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Distinct Roles of Hippocampus and Medial Prefrontal Cortex in Spatial and Nonspatial Memory

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ABSTRACT: In earlier work, patients with hippocampal damage successfully path integrated, apparently by maintaining spatial information in working memory. In contrast, rats with hippocampal damage were unable to path integrate, even when the paths were simple and working memory might have been expected to support performance. We considered possible ways to understand these findings. We tested rats with either hippocampal lesions or lesions of medial prefrontal cortex (mPFC) on three tasks of spatial or nonspatial memory: path integration, spatial alternation, and a nonspatial alternation task. Rats with mPFC lesions were impaired on both spatial and nonspatial alternation but performed normally on path integration. By contrast, rats with hippocampal lesions were impaired on path integration and spatial alternation but performed normally on nonspatial alternation. We propose that rodent neocortex is limited in its ability to construct a coherent spatial working memory of complex environments. Accordingly, in tasks such as path integration and spatial alternation, working memory cannot depend on neocortex alone. Rats may accomplish many spatial memory tasks by relying on long-term memory. Alternatively, they may accomplish these tasks within working memory through sustained coordination between hippocampus and other cortical brain regions such as mPFC, in the case of spatial alternation, or parietal cortex in the case of path integration. © **2016 Wiley Periodicals, Inc.**

KEY WORDS: working memory; long-term memory; path integration; spatial alternation; odor alternation

INTRODUCTION

The formation of declarative memory depends on the integrity of the hippocampus and related medial temporal lobe (MTL) structures (Squire, 1992; Eichenbaum and Cohen; 2001). These structures have also been associated with spatial cognition, including spatial navigation and path integration (Moser et al., 2008). These two perspectives are not entirely compatible (Eichenbaum and Cohen, 2014; Buffalo, 2015). The issue centers on the historic distinction between short-term (working) memory and long-term memory. Working memory refers to the limited amount of information that can be held in mind by active maintenance for a short time after learning (sometimes characterized as 7 digits, 4 objects, or 1 face) (Baddeley, 2003; Cowan, 2001; Warrington and Taylor, 1973). Working memory has been thought to be independent of the MTL and intact after MTL damage (Milner, 1972; Baddeley and Warrington, 1970; Jeneson and Squire, 2012). If so, tasks that can be managed within working memory, including spatial tasks, should be spared after MTL damage. Yet, if MTL structures support the computations needed for spatial tasks such as spatial navigation and path integration, then MTL damage should impair performance on these tasks regardless of the availability of working memory. Indeed, for spatial tasks the distinction between working memory and long-term memory might be irrelevant.

Several studies of patients with MTL damage have found intact performance on spatial memory tasks, including path integration, under conditions when working memory appears to support performance (Shrager et al., 2008; Jeneson et al., 2010; Kim et al., 2013). However, parallel tests of path integration in rats with hippocampal lesions yielded different results (Kim et al., 2013). In path integration, rats search for food in the dark and then attempt to return with the food to their start location. The finding was that rats performed at chance even with the simplest paths, e.g., when their outward path was only 1m in length, involved no turns, and was completed within 3s. If working memory can support successful path integration in humans with MTL damage, what might account for the inability of rats with hippocampal lesions to path integrate even in the simplest conditions when working memory might be expected to be available?

In the present study, we considered possible ways to understand these findings. Spatial working memory, as required for path integration, might be unavailable (or impoverished) because the rodent cortex is insufficient to construct and maintain a coherent spatial working memory of complex environments. If so, performance must depend on long-term memory (LTM), which depends on the hippocampus. Alternatively, as others have suggested, some forms of spatial working memory might depend on interaction between hippocampus and regions of neocortex such as medial prefrontal cortex (mPFC) (Jones and Wilson, 2005; Hyman et al., 2010; Gordon, 2011; Spellman et al., 2015). Hippocampal lesions would disrupt this

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interaction and impair working memory. To explore these ideas, we tested rats with either hippocampal lesions or mPFC lesions on three tasks of spatial or nonspatial memory: path integration, spatial alternation, and a novel, nonspatial task that required alternation between two different odor-scented cups.

MATERIALS AND METHODS

Experiment 1: Medial Prefrontal Cortex Lesions, T-Maze Alternation, and Path Integration

Subjects

Male Long Evans rats ($n = 35$) were prepared with bilateral lesions of the medial prefrontal cortex ($n = 18$) or served as sham-operated controls ($n = 17$). Cohort 1 (lesion = 12, control = 11) was tested on spatial alternation and then on path integration. Cohort 2 (lesion = 5, control = 6) was tested on path integration and then on spatial alternation. In cohort 2, one lesion animal did not complete path integration. In addition, two lesion animals and 1 control animal did not complete spatial alternation. These animals were excluded from the analysis of the relevant tasks. Rats were individually housed, maintained on a 12:12 h light:dark cycle, and tested in the light phase of the cycle. All procedures were approved by the University of California at San Diego, Institutional Animal Care and Use Committee.

Spatial Alternation Apparatus

A T-maze was constructed from wood and painted flat black. The stem of the maze was 52 cm long with a 20 cm start box and 32 cm runway. The left and right arms were 35 cm long. All the alleys of the maze were 10 cm wide, and the maze itself was 18 cm high. Plastic doors could be inserted to confine animals to the start box and to block entrance to either arm. A black plastic food cup (5 cm diameter) was held in place by Velcro at the end of each arm. The cup was 1.5 cm tall, enough to block the reward from view as the rat entered the arm, and it was located at a sufficient distance from the entrance to minimize odor cues from the reward itself.

Spatial Alternation Pretraining

During pretraining rats were maintained at ~80% of their free-feeding weight. Pretraining occurred over a five-day period (six trials per day). On the first three days, the rat was placed in the right or left arms six different times. One half of a Froot Loop was placed in a food cup at the end of the arm, and the rat had five minutes to consume the food. On the final two days, one arm was open, while the other arm was blocked off (forced-choice trial). The animal had five minutes to travel from the start box into the open arm to consume the food. The arm that was open on each trial was varied randomly to ensure that the rat spent equal time in each arm but did not learn any specific rule.

Surgical Procedure

Bilateral excitotoxic lesions of the medial prefrontal cortex were make using ibotenic acid (IBO; Biosearch Technologies). Isoflurane gas (delivered in O_2 at 1 l/min) was used to maintain anesthesia throughout the surgery and was varied from 0.8–2.0%. The rat was placed in a Kopf stereotaxic instrument, and the incisor bar was adjusted until Bregma was level with Lambda. IBO dissolved in 0.1 M PBS (concentration: 10 mg/ml, pH 7.4) was injected using a 10 μl Hamilton syringe attached to a Kopf microinjector (model 5000) mounted on the stereotax. The syringe was first lowered to the target depth and left in place for 1 minute. After injection at a rate of 0.1 μl/min, the syringe stayed at depth for 2 min to prevent the IBO from moving up the needle. Lesions were made at multiple locations (all coordinates are in millimeters and relative to Bregma): anteroposterior (AP) +2.0, mediolateral (ML) ±0. 7, dorsoventral (DV) −3.0; AP +3.0, ML ±0.7, DV −3.0, −4.0; AP +4.0, ML ±0.7, DV −3.0; AP +5.0, ML ±0.7, DV −3.0. At each site, 0.3 μl of IBO was injected.

Spatial Alternation Testing

Rats received seven trials each day for 10 days. Every trial began with the rat confined to the start box for 10 s. The first trial of each day was a forced-choice trial with one arm blocked (left or right arm equally often). On the subsequent six trials, both arms were open to allow the rat a free choice of which arm to enter. The open arm on the first trial was baited with reward, and on subsequent trials the baited arm was always opposite to the arm entered on the previous trial. A choice of arms was counted when all four paws were within one of the arms. The door was then lowered, the rat was allowed 10 seconds to either consume the food or to find an empty food cup, and the rat was then returned to the start box.

Spatial Alternation Data Analysis

Performance was measured as the percentage of trials on which a rat correctly alternated arm entrances (six possible alternations each day).

Path Integration Apparatus

The testing table was made of circular Plexiglas (2 m in diameter) elevated 64 cm above the floor and mounted on wheels around a fixed central platform that allowed the table to be easily rotated (Kim et al., 2013). Eight holes (12 cm diameter) were placed equidistantly around the table with plastic boxes mounted beneath them (Fig. 1). Each box was filled with used rat bedding. Wire mesh screens could be inserted between the box and the table in order to block entrance to a given hole. Infrared lights and an infrared camera were mounted above the table to track the animal's movements in darkness.

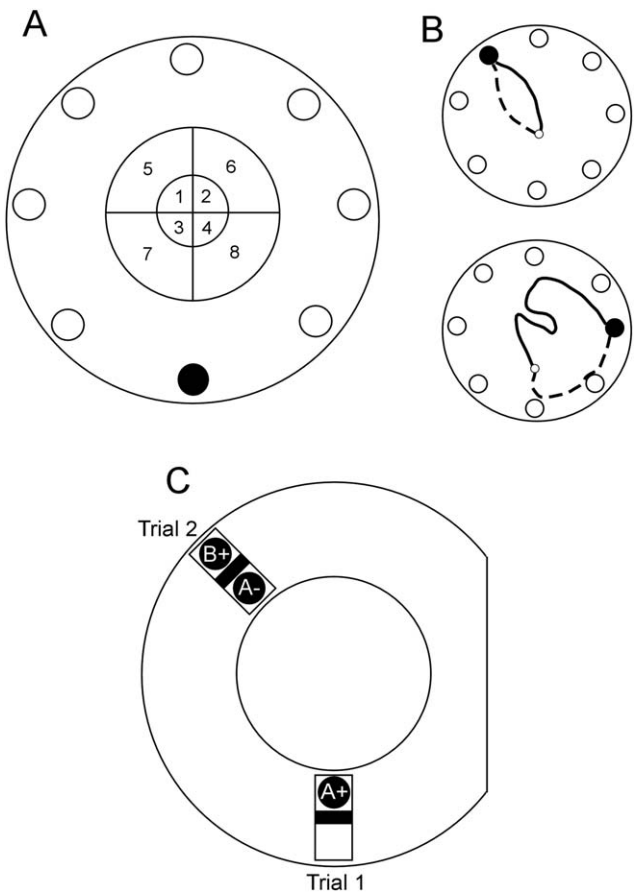

FIGURE 1. Path Integration and odor alternation. (A) The platform (2m diameter) used for path integration had eight escape holes, only one of which was open for any trial (indicated in black). The trial began when the rat left the escape hole to search for a food pellet and ended when the rat returned to the escape hole to eat the food. The accuracy of the return path was determined by the first hole that the rat visited after finding the food. A direct return to the open hole received a score of 0. A return to a hole adjacent to the open hole received a score of 1, and a return to holes further from the open hole received scores of 2, 3, or 4. (B) Two sample trials where the outward path to find the food is indicated in black, and the return path to the open hole is indicated by a dashed line (the food is represented by a white circle). The shorter path would receive a score of 0, and the longer path a score of 2. (C) The platform (91 cm diameter at widest point) used for odor alternation had a circular insert (48 cm diameter, 35 cm tall) in the middle, creating an annular corridor (24 cm wide). On trial 1, a cup of sand and scent A was placed in the annulus. After the animal successfully dug to retrieve the food reward, the first cup was removed, and two cups of sand with scents A and B were placed in a second location in the corridor. On trial 2, the cup with scent B was baited, and the presentation side of the baited cup was counterbalanced across trials. The animal was recorded as making a choice when it began to dig in one of the cups.

Path Integration Pretraining

During pretraining and testing, rats were maintained at ~80% of their free-feeding weight. Pretraining began with two days of exploration of the table in lighted conditions with all holes blocked off. After this initial period of exploration, five pellets of food (750-mg rodent pellets, Bio-Serv) were placed on the table for the rat to consume. After 3 or more pellets were consumed for two consecutive days, a hole was opened and the rat was placed inside. Once the rat exited the hole and consumed 3 or more pellets on each of two days, the procedure was repeated in the dark. The final phase began when the rat consumed 3 or more pellets in the dark condition. In the final phase the rat left the hole, found a single pellet, and brought it back to the hole to consume. Four trials were given each day with a 5-minute time limit per trial. Pretraining was complete when the rat successfully completed all four trials within the time limit on two consecutive days.

Path Integration Testing

Rats were given four trials per day with the lights off until they had successfully completed 50 trials. Rats were tested in groups of four, such that all four animals received one trial before receiving a second trial. A successful trial was one in which the rat left the open hole, found the food pellet on the table, and brought it back to the open hole to consume, all within a total of 5 minutes. Only one of the eight holes was open on each trial, and the open hole changed after each trial based on a pseudorandom sequence. The table was rotated three different times during each test session to change the position of the holes relative to the room (after all animals had received the first trial, after all had received the second trial, and after all had received the third trial). No hole was re-used in a test session until all the other holes had been used. The food was located at one of eight possible zones on the table (Fig. 1A). The zone used on each trial was pseudorandomized, and the placement of the food pellet within a zone varied. Once the rat returned to the open hole with the food, he was allowed to consume it before being returned to the home cage. If the rat consumed the food on the table or dropped the food before returning to the open hole, the trial was discarded (less than 1% of trials). One animal in the lesion group would not bring the food back to the open hole on the majority of trials and was excluded from analysis for this task.

Path Integration Data Analysis

Performance was measured by how accurately the rat returned to the open hole after finding the food. The animal could return to the start box itself (a score of zero), to one of the boxes adjacent to the start box (a score of 1), to one of the boxes 90° removed from the start box (a score of 2), to one of the boxes 135° removed from the start box (a score of 3), or to the box opposite from the start box (a score of 4) (Fig. 1). The accuracy of the return path was analyzed as a function of the amount of time taken to find the food (0-3, 4-6, >6 s), the number of 90° turns made on the outward path (0, 1, >1) or the length of the outward path (0-1, 1-2, >2 m).

EXPERIMENT 2: HIPPOCAMPAL LESIONS, T-MAZE ALTERNATION AND PATH INTEGRATION

Two groups of male Long Evans rats were tested. For spatial alternation, 8 animals were prepared with bilateral lesions of the hippocampus, and 8 animals served as sham-operated controls. One control animal did not complete spatial alternation. For path integration, 5 animals were prepared with bilateral lesions of the hippocampus, and 7 animals served as sham-operated controls. Data from these animals have been reported previously (Kim et al., 2013) and are included here for comparison. Rats were individually housed, maintained on a 12:12 h light:dark cycle, and tested in the light phase of the cycle. All procedures were approved by the University of California at San Diego, Institutional Animal Care and Use Committee.

Spatial Alternation and Path Integration

Spatial alternation and path integration testing followed the same procedure as in Experiment 1.

Surgical Procedure

Bilateral excitotoxic lesions of the entire hippocampus were performed using IBO under the same conditions as Experiment 1. Coordinates for the lesions were in millimeters relative to Bregma and are the same as in Clark et al., (2000): AP −2.4, ML ± 1.0, DV −3.5; AP −3.2, ML ± 1.4, DV −3.2, −2.3; AP −3.2, ML ±3.0, DV −2.7; AP −4.0, ML ±2.5, DV −2.8, −1.8; AP −4.0, ML ±3.7, DV −2.7; AP −4.8, ML ±4.9, ML −7.2, −6.4; AP −4.8, ML ±4.3, DV −7.7, −7.1, −3.5; AP −5.4, ML ±4.2, DV −4.4, −3.9; AP −5.4, ML ±5.0, DV −6.6, −5.9, −5.2, −4.5. Sham-operated control animals underwent the same surgical procedures up to the point of the craniotomy. Animals were given 14 days to recover after surgery before testing resumed.

EXPERIMENT 3: HIPPOCAMPAL AND MPFC LESIONS AND ODOR ALTERNATION

Male Long Evans rats ($n = 11$) were prepared with bilateral lesions of the hippocampus ($n = 6$) or served as sham-operated controls ($n = 5$).The two groups were matched based on performance before surgery. Later, the control group underwent a second surgery and received bilateral lesions of the medial prefrontal cortex. Rats were individually housed, maintained on a 12:12 h light:dark cycle, and tested in the light phase of the cycle. All procedures were approved by the University of California at San Diego, Institutional Animal Care and Use Committee.

Odor Alternation Apparatus

Testing took place on a platform (91 cm diameter at its widest point) with a 51 cm straight edge placed flush to a wall (Fig. 1C). A black plastic wall (9.5 cm high) was attached to the circular portion of the platform's perimeter. Initial training occurred on the platform. For testing, a plastic circular insert (48 cm diameter; 35 cm tall) was placed in the center of the platform to create an annular corridor 24 cm wide (narrower next to the wall). Testing took place within the 24 cm-wide portion of the annulus to insure that the two stimuli were equally accessible as an animal approached them.

Two 1% mixtures of scented play sand were prepared (one with cocoa and one with cinnamon). The scented sand was placed into two different glass cups (150 g each). One cup was 6.5 cm tall and 6 cm in diameter, with a smooth exterior design. This cup was used exclusively to hold cinnamon-scented sand. The other cup was 7 cm tall and 7 cm in diameter with a rough, pitted exterior design. This cup was used exclusively to hold cocoa-scented sand. The two cups were presented on holders constructed of .25 cm acrylic Perspex. The base of each holder was 22 cm X 10 cm, and a divider (10 cm high X 10 cm wide) was centered on the base between the cups. The cups were attached to the holder using Velcro. Ten cups of each design and holders were constructed so that all the odor pairs needed for a single testing session were available at the beginning of the session.

Odor Alternation Pretraining

During pretraining and testing, rats were maintained at ~80% of their free-feeding weight. Pretraining began with 150 g of unscented sand in a clear glass cup (6 cm diameter, 8 cm tall) placed in a plastic holder. 12 Froot Loop halves were placed at three different depths (4 fully buried, 4 half buried, 4 on top). The holder was placed in the home cage, and rats were allowed to consume the Froot Loops. This procedure was repeated after one hour and twice more on the following day. Beginning on the third day, 6 trials were presented on the platform, with a single glass cup on either the left or right side of the holder. Froot Loop halves were buried at varying depths, and each trial ended when the rat had eaten two halves. As the rat learned to dig across the six trials, the depth of the buried food was increased until the reward was fully buried in the sand (1.5-2.0 cm deep). Trials were separated by 10 sec, and there was a 20-min time limit to complete the 6 trials. This phase of pretraining ended when the rat completed the 6 trials within the 20-min time limit.

The rats were then trained on the odor alternation task in the open field of the platform. Rats received one trial with a single cup followed by 10 trials with two cups. On the two-cup trials, both scented cups were presented together. To receive a reward, the rat had to select the scented cup that was not selected on the previous trial. Thus, if the rat selected the cocoa-scented cup on a two-cup trial (regardless of whether the choice was correct), the rat needed to select the cinnamon-scented cup on the following trial in order to

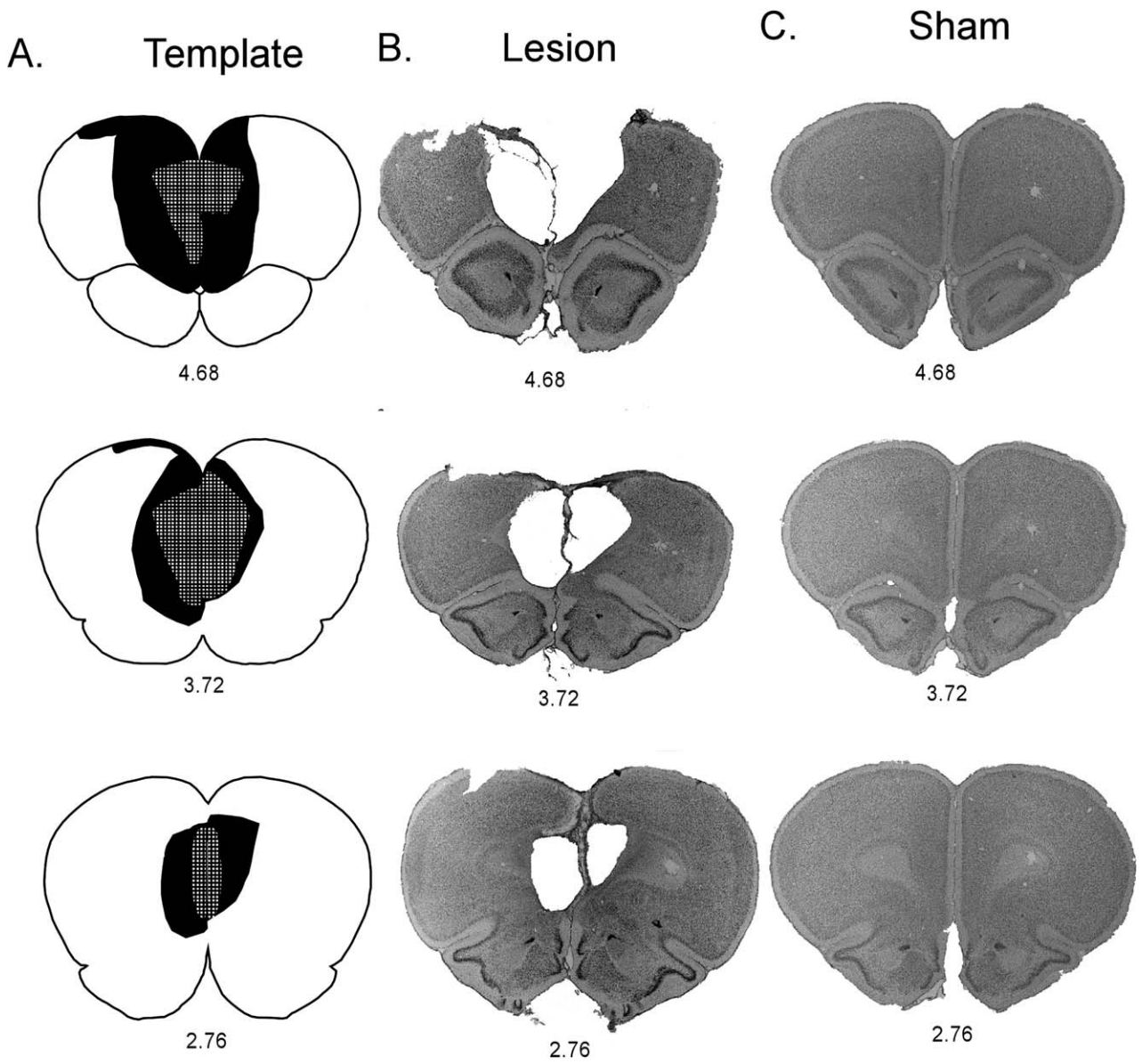

FIGURE 2. mPFC Histology. (A) A template showing the extent of the largest (gray) and smallest (black) lesions. Numbers below each template represent millimeters anterior to Bregma. Damage to the prelimbic and infralimbic cortices ranged from 85 − 100% (mean: 97%). There was also significant damage to the medial orbital cortex (mean: 93%), cingulate cortex (Area 1 mean: 68%, Area 2 mean: 47%), and the dorsal peduncular cortex (mean: 56%). (B). Representative histological sections from an animal with a medial prefrontal cortex lesion.

receive a reward (odor alternation). A choice was scored when the animal began to dig in one of the cups. If the choice was correct, the animal was allowed to find and consume the food reward. The holder was then removed. If the choice was incorrect, the holder was removed after the rat dug far enough to have obtained a reward had it been there. In either case, when the holder was removed, it was immediately replaced by another holder somewhere else along the corridor. The time between trials was determined by the rat's behavior and averaged about 10 sec. The left-right position of the correct cup was counterbalanced across trials.

Odor alternation testing was conducted within the annulus. On each trial a holder was placed within the annular corridor. Critically, spatial information was irrelevant to performance because the correct cup could appear at any location within the 24 cm-wide portions of the corridor. Between animals, the holders and platform were cleaned with 50% ethanol. After each day, all cups and holders were washed with water and cleaned with 50% ethanol. Scented-sand was remade weekly.

Surgical Procedure

Bilateral excitotoxic lesions of the hippocampus were performed using IBO under the same conditions as Experiment 1.

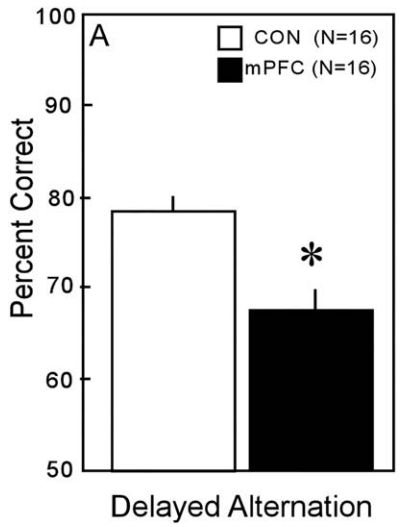

FIGURE 3. Spatial alternation after lesions of medial prefrontal cortex. Performance on the spatial alternation task was measured by the percentage of trials in which the animal correctly alternated arm entrances to receive food reward (percent correct). Animals received 7 trials a day for ten days. Animals with mPFC lesions were impaired relative to sham operated controls across ten days of testing. Error bars show the standard error of the mean (*$P < 0.001$).

Following the first round of post-surgical testing, the 5 animals that previously served as sham-operated controls received bilateral lesions of the medial prefrontal cortex under the same conditions and using the same coordinates as in Experiment 1.

Odor Alternation Testing

Rats received one single-cup trial and 10 two-cup trials per day until 10 days of testing were completed.

Odor Alternation Analysis

Performance was measured as the percentage of trials on which a rat correctly alternated odor choices (10 possible alternations each day). Testing occurred during 10 days following recovery from surgery.

RESULTS

Experiment 1: Medial Prefrontal Cortex Lesions, T-Maze Alternation and Path Integration

Neurohistological findings

All rats sustained bilateral damage to the prelimbic and infralimbic areas of the prefrontal cortex (Fig. 2) that ranged from 85% to 100% (mean 97%). All rats also sustained damage to surrounding areas of the prefrontal cortex, in particular the medial orbital cortex (mean 93%), cingulate cortex (Area 1 mean: 68%, Area 2 mean 47%) and the dorsal peduncular cortex (mean 56%).

Spatial alternation

The two cohorts of animals with mPFC lesion performed nearly the same, and were significantly impaired regardless of whether testing occurred before or after testing on path integration (ts > 2.5, ps < 0.05). Overall, the mPFC lesion group was impaired relative to the CON group across the 10 days of testing (mPFC mean $= 67.6 \pm 2.5\%$; CON mean $= 78.8 \pm 1.2\%$; $t(30) = 4.10$, $P < 0.001$; Fig. 3) and both groups performed better than chance (ts$(15) > 7.1$, ps < 0.001).

Path integration

The two cohorts performed similarly on all measures, and their data were combined (Fig. 4). Animals with medial prefrontal cortex lesions (mPFC) performed as well as control animals (CON) at path integration and were often numerically (but not significantly) better. The marginal advantage of the mPFC group is likely attributable to poor performance of the

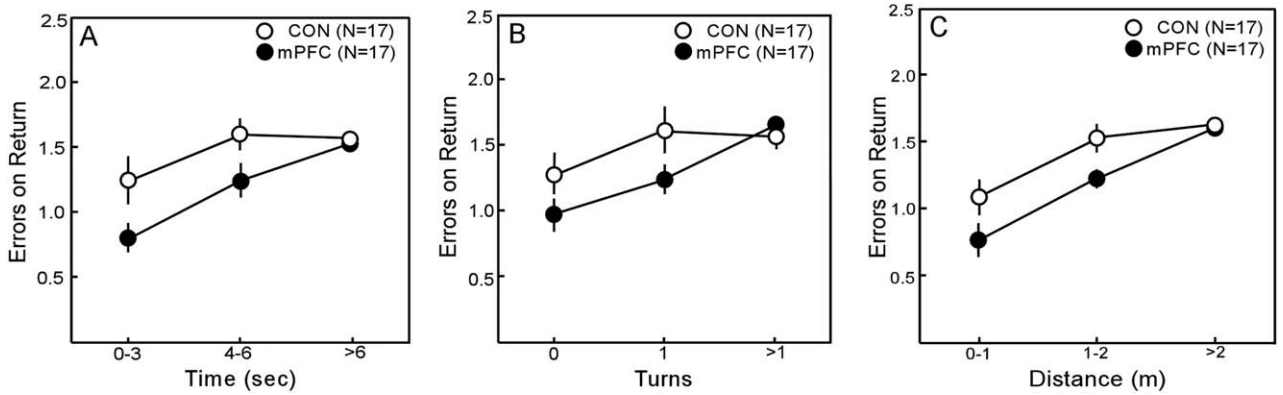

FIGURE 4. Path integration after lesions of medial prefrontal cortex. Trials were sorted according to the amount of time taken on the outward path (A), the number of turns taken on the outward path (B), and the distance traveled on the outward path (C). The accuracy of the return path (errors on return) was determined by the distance between the first hole visited and the open hole where the trial started. Lower scores indicate better performance. Animals with medial prefrontal cortex lesions (mPFC) performed as well as sham-operated controls (CON) in every condition and usually numerically better. Error bars show standard error of the mean.

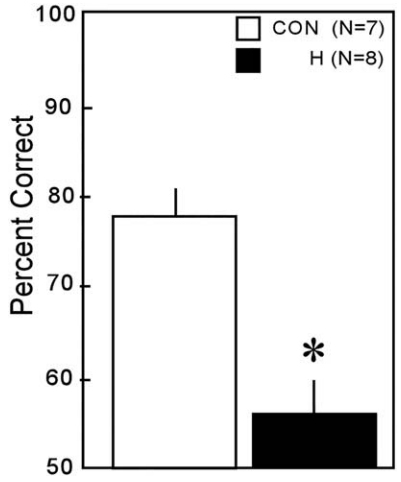

FIGURE 5. Spatial alternation after lesions of hippocampus. Performance on the spatial alternation task was measured by the percentage of trials in which the animal correctly alternated arm entrances to receive food reward (percent correct). Animals received 7 trials a day for ten days. Rats with hippocampal lesions were impaired relative to sham operated controls across the ten days of testing. Error bars show the standard error of the mean (*$P < 0.01$).

control group rather than to a facilitation of the mPFC group (see Fig. 6 for better control performance). The mPFC group performance declined as paths took more time, became more complex, and covered a longer distance (Time [0-3s] vs. >6 s: $t[16]=4.8$, $P<0.05$; Turns [0] vs. [>1]: $t[16]=4.2$, $P<0.05$; Distance [1m] vs. [>2m]: $t[16]=4.1$, $t<0.05$). Control performance showed a similar decline as paths grew longer (Distance [1m] vs [>2m]: $t[16]=3.0$; $P<0.05$), but this decline was not reliable for the other two measures. Finally, note that there was no difference between the two groups in any of the outward path measures between the two groups (Time: mPFC mean = 17.2 ± 1.1 s, control mean = 20.0 ± 1.6 s, $t[32]=1.5$, $P=0.15$; Turns: mPFC mean = 3.4 ± 0.2, control mean = 3.1 ± 0.2, $t[32]=1.0$, $P=0.3$; Distance: mPFC mean = 4.8 ± 0.3 m, control mean = 4.7 ± 0.2 m, $t[32]=0.22$, $P=0.8$).

Experiment 2: Hippocampal Lesions, T-Maze Alternation, and Path Integration

Neurohistological findings

Animals tested on spatial alternation sustained bilateral damage to all cell fields of the hippocampus. This damage included 82-100% of the hippocampus, with 96% mean damage. Animals tested on path integration sustained bilateral damage to all cell fields of the hippocampus. This damage included 85 − 97% of the hippocampus with 93% mean damage. Sparing occurred most frequently in the most medial aspects of the dorsal dentate gyrus and CA1 cell field, as well as the ventralmost region of the hippocampus. All rats had some damage to the cortex and fimbria overlying the dorsal hippocampus, which was associated with the placement of the syringe during surgery.

Spatial alternation

Animals with hippocampal lesions were impaired relative to CON animals across 10 days of testing (H mean = 55.2± 3.7%, control mean = 77.2 ± 2.9%, $t(13)=4.5$, $P<0.01$) (Fig. 5). The CON group performed better than chance ($t(6)=9.3$ $P<0.001$), but the H group did not ($t(7)=1.4$, $P>0.1$).

Path integration

Animals with lesions of the hippocampus (H) were impaired relative to sham operated controls (CON) (Fig. 6). This impairment was observed even in the simplest conditions:

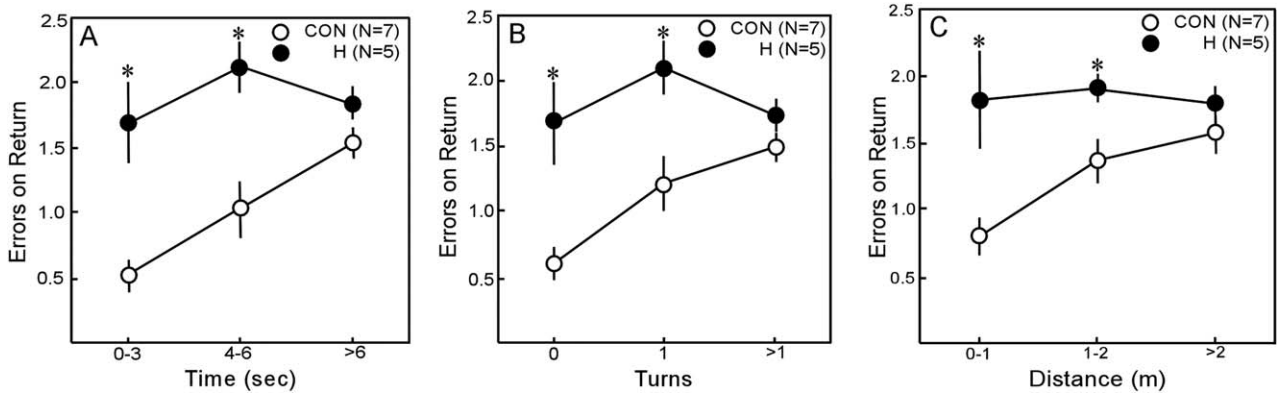

FIGURE 6. Path Integration after lesions of the hippocampus (from Kim, Sapiurka et al 2013). Trials were sorted according to the amount of time taken on the outward path (A), the number of turns taken on the outward path (B), and distance traveled on the outward path (C). The accuracy of the return path (errors on return) was determined by the distance between the first hole visited and the open hole where the trial started. Lower scores indicate better performance. Animals with hippocampal lesions (H) were impaired relative to sham operated controls (CON) on outward paths that took 6 seconds or less to complete (A), involved 0 or 1 90° turn (B), and were 2 meters or shorter (C). As trials became more complex, both groups performed poorly. Error bars show the standard error of the mean (*$P < 0.05$).

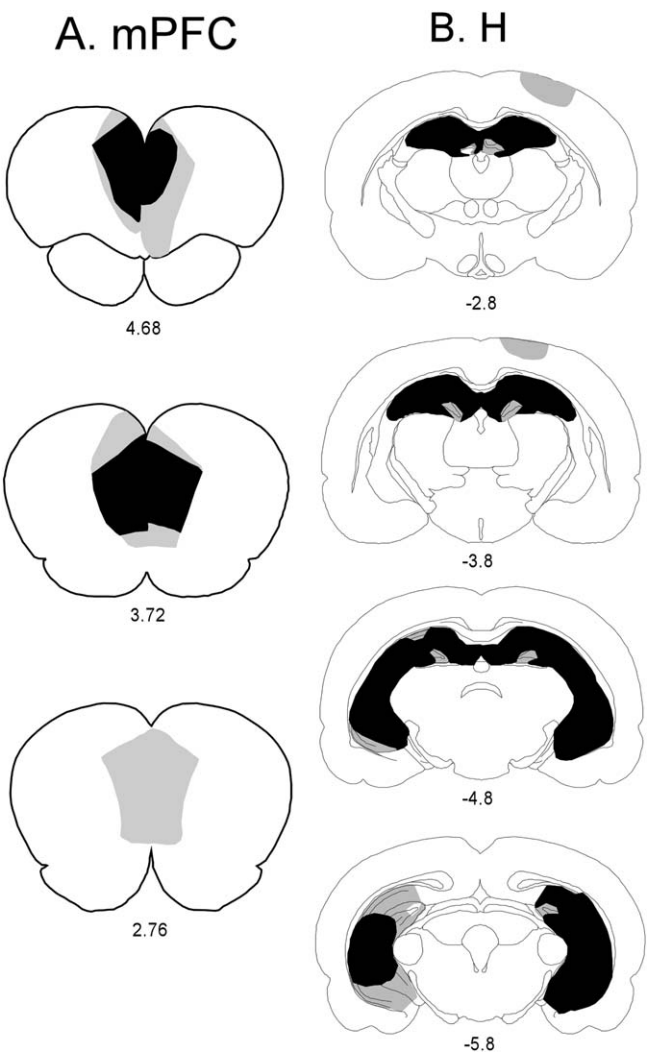

FIGURE 7. Odor alternation histology. (A) A template showing the extent of the largest (gray) and smallest (black) mPFC lesions. Numbers below each template represent millimeters anterior to Bregma. Damage to the prelimbic and infralimbic cortices ranged from 70%-100% (mean: 87%). There was also significant damage to the medial orbital cortex (mean: 70%), area 1 of cingulated cortex (mean: 46%), and the dorsal peduncular cortex (mean: 25%). B: A template showing the extent of the largest (gray) and smallest (black) H lesions. Numbers below each template represent millimeters anterior to Bregma. Damage to the hippocampus ranged from 83 to 96% (mean: 90%); sparing occurred most frequently in the most medial aspects of the dorsal dentate gyrus and the ventral-most region of the hippocampus.

when the food was found within 3 seconds ($t(10)=3.8$, $P<0.01$), when the outward path involved no turns ($t(10)=3.6$, $P<0.01$), or when it was less than 1 meter in length ($t(10)=2.9$, $P<0.05$). In addition, the H group performed no better than chance in any condition (calculated as an accuracy score of 2; H: all $ts<1.5$, all $ps>0.1$). For more complex outward paths involving $4-6$ s ($t(10)=3.6$, $P<0.01$), one turn ($t(10)=3$, $P<0.05$) or $1-2$ m ($t(10)=2.5$, $P<0.05$), the H animals were also impaired relative to the control group and performed no better than chance (H: all $ts<1$, all $ps>.1$; control: all $ts>5$, all $ps<0.01$). As expected, the control group exhibited poorer performance as outward paths became more complex (repeated measures ANOVA; All $Fs(2,12)>9.9$, all $ps<0.01$).

Experiment 3: Hippocampal and mPFC Lesions and Odor Alternation

Neurohistological findings

Animals that received hippocampal lesions sustained damage to all cell fields of the hippocampus (Fig. 7). This damage included $83-96\%$ of the hippocampus, with 90% mean damage. Sparing occurred most frequently in the most medial aspects of the dorsal dentate gyrus and CA1 cell field, as well as the ventral-most region of the hippocampus. All rats had some damage to the cortex and fimbria overlying the dorsal hippocampus, which was associated with the placement of the syringe during surgery.

All rats that received mPFC lesions sustained bilateral damage to the prelimbic and infralimibic areas of the prefrontal cortex (Fig. 7) that ranged from 70% to 97% (mean 87%). All rats also sustained damage to surrounding areas of the prefrontal cortex, in particular the medial orbital cortex (mean 70%), Area 1 of the cingulate cortex (mean 46%) and the dorsal peduncular cortex (mean: 25%).

Odor alternation results

During the last 10 days of preoperative testing, the 11 rats performed at 62.5% correct (Fig. 8A), which was above chance ($t(10)=6.1$, $P<0.001$). This group was then divided into two groups based on preoperative performance. This yielded a control group ($n=5$, mean $=62.4\pm3.2\%$) and a group to be given hippocampal lesions ($n=6$, mean $=62.6\pm3.0\%$; $t(9)=0.042$, $P>0.1$). After surgery (Fig. 8B), the rats with hippocampal lesions performed as well as controls (H mean: $63.8\pm2.8\%$; control mean: $60.9\pm2.4\%$; $t(9)=0.8$, $P>0.1$), and both groups performed above chance (H: $t(5)=4.9$, $P<0.01$; control: $t(4)=4.5$, $P<0.05$). Subsequently, after testing was complete for the H and CON groups, the control group received bilateral lesions of the medial prefrontal cortex (mPFC). These animals performed at chance ($t(4)=1.4$, $P>0.10$). They were impaired relative to the H group (mPFC mean: $51.6\pm1.1\%$, $t(9)=3.8$, $P<0.01$) and also relative to their own prelesion performance ($t(4)=4.9$, $P<0.01$).

DISCUSSION

In three experiments, we investigated the role of medial prefrontal cortex (mPFC) and hippocampus in two tests of spatial memory and one test of nonspatial memory. In Experiment 1, rats with bilateral lesions of the mPFC were impaired relative to controls on the spatial alternation task (Fig. 3). In contrast, animals with mPFC lesions performed as well as control

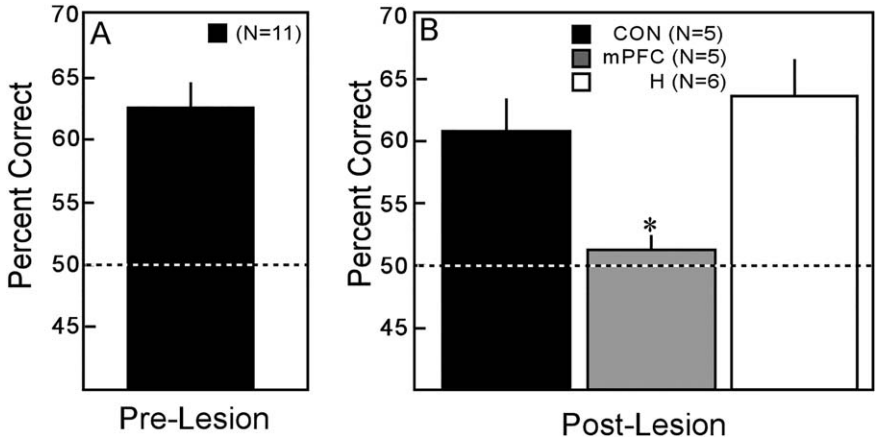

FIGURE 8. Odor alternation. Performance on the odor alternation task was measured by the percentage of trials in which the animal correctly alternated digging in scented sand to receive food reward (percent correct). A: The performance of all animals during the final two weeks of pretraining (mean = 62.5%). Animals were performing better than chance (50%). They were subsequently divided into two groups of matched performance and given either a bilateral hippocampal lesion (H) or sham surgery. The animals who received the sham surgery later received bilateral lesions of the medial prefrontal cortex (mPFC) (B). The performance of the animals in the two weeks of testing post-surgery. H animals performed as well as sham controls after surgery and both performed better than chance. By contrast, the mPFC animals were impaired relative to both the H and sham groups, and did not perform better than chance. Error bars show standard error of the mean (*$P < 0.01$).

animals on path integration (Fig. 4). Performance declined similarly in both groups as the outward paths became longer and more complex. In Experiment 2, rats with lesions of the hippocampus were impaired on spatial alternation and path integration, and they performed at chance levels on both tasks (Figs. 5 and 6). In Experiment 3, animals with mPFC lesions or hippocampal lesions were tested on odor alternation, a novel nonspatial alternation task. This task was designed to resemble the spatial alternation task but without a spatial component. mPFC lesions abolished the capacity for odor alternation (Fig. 8). However, rats with hippocampal lesions performed like controls on this task and above chance (Fig. 8). Thus, mPFC lesions spared path integration but impaired both spatial and odor alternation. Hippocampal lesions impaired path integration and spatial alternation but spared odor alternation.

All the tasks in the present study are putative tasks of working memory because animals were required to maintain information in memory for a span of a few seconds. Yet even when tasks span only a few seconds, working memory has a limited capacity. One way to understand the effects of hippocampal lesions and mPFC lesions is to suppose that the information needed to guide performance in spatial tasks exceeds the working memory capacity of the rodent (Kim et al., 2013). Because of this limitation, rodents may be unable to construct an effective working memory of spatial environments, which requires representing multiple cues and their relationships. Accordingly, performance on the spatial tasks (i.e., path integration and spatial alternation) may need to depend substantially on long-term memory, even in intact animals. If so, hippocampal lesions would be expected to impair performance on these tasks, because the ability to form long-term memory is impaired. These circumstances do not apply to nonspatial tasks, such as odor alternation, because the information that must be maintained in working memory is simpler than in spatial tasks. Thus, we suggest that hippocampal lesions spared odor alternation because the task could be managed within working memory, just as hippocampal lesions in earlier studies spared tasks of object recognition when the retention delay was very short (Clark et al., 2000, 2001).

It is also possible, in the case of path integration and spatial alternation, that hippocampal lesions directly impaired spatial working memory. In humans, cortical processing may be sufficient to support the representation of a mental map and its maintenance in working memory. Hence, hippocampal lesions in humans spared path integration (Kim et al., 2013). However, in rats (as suggested above) these cortical mechanisms may be insufficient to represent a spatial environment. Accordingly, for tasks such as path integration and spatial alternation, working memory cannot depend on neocortex alone. Accomplishing these tasks might require sustained interaction between hippocampus and other brain regions such as mPFC, in the case of spatial alternation, or parietal cortex in the case of path integration (Whitlock et al., 2008). If so, hippocampal lesions would impair performance because of this requirement for coordination with neocortex.

An alternative interpretation is that the hippocampus is especially important for spatial information processing. Thus, hippocampal lesions impaired performance in path integration and spatial alternation but not odor alternation. Note though that rats with hippocampal lesions exhibit intact spatial alternation performance in a continuous alternation version of the task (i.e., when no delay is imposed; Ainge et al., 2007). An impairment emerges only when a delay is introduced. Thus, the spatial processing demands are identical in these two

versions of the task, and the critical difference is the memory demand created by the delay. From this perspective, impaired path integration and impaired spatial alternation after hippocampal lesions may reflect the relational information that must be processed (the spatial layouts of the alternation and path integration environments), together with a need to remember the information. Odor alternation was intact because this task does not have the same requirement for relational information processing and (as suggested above) can be supported by working memory.

In contrast to the findings for hippocampal lesions, mPFC lesions spared path integration. mPFC has often been associated with spatial working memory (Horst and Laubach, 2009; Hyman et al., 2010; Gordon, 2011; Spellman et al., 2015). If path integration can in fact be supported by spatial working memory in coordination with mPFC, then mPFC lesions should have impaired path integration. However, if (as proposed here), path integration in the rodent depends substantially on long-term memory, then animals with mPFC lesions could have succeeded at path integration by relying on long-term memory. Alternatively, rather than suppose that the mPFC supports spatial working memory, some have emphasized the importance of mPFC in tasks with high interference, where animals must manage the separation of multiple, similar events (Granon et al., 1994; Kane and Engle, 2002). From that perspective, mPFC lesions impaired spatial alternation and odor alternation (a nonspatial task) because of the interference that develops in the alternation tasks. Unlike path integration, each trial of the alternation tasks was dependent on information obtained from the previous trial, and the same response was repeated multiple times in each session.

There is evidence that an interaction between hippocampus and mPFC is important for spatial working memory (Jones and Wilson, 2005; Hyman, 2010; Spellman et al., 2015), though perhaps only in tasks with high interference like spatial alternation. For example, successful performance on a nonmatching-to-position task was related to the degree of synchrony between unit activity in mPFC and hippocampal theta (Hyman, 2010). In addition, optogenetic inhibition of the projection from hippocampus to mPFC impaired performance in a spatial alternation task (Spellman et al., 2015). A hippocampus-mPFC interaction is apparently not pertinent to path integration, given that mPFC lesions spared performance in this task.

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