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Lesions of the medial or lateral perforant path have different effects on hippocampal contributions to place learning and on fear conditioning to context

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Abstract

The axons of the neurons in the medial and lateral components of the entorhinal cortex (MEC and LEC) form the medial and lateral perforant paths (MPP and LPP) which represent the major source of cortical input to the hippocampus. Anatomical, physiological, and pharmacological studies have shown that MPP and LPP are distinct. Unfortunately, assessment of the functional significance of damage to either of these pathways has not used tasks known to be sensitive to hippocampal function in the rodent. In this study, we performed dissociated lesions of MPP and LPP using a combined physiological and anatomical method. Rats with lesions of either the MPP or the LPP were tested on place learning in the water task and on a discriminative fear conditioning to context task. The results indicated that the MPP, but not LPP, lesions resulted in impaired place learning. The context discrimination data revealed an amygdala-like, reduced fear effect of MPP lesions and an enhanced discriminative fear conditioning to context effect of LPP lesions. Consistent with a two-stage model of spatial learning proposed by Buzsáki (Buzsáki G, Two-stage model of memory trace formation: a role for 'noisy' brain states. Neuroscience 1989;31(3):551–570), the impairment in the water task can be interpreted as reflecting the higher efficiency of the MPP synapses in activating hippocampal neurons. The context discrimination results can be explained by either a dissociation of sensory information that reaches the MEC and LEC, or alternatively, by a dissociation between the limbic nature of the MEC and the sensory nature of the LEC.

Keywords: Hippocampus; Amygdala; Entorhinal cortex; Electrophysiology; Dentate gyrus

1. Introduction

Hippocampal function has become synonymous with learning and memory processes. This association is based on strong historical [62], theoretical [23,31,53,60,61,67] and empirical grounds [6–8,46,49,-52,54,69]. Despite increasing support for this view, an understanding of the precise role of the hippocampus remains elusive. One approach towards solving this issue is to systematically assess the contribution of different input pathways to the hippocampus, one of which is the perforant path (PP).

PP originates in the entorhinal cortex (EC) and projects to the dentate gyrus (DG), CA3, CA1, and the subiculum (S). Based on histological criteria, the EC has been divided into MEC and LEC which in turn generate the MPP and the LPP (for review, see Ref. [75]). Anatomical data indicate a topographical organization of these projections on the transversal axis [2]. In both the DG and CA3, PP input is segregated horizontally, with the LPP forming synapses on the outer third and the MPP on the middle third of the dendritic tree

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[32]. In both the CA1 and S, the PP projections are segregated vertically, with the MPP inervating the proximal part of CA1 and distal S, and LPP inervating distal CA1 and proximal S (proximal and distal are defined relative to the DG). This arrangement suggests that the distinctiveness of MEC and LEC input is (at least partially) preserved within the hippocampal circuit.

Electrophysiological evidence is in agreement with the idea that MPP and LPP supply selective input to the hippocampus. McNaughton and Barnes [43] found that excitatory postsynaptic potentials (EPSPs) elicited with LPP stimulation had comparatively higher peak latencies, half widths, and rise times, indicating activation at remote synaptic sites and slow depolarization of the cell population. MPP stimulation resulted in shorter latencies and higher amplitudes of the population spike (PS), reflecting a higher efficiency of this pathway in activating dentate granule cells. Further studies [1,42] suggested that the activation characteristics of MPP and LPP are not due to differential passive decay of the dendritic depolarization resulting from the variation of synaptic sites distance from the granule cells' soma, but to active properties of synaptic transmission. Colino and Malenka [21] reported LPP-based EPSP facilitation and MPP-based EPSP suppression, while induction of long-term potentiation (LTP) was more successful with MPP than LPP stimulation, suggesting thus the possibility of differential involvement of the two pathways in learning and memory processes.

A third line of evidence arguing for the different nature of MPP and LPP comes from neuropharmacological studies. LPP presents immunoreactivity to enkephalin while MPP is immunoreactive to CCK [27]. Enkephalin applied to slices interacts with LPP-elicited LTP [11], while CCK facilitates the induction of MPP-elicited LTP [22]. Induction of LTP in the LPP requires opioid receptor activation, particularly of the δ-1 and μ subtypes [10,12,13].

Although the anatomical, physiological, and neuropharmacological differences between MPP and LPP have been known for some time, little effort has been directed towards understanding their behavioral correlates. Myhrer found increased rearing [50] and decreased investigation of novel objects [51] in rats with selective LPP lesions when compared to rats with MPP lesions, suggesting that lesions of the MPP or LPP input might have differential relevance to behavior. However, we believe that some of the testing paradigms employed in these studies are not directly relevant to hippocampal function [70]. Thus, the present experiments investigated the possibility that the two pathways play selective roles in hippocampally-dependent learning and memory processes. A prediction based on anatomical data was difficult to formulate at the beginning of this experiment, but electrophysiological data suggested that MPP lesions would result in behavioral modifications similar to the ones obtained following hippocampal lesion, while LPP lesions would not be as effective. We used testing paradigms known to be relevant to hippocampal function: a modified version of the water task [38,67] and a discriminative fear conditioning to context task [41]. The first test requires integration of information from a constellation of cues to locate a platform submerged in water. Interference with hippocampal input presumably alters the formation of a cognitive map that the organism uses to solve this problem [53]. The second task requires the subject to discriminate between two distinct environments. It has been postulated that the hippocampus is involved in learning relationships among cues [23,61,68]. Consequently, alterations in its input may prevent successful discriminative fear conditioning to context.

2. Materials and methods

2.1. Subjects

Male Long Evans rats (Charles River colonies) weighing between 250 and 300 g were used for this experiment. The animals were housed individually in clear plastic cages with food and water ad libitum. The subjects were assigned randomly to one of the following groups: (a) pilot ($n = 5$); (b) control ($n = 12$); (c) MPP lesions ($n = 12$); (d) LPP lesions ($n = 13$); (e) MPP sham ($n = 6$); and (f) LPP sham ($n = 6$). The pilot group data were used towards developing the lesioning and histological procedures. Two animals, both with LPP lesions, developed epileptic seizures during testing and were excluded from this analysis.

2.2. Surgical procedures

Sham and lesioned animals underwent the same general procedure, except that the former group received no lesion. Each subject was anesthetized using a dose of 1 ml/kg of sodium pentobarbital (Somnotol, MTC Pharmaceuticals) after prior administration of 1% solution of atropine, 5 mg/kg (Sigma). During surgery, the eyes of the animals were protected with a drop of mineral oil. The rats were placed in the stereotaxic apparatus and an incision was made on the midline of the scalp. Haemosthasis tweezers were used to deflect the skin. The skull was exposed, cleaned, and positioned horizontally by adjusting the mouth piece (bregma and lambda within 0.3 mm on dorso-ventral axis). On each side, the positions of the DG and MPP or LPP were marked on the skull. The coordinates (all with respect to bregma) were: for the DG, 3.5 mm posterior and 2.2 mm lateral; for MPP, 7.6 mm posterior and 4.1 mm lateral; and for LPP, 7.7 mm posterior

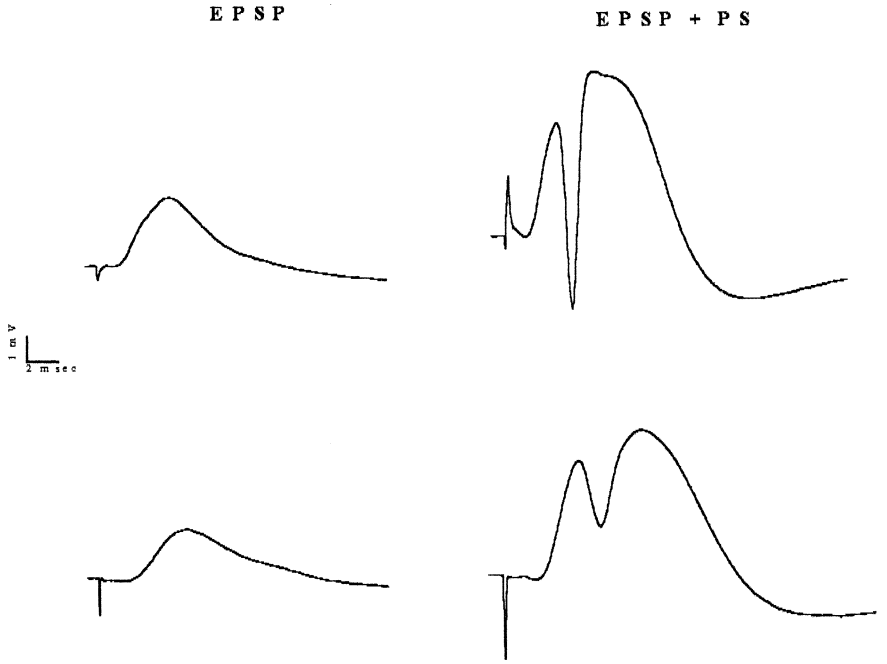


Fig. 1. Field potentials recorded from MPP (top) and LPP (bottom) by using placements identical to the lesion sites in this study. Similar to results previously reported by McNaughton and Barnes [43], the EPSPs elicited with LPP placements had slower onset latency, slower rise time, and larger half width than the ones elicited by MPP stimulation. MPP in turn was more efficient in firing the granule cells, as it can be seen from the higher amplitude of the PS obtained with stimulation of this pathway.

and 5.0 mm lateral. These coordinates were used in previous electrophysiological studies of MPP and LPP [12,45]. After the desired positions were marked, burr holes were drilled in the bone using an electrical drill. Care was taken that there would be no injury to the surface of the brain during the drilling procedure.

Double twisted stimulating electrodes manufactured from Teflon-coated stainless steel wire, 195 μm in diameter (A-M Systems) were lowered in either the MPP or the LPP location. A concentric tungsten monopolar electrode, 25 μm inner pole/200 μm outer pole diameter (Frederick Haer), was used for recording. The final positions of the electrodes were chosen as the ones rendering maximum amplitude of the PS; for details see Section 2.3 below. After final placements were achieved, the stimulating electrode was disconnected from the stimulator, connected with both tips to the positive source of a DC lesion maker (LM 100, The Superior Electric), and a 1.5-mA current was delivered for 15 s. The pilot work indicated that these current parameters would result in a lesion most likely affecting the fibers in one pathway while sparing the fibers in the other. As mentioned above, no lesion was performed in the sham group.

At the end of the lesion procedure in the lesion groups or at the end of the electrophysiological procedures in the sham groups, both electrodes were removed and the wound was sutured using wound clips. An antibiotic ointment (Hibitane, Wyeth-Ayrest Canada) was applied to the wound. All animals received intramuscular injections of 0.15 ml of penicillin (Penlong, Rogar/STB) and 0.15–0.2 ml of buprenorphine hydrochloride (Temgesic, Reckitt & Colman Pharmaceuticals) at the end of the surgical procedure. The animals were left in a heated environment to recover and were returned to the colony the next day.

2.3. Electrophysiological procedures

Previous work [42,43] demonstrated that the characteristics of the field potentials elicited by MPP and LPP activation are different. Compared to MPP, LPP stimulation generates EPSPs with longer peak latency, higher half width, and slower rise time, and PSs with smaller amplitude and longer latency. Previous work using the same coordinates (Ferbinteanu, Srebro and Milgram, unpublished observations) indicated a mean EPSP half width of 3.6 ms (range 2.49–4.44 ms) obtained following MPP stimulation versus a mean half width of 5.8 ms (range 5.38–6.83 ms) after LPP stimulation. The EPSP mean onset latency in the MPP group was 4.9 ms (range 4.69–5.22 ms) while in the LPP group the corresponding value was 6.0 ms (range 5.28–6.65 ms). Fig. 1 shows examples of signals recorded upon stimulation of the MPP and LPP that were included in the analysis. In the present study, similar parameters could not be calculated, due to experimental set-up and to stimulating electrode properties (see below).

The tip of the stimulating electrode was cut at an angle and the insulation was removed on a length of 0.5

mm measured from the tip. This was done so that the lesion would extend over the distance between 3.0 and 3.5 mm ventral coordinates corresponding to the limits within which the PP is expected to be found. Due to this procedure, the number of PP fibers stimulated concomitantly increased; this was desirable for the lesion purposes, but modified the electrophysiological parameters of the signal. First, the amplitude of the PS increased up to 8–10 mV with LPP stimulation, and up to 30 mV with MPP stimulation. Second, the amplitude of the EPSPs also increased, although not as markedly. A third modification was represented by a drop in the threshold of the stimulation level eliciting a PS. Fourth, the width of the signal could no longer be used as a discriminating parameter. In these conditions, the height of the PS and the overall configuration of the signal became the two criteria used for determining the location of the stimulating electrode: a PS larger than 12 mV or reaching high levels of negativity with respect to the baseline was interpreted as resulting from MPP stimulation. Signals with these characteristics were also consistently associated with occurrence of the PS on the rising phase of the EPSP (short PS onset). LPP stimulation resulted in smaller PSs with longer onset latencies and which did not reach high levels of negativity relative to the baseline.

As described above, the surgical placement of the electrodes was accomplished under electrophysiological guidance. Monophasic pulses 0.1 ms in duration (Grass SD9 stimulator, Grass Instrument) were delivered at intervals of at least 10 s, to avoid inhibition of the pathway. The signal was passed though a Grass amplifier (model P15, Grass Instrument), filtered between 1 Hz and 3 kHz, and displayed on an oscilloscope. An input-output curve procedure was performed by delivering stimuli of increasing intensities to either the MPP or the LPP. The configuration of the signal, amplitude of pure (no superimposed PS) EPSPs, and the maximum amplitude of the PS were recorded.

Both electrodes were lowered in steps of 0.2 mm into the brain up to a ventral co-ordinate of 2.0–2.2 mm. At this point, only the stimulating electrode continued to be lowered until a reliable signal (typical for the CA1 field of the hippocampus) was detected by the recording electrode. Once the signal was detected, the lowering of the stimulating electrode ceased and the recording electrode was moved ventrally approximately 0.2 mm past the point of signal reversal that represents the border between the molecular and granular layers of the DG [3]. This position was considered as being in the vicinity of the soma of the granule cells and therefore allowing close to maximal signal recording. The stimulating electrode advancement was resumed until the field potential with a maximum PS was recorded from the granule cells. This point was chosen as the lesion site.

2.4. Behavioral testing

Animals underwent three types of behavioral testing: place learning, timing, and discriminative fear conditioning to context. The first two experiments were run according to a counterbalanced design. Unfortunately, this procedure did not allow sufficient time for the rats to learn the timing paradigm. Accordingly, data from only the first and last experiments will be presented in this paper.

The timing procedure was described by Olton et al. [55]. Briefly, animals were food deprived to 85% of their ad libitum weight during the whole training period. Each subject was placed in an operant cage where a lever press would deliver three food pellets after the elapse of a 50-s interval from the presentation of a salient cue (light). With training, normal animals increase their rate of bar pressing around the point in time when food becomes available. As mentioned above, we found that a 3-week interval was not enough to bring normal animals to the criterion described in the literature [55].

Because the place learning and timing tasks were counterbalanced, the interval between lesion and testing was 1–2 weeks for half the subjects, and 5–6 weeks for the other half of the subjects. Of the six animals that were selected for the final analysis in the MPP and LPP lesion groups, four were first tested in the place learning paradigm and the remaining two were first tested in the timing paradigm. Testing in the context discrimination paradigm was conducted approximately 5 months post lesion.

Due to the time requirements imposed by the lesion procedure, the subjects were divided into subgroups of six animals each, except for the first two subgroups which had four and eight subjects, respectively. Each subgroup included control, sham, and lesioned animals.

2.4.1. Place learning

2.4.1.1. Apparatus. A modified version of the Morris water task was used. This procedure was initially used by McDonald and White [38] to dissociate between two learning and memory systems, one involving the hippocampus and the other the dorsal striatum. Briefly, a white plastic pool 180 cm in diameter was filled with water to which white, non-toxic paint was added to provide a uniform surface and enhance the contrast between the dark animal and the water. A clear Plexiglas platform with a 12×12-cm surface was submerged approximately 2–3 cm under water. The invisible platform was mounted on a column made of the same material and was connected to a base which provided support and served as a means of maintaining the platform in place. A visible platform constructed from wood and painted black on the sides and white on the

top could be attached to the invisible platform; following attachment, the visible platform protruded 2–3 cm above the water surface.

A computer assisted tracking system (VP118, HVS Image) was used for data collection. An overhead camera tracked the movements of the rats in the pool. The experimenter used an air-operated device to signal the beginning and the end of the trial to the computer. Extramaze cues were represented by three posters (different sizes and orientations) mounted on the walls, the computer rack, the experimenter, and the door.

2.4.1.2. Procedure. The platform was placed in the SE quadrant and was maintained in the same position for the duration of the experiment. On days 1–3, the subjects were trained with the visible platform. On day 4, the visible platform was removed and the animals were required to find the invisible platform. This cycle was repeated three times. On day 13 (the final day), the platform with the visible top attached was moved to a position in the NW quadrant that was diametrically opposed to the one that the animals had been trained with during days 1–12. The old location and the new location were situated at equal distances from the starting positions.

During days 1–12, subjects received four trials, each trial starting at a different cardinal compass point of the room. The sequence of the starting points was randomized within each day and was the same for all subjects. The rats were removed from their individual holding cages and placed in the pool facing the wall. Trials were stopped when the animal reached the platform or after 30 s. At the end of each trial, the animal was left on the platform for 10 s. Except during testing, the subjects had no access to visual cues for orientation, as each cage was kept covered with a towel. The towel was also used to prevent the animal from seeing the room while being transported to the pool. During each day, the animals were all given their first trial, then all were given the second trial, etc. As a consequence, the intertrial interval varied depending on the type of trial (visible or invisible) and on the amount of training. For the final test (day 13), only two trials were run: the first starting at the NE position, and the second starting at the SW position.

The computer software calculated five parameters for each swim: (a) the latency to escape; (b) the time spent in each quadrant; (c) the length of the trajectory described by the animal; (d) the speed of swimming; and (e) the heading angle. The latter was defined as the angle between a straight line connecting the starting position and the platform on one hand, and the starting position and a point located at 36 cm from it on the animal's trajectory on the other hand. At the end of each day, the results of the four trials were averaged and reported as one data point.

2.4.2. Context discrimination

2.4.2.1. Apparatus. The testing apparatus consisted of two large Plexiglas chambers connected by an alley (Fig. 2). The two chambers differed in shape and color thus providing visual information that could be used for discrimination. The black chamber was a triangular prism 30 cm tall having as a base an equilateral triangle with a 61-cm edge. The white box was a rectangular prism of similar height having as a base a square with a 41-cm edge. Both chambers had a metallic grid floor connected to the output of a shock generator (Grason-Stadler), and a removable top manufactured of translucent Plexiglas that prevented access to external spatial information. Each chamber was connected with the middle alley by a wall opening located in a similar position within the overall configuration of the wall. Cylindrical containers (approximately 3 cm in diameter) with a pierced base were passed through circular openings placed in the alley wall; the opposite end of the container, which was protruding outside the apparatus, was closed. The containers had one of two different odors (eucalyptus for the white box and amyloacetate for the black box) providing olfactory cues that could also be used for discrimination.

The connecting alley was a $16.5 \times 11 \times 11$-cm rectangular prism manufactured from Plexiglas painted grey and with a roof that could revolve around a hinge. The openings of the connecting alley into either box could be closed by using transparent Plexiglas doors. The whole apparatus was placed on a table with a transparent 124×124-cm Plexiglas top. A mirror installed under the table was angled to approximately 45° from the floor. This allowed tape recording of the subjects' behavior to be made via a video camera.

2.4.2.2. Procedure. The animals were brought on a cart from the animal housing facility and kept in a hallway outside of the testing room but within the main laboratory during testing. They were familiarized with the apparatus during the first day by being placed through the pivoting lid in the alley and being allowed to freely explore for 10 min. Records were kept of the time spent in each box.

On days 2 and 3 (training days) the communication doors were obstructed. Each subject received shock in the paired chamber on one day, or was simply exposed to the unpaired chamber during the other day. The identity of the chamber associated with the shock and the order of presentation (day 2 or day 3) were counterbalanced within the four groups (the shams were combined into one group). The rats were placed through the removable top in the assigned chamber and confined there for 5 min. During this time they received three shocks (0.6-mA current for 2 s each time) at an interval of 1 min; shock delivery occurred at minutes 2,

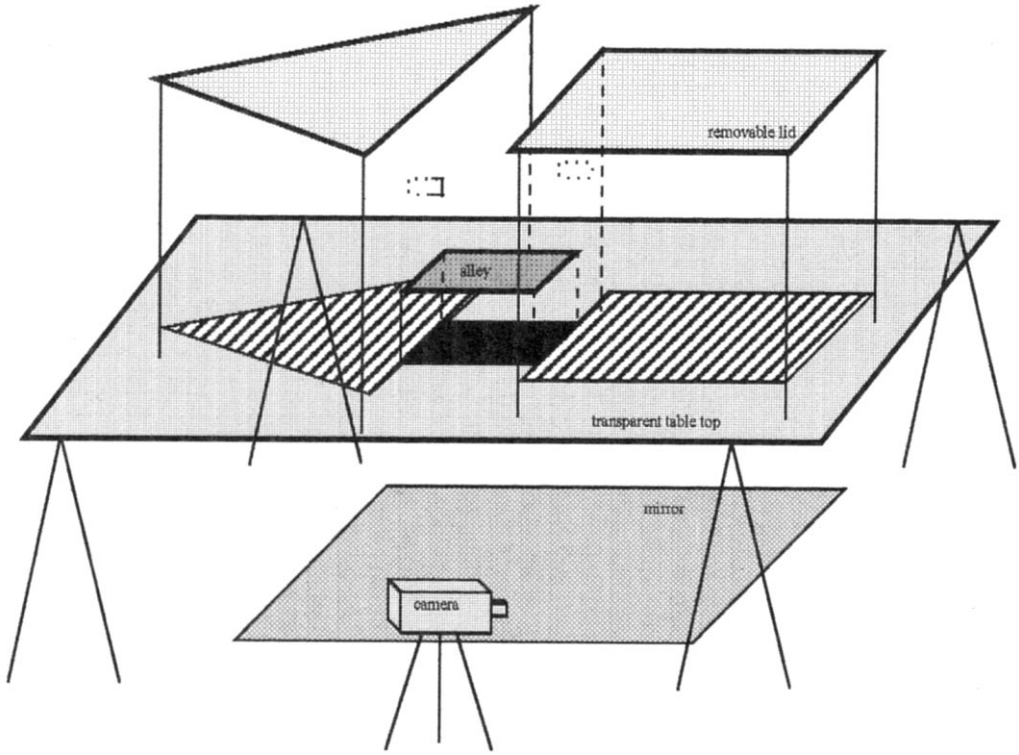


Fig. 2. The apparatus used for the discriminative fear conditioning to context task. The two chambers were of different shape and color and were associated with different odors. Translucent lids prevented the rats from gaining access to cues in the room. The two chambers are connected by a middle alley that has a pivoting lid. During pre-exposure and final preference test, the animals were introduced into the apparatus through the pivoting lid and were allowed free exploration. Otherwise, the subjects were introduced into one of the chambers through the corresponding removable roof and were confined inside for 5 min.

3 and 4. On the other day, the animals were placed in a similar way in the second chamber and kept there for 5 min, but no shock was delivered. In two cases, two of the control animals did not display any signs of distress (flinch, vocalization, or freezing) and it was therefore deduced that they did not receive the shock. Data from these subjects were not included in the analysis.

On days 4 and 5, the animals were again placed for 5 min in one of the chambers in a similar way as described for days 2 and 3. The order of presentation was counterbalanced (paired versus unpaired). The subjects' behavior was recorded on tape during this interval. For each chamber, later analysis determined the amount of freezing (defined as absence of any movement except breathing) exhibited by each subject. The amount of defecation upon presentation of each chamber was also recorded.

On day 6, the doors obstructing the openings of the connecting alley were removed, the animals were placed in the apparatus through the roof of the connecting alley, and were given a preference test in which they were allowed to freely explore the testing apparatus for 20 min. For every animal, a video recording was made together with a hard copy recording of the time spent in each box. In one case, while administering the shock, the animal attempted to jump out of the box through the removable roof. During the preference test this animal spent a significant amount of time in the paired box trying to escape in a similar way; the data from this subject were not considered for the final analysis.

2.5. Histology

At the completion of the experiment, animals were deeply anesthetized and perfused intracardially with saline and 10% formalin. The brains were removed and placed in 10% formalin for at least 1 week, after which they were placed in 20% sucrose for a minimum of 24 h. They were sectioned horizontally (40 μm) and stained with a metachromatic stain that rendered the cell bodies purple and fiber pathways red. A microscopical examination was performed to assess the location and size of the lesion; the assessment was done blindly with respect to the electrophysiological data. This anatomical analysis, together with the electrophysiological records that were made during the surgery, determined which animals were included in the lesion groups.

2.6. Data analysis

As indicated above, in both experiments we considered multiple parameters and a separate analysis was conducted per parameter. For each experiment, repeated measures ANOVAs were performed on a set including only the control and sham groups. If no main effects or interactions were found significant, the data were combined into a single group which we referred to as 'control'. Subsequently, repeated measures ANOVAs were performed on data sets including the combined control data and the two lesion groups. Student-Newman-Keuls post hoc tests were used for further investigation where main effects of lesion were found significant. In the analysis of the context conditioning data, a difference score for each animal was calculated; these difference scores were investigated as described. Additionally, freezing and defecation were investigated within each chamber (paired versus unpaired), and an analysis was also run on the average defecation score.

3. Results

3.1. Histology

3.1.1. Results

Fig. 3a shows the individual lesions and their maximum/minimum extents for each group. As mentioned above, the electrophysiological characteristics of the LPP and MPP signals were defined in agreement with previous work [1,42,43]. The anatomical placement of the lesion was assessed based on drawings published by Steward and Scoville [65] and in agreement with more recent studies [27,56]. Based on the combined anatomophysiological criterion, six MPP- and six LPP-lesioned animals were included in the final analysis. Data from rejected subjects were not included in the analysis and no animals were reassigned to lesion groups.

Fig. 3b is a reproduction of Fig. 1 from Fredens et al. [27] and it shows the distribution of the MPP and LPP as revealed by immunostaining. When compared to Fig. 3a, it is clear that our MPP lesions did not interfere with LPP fibers, as they cross the fimbria on their way from the EC to the DG. Fig. 3c shows photographs of the lesions in one LPP- (left) and one MPP-lesioned (right) animals; both of these animals had medium size lesions within their group. As can be seen from this evidence, there is little or no overlap between the lesion sites. Regarding size, with one exception (the largest MPP lesion), the lesions were of comparable extent. To confirm this inspection, we performed a quantitative analysis of the data. The area delineated between 6.2 and -10.0 mm posterior from bregma and 2.4 and 6.0 mm lateral from bregma (as defined by the Paxinos and Watson atlas) was considered reference area. For each animal, the lesion area was expressed as a percentage out of the reference area and subsequently, we averaged across plates. Using this procedure, the mean MPP lesion size was 7.65% ($\pm$ 0.74 S.E.M.) and the mean LPP lesion size was 7.26% ($\pm$ 0.47 S.E.M.). A two-tailed t-test performed on these data indicated lack of significant differences between the two groups ($t_{22} = -0.467$, $P = 0.64$).

Inspection of the MPP lesions indicated that there was limited damage at the dorsal site of electrode implant, in a transition area between the perirhinal cortex and the retrosplenial cortex. Out of the six animals that met the double selection criterion, one had no damage to either pre- (PreS) or parasubiculum (ParaS), while four had unilateral partial damage and one had bilateral partial damage to these structures. Regarding the postsubiculum (PostS; defined as the dorsal PreS, between 2.4 and 4.2 ventral location from bregma), three animals did not have any damage, two showed unilateral partial damage, and one showed bilateral partial damage. Small damage to the transition area between the PreS and ParaS on one hand and the EC on the other hand was present in all animals. We attributed this area to the most posterior part of the S.

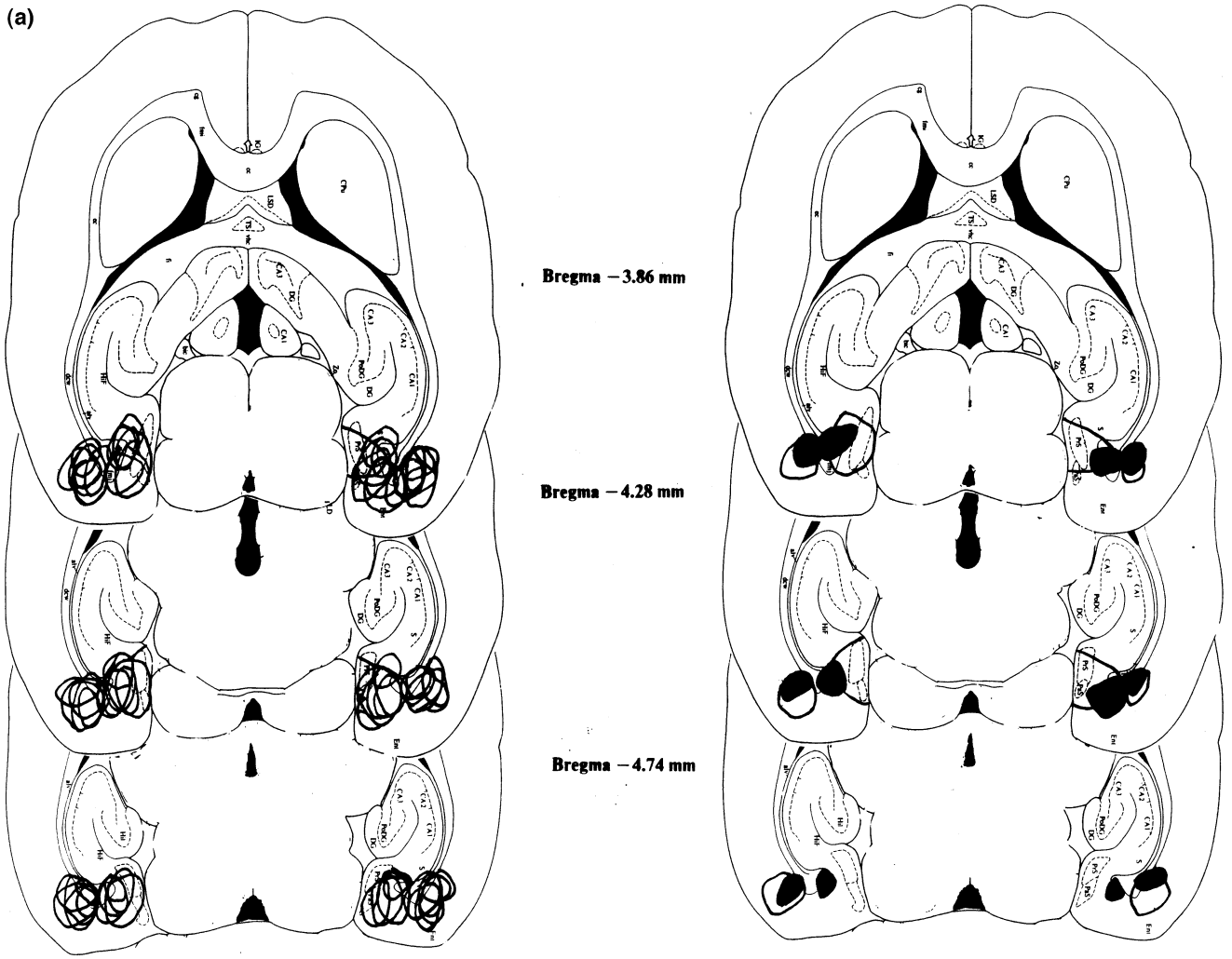
Inspection of the LPP lesions revealed that, similar to the MPP lesions, there was limited damage at the dorsal site of implant, in a transition area between perirhinal and occipital cortex. At a deeper level, the damage was limited to LPP and the deep layers of the EC. Compared to MPP lesions, the LPP lesions were somewhat smaller and more homogenous in size. The implant site was remarkably consistent.

Although the lesions could not eliminate completely the MEC or LEC input to the hippocampus, we consider that a large number of fibers was lesioned and that only the most ventral components of MPP or LPP were spared.

3.2. Experiment 1—place learning in the modified water task

As mentioned, the water task employed in this experiment, which contains both visible and invisible platform training, was designed by Sutherland and Rudy [67] and used, in a modified version, by McDonald and White [38]. Training with the visible platform served two functions: first, to investigate if any motric or motivational differences existed among groups, and second, to help the subjects overcome any procedural impairments that might have occurred. Thus, before the subjects were submitted to an invisible platform trial, they were familiar with the requirement of swimming to a platform located in the pool.

For each trial, five measurements were taken: latency, time spent in each quadrant, trajectory length, swimming speed, and heading angle. Of the quadrant



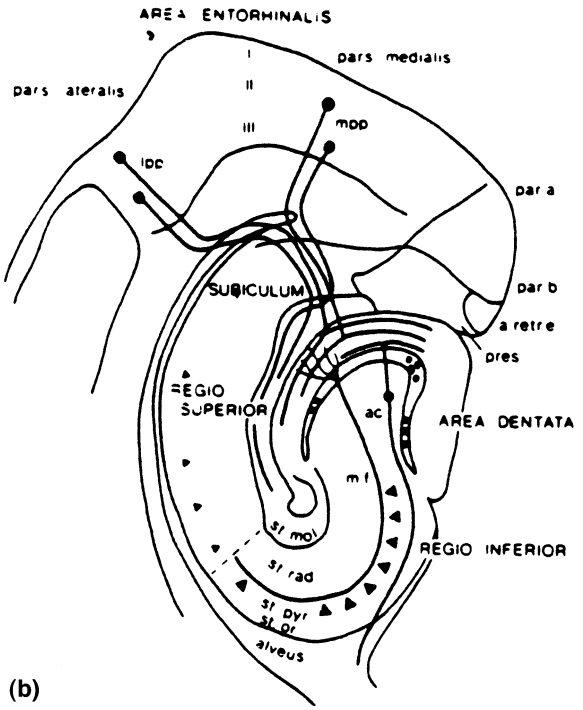
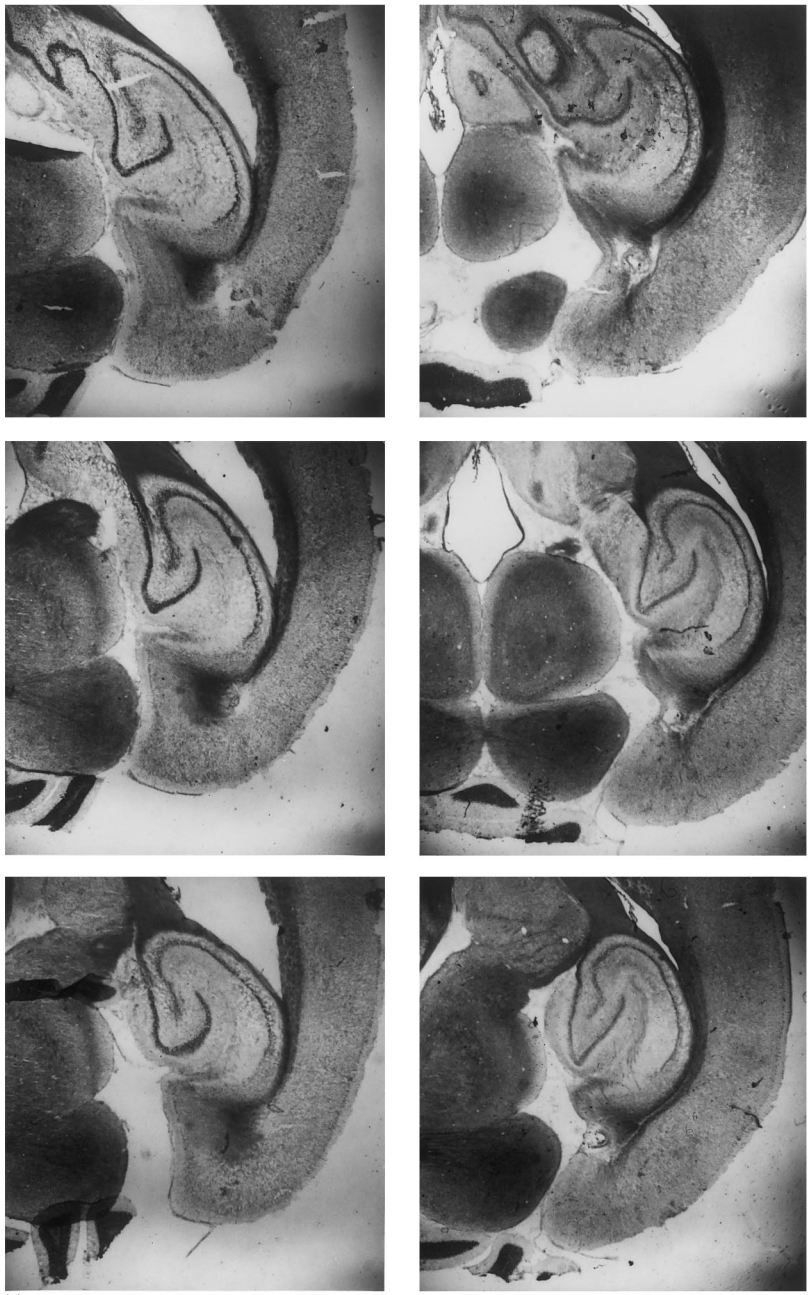


Fig. 3. (a) Drawings indicating individual lesions (left), as well as the maximum and minimum lesion extent (right) in both the LPP and the MPP groups. The anatomical assessment was based on drawings published by Steward and Scoville [64], Peterson et al. [56], and Fredens et al. [26]. (b) Reproduction of Fig. 1 in Fredens et al. [27]. When compared to (a), it can be seen that the MPP and LPP lesions did not interfere with the other pathway fibers, respectively. (c) Photographs of one LPP (left) and one MPP (right) lesion. Medium size lesions were selected in both cases.



(c)

Fig. 3. (*Continued*)

data, we report here only results pertaining to the platform quadrant. The disorientation created by the absence of cues during transport from the holding cage to the pool most likely affected the heading angle parameter [29,37,73], and possibly the swim speed as well. Therefore, we consider that for this experiment, these measurements were not reliable learning indicators.

The data were divided in two groups according to the status of the platform (visible or invisible) and analyzed separately.

3.2.1. Visible platform

Figs. 4–6 show the group means of latency, trajectory length, and quadrant preference for the first 12 days of testing. All groups learned this task equally well, as indicated by the similarity of latencies and trajectory lengths obtained after several days of testing. The differences in preference for the platform quadrant do not argue for impairment in the lesion groups because in these cases, the values of this parameter are higher than the ones obtained from the control group.

For each of the three parameters, a day × lesion

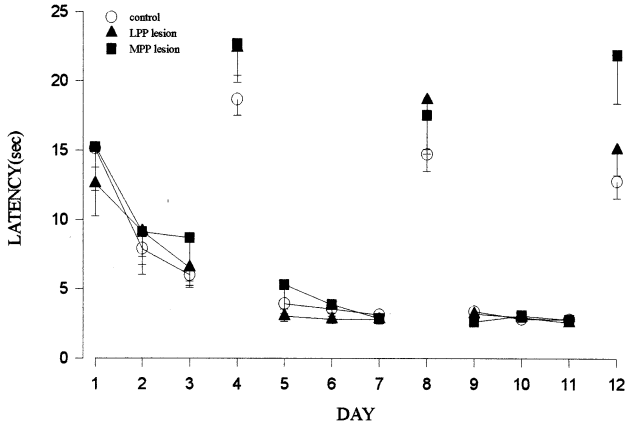


Fig. 4. Mean latency to escape for all experimental groups across the first 12 days of water task training. Differences in results for the visible platform trials disappeared in the last 6 days of testing. The invisible platform data (days 4, 8, and 12) indicate considerably higher latencies, reflecting the different nature of this task. Across testing, MPP lesioned animals required more time to escape in the invisible platform task.

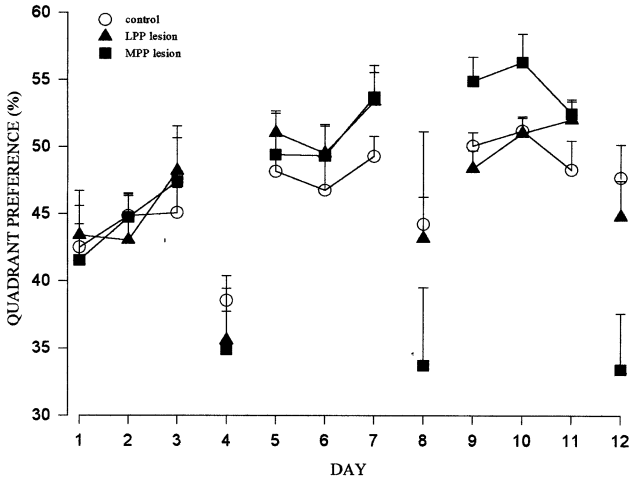


Fig. 6. Preference for the platform quadrant for all experimental groups across the first 12 days of water task training. The differences present during the visible platform trials do not support impairment in any of the lesioned groups. All groups but the MPP lesioned ones improved on the invisible platform tests.

ANOVA for unbalanced designs was performed. In all cases, the results revealed a significant main effect of day ($F_{8,264} = 45.67$, $P < 0.001$; $F_{8,264} = 40.25$, $P < 0.001$; $F_{8,264} = 5.68$, $P < 0.001$), but no significant effect of lesion group, and no significant interaction. This analysis confirmed that there were no sensory, motor, or motivational differences between the groups.

3.2.2. Invisible platform

Figs. 4–6 also show the results of invisible platform testing. There was a noticeable increase in latency and trajectory length for all groups when compared to the visible platform trials, reflecting the different nature of the two tasks. The data suggested impairment in the MPP lesion group, as the values of these parameters did not decrease with training as much as for the rest of the subjects, while the quadrant preference did not improve at all.

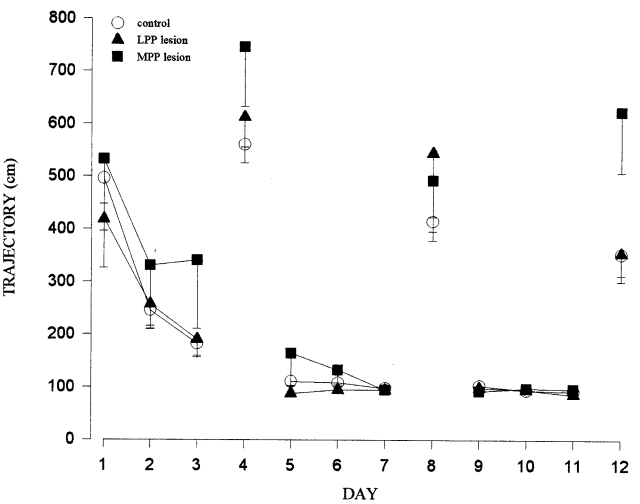


Fig. 5. Mean trajectory length for all experimental groups across the first 12 days of water task training. Similar to latency, the values of this parameter decreased as the subjects learned the task. The visible platform data indicate no differences among groups, while the invisible platform trials reveal that the MPP lesioned animals consistently swam longer distances.

The data from the control and both sham groups were analyzed separately using a repeated measures day × lesion ANOVA on latency, trajectory length, and quadrant preference as described. Because no main effects or interaction were found significant, the data from these groups were combined in one set that we refer to as 'control'. A day × lesion ANOVA for unbalanced designs was then performed on the combined data set. The latency data indicated a significant lesion effect ($F_{2,33} = 3.44$, $P < 0.05$) and of day ($F_{2,66} = 4.26$, $P < 0.05$). Comparisons among the lesion groups revealed no significant differences on days 4 and 8, but significant differences on day 12 between control and MPP lesion groups, as well as between the MPP and LPP lesion groups, but not between LPP lesion and controls. The trajectory length data indicated a significant main effect of day ($F_{2,66} = 6.86$, $P < 0.001$), and a main effect of lesion approaching significance ($F_{2,33} = 3.07$, $P = 0.059$), and no significant interaction between lesion and day of testing. No further comparisons were performed in this case. The quadrant preference data showed a main effect of lesion group ($F_{2,33} = 5.01$, $P < 0.05$). As in the case of the latency data, pairwise comparisons indicated no significant differences within day 4 or within day 8, but significant differences between controls and the MPP lesion group, and between the two lesion groups, but not between the controls and the LPP lesion groups.

3.2.3. Visible versus invisible platform trial (day 13)

Previous work [38] demonstrated that in a group of normal animals, half of them would choose the old platform location while the other half would go to the new location. This result demonstrates that there are individual differences among animals, some being better place learners, while some others are better at performing a stimulus-response behavior. In this experiment, only three out of the 12 control rats swam to the old platform location, while all the other subjects chose the visible platform. As in the case of heading angle and speed parameters, we interpreted this result as a consequence of the experimental procedure. The lack of access to visual cues except during testing likely favored a stimulus-response type of behavior over a navigational one when a stimulus-response solution was available.

To summarize, the visible platform testing argued for equal sensory and motor abilities, equal motivation, and no procedural impairment across groups. The invisible platform trials demonstrated impairment in place learning following MPP lesions, but not LPP lesions. This conclusion was based on results indicating significantly higher latencies and lack of increased preference for the platform quadrant for the MPP lesion group. The trajectory length data were also in agreement with this conclusion, as the overall pattern of results indicated a tendency for longer paths for the MPP lesion group. The level of impairment following the MPP lesion was almost identical to the one obtained in rats with fornix lesions [38] and in rats with hippocampal damage [67].

3.3. Experiment 2—discriminative fear conditioning to context

Both the amygdala and the hippocampus can form representations that are potentially used in dissociating between similar environments presented in the context conditioning task, but the nature of these representations might be different. Thus, the amygdala is involved in forming an association between the affective state generated by the stimulus (shock) and the apparatus [35,57], while the hippocampus enables the organism to perform the fine discrimination necessary for dissociating the 'dangerous' from the 'safe' chamber [41]. The design of this experiment originally proposed by McDonald et al. [41] does not involve spatial learning (the chambers are covered with translucent plastic) but seems to require synergistic involvement of amygdalar and hippocampal functions. In this previous study, amygdala lesions resulted in the absence of a fear response to any of the two chambers, indicating a lack of association of the apparatus with shock. Hippocampal lesions were followed by a display of high fear response in both chambers, indicating memory of the shock-apparatus association, but lack of discrimination between environments. Within this framework, reference will be made to amygdala-like and hippocampal-like behaviors. We consider the present fear conditioning to context task to be quite different than the one chamber version, as additional to the association of a negative emotional valence to the apparatus, the subjects are required to perform a discrimination task.

Assessment of the fear conditioning to context was based on amount of freezing and defecation upon confinement in each chamber (days 4 and 5) and on time spent in the paired and unpaired chambers when a choice was available (last day). Fig. 7 shows that the LPP lesion group, but not the others, exhibited discriminative freezing. Visual inspection of the data suggested that the MPP lesion group showed low overall freezing, similar to the LPP lesion group in the unpaired chamber (amygdala-like behavior). Together with the fear response exhibited by the MPP lesioned animals during exposure to shock (defecation, freezing, flinch, and vocalization) this argues against a general inability to express fear following MPP lesions. The controls showed freezing similar to the LPP group in the paired chamber (hippocampal-like behavior), arguing against an abnormally high fear response associated with LPP lesions.

The statistical analysis confirmed the effects suggested by the visual inspection of the data. As in the analysis of the water task the data from the controls and the two sham groups did not show significant differences and were combined into one control group. A lesion × chamber ANOVA for unbalanced designs indicated a significant lesion effect ($F_{2,31} = 4.67$, $P < 0.05$) and a significant lesion × chamber interaction

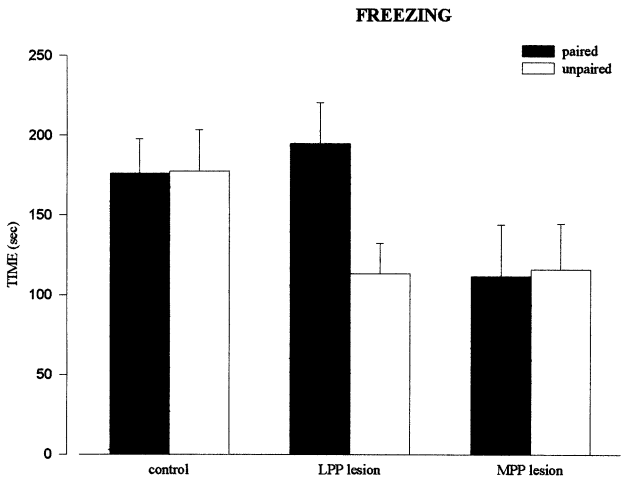


Fig. 7. Amount of freezing after shock administration. The LPP lesion group uniquely discriminated between the two chambers while the MPP lesion group showed less freezing overall. The control and sham groups showed high, non-discriminative freezing.

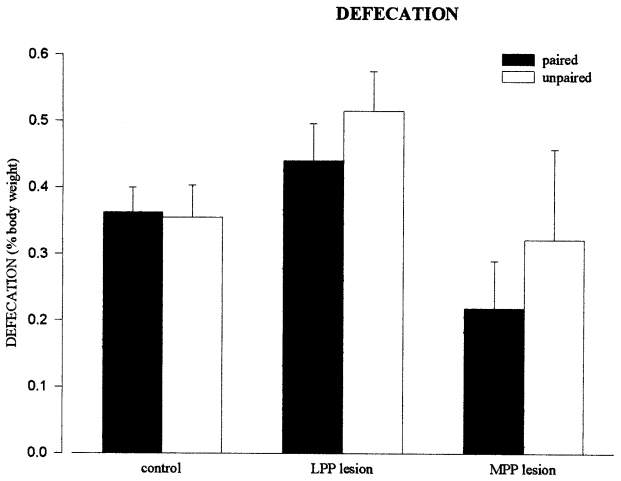


Fig. 8. Amount of defecation after shock administration upon chamber presentation. The LPP lesion group showed a tendency towards increased defecation when compared to the controls, while the MPP lesion group showed the opposite effect.

($F_{2,31} = 4.13$, $P < 0.05$). A difference score was calculated for each animal between the freezing time in the paired versus the unpaired chambers. One way ANOVA analysis of this score indicated a significant effect of lesion ($F_{2,31} = 4.13$, $P < 0.05$) and pairwise comparisons showed significant differences between LPP lesion group and both the controls and MPP lesion group, while no significant differences were found between the last two. Analysis limited to the data in the paired chamber indicated significant differences between controls and MPP lesion group as well as between the two lesion groups, but no differences between LPP lesion group and controls.

Fig. 8 shows the defecation data. Combination of the data from the control and the two sham groups was performed after no significant differences were found among these groups. Two-way ANOVA analysis indicated a significant main effect of lesion ($F_{2,31} = 3.64$, $P < 0.05$). Defecation was not discriminative between the two chambers in any of the groups, but in agreement with the pattern described above, the MPP lesion group showed less defecation overall than the LPP lesion group, while in the paired box the group showed significantly less defecation than both the LPP lesion and the control groups.

Fig. 9 shows the mean amount of time each group spent in the paired and unpaired chambers during pre-exposure and during the final preference test. After shock administration, all groups preferred the unpaired chamber, demonstrating retention of the identity of the one associated with shock when presented with the whole apparatus. In agreement with the pattern described by the freezing and defecation data, the preference test suggested a trend towards larger difference between the time spent in the paired versus the unpaired chamber in the LPP lesion group.

To reduce error variance, a logarithmic transformation was performed on this data set. An overall lesion × chamber × time of testing (pre-exposure versus final preference) ANOVA indicated a significant effect of chamber identity (paired/unpaired; $F_{1,60} = 16.12$, $P < 0.001$) and a significant effect of time of testing (pre-shock/post-shock; $F_{1,60} = 20.44$, $P < 0.001$), as well as a significant chamber × time of testing interaction ($F_{1,60} = 23.05$, $P < 0.001$). However, there were no differences among lesion groups.

To address the issue of hyperactivity following lesion, we analyzed the number of entries per chamber during the pre-exposure period. An ANOVA for unbalanced design on a data set composed of only the control, MPP sham, and LPP sham subjects indicated no significant differences among groups; the data from these animals were subsequently combined. A similar procedure performed on the complete data set (the combined control group, the MPP lesion, and the LPP lesion animals) indicated no significant differences among groups. This result argued against the hypothesis that the MPP lesion group showed low freezing because of hyperactivity. The similar levels of freezing the MPP and LPP lesion group exhibited in the unpaired box were in agreement with this interpretation, as well as the fact that while the MPP lesion group froze an average of about 100 s out of a maximum of 300 s, the control group did not freeze more than an average of about 175 s. We concluded therefore that hyperactivity was an unlikely explanation for the freezing results. This interpretation is in agreement with previous reports [25,26] which indicated that hippocampal lesions are associated with specific effects on freezing because they affect context, but not tone conditioning.

We interpreted these results as demonstrating that the MPP lesions resulted in an amygdala-like, low fear response as assessed by both freezing and defecation. The control group showed high, non-discriminative fear response, similar to a hippocampal-like behavior previously reported by McDonald et al. [41]. The LPP lesions were followed by enhanced discriminative freezing combined with a lack of discriminative defecation, thus providing an argument for different learning rates for different fear parameters; this hypothesis is sustained by data obtained in our laboratory [4,5] showing that acquisition of discriminative defecation requires repeated training drills. The preference data indicated that the lack of discriminative response was not due to lack of sensory-related discrimination between the two chambers. Taken together, these results suggest enhanced discriminative fear conditioning to context following LPP lesions and decreased learning of fear response to context following MPP lesions. The MPP lesions do not result in lack of discrimination between contexts per se.

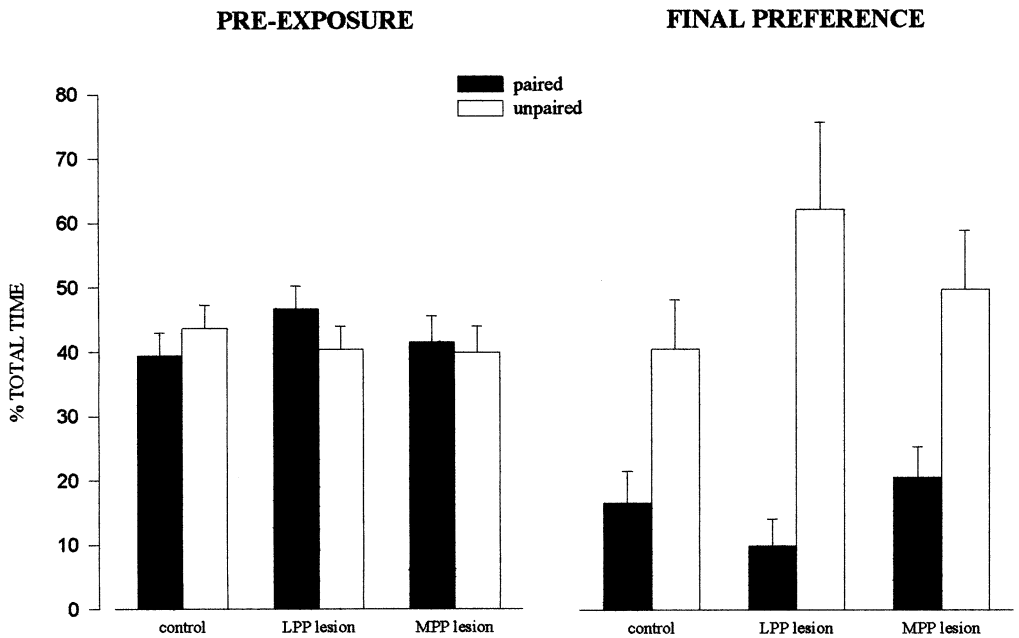


Fig. 9. Results of the initial exploration and final preference tests. Pre-exposure data show no preference for any of the chambers. Following shock administration, all groups preferred the unpaired chamber, indicating that all subjects learned the chamber identity. In agreement with the rest of the data, the LPP lesion group showed the largest discrimination between chambers.

4. Discussion

The results show that the MPP and the LPP have selective contributions to hippocampal function. The results of the water task are in agreement with previous reports [64] and demonstrated that the MEC, but not LEC, input is essential for successful place learning. The context task revealed an amygdala-like, low fear effect following MPP lesion and enhanced conditioning to context following LPP lesion.

This experiment, to our knowledge, is the first investigating the behavioral relevance of the differences between MPP and LPP using hippocampally-specific behavioral tasks. Place learning and context discrimination are paradigms extensively used to assess deficits following interference to hippocampal function. Our water task data are in agreement with previously reported results [38] showing that a solution to the invisible platform problem requires an active hippocampus, while navigation towards a visible cue is a task involving the dorsal striatum. Additionally, the present experiment demonstrated that successful place learning specifically requires the MEC input, while the LEC input does not seem to be essential.

The discriminative fear conditioning to context task revealed a unexpected amygdala-like effect (for definition of this term, see the introductory paragraph to Section 3.3 regarding the context discrimination task) following the MPP lesion, and an enhancement of discriminative freezing following the LPP lesion. The control group showed high freezing and defecation in both chambers, and all groups spent more time in the unpaired chamber in the final preference test.

In the following discussion, we will first address the issues of lesion procedure, alternative interpretations of the lesion effects on the water task, and methodological aspects of the discriminative fear conditioning to context. We will then offer an interpretation of our data within the context of current anatomical and physiological research on the hippocampal formation, together with broader methodological and theoretical implications we believe our study indicates.

4.1. Lesion procedure

In this study, we employed electrolytical lesions because they allowed us to create a combined anatomophysiological assessment criterion. We considered that given the close proximity of the targeted structures, the electrophysiological data would greatly increase our confidence that the lesion was performed in the intended location. The histological assessment was performed by one of us without previous knowledge of the electrophysiological data. We consistently found that good anatomical placements were associated with good electrophysiological signals, and vice versa, poor anatomical placements resulted in poorer signals. It should be taken into consideration that a good electrophysiological signal occurs when both the stimulating and recording electrodes are well placed; therefore, we consider that our assessment was rather conservative.

The damage that occurred to structures other than MPP or LPP and its implications are discussed in the following section.

A second issue is the proximity of the LPP and MPP lesion sites, as well as the lesion size. As can be seen from Fig. 3a, there was little or no overlap between the two sets of lesions. Fig. 3b, together with the characteristics of the field potentials (see above), indicate that the lesions were on target and did not interfere substantially with the other pathway's fibers. Regarding the lesion size, the quantitative analysis indicated lack of significant differences between the two lesion groups. Therefore, the behavioral differences found between the two lesion groups cannot be attributed to differences in lesion extent. Note however that if MPP and LPP are structures with different anatomy, physiology, and behavioral relevance, the size of the lesion may or may not be related to the magnitude of the behavioral effects. By analogy, a large lesion of area A might result in no behavioral modification, while a small area B lesion might have considerable effects.

Different techniques that we could have used to accomplish the MPP/LPP dissociation were knife cuts and heat lesions. However, neither of these procedures allows for electrophysiological recording. There are no well-established pathway-specific neurotoxic lesion procedures that we were aware of. Beside specificity, one advantage of this procedure would have been the possibility of electrophysiological recordings. However, a disadvantage would have been the possible diffusion of the lesioning agent.

A different approach for our investigation would have been selective lesions of the LEC and MEC. However, beside the similar problems of close proximity and of sparing of input, EC lesions would have eliminated the inhibitory interneurons present in this area (for their functional significance, see Refs. [18,20]), as well as other pathways that relay in the EC, but which are not part of MPP or LPP.

We therefore conclude that, although not perfect, our approach was the best method of targeting specifically the MEC versus the LEC input to the hippocampus.

4.2. Alternative interpretations of the lesion effects on the water task

Taube et al. [71] found that rats with NMDA or electrolytic lesions of the PostS showed impairment in spatial tasks. The NMDA lesions were localized to the neuronal population of the targeted structure and were followed by a significantly smaller deficit when compared to the effect of electrolytic lesions. The authors interpreted this difference as an effect of damage to perforant path fibers following the electrolytic procedure. Due to the location of the lesion (6.8–7.8 posterior and 3.1 lateral to bregma), we believe that a majority of the perforant path fibers were in fact MPP. Thus, the results reported by Taube et al. [71] strongly support our conclusion that MPP lesion impairs spatial learning. Given the minimal damage to the PostS incurred by the MPP lesion group, we consider that lesions of this structure could not provide an explanation for the reported behavioral deficit.

Bouffard and Jarrard [9] reported that damage of the S and EC did not impair performance in the radial maze. The lack of impairment could be explained by the sparing of the deep layers of the EC at the dorsal level. Of importance to this study is that removal of the entire S was not followed by impairment in this task. A different study [47] indicated that complete damage of the S (20 injection sites) was followed by a temporary deficit in spatial learning which was overcome by extensive training. We consider that the small damage to the most posterior part of the S associated with the MPP lesions was not sufficient to provide a satisfactory explanation for the deficit in the water task demonstrated by the MPP lesion group.

4.3. Discriminative fear conditioning to context task.

In this section, we will address two issues: (a) the lack of discriminative freezing and defecation, combined with discriminative preference shown by the control group, and (b) the relevance of this task to hippocampal function. The former is explained by the difficulty of the discrimination task as dictated by the overall ambiguity level of the context, and by the different learning rate for different fear parameters. The latter is demonstrated by data obtained from HPC lesioned, as well as $Nfl^{+/-}$/Nmdar $1^{+/-}$ mutant mice [26].

On first view, the lack of discriminative freezing and defecation combined with discriminative chamber preference shown by the control group might seem unusual. However, this is not necessarily so if one considers that the ambiguity level of the context influences behavior and that different fear parameters have different learning rates and possibly even different neural substrates. Thus, data from a different experiment run in our lab [4,5] indicate that when normal animals are conditioned in a paradigm identical to the one used in the present experiment (high context ambiguity—both chambers are placed in the same room), they do not exhibit discriminative freezing; however, when the shock is administered in a different room (low context ambiguity), the animals freeze discriminatively. This means that with one conditioning trial, discriminative freezing is exhibited only if the context ambiguity is low.

Second, data from the same experiment indicate that different fear behaviors have different acquisition rates: context preference is acquired fastest, freezing, modification in locomotion, and urination, are affected next, while the last modifications are seen in heart rate,

ultrasonic vocalizations, body temperature, and defecation. It is important to note that the preference task is, by definition, a recognition task, while the freezing and defecation parameters are measured in a recall task. Given the large body of literature indicating that these two memory tasks require different cognitive processes, it is not all that surprising that they exhibit different rates of acquisition (also, see Ref. [26] for the relevance of hippocampal function to recognition processes). A second point is that previous work [66] indicated that in certain circumstances, the hippocampus and not the amygdala, controls defecation, while McDonald et al. [41] and Frankland et al. [26] showed that in this task, the hippocampus interferes with discriminative freezing. Together, this explains why in the present experiment (a) the control group showed preference for the unpaired chamber, but did not show discriminative freezing or defecation; (b) the LPP lesion group showed preference for the unpaired chamber and discriminative freezing, but not discriminative defecation; and (c) the control and LPP groups were different on freezing, but not on preference and defecation.

Regarding the issue of context conditioning, some studies reported HPC involvement [19,35,36,58,63,66], and some did not [28,58]. We consider that the task we used in the present experiment is sensitive to HPC function. McDonald et al. [41] and Frankland et al. [26] indicated that pre-training lesions of the HPC interfere with context discrimination (as assessed by freezing), while context recognition is supported by different neurological substrates. The dissonance present in the literature very likely reflects heterogeneous ambiguity levels of the context conditioning paradigms employed by different researchers. Tasks that have a high level of ambiguity increase demands on the HPC function. A paradigm similar to the present one, based on discrimination between two chambers situated in the same room, likely requires more HPC involvement than a paradigm that employs two different chambers in two different rooms, which in turn requires more HPC involvement than a paradigm using only one chamber. Combined with the factor of separate learning rates for different fear parameters, these two aspects explain why in some, but not all, situations context conditioning is HPC sensitive and why some, but not all, fear measurements indicate context conditioning.

4.4. What can current views of hippocampal anatomy contribute to the interpretation of the present behavioral results?

Witter et al. [75] suggested that the LEC is part of a mainly sensory circuitry that connects different cortical areas with the hippocampal loop, while the MEC receives input from limbic areas and sends projections to the LEC itself. Fig. 10a presents a diagram based on anatomical data summarized by Witter et al. [75] and by Amaral and Witter [2]. The perirhinal (PER) and postrhinal (POR) cortices project predominantly to LEC and receive unimodal and polymodal associational input representing highly processed sensory information. The information reaching the MEC on the other hand is mainly non-sensory, non-specific, and comes from the PreS and ParaS, the amygdala, and the cingulate and the retrosplenial cortices; POR also contributes to the input. It is important to note that the selectivity of input between LEC and MEC is relative rather than absolute, but it is less clear what exactly the partition is [2,15](also Ref. [75], p. 224). Within the hippocampal formation, the EC-CA1/S connections are organized in parallel loops (see Ref. [75], pp. 480–481) suggesting the possibility of input channeling (Fig. 10b). The information circulated in these parallel loops has multiple opportunities to be cross-referenced, once inside of the hippocampus (DG and CA3), and once at the level of cortical interconnections.

A second possible factor dissociating between the MEC and LEC is the segregation of sensory input. Although sensory information reaches both LEC and MEC, it has been argued that in the rat, the LEC receives most of the olfactory input, while the MEC receives most of the visual input [2,75]. The evidence supporting this idea is based on anatomical (see Refs. [2,75] for review) and physiological data. Wilson and Steward (1978) (cited by Ref. [75]) demonstrated that lesion of the LEC is followed by abolishment of hippocampal activity initiated by olfactory input. Because a total selectivity of projections is not supported by the anatomical data (for review, see Ref. [15]), it is probably safe to assume that, as in the case of sensory versus limbic nature of LEC and MEC, the segregation of sensory input is relative and not absolute.

Consequently, two main hypotheses can be formulated to explain the results of the discriminative fear conditioning to context task. The first centers around the different contributions the LEC and MEC provide to the representations the animals form in the context task. The second is related to the segregation of sensory input within the hippocampal formation.

If LEC is part of a circuit involving communication with other cortical areas, then it is possible that functional alterations of LEC may result in degraded activity within networks storing memory traces. We can envision two consequences of this degradation. The first possibility is that in circumstances where all cues are present (as for instance in the water task), there is little demand on cued recall and MEC input is sufficient for successful performance. In the two chamber context conditioning paradigm, damage of the LEC input could result in a degraded memory trace normally formed during pre-exposure [24,30,39,74]. During training, this degradation may prevent the animal confined to one

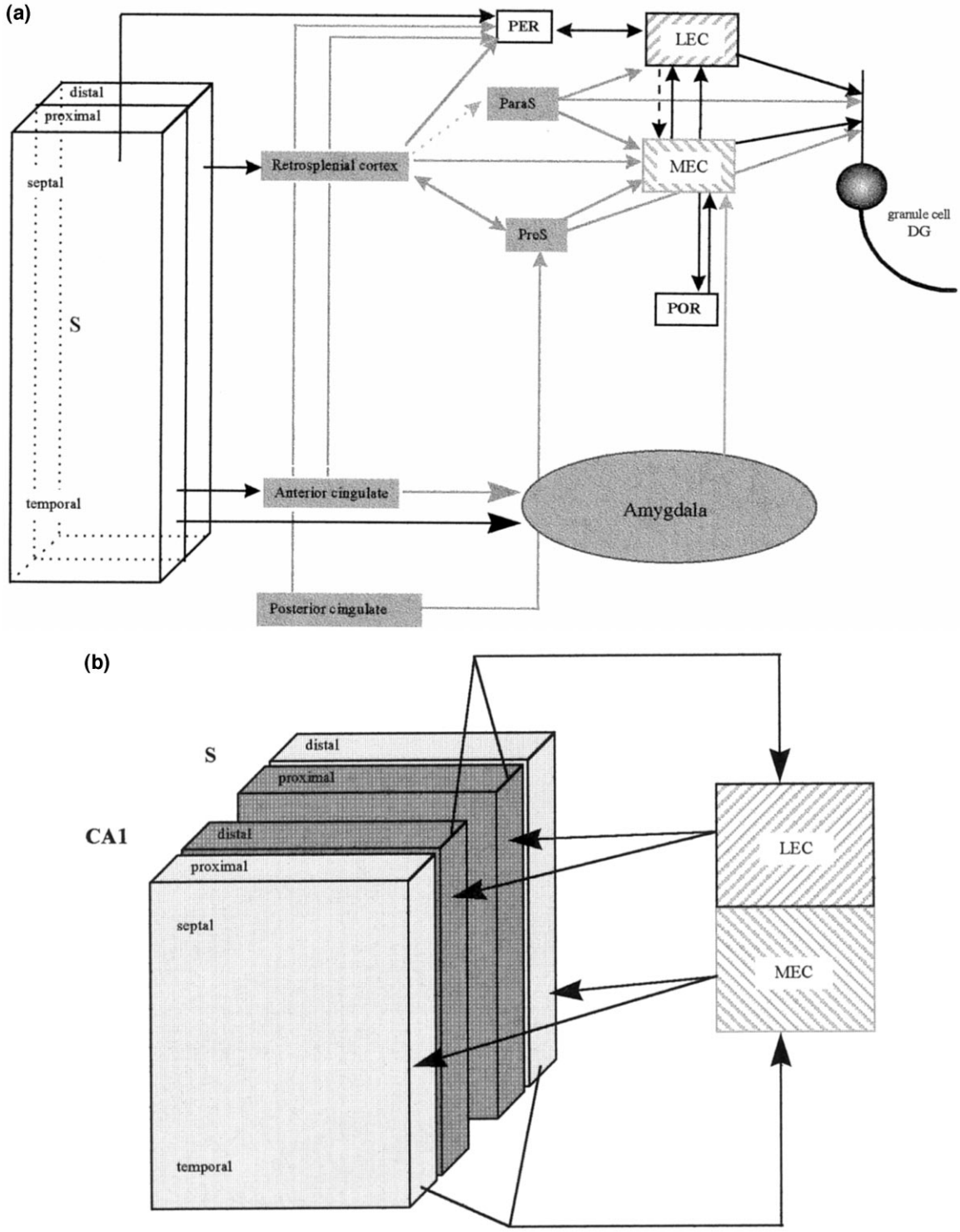


Fig. 10. (a) Diagram illustrating the sensory nature of the projections to lateral entorhinal cortex (LEC; in black) as opposed to the limbic nature of the medial entorhinal cortex MEC input (gray). The perirhinal (PER) and postrhinal (POR) cortices project strongly to LEC, while the presubiculum (PreS) and parasubiculum (ParaS) project mainly to MEC. The segregation of input is relative rather than absolute, as POR projects to both MEC and LEC, and ParaS projects to both MEC and LEC. The MEC/LEC input can be cross-referenced intrahippocampally at the level of the dentate gyrus (DG) and extrahippocampally at the level of the cingulate and retrosplenial cortices. The output of the subiculum (S) is topographically organized on the septo-temporal axis and on the transversal axis. Proximal and distal indicate relative position to the dentate gyrus. For details, see Section 4. (b) Parallel loops within CA1 and S could support channeling of information within the hippocampus. The monosynaptic pathway from the EC to the CA1 entrains fast frequency oscillations. From the EC, the activity may spread to cortical networks where the information storage takes place. See Section 4 for details.

chamber to recall the rest of the apparatus, while control animals, with an intact two-chamber representation, could attribute the negative valence to the entire complex. Thus, in the LPP lesion group, only cues present during shock delivery and not others would elicit fear, explaining the enhanced discriminative context conditioning. On the other hand, the limbic nature of the MEC connections renders the MPP input important for forming associations between shock and specific cues within the apparatus. In its absence, no cues can elicit an appropriate fear behavior (amygdala-like function).

An alternative explanation is suggested by empirical data showing that the hippocampal function actively suppresses the formation of amygdala-based representations. It has been reported that fornix lesions improve acquisition of a conditioned place preference (CPP) task [74]. The information acquired by the hippocampus during pre-exposure to an environment is specifically involved in this suppression and likely involves the EC-hippocampus connections [39]. Interference with the LEC function could result in a diminution of the active suppression that the hippocampus exerts over the amygdala, enabling the latter to support a strong specific association between the cues directly paired with shock and the fear response. MPP lesions could result in enhancement of this active suppression by freeing the LEC activity and hindering the amygdala-based learning.

Therefore, damage of the LEC input could play a role in the enhancement of context conditioning either actively by preventing recall, or passively by removing the suppression exerted by the hippocampus on the amygdala. The MPP lesion effect could be explained by disruption of the MEC-amygdala interaction or by disinhibition of the LEC suppressive activity.

Segregation of sensory input provides a second possible direction in explaining the context discrimination data. In this experiment, rats were presented with both visual and olfactory cues. The exposure to visual information is well controlled, but the same is not true in the case of odors. Due to the unique characteristics of the olfactory cues, the process of forming the corresponding representations may be more complex. Ambiguity could result from mixture of the molecules in the air or from activation of similar receptors; in either case, the rat would form a single odor representation rather than two independent ones. In agreement with the idea of selective sensory input to the EC, LPP lesions might prevent the olfactory input from reaching the hippocampal circuit. This in turn could result in overall disambiguation of the discrimination task and consequently, enhanced context conditioning. The amygdala-like behavior of the MPP group could be explained as an inability of the olfactory information in directing behavior.

4.5. What can current knowledge of hippocampal physiology contribute to the interpretation of the present behavioral results?

Based on electrophysiological studies, Buzsáki [16] has proposed a two-staged model for the formation of memory traces in the hippocampus. During exploratory behavior, the rhythmical firing of granule cells is recorded as theta waves, while the pyramidal cells of the CA3-CA1 field are relatively silent. At the end of exploration, the synchronized firing of CA3, CA1 and subicular neurons generates the EEG sharp waves (SPW) and the granule cells' activity decreases. According to Buzsáki's model, the rapid firing in the DG produces a transient heterosynaptic potentiation of the CA3 pyramidal cells that provides the neural basis of a fragile memory trace. The decay of this trace is prevented by the SPW bursts because as the CA3 cells engage in repeated synchronous activity, they increase the synaptic efficacy of the autoassociative CA3 network and of the CA3-CA1 connections. According to this theory, formation of enduring memory traces therefore requires both theta and SPW waves.

The SPW bursts entrain the CA3 and CA1 neurons thus generating, through interaction with the inhibitory interneurons [78], a high frequency oscillation (ripple) transmitted to the EC [20]. Buzsáki [16] speculated that from EC the bursts engage cortical networks where information is ultimately stored. It was subsequently found that EC participates in the generation of gamma oscillations which may synchronize the hippocampal and cortical networks [14]. In agreement with the channeled input hypothesis proposed by Amaral and Witter [2], physiological studies indicated that stimulation of the PP results in activation of its origin back in the EC [17] and indicated the presence of reverberations in the EC-CA1/S loops [33] controlled by the inhibitory network of the superficial EC layers [20]. This evidence thus supports the possibility that the MEC/LEC information dissociation is maintained through the hippocampal loop and selectively stored in cortical networks.

Second, the EC-CA1 connections are both mono- and polysynaptic, the latter being represented by EC-DG-CA3-CA1 and EC-CA3-CA1 pathways. The monosynaptic pathway may be the means by which the EC entrains fast oscillations in CA1 [18]. The first polysynaptic pathway is active during exploration, while the second is active at the end of exploration. Integrated with Buzsáki's model, this explains why destruction of the dentate granule cells does not interfere with the activity of the CA1 place cells, but disrupts spatial learning [44] and why spatial learning does not occur in the absence of active movement within a novel environment [39,40,74]. Jones [34] summarized evidence indicating that the EC-DG circuit is active

only in the presence of fast activity in the PP, thus supporting the filter role proposed for DG by Treves and Rolls [72] and agreeing with the revised version of the configural association theory [62] which postulates that the hippocampus increases the salience of relevant cues. Additionally, Moser [48] demonstrated that the inhibition on the granule cells of the DG is lowered during spatial learning and exploration. All this evidence points towards an essential role of the DG in place learning.

In conclusion, it seems that EC activation of the DG is more likely to happen during spatial learning or related behaviors, is necessary for learning to occur, and generates activity that could modulate the function of the parallel EC-CA1/ S loops and the related cortical networks. We can explain the results of the water task as a consequence of the different characteristics of MPP and LPP functionality. Because MPP is more apt than LPP in activating the DG neurons [1,43], it is likely that this pathway is predominantly responsible for processes leading to place learning. Subsequently, MPP lesions result in spatial learning deficits, while LPP lesions have little or no effect. While there is no direct evidence that we are aware of concerning dissociated sensitivity of MEC and LEC neurons to spatial information, one study by Quirk et al. [59] showed that the MEC cells have similar location sensitivity as the hippocampal place cells, although their activity is less specific and more sensory driven.

4.6. Methodological implications

While the invisible platform test is widely used for assessment of hippocampal function, less agreement exists regarding behavioral tests using context conditioning. The data obtained in this study emphasizes the importance of two-chamber designs in which the presence of the unpaired chamber allows dissociation of specific context conditioning from conditioning to other factors. While a one-chamber design similar to the one used by Phillips and LeDoux [58] does permit to evaluate whether the animal remembers a significant event, it does not allow to specify what triggers the remembrance: the removal from the animal colony or housing cage, the experimenter's handling, or indeed the combination of cues referred to as 'context' in the literature.

The lack of preference for a chamber during pre-exposure combined with the clear preference of the unpaired chamber after shock administration demonstrates that this experimental design is unbiased: any behavioral modifications are due to the experimental manipulation and not to other factors. We also consider the two chamber procedure to be more appropriate than using passive or active avoidance by placing the animal in one chamber [76,77] because this procedure would not require the animal to make its own choice. A simple preference test [63] also seems to result in an incomplete picture because in our study although all groups dissociated between the two chambers, the freezing and defecation data revealed important differences.

4.7. Theoretical implications

Despite a large number of studies investigating the functional significance of the hippocampal formation, there is still controversy in the literature regarding the theoretical interpretations of the data. O'Keefe and Nadel [53] postulated that the hippocampus is responsible for the formation of a cognitive map that enables the organism to navigate through the environment. In agreement with a large body of evidence, the present experiment indicates that selective disruption of MEC, but not LEC hippocampal input affects place learning. The results of the discriminative fear conditioning to context task however cannot be explained by this theory and indicate that the hippocampal function is not uniquely relevant to spatial behavior.

A different standpoint that can explain the pattern of results presently reported is represented by the so-called relational or configural theories [23,61] which postulate that the function of the hippocampus is necessary for the acquisition of significant relationships among initially neutral cues. Such a function would indeed be relevant not only for successful navigation in the invisible platform task, but also in the identification of the safe chamber in the context conditioning paradigm.

4.8. Conclusion

The data presently reported indicate that (a) the anatomical, physiological, and neuropharmacological differences between MPP and LPP bear relevance to behavior, (b) the MEC but not LEC conveys information essential for spatial navigation, and (c) LEC function seems to have a suppressive effect on context discrimination. The current approach in investigating the behavioral contributions of specific hippocampal input might be useful in understanding the relevance of different medial temporal lobe areas to learning and memory processes.

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