Navigation using global or local reference frames in rats with medial and lateral entorhinal cortex lesions.

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Abstract

The medial (MEC) and the lateral (LEC) regions of the entorhinal cortex send a major input to the hippocampus and have been proposed to play a foremost role in combining spatial and non-spatial attributes of episodic memory. In addition, it has been recently suggested that the MEC is involved in the processing of information in a global reference frame and the LEC in the processing of information in a local reference frame. Whether these putative functions could be generalized to navigation contexts has not been established yet. To address this hypothesis, rats with MEC or LEC NMDA-induced lesions were trained in two versions of a navigation task in the water maze, a global cue condition in which they had to use distal room cues and a local cue condition in which they had to use 3 objects placed in the pool. In the global cue condition, MEC-lesioned rats exhibited slower acquisition and were not able to **precisely** locate the submerged platform during the probe trial. In contrast LEC-lesioned rats exhibited control-like performance. In the local cue condition, navigational abilities were spared in both lesion groups. In addition when the 3 different objects were replaced by 3 identical objects, all groups maintained their navigation accuracy suggesting that the identity of objects is not crucial for place navigation. Overall, the results indicate that the MEC is necessary for place navigation using a global reference frame. In contrast, navigation using a local reference frame does not require the LEC nor the MEC.

1. Introduction

It is broadly acknowledged that the entorhinal-hippocampal network is the core of a system participating in the encoding and storage of episodic memory in the brain [1-3]. More specifically, the network would be involved in the formation of a spatial framework necessary to encode the non-spatial characteristics of events therefore preserving the specificity of episodic memories. The entorhinal cortex (EC) that provides major anatomical input to the hippocampus from the medial EC (MEC) and lateral EC (LEC) [4-9] has been suggested to play a key role in these processes. In agreement with anatomical data, electrophysiological and inactivation studies in rodents support the view that the MEC and the LEC have distinct functions [10-22].

The MEC has been suggested to play a role in the processing of self-motion (idiothetic) cues [23,24] on the basis of the firing properties of grid cells, spatially-tuned neurons with periodically-distributed firing fields [25-28], and of the deficits resulting from lesions in path integration [17] and motion-based distance estimation [29] tasks. The MEC is also involved in the processing of environmental (allothetic) cues as shown by the disruptive effect of MEC inactivation in spatial navigation tasks [10,17,30,31]. Interaction between idiothetic and allothetic information processing is observed in grid cells whose self-motion-dependent activity is modulated by external landmarks [28,32-38]. Non spatial correlates have also been found in MEC neurons that show object-dependent spatial firing, thus suggesting vector-coding properties [39].

In contrast, the LEC has been suggested to be primarily not involved in the processing of spatial information. LEC lesions disrupt the detection of a novel object [15,17,40,41], and recognition of objects-context associations [42] but do not affect navigation in the Morris water maze [17,43]. Unit recordings in an empty recording chamber did not reveal spatially-tuned neurons in the LEC [11,13]. However, when the environment contains discrete objects, LEC neurons fire in specific places relative to these objects or their previous location [12,19,44-46].

Thus, if the role of the MEC appears to be mostly involved in the processing of spatial information and the LEC in the processing of non-spatial information, the notion of such a clear division of labor between the two subregions, is seemingly oversimple. It has been then proposed that the MEC is involved in the processing of global spatial information (provided by distant cues) whereas the LEC is involved in the processing of local information (provided by local cues) [12,16,20,45,47]. Both distant and local cues provide spatial and non-spatial information (location and identity of items). However,

given the multimodal nature of local cues, i.e. objects located in the animal's sensorimotor space, and the importance of potential interactions with these objects, the processing of local cues would result in a strong combination of spatial and non-spatial information. Accordingly, these functions would be necessary for animals to perform accurate navigation as they have to manage different spatial scales in their natural environment. However, no behavioral studies have examined this hypothesized MEC-LEC dissociation using a navigation task. In this perspective, we trained rats with medial or lateral entorhinal cortex lesions in two versions of the place navigation task in the Morris water maze, a global reference frame version in which rats were required to use extra-maze cues and a local reference frame version in which rats had to use objects located directly in the pool to locate the hidden platform. According to the hypothesis, rats with MEC lesions would be impaired in the global cue condition whereas rats with LEC lesions would be impaired in the local cue condition.

2. Materials and Methods

2.1. Subjects

Subjects were 23 male Long-Evans rats (Janvier, Le Genest-St-Isles, France) weighing between 300 and 350 g, housed in individual cages (40 cm long x 26 cm wide x 16 cm high) with ad libitum food and water and kept in a temperature-controlled room (20 ± 2°C) with 12h light/dark cycle. One week after arrival, animals were handled daily by the experimenter for 7 days. Prior to surgery, they were arbitrarily assigned to 3 groups: MEC-lesioned rats (MEC, n = 8), LEC-lesioned rats (LEC, n = 8), and sham-lesioned rats (SHAM, n = 7). The sham-lesioned group included sham MEC-lesioned (n = 3) and sham LEC-lesioned rats (n = 4). Subsequently, sham MEC and sham LEC rats were pooled in a single SHAM group as they were not different in acquisition of all tasks. The rats from the 3 groups were submitted to two versions of a place navigation task in the Morris water maze, a global cue version and a local cue version. Following surgery, animals were allowed to recover for 10 days before training was started. The experiments were performed in accordance with the European guidelines (European Community Council Directive, 2010, 2010/63/EU) and National guidelines (Council directive n°87848 of the Direction des Services Vétérinaires de la Santé et de la Protection Animale permission n° 13.24 from the Ministère de l'Agriculture et de la Pêche to E.S., local ethic committee and national authorization n° A8-12-12). Behavioral testing was carried out during the light phase.

2.2. Surgery

Rats were deeply anesthetized by an intramuscular (i.m.) injection of medetomidine (25 mg/kg; Domitor, Pfizer, Paris, France) and ketamine (75 mg/kg; Imalgène, Merial, France) and placed in a Kopf stereotaxic apparatus (Kopf instruments, Tujunga, CA). A midline incision of the scalp was made and the skin and muscles were carefully retracted to expose the skull. Holes were drilled above the target regions. Bilateral lesions of the MEC or LEC were made by infusing a solution of N-methyl-D-aspartate (NMDA, Sigma) (20 mg/ml) dissolved in phosphate buffered saline (pH 7.4; 0.1 mol/L) [30,41]. The solution was injected at a rate of 0.05 µL/min (CMA 100 microinjection pump, CMA, Sweden) via a 25 gauge stainless-steel cannula connected by a flexible polyethylene tube to a Hamilton syringe (10 µL). Lesions of the MEC or LEC were made in each hemisphere, infusing the following volumes of NMDA at the coordinates relative to bregma: MEC (3 lesion points): 0.20 µL, AP: –7.5 mm, L: ±4.6 mm; 0.35 µL, AP: –8.2 mm, L: ±4.8 mm; 0.25 µL, AP: –8.8 mm, L: ±5.0 mm; LEC (2 lesion points): 0.30 µL, AP: –6.6 mm, L: ±6.4 mm; 0.35 µL, AP: –7.3 mm, L: ±6.2 mm. For each lesion point, the cannula was lowered very slowly until its tip touched the floor of the brain or calvarium and then raised 1 mm [41,48]. This position was taken as the dorsoventral coordinate of the lesion. Due to the anteroposterior curvature of the calvarium, this coordinate was different for each lesion point. Sham-operated rats were treated the same way as lesioned rats except that the injected solution contained only phosphate buffered saline (pH 7.4; 0.1 mol/L). After lesions, the skin was sutured and the rats received an injection of long-acting antibiotic (Amoxycilline, s.c., Pfizer, France) and analgesic (Tolfédine, 0.06 mg/kg, s.c., Vetoquinol, France) as postoperative treatment. They were placed back in their home cage for recovery in a temperature-controlled room at 22°C for at least 48h before they were put back in the colony room.

2.3. Apparatus and Behavioral Procedure

The water maze was an elevated circular pool (1.80 m diameter; 30 cm deep, 50 cm above the floor) located in a well-lit room containing numerous extra-maze cues (door, cupboards, shelves, pieces of equipment, posters, etc.) and filled with 20°C $\pm$ 2°C water made opaque by addition of chalk powder. A transparent Plexiglas circular platform (diameter, 10 cm) was placed inside the pool, 40 cm away from the wall. Its top surface was 1 cm below the surface of the water and was therefore invisible to the animals. An overhead camera was connected to a computer. A radio set fixed to the ceiling in a central position produced background noise > 70 dB to mask non controlled directional sounds. The rats were trained in a reference memory version of the navigation task in which the platform was held in a constant

position throughout training. The rats were trained to reach the submerged platform in two conditions, a global cue condition and a local cue condition (see [49,50] for a similar procedure).

In the global cue condition, extra-maze room cues were available. Rats received 4 daily trials during 20 days with a 30-sec inter-trial interval (rat left on the platform) from four possible starting places at the pool periphery (N, E, S, and W) used in a pseudo random order. A typical trial consisted of gently releasing a rat in the water, its head facing the wall, Maximum duration for a trial was 1 min. If the animal did not reach the platform within 1 min, it was guided toward it by the experimenter. After completion of the 4 trials in the last training day (day 6), the rats received a 1-min probe trial during which the platform has been removed.

In the local cue condition (Figure 1), the pool was surrounded by an opaque white circular curtain. Three objects, the local cues, were placed in the pool in an isosceles configuration: a black and white vertically stripped cylinder (diameter 16 cm, total height 41 cm, the stripes were made by juxtaposing black and white thick tape), a black and white horizontally stripped cylinder (diameter 11 cm, total height 60 cm, the stripes were made by juxtaposing black and white thick tape), a black cone (total height 48 cm, diameter at the base 19 cm, diameter at the top, 4.5 cm, the surface was textured by plastic edges on the circumference). Objects were placed at 8 cm (edge) from the wall allowing rats to swim around them. The platform was located 40 cm away from the wall as in the global cue condition (Figure 1b). However, to neutralize the influence of room cues, both the objects and the platform were rotated 120° in an arbitrary direction relative to the pool center between days. The objects were not cleaned between trials to preserve potential olfactory cues. Three starting places at the pool periphery (S, NW, NE) were used on each daily session. Rats received 6 daily trials (2 per starting place) during 7 days. The kind of information used by the animals to successfully navigate in this task is ambiguous however. They can rely on the combination of spatial (configuration) and non-spatial (identity of objects) information but successful navigation is also possible if the animals use only the spatial configuration of objects and disregard their identity. Thus in order to address this issue, we submitted the animals to an additional local cue condition in which all 3 objects were identical so that the only relevant information to locate the platform was the spatial configuration of objects. The 3 objects were identical gray polyvinylchloride cylinders (diameter 10 cm, total height 60 cm). These objects occupied the same location as the distinct objects and the platform was at the same location relative to the objects as in the distinct object condition. The rats received 6 daily trials during 5 days and the procedure was similar to the previous

distinct object situation. In both local cue conditions, i.e. distinct objects or identical object, the rats received a 1-min probe trial after completion of the 6 trials in the last training day during which the platform has been removed

Finally we tested the animals in a visually-guided task to ascertain that the deficits were dependent on the cue condition. The water maze was still surrounded by the curtains but no objects were placed in the pool. The platform was cued by a black tennis ball fixed at the end of a 20 cm metal rod which was attached to the platform side. The rats received 4 daily trials during 2 days from four possible starting places (N, S, W, E) in pseudorandom order similar to those used in the distal cue condition.

The order of the global cue and local cue conditions were not counterbalanced as it has been previously shown that changing the order of the behavioral tasks does not modify the lesion effects on performance [49]. All rats were therefore submitted to the sequence: global cue condition, local cue condition (different objects, identical objects), and visually-guided condition.

2.4. Histology

Immediately after the last behavioral testing, rats were injected with a lethal dose of pentobarbital sodium (Doletal 6%, Sanofi Santé France) and decapitated. The brains were rapidly removed, frozen in dry ice and kept at -80°C until cryostat sectioning. Coronal 20-µm-thick tissue sections were cut at -20°C, at the level of the entorhinal cortex (between interaural coordinates A 3.96 and -0.48 mm based on the stereotaxic atlas of Paxinos et Watson [51].Tissue sections were thaw mounted onto SuperFrost plus glass slides (Fisher Scientific, Elancourt, France) and stored at -80°C until utilization.

Entorhinal cortex sections were stained using 0.5% cresyl violet dye and differentiated in a jar of 70° alcohol (Nissl Staining). Then, these sections were dehydrated in several rinses of increasing alcohol concentration (80°, 95° and 100°), delipidated in xylene and embedded in Depex (Gurr®, England).

Sections were observed by transmission optical microscopy using an Eclipse E600 microscope (Nikon, France).

To further quantify the lesion extent, the proportion of damaged brain tissue in MEC and LEC rats was estimated using the open source ImageJ software. The lesion outline was manually determined at five levels of the Paxinos and Watson atlas (those shown on Figure 2) and the lesion area in the two sides, left and right, was measured (for the lesions extending the MEC or LEC area, only the portion circumscribed to the targeted area was taken into account). For each animal the lesion area was

summed up across stereotactic levels and sides. Using the total MEC or LEC surface, we then calculated for each animal a lesion ratio (lesioned area*100/total area), termed the lesion index, that was averaged in each group. In addition, using the median value, we split each lesion group into two subgroups: rats with small lesions (i.e. lowest lesion indexes) and rats with large lesions (i.e. highest lesion indexes) which were compared to each other.

2.5. Data analysis

A videotracking system (Videotrack 1.7, Viewpoint, France) was used to record and analyze the rats' spatial behavior. Navigational abilities were evaluated by measuring escape latency and distance. For the probe trial, the time spent in the four quadrants was measured, i.e. the quadrant where the platform was located and the other three quadrants. In addition, to precisely describe the navigation and place learning performance during probe trials, we calculated the time spent by the animals in a circular (diameter 20 cm) area, the goal area, centered on the platform location and in equivalent areas located in the three other quadrants. Using custom-made programs, we also computed the average distance to the platform (cm, sampling rate 25 Hz), the latency (s) and distance (cm) for the first crossing of the goal area, and the searching error, i.e. $\delta = [\Sigma_i d_i^2 / (2n)]^{1/2}$, where d_i is the distance (in cm) of the animal to the goal platform on each sample and n is the total number of samples used for the calculation, and which reflects the dispersion of search behavior independently from the distance swum by the animal. We also evaluated exploration of the intra-maze objects by measuring the time spent in a circular area centered on each of the three objects. The size of this area was adjusted so that detection of the animal's snout in this area would correspond to an actual contact with this object. We used the time spent in the object area, i.e. the duration of contacts with the object, to compute an exploration index for each rat. This index involved normalization of the time spent in the object area by the distance swam during training according to the following formula: Object exploration index = t x 100/d, where t is the total time spent in the object area for the 3 objects during each trial of a training session and d the mean distance swam by this rat during this training session. This index was averaged over trials in a session and over sessions to provide a single value per rat. One-factor, two-factor, or repeated measures ANOVAs were used with post-hoc Newman-Keuls tests for pairwise comparisons.

To examine whether the behavioral performance was correlated to the lesion size, we calculated the Pearson's correlation coefficient r between the lesion index (see Materials and methods/histology section) and the measures of place searching during the probe trial including time spent in the goal

quadrant and the goal area (annulus). We also calculated the correlation between the lesion index and the amount of contacts with the intramaze objects in the last session of the local cue condition. In addition, performance of rats with small lesions and rats with large lesions were compared using a Mann-Whitney test.

3. Results

3.1. Histology

Figure 2 shows the extent of lesions for individual rats in the MEC and LEC groups.

LEC lesions. All rats had bilateral lesions of the LEC within the -5.94 mm to – 8.28 mm stereotaxic level range including the DLEnt (Dorsolateral Entorhinal cortex), DIEnt (Dorsal Intermediate Entorhinal cortex) and VIEnt (Ventral Intermediate Entorhinal cortex) areas according to the Paxinos and Watson's nomenclature [51] or LEA (Lateral Entorhinal Area) region according to the Dolorfo and Amaral's nomenclature [52]. In some rats, unilateral damage was seen in the most posterior or anterior levels. Lesion invaded more ventral or dorsal regions outside the LEC but this was not consistent across animals or across levels: ventrally, partial damage of the amygdalopiriform transition area, which has been often considered part of the lateral entorhinal cortex but is now viewed as a separate anatomical area [54] could be found. Dorsally, damage to the perirhinal cortex and, in all cases unilaterally, to temporal auditory areas was seen.

MEC lesions. All rats had bilateral lesions of the MEC within the - 6.96 mm to - 8.76 mm stereotaxic level range including the MEnt (Medial Entorhinal Cortex) and CEnt (Caudomedial Entorhinal Cortex) areas according to the Paxinos and Watson's nomenclature [51] or MEA region according to the Dolorfo and Amaral's nomenclature [52]. Damage to MEC was mostly unilateral at the most anterior level (- 6.96 mm) but bilateral at posterior levels. In some cases, damage to neighboring areas was seen: laterally lesions encroached the VIEnt area at posterior levels and portions of DLEnt areas was lesioned at anterior levels in one rat (M3). Medially, some damage to the parasubiculum was seen in 3 rats (M1, M2, M4) on one side at the most anterior level only (- 6.96 mm).

The proportion of damaged tissue, i.e. the lesion index, was 19.95 $\pm$ 1.027 % in MEC rats and 30.67 $\pm$ 5.39 %.in LEC rats.

3.2. Global cue condition

Training. MEC rats exhibited faster swimming speed than both SHAM and LEC rats (SHAM, 24.01 $\pm$ 0.46 cm/s; MEC, 30.18 $\pm$ 1.0 cm/s; LEC, 23.94 $\pm$ 0.71 cm/s, one-factor ANOVA, $F(2,20) = 21.29$, $p =$

1.1 10^{-5}, NK tests; MEC > SHAM, p = 1.6 10^{-4}; MEC > LEC, p = 1.8 10^{-4}, SHAM = LEC, p = 0.95). The distance swam to reach the platform decreased in the 3 groups, albeit differentially, across sessions indicating effective learning (repeated measure ANOVA, session effect, $F(19,380) = 25.61$, $p < 0.0001$; session x group interaction, $F(38,380) = 1.61$, $p = 0.015$; Figure 3A). In addition, MEC rats swam longer distances than both LEC and SHAM rats (group effect, $F(2,20) = 24.07$, p = 0.5 10^{-5}; MEC > SHAM, p = 1.58 10^{-4}; MEC > LEC, p = 1.59 10^{-4}; SHAM = LEC, p = 0.881). A one-factor ANOVA on the last day showed that the MEC rats were still impaired at the end of training (effect of group, $F(2,20) = 4.72$, $p = 0.021$; NK tests, MEC > SHAM, p = 0.021; MEC > LEC, p = 0.034; SHAM = **LEC**, p = 0.834; Figure 3A).

Probe trial. Figures 3B and 3C show the performance of the 3 groups during the probe trial. First we looked at the time spent in the four quadrants (Figure 3B). A group x quadrant (goal quadrant vs. non goal quadrants) ANOVA indicated a quadrant effect ($F(1,40) = 108.39$, $p < 0.0001$), a group x quadrant interaction ($F(2,40) = 8.85$, p = 0.66 10^{-3}) but no group effect ($F(2,40) = 1.78$, $p = 0.181$). All 3 groups spent more time in the goal quadrant than in the non-goal quadrants (NK, SHAM, p = 1.39 10^{-4}; MEC, p = 0.012; LEC, p = 1.63 10^{-4}), but SHAM and LEC rats showed greater time spent in the goal quadrant than MEC rats (NK, SHAM > MEC, p = 0.0018; LEC > MEC, p = 0.95 10^{-3}; LEC = SHAM, p = 0.91). However, although MEC rats spent more time in the goal quadrant, they may not be able to search for the platform at the exact location. We therefore analyzed the time spent in the annulus which corresponds to the precise platform location (Figure 3B). A group x area (goal area vs. non goal areas) ANOVA revealed a significant effect of group ($F(2,40) = 6.86$, $p = 0.0028$), area ($F(1,40) = 50.63$, $p < 0.0001$) and group x area interaction ($F(2,40) = 8.45$, $p = 0.00087$). Both SHAM and LEC rats spent more time in the goal area than the non-goal areas (NK, SHAM, p = 1.3 10^{-4}; LEC, p = 1.31 10^{-4}) whereas MEC did not show any preference for the goal area (p = 0.43). In addition, both SHAM and LEC rats spent more time in the goal area than the MEC rats (NK, SHAM vs. MEC, p = 1.51 10^{-4}; LEC vs. MEC, p = 1.77 10^{-4}; SHAM vs. LEC, p = 0.85). The distance swum before the first crossing of the annulus was not different across groups (p = 0.17). However, the average distance to the goal area during the probe trial was greater in MEC rats relative to both SHAM and LEC rats (one factor ANOVA, $F(2,20) = 5.006$, $p = 0.017$; NK, MEC > SHAM p = 0.016, MEC > LEC, p = 0.038, LEC = SHAM, p = 0.41) indicating less accurate searching behavior in MEC rats. More specifically, such inaccuracy was observed during the first half (0-30 s) of the probe trial (one factor ANOVA, $F(2,20) = 8.199$, $p = 0.002$; NK, MEC > SHAM, p = 0.003, MEC > LEC, p = 0.008, LEC = SHAM, p = 0.39) but not during the second half (30-60 s) (one

factor ANOVA, F(2,20) = 2.273, p = 0.129). In addition, a greater searching error was found in MEC rats relative to both SHAM and LEC rats during the 0-30 s period (one-factor ANOVA, F(2,20) = 7.722, p = 0.003; MEC > SHAM p = 0.004; MEC > LEC p = 0.009; SHAM = LEC p = 0.399).

We looked at potential correlations between the lesion size and the behavioral deficit during the probe trial in the MEC group. However, we failed to find any correlation between the lesion index and the time spent in the goal quadrants or goal annulus in the global cue condition (quadrant: r = -0.301; annulus: r = 0.215, all ps > 0.05). No difference between rats with small or large lesions was found for these two measures (Mann Whitney, goal quadrant, U = 4, annulus, U = 6, all ps > 0.05).

These results indicate that the MEC rats were not totally disoriented and concentrated their swim in the correct quadrant of the pool. However, they had not learned the precise platform location when using global cues. No deficit was found in LEC rats.

3.3. Local cue condition

3.3.1. Different objects

Training. Like in the global cue condition, rats from the 3 groups did not swim at same speed (one-factor ANOVA on average speed over training, F(2,20) = 3.84, p = 0.039; NK, MEC > LEC, p = 0.037; all other comparisons p > 0.05). A repeated-measure ANOVA on distances revealed significant effects of group (F(2,20) = 9.66, p = 0.0012), session (F(6,120) = 2.79, p = 0.014) but no group x session interaction (F(12,120) = 0.34, p = 0.97). MEC rats swam on longer distances than both SHAM and LEC rats (NK, MEC > SHAM, p = 0.0016; MEC > LEC, 0.0031; Figure 4A, left panel). On the last training day, MEC rats were still impaired relative to both SHAM and LEC (one factor ANOVA, F(2,20) = 8.49, p = 0.002; MEC > SHAM, p = 0.003; MEC > LEC, p = 0.0041). MEC rats did not show a greater object exploration index than SHAM rats during training. In contrast, LEC rats explored more the objects placed in the pool than both SHAM and MEC rats (one-factor ANOVA, F(2,20) = 9.51, p = 0.0012; MEC = SHAM, p = 0.752; LEC > SHAM, p = 0.0026; LEC > MEC, p = 0.0021; Figure 4B, left panel). This suggests that LEC rats needed to collect a greater amount of information on their environment which could be part of a compensation strategy that allowed them to improve navigation and place location finding. MEC were not able to use this strategy. A significant correlation was found between the lesion index and the exploration index in the LEC group during the last training day (J7) (r = 0.812, p = 0.014) and consistent with this correlation, LEC rats with small lesions exhibited lower exploration index than rats with large lesions (Mann-Whitney, U = 0, p = 0.021).

Probe trial. Figure 4C and 4D left panels shows the performance of the 3 groups during the probe trial. A group x quadrant (goal quadrant vs. non goal quadrants) ANOVA indicated a quadrant effect ($F(1,40) = 238.17$, $p < 0.0001$), but no group x quadrant interaction ($F(2,40) = 2.16$, $p = 0.13$) and no group effect ($F(2,40) = 0.53$, $p = 0.59$). All 3 groups spent more time in the goal quadrant than in the non-goal quadrants (NK, SHAM, $p = 1.25\ 10^{-4}$; MEC, $p = 1.16\ 10^{-4}$; LEC, $p = 1.25\ 10^{-4}$). A group x area (goal area vs. non goal areas) ANOVA on the time spent in the annulus showed significant effects of group ($F(2,40) = 7.81$, $p = 0.0014$), area ($F(1,40) = 190.55$, $p < 0.0001$) and group x area interaction ($F(2,40) = 9.20$, $p = 0.00052$). All groups spent more time in the goal area than in the non-goal areas (NK, SHAM, $p = 1.4\ 10^{-4}$; MEC, $p = 1.2\ 10^{-4}$, LEC, $p = 1.6\ 10^{-4}$) but SHAM rats spent more time in the goal area than both MEC and LEC rats (SHAM > MEC, $p = 1.2\ 10^{-4}$; SHAM > LEC, $p = 1.2\ 10^{-4}$; LEC = MEC, $p = 0.07$; Figure 4**D**, left panel). This effect cannot be accounted for by the fact that MEC- and LEC-lesioned rats spend time exploring the objects than SHAM rats since all groups exhibited similar object exploration (one-factor ANOVA, $F(2,20) = 0.31$, $p = 0.73$). Additional analyses showed that all 3 groups displayed similar latency and distance for first crossing of the goal area (one-factor ANOVA, Latency, $F(2,20) = 2.29$, $p = 0.126$; Distance, $F(2,20) = 1.98$, $p = 0.164$) and average distance to the goal (one-factor ANOVA, $F(2,20) = 0.923$, $p = 0.41$). The comparison of average distance to the goal was marginally significant for the 0-30 s period ($F(2,20) = 3.34$, $p = 0.056$) due to MEC rats (MEC > SHAM, $p = 0.056$; all other comparisons $p >> 0.05$). The groups were not different in the 30-60 s period ($F(2,20) = 0.75$, $p = 0.485$). No difference between groups for searching error was found for the whole trial (one-factor ANOVA, group, $F(2,20) = 0.47$, $p = 0.63$) or for each of the two periods (0-30 s, $F(2,20) = 2.10$, $p = 0.148$; 30-60 s, $F(2,20) = 0.86$, $p = 0.438$).

3.3.2. Identical objects

Training. Rats were given 6 daily trials for 5 days in the local cue conditions with 3 identical objects to determine whether the identity of objects is a critical factor for place learning. All groups swam at same speed (one-factor ANOVA, $F(2,20) = 1.927$, $p = 0.172$). Again, MEC rats swam on longer distances than the LEC and SHAM rats (Repeated-measure ANOVA, significant effect of group ($F(2,20) = 1.927$, $p = 0.00019$; MEC > SHAM, $p = 0.000637$; MEC > LEC, $p = 0.000459$; Figure 4A right panel). The rats from all groups showed a stable performance across sessions likely due to their initial navigation experience acquired in the different object condition (no effect of session, $F(4,80) = 1.738$, $p = 0.014$ and no group x session interaction ($F(8,80) = 1.358$, $p = 0.228$). On the last training day (J5),

all groups exhibited similar performance (One factor ANOVA, $F(2,20) = 2.581$, $p = 0.101$). The 3 groups did not show different object exploration index during training (one-factor ANOVA, $F(2,20) = 0.551$, $p = 0.585$; Figure 4B, right panel).

Probe trial. As shown in Figure 4C right panel, all groups spent more time in the goal quadrant than in the non-goal quadrants (group x area ANOVA, effect of quadrant, $F(1, 40) = 218,03$, $p < 0.0001$; no effect of group, $F(2,40) = 0.41$, $p = 0.67$, and no group x quadrant interaction, $F(2,40) = 1.65$, $p = 0.21$; NK, goal quadrant vs. non-goal quadrant, SHAM: $p = 1.39\ 10^{-4}$; MEC: $p = 1.16\ 10^{-4}$; LEC: $p = 1.63\ 10^{-4}$). Analysis of the time spent in the annulus showed that all groups had a preference for the goal area relative to the non-goal area (group x area ANOVA, effect of area, $F(1, 40) = 89, 42$, $p < 10^{-5}$; NK, goal area vs. non-goal area, SHAM: $p = 0.00016$; MEC: $p = 0.046$; LEC: $p = 0.00126$). In addition, the 3 groups exhibited different performance due to a greater amount of visits to the goal area in SHAM and LEC rats than in MEC rats (ANOVA, effect of group, $F(2,40) = 7.63$, $p = 0.0016$; group x area interaction, $F(2,40) = 7.38$, $p = 0.0018$) during the probe trial (distance in goal area, SHAM > MEC, $p = 0.00013$, SHAM = LEC, $p = 0.091$, LEC > MEC, $p = 0.00089$). Like in the different object condition, MEC rats did not explore more the objects than SHAM and LEC rats (one factor ANOVA, $F(2,20) = 0.603$, $p = 0.557$). All 3 groups were similar in terms of latency and distance for first crossing of the goal area (one-factor ANOVA, Latency, $F(2,20) = 2.15$, $p = 0.143$; Distance, $F(2,20) = 2.066$, $p = 0.153$), average distance to the goal area (one-factor ANOVA, 0-60s: $F(2,20) = 1.89$, $p = 0.177$; 0-30s: $F(2,20) = 2.50$, $p = 0.108$; 30-60s: $F(2,20) = 0.820$, $p = 0.455$), and searching error (one-factor ANOVA, 0-60s: $F(2,20) = 1.19$, $p = 0.325$; 0-30s: $F(2,20) = 2.06$, $p = 0.154$; 30-60s: $F(2,20) = 0.407$, $p = 0.671$).

3.3.3. Cued-platform condition

After the local cue condition, animals were trained in a cued-platform condition to assess their ability to navigate to a visible goal. On the last training day (day 2), all 3 groups were similar in terms of swimming speed (one factor ANOVA, $F(2,20) = 0.383$, $p = 0.687$) and latency to reach the platform (one factor ANOVA, $F(2.69$, $p = 0.092$).

3.3.4. Summary

Overall, the results show that when global cues were used during training MEC rats exhibited acquisition and place learning deficits. In contrast LEC rats were not impaired. When local cues, either different or identical objects, were available in the pool, MEC rats were impaired during acquisition but eventually learnt the platform location as observed in the probe trial. In the local cue condition, LEC rats

showed no impairment during acquisition or probe trial. However, when the objects were different, they made more contacts with the objects than both SHAM and MEC rats during acquisition. This effect on object exploration was not seen with identical objects. Finally all three groups showed similar performance in the cue-platform condition.

4. Discussion

In the present study, we addressed the hypothesis that the MEC is involved in the processing of global spatial information (provided by distant cues) and the LEC in the processing of local information (provided by proximal cues). In particular, whether such functional dissociation could be generalized to all navigation contexts has not been established yet. For that purpose, we used two versions of a place navigation task in the water maze in which rats had to use either a global reference frame or a local reference frame.

In the global reference frame task, i.e. when only distant cues were available, we found that MEC lesions affected acquisition (longer trajectories to the platform) and disrupted accurate place learning (with a bias for the goal quadrant but no preference for the goal area), an effect which is consistent with previous results using similar NMDA lesions [31] (note however different effects in rats with mechanical lesions [17]). This is in contrast with the unaffected performance of rats with LEC lesions, in both acquisition and probe trial. These results therefore show a dissociation of the roles of MEC and LEC and are consistent with the view that the MEC plays a prominent role in the processing of spatial information within a global reference frame whereas the LEC is not involved.

The theory predicts that LEC-lesioned rats would be impaired in the local reference frame task, i.e. when only local objects are available, and that MEC-lesioned rats would be unimpaired. This is not what we obtained however. In this task, LEC lesions did not alter acquisition or place learning. Nevertheless, when different objects were in the pool, LEC-lesioned rats spent more time near the objects during acquisition, presumably collecting spatial and non-spatial information about them. This may be part of a compensatory strategy that would reflect difficulties in using the 3 different objects for building a representation of the environment during acquisition. Furthermore, we found that this increase was not observed when the animals had to use similar objects to navigate to the platform.

Even if object exploration in the water maze is more limited than in a dry arena, the results show that the rats interacted with these objects and were able to use them for navigation. Swimming along these objects allowed the rats to collect somatosensory and undoubtedly olfactory information (the

objects were textured and not cleaned during training and test) in addition to visual information so that the objects provided multimodal sensory information that could be combined with spatial information. It is hypothesized that the LEC would be important for such processing [15,18].

The results are consistent with our previous work showing that the involvement of the LEC and the MEC in the processing of local objects is modulated by the number and diversity of objects. Rats submitted to a spontaneous exploration task were impaired to detect a spatial change in the object configuration when all objects were different but this deficit was reversed when the objects were similar [42]. In the present study, we hypothesized that processing the identity of local objects would be more critical in a place navigation task because it would allow more accurate platform reaching. The results however demonstrate that the identity of objects is not essential for navigation as rats exhibited similar performance when identical or different objects were in the pool during acquisition and during the probe trial. We found that SHAM rats performed accurate navigation after training, suggesting that they used the geometric arrangement of the objects as previously described [54-58] because it provided unambiguous spatial information. As LEC rats were not different from SHAM rats, there are two possible explanations. First, LEC-lesioned rats relied on the geometric arrangement of objects (isosceles triangle) in the identical condition (like SHAM rats) because the identity of objects was irrelevant. Second, LEC rats were unable to process the non-spatial characteristics of discrete items in particular spatial contexts [13,16,20,42,45,46,59,60] and used geometry to navigate in both conditions. Accordingly, it would be because they used geometry that LEC rats exhibited SHAM-like performance in the different object condition. As LEC rats explored more the objects than SHAM and MEC rats, we suggest that they were impaired in using the objects and therefore used geometry to compensate for this impairment and maintain navigation, a hypothesis that needs to be investigated in future studies. Overall the results indicate that the LEC is not necessary for processing a local reference frame for navigation and place learning.

In the local reference frame condition, MEC-lesioned rats swam on longer distance to reach the platform than the two other groups during acquisition. Nevertheless, they exhibited accurate place learning behavior in both different and identical object conditions during the probe trial. To account for this seemingly paradoxical effect, it is relevant to refer to the distinction between “knowing where” and “getting there” proposed by Whishaw et al. [61]. They suggested that learning to navigate to a hidden platform involves two distinct processes, namely learning the platform location on the basis of allothetic

cues and learning how to get to the platform which involves processes related to the active monitoring of movements such as path integration. Moreover, using local landmarks to navigate requires that the animals thoroughly combine motion-related information and allothetic cue information to compensate for self-induced motion parallax and extract spatial invariants [49,55]. Thus, dissociating these two computations, knowing where and getting there, is particularly relevant in the local reference frame condition and is clearly consistent with the putative function of the MEC in the processing of motion-related information through the grid cell system [17,26,27,29]. Accurate performance during the probe trial nevertheless indicates that MEC rats learnt the platform location as did SHAM and LEC groups therefore suggesting that the MEC is not critically involved in the processing of spatial information in the local reference frame condition.

The deficit seen in rats with MEC lesions during acquisition of place navigation using local objects may be related to alteration of a recently discovered neuron population termed object-vector cells that fire at specific distances and directions from salient objects suggesting allocentric vector coding [39]. How these neurons contribute to navigation is not known yet. In the MEC, the object-vector cells coexist with other types of neurons, such as grid cells, border cells, head-direction cells that together contribute to determination of the animal's location in the environment. The presence of 3 salient objects forming an isosceles configuration in the local cue condition may change the geometrical aspects of the environment, and therefore possibly affect the grid cell pattern as previously shown [35]. Degraded grid cell pattern may disrupt spatial navigation as seen during acquisition [27].

Overall, our results support the view that global and local references frames are processed by distinct systems encoding spatial representations at different scales. Using a global reference frame would be more appropriate for large-scale navigation, distal cues providing reliable directional information. Using a local reference frame on the basis of proximal landmarks would be more appropriate for small-scale navigation and for incorporating individual items into the spatial representation as a framework for episodic memory. Both systems incorporate self-motion information and allothetic cues (mainly visual) to form local or global representations. However, the weight of self-motion information may be greater in the local reference frame to compensate for self-induced motion parallax and extract spatial invariants [49,55]. Lesion and unit recording studies suggest that the EC and the hippocampus are part of the network that generate multiscale spatial representations. Indeed, firing field size and inter-field spacing of grid cells in the MEC, firing field size of place cells in the hippocampus

increase from dorsal to ventral axis of these two areas (EC: [25,50,62-64], Hippocampus: [49,65,66]). In addition, evidence has accumulated that the MEC and the LEC underlie different functions regarding their implication in the global and local processing systems [16,20,47]. Our results pinpoint the possibility that the LEC is not necessarily involved in the processing of a local reference frame when the animal has to navigate to a hidden platform. Further studies may therefore consider the possibility that different behavioral and/or environmental contexts can modulate the LEC implication in such processing. Interestingly, the function of the MEC seems to be less dependent on such factors (see [41]). That there is a dissociation between a global and a local processing system, involving different neural networks clearly emerges from the navigation literature [67] but this may almost be seen as paradoxical since smooth navigation should require close interaction and coordination of these systems. The hippocampus may play a key role in this coordination.

5. References

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6. Figure captions

Figure 1: Local cue condition. Apparatus. In the right corner, view from above where the objects are shown as light grey triangle, circle and square. The platform location is shown as a black circle. See text for details.

Figure 2: Extent of NMDA lesions in LEC and MEC groups. Lesions of individual rats are shown using Paxinos and Watson atlas' drawings (2007). On the right, photographs show examples of lesions at the corresponding coordinates. M1-M8: rats with MEC lesions, L1-L8: rats with LEC lesions.

Figure 3: Global cue condition. A. Acquisition. Average time-course of distance swum $\pm$ SEM (s) during learning. B. Probe trial. Upper graph: average time $\pm$ SEM (s) spent in the quadrant where the platform was located and the averaged 3 other quadrants. Lower graph: average time $\pm$ SEM (s) spent in the annulus corresponding to the goal area (where the platform was precisely located) and in the non-goal areas (3 geometrically identical areas). Asterisks indicate significant difference *** $p < 0.001$, ns: non significant. C. Representative swim paths during the probe trial for each group. The light grey circle corresponds to the goal area (annulus centered on the platform location) and the white circles correspond to non-goal areas.

Figure 4: Local cue condition. A. Acquisition. Average time-course of distance swum $\pm$ SEM (s) during learning in the different and identical object conditions. B. Object exploration index $\pm$ SEM during acquisition. C. Probe trial. Upper left and right graphs: average time $\pm$ SEM (s) spent in the quadrant where the platform was located and the averaged 3 other quadrants. Lower left and right graphs: average time $\pm$ SEM (s) spent in the annulus corresponding to the goal area (where the platform was **precisely** located) and in the non-goal areas (3 geometrically identical areas). Asterisks indicate significant difference *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: non significant. D. Representative swim paths during the probe trial for each group. The light grey circle corresponds to the goal area (annulus centered on the platform location) and the white circles correspond to non-goal areas. The objects are shown as light grey triangle, circle and square against the pool wall.

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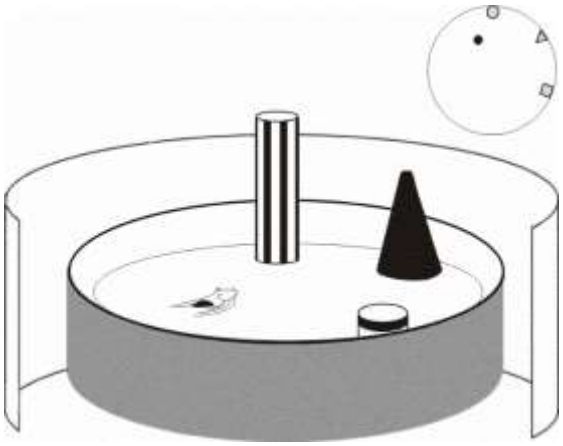

Figure 1

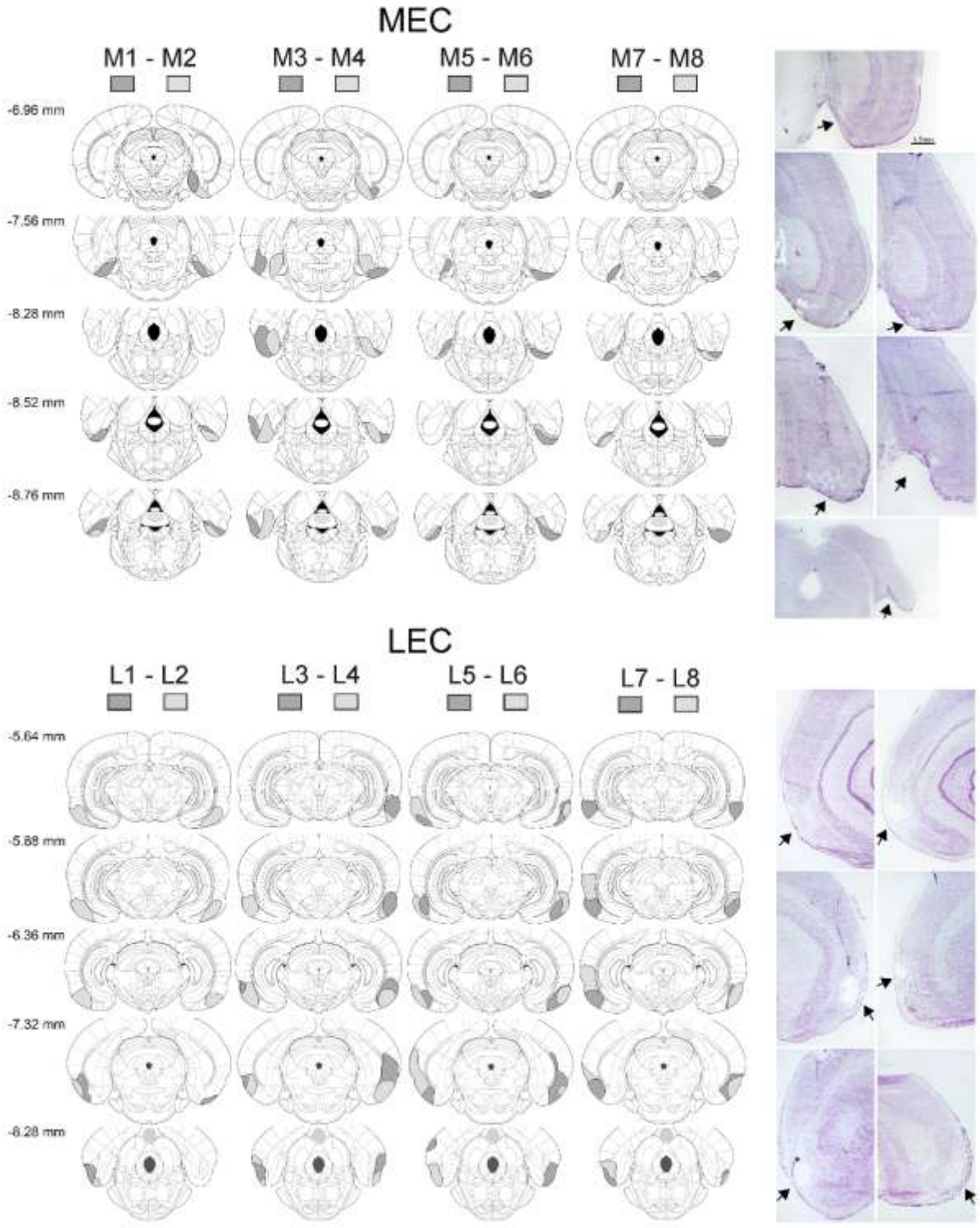
MEC
M1 - M2
M3 - M4
M5 - M6
M7 - M8
-6.96 mm
-7.56 mm
-8.28 mm
-8.52 mm
-8.76 mm
LEC
L1 - L2
L3 - L4
L5 - L6
L7 - L8
-5.64 mm
-5.88 mm
-6.36 mm
-7.32 mm
-8.28 mm

Figure 2

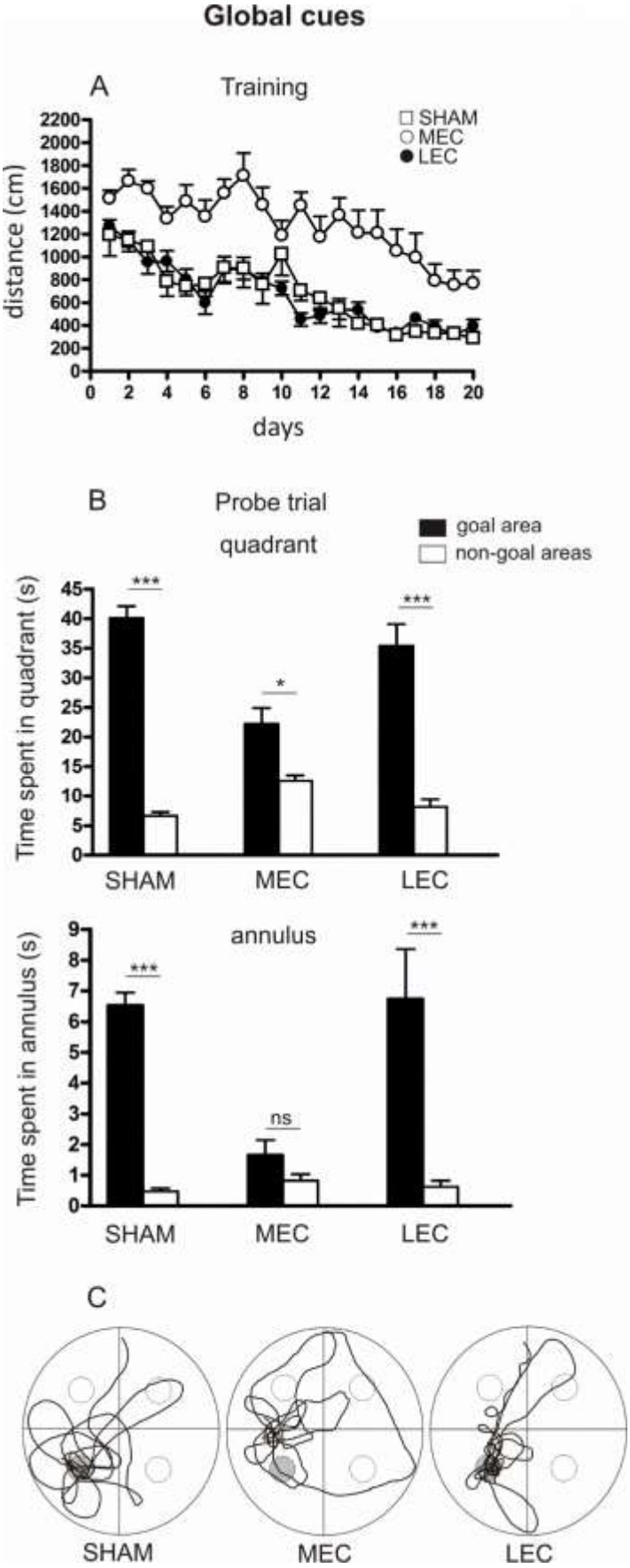
Global cues
A
Training
SHAM
MEC
LEC
distance (cm)
days
B
Probe trial
quadrant
goal area
non-goal areas
Time spent in quadrant (s)
SHAM
MEC
LEC
annulus
Time spent in annulus (s)
ns
C
SHAM
MEC
LEC

Figure 3

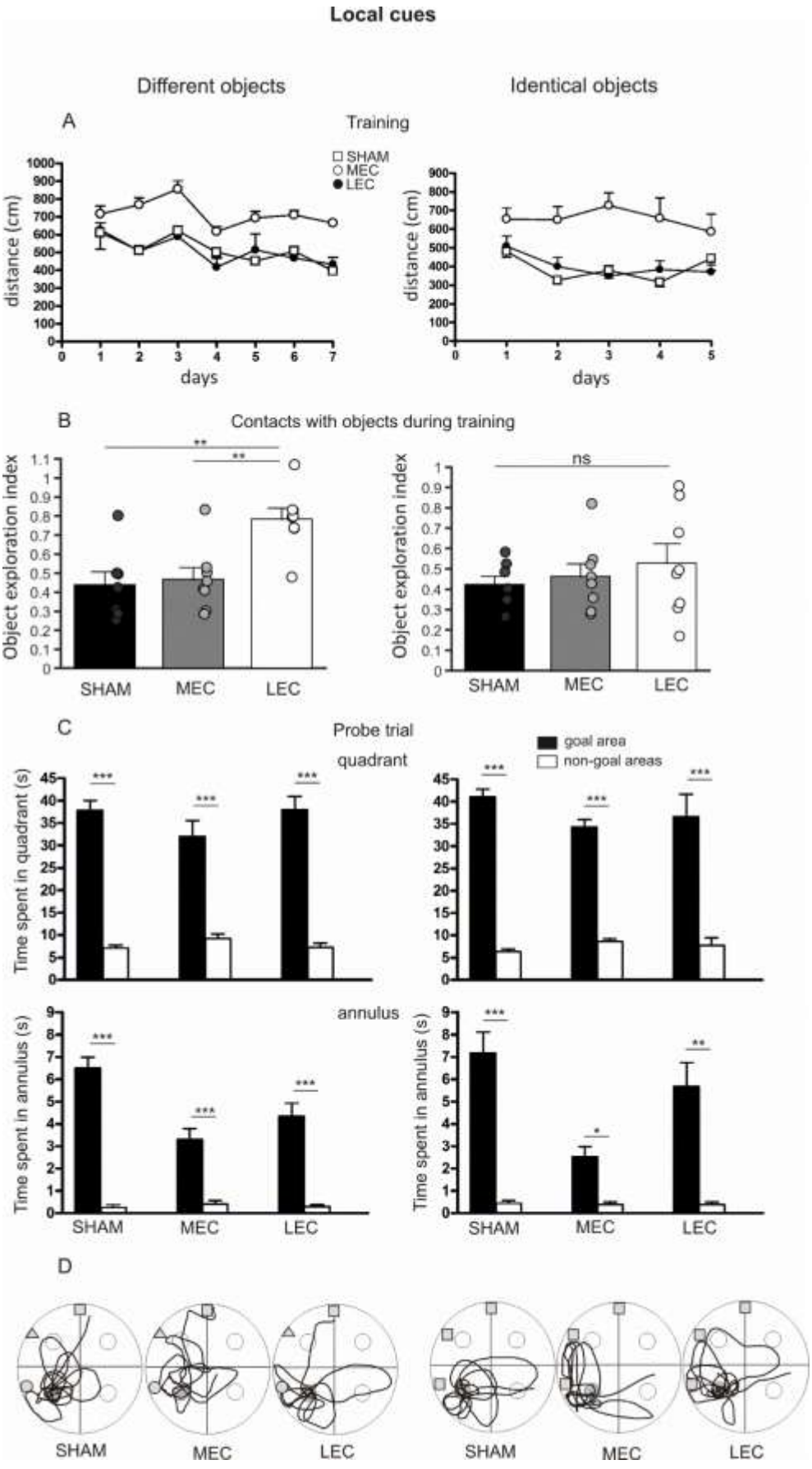
Local cues
Different objects
Identical objects
A
Training
SHAM
MEC
LEC
distance (cm)
days
B
Contacts with objects during training
Object exploration index
ns
SHAM
MEC
LEC
C
Probe trial
quadrant
goal area
non-goal areas
Time spent in quadrant (s)
annulus
Time spent in annulus (s)
D

Figure 4