

Functional interaction between the associative parietal cortex and hippocampal place cell firing in the rat

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Abstract

The hippocampus and associative parietal cortex (APC) both contribute to spatial memory but the nature of their functional interaction remains unknown. To address this issue, we investigated the effects of APC lesions on hippocampal place cell firing in freely moving rats. Place cells were recorded from APC-lesioned and control rats as they performed a pellet-chasing task in a circular arena containing three object cues. During successive recording sessions, cue manipulations including object rotation in the absence of the rat and object removal in the presence of the rat were made to examine the control exerted by the objects or by non-visual intramaze cues on place field location, respectively. Object rotations resulted in equivalent field rotation for all cells in control rats. In contrast, a fraction of place fields in APC-lesioned rats did not rotate but remained stable relative to the room. Object removal produced different effects in APC-lesioned and control rats. In control rats, most place fields remained stable relative to the previous object rotation session, indicating that they were anchored to olfactory and/or idiothetic cues. In APC-lesioned rats, a majority of place fields shifted back to their initial, standard location, thus suggesting that they relied on uncontrolled background cues to maintain place field stability. These results provide strong evidence that the hippocampus and the APC cooperate in the formation of spatial memory and suggest that the APC is involved in elaboration of a hippocampal map based on proximal landmarks.

Introduction

Recent studies on the neural basis of spatial behaviors have shown that the processing of spatial information requires the interaction of a large number of brain structures (e.g. Aggleton *et al.*, 2000). Thus, the behavioral deficits observed after damage to different brain regions allow the identification of some of the structures involved in spatial perception, cognition and action. However, such an approach does not directly address the issue of the interplay between these structures. Among these putative interactions, the functional relationships between the associative parietal cortex (APC) and hippocampus have raised a great deal of interest (McNaughton *et al.*, 1989; Arbib, 1997; Save & Poucet, 2000b). The hypothesis that the APC is involved in spatial perception and cognition is supported by human lesion data (Jeannerod, 1985; Vallar, 1997), single-unit recording in monkeys (Lynch, 1980; Steinmetz, 1998) and rat lesion data. Confirming earlier work by Krieg (1946), a number of neuroanatomical studies allowed delimitation of the APC in the rat, a region distinct from purely visual or somatosensory areas, on the basis of cytoarchitectonic organization, thalamo-cortical and cortico-cortical connections (Kolb & Walkey, 1987; Reep *et al.*, 1994). Projections from various sensory cortical areas, including visual, somatosensory and auditory cortices, converge on the APC, therefore suggesting that this structure performs multimodal association (Reep *et al.*, 1994). Main outputs include the retrosplenial and parahippocampal cortices which allow the APC to be (indirectly) connected with the hippocampus via the entorhinal cortex (Lavenex & Amaral, 2000). Based on neuroanatomical and

behavioral work, it is now considered that the APC in the rat is analogous, if not homologous, to the posterior parietal cortex of primates (Kolb & Walkey, 1987; Bucci *et al.*, 1999).

Bilateral damage to the APC in rats induces deficits in a range of spatial tasks (Kolb & Walkey, 1987; DiMattia & Kesner, 1988; Save *et al.*, 1992, 2001; Save & Moghaddam, 1996; Save & Poucet, 2000a; Alexinsky, 2001), thus raising the possibility of a close functional interaction with the hippocampus. However, it has never been demonstrated that the APC exerts some influence over hippocampal functioning.

The contribution of the hippocampus to spatial cognition has been shown mainly by lesion and unit recording studies. The dramatic effects of hippocampal lesions on the performance of rats in allocentric spatial tasks have been shown repeatedly (e.g. Morris *et al.*, 1982). In addition, single-cell recording studies have revealed the existence of pyramidal cells in the CA1 and CA3 fields that are characterized by location-specific firing, i.e. these 'place' cells fire in a consistent manner relative to the location of the animal in space (O'Keefe & Dostrovsky, 1971; Muller *et al.*, 1987). It has been demonstrated that place cell activity is controlled by environmental (allothetic) and motion-related (idiothetic) cues (Muller & Kubie, 1987; Gothard *et al.*, 1996a). This suggests that activity is strongly dependent on the processing of sensory information performed upstream by other brain regions. We hypothesize that the APC is part of such a functional network that provides stable sensory information to the hippocampal place cell system.

The main objective of the present study was to provide direct evidence of the interaction between the APC and hippocampus and to examine the nature of the processes requiring such interaction. Accordingly, we recorded hippocampal place cells in rats with

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bilateral parietal cortical lesions. We assumed that, if these two structures cooperate, alteration of the APC should elicit functional modifications of hippocampal place cell firing.

Materials and methods

Subjects

Hippocampal place cells were recorded in eight naive Long-Evans male rats purchased from a commercial supplier (Janvier, Le Genest-St-Isles, France). Upon arrival, they were housed in individual cages (40 cm long \times 26 cm wide \times 16 cm high) with food and water available *ad libitum*. They were kept in a temperature-controlled room (20 ± 2 °C) with a natural light/dark cycle. Before electrode implantation, the animals were submitted to a progressive food deprivation schedule until they reached 85% of their initial *ad libitum* body weight. They were then trained to forage for randomly located 20-mg food pellets in a small arena (pellet-chasing task). The experiments were performed in accordance with the NIH guide for the care and use of laboratory animals (NIH publication no. 86-23, revised 1987), European guidelines (European Community Council Directive, November 24, 1986, 86/609/EEC) and national guidelines (permission no. 13.24 from the Ministère de l'Agriculture et de la Pêche to E.S.).

Electrode implantation and lesions

Surgery was performed at the end of training. Rats were deeply anesthetized by injection of sodium pentobarbital (40 mg/kg i.p.; Sanofi Santé Animal, Libourne, France) preceded by atropine sulfate (0.25 mg/kg, i.p.). Additional injections of ketamine (50 mg/kg i.p.; Imalgène, Merial, France) were occasionally administered to maintain appropriate anesthesia throughout surgery. The rats were placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). A midline incision of the scalp was made and the skin and muscles were carefully retracted to expose the skull. Bilateral lesions of the APC were performed in five rats and were produced by thermocoagulation of the dura, a technique successfully used in previous research to produce lesions limited to the cortical mantle (Baunez *et al.*, 1998; Save *et al.*, 2001). Two windows were opened in the skull to expose the brain at the following coordinates relative to bregma: AP, from -2 to -6 mm and L, from ± 1.5 to ± 5.5 mm (Kolb & Walkey, 1987). Lesions were made by applying the tip (diameter, 0.5 mm) of a calibrated soldering iron (temperature 120 °C) directly on the dura for 0.5 s at each of five points on the cortical surface within the exposed brain area. A piece of sterile gelfoam was placed in the openings.

After lesions were made, we implanted the electrodes in the dorsal hippocampus of the right hemisphere. Electrodes were 25- μ m nichrome wires. A bundle of 10 of these was inserted in a 30-G stainless steel guide cannula that was secured to the central pin of a circular connector (Kubie, 1984). Each wire was connected to a peripheral pin of the connector. Three drive screws with nylon cuffs were attached to the connector by acrylic. When implanted, turning the screws allowed the electrodes and cannula to be moved down in the brain. Miniature screws were placed over the right olfactory bulb, left frontal cortex and left cerebellar hemisphere to anchor the headstage. The guide cannula and electrodes were lowered into the brain at the following coordinates relative to bregma: AP, -3.8 mm, L, -3.0 mm and DV, -1.5 mm (Paxinos & Watson, 1986). The bottom (nylon cuffs) of the three drive screw assembly was then cemented to the skull. The rats were sutured and received an injection of antibiotic (Terramycine, 60 mg/kg, i.m.; Pfizer, Paris, France) and of analgesic

(Tolfedine, 0.06 mg/kg, s.c.; Vetoquinol, Lure, France). Three control rats received the same surgical treatment except that no lesion was made. The animals were allowed to recover for at least 7 days before cell screening started.

At the completion of the experiment, APC-lesioned and sham-operated rats were anaesthetized with a lethal dose of sodium pentobarbital. Just before death, a positive current (15 μ A for 30 s) was passed through one of the microwires to deposit iron that could be visualized after reaction with potassium ferrocyanide (Prussian blue). Rats were transcardially perfused with a 10% formalin solution and the brains were removed and stored for 1 day in a 4% formalin solution with 3% ferrocyanide. They were then stored in a 4% formalin solution. Later, coronal 40- μ m-thick sections were made. Every fifth section was mounted and stained with cresyl violet. The slides were observed under the microscope to determine the lesion extent and electrode placements.

Recording apparatus

The recording apparatus was a grey cylinder 50 cm high and 76 cm in diameter. As in our previous studies (Save *et al.*, 1998; Paz-Villagrán *et al.*, 2002), the cylinder was homogeneously painted grey and was visually isolated from the rest of the laboratory by a concentrically placed cylindrical curtain 250 cm in diameter and height with three possible entrances. The apparatus was lit by indirect light provided by four 25-W bulbs placed on the ceiling in symmetrical positions. During all phases of the study, a radio tuned to an FM station was fixed to the ceiling in a central position relative to the cylinder, producing background noise >70 dB to mask non-controlled directional sounds. Three cue objects similar to those used by Save *et al.* (1998) and Paz-Villagrán *et al.* (2002) were used. The objects differed from each other in color, size, shape and texture. There was a glass bottle (29 cm high), a white plastic cylinder (21 cm high) and a black wooden cone (20 cm high). Their locations were fixed relative to each other. Each object was placed against the wall of the cylinder and their arrangement formed an isosceles triangle (Fig. 1).

The computer and monitoring and recording equipment were located in a room adjacent to the room containing the cylinder.

Recording methods

Starting 1 week after surgery, the activity from each electrode was screened daily while the rats performed the pellet-chasing task in the cylinder. The electrodes were lowered (by 25–50- μ m steps) over a period of several weeks while searching for unit waveforms of sufficient amplitude (>100 μ V, i.e. approx. three times the background noise). Once such a waveform was isolated, successive recording sessions were run (see below). For screening and recording, the animal was connected to a headstage comprising a connector that mated with the electrode connector cemented to the skull, unity gain preamplifiers, one for each electrode, a light-emitting diode for tracking the rat's head position and a multiwire cable to lead the signals to a commutator that allowed the rat to move freely. The fixed side of the commutator was connected to a distribution panel and amplifiers. Signals were amplified 10 000 times, band-pass filtered between 0.3 and 10 kHz and sent to a 250-kHz analog-to-digital board in a PC computer. Using a DataWave acquisition system (DataWave, Longmont, CO, USA), waveforms of identified units were sampled at 32 kHz (1-ms burst of 32 samples each time the voltage exceeded an experimenter-defined threshold) and stored. Before the initial recording session, spike discharges of single units were separated using

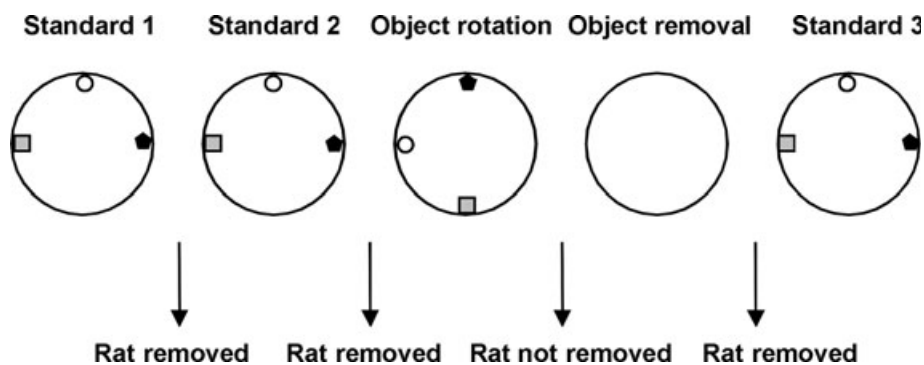


FIG. 1. Schematic representation of the protocol. Five successive 16-min recording sessions were run. Between sessions, the rat was removed from the cylinder and the floor was cleaned, except between Object rotation and Object removal sessions.

online clustering software (Discovery; DataWave) to simplify later offline separation. The rat's head position in the apparatus was obtained by locating a light-emitting diode that was secured ≈ 1 cm above the head. The light-emitting diode was tracked at 50 Hz with a digital spot follower that received RGB signals from a CCD color camera fixed above the apparatus. The light-emitting diode was detected in a grid of 64×64 square regions (pixels) 25 mm per side.

Testing protocol

Selected extracellular signals were recorded during a sequence of five 16-min recording sessions (Fig. 1). In the first session (Standard 1), the object cues were at locations used during screening, allowing for the establishment of initial place field location and characteristics. The second session (Standard 2) was similar to Standard 1 and was performed to check for place field constancy in stable conditions. The third session (Object rotation) was run with the three objects rotated 90° (counterclockwise according to the camera view displayed on the monitor) as a rigid set around the arena center to examine the control which they exerted on place field location. Between the sessions, the rat was disconnected, removed from the arena and placed back in its home cage. In addition, the floor was cleaned with a wet sponge to neutralize olfactory cues. After session 3 (Object rotation), while the animal remained in the apparatus, the three object cues were removed and the floor was not cleaned. The purpose of the fourth session (Object removal) was to examine place field stability in the absence of the object cues. Previous work has shown that place fields remain stable after the objects are removed only if the floor is not cleaned. This suggests a role for olfactory cues associated with movement-related cues in stabilization of spatial representation (Save *et al.*, 2000). A last standard session (Standard 3) was finally run to check for cell-recording stability across sessions.

Data analysis

Behavioral analysis

For each recording session, object exploration was measured by accumulating the time spent by the animal in a 4-pixel diameter area centred on the objects (Save *et al.*, 1998).

Unit analysis

Only well-isolated cells with clear location-specific activity were included in the data set. The first step in offline analyses was to refine boundaries for waveform clusters that were set before recording. Using Discovery (DataWave) software, scatterplots of the most characteristic

waveform parameters (e.g. peak voltage and waveform duration) were generated from the signals emanating from putative units recorded on each channel. Discrimination of candidate waveforms was based on at most eight possible parameters, including minimum and maximum spike voltages, spike duration, spike amplitude (from peak to trough), time of occurrence of minimum and maximum spike voltages, and analog-to-digital values at two selected points of the waveform were used. This ensured that the signals were produced by single cells rather than by several cells with similar waveforms. The cluster boundaries established for the first session were used for subsequent sessions. In addition, only cells with similar spatial discharge properties during initial and final standard sessions (see below) were kept for the analysis so as to ensure that the same cell or set of cells was studied across the whole sequence of environmental manipulations.

Once single units were well separated, the positional firing rate distributions were calculated. The total time that the light was detected (dwell time) and the total number of spikes in each pixel were accumulated for the session duration (16 min). Dividing the total number of spikes by the dwell time in each pixel allowed the construction of a firing rate map for the session to visualize the positional firing distribution (Muller *et al.*, 1987). In such maps, pixels that were not visited by the rat are displayed as white and pixels that were visited, but in which no spike occurred during the session, are displayed as yellow. The highest firing rate is coded as purple and intermediate rates are coded as blue, green, red and orange pixels from high to low. The values used as boundaries between categories were determined for the first (standard) session and applied to subsequent sessions of a recording sequence to allow for comparison between these sessions for a given cell.

A place field was defined as a set of at least nine contiguous pixels with a firing rate above the mean firing rate (i.e. above the total number of spikes divided by the session duration). In addition to the qualitative description of place cell firing provided by examination of the maps, several numerical measures were used to describe the positional firing patterns: (i) in-field mean firing rate was the total number of spikes emitted by the cell while the rat was in the place field divided by the total time spent in the field; (ii) in-field peak firing rate; (iii) spatial coherence was a computed autocorrelation between the rate for each pixel and the average rate of the eight neighboring pixels; it measures the local smoothness of firing rate contours and is a way to quantify the strength of spatial firing for a cell (Muller & Kubie, 1989) and (iv) information content measured the amount of information (in bits) conveyed about spatial location by a single action potential emitted by a single cell (Markus *et al.*, 1994). This was calculated according to the formula: $I = \sum_i (\lambda_i/\lambda) \times \log_2 (\lambda_i/\lambda) \times P_i$ where λ_i is the mean firing rate in each pixel, λ is the overall mean firing rate and

P_i is the probability of the animal being in pixel i (i.e. dwelling time in pixel/total dwelling time). The minimal value of positional information content is 0 for a cell which does not provide any information about location.

To estimate numerically place field stability after environmental manipulations (Object rotation and Object removal), spatial correlations were made between pairs of firing rate arrays. For object rotation, we calculated a pixel-by-pixel cross-correlation as the positional firing pattern for the first session (Object rotation) was rotated in 6° steps relative to the positional firing pattern for the second session (Standard 2). For object removal, we calculated a pixel-by-pixel cross-correlation as the positional firing pattern for the first session (Object removal) was rotated in 6° steps relative to the positional firing pattern for the second session (Object rotation). The angle associated with the highest correlation (R_{Max}) was taken as the rotation angle of the place field between the two sessions. That R_{Max} was found for a $0 \pm 30^\circ$ rotation indicated that the place field was roughly at the same location in the two sessions. For Object rotation, the field was considered to rotate with the objects if the angle associated with R_{Max} was $90 \pm 30^\circ$ (the objects were rotated 90°). To normalize the distribution, each

R_{Max} value was transformed into a Z_{Max} score. These Z_{Max} scores were used for calculation of the means and SEs and for statistical analyses.

Results

Histology

The extent of the APC lesion is shown in Fig. 2. Figure 2A represents a view of a rat brain from above when the damaged areas in all five rats are superimposed. Lesions were consistently placed from one animal to the other. Figure 2B shows the maximal and minimal extent of parietal lesions at five coronal levels (adapted from Paxinos & Watson, 1986). The neocortical area described as 'area 7' by Krieg (1946), Kolb & Walkey (1987), Reep *et al.* (1994) and assumed to be an associative area was damaged. No damage to the underlying hippocampus was observed in any of the brains (Fig. 2C). Fiber bundles, such as the corpus callosum, cingulum and alveus, were spared. The external capsule was generally damaged. However, damage was limited to its dorsal part and with a maximal antero-posterior extent of 2.5 mm within the lesion site. At this level, the

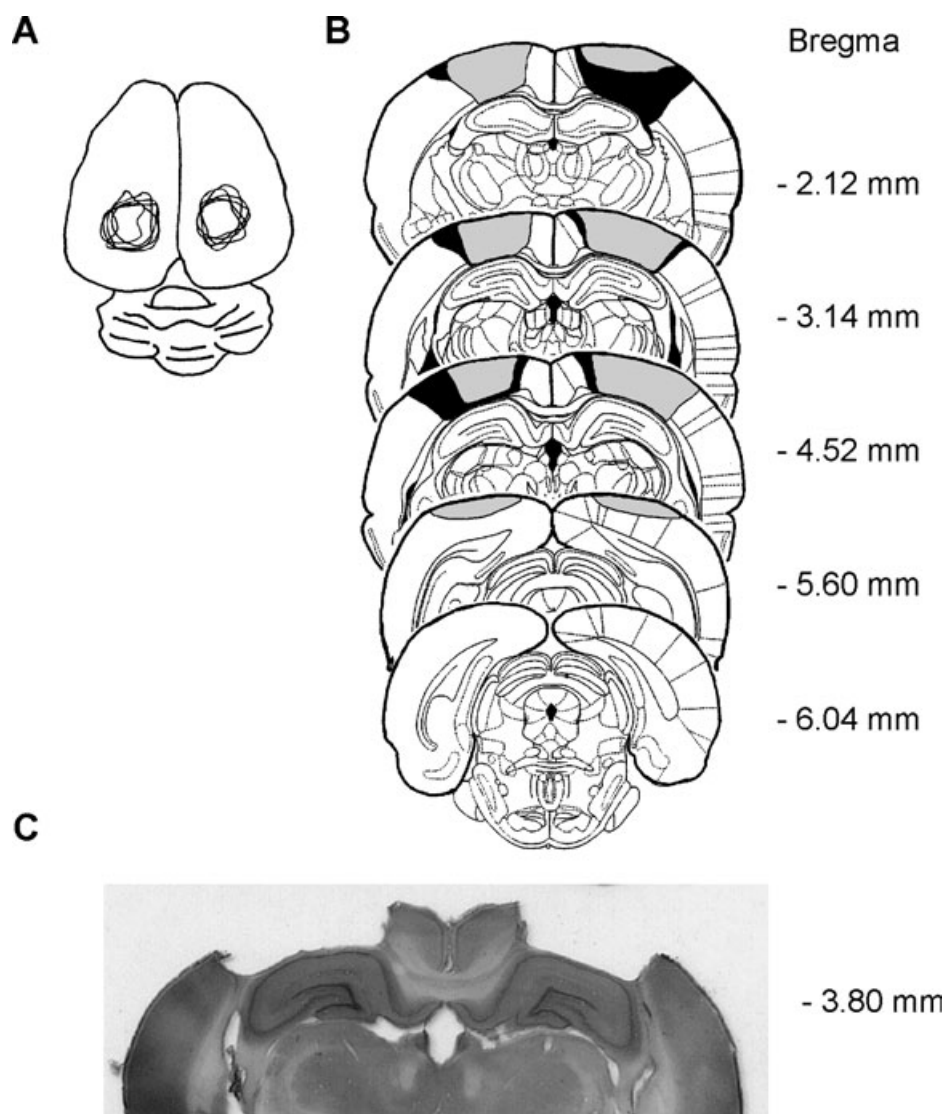


FIG. 2. (A) Top view of a brain with parietal lesions in all five rats superimposed. (B) Reconstruction of parietal lesions on coronal sections of a brain. Maximal (black) and minimal (grey) extent of the lesions is displayed at five levels relative to Bregma. (C) Photomicrograph of a coronal section showing associative parietal cortex lesions at -3.80 mm relative to Bregma.

external capsule contains fibers originating from the neocortex (in particular the parietal areas) that interconnect contralateral cortical areas via the corpus callosum (Jacobson, 1965). They also project to thalamic nuclei (Lashley, 1941) and to various subcortical targets. Thus, damaging the external capsule probably disrupted the inputs and outputs of the APC.

Behavior

Comparison of object exploration averaged over the four sessions with objects in control and parietal rats did not yield a significant difference (mean duration of exploration: control rats, 48.0 ± 2.57 s; parietal rats, 44.3 ± 2.03 s, $t_{36} = 1.3$, NS), thus showing that the two groups displayed similar exploratory activity.

Basic firing properties of place cells in associative parietal cortex-lesioned rats

A total of 92 CA1 pyramidal cells with large amplitude waveforms (background noise level < 30 μ V), recorded in 39 complete session sequences, were included in the study for analysis. As the testing protocol required that the same cells were recorded across successive sessions, data from unreliable cells were discarded. Included in this category are cells whose waveforms changed so much in shape or amplitude that we could not be confident that they were the same unit. We were very conservative about analysing only well-separated waveforms; the same settings for waveform clustering were used throughout the session sequence. In this way, it is very unlikely that similar waveforms from two cells recorded across successive sessions were erroneously assigned to a single cell that changed its firing pattern. In addition, that the same cells were recorded across a sequence of sessions was supported by a high similarity, measured by the session-to-session correlation, between the first standard and last (session 5) standard sessions (parietal rats, Z_{Max} , 0.606 ± 0.07 , mean angle, $1.24 \pm 1.72^\circ$; control rats, Z_{Max} , 0.548 ± 0.04 , mean angle, $1.16 \pm 0.92^\circ$).

All accepted cells were characterized by stable place fields across standard sessions (1 and 2). Fifty-six cells recorded in 15 sequences were obtained from three control rats and 36 cells recorded in 23 sequences were obtained from five parietal rats. The lower number of cells recorded in parietal rats was due to the fact that these rats lost their headstage sooner than control rats. This occurred because the surface of the skull supporting the headstage was much smaller in parietal rats as a consequence of the opening of the two bone windows for parietal lesions. In only a small number of sequences were ensembles of more than two cells recorded simultaneously (parietal rats, two sequences; control rats, 10 sequences). Such low numbers did not allow us to run analyses of the coherence of individual cell responses (discordant or concordant) within cell ensembles after cue manipulation.

Table 1 shows the basic firing properties of place cells in parietal and control rats averaged over the first two standard sessions. Two-

tailed *t*-tests for independent samples revealed that there was no difference between the two groups for all parameters except field size (in-field mean firing rate, t_{90} , 1.66, NS; in-field peak firing rate, t_{90} , 1.21, NS; coherence, t_{90} , 0.887, NS; information content, t_{90} , 0.662, NS). Place fields were larger in parietal rats than in control rats (t_{90} , 3.39, $P < 0.01$).

Effects of object manipulations

Object rotation

Figure 3 shows the distribution of rotation angles (i.e. associated with R_{Max}) for all cells in control and parietal rats. In control rats, the great majority of rotation angles were clearly clustered around the 90° position indicating an accurate control of place field location by the objects. This result, consistent with previous work (Cressant *et al.*, 1997; Save *et al.*, 1998), shows that three object cues placed at the periphery of the cylinder can ideally control place cell activity. In contrast, the distribution of angles was slightly different in parietal rats. This was confirmed by a *t*-test showing that the rotation angles were different in control and parietal rats (control rats, mean Z_{Max} , 0.539 ± 0.031 , mean angle, $93.29 \pm 1.26^\circ$; parietal rats, mean Z_{Max} , 0.679 ± 0.06 , mean angle, $67.97 \pm 6.10^\circ$, $t_{90} = 4.94$, $P < 0.001$). This difference was due to eight cells (out of 36 cells recorded in parietal rats) whose fields did not rotate with the objects but remained stable relative to the room frame of reference (rotation angle close to 0°) (Fig. 3). These eight cells were recorded in three sequences and in three different rats showing that the response was not idiosyncratic. Note that the stable place fields in parietal rats remained at their initial location after object removal (session 4). This suggests that place cell activity was not controlled in these cases by the intramaze object cues but by some uncontrolled background cues. Thus, place cells with stable place fields during Object rotation were discarded from the analysis of Object removal session. Table 2 shows the Z_{Max} and rotation angles between Object rotation and Standard 2 firing rate maps for both rotated and stable fields. Z_{Max} was greater for stable than for rotated fields (t_{34} , -2.58 , $P < 0.05$). This difference may be due to the fact that stable fields had greater information content than fields that did rotated ones (rotated fields, information content, 1.340 ± 0.098 ; stable fields, information content, 2.165 ± 0.270 , t_{34} , -3.52 , $P < 0.01$). An alternative explanation is that rotation of the fields resulted in subtle modifications of their shape, thereby decreasing the pixel-by-pixel correlation.

Object removal

After Object rotation, the rat was left in the arena and the three object cues were removed. In order to maintain place field stability, the animal could rely on idiothetic cues and/or self-deposited olfactory cues as the floor was not cleaned (Quirk *et al.*, 1990; Save *et al.*, 2000). First, we found that nine of 56 cells in control rats and one of 28 cells in parietal rats stopped firing (see Save *et al.*, 2000 for similar results). Note that, for all these cells, the field reappeared in the last standard session. No correlation could be computed for these cells.

TABLE 1. Firing parameters of place cells in control and parietal rats

	Number of cells	In-field mean firing rate (Hz)	In-field peak firing rate (Hz)	Coherence	Information content	Field size (pixels)
Control rats	56	2.57 ± 0.25	5.88 ± 0.71	0.64 ± 0.02	1.56 ± 0.08	$54.05 \pm 3.69^*$
Parietal rats	36	3.24 ± 0.32	7.23 ± 0.85	0.66 ± 0.02	1.47 ± 0.11	77.15 ± 6.27

Data are presented as means \pm SEM. * $P < 0.01$, comparing control and parietal rats (*t*-test for independent samples).

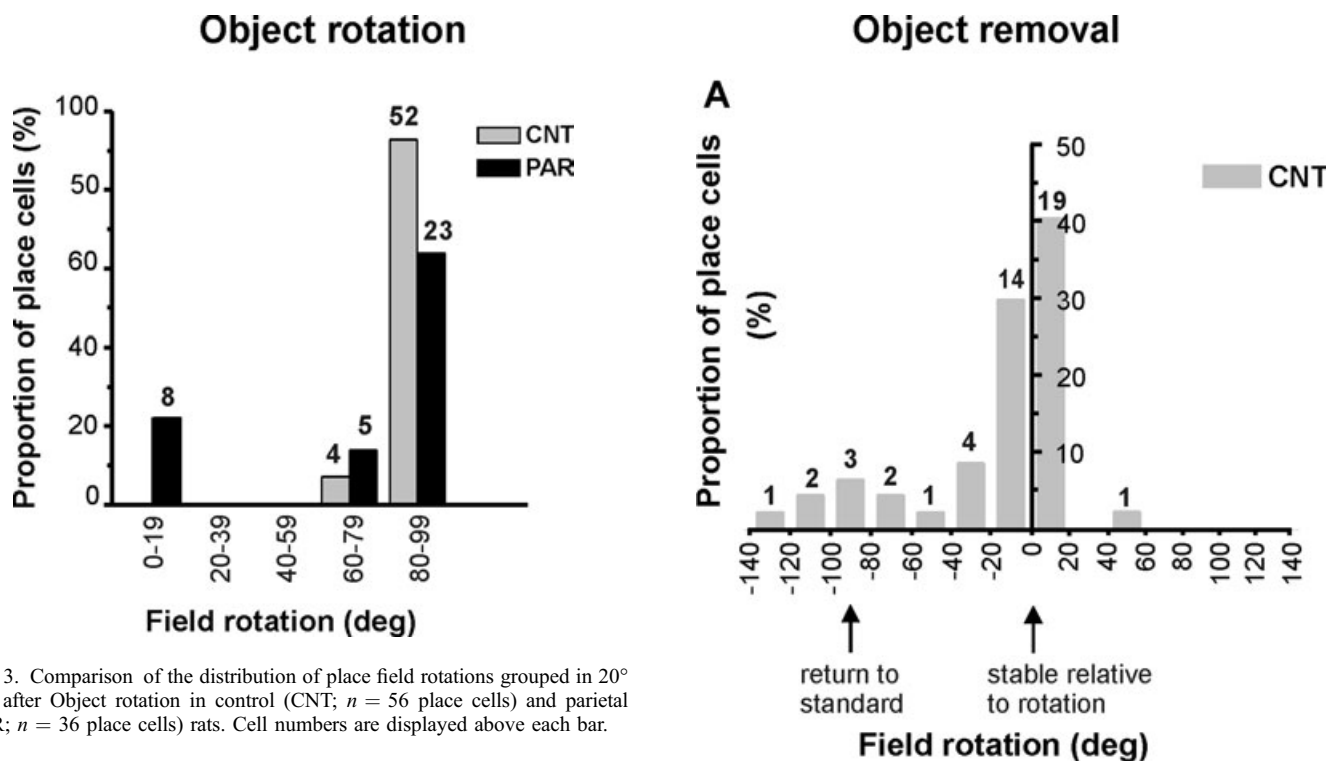


FIG. 3. Comparison of the distribution of place field rotations grouped in 20° bins after Object rotation in control (CNT; $n = 56$ place cells) and parietal (PAR; $n = 36$ place cells) rats. Cell numbers are displayed above each bar.

TABLE 2. Correlation between Object rotation session and Standard 2 session firing rate maps

Object rotation	Cells (n)	Z_{Max}	Rotation angle (°)
Control rats			
Rotated fields	56	0.539 ± 0.031	93.3 ± 1.3
Parietal rats			
Rotated fields	28	0.602 ± 0.053	86.6 ± 2.0
Stable fields	8	$0.949 \pm 0.141^*$	2.6 ± 1.9

Data are presented as means \pm SEM. * $P < 0.05$, comparing stable and rotated fields in parietal rats.

Second, considering the cells that still fired after object removal (control rat, $n = 47$; parietal rats, $n = 27$), we showed that the distribution of the rotation angles was different in control and parietal rats (control rats, mean Z_{Max} , 0.434 ± 0.040 , mean angle, $-15.53 \pm 5.66^\circ$; parietal rats, mean Z_{Max} , 0.455 ± 0.044 , mean angle, $-64.23 \pm 6.22^\circ$; comparison of the angles, t_{72} , -4.83 , $P < 0.001$) (Fig. 4). This suggested that the two groups exhibited different responses to object removal. In control rats, the great majority of the place fields (37 of 47) remained stable relative to the rotation session after object removal (Fig. 4A). The remaining fields (nine cells) exhibited various rotating angles ranging between -40° and -120° . One field exhibited a positive rotation angle. In contrast, the distribution of rotation angles was different in parietal rats (Fig. 4B). For a number of cells (17 of 27 cells), the fields were found to rotate -90° (in the -120° to -60° range; mean Z_{Max} , 0.396 ± 0.054 , mean angle, $-83.90 \pm 15.40^\circ$), thus returning to their initial location as in the standard session. This was confirmed by additional correlations made between the Object removal and the Standard 2 sessions (mean Z_{Max} , 0.556 ± 0.070 , mean angle, $2.4 \pm 1.80^\circ$), thus showing that place field locations were similar in the two sessions. A few cells had place fields (nine of 27) that rotated in the -20° to -60° range (Fig. 4). In only one cell was the field found

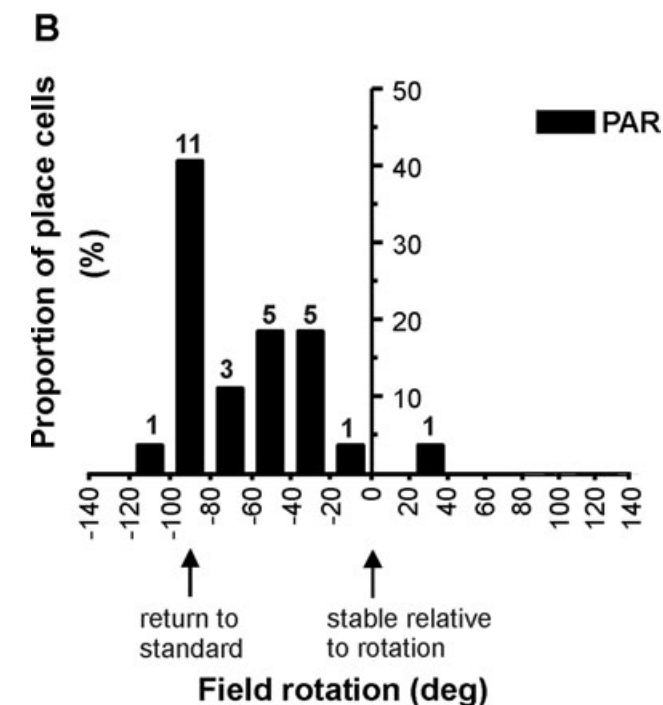


FIG. 4. Distribution of place field rotations grouped in 20° bins after Object removal in (A) control (CNT; $n = 47$ place cells) and (B) parietal (PAR; $n = 27$ place cells) rats. Cell numbers are displayed above each bar.

to rotate in the $0 \pm 20^\circ$ range and thus remained stable relative to the rotation session.

Examples of different place cell responses to Object removal in control and parietal rats are displayed in Fig. 5.

Overall, the results of Object removal suggest that most place fields in control rats remained at the same location as during the immediately

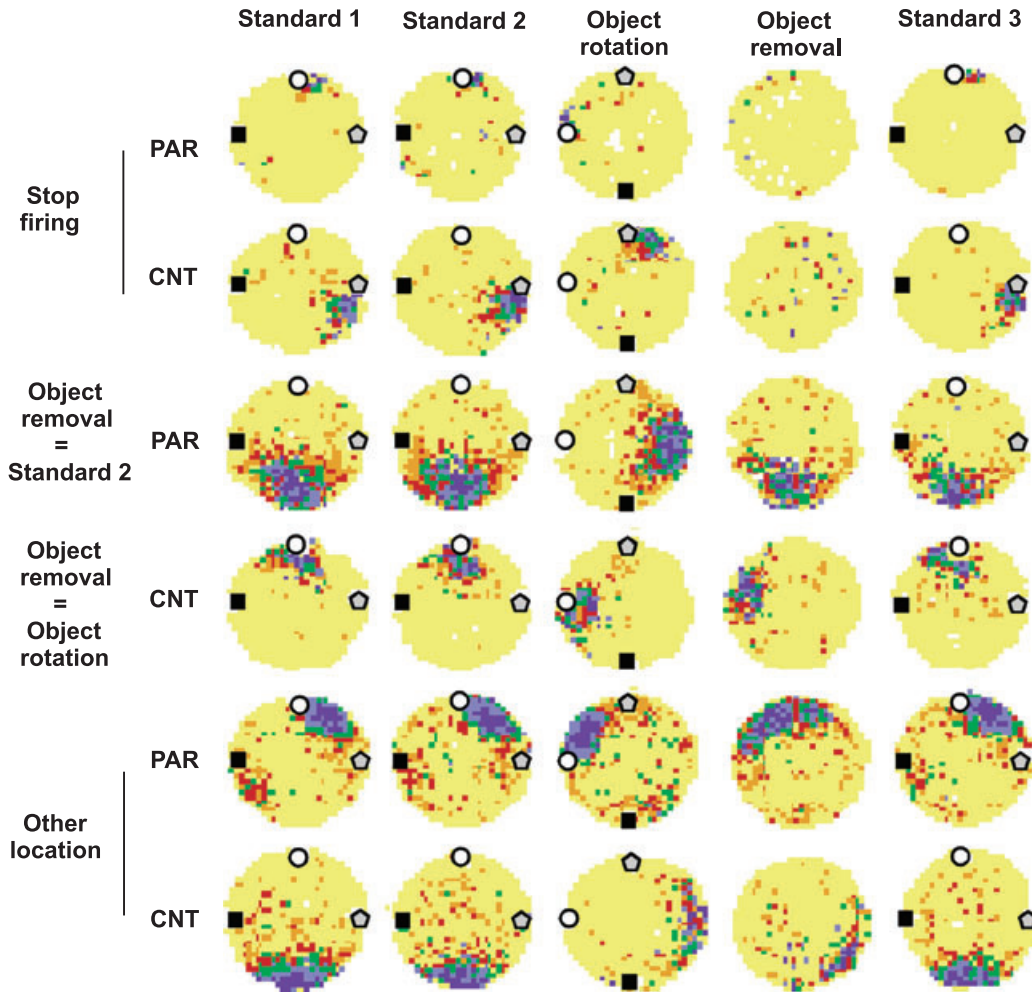


FIG. 5. Examples of firing rate maps in control and parietal rats in a recording sequence. Object removal resulted in different responses. In the two groups, a number of cells stopped firing. In control (CNT) rats, most place fields remained stable relative to the rotation session (Object removal = Object rotation). Note that only one cell in parietal (PAR) rats was found to display such a response. In parietal rats, most place fields shifted back to their initial location (Object removal = Standard 2). Other responses included intermediate placement of the fields.

preceding Object rotation session whereas, in parietal rats, a majority of cells had place fields that shifted back to the initial location seen during session Standard 2.

Discussion

In this study, we have shown that damaging the APC modified hippocampal place cell activity. This demonstrates that the APC and hippocampus are functionally related and that the APC contributes fully to spatial mapping.

Although the basic firing characteristics of place cells in parietal rats were mostly unaffected, the results indicate that cue manipulations, namely object rotation and object removal, produced different effects in parietal and control rats. More specifically, the object cues were found to exert less control on the location of place fields in parietal than in control rats. First, only in parietal rats were some fields observed to remain stable relative to the room reference frame after object rotation. Second, among those fields that rotated, most switched back to their initial location after object removal, suggesting a realignment of the representation relative to the room frame. In control rats, only a minority of cells displayed such a response. These results

suggest that parietal rats were able to rely, to some extent, on uncontrolled background cues to maintain a stable representation of space after object removal. These cues may possibly be auditory (remote ultrasounds) or, more likely, low spatial visual frequencies such as shades in the curtain (Zugaro *et al.*, 2001).

Previous work has shown that, when the visual cue is removed in the presence of the animal, place fields remain stable as long as olfactory cues are still available (Save *et al.*, 2000). Thus, in the absence of visual information, rats may use the immediate memory of object cue location and a combination of olfactory and idiothetic cues to maintain stable place fields. That a large fraction of place fields remained stable after object removal in control rats during this experiment is consistent with the results of Save *et al.* (2000) and suggests that the animals relied on non-visual information to anchor place field stability. In parietal rats, a failure to maintain field stability after object removal may be a consequence of a deficit in using idiothetic cues (Save & Moghaddam, 1996; Save *et al.*, 2001). Another hypothesis is that parietal rats had a memory deficit of the object cue location. However, the observation that most place fields were realigned relative to the room frame cannot simply be accounted for by these two hypotheses. Such a realignment can only be explained by the capability of rats to use room cues to anchor place fields.

Overall, these results suggest that the main effect of APC lesions is an alteration of the balance between the use of distal cues (room frame) and proximal cues (arena frame). This effect appears paradoxical because the room frame was devoid of large, experimenter-controlled cues whereas the arena frame contained three conspicuous objects, different in contrast, size and texture. This difference in cue saliency may explain how a large fraction of place fields was nonetheless controlled by the object cues after object rotation in parietal rats. However, when the objects were removed from the arena, the discrepancy in cue saliency was abolished, thus resulting in rats realigning their hippocampal map with the room frame. Interestingly, the capability of rats to neglect nearby cues to the benefit of uncontrolled background cues has recently been reported by Zugaro *et al.* (2001) for head direction cells.

Our hypothesis is that parietal rats used uncontrolled background cues because they had difficulties in using the object cues to form a map and to navigate. This hypothesis is consistent with recent lesion work showing that rats with APC damage had a place-learning deficit in the Morris water maze task when they had to rely on object cues placed directly in the pool. In contrast, no deficit was found when the animals had to use room cues (Save & Poucet, 2000a). Parietal rats also exhibited a deficit in establishing a representation based on an arrangement of proximal objects during exploration (Save *et al.*, 1992). Thus, there is converging evidence that the APC plays an important role in the formation of a hippocampal spatial map based on proximal objects.

Hypothetically, the contribution of the APC to the balance between proximal and distal cue processing may depend on the importance of these cues relative to the rat's behavior. This possibility, however, was not bolstered by the results of a recent lesion study (Parron *et al.*, 2004) which revealed that rats with APC lesions were impaired in place navigation based on the use of proximal objects while still being able to use distal cues. Thus, the APC seems to be required for processing proximal objects irrespective of the task.

Together with previous data, the present results support the hypothesis that map-based navigation depends, at least, on two processing systems that allow formation of spatial representations. One system is devoted to the processing of proximal landmarks and the other to the processing of distal landmarks. This hypothesis is akin to the parallel map theory recently proposed by Jacobs & Schenk (2003). The model postulates that the hippocampus contains an integrated map of the environment which results from simultaneous activation of two maps, the bearing and sketch maps. The bearing map is constructed from directional landmarks, i.e. distal landmarks, and idiothetic cues and the sketch map is constructed from positional landmarks, i.e. proximal landmarks. Positional landmarks are typically objects that are located in the animal's movement space, which was the case in the present study. Thus, our results suggest that the APC contributes to the formation of a map based on an arrangement of proximal landmarks (i.e. a sketch map) but is relatively less involved in the formation of a bearing map. The latter would require activation of other brain regions such as the entorhinal cortex (Parron *et al.*, 2004). A dorsal hippocampal lesion was found to disrupt the use of both proximal and distal landmarks (Save & Poucet, 2000a), thus indicating that the two systems, relatively independent at the neocortical level, eventually converge on the hippocampus. Interestingly, a recent report from the Mosers' group revealed that an allocentric spatial representation is already formed in the entorhinal cortex (Fyhn *et al.*, 2004). Thus, convergence of the two processing systems may even occur upstream of the hippocampus. However, how the hippocampal and entorhinal cortical representations contribute to spatial memory remains poorly understood. Following our results, we

propose that the hippocampus would play a role in managing the proximal and distal reference frames. Supporting evidence comes from several studies showing that place cells are able to use spatial reference frames based on proximal and distal cues to anchor their location-specific activity (Gothard *et al.*, 1996b; Bures *et al.*, 1997; Tanila *et al.*, 1997; Brown & Skaggs, 2002; Knierim, 2002). These reference frames usually coincide and provide redundant information as to the animal's location, unless they provide conflicting information.

The evidence of a functional interaction between the APC and hippocampus raises the issue of the circuits that underlie their cross-talk. Indeed, these two structures are not directly connected. Various possible pathways may convey information processed by the APC to the hippocampus including direct projections to the entorhinal cortex or more indirect projections through the retrosplenial or postrhinal cortices (Reep *et al.*, 1994; Burwell & Amaral, 1998). However, whichever pathways are activated, it is important to emphasize that functional interactions between the APC and hippocampus involve the participation of other brain regions. Interestingly, a number of recent studies have revealed that these regions exert some influence on place cell activity (retrosplenial cortex, Cooper & Mizumori, 2001; entorhinal cortex, Brun *et al.*, 2003) or contain neurons with location-specific firing (postrhinal cortex, Burwell & Hafeman, 2003). This suggests that a network of cortical areas contributes to formation of a hippocampal spatial map. The role of each cortical region in the processing of the specific reference frame remains to be investigated.

Overall, our results demonstrate that, even if the APC is not directly connected to the hippocampus, it plays an important role in establishing the functional properties of the hippocampal place cell system. In particular, the APC may be involved in the processing of spatial information based on the use of proximal landmarks. It is suggested from a number of results that hippocampal place cell firing results from an integration of two processes, one devoted to the processing of a reference frame based on distal landmarks and the other to the processing of a reference frame based on proximal landmarks.

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Abbreviations

APC, associative parietal cortex.

References

- Aggleton, J.P., Vann, S.D., Oswald, C.J.P. & Good, M. (2000) Identifying cortical inputs that subservise allocentric spatial processes: a simple problem with a complex answer. *Hippocampus*, **10**, 466–474.
- Alexinsky, T. (2001) Differential effect of thalamic and cortical lesions on memory systems in the rat. *Behav. Brain Res.*, **122**, 175–191.
- Arbib, M. (1997) From visual affordances in monkey parietal cortex to hippocampo-parietal interactions underlying rat navigation. *Phil. Trans. R. Soc. London B*, **352**, 1429–1436.
- Baunez, C., Salin, P., Nieoullon, A. & Amalric, M. (1998) Impaired performance in a conditioned reaction time task after thermocoagulatory lesions of the fronto-parietal cortex in rats. *Cereb. Cortex*, **8**, 301–309.
- Brown, J.E. & Skaggs, W.E. (2002) Concordant and discordant coding of spatial location in populations of hippocampal CA1 pyramidal cells. *J. Neurophysiol.*, **88**, 1605–1613.

- Brun, V.H., Leutgeb, S., Wu, H., Schwarcz, R., Witter, M.P., Moser, M.B. & Moser, E.I. (2003) Place representation in CA1 after selective lesions of entorhinal cortex layer III. *Soc. Neurosci. Abstr.*, Online, 91.4.
- Bucci, D.J., Conley, M. & Gallagher, M. (1999) Thalamic and basal forebrain cholinergic connections of the rat posterior parietal cortex. *Neuroreport*, **10**, 941–945.
- Bures, J., Fenton, A.A., Kaminsky, Y. & Zinyuk, L. (1997) Place cells and place navigation. *Proc. Natl Acad. Sci.*, **94**, 343–350.
- Burwell, R.D. & Amaral, D.G. (1998) Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *J. Comp. Neurol.*, **398**, 1–27.
- Burwell, R.D. & Hafeman, D.M. (2003) Positional firing properties of postrhinal cortex neurons. *Neuroscience*, **119**, 577–588.
- Cooper, B.G. & Mizumori, S.J.Y. (2001) Temporary inactivation of the retrosplenial cortex causes a transient reorganization of spatial coding in the hippocampus. *J. Neurosci.*, **21**, 3986–4001.
- Cressant, A., Muller, R.U. & Poucet, B. (1997) Failure of centrally placed objects to control the firing fields of hippocampal place cells. *J. Neurosci.*, **17**, 2532–2542.
- DiMattia, B.V. & Kesner, R.P. (1988) Spatial cognitive maps: differential role of parietal cortex and hippocampal formation. *Behav. Neurosci.*, **102**, 471–480.
- Fyhn, M., Molden, S., Witter, M.P., Moser, E.I. & Moser, M.B. (2004) Spatial representation in the entorhinal cortex. *Science*, **305**, 1258–1264.
- Gothard, M.G., Skaggs, W.E., Moore, K.M. & McNaughton, B.L. (1996a) Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task. *J. Neurosci.*, **16**, 823–835.
- Gothard, M.G., Skaggs, W.E. & McNaughton, B.L. (1996b) Dynamics of mismatch correction in the hippocampal ensemble code for space: interaction between path integration and environmental cues. *J. Neurosci.*, **16**, 8027–8040.
- Jacobs, L.F. & Schenk, F. (2003) Unpacking the cognitive map: the parallel map theory of hippocampal function. *Psychol. Rev.*, **110**, 285–315.
- Jacobson, S. (1965) Intralaminar, interlaminar, callosal and thalamocortical connections in frontal and parietal areas of the albino rat cerebral cortex. *J. Comp. Neurol.*, **124**, 131–146.
- Jeannerod, M. (1985) The posterior parietal area as a spatial generator. In Ingle, D.J., Jeannerod, M. & Lee, D.N. (Eds), *Brain Mechanisms and Spatial Vision*. NATO ASI Series. Martinus-Nijhoff Publishers, Dordrecht, Netherlands. pp. 279–298.
- Knierim, J.J. (2002) Dynamic interactions between local surface cues, distal landmarks and intrinsic circuitry in hippocampal place cells. *J. Neurosci.*, **22**, 6254–6264.
- Kolb, B. & Walkey, J. (1987) Behavioral and anatomical studies of the posterior parietal cortex in the rat. *Behav. Brain Res.*, **23**, 127–145.
- Krieg, W.J.S. (1946) Connections of the cerebral cortex. I. The albino rat. A. Topography of the cortical areas. *J. Comp. Neurol.*, **84**, 221–275.
- Kubie, J.L. (1984) A drivable bundle of microwires for collecting single-unit data from freely moving rats. *Physiol. Behav.*, **32**, 115–118.
- Lashley, K.S. (1941) Thalamo-cortical connections of the rat's brain. *J. Comp. Neurol.*, **75**, 67–121.
- Lavenex, P. & Amaral, D.G. (2000) Hippocampal–neocortical interaction: a hierarchy of associativity. *Hippocampus*, **10**, 420–430.
- Lynch, J.C. (1980) The functional organization of posterior parietal association cortex. *Behav. Brain Sci.*, **3**, 485–534.
- Markus, E.J., Barnes, C.A., McNaughton, B.L., Gladden, V.L. & Skaggs, W.E. (1994) Spatial information content and reliability of hippocampal neurons: effects of visual input. *Hippocampus*, **4**, 410–421.
- McNaughton, B.L., Leonard, B. & Chen, L. (1989) Cortical–hippocampal interactions and cognitive mapping: a hypothesis based on reintegration of the parietal and inferotemporal pathways for visual processing. *Psychobiology*, **17**, 236–246.
- Morris, R.G.M., Garrud, P., Rawlins, J.N.P. & O'Keefe, J. (1982) Place navigation impaired in rats with hippocampal lesions. *Nature*, **297**, 681–683.
- Muller, R.U. & Kubie, J.L. (1987) The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *J. Neurosci.*, **7**, 1951–1968.
- Muller, R.U. & Kubie, J.L. (1989) The firing of hippocampal place cells predicts the future position of freely moving rat. *J. Neurosci.*, **9**, 4101–4110.
- Muller, R.U., Kubie, J.L. & Ranck, J.B. (1987) Spatial firing pattern of hippocampal complex-spike cells in a fixed environment. *J. Neurosci.*, **7**, 1935–1950.
- O'Keefe, J. & Dostrovsky, J. (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely moving rat. *Brain Res.*, **34**, 171–175.
- Parron, C., Poucet, B. & Save, E. (2004) Entorhinal cortex lesions impairs the use of distal but not proximal landmarks during navigation in the rat. *Behav. Brain Res.*, **154**, 345–352.
- Paxinos, G. & Watson, C. (1986) *The Brain in Stereotaxic Coordinates*. Academic Press, New York.
- Paz-Villagrán, V., Lenck-Santini, P.-P., Save, E. & Poucet, B. (2002) Properties of place cell firing after damage to the visual cortex. *Eur. J. Neurosci.*, **16**, 771–776.
- Quirk, G.J., Muller, R.U. & Kubie, J.L. (1990) The firing of hippocampal place cells in the dark depends on the rat's recent experience. *J. Neurosci.*, **10**, 2008–2017.
- Reep, R.L., Chandler, H.C., King, V. & Corwin, J.V. (1994) Rat posterior parietal cortex: topography of corticocortical and thalamic connections. *Exp. Brain Res.*, **100**, 67–84.
- Save, E. & Moghaddam, M. (1996) Effects of lesions of the associative parietal cortex in the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat. *Behav. Neurosci.*, **110**, 74–85.
- Save, E. & Poucet, B. (2000a) Involvement of the hippocampus and associative parietal cortex in the use of proximal and distal landmarks for navigation. *Behav. Brain Res.*, **109**, 195–206.
- Save, E. & Poucet, B. (2000b) Hippocampal–parietal cortical interactions in spatial cognition. *Hippocampus*, **10**, 491–499.
- Save, E., Poucet, B., Foreman, N. & Buhot, M.C. (1992) Object exploration and reactions to spatial and non-spatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behav. Neurosci.*, **106**, 447–456.
- Save, E., Cressant, A., Thinus-Blanc, C. & Poucet, B. (1998) Spatial firing of hippocampal place cells in blind rats. *J. Neurosci.*, **18**, 1818–1826.
- Save, E., Nerad, L. & Poucet, B. (2000) Contribution of multiple sensory information to place field stability in hippocampal place cells. *Hippocampus*, **10**, 64–76.
- Save, E., Guazzelli, A. & Poucet, B. (2001) Dissociation of the effects of lesions of the dorsal hippocampus and parietal cortex on path integration in the rat. *Behav. Neurosci.*, **115**, 1212–1223.
- Steinmetz, M.A. (1998) Contributions of posterior parietal cortex to cognitive functions in primates. *Psychobiology*, **26**, 109–118.
- Tanila, H., Shapiro, M.L. & Eichenbaum, H. (1997) Discordance of spatial representation in ensembles of hippocampal place cells. *Hippocampus*, **7**, 613–623.
- Vallar, G. (1997) Spatial frames of reference and somatosensory processing: a neuropsychological perspective. *Phil. Trans. R. Soc. London B*, **352**, 1401–1409.
- Zugaro, M., Berthoz, A. & Wiener, S.I. (2001) Background, but not foreground, spatial cues are taken as references for head direction responses by rat anterodorsal thalamus neurons. *J. Neurosci.*, **21**, RC154.