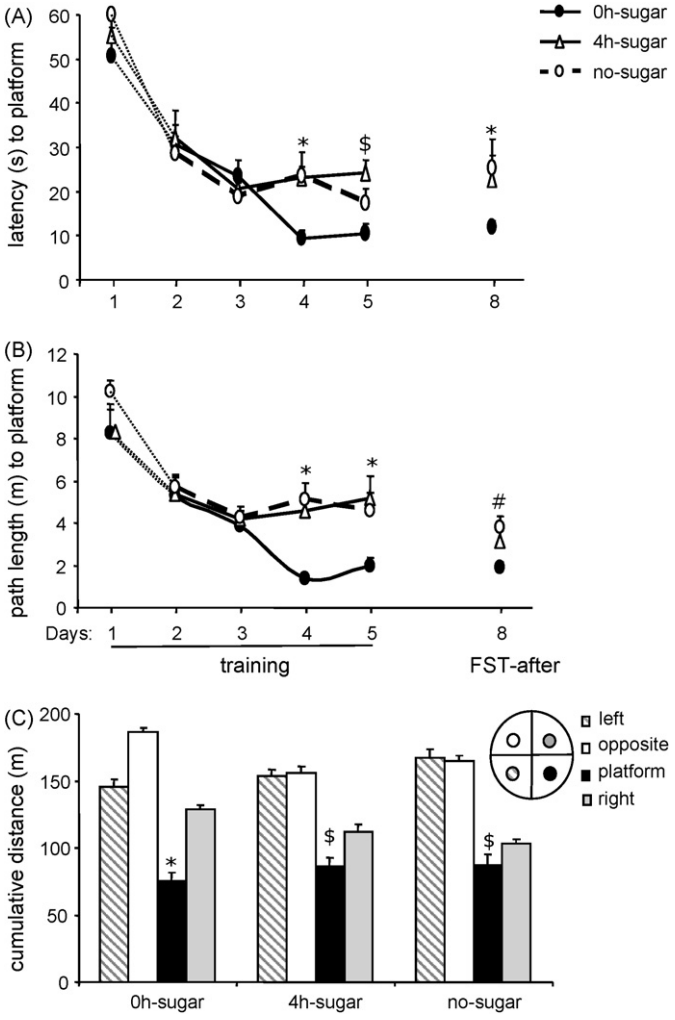

Fig. 1. Water maze: (A) latency in seconds (s) and (B) distance swum in meters (m) to the platform during spatial training trials on day 1 (1 trial), days 2 and 3 (4 trials), days 4 and 5 (3 trials). For the free swim trial after training (FST-after; day 8), latency and distance are calculated based on the first visit of the former platform location. Mice consumed sugar in their home cage immediately after the last training trial of the day (0 h-sugar) or 4 h later (4 h-sugar) or no-sugar. (C) Free swim trial after spatial training: Cumulative distance in meters to the former platform location (black bar) and virtual platform locations in adjacent and opposite quadrants (see inset). Less distance indicates more specificity towards the platform location. Data represent mean ± S.E.M. (A, B) $p < 0.05$ * 0 h-sugar vs. 4 h- and no-sugar groups; $ 0 h-sugar vs. 4 h-sugar group; # 0 h-sugar vs. no-sugar group. (C) $p < 0.05$ * platform location vs. the three virtual platform locations; $ vs. left and opposite virtual platform locations.

2.4.2. Schedule and procedure

Day 1 started with FST-before, which allowed estimation of the swimming ability and to determine the pre-training exploratory strategy. One hour later, the first spatial training trial took place. On consecutive days, mice received four trials on days 2 and 3, followed by three training trials on days 4 and 5. Spatial training thus consisted of 15 trials over five consecutive days. Three days after the last spatial training trial, goal-directed search strategy was assessed in FST-after (day 8).

Spatial training trials were analyzed for: latency (s) and distance swum (m) to climb on the platform, swim speed (cm/s), cumulative distance to platform (m). To allow comparison, both free swim trials were analyzed for the first 60 s. General activity was represented by total distance swum (m) and velocity (cm/s). Swim patterns were quantified on: time spent in platform quadrant (percentage), latency (s), crossings (number) and cumulative distance to former platform location, relative to

Table 1
Latency to platform in seconds during the first trial of the day for the 0 h-sugar, 4 h-sugar and no-sugar groups.

	Water maze: the first trial of the day			
	Day 2	Day 3	Day 4	Day 5
0 h-sugar	48.9 ± 7.2	25.3 ± 9.1	9.3 ± 2.9*	6.7 ± 1.9*
4 h-sugar	45.1 ± 7.2	24.9 ± 6.1	21.6 ± 6.3	16.9 ± 5.1
No-sugar	46.3 ± 9.0	29.0 ± 8.4	22.1 ± 7.7	18.8 ± 6.4

Data are presented as mean ± S.E.M.; * $p < 0.05$ vs. other groups, same day.

the other possible three positions [7,10]. Thigmotaxis was expressed as time spent (%) close to the wall (RIM zone = 10 cm).

2.5. Circular hole board

2.5.1. Apparatus

The circular hole board is a revolvable white Plexiglas plate (diameter: 110 cm) with 12 holes (diameter: 5 cm) at equal distance to each other, 10 cm from the rim. It was situated 1 m above the floor. In the original circular hole board set-up [4] bright light and loud noise were used as aversive stimuli to motivate the animals to search for the exit. We performed the task under dim light conditions (120 lux on the surface of the board), in a quiet surrounding and with numerous distal cues in the room which allowed spatial orientation. The holes on the CHB could be closed by a lid at a depth of 5 cm. Whether a hole was open or not could be recognized by the mouse if it put its head over the edge of the hole. If open, the hole provided access to the home cage of the mouse via an s-shaped 15 cm long tunnel (diameter: 5 cm). Mice were 'pre-trained' to climb through the tunnel 3 times every other day. This was performed in the week preceding familiarization to sugar corns, during weighing of the mice.

2.5.2. Training trials

Before each trial started, the board was swept clean with 1%HAc. Next, the board was turned clock- or anti-clockwise until the randomly determined hole was at the fixed location of the exit (spatial training). The home cage was placed underneath the exit tunnel (not visible for the mouse), and the mouse was placed in a non-transparent cylinder (PVC, diameter 10 cm, 25 cm high) at the center of the board. After 10 s the cylinder was lifted and the mouse could explore the board. There was just one open hole during spatial training trials which was at the same location in all trials. As a control for possible odor cues, we turned and cleaned the board between trials, and placed the home cage underneath the tunnel, opposite to the exit hole, during the free exploration trial after training (FET-after). A free exploration trial preceded and followed the spatial training trials (all holes closed; FET-before: 300 s; FET-after: 120 s).

2.5.3. Schedule and procedure

Day 1 started with FET-before, which allowed to determine the pre-training exploratory strategy. After 5 min of exploration the animals were guided using a grid (20 cm × 6 cm), to the exit tunnel that they would need to search for during spatial training. Upon entering their home cage, they had free access to sugar (30 mg). Spatial training was given on days 2–5: one exit hole was accessible in a fixed position. Mice received two trials per day with an inter-trial-interval of 15 min. If the mouse did not find the exit hole within 120 s, it was guided there by a grid. Three days after the last training, FET-after (exit hole closed) was performed to determine whether spatial learning had altered the exploration into a goal-directed search strategy.

Spatial training trials were analyzed for latency (s), path length (m), velocity (cm/s) and time (s) before leaving the start area in the center (diameter 30 cm). For the analysis of FET-before and FET-after, the CHB was divided in several zones of interest: (i) total arena: path length, velocity, (ii) start center: latency to leave center, percentage time spent, (iii) holes zone: latency hole area, hole visits, percentage time spent near exit and left/right adjacent hole, (iv) RIM zone: path length, velocity of moving, latency to RIM, percentage time spent. The latency (s) and path length (m) to the location of the hole used during spatial training were measured. The search strategies are described as *perseveration*: i.e., repeated visits of the same hole or alternately visiting two neighboring holes, and *serial*: i.e., more than two holes visited in sequence; calculated in relation to the total number of hole visits. A hole visit was detected if the animal had at least its nose over the rim of the hole. Detections by the image-analysis system were additionally cross-checked with manual protocols. To compare behaviour during free exploration trials we analysed both trials 120 s.

2.6. Statistical analysis

Data were subjected to ANOVA (factors: time, condition: 0 h-sugar, 4 h-sugar and no-sugar), when appropriate with repeated measures followed by a post-hoc *Tukey*-test. Time in quadrants and platform crossings of the free swim trials were analysed with Friedmans Analysis of Variance (FR: per group) and Wilcoxon test (W: within group). Other parameters were compared with Student's *T*-test. We lost the data of the CHB 4 h-sugar group due to computer problems. Data are presented as mean ± S.E.M. Significance was accepted at $p < 0.05$.

3. Results

3.1. Water maze: spatial training

All mice learned to locate the platform as indicated by a decrease in latency (Fig. 1A) and path length (Fig. 1B) to platform over days (latency: $F_{(4, 84)} = 52.508$; $p = 0.001$; distance: $F_{(4, 84)} = 29.014$, $p = 0.001$), with significant differences between the groups (latency: $F_{(2, 21)} = 5.145$; $p = 0.015$; distance: $F_{(2, 21)} = 5.706$; $p = 0.01$). Mice with post-training sugar administration in close context (0 h-sugar) had the shortest latencies and distance to platform from day 4 onwards (compared to the 4 h-sugar group on days 4, 5 and 8: $p < 0.01$; no-sugar group on days 4 and 8: $p = 0.01$). After day 3, the course of performance in latency and distance differed significantly between the groups ($F_{(8, 84)} = 2.397$, $p = 0.022$). While the latency and distance to platform continued to decrease in the 0 h-sugar group, it remained at the same level in the other two groups. Swim speed remained constant over the course of training and did not differ between the groups (data not shown). Interestingly, the latencies to platform of the first trial on days 4 and 5 were significantly lower in the 0 h-sugar mice than in the 4 h-sugar and no-sugar controls (Table 1). The performance in the other training trials of the day was variable. Performance in trials within 1 day (trial-to-trial performance) did not differ between the groups.

3.2. Water maze: search strategies during free swim trials before and after spatial training

Before spatial training, mice of all groups behaved comparable regarding total distance swum, swim velocity and percentage time spent in RIM zone (Table 2a). After spatial training, on day 8, general activity between groups was again similar, but the 0 h-sugar group was more active than before training (paired *T*-test: distance and velocity, $p = 0.033$). All groups spent less time in the RIM zone of the pool, indicating a shift in their swim strategy towards the open area of the pool where the platform was positioned during spatial training.

Table 2a
General activity expressed as path length swum, swim speed and percentage of time spent along the wall (RIM) in the water maze during the free swim trials before and after spatial training.

	0 h-sugar		4 h-sugar		No-sugar	
	Before	After	Before	After	Before	After
Path length (m)	9.3 ± 0.4	10.1 ± 0.4#	9.4 ± 0.5	10.4 ± 0.9	10.2 ± 0.4	11.1 ± 0.6
Swim speed (cm/s)	15.6 ± 0.7	16.8 ± 0.9#	16.3 ± 0.9	17.4 ± 0.9	17.1 ± 0.7	18.6 ± 1.0
% Time in RIM	59.9 ± 4.4	31.1 ± 3.8#	58.0 ± 2.7	21.1 ± 2.8#	65.2 ± 4.7	33.1 ± 8.3#

Data are presented as mean ± S.E.M.
# $p < 0.05$ within group.

Table 2b
Free swim trial after spatial training: latency to and crossing of the former platform position; increase in percentage of time spent in the platform quadrant (free swim trial before = 100%).

	0 h-sugar	4 h-sugar	No-sugar
Latency (s)	11.8 ± 1.7*	22.7 ± 5.6	25.3 ± 6.6
Crossings	3.6 ± 0.4$	2.9 ± 0.6	2.1 ± 0.5
% Time spent in platform quadrant	236.3 ± 22.0*	124.1 ± 23.3	182.6 ± 16.0

Data are presented as mean ± S.E.M.; $p < 0.05$; * vs. other groups; $ vs. no-sugar.

Spatial training altered the search strategy (Table 2b): latency to the former platform location was shortest in 0 h-sugar mice and their time spent in the platform quadrant was longer than in the other two groups. The number of platform crossings increased from FST-before to FST-after (paired *T*-test: $p < 0.05$), but did not differ between the groups. All groups directed their behaviour towards the area of the platform location, but it was most specific for mice of the 0 h-sugar group. They spent more time near the platform location, indicated by: (i) the lowest cumulative distance (Fig. 1C; Friedman $p < 0.05$ vs. the virtual platform locations in the other three quadrants) and (ii) the increase in percentage of time spent in platform quadrant compared to FST-before (Friedman-Wilcoxon $p < 0.05$ vs. other quadrants). Also 4 h-sugar mice had a significant lower cumulative distance to platform vs. the other three virtual locations. The no-sugar controls had a similar low cumulative distance to the platform and one virtual adjacent platform location, indicating less specificity of search patterns.

3.3. Circular hole board: spatial training trials

Latency and distance to the exit tunnel differed significantly between groups (Fig. 2; main effect latency: $F_{(1, 18)} = 19.652$, $p = 0.001$) with significantly shorter latencies for the 0 h-sugar group from days 2 to 5. In both groups, latency and path length decreased over days (latency: $F_{(3, 54)} = 36.148$; $p = 0.001$; distance: $F_{(3, 54)} = 4.053$; $p = 0.011$), indicating learning of the task. Velocity of movement increased accordingly ($F_{(3, 54)} = 20.689$; $p = 0.001$). Mice left the start area faster. This was group dependent (interaction time × condition: $F_{(3, 54)} = 4.749$, $p = 0.005$). On days 2 and 3 of training, mice of the 0 h-sugar group had significantly shorter latencies to leave the start ($p < 0.001$) than no-sugar controls.

The slope and course of the learning curve for latency, path length and velocity over days was comparable between groups (interaction time × condition: latency, $F_{(3, 54)} = 0.370$, $p = 0.774$; distance, $F_{(3, 54)} = 0.316$, $p = 0.814$; velocity, $F_{(3, 54)} = 1.494$, $p = 0.226$).

3.4. Circular hole board: spatial training trials

In addition to the mean daily performance, trial-to-trial performance within the day (short-term/working memory) revealed distinct differences. The first trial of the day of the 0 h-sugar mice had the shortest latencies to the exit tunnel (Fig. 3A; trials with odd numbers: trial 1, $p = 0.012$; trial 3, $p = 0.004$; trial 5, $p = 0.016$; trial 7, $p = 0.046$). The second trial of the day was always comparable to the no-sugar control group. In the 0 h-sugar mice, time to leave the start center was significantly lower for trials 1 and 3 (all $p < 0.01$; Fig. 3B). While mice of the 0 h-sugar group have similar velocities in the first and second daily trial and keep their velocity constant from trial 1 to 7, no-sugar mice have lower velocity in trials 1 and 3 ($p < 0.05$; Fig. 3C) and increase their velocity during their second trial of the day above the 0 h-sugar mice ($p < 0.05$ trials 4 and 6). Path length was not significantly different between the trials (Fig. 3D).

3.5. Circular hole board: general activity, exploration and search strategies

Before spatial training, the behavioural response, i.e., sum of analysed parameters, on the circular hole board was similar between groups (MANOVA: $F_{(14, 5)} = 1.281$; $p = 0.420$). After spatial training, the behavioural response was not only different from before training, but also between groups (MANOVA: $F_{(14, 5)} = 6.635$; $p = 0.024$; Table 3). Now, both groups were more active (increase in path length, velocity, total hole visits) and left the start centre quicker resulting in shorter latencies to the hole and RIM zones (all $p < 0.05$). The 0 h-sugar mice had the lower latencies to leave the start center ($p = 0.002$), to make the first hole visit ($p = 0.002$) and arrive at the RIM zone ($p = 0.040$). In both groups, the use of the perseveration strategy dropped dramatically from about 70% to 30%, while the use of the serial strategy increased from about 20% to 80% (both variables $p < 0.01$). In addition, time spent near the exit hole and adjacent holes increased specifically for the 0 h-sugar group from FET-before to FET-after (208.7 ± 21.4%; paired *T*-test, $p = 0.001$) and was significantly higher than in no-sugar controls (134.9 ± 20.6%; $p = 0.023$).

4. Discussion

Post-training sugar reward facilitated the cognitive performance of mice in two spatial learning tasks: the water maze and the circular hole board (CHB). The memory facilitating effects are expressed in a task-dependent pattern.

Table 3
General activity parameters measured on the circular hole board during the free exploration trials before and after spatial training.

		0 h-sugar		No-sugar	
	Parameter	Before	After	Before	After
Total	Path length (m)	4.2 ± 0.7	7.7 ± 0.3#	3.4 ± 0.4	6.7 ± 0.5#
	Velocity (cm/s)	9.1 ± 0.7	13.2 ± 0.3#	7.3 ± 0.6	13.4 ± 0.5#
Center	Latency to leave center (s)	3.7 ± 0.5	1.7 ± 0.1*#	4.7 ± 0.4	4.3 ± 0.7
	% Time	3.5 ± 0.6	2.0 ± 0.3	5.0 ± 0.4	4.4 ± 0.9
Holes	Latency to hole area (s)	7.9 ± 0.7	3.4 ± 0.2*#	10.5 ± 1.7	6.3 ± 0.8#
	Hole visits (number)	12.6 ± 1.6	28.7 ± 1.6#	9.3 ± 1.1	23.2 ± 2.4#
	% Time near exit and adjacent holes	100%	208.7 ± 21.4*#	100%	134.9 ± 20.6#
RIM	Path length (m)	0.9 ± 0.3	0.7 ± 0.1	0.6 ± 0.2	0.5 ± 0.1
	Velocity (cm/s)	7.0 ± 0.7	10.1 ± 0.6	5.4 ± 0.6	8.9 ± 0.9
	% Time	23. 8 ± 4.3	11.3 ± 1.4#	28.1 ± 5.0	12.8 ± 1.6#
	Latency (s)	23.0 ± 4.7	14.9 ± 2.9*	30.7 ± 8.0	27.0 ± 4.7
Search pattern	% Serial hole visits	20.6 ± 6.7	80.4 ± 4.1#	21.0 ± 6.5	80.6 ± 5.4#
	% Perseveration of hole visits	70.9 ± 4.4	30.9 ± 3.7#	63.8 ± 3.1	32.5 ± 5.6#

Data are presented as mean ± S.E.M.; $p < 0.05$; *between groups; # within group.

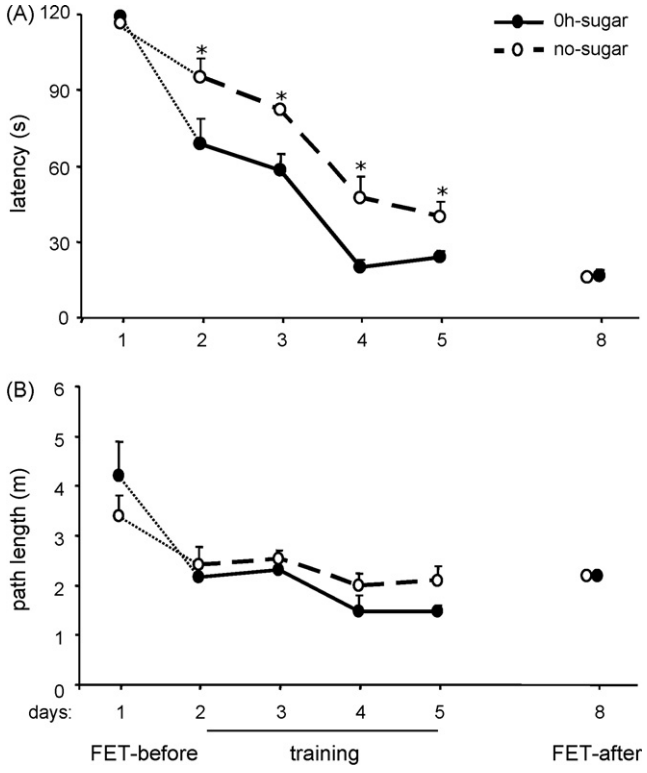

Fig. 2. Circular hole board: (A) latency in seconds (s) and (B) distance walked in meters (m) to the exit hole during spatial training trials on days 2, 3, 4, 5 (2 trials per day) and during free exploration trials (FET) before (day1) and after (day 8) spatial training. Mice had received sugar in their home cage immediately after passing through the exit hole at the end of the FET-before and each day after the last training trial (0 h-sugar) or no-sugar. Latency and path length during FET: FET-before indicates the distance walked during 120 s; FET-after indicates the latency and distance to the first visit of the exit hole. Data represent mean ± S.E.M. * $p < 0.05$ between groups.

4.1. Post-training sugar reward and cognitive performance in the water maze and circular hole board

The WM and the CHB were originally designed for rats [4,23]. Studies comparing the behaviour of rats and mice in the WM and CHB reported that the WM is less suited for testing spatial learning and memory in mice [26,27,29]. Dry-land mazes like the CHB, take into account the predominant dry-land activity of mice and their aversion of water. Our data support that task-inherent properties differentially affect cognitive performance. For example, within-day performance largely varied for mice trained in the WM, while the exit hole was always faster located on the second trial of the day in the CHB task. Both tasks provide behavioural parameters related to general activity, and possible emotional and motivational states. However, the CHB contributes more data for short-term memory, emotional and motivational processes [12] than the WM paradigm. Of course, the choice of the spatial learning task should be hypothesis driven. Modulation of consolidation was achieved by allowing mice free access to sugar in the home cage after the last training trial of the day. As expected, sugar reward in close-context with training (immediately, but not 4 h later) facilitated memory in both spatial tasks, albeit within different time domains.

In the WM, the effect of sugar reward was expressed in latency and distance to platform from the fourth day of training onwards, i.e., after 12 trials, when 0 h-sugar rewarded mice swam shorter distances to locate the exit platform during the first trial of the day. The superior performance was still expressed in the free swim trial 3 days following the last spatial training. These mice were more precise in navigating towards the previously learned location of the platform, spent most time around the former platform location, i.e. behavioural persistence. We may argue that memory for the platform location has been strengthened and/or it is less susceptible to extinction in the free swim trial. Swimming speed as indicator for increased motivation to reach the platform is less likely as it was comparable between groups. Out-of context rewarded mice (receiving sugar with a 4 h delay) behaved more similar to no-sugar mice, further underlining the importance of close-context reward and its effect on consolidation. We conclude that post-training sugar in close-context results in improved performance via modulation of consolidation processes.

In the CHB task, memory improvement by sugar-reward was evident already on the first training day. How is this possible? The free exploration trial before training, is actually the first sugar-rewarded trial: At the end of the free exploration trial, mice are guided to the exit hole, enter their home cage and get free access to sugar. The superior performance was maintained over the course of training. Whereas the learning curves for both groups run in parallel, sugar-rewarded mice reach their maximum performance on day 4, while control mice are still improving. Remarkably, sugar-rewarded mice had shorter latencies in the first trial of the day than control mice, while the second trial was comparable between groups. Parameters of the free exploration trial after training indicate that sugar-rewarded mice are more persistent in their search for the exit, spending more time in that area. We conclude that sugar in close-context to training affects long-term memory, but does not shift performance parameters in general. Mice of the no-sugar group require more training to reach a similar level of performance.

4.2. Emotion, motivation and memory

To differentiate effects on memory from motivational and emotional components, the CHB provides several parameters. For example, an increase in velocity to the exit hole might be indicative for motivational effects. Indeed, in the first trials on days 2 and 3, sugar-rewarded mice had shorter latencies to the exit holes and a higher velocity than no-sugar controls. However, on the following days short latencies remained in the face of comparable velocity in the first trial of the day. Moreover, in the second trial of the day velocity of sugar-rewarded mice was lower than in no-sugar controls. No-sugar mice moved faster on the second trial of the day. If velocity is an indicator for motivation, we have to consider a "trial-dependent" motivation that is apparent in the no-sugar control mice.

Spending more time in the central, most unprotected area is generally accepted as reduced anxiety-like behaviour [3,5]. On the CHB this will increase the latency to the exit hole. Indeed, no-sugar mice remained longer in the center during the first trial of the day. In the second trial, latency to leave the center was comparable between groups. There is no argument that receiving sugar the day before will change anxiety-related behaviour. It is more likely that no-sugar control mice take more time for orientation, than being less anxious. Anyhow, the shorter time in center contributes to, but does not explain the shorter latencies to the exit hole in the sugar-rewarded mice. In relation to latencies and velocities, distance to exit hole indicates that sugar-rewarded mice move more goal-directed than the no-sugar control mice.

We conclude that post-training sugar-reward in the CHB affects memory consolidation, most clearly expressed in the performance of the first trial of the day. Motivational and emotional aspects play a minor role.

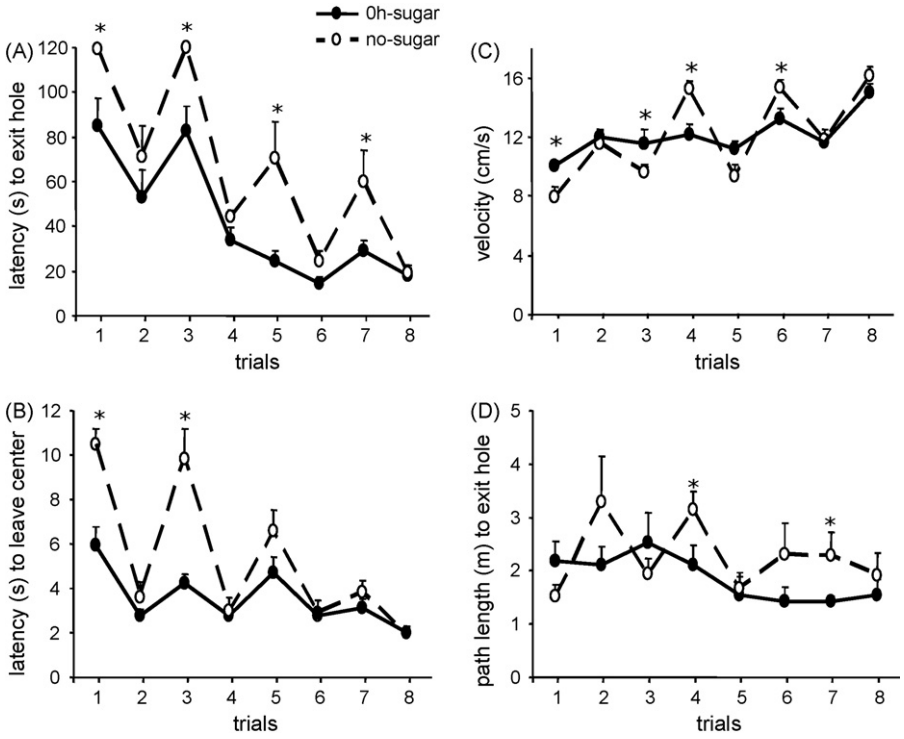

Fig. 3. Circular hole board: performance per trial during spatial training to locate the exit hole (days 2–5, i.e., trials 1–8) for mice that received sugar immediately after training (0 h-sugar) or no-sugar. Odd numbers present the first trial of the day. (A) Latency in seconds (s) to the exit hole, (B) latency to leave the center, (C) velocity in cm/s and (D) distance walked in meters (m) to the exit hole. Data represent mean ± S.E.M. * $p < 0.05$ between groups.

4.3. Task-inherent activation of the stress system and glucose administration

Learning tasks present novelty to mice, with often rather aversive properties that activate the stress system, leading to the secretion of adrenal stress hormones: epinephrine and glucocorticoids. Facilitation of memory is a commonly reported effect, specifically when stress hormones are elevated in close-context with learning trials, i.e., during acquisition and specifically post-training [9,11,20]. Dose-dependent manipulation of corticosterone concentrations during and after training, either by lowering the water temperature or injecting the hormone, facilitates spatial learning in rats [1,9,16,25]. In a parallel study using the same training protocols for WM and CHB, we found corticosterone concentrations 20 min after the start of spatial training on day 5 to be higher in WM than in CHB trained mice (±100 and ±30 ng/ml respectively; own unpublished data). This task-dependent corticosterone response might affect the slope of the learning curve in the WM and CHB task, interacting with the effect of sugar-reward.

Studies on the effect of sugar reward and other drugs on learning include handling, restraining and injecting the animal and thereby, additionally increasing stress-hormone secretion [21]. This task-independent activation of the stress system may contribute to the modulation of memory. Giving mice free access to sugar in close context with their performance in the learning task, we introduce a non-invasive method for sugar reward that is devoid of possible interfering effects of stress hormones on memory processes.

4.4. Reinforcement of behaviour or reinforcement of a memory trace

Traditional reinforcement theory considers memory as something that is somehow determined by reinforcement and, thus, takes place after reinforcement. Reinforcers are thought to increase the probability of behavioural responses. This separation between theories of memory and theories of reinforcement, had been challenged by Huston and colleagues [13–15] proposing an integrated theory of memory and reinforcement. After the performance of a learning task (i.e., during the post-trial, post-training period) memory remains susceptible to disruptive or facilitating treatments. Memory is still in a labile form prior to being fixed or consolidated in a more permanent form [19]. Consequently, positive reinforcers (reward; for a discussion on the difference of reward and reinforcement: see [28]) presented after the learning trial during periods of labile memory should also promote learning. In a first study by Huston and colleagues [13], mice received an aversive electric footshock when stepping down from a platform. Should the reinforcer facilitate the behavioural response, mice are expected to step-down faster in the test trial. On the contrary, post-trial presentation of food facilitated inhibitory avoidance learning: the animals remained on the platform longer than controls. This finding and a series of studies using other aversive, but also appetitive tasks (summarized in [15]), support the theory that the reinforcer (food, electrical brain stimulation, substance P) acts on the central consequences of behaviour, i.e., a memory trace; and not the behavioural response itself.

In the present study, mice had access to sugar after the last training trial of the day. Long-term memory is improved by sugar-reward in both spatial tasks, expressed as superior performance in the first trial of the following day. Whereas the memory facilitating effect in the CHB is observed already after the first contingency: location of and moving through the exit hole and sugar consumption, it takes several days until it is obvious in the WM. As suggested before, this time-related effect of the reinforcer is most likely due to task-inherent properties. However, common to both tasks is that goal-directed behaviour during training trials and the persistence of the search pattern in the area of the platform and exit hole are strengthened. General activity and velocity as behavioural

responses to the task are not reinforced. Thus, it is the memory trace of: how to locate the platform or exit hole, that is strengthened by sugar reward. The memory facilitating effects of sugar are most obvious in the earlier phases of learning. We conclude that our findings substantiate the theory of an integrated reinforcement and memory process.

5. Conclusion

Post-training sugar facilitates spatial memory in mice. The pattern of the memory facilitating effects depends on the task-inherent properties of the WM and CHB. In line with others [26,29], we consider the CHB better adapted to the species-specific needs of mice. Moreover, it allows to collect a broader set of variables related to motivation and emotional expression than the present WM paradigm. The limited number of training trials in the CHB task gives way to pharmacological interventions in close context with training events. The non-invasive administration method of sugar discarded the generally adverse effects related to the method of treatment. Post-training self administration of sugar proved to be an exciting approach to reveal the effects of reinforcers on the formation of memories. Since changes in the reward processing system belong to the main symptoms of stress-related diseases like depression (e.g., anhedonia), we propose that our test-paradigm will be a valid tool to test reinforcement processes in animal models of such stress-related diseases.

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References

[1] Akirav I, Kozenicky M, Tal D, Sandi C, Venero C, Richter-Levin G. A facilitative role for corticosterone in the acquisition of a spatial task under moderate stress. Learn Mem 2004;11:188–95.
[2] Akirav I, Sandi C, Richter-Levin G. Differential activation of hippocampus and amygdala following spatial learning under stress. Eur J Neurosci 2001;14:719–25.
[3] Archer J. Tests for emotionality in rats and mice: a review. Anim Behav 1973;21:205–35.
[4] Barnes CA. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychol 1979;93:74–104.
[5] Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 2001;25:235–60.
[6] Dalm S, Brinks V, van der Mark MH, de Kloet ER, Oitzl MS. Non-invasive stress-free application of glucocorticoid ligands in mice. J Neurosci Methods 2008;170:77–84.
[7] Dalm S, Grootendorst J, de Kloet ER, Oitzl MS. Quantification of swim patterns in the Morris water maze. Behav Res Methods Instrum Comput 2000;32:134–9.
[8] Dawson RG, McGaugh JL. Modification of memory storage processes. Behav Sci 1971;16:45–63.
[9] de Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. Endocr Rev 1998;19:269–301.
[10] Gallagher M, Burwell R, Burchinal M. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav Neurosci 1993;107:618–26.
[11] Gold PE. Glucose modulation of memory storage processing. Behav Neural Biol 1986;45:342–9.
[12] Grootendorst J, de Kloet ER, Vossen C, Dalm S, Oitzl MS. Repeated exposure to rats has persistent genotype-dependent effects on learning and locomotor activity of apolipoprotein E knockout and C57Bl/6 mice. Behav Brain Res 2001;125:249–59.
[13] Huston JP, Mondadori C, Waser PG. Facilitation of learning by reward of posttrial memory processes. Experientia 1974;30:1038–40.
[14] Huston JP, Mueller CC, Mondadori C. Memory facilitation by posttrial hypothalamic stimulation and other reinforcers: a central theory of reinforcement. Biobehavioral Reviews 1977;1:143–50.
[15] Huston JP, Oitzl MS. The relationship between reinforcement and memory: parallels in the rewarding and mnemonic effects of the neuropeptide substance P. Neurosci Biobehav Rev 1989;13:171–80.
[16] Joels M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ. Learning under stress: how does it work? Trends Cogn Sci 2006;10:152–8.
[17] Karami KJ, Coppola J, Krishnamurthy K, Llanos DJ, Mukherjee A, Venkatachalam K. Effect of food deprivation and hormones of glucose homeostasis on the acetyl CoA carboxylase activity in mouse brain: a potential role of acc in the regulation of energy balance. Nutr Metab (Lond) 2006;3:15.
[18] Makimura H, Mizuno TM, Isoda F, Beasley J, Silverstein JH, Mobbs CV. Role of glucocorticoids in mediating effects of fasting and diabetes on hypothalamic gene expression. BMC Physiol 2003;3:5.
[19] McGaugh JL, Herz MJ. Memory consolidation. San Francisco: Albion Publishing Company; 1972.
[20] McGaugh JL, Roozendaal B. Role of adrenal stress hormones in forming lasting memories in the brain. Curr Opin Neurobiol 2002;12:205–10.
[21] Meijer MK, Spruijt BM, van Zutphen LF, Baumans V. Effect of restraint and injection methods on heart rate and body temperature in mice. Lab Anim 2006;40:382–91.
[22] Messier C. Glucose improvement of memory: a review. Eur J Pharmacol 2004;490:33–57.
[23] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11:47–60.
[24] Sandi C, Borrell J, Guaza C. Behavioral, neuroendocrine, and immunological outcomes of escapable or inescapable shocks. Physiol Behav 1992;51:651–6.
[25] Sandi C, Loscertales M, Guaza C. Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. Eur J Neurosci 1997;9:637–42.
[26] Whishaw IQ. A comparison of rats and mice in a swimming pool place task and matching to place task: some surprising differences. Physiol Behav 1995;58:687–93.
[27] Whishaw IQ, Tomie JA. Of mice and mazes: similarities between mice and rats on dry land but not water mazes. Physiol Behav 1996;60:1191–7.
[28] White NM. Reward or reinforcement: what's the difference? Neurosci Biobehav Rev 1989;13:181–6.
[29] Wotjak CT. Of mice and men. Potentials and caveats of behavioural experiments with mice. B I F Futura 2004:158–69.