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Directional learning, but no spatial mapping by rats performing a navigational task in an inverted orientation

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Abstract

Previous studies have identified neurons throughout the rat limbic system that fire as a function of the animal's head direction (HD). This HD signal is particularly robust when rats locomote in the horizontal and vertical planes, but is severely attenuated when locomoting upside-down (Calton & Taube, 2005). Given the hypothesis that the HD signal represents an animal's sense of its directional heading, we evaluated whether rats could accurately navigate in an inverted (upside-down) orientation. The task required the animals to find an escape hole while locomoting inverted on a circular platform suspended from the ceiling. In experiment 1, Long-Evans rats were trained to navigate to the escape hole by locomoting from either one or four start points. Interestingly, no animals from the 4-start point group reached criterion, even after 30 days of training. Animals in the 1-start point group reached criterion after about 6 training sessions. In Experiment 2, probe tests revealed that animals navigating from either 1- or 2-start points utilized distal visual landmarks for accurate orientation. However, subsequent probe tests revealed that their performance was markedly attenuated when required to navigate to the escape hole from a novel starting point. This absence of flexibility while navigating upside-down was confirmed in experiment 3 where we show that the rats do not learn to reach a *place*, but instead learn separate *trajectories* to the target hole(s). Based on these results we argue that inverted navigation primarily involves a simple directional strategy based on visual landmarks.

Keywords

spatial navigation; vestibular system; head direction cells; spatial task

1. INTRODUCTION

The different strategies used by humans and animals to navigate accurately in their environment have been studied extensively by neuroscientists (Aguirre, Zarahn, & D'Esposito, 1998; Hartley, Maguire, Spiers, & Burgess, 2003; Maguire, Burgess, Donnett, Frackowiak, Frith, & O'Keefe, 1998; O'Keefe & Nadel, 1978). The theories postulated to account for successful

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navigation have been shaped, in part, by the discovery of different types of spatially tuned neurons that code for location (place cells), directional orientation (head direction cells), and grid of the environment (grid cells) using an allocentric frame of reference (Ekstrom, Kahana, Caplan, Fields, Isham, Newman, & Fried, 2003; Hafting, Fyhn, Molden, Moser, & Moser, 2005; O'Keefe & Dostrovsky, 1971; Taube, Muller, & Ranck, 1990). Indeed, these findings, combined with animal lesions studies showing that disruption of brain areas containing these neurons prevents animals from solving allocentric spatial tasks (Morris, Garrud, Rawlins, & O'Keefe, 1982), led to the idea that these neurons, primarily found in the limbic system, were the neural substrate of a flexible representation of space or *cognitive map* (O'Keefe & Nadel, 1978). On the other hand, when facing a spatial problem, rats do not always rely on a cognitive map-based strategy, but may also use: 1) a specific landmark situated close to the goal (beacon navigation), 2) a small number of distal cues that provides them the direction to head in order to find the goal (direction strategy), or 3) simply learn a sequence of turns (e.g., left-right-left) and thus use a body-centered (egocentric) frame of reference (response strategy) (McDonald & White, 1993; Packard & McGaugh, 1996; Skinner, Etchegary, Ekert-Maret, Baker, Harley, Evans, & Martin, 2003).

Most studies on mammalian navigation have involved two-dimensional terrestrial environments. However, many species live and move about in a three-dimensional (3-D) environment - the most obvious species include marine and arboreal mammals, as well as rodents that live in subterranean environments. But humans also frequently navigate in 3-D environments, particularly air pilots and astronauts, many of whom frequently encounter periods of disorientation that often lead to motion sickness, which can have serious consequences during flight. Thus, determining which strategies and reference frames are used during navigation in a three-dimensional (3-D) environment is crucial for understanding how animals live and orient in a 3-D world.

Previous experiments have studied how head direction (HD) cells respond when a rat locomotes in a vertical plane or upside down in 1-g (Calton & Taube, 2005; Stackman, Tullman, & Taube, 2000) and in 0-g (Taube, Stackman, Calton, & Oman, 2004). In these experiments, it was observed that the vertical plane was treated as an extension of the floor and that HD cells maintained their direction-specific firing when the rat locomoted on the wall. However, when the rats locomoted upside-down on the ceiling, HD cells showed dramatic change in their activity, including a complete loss of directional-specific firing in two-thirds of the cells, and a marked reduction in directional activity in the remaining cells. Furthermore, in cells that maintained some semblance of directional firing, there was frequently a shift in their preferred firing direction. However, even with this distorted directional signal, rats performed quite well in the task - climbing a vertical wall to a ceiling, then traversing the ceiling inverted, and finally down another wall in order to reach a reward compartment. Observations of the rats appeared to indicate that they knew which direction to go in order to obtain the reward. This accuracy in the task was attributed to the low navigational demand and may not have required a functional HD system. To date, there are no experiments that have tested the extent to which rats are capable of accurate navigation in an inverted orientation. In the present study we devised a more demanding spatial paradigm in order to examine whether rats are capable of accurate navigation in an inverted orientation, and if so, what spatial strategies they use to perform the task.

2. GENERAL METHODS

2.1 Subjects

Experimentally naive female Long-evans rats served as subjects for all three experiments. The animals were housed singly in clear plastic cages in a room maintained on 12 hr light/dark

cycle. Water and food were available *ad libitum* in the home cage. All procedures were conducted under an institutionally approved animal care and use protocol.

2.2 Apparatus

The “inverted maze” apparatus consisted of a 190-cm diameter circular wire mesh that was suspended ~50 cm from the ceiling, with four evenly spaced holes that formed four possible escape exits from the maze (Fig. 1). Each one of these 15-cm holes could be closed with a piece of wood. The wire mesh was composed of metal screening with square size of 0.5×0.5 cm. The maze was in a room containing many salient visual cues including posters, a TV, a door, a blackboard, two windows along one wall, and a desk with a computer on it.

2.3 Behavioral procedure

Prior to the commencement of training all rats were handled 3 min per day for 3 days. The basic task involved the rat being placed in an inverted position, either on the periphery of the maze or at the maze center (see below), and because rats contain a natural proclivity to remain in an upright position they were highly motivated to find a hole to escape through in order to enable themselves to return to an upright position on top of the circular board. For all experiments three of the four holes were blocked with a piece of wood - thus, only one hole was open to escape through. Although the rat could see the small depression made by the four holes, it was not possible for it to determine which hole was the correct one to escape through without being close to it because of the small angle between its head and the wire mesh surface. This point is firmly demonstrated by noting the great difficulty rats had in finding the correct hole in the four start position condition (see results below). Throughout all the experiments there was no attempt to disorient the rat when bringing it into the room or when placing it on the inverted maze.

2.3.1 Pretraining—Before being trained to solve the spatial task in the maze, the rats were trained to climb short distances in an inverted position on the inverted maze. More specifically, pretraining consisted of 8 trials per day where the rats were released on the maze (upside-down) in close proximity to one of the four escape holes. This procedure enabled them to learn how to locomote inverted on the maze and to understand how to exit from the maze by passing through the hole and returning to a normal upright position on top of the maze. The target hole and the release direction of the rats were varied each trial. Depending on the improvement of the rats, the experimenter progressively increased the distance between the release point and the hole until the animals were able to reach the target hole when released 20 cm away from it. When animals were able to climb to the open hole from that distance, the animals were assigned to a testing group and the training phase started. The rats that could not complete this pretraining phase after 8 days were considered “bad climbers” and excluded from the experiments.

2.3.2 Training—At the start of training the rats’ cages were brought into the maze room on a wheeled cart and the cart was placed 2 m away from the maze on one side of the room. A typical training trial consisted of releasing the rat from one of the four start points (north, south, west and east) along the perimeter of the maze and allowing it to locomote inverted to the correct uncovered escape hole. In cases when the rats could not reach the hole after 30 sec, they were led to it by the experimenter. We could not allow for more search time because the rats frequently became tired while suspended and would inevitably fall off the maze after a minute. Moreover, we observed that well-trained rats were proficient at navigating to the escape hole in less than 30 sec, regardless of whether the W, E, S, or N start point was used (see Fig. 1 right panel). At the end of each trial the rat was returned to its home cage while the other rats were given a training trial. Each training session consisted of four trials and animals were given one training session per day. The latency to find the target hole was timed by the experimenter.

standing at different locations around the edge of the circular platform. All training trials were videotaped via a video camera placed on the floor below the maze and aimed upward at the inverted maze.

2.3.3 Criterion—In all three experiments, the animals were considered to have learned the task when they accurately reached the target hole (in < 30 sec) on 3 out of 4 trials for two consecutive days.

2.4 Statistical analysis

In experiment 1, an ANOVA with repeated measures for the factor *session* was performed to analyze the performance of animals trained from four start points (4SP). In all other cases learning comparisons were performed on the mean group value ($\pm$ SEM) of training days necessary to reach criterion, which were analysed using Student's *t* test. All tests comparing the performance of animals in a specific testing situation with their performance in their last regular training session were analysed using a two-way ANOVA (*session* $\times$ *group*), with repeated measures for the factor *session*.

3. EXPERIMENT 1

The aim of the first experiment was to test whether, given the marked disruption of the head direction signal during inverted locomotion, the rats were able to learn a spatial task that required them to navigate accurately upside-down. This task resembled the Morris water maze task in that the rats had to locate a spatial location relative to room cues in order to avoid an aversive stimulus, which in this case was the continuous state of being in an inverted position.

3.1 Method

Two groups of animals were trained to two different versions of the task: (1) 11 animals were trained to find the target hole from four start points (4SP) and (2) 10 animals were trained from one start point (1SP). For each animal, the target hole was kept constant during the entire training procedure.

Following training on the inverted hole board escape task, the 4SP group was trained in a Morris water maze task. The pool (180 cm diameter, 50 cm high) was positioned 14 cm above the floor. The water, which was ~23 cm in depth, was made opaque with white powder and a 13 cm diameter plastic platform was located in one quadrant of the pool, such that its top was submerged ~1 cm beneath the surface. The platform's location was fixed for all trials. Each trial was videotaped from an overhead camera. For each trial we measured the time it took for them to find and climb onto the platform (escape latency). Animals received 4 trials per day until they reached the learning criterion, which was defined as finding the platform in less than 10 sec in 3 out of 4 trials on two consecutive days.

3.2 Results and discussion

The rats trained from one start point reached criterion after a mean of 5.9 ± 0.57 (SEM) training sessions. The mean latency to reach the escape hole on their last training day was 16.2 ± 1.41 sec, range 9-22 sec. In contrast, none of the 11 rats trained in the 4SP condition could reach criterion even after 29 training sessions, although there was a small improvement over time. As shown in Figure 3, these rats improved their mean latency to the target hole with training days, and the ANOVA confirms this slight improvement over training sessions; $F(28,280) = 3.864$, $p < 0.0001$, but none of the rats met criterion. Although statistically there was an improvement over time, the graph in Figure 3 clearly indicates that this improvement was minimal, particularly after training Day 6 where subsequent learning is virtually absent.

Part of the overall small improvement is due to correct responses on some trials when the start point was at one of the two locations adjacent to the target hole. On the last 3 days of testing, the 4SP rats needed an average of 20.9 ± 1.6 sec to reach the target hole when released from the nearer start points. However, they apparently could *not* use this knowledge to improve their performance from the farther start points, as the average time to reach the escape hole was 29.7 ± 0.3 sec, which is almost the entire 30 sec length of the trial. This latency difference could be explained by the longer distances the rats had to walk when the escape hole was on the opposite side of the platform from the release point. Furthermore, because it was difficult to match the distances the rats had to locomote in the two versions of the task (1SP vs. 4SP), we cannot exclude the possibility that this distance factor contributed to the observed learning deficit (i.e., longer escape latencies) in the 4SP version of the task. However, analysis of heading trajectory (measured after the rat had walked a body length from their release point), revealed that the poor performance the rats showed from the farther start points was more likely due to an initial poor directional heading. Indeed, when released from the closer start points, the rats directional heading only deviated $12.9 \pm 3.7^\circ$ away from the correct direction to the target hole, but deviated by $53.1 \pm 4.9^\circ$ when released from the farther start points. Thus, 4SP animals were modestly able to learn how to reach the escape hole from the two close start points, but even after 29 training sessions, this knowledge did not appear to be sufficiently flexible to transfer to the two other more difficult start points that were distant to the escape hole.

In order to verify that these animals were able to learn a more normal spatial task under similar recording room conditions, we trained them in a comparable version of the standard water maze task. The results indicate that these animals display normal performance in this task and readily acquire the task. On average, the animals only required 3.9 ± 0.5 sessions to reach the learning criterion in finding and escaping onto the submerged platform. This finding indicates that their poor performance in the 4SP inverted maze cannot be attributed to 1) a general learning deficit, 2) an inability to see the surrounding visual landmark cues, or 3) the stressful characteristic of the task, because they showed normal performances in the water maze task. Moreover, the fact that the rats were able to learn the inverted escape task in the 1SP version of the task also supports this view.

In summary, although we cannot be certain that distance did not play a role in the difficulty the animals had in learning the 4SP condition, it is noteworthy that the spatial knowledge they have is not sufficiently flexible to enable them to reach criterion. Thus, our hypothesis is that the learning deficit is due to an inability to form a flexible, map-like representation of the environment when in an inverted position. To test this hypothesis, in Experiments 2 and 3 we test whether the deficit is due to the increased spatial demand of the 4SP condition.

4. EXPERIMENT 2

The aim of the second experiment was to test whether the rats had a flexible representation of the environment when navigating in the inverted maze. Experiment 1 showed that they could solve the task when they are released from a unique (single) start point. What type of strategy did they use in this situation? Did they learn the location using simple response learning (i.e., turn right), or did they learn the location using a more cognitive map-like representation? We also wanted to determine whether the rats could learn a more demanding version of the task by using two start points. Using this procedure and several probe trials enabled us to determine what strategies and cues the rats were using to solve the task.

4.1 Method

4.1.1 Training—Two new groups of rats were trained to reach the escape hole in the inverted maze, either from one start point (1SP, $n=7$), or from two start points (2SP, $n=11$). In order to be able to directly compare these two learning conditions, and to reduce the non-specific

difficulties of the task, we trained the 2SP rats from the two closest start points of their assigned hole (e.g., Fig. 2A). After reaching criterion the rats were submitted to a series of probe tests. In between each of these probe tests the rats were trained again to criterion. Analysis of the percentage of correct trial revealed no differences between groups for any of the criterion performance scores on days preceding probes ($p > 0.05$).

4.1.2 Probe Tests—As in the training sessions, the rats had 30 sec to reach the hole, but when it failed to find the hole, in order to prevent learning, it was not led to the hole, and the trial was scored 30 sec.

Center probe: The rats were released from the center of the maze facing one of the four cardinal points (4 trials). The target hole was open and the rats had 30 sec to reach it. The experimenter scored the latency they needed to reach the hole when released from this novel start point. This test enabled us to determine whether the rats had learned the task using a cognitive map-like strategy - if so, then their performance should be good when released from the novel center point. In contrast, if they had learned a more rigid strategy (*response* or *directional* strategy), then they should be impaired on this probe.

Olfactory probe: In order to test whether rats used olfactory cues to find the target hole, they were submitted to a four trial test after the maze had been rotated 90° and cleaned with alcohol. If they were using olfactory cues to locate the correct target hole, then their behavior should be altered by the suppression of these cues during the maze rotation trial.

Reversal probe: To make sure that the rats could not see the correct escape hole from the start point, they were submitted to a training session (4 trials) with a new target hole located in the opposite quadrant of the maze and released from new starting points that remained in the same relative position to the escape hole for both the 1SP and 2SP conditions. Good performance on the reversal probe would suggest that they could see the correct target hole from their start position and/or were using a simple response strategy.

Blind start probe: In this four trial test, the rats were given a regular training session (same hole and start point), except that during the last two trials the experimenter used his lab coat to shield them from viewing visual cues within the room until they were released on the maze. This probe tested the extent to which rats used spatial information they derived from room cues before being placed upside-down on the maze periphery.

Curtain probe: Finally, in order to verify that distal visual cues were essential for performing the task accurately, we suppressed these cues by hanging a black floor-to-ceiling curtain around the maze. To completely eliminate any visual cues, this test was performed under infrared light. The rats were given four regular training trials in these conditions.

4.2 Results and discussion

4.2.1 Training—Both 1SP and 2SP groups reached criterion. As shown in Figure 4A the rats trained from one start point were much faster to learn the task than those trained from two start points with the mean number of training sessions to reach criterion: 1SP = 5 ± 0.7 sessions; 2SP = 13.3 ± 1.6 sessions. Statistical analysis confirms that the 1SP group needed significantly less training sessions to learn the task [$t(16) = -3.899$, $p < 0.01$]. It is noteworthy that the 2SP rats required more than twice as many training sessions as the 1SP rats to reach criterion. This difference suggests that learning to reach the target hole from a second start point does not benefit from knowing how to reach it from one start point. One possible explanation for this deficiency is that the 2SP rats are required to build a more complex representation of the environment to encode the target hole location compared to the 1SP rats, which could have

used a simple response strategy. Thus, several probe tests were conducted to determine what kind of spatial strategy each group used to solve the task.

4.2.2 Center probe—This probe determined whether the animals used a flexible, map-like representation of the environment that permits them to reach the target hole from a new start point in the maze – in this case the center of the maze. Figure 4B shows that the performance of both groups was severely altered in this testing condition. Indeed, there is a clear increase in the mean latency to reach the target hole during the probe session (mean = 28.38 ± 0.43 sec) compared with the mean latency observed on the last regular training session (mean = 15.60 ± 0.58 sec). A two-way ANOVA with repeated measures confirms this strong *session* effect; $F(1,16) = 85.342$, $p < 0.0001$. This analysis also reveals a global *group* effect [$F(1,16) = 5.088$, $p < 0.05$], reflecting the fact that the 1SP group is on average faster to reach the hole than the 2SP group. But more importantly for our question of whether the animals used a map-like representation, there is no *session* $\times$ *group* interaction ($p > 0.05$). This result indicates that both groups were similarly affected by this testing situation, where both groups displayed poor performances when released from a new start point. This absence of flexibility may account for the poor performance we observed in the 4SP condition in Experiment 1. However, the question now arises as to what strategy they used when performing the 1SP and 2SP versions of the task.

4.2.3 Olfactory probe—One possibility is that the rats navigated to the escape hole using surface cues (e.g., olfactory or surface texture). In order to test this possibility, we cleaned the maze with alcohol and rotated it 90°. As shown in Figure 4C, this procedure did not have any effect on the performance of the animals. Indeed, analyses did not reveal any effect for *session* ($p > 0.05$) or an interaction between *session* $\times$ *group* ($p > 0.05$). As in the center probe, the only difference is a global *group* effect [$F(1,16) = 11.875$, $p < 0.01$] showing that the 1SP group was faster to reach the escape hole. This result clearly shows that the rats did not simply learn to use olfactory or surface cues to reach the escape hole, since the suppression of these cues did not alter their performance.

4.2.4 Reversal probe—A second possibility for how the rats learned the task could be that they were able to see the target hole. To test this hypothesis, they were given a training session with a new target hole, and released from new starting points that remained in the same relative position to the escape hole. Figure 4D shows an increase in the latencies to reach the hole in this condition compared with their last regular training session for both the 1SP and 2SP conditions. The analysis again indicates a global effect for *group* [$F(1,14) = 9.019$, $p < 0.01$], but more importantly, confirms the *session* effect [$F(1,14) = 60.029$, $p < 0.0001$], with no interaction for *session* $\times$ *group* ($p > 0.05$). These results suggest that the rats were not able to see the target hole. It is also noteworthy that even if, as shown by the *session* effect, the performance of 1SP animals was disrupted by the testing conditions, they still showed a small improvement in performance (mean latency: 19.0 ± 2.4 sec) over the course of the reversal session (Fig. 4E). The analysis reveals that this latency is significantly different from 30 sec [$t(6) = -4.64$, $p < 0.01$], while the mean latency for the 2SP group (27.0 ± 4.5 sec) is not significantly different from the 30 sec maximum allowable time. It is also noteworthy that analysis of the improvement in performance during this test session (two-way ANOVA) reveals a group effect $F(1,14) = 8.85$, $p < 0.01$, a global trial effect $F(3,42) = 3.02$, $p < 0.05$, and interestingly, a strong trend for interaction $F(3,42) = 2.23$, $p = 0.09$. Taken together, these results indicate that the 1SP group may have used a response strategy to find the hole in this new set of conditions. However, using a response strategy cannot be the sole explanation for the improved performance in the 1SP group, because if their behavior was based entirely on a response strategy, then their performance should not have declined at all when moving both

the target hole and the release point together, but Figure 4E shows that there was a significant decrement in performance on the first reversal trial.

4.2.5 Blind start probe—Another strategy the animals may have used to solve the task is that they may have learned where to navigate before being placed upside-down on the inverted maze by associating a particular set of visual cues at the start point with a trajectory to the target hole. If this process was true, then blindfolding them until they were on the maze (inverted) should disrupt their performance. On the day of the blind start probe the animals were given 4 trials, with the first two trials identical to the training procedures on preceding days and the last two trials conducted with the rats visually shielded on the trip from their home cage to the release point on the maze. Figure 4F plots the escape latency for the first two regular trials of this probe session (Regular Trials) compared with the performances for the last two trials when they were blindfolded (Blindfold Trials). The plot shows that blindfolding the rats before their inverted trial did not disrupt their performance. The only statistical effect is a *group* effect, but no *block* effect, or interaction between these two factors ($p > 0.05$). This result indicates that the rats did not use visual information available before their inverted trial either at their cage or in transit to the maze in order to perform the task. Thus, it appears that both groups have all the information they need to perform the task while inverted at the release point.

4.2.6 Curtain probe—The results from the above probes suggest that the rats in the 1SP and 2SP conditions are using a combination of distal room cues and trajectory learning in order to perform the task. To test whether visual information about the distal room cues was necessary, we suppressed all distal visual cues by enclosing the maze in a floor-to-ceiling curtain and conducted a regular training session under these conditions. Figure 4G shows that the performances of both 1SP and 2SP groups were severely disrupted in this testing condition, with a clear increase in the mean latency to reach the hole. In addition to the *group* effect [$F(1,15) = 5.67, p < 0.05$], the analysis reveals a clear *session* effect [$F(1,15) = 23.595, p < 0.001$], and no interaction between these factors. These results indicate that viewing the distal room cues was critically important for accurate performance. It is important to note, however, that the novelty of the testing conditions (i.e., the darkness) may have been stressful for the animals. But this possibility is unlikely when considered with the findings that the rats performances were unaffected by alterations to the environment in other probe tests (blind start and olfactory probe), as well as the observation made by others that rodents demonstrate less stress related behaviors in darkness (Alstott & Timberlake, 2009; Clark, Hamilton, & Whishaw, 2006; Eilam, 2004; Nasello, Machado, Bastos, & Felicio, 1998).

4.2.7 Summary—Taken together, these results suggest that both groups are able to orient themselves relative to distal room cues and learn to perform the spatial task. The results from the center probe also indicates that the spatial information they used was not a flexible, map-like representation of the environment, since the rats were not able to reach the target hole when released from a novel start point. Experiment 3 was designed to confirm this finding and demonstrate the rats use a *directional strategy* based on visual landmarks to solve the task, which is similar to the strategy others have suggested for the Morris water task (Hamilton, Akers, Johnson, Rice, Candelaria, Sutherland, Weisend, & Redhead, 2008) and appetitive spatial tasks in an open field and on a T maze (Skinner et al., 2003). In essence, the animals learn to associate a small set of distal visual cues with a start point, and then simply learn a trajectory from the start point to the target hole.

A second issue we wanted to address in Experiment 3 is whether both 1SP and 2SP groups were using this *directional strategy*. Interestingly, when training the 1SP and 2SP groups we noticed two differences between the two learning conditions: 1) the large difference in the mean number of sessions to reach criterion (~5 vs. 13 for 1SP and 2SP, respectively) and 2)

the *group* effect in the latency to reach the hole, that was significant for all testing conditions. In addition, we also noticed that 1SP animals showed a small improvement in the reversal test, which suggests that even if they used visual cues, they may also have encoded an egocentric strategy.

5. EXPERIMENT 3

Experiment 3 was designed to test two hypotheses. First, 2SP rats did not learn the location of the target hole relative to distal spatial cues, but rather learned distinct trajectories (directional strategy) based on simple associations between its start point and the set of distal visual cues it viewed from the start point. The second hypothesis was that even if 1SP and 2SP groups both showed comparable behavior in different probes (center probe, curtain probe), several differences (learning speed, reversal probe) suggest that they could also be using other strategies to solve this task. As mentioned above, we hypothesized that 2SP rats had learned a directional strategy, but that 1SP rats were at times learning a response strategy.

5.1 Method

5.1.1 Training—After handling and pretraining, the rats were assigned to one of the three training conditions (Fig. 2). The first group ($n=8$) was trained to reach a target hole from two start points in a similar way as in experiment 2 (Fig. 2A). The second group ($n=8$) was also released in the maze from two start points, but in this condition the rats had to reach one of two distinct target holes depending on their release point in the maze (Fig. 2B). In this condition they had to make a left turn from one start position and a right turn from the other start position. The third group ($n=8$) was also trained to reach two different target holes, but in this case the holes were in the same relative position to their start point (e.g., always to the right) (Fig. 2C). The rationale was that if the rats had to learn about the locations of the target holes, then it would be more difficult for them to learn the task when they had to encode two places (Fig 2A: 2SP–Different place–Opposite turn), than when they only had one location to learn (Fig 2B: 2SP–Same place–Opposite turn). On the other hand, if they simply learn two trajectories (directional strategy), then both conditions would necessitate the same amount of training to reach criterion. Finally, our last prediction was that if the animals showed a tendency to learn a response strategy in the inverted maze, then our third group (Fig 2C: 2SP–Different place–Same turn), should reach criterion earlier than animals that had to learn opposite turns (Fig 2B).

5.1.2 Probe Tests—When they had reached the learning criterion, the three groups were given a *center probe* test where, as previously described, they were released from the center of the maze – a novel release point for them. They were then trained to criterion again and tested with the curtain surrounding the maze (*curtain test*). Analysis of the percentage of correct trial reveals no significant difference between groups on performance scores for any of the criterion days preceding probe trials ($p>0.05$).

5.2 Results and discussion

Figure 5A depicts the number of sessions to reach criterion for the three groups. Given that we wanted to test the effect of (1) the *place* factor and (2) the effect of the *turn* factor, planned comparison tests were conducted to analyse these variables.

5.2.1 2SP-Same place-Opposite turn vs. 2SP-Different place-Opposite turn—A planned comparison between these two groups revealed no significant difference for the number of sessions the rats needed to reach criterion ($t(14) = 2.125$, $p>0.05$) with the mean number sessions = 13 ± 1.12 and 10.88 ± 1.37 for the same place and different place conditions, respectively. This result suggests that 2SP rats did not learn to reach a unique location (the

escape hole) from two start points, but instead learned two separate trajectories without benefiting from the fact that these routes lead to the same location.

Figure 5B demonstrates that both groups showed an increase in their latency to find the target hole when released from the center of the maze (center probe). This finding is confirmed by the ANOVA, which revealed a significant effect for *session* [$F(1,14)=258.48$, $p<0.0001$], a global effect for *group* [$F(1,14)=4.968$, $p<0.05$], but more importantly, no interaction between these factors ($p>0.05$). This result confirms our previous observations in experiment 2 and indicates that the rats used a rigid representation to solve the task, although from this experiment alone, we cannot determine whether the strategy was more route-based or response-based.

Finally, when tested with a curtain surrounding the maze, these two groups showed a similar alteration in their performance (Fig. 5C), with an increase in latencies to find the target hole, which is confirmed by the analysis revealing an effect for *session* [$F(1,14)=172.372$, $p<0.0001$], but no other significant difference ($p>0.05$).

Thus, these two training conditions lead to a similar behavioral profile. As hypothesized, the rats do not benefit from learning to go to the same location, which suggests that they do not encode a “place,” but more likely encode two trajectories to find the escape hole. The results of the center probe confirm the rigid character of their representation and support the view that 2SP rats use a simple association between a set of visual cues (curtain probe) and a start point for orientation in the inverted maze (directional strategy).

5.2.2 2SP-Different places-Same turn vs. 2SP-Different places-Opposite turn—

The second issue we wanted to address was whether the rats preferentially use a response strategy (e.g., always turn right) if given the opportunity, compared to a *directional strategy*, which they have to use in the regular 2SP condition. As suggested by Figure 5A, these two groups were not significantly different in the number of sessions they needed to reach criterion ($p>0.05$). This result suggests that even if a response strategy is possible, the rats do not preferentially use this strategy in the inverted maze.

Figure 5B plots the latencies for both groups when released from the center of the maze, compared to their performance on their last regular training session. Consistent with what we observed in experiment 2, the only significant difference revealed by the analysis is a *session* effect [$F(1,14)=162.187$, $p<0.0001$], confirming the rigidity of the representation used by both of these groups. Finally, Figure 5C shows the results for the curtain probe. The analysis reveals a global *group* effect [$F(1,14)=6.631$, $p<0.05$], indicating that the 2SP-Same turn group is faster to reach the escape hole than the 2SP-Opposite turn group, a global *session* effect [$F(1,14)=89.32$, $p<0.0001$], but not the expected interaction between these two factors ($p>0.05$). This result suggests that both groups were similarly affected by the suppression of distal cues.

Although the 2SP-Same turn and 2SP-Opposite turn groups performed similarly on the probe tests, it is possible that a significant difference between the two groups could be artificially masked by the 30 sec cut-off applied to the failed trials, since the mean latencies were close to this ceiling value. Another way to analyze the curtain test that avoids this problem is to plot the percentage of successful trials performed by each group. A two-way ANOVA comparing the percentage of correct trials on the probe test with their performance on criterion day reveals a global *group* effect $F(1,14)=8.40$, $p<0.05$, a *session* effect $F(1,14)=109.87$, $p<0.0001$, but also a trend for an interaction between these factors $F(1,14)=2.74$, $p=0.12$. Even if this interaction does not reach significance it is interesting to note that there is a clear difference in the performance between the 2SP-Same turn ($46.875 \pm 7.38\%$) and 2SP-Opposite turn animals ($18.75 \pm 7.83\%$), with the 2SP-Same turn animals clearly being more successful without visual cues [$t(14)=-2.614$, $p<0.05$] (Fig. 5D). This analysis is also confirmed by further evaluation

of the latencies displayed by the animals in the course of the curtain test session. Indeed, it is noteworthy that 2SP-Same turn animals show better performance scores when deprived of visual cues (mean: 21.75 ± 1.67 sec) compared to 2SP-Opposite turn animals [mean: 26.90 ± 1.55 sec, $t(14) = 2.262$, $p < 0.05$] and from the cut-off value (30 sec) [$t(7) = -4.95$, $p < 0.01$]. Thus, even if not very strong, the egocentric strategy appears to have been utilized by the 2SP-Same turn animals since they appear to be able to solve the task without visual cues.

In summary, the third experiment demonstrates that 2SP animals do not learn to reach a *place* from two different start points, but rather learn two independent *trajectories*, and do not benefit from whether the two places are the same or different. In addition, this experiment suggests that when an egocentric strategy is possible, the animals appear to encode a body-centered strategy, and are able to use this strategy when distal cues are unavailable.

6. DISCUSSION

Can rats form a flexible, map-like representation when inverted?

Experiment 1 examined whether rats, that presumably have a distorted HD signal when locomoting upside-down (Calton et al., 2005) would be able solve a spatial task that required a flexible, map-like representation of the environment while in an inverted position. The task used by Calton and Taube required a rat to traverse a 30 cm wide path that started on the floor, then involved climbing a 4 foot wire mesh to the ceiling, traversing a 4 foot ceiling in an inverted position to another wall, and then climbing down the 4 foot wall into a second compartment on the floor to retrieve a food reward. In this task Calton and Taube made two apparently incompatible observations: 1) on the one hand, HD signals recorded in the anterior thalamus were clearly disrupted when the rat was locomoting upside down, and 2) on the other hand, the authors did not notice any behavioural sign of disorientation as the rats performed the task. Indeed, the rats were able to climb and walk on the ceiling of this square ring shuttle box to reach the reward compartment without any apparent difficulty. To account for the accurate performance, but poor HD cell signals, the authors postulated that the rats may have used a simple associative strategy to compensate for their supposed loss of allocentric orientation caused by the absence of a strong directional signal. Using a simple associative strategy to perform the task would not have required the rats to remain adequately oriented when in an inverted position. Indeed, all the rats had to do once they entered the vertical plane was simply keep moving straight ahead and they would eventually reach the reward compartment.

The present experiments support their hypothesis and reconcile the two observations, since we show that even if the rats were able to solve a spatial task locomoting upside-down, they cannot build an allocentric representation of the environment. We observed that the rats used a simple and rigid orientation strategy. More specifically, our results indicate that the rats encode trajectories to find their way to the target hole in the inverted hole board escape task. In other words, they display either a directional strategy or a response strategy when it is possible, but in none of our testing conditions did the rats show the flexibility associated with place learning. Experiment 1 showed that the rats could learn to reach a target location when released from one start point, but not when they have to find the escape hole from four different start points, even after extensive training (116 trials over 29 days of testing). Experiment 2 tested whether in a less demanding version of this spatial task (2 start points), the rats would be able to build a flexible representation of the environment to perform accurately in the task. The results show that even if the animals were able to learn this task, they were severely impaired when required to reach the target hole from a new start point, indicating that, as suggested by their incapacity to perform the 4SP version of the task, they have difficulty forming rich, flexible spatial representations of their environment when they are upside-down. Further, the different probe trials revealed that they do not use a beacon strategy (olfactory or surface cues), but instead require distal visual cues. The aim of the third experiment was to clarify this point and test the

hypothesis that 2SP animals were using a simple directional strategy. The results confirmed the rigid character of their spatial representation, and more importantly, they showed that the rats did not learn to reach a specific location in space, but simply headed toward a small set of visual cues. In other words, they encode a simple association between a start point and a set of cues that indicates the goal location - they learn a *direction*, but not a *place*.

A second issue arising from experiment 2 was the large difference in learning rate between the 1SP and 2SP animals, wherein under all of the testing conditions 1SP animals had faster escape latencies than 2SP animals. This finding suggests that 1SP animals may pay less attention to distal cues and have an automatic motor response when released onto the inverted maze. We also tested whether the rats tended to use an egocentric strategy to solve the task. Although we didn't observe the expected difference in learning rate between 2SP-Different place-Same-turn and 2SP-Different place-Opposite turn groups, the curtain probe test showed that even when deprived of distal visual cues, the 2SP-Different place-Same-turn animals were able to find the target hole more accurately than animals that were not given the opportunity to learn a *response* (2SP-Different place-Opposite turn). This result suggests that under inverted conditions, a direction strategy is most likely the animals first strategy choice, but that are capable of using an egocentric strategy as well.

As discussed above, HD cells are known to encode the directional heading of the animal in an *allocentric* frame of reference (Taube, 1995; Taube et al., 1990). This characteristic is shared with hippocampal place cells and entorhinal grid cells (Hafting et al., 2005; O'Keefe, 1976). Numerous studies have demonstrated that these allocentric signals, as well as the brain areas where they are found, support the formation of cognitive maps and the flexible spatial representations that are then used to solve navigational tasks (Eichenbaum, Stewart, & Morris, 1990; Steffenach, Witter, Moser, & Moser, 2005; Taube, Kesslak, & Cotman, 1992). The behavioral criterion often used to define flexible, or *map-like* navigation, is the ability of animals to perform short-cuts and find new ways to a goal they want to reach in a familiar environment. This type of spatial representation is classically opposed to *response* and *directional* strategies, which instead, are characterized by a simple association between a stimulus and a motor response (e.g., a turn for the response strategy), or on an association between two simple set of stimuli (a start point and a salient visual cue for the directional strategy).

HD signal and response strategy

A large number of studies have shown that a response strategy (learning a motor response) and allocentric spatial mapping rely on different memory systems, which interact with each other, but can function independently, too (Gold, 2004; McDonald & White, 1993; Poldrack, Clark, Pare-Blagoev, Shohamy, Creso Moyano, Myers, & Gluck, 2001; Squire, 2004). The importance of motor information in the control of the HD signal has been demonstrated in experiments, where in the absence of reliable visual cues, motor cues permit an accurate update in the preferred firing direction of HD cells (Stackman, Golob, Bassett, & Taube, 2003). However, one important issue is whether the reverse is also true. In other words, does learning a motor response necessitate the presence of an accurate HD signal? The fact that HD cells have been recorded in the dorsal striatum of rats (Ragozzino, Leutgeb, & Mizumori, 2001; Wiener, 1993), a brain structure that has been shown to be critical for motor response learning (Knowlton, Mangels, & Squire, 1996; Packard & McGaugh, 1996; Yin, Ostlund, Knowlton, & Balleine, 2005) would suggest that, at least for motor responses in spatial tasks, the HD signal could be important. However, the relationship between the striatal HD signal and the limbic HD signal is poorly understood, and there is no evidence to support the view that striatal HD cells and limbic HD system operate independently from one another. Thus, it is possible that the striatum functions relatively normally even without a functional HD cell signal

originating from the limbic system. In contrast, several lesion studies in rats have shown that lesions of the anterodorsal thalamic nucleus (a key structure in the HD limbic network) severely alters the ability of rats to solve allocentric spatial tasks, but, interestingly, has no effect on egocentric variants of these tasks (Aggleton, Hunt, Nagle, & Neave, 1996; Sziklas & Petrides, 1999; Warburton, Baird, & Aggleton, 1997; Wolff, Gibb, Cassel, & Dalrymple-Alford, 2008). These findings support the view that the thalamic HD signal is independent of the circuit responsible for the orientation based motor response (as well as the circuit involved in a directional strategy, which is discussed below). This view would be in agreement with both our observations in the present study and consistent with the findings of Calton and Taube (2005) in their inverted HD cell study.

HD signal and directional strategy

At present very few studies have tested whether a directional strategy necessitated a functional HD network. Indeed, a directional strategy was first suggested as an alternative for flexible map-like navigation in Tolman's classic experiments (Tolman, Ritchie, & Kalish, 1946), but until recently, this spatial strategy has been neglected by neuroscientists. A few studies, however, have shown that this strategy is altered (disrupted) by hippocampal lesions (Stringer, Martin, & Skinner, 2005) and another study revealed that rats do not need a HD signal to perform a directional task accurately (Hamilton, Clark, Rice, Johnson, Akers, & Taube, 2009). In the latter study Hamilton et al. trained rats with lesions of the dorsal tegmental nucleus (DTN) in a directional version of the water maze task. DTN lesions have been shown to abolish the HD signal in the anterodorsal thalamus (Bassett, Tullman, & Taube, 2007). Hamilton showed that DTN lesioned animals were initially impaired in the direction task, but after further training they ultimately performed as well as controls. This result indicates that learning to associate a start point with distal visual cues, followed by heading in the correct direction, does not rely on an intact limbic HD signal.

Why was the 4SP condition difficult for the inverted rats to learn? Although we did not record HD cells during the task, based on previous studies it is likely that the limbic HD signal was disrupted when the animals were in an inverted position. This situation made it difficult for the animals to construct a flexible, map-like representation of the environment, and this lead to poor performances from multiple start points. There is, of course, the 'chicken and egg' issue of which came first: did the loss of the HD signal because of altered otolith input lead to the inability to form a cognitive map? Or, did the inability to construct a cognitive map, because of a presumed disorientation when inverted, lead to a disrupted HD signal? A similar set of questions could be posed for the influence of inverted orientation on place and grid cell signals, although currently there have been no studies that have examined the effects of inverted orientation on place or grid cell activity. Although the current study does not shed light on these questions, it might be possible to address it by determining whether rats are capable of learning the 4SP condition if a more progressive training method, comparable to what is used in "transverse patterning" or "transitive learning" paradigms, was used. In these protocols animals usually learn one separate problem at a time and eventually learn to string together a number of representations (Eichenbaum, Mathews, & Cohen, 1989). In our case, the rats would be trained to reach criterion from 1 start point, and then to a second one, and so on. After reaching criterion from a number of start points, it is possible that this type of training would help them form a more flexible spatial representation of the environment and possibly enable them to make inferences about a novel start position based on their extensive experience. Under this training regimen, it would be interesting to determine if accurate performances when started from novel entry points coincided with the return of an intact HD signal or if there was a dissociation between the two parameters. Of course, it is possible that even with extensive training the animals may still only learn a set of associations that entail entry points plus trajectories, as do fornix-lesioned rats in odor-discrimination tasks (Eichenbaum, Fagan,

Mathews, & Cohen, 1988; Eichenbaum et al., 1989). Regardless of whether the animals can eventually learn accurate routes from multiple start points, our results demonstrate that the first strategy of choice under the present inverted conditions is to use a directional strategy.

Summary

In summary, our study confirms the prediction made by Calton and Taube (2005), showing that rats locomoting upside-down have difficulty building a flexible spatial representation of the environment, but are still capable of simple associative strategies (egocentric or directional). This finding is consistent with other studies showing an independence of these two simple orientation strategies from the HD signal (Golob, Stackman, Wong, & Taube, 2001). Interestingly, our findings are consistent with observations in 0-g conditions where astronauts frequently have great difficulty forming a three dimensional representation of their spatial environment and often report a profound sense of disorientation (Oman, 2007). Under 0-g conditions, there is an alteration in the normal otolith signal experienced by astronauts and this unfamiliar situation (in an unknown way) impedes the astronaut's ability to form an accurate representation of their spatial environment. Using non-immersive virtual reality tasks that simulate navigational problems, several studies have demonstrated that subjects have difficulty in building an accurate mental representation of their route whenever the path requires a body rotation other than in yaw (Aoki, Ohno, & Yamaguchi, 2005; Aoki, Ohno, & Yamaguchi, 2003; Vidal, Amorim, & Berthoz, 2004). Further, when performing three-dimensional mental rotation tasks, subjects appear to use simple strategies to navigate, rather than a flexible cognitive map-like representation: either remembering the series of moves they have to make in order to reach the goal, or, by learning each module's landmark arrangement from a standard viewpoint (Oman, 2007; Oman, Shebilske, Richards, Tubre, Beall, & Natapoff, 2002; Richards, Oman, Shebilske, Beall, Liu, & Natapoff, 2003). The latter explanation is similar to the directional strategy used by the rats in the inverted hole board escape task. Along different lines a similar pattern of results was also observed in patients with unilateral vestibular loss (Peruch, Borel, Magnan, & Lacour, 2005). In their study they reported that vestibular patients were as accurate as controls when they had to reproduce a previously explored route, but showed significantly poorer performance when the task was more complex and required them to return to their point of origin after walking a path formed by two legs of a triangle (path completion task). These results are consistent with the view that an intact vestibular system was necessary to form an accurate, map-like representation of the environment.

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Figure 1.

The test room and the inverted maze apparatus. The apparatus consisted of a 190-cm diameter circular wire mesh that was suspended ~50 cm from the ceiling, with four evenly spaced holes that formed four possible escape exits from the maze. Each one of these 15-cm holes could be blocked with a piece of wood. The wire mesh was composed of metal screening with square size of 0.5×0.5 cm.

Figure 2.

Experimental protocol for the two start points (2SP) training conditions. A) 2SP-Same place-Opposite turn: the rats had to learn to reach a single target hole from two different start points (e.g., South and East) that required two opposite turns (right from South, and left from East). B) 2SP-Different place-Opposite turn: the rats were also released in the maze from two start points, but in this condition they had to reach one of two distinct target holes (different place) depending on their release point in the maze. The start points were selected such that the rats had to learn two opposite turns, similar to the conditions in A. C) 2SP-Different place-Same turn: the rats were trained to reach two different escape holes (different place) from two start points, but in this case, both escape holes were in the same relative position from the start point. In this condition, learning a particular egocentric turn (e.g., always turn right) could be a successful strategy for the rats.

Figure 3.

Escape latencies for the 4SP and 1SP conditions in experiment 1. *A*) Mean escape latencies for the 29 training sessions (4 trials per session) for the animals trained to reach the escape hole from 4 start points. *B*) Mean escape latencies for the animals trained to reach the escape hole from a single start point (1SP condition). Because the two groups did not reach criterion in the same amount of time, the graph plots the individual learning curve for each animal.

Figure 4.

Results from experiment 2. *A)* Mean number of sessions to criterion for the animals trained to reach the escape hole from 1 start point (1SP) and 2 start points (2SP). *B)* Mean escape latency that 1SP and 2SP animals needed to reach the escape hole when released on the maze from a novel starting location (center of the maze), compared with the mean escape latency on their last regular training session (Criterion). *C)* Mean escape latency that 1SP and 2SP animals needed to reach the hole after the maze was cleaned with alcohol and rotated 90° (Olfactory probe), compared with the mean escape latency on their last regular training session (Criterion). *D)* Mean escape latency that 1SP and 2SP animals needed to reach a new target hole (Reversal probe), compared with the mean escape latency on their last regular training session (Criterion). *E)* Mean escape latency that 1SP and 2SP needed to reach the new target hole over the course of the reversal session (4 probe trials). *F)* Mean escape latency that 1SP and 2SP animals needed to reach the escape hole (2 last trials) when the rats were shielded from viewing visual cues within the room until they were released on the maze (Blindfolded trials), compared with the mean escape latency on the first two regular trials of the same session (Regular trials). *G)* Mean escape latency that 1SP and 2SP animals needed to reach the escape hole when deprived from distal visual cues (Curtain probe), compared with the mean escape latency on their last regular training session (Criterion).

Figure 5.

Results from experiment 3. *A*) Mean number of sessions to reach criterion for animals trained to reach a single escape hole from 2 start points (2SP), 2 different escape holes requiring opposite turns (Different place - Opposite turn), and 2 different escape holes requiring turns in the same direction (Different place - Same turn). *B*) Mean escape latency the three groups needed to reach the escape hole when released from the center of the maze (Center Probe), compared with the mean escape latency on their last regular training session (Criterion). Note that the values for the Different place - Same turn group are hidden from view by the values from the 2SP group. *C*) Mean escape latency the three groups needed to reach the target hole when deprived from viewing distal visual cues (Curtain Probe), compared with the mean escape latency on their last regular training session (Criterion). *D*) Percentage of correct trials for 2SPDiff place-Opposite turn animals (Opposite Turn) and 2SP-Diff place-Same turn animals (Same Turn) during the curtain probe.