A spatial paradigm, the allothetic place avoidance alternation task, for testing visuospatial working memory and skill learning in rats

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ABSTRACT

We present a paradigm for assessing visuospatial working memory and skill learning in a rodent model, based on the place avoidance test. In our allothetic place avoidance alternation task (APAAT) the paradigm is comprised of minimal training sessions, tests various aspects of learning and memory and provides a rich set of parameters. A single working memory session consists of four conditions: habituation (no shock), two place avoidance training intervals (shock activated) and a retrieval test (shock inactivated). The location of the shock sector is alternated for each training day which initially requires extinction of previous representations and further working memory to achieve effective place avoidance across sessions. Visuospatial skill memory was evaluated by the shock/entrance ratio by tracking locomotor activity which is essential to execute a place avoidance strategy. For each day rats learned to avoid a new place with shock, as shown by a decreased number of entrances, and an increased time to the first entrance and maximum avoidance time. Skill learning improved according to the decreased number of shocks per entrance across conditions. These results indicate that complex cognitive functions are captured by this behavioral method. This APAAT paradigm expands and complements existing tools for studying hippocampal-prefrontal dependent functions to support development of treatment interventions.

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1. Introduction

Working memory underlies effective problem solving and planning abilities, and efficiency of processing and response accuracy improve with practice (Berry et al., 2009). More specifically, successful visuospatial working memory ability is associated with increased frontal, parietal and hippocampal activity in humans (Westerberg and Klingberg, 2007) and with changes in synaptic plasticity via long-term potentiation (LTP) in humans and rats (Goto et al., 2009). The radial arm maze (Lee and Kesner, 2002) and the Morris water maze (Khakpour-Taleghani et al., 2009) are favored tests to study long-term memory in rats and are utilized to study spatial working memory (de Saint Blanquat et al., 2010). In a place preference task, working memory is assessed by alternating the location of a to be remembered place reinforced by reward (e.g. food or platform) from trial to trial; an experimental session includes a training trial for acquisition and a test trial to evaluate retrieval (Rossier et al., 2000; Hok et al., 2007). These procedures require a long lasting habituation and an extensive period for acquisition of the task rules before a probe test of retrieval. These tasks engage working memory by processing visuospatial representa-

tions, which are accompanied by proximal and self-motion stimuli. Sensory input from useful stimuli is needed to build proper representations of space and dictates the preferred mode of navigation (rats (Cimadevilla et al., 2000a); humans (Stankiewicz et al., 2006)). Essential components of working memory in humans and animals include acquisition, maintenance and retrieval of information. Spatial working memory engages the dorsal hippocampus (Yoon et al., 2008) and also the prefrontal cortex in regard to attention processes, short- and long-term memory (Blumenfeld and Ranganath, 2007), and furthermore in regard to goals associated with path planning (Hok et al., 2005).

Place avoidance in the active variant has been shown to depend on the integrity of the hippocampus in trained rats and to require more than a stimulus response strategy to solve correctly (Cimadevilla et al., 2000a). In the active place avoidance task, avoidance of a shock sector requires on-going segregation of distal allothetic visual information from the proximal allothetic and self-motion information, a process described as cognitive coordination (Wesierska et al., 2005). Training with the position of the shock sector changed daily showed a lack of improvement in active place avoidance across days, as no effect of short-term memory functioning was found (Cimadevilla et al., 2000a; Cimadevilla et al., 2000b; Cimadevilla et al., 2001). For performance with a stable shock sector location, the recall engages long-term memory (Pastalkova et al., 2006) which improved over consecutive sessions,

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and was found to be better for males than females (Cimadevilla et al., 2001).

We herein introduce the allothetic place avoidance alternation task (APAAT), a variant of the active place avoidance test (Bures et al., 1997). Recollection of a new place via avoidance reflects working memory functioning and is tested during the training intervals (active shocks) and, after a 5 min delay during a retrieval test (shocks inactivated). The main difference between our procedure and those used by the above cited authors is that over consecutive sessions, the position of the place where shocks are dispensed is altered, so that a previous representation must be extinguished and a new representation processed in order for successful avoidance to occur in a short time period. Additionally, the memory of the new shock location is tested after a short delay during the retrieval test. The paradigm therefore more closely represents the working memory processes engaged daily and further, also models the associated carry-over of previously established skill ability whereby a long-term follow-up session allows for the evaluation of visuospatial skill retention.

Thus, the aim of APAAT use is to test working memory and skill learning, and in future studies may be used to further our understanding of how hippocampal-prefrontal circuitry supports such behavior on a cellular and structural level.

2. Materials and methods

2.1. Animals

The experiments were performed with 12 naive male Long Evans rats (300–320 ± 5 g) that were housed in standard conditions, with food and water available ad libitum and normal light–dark cycles (light from 8:00 a.m. to 8:00 p.m.). The animals were handled for 4 days prior to experiment onset. The experiment was conducted in accordance with both the regulations of the Polish Communities Council for the care and use of laboratory animals and the European Community Directive for the ethical use of experimental animals.

2.2. Place avoidance apparatus

As described by Wesierska et al. (Wesierska et al., 2009), the place avoidance apparatus was comprised of a flat, circular metal arena (diameter 80 cm), which rotated at a speed of 1 rpm, and was rimmed by a metal lip on the periphery (2 cm). The elevated arena was located in the center of a 3 m × 4 m room. Dim light (±20 lux) was produced from a floor lamp and visual landmarks marked the walls. A commercially available place-avoidance system (Bio-Signal Group, Brooklyn, New York), which allowed automatic calculation of the position of freely moving rats on the arena according to the location of the shock sector, was employed for data collection and analysis. By use of infrared light-emitting diodes (LED) fixed on the periphery of the arena and between the shoulders on a latex harness, the position of the rat was tracked at 20 ms intervals using an infrared-sensitive television camera attached to a computer system. Whenever the rat entered the 45° shock sector, a computer-triggered, constant-current (50 Hz, 0.5 sec) shock was delivered across the low- and the high-impendence electrodes. The low-impendence (~100 Ω) shock electrode was clipped to a pin on the back of the rat, while the high-impendence (~100 KΩ) electrode was produced by contact of the rat's feet to the grounded arena surface. In order to affect a moderate response to the shock (e.g. not freezing or escaping from the arena) the current amplitude (0.2–0.5 mA) was adjusted individually for each rat. If the rat did not escape from the shock sector, the shock was repeated every 1.5 s. The experiment was monitored in an adjacent room in

which the computer-controlled recording equipment was located (see Fig. 1).

2.3. Behavioral procedures

The experiment started with habitation on day "0" (D0ha) when rats were first exposed to the rotating arena for 5 min without an active shock sector ("dummy shock" sector). The position of the shock sector was defined by room-frame coordinates and was changed each day according to the following random order: D1 – Northwest, D2 – Northeast, D3 – Southwest and D21 – Southeast. The position of the dummy shock sector was the same as for the active shock sector of the respective day. For D0, the dummy sector was the same as for D1. On Day 1 (D1) rats began working memory place avoidance training which included a 5-min habituation (rotating arena, no shock) (D1ha), directly followed by two consecutive 5-min training intervals of acquisition (rotating arena, shock) (D1tr1; D1tr2). The rats completed acquisition training after 10 min if they showed continuous avoidance for a minimum of 90 s. For those which did not meet this criterion before the end of the 10 min (10.5% per entire experiment), training was extended for 5 min. Data analysis was made with the first and final training intervals. Following the training condition, the rats were removed from the arena and placed in a cage next to the experiment room for a 5-min break. For the retrieval test, the rats were then returned to the rotating arena for 5 min to be tested with the original shock sector inactivated (D1ts). Rats underwent three days of working memory training which consisted of the four conditions described (ha, tr1, tr2, and ts) (Fig. 1). To retest the long-term influence of previous exposure and training on skill learning, and to test working memory in a fourth session on D21, the rats underwent the same procedure with the shock sector located in a novel position (see Fig. 1).

2.4. Learning and memory measures and data analysis

The rats' ability to learn and form internal representations or memories of a particular place location are elucidated by various parameters measured by the tracking system which include: (1) number of entrances into the shock sector (active or inactive) (E), (2) number of shocks per entrance (SH/ENTR), (3) time to the first entrance (T1) and (4) maximum avoidance time (Tmax) (in seconds). For habituation, when shock was not presented, the values of the above parameters were calculated for the dummy shock sector according to D1.

The above described measures, the values of which were normally distributed, were statistically estimated. The performance during habituation over days (D0, D1ha, D2ha, D3ha, D21ha) or the performance within the session conditions (ha, tr1, tr2, ts) were evaluated independently using a one-way ANOVA followed by a Tukey HSD post hoc test. For between-session performance, a two-way ANOVA (day (D1, D2, D3, D21) × condition (ha, tr1, tr2, ts): 4 × 4; with repeated measures on the last factor) was performed followed by a Tukey post hoc test. Significance was accepted at $P < 0.05$ level. Statistical analysis was performed with STATISTICA 7.1. Results are reported as mean ± S.E.M.

3. Results

3.1. Habituation (ha) to novelty (D0ha) and exposure to the neutral arena (D1ha, D2ha, D3ha, D21ha)

During the habituation session the shock was not active. The performance of rats during habituation for D0, D1, D2, D3 and after the long delay on D21 was comparable because despite prior training, through 5 min of exposure to the neutral arena extinction of
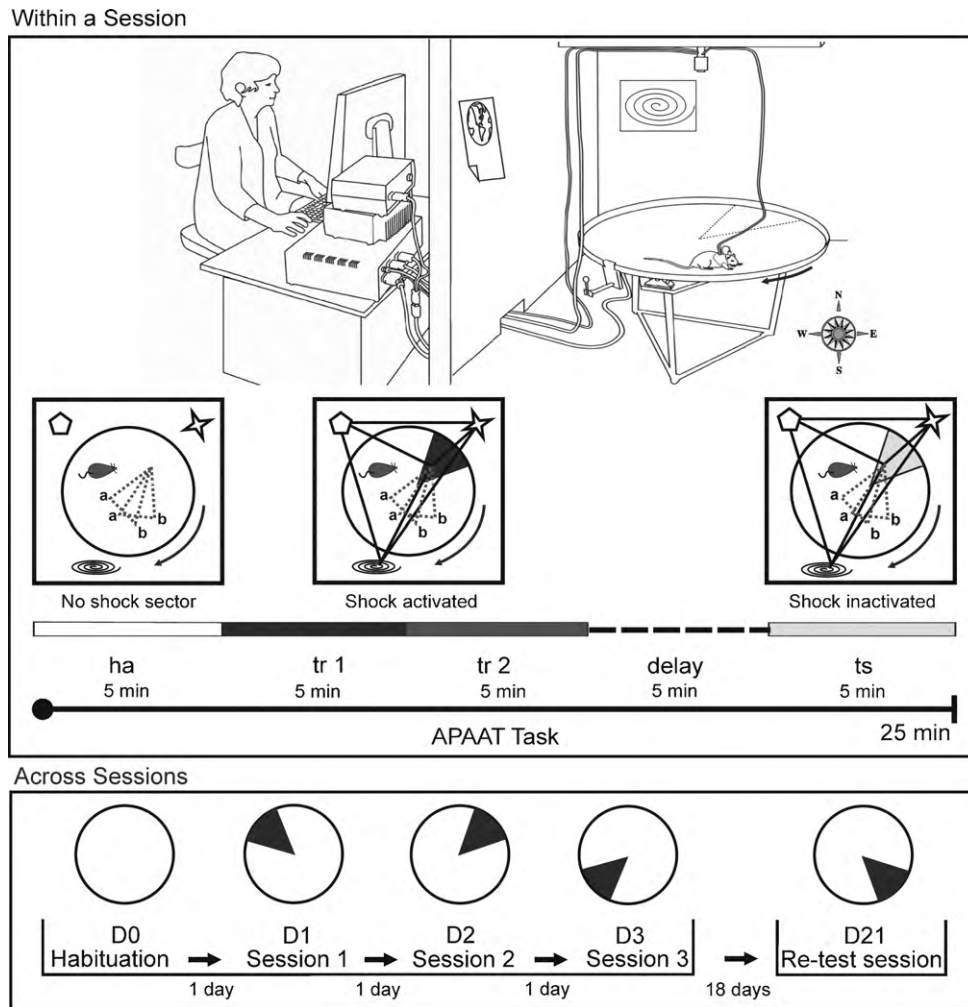
Fig. 1. Experimental design of the allothetic place avoidance alternation task (APAAT). Experimental set-up for the visuospatial working memory place avoidance test is presented in the upper part of the figure. In the APAAT task, within a session rats underwent a habituation interval (ha), two consecutive 5-min training intervals (tr1, tr2), a 5-min delay, followed by a retrieval test (ts). Across sessions, rats underwent a habituation day (D0), followed by a training + test period across three consecutive sessions (D1, D2, D3). The rats learned to solve place avoidance in spite of alternating locations of the shock sector for each of the training days. For the retest session (D21), the location was again changed in order to test the robustness of the rats' skill to avoid. The shock sector was active only during the training intervals, but not during D0 habituation, or for the habituation or retrieval test intervals.

place avoidance occurred, in which the rats reverted to freely moving behavior and did not avoid. The one-way ANOVA with repeated measures for the habituation condition across days showed no significant effect of number of entrances into the sector where shocks had occurred (ENTR $F4,44 = 0.60$; $P = 0.66$), time to the first entrance (T1 $F4,44 = 2.23$; $P = 0.08$) and maximum avoidance time (Tmax $F4,44 = 0.28$; $P = 0.89$). The distance covered by rats during the habituation period differed across days as confirmed by the same ANOVA for total path length (TPL $F4,44 = 10.48$; $P < 4.7 \times 10^{-6}$). A post hoc test confirmed that rats walked more during D0, when they were exposed to the rotating arena for the first time than on the other days ($P < 0.002$), while the distance was similar for the three consecutive training sessions (D1, D2, D3) (see Fig. 2).

3.2. Working memory and short-term memory; Day 1 only – within-session comparisons

Already within the first session of working memory training rats learned to avoid the sector with shocks. Not surprisingly the number of entrances into the shock sector changed dependent on conditions (ha, tr1, tr2, ts) as confirmed by a one-way ANOVA with repeated measures ($F3,33 = 17.53$; $P < 5 \times 10^{-6}$). In comparison to habituation (ha) the number of entrances decreased rapidly from

the first training interval (tr1) to the second training interval (t2) of place avoidance in response to active delivery of shocks and remained low in the test for retrieval (ts) despite that the shock was inactivated (ha > tr1 > tr2 = ts; post hoc test; $P < 0.0004$) (see Fig. 3 ENTR). Interestingly, for the retrieval test rats also made significantly fewer entrances compared to the habituation interval ($P < 0.0004$). Essentially the two experimental conditions were the same, except that the retrieval interval followed training, while habituation preceded it. The same ANOVA also showed a significant main effect of condition on T1 ($F3,33 = 7.79$; $P < 0.0005$) and Tmax ($F3,33 = 14.14$; $P < 4 \times 10^{-3}$). This was reflected by a long time to the first entrance into the sector, indicative of active avoidance, during the retrieval test when shocks were inactivated compared to tr1 (post hoc test; $P < 0.0004$) (see Fig. 3 T1, Tmax). The benefits of continued training were shown by the increased T1 and Tmax in the latter training interval (tr2) and also for ts compared to ha and tr1 (post hoc test; $P < 0.003$). For D1, the experimental conditions had no effect on total path length (see Fig. 3 TPL).

3.3. Learning and skill retention – between-session comparisons

In the consecutive training intervals, rats learned to actively avoid the place with the shock sector even as its location was
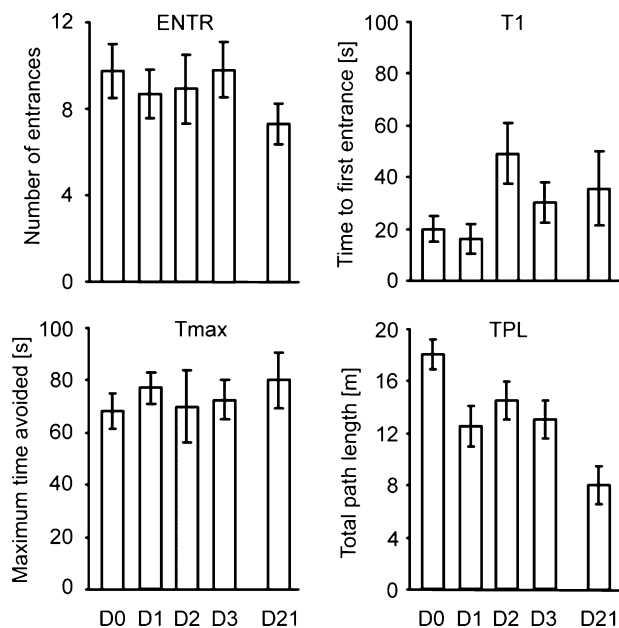
Fig. 2. Behavior during the habituation (ha) condition across days. During the habituation condition no differences were found across days (D1, 2, 3, 21) for the number of entrances (ENTR), time to the first entrance (T1) and maximum avoidance time (Tmax) of the defined 45° sector. For the total path length (TPL), rats walked more on D0. For each panel, $n = 12$, and data are mean ± S.E.M.

alternated from session to session and this avoidance was maintained during the retrieval test (shock inactivated) (see Fig. 4). The two-way ANOVA (day × condition; 4 × 4; with repeated measures on the last factor) for ENTR confirmed a main effect of condition ($F_{3,30} = 25.80$; $P < 1 \times 10^{-6}$) and a day by condition interaction ($F_{9,90} = 2.31$; $P < 0.02$). Rats made fewer entrances into the shock sector later in the session during tr2 and ts than during ha and tr1 (post hoc test; $P < 0.01$). A post hoc test for the interaction confirmed that during the retest day (D21) all rats made more entrances during tr1 of avoidance training and for the memory retrieval test (ts) than in the same conditions for the previous days ($P < 0.03$).

The same ANOVA for T1 showed a significant effect of condition ($F_{3,30} = 14.66$; $P < 4 \times 10^{-4}$), whereas an effect of day and an interaction of day by condition were not significant. Naturally, after exposure to the shock rats then entered into the sector after a longer delay during tr2 and ts (shock inactivated) (post hoc test; $P < 0.004$) than during tr1 or ha.

Due to the changing location of the shock sector over sessions, the long maximum avoidance time (Tmax) expressed working memory during the place avoidance training. During training
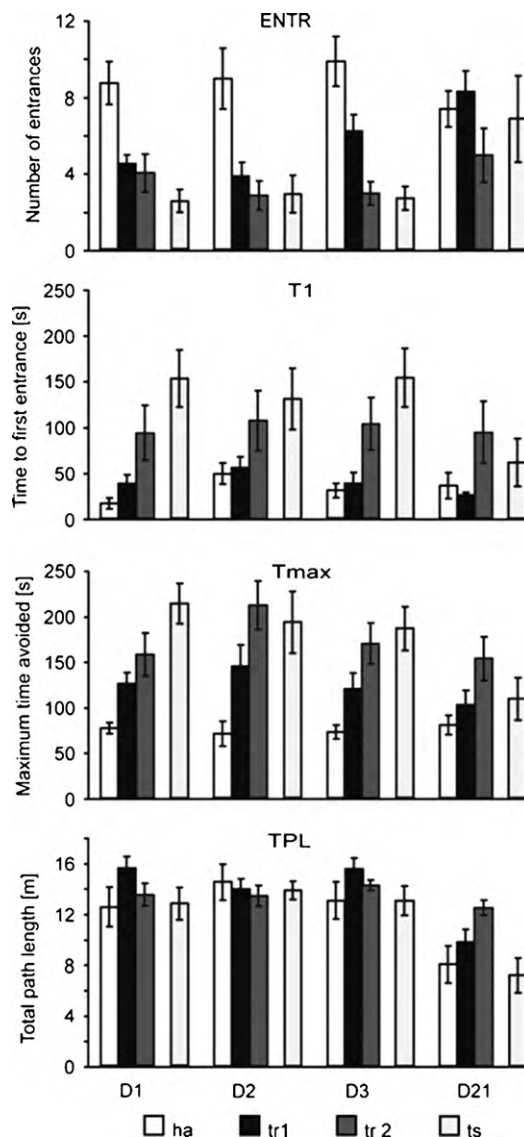

Fig. 4. Between-session learning and task performance. Rats engaged working memory as shown by a decreased number of entrances (ENTR) into the shock sector even though the location changed from session to session. Working memory is also expressed by the long maximum avoidance time (Tmax) of the location of the shock sector during the place avoidance training, when the sector was reinforced by shock administration. The long delay to the first entrance (T1) during the retrieval test (ts) can be taken as an index of short-term memory function. In all sessions locomotor activity was stable across conditions; however on D21 the overall locomotor activity was suppressed. For each panel, $n = 12$, and data are mean ± S.E.M.
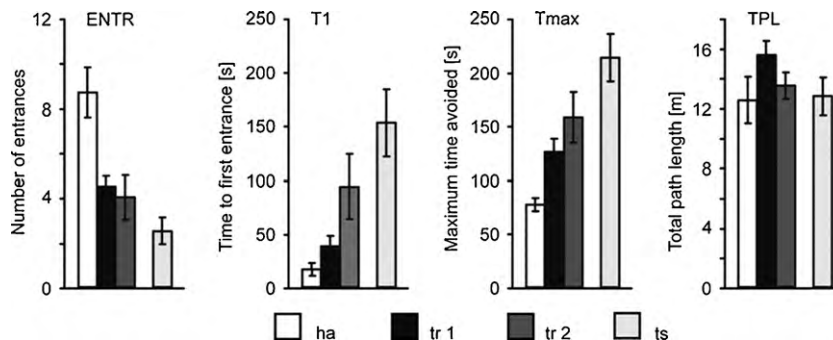

Fig. 3. Working memory within one session. In the first session of place avoidance training (D1) rats employed working memory to avoid the shock sector (training 1 and 2) and maintained the representation during the retrieval test (ts). This was demonstrated by a decreased number of entrances and an increase in the T1 and Tmax during avoidance training (tr1, tr2) and during the retrieval test compared to habituation (ha) ($P < 0.004$). The experimental conditions had no effect on the total path length (TPL). For each panel, $n = 12$, and data are represented by mean ± S.E.M.
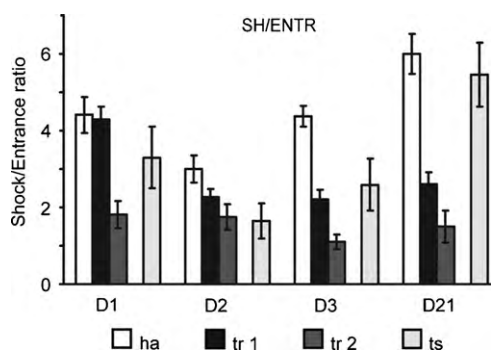
Fig. 5. Skill learning. The ratio of shocks per entrance was high for training 1 (tr1) during the first session only, when rats escaped from, rather than avoided the shock. Acquisition of skill learning improved performance which was demonstrated by a decrease in the ratio during training sessions (tr1 and tr2) across days. For each panel, $n = 12$, and data are mean ± S.E.M.

(shocks activated) short-term memory (STM) was evaluated following the 5-min delay outside the arena by the retrieval test (shocks inactivated). The same ANOVA for Tmax confirmed a significant effect of condition (F 3,30 = 22.64; $P < 8 \times 10^{-5}$) and an interaction for day by condition (F 9,90 = 2.48; $P < 0.01$), whereas there was no significant effect of day. During tr1 and tr2 of place avoidance training, as well as during the ts, the Tmax was longer than during habituation because of actual or expected aversive reinforcement (post hoc test; $P < 0.01$). On D2 when rats already have experience with place avoidance, but before the maximal task load due to alternation of the shock sector, the Tmax was longer during the latter training interval (tr2) than during the same training interval on the other days (post hoc: $P < 0.03$). On D21, after a long break without exposure to this now familiar task, the trained rats showed a shorter Tmax than during the other days (post hoc test; $P < 0.003$). Rats walked a similar distance (TPL) in all conditions, however this distance decreased on D21 as confirmed by a significant effect of day (ANOVA; F 3,30 = 13,16; $P < 1 \times 10^{-3}$); post hoc test $P < 0.0004$), whereas an effect of condition and the day by condition interaction were not significant.

3.4. Skill training – within- and between-session comparisons

Skill ability as estimated by the ratio of shocks per entrance (SH/ENTR) changed across conditions and sessions. It was learned rapidly (within the first session) and maintained over the long-term (between sessions) as shown by the equally low ratio for tr2 from the first to the retest sessions (D1 vs D21) (see Fig. 5). A two-way ANOVA (day × condition; 4 × 4; with repeated measures on the last factor) for the SH/ENTR ratio confirmed a significant effect of days (F 3,33 = 13.52; $P < 1 \times 10^{-4}$), conditions (F 3,33 = 15.09; $P < 1 \times 10^{-4}$) and an interaction for day by condition (F 9,99 = 6.03; $P < 1 \times 10^{-4}$). The SH/ENTR ratio was the same for D2 and D3 which differed from D1 and D21 ($P < 0.03$). A post hoc test for condition confirmed a high ratio during ha ($P < 0.04$) when rats moved freely but the lowest ratio during tr2 when rats had the most training, whereas the ratio for tr1 was the same as for the ts ($P < 0.04$). A post hoc test for the day by condition interaction confirmed that only on D1 the ratio for ha and tr1 was the same, but decreased for tr2 in which rats could more quickly escape the shock sector. The SH/ENTR ratio for tr1 on D1 was higher than for all other days (D2, D3 and D21) which shows that the most essential learning for successful place avoidance occurs during D1 ($P < 0.04$). For all days, the SH/ENTR ratio for tr2 was equally low showing that the skill to avoid, once learned, is robust despite the alternating shock sector location and after a long-term break in training.

Rats show equal distribution of time spent in all quadrants of the arena during habituation (see Fig. 6a). In habituation rats are not forced to move as a consequence of an active shock sector. This equal percentage of time spent may be an effect of autonomous walking and/or passive transport by the rotating arena (continuous trajectory, see Fig. 6b). Behavior changes when the shock sector is activated. Avoidance of the place with shocks requires an instrumental response – by active avoidance. However on the 80 cm Ø arena with a 45° shock sector rats can use different strategies in response to the various conditions (ha, tr1, tr2, ts, e.g. during working memory training or in the retrieval test) but also according to their level of experience (e.g. training day). During avoidance training and the retrieval test rats spend more time out of the shock sector (see Fig. 6a narrow black triangle). As training begins, rats spend the most time counter-clockwise to the shock sector. With more exposure to the arena, rats spend an increasing amount of time in the quadrant opposite to the shock sector (Fig. 6a). This strategy is visualized by the path trajectory of the rat's movement (Fig. 6b).

4. Discussion

The purpose of this study was to develop a novel paradigm for testing visuospatial working memory and skill learning in rats. By optimizing models, understanding about the relationship between function and neurobiology can be improved and thereby further support development of potential treatment options available for clinical use. The model presented herein allows for the evaluation of on-going visuospatial working memory and experience-dependent skill learning expressed by active avoidance (McNab et al., 2009). Moreover, the processes inherent to transforming novel representations into lasting processes (Blumenfeld and Ranganath, 2007) can therefore also be tested by this model. This is important according to the study of memory function and for development of interventions for learning and memory disorders.

4.1. Working memory and short-term memory

For Day 1 only, fast learning is already apparent by the rat's ability to not only escape from the shock sector (D1tr1), but more so by their ability to avoid the sector (D1tr2). The number of shocks per entrance (see Fig. 5) is an index of extremely early learning. This is also apparent by the number of entrances, an increased time to the first entrance (tr1) and a higher maximum avoidance time (see Fig. 3). Working memory is shown not only by the rats ability to maintain avoidance during training, but furthermore they maintained the representation from the training (tr2) to the retrieval condition (ts). It is difficult to distinguish working memory from short-term memory processes. Working memory includes processes and mechanisms which are involved in the processing of short-term memory; however differences between them are anchored in their functions. STM sustains a limited amount of information over a short time period, in contrast to working memory which is an effect of several functional components working together and is sensitive to task complexity (Cowan, 2008). This working memory and short-term memory pattern of proficiency was also expressed across all experimental days (see Fig. 4). Such fast acquisition does not exist in other prominent working memory tasks.

Formation of the representation of the shock location is hippocampal-dependent and based on useful distal information from the surround. Expression of it after a short time delay (retrieval test) engaged short-term processes. From an anatomical perspective, in animals and humans, prefrontal cortex activity supports attentional processes, working memory, short-term memory
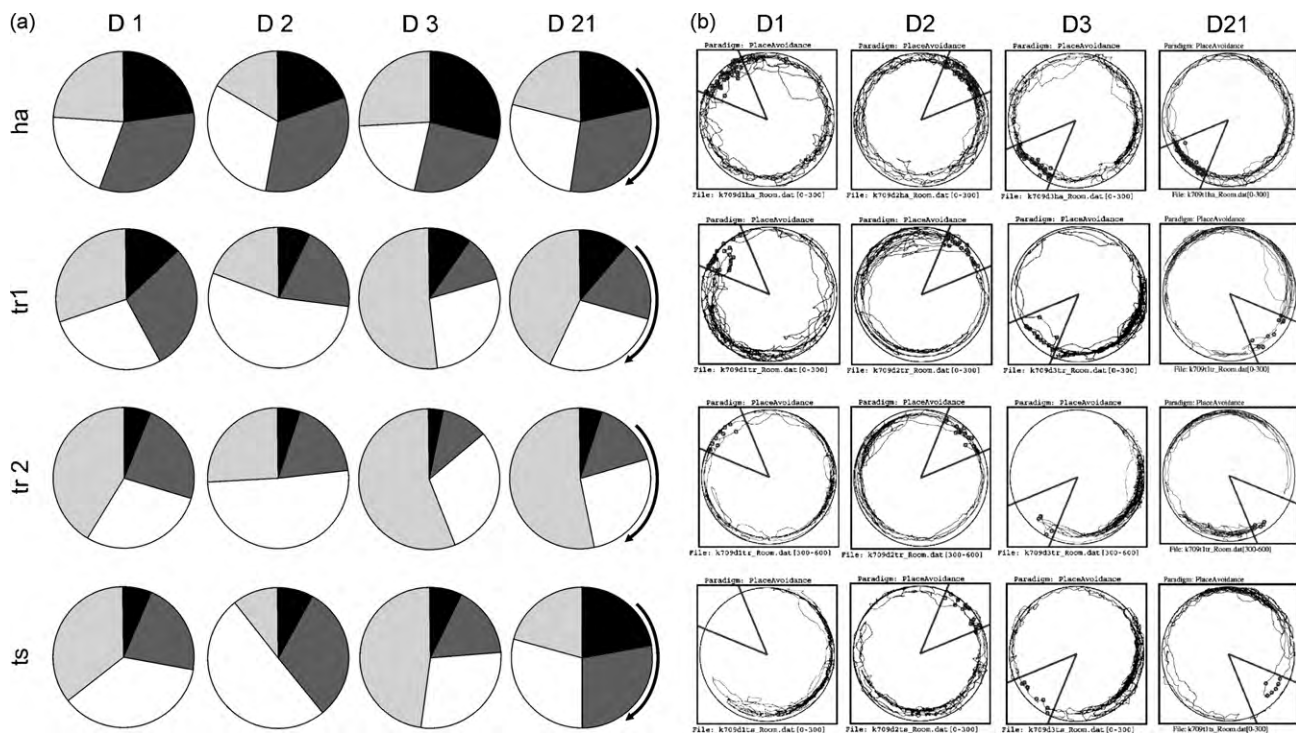
Fig. 6. Rats' performance during the APAAT test. (a) Percentage of time spent by rats (average) in the quadrant where shocks were presented ("TARGET," black), in the quadrant opposite to the target ("OPP," white), in the clockwise quadrant ("CW," dark grey) and the counter-clockwise ("CCW," light grey) position relative to the direction of the arena rotation (see arrow on left side); (b) representative path trajectories on the arena.

and executive function (Dalley et al., 2004; Funahashi, 2001), whereas the hippocampal formation is established as the main structure involved in spatial memory processes (Liang et al., 1994; Niewoehner et al., 2007) and for long-term memory formation (Yanike et al., 2004). Acquisition of the place avoidance task has been shown to engage attentional processes (Wesierska et al., 2005) besides spatial memory and cognitive processes.

In order to preserve the hippocampal-prefrontal nature of this visuospatial working memory task, it was important that a simple stimulus response association (Tulving and Schacter, 1990) was not employable to solve avoidance. A previous study evaluated the role of sex differences under long-lasting acquisition (20 min) and extinction (20 min) in which the punished sector was alternated daily, however habituation was performed on the first day only, the shock sector was larger (60°), no retrieval test was performed and sessions were repeated for 8 days (Cimadevilla et al., 2000b). Our test is dedicated to test memories acquired over a short time period, in which every session included a habituation condition and a retrieval test. This means that for every new representation built, the rats had to extinguish previous representations. This increased the difficulty, or the load of the on-going memory process with each consecutive session. Even though the location of the sector changed every day, the pattern of learning and memory apparent in a given session was preserved across sessions. Despite spatial alternation of the shock sector to a novel place on the arena, the working memory necessary to continuously avoid was maintained.

While learning and memory indexes were the same for all days, questions remain open concerning the differences in particular conditions evident for D21. In this retest session, rats showed normal learning and working memory according to increased avoidance comparable to the other days; however the performance differed in regard to the retrieval test. Specifically during the retrieval test, unlike for other days, they displayed a higher number of entrances, lower time to first entrance and a lower max-

imum time avoided, in addition to a lower total path length. This change from maintaining avoidance in the absence of shock to non-avoidance is in stark contrast to the rats' performance during the initial training sessions (see Fig. 4). One explanation is that in early exposure, the rats were highly motivated due to the novelty and saliency of the shock as a reinforcer. Higher vigilance and higher anxiety (e.g. more defecation and a more peripheral location on arena–unpublished data) would likely support maintained avoidance in the retrieval test. Another possible explanation relates to the consolidation and transference of memory from the short- to the long-term. In this case they do not transfer the shock location per se; however they maintained the ability to avoid as shown by the low SH/ENTR ratio during the training intervals (see Fig. 5).

4.2. Skill learning, long-term memory and strategy

A novel feature of this paradigm is that it captures more complex cognitive functions such as skill learning. It is apparent that avoidance is generally fast and easy to learn, because it is built via an aversive reinforcer (e.g. foot shock) contrary to the appetitive reinforcers used in maze tasks. Initially when rats do not know the location of the shock sector or how to avoid it, they enter, halt and thereby receive several shocks before escaping. This results in a high shock per entrance ratio (see Fig. 5). Learning is shown by a decrease in this ratio as training accumulates. In order to successfully avoid, rats must recognize the shock sector via distal cues and re-position themselves counter-rotation. Even though the location of the shock sector is altered, rats show a very low number of shocks per entrance. The decrement in this ratio (e.g. faster escape, fewer shocks) over time increases within and across sessions, suggesting cognitive skill learning. Rats learned and clearly decreased error with exposure to the task or via "practice." Despite non-avoidance for the retrieval trial and a higher SH/ENTR ratio during ha and ts, the most striking feature about the D21 retest is that the rats

showed an equally low number of shocks per entrance as for D2 and D3, which were all significantly less than for D1. This means that even with a long delay and a new shock sector location they did not revert simply to escape behavior (see D1tr1), but they maintained the skill to avoid.

In maze learning rats undergo forced choice which limits the potential employment of varied strategies. Our task is different, as the rotation of the arena adds an element of influence on how the rats move and part of successful avoidance requires learning to move against the direction of rotation. Therefore relevant information to construct accurate spatial representations is derived from distal visual cues which must be segregated from proximal allothetic information from the arena (e.g. odorant cues, feces). The latter stimuli have been found to be a source of error in path integration (Stuchlik et al., 2001). In our procedure each working memory session started with a habituation during which all quadrants of the arena were visited for the same amount of time. In early acquisition rats enter into the shock sector and escape. With training, rats spend an ever decreasing amount of time in the shock sector with associated increases in the counter-clockwise and opposite quadrants (see Fig. 6a). However since the rats can move freely on the arena and walking is a natural activity, they have a higher degree of freedom in how they solve avoidance. Further, as training progressed across days, due to prior exposure to the arena and the changing of the punished area to a relatively close location, their experience with prior shock sectors can influence their response to avoid the new sector. Conceivably this would require a kind of behavioral inhibition which is known to be regulated by the prefrontal cortex (Sakagami et al., 2006). An effect of the position of the shock sector on place avoidance was not observed during acquisition when place avoidance sessions were followed by a long lasting extinction (Cimadevilla et al., 2000b).

Cognitive skill learning is the effective integration, development and application of the rules, procedures and strategies that support successful performance (Beauchamp et al., 2003). This learning occurs implicitly, is engaged with repetition and requires frontostriatal circuitry (Beauchamp et al., 2008). In rodent models, the study of cognitive skill learning has not been explored in depth.

5. Conclusions

This novel paradigm for rats contains critical elements of cognitive interest, namely visuospatial working memory and skill learning, which are functions supported by hippocampal-prefrontal circuitry (Yoon et al., 2008). Hence, we aimed to advance the suitability, efficacy and validity of a rat behavioral model for the purpose of both basic research of cellular mechanisms and for preclinical treatment. The impact of this new paradigm is that the design provides highly sensitive measures which allow for disassociation between cognitive, learning, sensorimotor, olfactory or motivational states under pharmacological, lesion and stimulation interventions. The authors have successfully used this paradigm to test the influence of transcranial direct current stimulation on these functions (paper in preparation).

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