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Research report

The role of rat posterior parietal cortex in coordinating spatial representations during place avoidance in dissociated reference frames on a continuously rotating arena (Carousel)

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HIGHLIGHTS

- Posterior parietal cortex (PPC) lesioned rats were tested in Carousel maze.
- PPC rats were impaired in flexible use of extramaze cues.
- PPC rats were impaired in proximal, but not distal, cues navigation in water maze.
- PPC lesion impairs flexible use of behavioral strategies under high cognitive load.

ARTICLE INFO

ABSTRACT

On the Carousel maze, rats are trained to avoid a sector of a circular rotating arena, punishable by a mild electric foot-shock. In the room frame (RF) variant, the punishable sector remains stable relative to the room, while in the arena frame (AF) version, the sector rotates with the arena. The rats therefore need to disregard local olfactory, tactile and self-motion cues in RF condition and distal extra-maze landmarks in the AF task. In both primates and rodents, the coordination of various spatial reference frames is thought to depend on the posterior parietal cortex (PPC). We have previously shown that PPC-lesioned rats can solve both variants of the Carousel avoidance task. Here we aimed to determine the effects of bilateral thermocoagulation lesion of the PPC in Long–Evans rats on the ability to transition between multiple spatial strategies. The rats were first trained in five sessions in one condition and then another five sessions in the other. The following training schemes were used: RF to AF, RF to RF reversal (sector on the opposite side), and AF to RF. We found a PPC lesion-associated impairment in the transition from the AF to RF task, but not vice versa. Furthermore, PPC lesion impaired performance in RF reversal. In accordance to the literature, we also found an impairment in navigation guided by intra-maze visuospatial cues, but not by extra-maze cues in the water maze. Therefore, the PPC lesion-induced impairment is neither specific to distant cues nor to allocentric processing. Our results thus indicate a role of the PPC in the flexibility in spatial behaviors guided by visual orientation cues.

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1. Introduction

Locations in an environment can be encoded with respect to different frames of reference. Usually, we consider two principal referencing systems: egocentric (body-centered), further specified as retino-, head-, or trunk-centric; and allocentric (body independent) which can be arbitrarily centered on any point within the environment. Behavioral activities, such as spatial navigation, reaching and grasping require translation of information coded in distinct spatial frames into purposeful thinking and behavior. In primates, it is believed that the principal brain structure providing these computations is the posterior parietal cortex – PPC [1].

Rodent associative cortex located between occipital and somatosensory cortices considered an analog of the primate PPC

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Table 1

Group averages (±S.E.M.) of maximum inter-entrance interval (MaxT) and the latency to the first entrance (Lat1E) for the first and last session in a particular phase of a given experiment.

	MaxT (s)		Lat1E (s)	
	Sham-operated	Lesioned	Sham-operated	Lesioned
Exp. 1				
RF avoidance (session 1–5)	$382.5 \pm 52.2 – 654.3 \pm 61.6$	$300.4 \pm 78.0 – 587.5 \pm 83.5$	$37.9 \pm 11.5 – 293.9 \pm 105.6$	$4.86 \pm 2.02 – 109.1 \pm 30.7$
AF avoidance (session 6–10)	$235 \pm 49.9 – 531.2 \pm 54.5$	$425.7 \pm 100.3 – 548.7 \pm 59.4$	$44.9 \pm 9.1 – 162.6 \pm 59.3$	$150.5 \pm 120.4 – 159.9 \pm 82.7$
Exp. 2				
RF avoidance (session 1–5)	$362.9 \pm 64.7 – 952.1 \pm 67.3$	$229.6 \pm 41.1 – 617.0 \pm 101.4$[*]	$13.7 \pm 6.6 – 625.5 \pm 158.7$	$18.2 \pm 11.1 – 414.4 \pm 129.1$
RF reversal (session 6–10)	$504.0 \pm 73.9 – 767.0 \pm 113.6$	$226.4 \pm 57.2 – 661.4 \pm 124.6$[*]	$5.0 \pm 1.8 – 299.3 \pm 160.5$	$8.3 \pm 4.7 – 269.9 \pm 148.9$
Exp. 3				
AF avoidance (session 1–5)	$495.7 \pm 78.2 – 618.6 \pm 58.3$	$495.7 \pm 78.2 – 618.6 \pm 58.3$	$16.5 \pm 4.9 – 312.1 \pm 68.2$	$11.6 \pm 5.6 – 625.0 \pm 192.0$
RF avoidance (session 6–10)	$307.6 \pm 69.3 – 589.1 \pm 64.2$	$183.4 \pm 21.3 – 419.3 \pm 46.5$[*]	$32.3 \pm 6.7 – 32.0 \pm 12.8$	$45.0 \pm 13.2 – 55.9 \pm 19.7$

[*] Significant ($p < 0.05$) group effect.

[2,3] covers a substantially smaller surface of the telencephalon. It has been shown to play an important role in spatial representations, which is, however, not yet fully understood [2,4–7].

The first attempts to explain parietal function in terms of allocentric processing [8,9] need to be revised since both ablation experiments and electrophysiological recordings showed that the PPC is involved in both egocentric [10–12] and allocentric processing [2,13–15]. Moreover, lesions of the PPC do not prevent the use of egocentric [16] or allocentric information [15] per se. Current theories of rodent PPC function focus on integration of different reference frames into goal-directed behavior [7,12,14]. One of the explanations is based on a hypothesis that the PPC encodes information in both frames and deficits manifest as the load for the translator increases [6,15].

Active place avoidance on a Carousel requires the rats to avoid an unmarked place on a continuously rotating (1 rpm) circular arena [17–19] and allows assessment of the ability to segregate and coordinate spatial information bound to a particular reference frame. The rotation dissociates spatial cues in the environment into two independent reference frames of the rotating arena and stationary room. The to-be-avoided place can be defined in either the arena- or room-frame. Thus, in principle, we can use two variants of this task: a room-based task in light with emphasis on visual cues and arena-based task in darkness where navigation is guided by, largely self-generated, local cues on the arena floor. Notably, both tasks require segregation of stimuli room- and arena-cues into coherent subsets [20].

Our previous work found no effect of PPC lesions on navigation in either the arena- or room-frame [21]. However, the ability to select the relevant frame for navigation and switch to the other frame was not systematically analyzed in that study. Rodent PPC has been implicated in attention processes [22] and cognitive flexibility, particularly in extra-dimensional shift [23]. Our pilot data suggested that PPC lesions might affect the way rats use a relevant frame in the presence of cues from both frames. Therefore, we aimed to determine how PPC lesions affect segregation and flexibility in the use of the frame relevant for navigation. We hypothesized that PPC is involved in flexible switching from one frame of reference to the other and that deficit induced by PPC lesions will be most prominent during the first session after the switch and independent of its direction, i.e. from arena to room frame avoidance or vice versa (Table 1).

2. Materials and methods

2.1. Animals

Forty-eight male adult Long–Evans rats from the breeding colony of the Institute of Physiology were used in the study. They were three months old upon arrival and weighed 250–320 g. They were housed in groups of two or three per cage in a temperature-controlled animal room (21 °C) with a 12/12 h light/dark cycle (lights on at 7:00). Food and water were freely available until three days prior to behavioral training when the rats were food-restricted to maintain their weight at 90% of their free-feeding weight. All the experimental procedures were carried out during the light period of the day with the emphasis to test each animal in the same time period. The testing order of the animals was kept constant throughout the training and remained counterbalanced across groups. All the manipulations complied with the Czech Animal Protection Act, directive of the European Community Council (2010/63/EC), and NIH guidelines.

2.2. Surgery

Rats were randomly assigned to sham-operated (controls, $n = 24$) or posterior parietal cortex lesion (parietal, $n = 24$) groups. They were anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (40 mg/kg, i.p.) and gently fixed in a stereotaxic frame (TSE systems). The head was shaved and the scalp was incised and retracted. The skull was cleared and two trephine openings were drilled into the skull to expose the brain at the following coordinates relative to the *bregma*: AP −2 to −6 mm, ML ±1.5 to ±5.5 mm. Bilateral thermocoagulation lesions of PPC were induced by applying the tip (diameter = 0.5 mm) of a calibrated soldering iron (temperature 180 °C) directly to the *dura mater* for 0.5 s at various points within the exposed area until the entire surface was lesioned. Sham-operated rats underwent the same procedure except for applying the soldering tip to the *dura*. Thermocoagulation lesions have been shown to be effective and safe for the animals [15,21]. Sterile gelfoam was then placed in the openings and the wound was sutured, followed by a local application of lidocaine and antiseptic. The rats were left to recover for at least 14 days. One sham-operated animal died during recovery period. A day before the start of the behavioral pretraining, awake animals were gently implanted with a hypodermic needle connector, which pierced the skin between rat's shoulders. The tip was blunted and swirled to prevent slipping out and to provide a purchase for an alligator clip connecting a shock-delivering wire.

2.3. Behavioral apparatuses

2.3.1. Carousel

The Carousel [17,19,21,24] consists of a smooth metallic circular arena (82 cm in diameter) enclosed by a 30 cm high transparent Plexiglas wall. The arena is elevated 1 m above the floor of a 2 m × 3 m dimly illuminated room containing an abundance of extra-maze cues. An infrared light-emitting diode (LED) located

on the arena circumference was used to monitor arena rotation and another LED was worn by the rat between its shoulders. At the beginning of each experimental session, a rat was placed onto the slowly rotating arena directly opposite to the to-be-avoided place and tracking was switched on. A computer-based tracking system (Tracker; Biosignal Group, USA) located in an adjacent room recorded the position of both the rat and the arena every 40 ms. The time series of position coordinates were stored for off-line analysis (TrackAnalysis; Biosignal Group, USA). A cable delivering footshocks was connected to the needle implanted on the animal's back via a small alligator clip. Animals had to avoid an unmarked 60-deg sector of the arena. The sector was defined in either the room-frame (RF variant) or in the arena-frame, i.e. rotated with the arena (AF variant). Whenever the rat entered the sector for more than 500 ms, the tracking system delivered a mild shock (50 Hz, 0.5 s, 0.3–0.7 mA) and counted an entrance. The shock intensity was set individually for each for each rat to elicit a rapid escape response but to prevent freezing. In most cases, animals responded appropriately to 0.4 mA. If the rat did not leave the sector, additional shocks were given every 1200 ms, but no more entrances were counted until the rat left the sector for more than 300 ms. Shocks were delivered in a current-controlled circuit via the implanted needle and the arena floor (the highest voltage drop was between rats' paws and grounded floor). To ensure sufficient locomotor activity, which is a prerequisite for meaningful evaluation of arena-frame avoidance, animals were trained to forage for barley grains randomly dispersed from an overhead feeder (approximately one grain per 10 s). The arena floor was cleaned with wet towel between rats, ensuring the rats could not use inter-trial scent marks.

2.3.2. Morris water maze (MWM)

A blue painted water tank (d = 193 cm) situated in 4 m × 5 m room with abundance of salient extramaze cues was filled up to 40 cm from the edge with water (22 °C) made opaque by non-toxic paint (Primalex, PPG Deco). The top of a cylindrical platform (d = 10 cm), made of transparent Plexiglas, remained submerged 1–2 cm below the water surface. Illumination was provided by a 30 W compact fluorescent light bulb located on the ceiling above the center of the pool, just next to an analog B/W camera. The video signal was passed to a tracking program (Tracker, Biosignal Group, USA), which sampled the position of the rat at 25 Hz. In the proximal cues version, the pool was surrounded by a black curtain hiding the extramaze cues in the room and three objects provided intramaze cues: a vertical metal rod (2 cm in diameter, 50 cm long), fixed floating plastic duck (10 cm × 6 cm × 8 cm), and another vertical metal rod (3 cm in diameter, 50 cm long) with a white polystyrene ball (8 cm in diameter) on the top. Both rods were coated with vaseline to prevent rats from hanging on them.

2.4. Behavioral procedures

2.4.1. Behavioral pretraining

All rats underwent two days of habituation to the apparatus prior to Carousel testing. Each rat collected randomly scattered barley pellets on a stable arena for 20 min, with no foot shocks. To motivate rats to move in arena-frame training and to prevent bias while switching from room-frame to arena-frame, we used foraging motivation, throughout all subsequent training although the room-frame task itself does not require such a motivation.

2.4.2. Place avoidance training

In the first experiment (Fig. 1, Exp. 1), eight parietal rats and eight sham controls were trained in five sessions to avoid a place defined in the reference frame of the room (RF) while arena slowly rotated. During session six to ten, the sector was defined in the AF and rotated with the arena, therefore rats had to change their
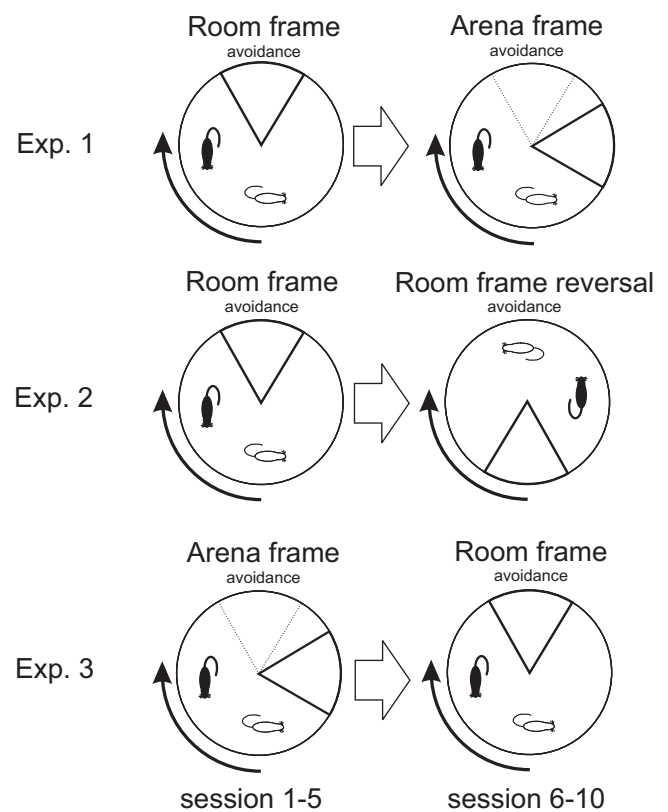

Fig. 1. Scheme of experiments in the Carousel apparatus. In Exp. 1 (top panel), rats switched from the RF to AF, in the Exp. 2 (middle panel), rats switched from RF-sector in North to RF sector in South. In the Exp. 3 rats switched from the AF to RF-based avoidance.

avoidance behavior accordingly and switch to using proximal orienting cues. In this case, both phases were reinforced. In the second experiment, eight lesion and eight sham operated animals were first trained in room-frame avoidance in five sessions (sector located in the North; Fig. 1, Exp. 2). During sessions six to ten the sector was moved to the South and remained in room coordinates. In the third experiment, 8 lesioned and 8 sham-operated controls were first trained to avoid a sector rotating with the arena using intramaze cues (AF) and subsequently to switch to room-frame (RF) avoidance using extramaze cues (Fig. 1, Exp. 3). Each place avoidance lasted 20 min and each rat was trained in a single session a day.

2.4.3. Distal cues testing in the MWM

Eight lesion rats and eight sham-operated rats randomly chosen after completion of the place avoidance training (4 lesion and 4 sham rats from RF–AF experiment; 4 lesion and 4 sham rats from RF–AF experiment) were then trained during six sessions in the distal-cue version of the water maze. Rats received two four-trial sessions per day. Within a session, rats were consecutively released from four starting points (which changed in a pseudorandom order), arbitrarily assigned to the four cardinal directions (N, S, W, E), while the location of the submerged platform remained in the center of NE quadrant. If a rat did not find the platform within 60 s (maximum trial time), it was gently guided to it and left there for 15 s. Rats were always transferred to a drying cage outside the pool for at least 30 s before the next trial.

2.4.4. Proximal cues testing in the MWM

Different groups of randomly chosen lesion (n = 8) and sham-operated (n = 8) rats, which completed the place avoidance training
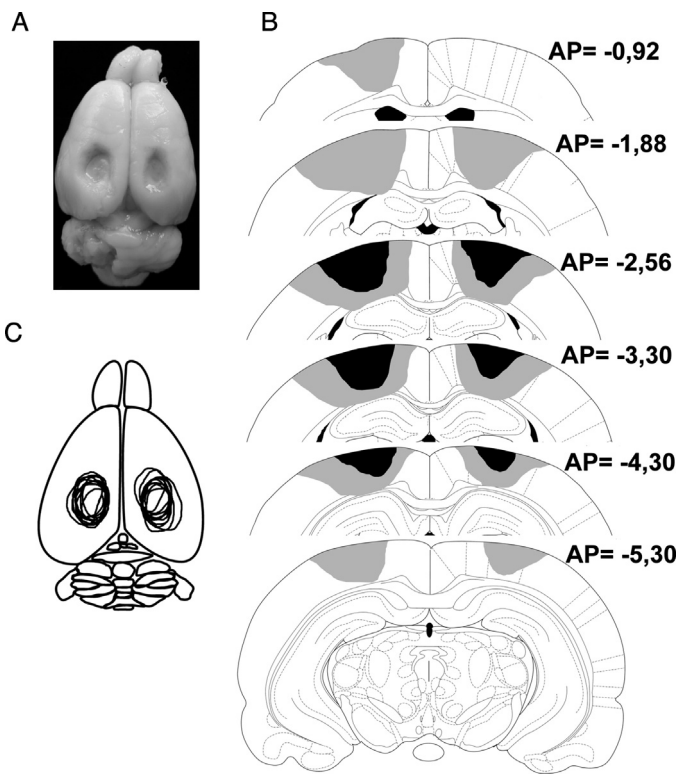
Fig. 2. Extent of PPC lesion. Depiction of a representative lesion (A). Reconstruction of the extent of minimal (black) and maximal (gray) lesions on the coronal sections of the brain adopted from Paxinos and Watson [38] (B). Top view of brain scheme illustrating variability of the lesions (C).

(4 lesion and 4 sham rats from RF–AF experiment; 4 lesion and 4 sham rats from AF–RF experiment) were trained in eight sessions in the proximal cues version of the Morris water maze. The training procedure was the same as previously described except that a black curtain surrounded the pool so that the position information could only be derived from the three objects placed inside the pool. Their constellation and their relation to the submerged platform were identical to that in the study of Poucet and Save [15]. Once a session had finished, the reference frame consisting of the three objects rotated randomly (and accordingly did the platform) to eliminate bias from background cues.

2.5. Lesion verification

After finishing behavioral testing, lesion animals were overdosed with ketamine and then transcardially perfused by saline and subsequently with 4%-formaldehyde solution. The brains were frozen and coronally sectioned into 50-μm sections, stained with cresylviolet and the extent of PPC lesion was verified (Fig. 2).

2.6. Data analysis

Place avoidance was evaluated using the number of entrances (Ent) into the to-be-avoided sector as the principal parameter reflecting avoidance efficiency. Other parameters included maximum inter-entrance interval (MaxT), and the latency to the first entrance (Lat1E) in a given session. The former reflects within-session learning, whereas the latter is an index of between-session long-term memory. Active locomotor activity was assessed as the total distance (DIST) traveled during a session and measured as the sum of distances between points sampled each second in the arena-frame coordinates. In the MWM, we evaluated latencies to escape on the platform. Results were analyzed separately for

each task variant using a two-way ANOVA (LESION vs. SESSION) with repeated measures on sessions and a Tukey's HSD post hoc test when appropriate. If the data were not normally distributed (assessed by Shapiro–Wilk test) or did not meet the assumption of homogeneity of variance (Bartlett's test), logarithmic transformations were conducted. Significance was accepted at $p < 0.05$. All data are presented as box plots. Calculations were performed using Statistica 7.1 (Statsoft, Inc.).

3. Results

3.1. Experiment 1: room-frame to arena-frame switch (RF → AF)

Rats readily acquired the room-defined place avoidance (Fig. 3A, Table 1). The ANOVA revealed a highly significant effect of sessions on all parameters of avoidance (Ent: $F_{(4,56)} = 22.51$; MaxT: $F_{(4,56)} = 9.26$; Lat1E: $F_{(4,56)} = 11.98$; all p's < 0.001). In contrast, the effect of lesion did not reach statistical significance on any of the place avoidance parameters (Ent: $F_{(1,14)} = 2.75$, $p = 0.12$; MaxT: $F_{(1,14)} = 3.78$, $p = 0.072$; Lat1E: $F_{(1,14)} = 3.45$, $p = 0.084$) and no significant interaction was found either. Similarly, the locomotor activity changed across days ($F_{(4,56)} = 8.82$, $p < 0.001$), but no effect of lesion ($F_{(1,14)} = 0.12$, $p = 0.74$) or significant SESSION vs. LESION interaction ($F_{(4,56)} = 2.45$, $p = 0.056$) was found.

Next, we evaluated the ability to switch to AF-based avoidance. The ANOVA found a significant effect of session (Ent: $F_{(4,56)} = 8.82$, $p < 0.001$), but no effect of lesion ($F_{(1,14)} = 1.23$, $p = 0.29$) indicating that all rats improved their avoidance over time. The graph indicates that lesioned rats avoided slightly better than controls, which might be due to one sham-operated animal perseverating tenaciously in room-frame avoidance. No effect of lesion was observed on the other measures of avoidance (MaxT: $F_{(1,14)} = 1.63$, $p = 0.22$; Lat1E: $F_{(1,14)} = 0.57$, $p = 0.46$). Analysis of locomotion found no effects of session ($F_{(4,56)} = 1.69$, $p = 0.16$), lesion ($F_{(1,14)} = 0.1$, $p = 0.76$), or interaction ($F_{(4,56)} = 1.73$, $p = 0.16$). These results clearly show that PPC lesions did not prevent switching from efficient RF to AF place avoidance.

3.2. Experiment 2: room-frame learning and reversal (RF spatial reversal)

Different group of lesioned and sham rats underwent training that involved an "intraframe" reversal. After five sessions of training to avoid a place located in the North in the RF coordinates the to-be-avoided place was relocated to the South, still in the RF (Fig. 3B, Table 1)). One sham-operated animal died at the beginning of the training.

Similarly to the Experiment 1, both groups readily acquired the place avoidance (Ent: $F_{(4,52)} = 46.9$, $p < 0.0001$) while the PPC lesions did not significantly affect the performance (Ent: $F_{(1,13)} = 2.31$, $p = 0.15$; interaction NS). The same pattern was observed on the time to the first entrance (Lat1E: SESSIONS: $F_{(4,52)} = 21.67$, $p < 0.0001$; LESION: $F_{(1,13)} = 2.25$, $p = 0.16$; interaction NS), but the lesioned animals did not maintain their avoidance within session as long as controls (MaxT: $F_{(1,13)} = 4.7$, $p < 0.049$). Importantly, the lesion did not affect locomotion, which, however, varied across sessions ($F_{(4,52)} = 6.9$, $p < 0.001$).

All rats improved their avoidance in response to "intraframe" reversal to a novel position (Ent: $F_{(4,52)} = 14.1$, $p < 0.001$; Lat1E: $F_{(4,52)} = 6.93$, $p = 0.0001$) with no significant effect of lesion (Ent: $F_{(1,13)} = 3.55$, $p = 0.08$; Lat1E: $F_{(1,13)} = 3.55$, $p = 0.5$; both interactions NS). Significant improvement in MaxT across sessions ($F_{(4,52)} = 7.85$, $p < 0.001$) was accompanied with moderate deficit in lesioned group ($F_{(1,13)} = 5.94$, $p = 0.03$) with no interaction ($F_{(4,52)} = 1.62$,
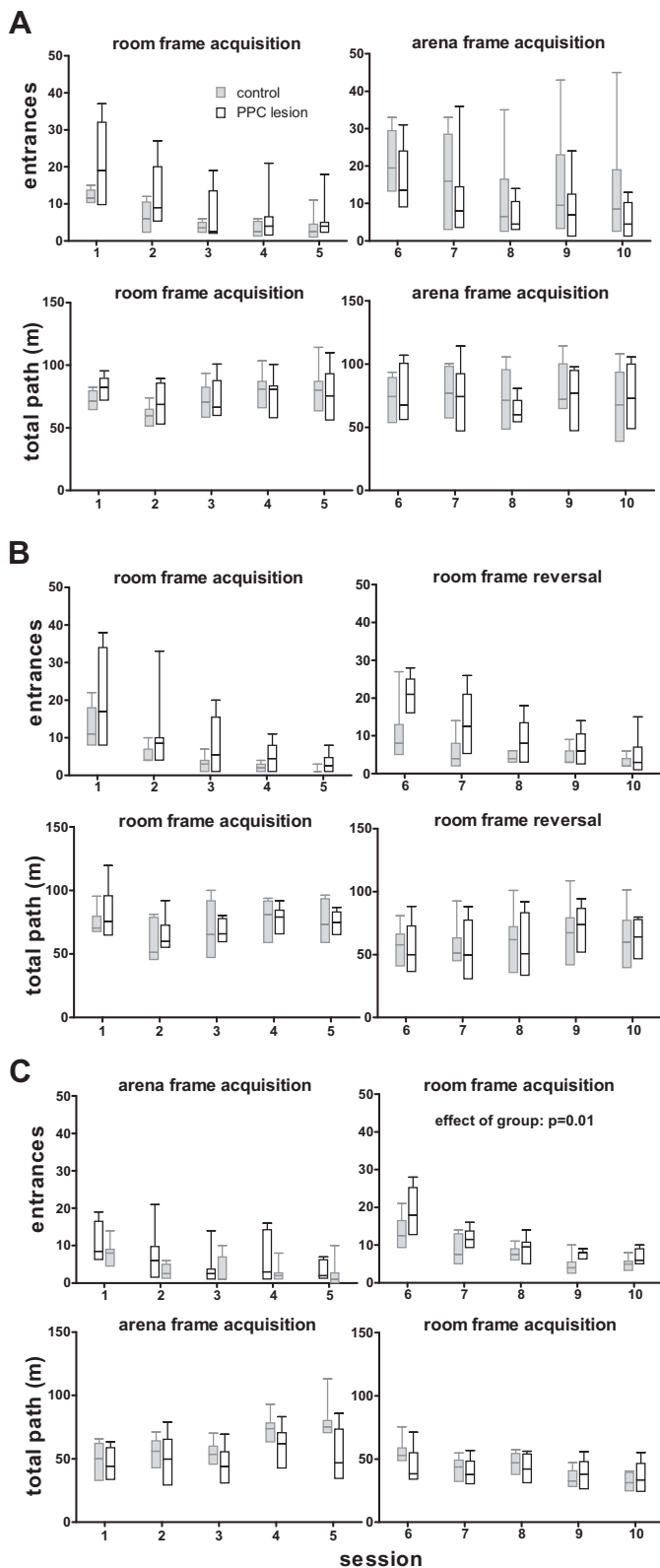
Fig. 3. Performance on the Carousel demonstrated by number of entrances into the punishment sector and by total path elapsed during a session. In the first experiment (A) rats were trained in five daily sessions to avoid a sector defined in the room frame, followed by five sessions of training to avoid arena frame bound sector. In the second experiment (B) the sector was defined in the room frame throughout training but it was moved to the opposite side beginning in session 6. In the third experiment (C), rats were first trained under room frame conditions and then they switched to arena frame conditions. Data represented as box plots. Group effect shown if reached significance.

$p = 0.18$). Locomotion fluctuated during training ($F_{(4,52)} = 4.25$, $p < 0.005$), independent of lesion ($F_{(1,13)} = 0.037$, $p = 0.85$).

To investigate the observed deficit in more detail, we evaluated the temporal distribution of the entrance events. For this purpose, we split each session into halves and analyzed the halves separately across all sessions (Fig. 4A). We found that the early session performance was impaired in lesioned animals throughout the training (Ent: $F_{(1,13)} = 4.81$, $p = 0.047$). In contrast, late session performance was affected by the lesion only in the first two sessions after the switch as indicated by Tukey's post hoc tests that followed a significant SESSION vs. LESION interaction ($F_{(4,52)} = 3.06$, $p = 0.024$).

3.3. Experiment 3: arena-frame to room-frame switch (AF → RF)

In Experiment 3, we trained a new set of lesioned and sham rats to avoid a place in the AF in 5 sessions and then to switch to RF-based avoidance in 5 more sessions (Fig. 3C, Table 1)). All rats improved their initial AF-based avoidance (Ent: $F_{(4,56)} = 11.65$, $p < 0.0001$), Lat1E: ($F_{(4,56)} = 18.97$, $p < 0.0001$) although the effect on MaxT did not reach significance ($F_{(4,56)} = 2.39$, $p = 0.062$). No effect of group was observed on any of the parameters. Locomotion increased over time ($F_{(4,56)} = 7.89$, $p < 0.0001$) with no effect of lesion ($F_{(1,13)} = 2.37$, $p = 0.15$).

The switch to RF-based avoidance revealed a deficit in the lesioned rats (Ent: $F_{(1,14)} = 8.45$, $p = 0.012$; MaxT: $F_{(1,14)} = 8.7$, $p = 0.01$), although both lesioned and sham rats improved their avoidance over time (Ent: $F_{(4,56)} = 23.66$, $p < 0.0001$; MaxT: $F_{(4,56)} = 12.6$, $p < 0.0001$) and no interactions were found (Ent: $F_{(4,56)} = 0.77$, $p = 0.55$; MaxT: $F_{(4,56)} = 0.22$, $p = 0.92$). Locomotion varied across sessions ($F_{(4,56)} = 4.09$, $p < 0.006$), but there was no effect of lesion ($F_{(1,13)} = 0.64$, $p = 0.44$) nor interaction ($F_{(4,56)} = 0.58$, $p = 0.68$). Detailed analysis of temporal distribution of entrance events (Fig. 4B), again, revealed a lesion-induced deficit early in the sessions ($F_{(1,14)} = 5.77$, $p = 0.03$), but here, the deficit extended also into the second halves of sessions ($F_{(1,14)} = 8.37$, $p = 0.012$).

We further sought to determine whether PPC lesions affected spatial perseveration by measuring the extinction of previously acquired spatial response in session 6. We examined how many times animals entered the no longer reinforced place. To evaluate the temporal dynamics of the extinction in more detail, we split the switch session into halves (Fig. 5). During RF to AF avoidance switch, both lesioned and sham animals entered the old place in the room-frame less frequently during the first than during the second half of the session ($F_{(1,14)} = 9.36$, $p = 0.009$), but the lesioned rats entered the place more often ($F_{(1,14)} = 6.48$, $p < 0.023$). During the "intraframe" switch (RF reversal), all rats committed more entrances during the second half of the session ($F_{(1,13)} = 13.85$, $p = 0.003$), but the lesion had no effect. Similarly, when rats switched from AF to RF, more entrances occurred late in the session ($F_{(1,14)} = 6.13$, $p = 0.027$) with no significant effect of lesion. Fig. 5 clearly illustrates the low number of entrances throughout the session compared to Experiments 1 and 2, indicating rats' inability to quickly abandon AF navigation.

The above data demonstrate that PPC lesion impaired the ability to switch from AF to RF place avoidance, i.e. from utilizing proximal to using distal spatial cues. This is in contrast to findings in the MWM by Save and Poucet [15]. However, in that study, rats were required to use purely visual cues to navigate to a hidden goal in a pool. To address the question whether our PPC lesion generally impaired processing of distal cues or if this deficit depends on the substrate and cue modality, we aimed to replicate the work of Save and Poucet [15].
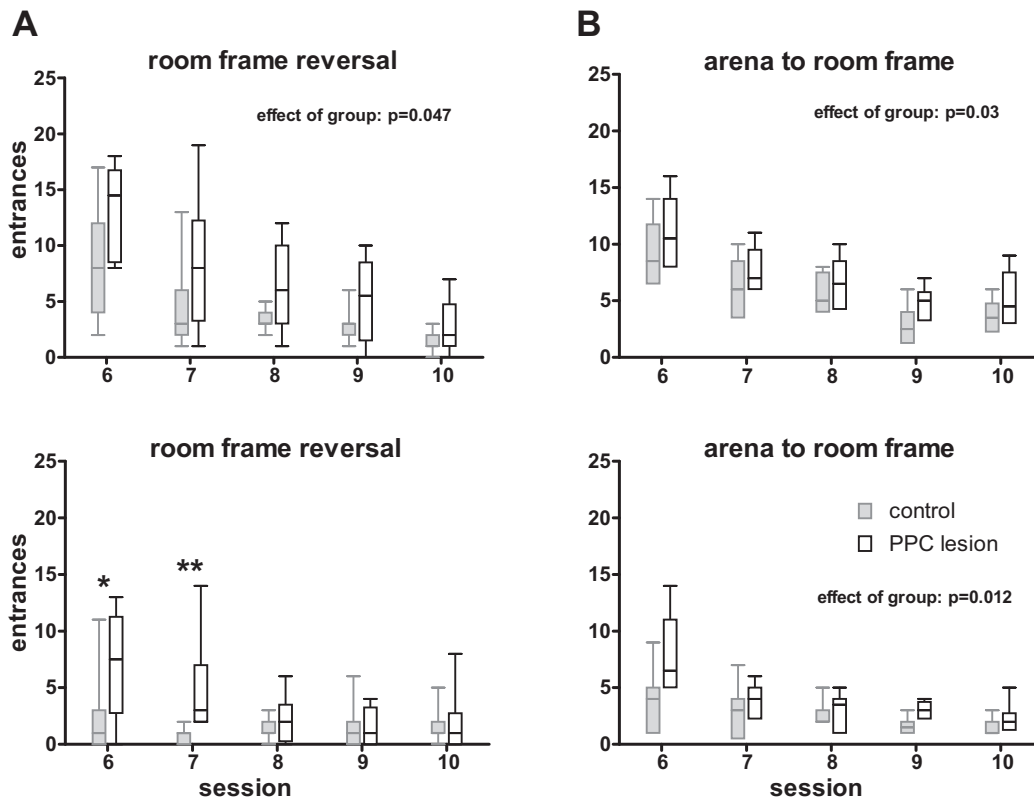
Fig. 4. Number of entrances assessed in the first (upper graphs) and second (lower graphs) half of the "switched" sessions of the experiments in which group effect was revealed. Data represented as box plots. Group effect shown if reached significance. Between group significance within a single session indicated by asterisks: * $p < 0.05$ and ** $p < 0.01$.
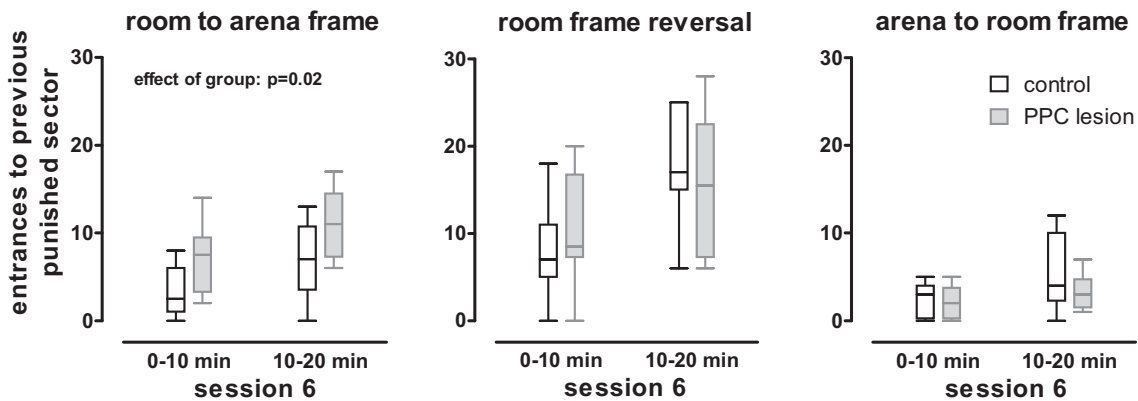

Fig. 5. Number of entrances into the location of the previous punishment sector in the first half and the second half of the sixth session of each experiment. Data represented as box plots. Group effect shown if reached significance.

3.4. Morris water maze

All rats decreased their escape latency over the course of training ($F_{(5,70)} = 42.6$, $p < 0.0001$) in the classic incremental version of the water maze with distal cues available (distal cues version, Fig. 6A). Although the PPC lesion resulted in slightly higher latencies, this difference fell short of significance ($F_{(1,14)} = 4.39$, $p = 0.055$). In the proximal version MWM (proximal cues version, Fig. 6B) where navigation was based on three intramaze objects, the improvement was a bit slower. This was partly due to apparently different strategy since animals often swam close to a landmark before turning toward the platform (Fig. 6B). The ANOVA revealed not only significant effect of session ($F_{(7,98)} = 14.2$, $p < 0.0001$), but also an effect of lesion ($F_{(1,14)} = 10.53$, $p = 0.006$). These results fully correspond

to those of Save and Poucet [15], demonstrating that a PPC lesion disrupts navigation based on intramaze, but not extramaze, visual objects.

4. Discussion

The present study reports three principal findings: first, we found that posterior parietal cortex (PPC) lesioned rats had unchanged performance in arena-frame (AF) based navigation on the Carousel arena, regardless of the order of tasks in the training phase. Second, we observed a deficit in room-frame (RF) based navigation in PPC-lesioned animals following a switch from AF-based avoidance or following a change in the position of the target sector (RF reversal). Third, we were able to replicate the results
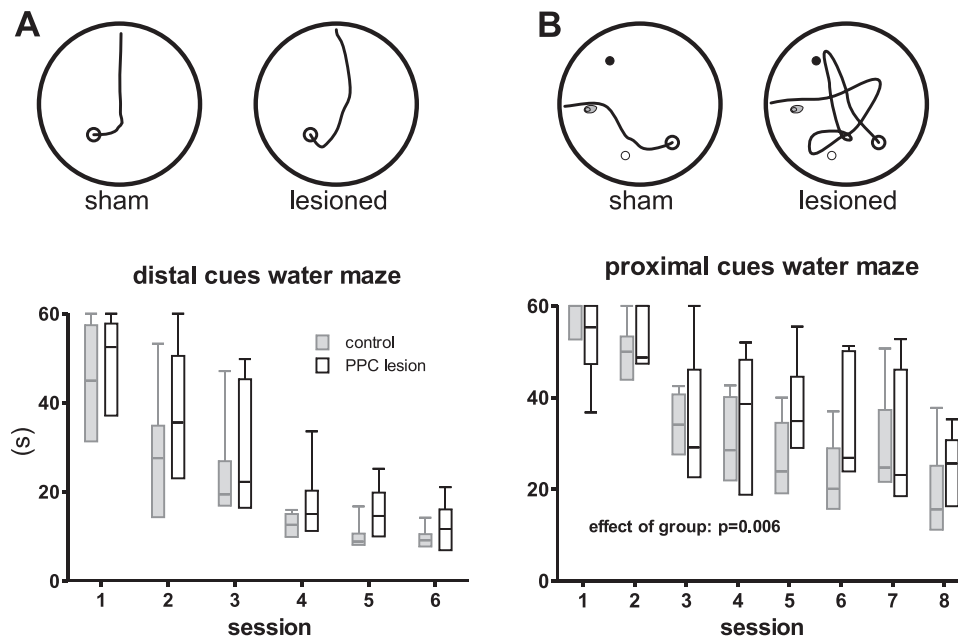
Fig. 6. Representative trajectory of a well-learned rat and performance in distal (A) and proximal (B) versions of the Morris water maze assessed by latency to reach the platform. Data represented as box plots. Group effect shown if reached significance.

of an earlier study by Save and Poucet [15], where PPC-lesioned rats exhibited a specific spatial cognition impairment in navigation using local intra-maze cues in the MWM.

Unlike traditional stationary spatial tasks such as the Morris water maze or Barnes maze, spatial information available to the animal on the Carousel maze is significantly more complex. The arena rotation dissociates the spatial environment into two distinct reference frames: "room frame" consisting of extra-maze visuospatial cues, and "arena frame" defined primarily by egocentric self-motion cues. It has been demonstrated that successful RF-avoidance on the Carousel relies upon a hippocampus-dependent ability to segregate relevant spatial cues from irrelevant ones, and to use the relevant subset of spatial stimuli to construct allocentric spatial representations [25]. However, the exact neural circuitry underlying AF-based avoidance as well as the neural mechanisms of selection of and directing one's attention to the appropriate task-relevant frame remain nebulous. Several studies have shown that lesions of the rat PPC impair egocentric spatial processing [11,26,27]. Originally, these findings led us to hypothesize that AF-navigation would be dependent upon the PPC and that the selection of task-appropriate reference frame might involve 'competition' between allocentric spatial representation in the hippocampus and egocentric in the PPC and subsequent 'suppression' of the irrelevant frame. However, our earlier reported findings [21] contradicted this thesis, showing that neither allocentric (RF-based avoidance in light) nor egocentric (AF-based avoidance in darkness) navigation on Carousel is impaired in PPC-lesioned rats. Furthermore, several studies suggested that a critical role of the PPC in both primates and rodents might consist in providing coordination among multiple reference frames in complex tasks, rather than representing a specific reference frame per se [1,5]. Hence, in the present study we re-stated our original hypothesis to suggest that the PPC's role subsists in flexible coordination of allocentric and egocentric reference frames. According to this hypothesis, PPC lesion would leave navigation in either reference frame unimpaired when learned separately, but it would have caused a comparative difficulty when switching between RF- and AF-based tasks. This presumed impairment would likely be attributable to latent learning in the PPC-intact

group. This group would have been able to switch between both frames regularly and therefore acquire representations of both reference frames within the initial training phase, while the PPC-lesioned rats would have to disregard the task-irrelevant frame during the initial phase. This difference in learning strategies would be reflected in a slower acquisition of avoidance behavior in the frame transition phase. However, this hypothesis does not predict any significant differences in the RF reversal task, as this task does not involve transition between allocentric and egocentric reference frames.

Consistent with this hypothesis, we found no impairment of PPC-lesioned rats in the AF-based avoidance on Carousel in light during the initial training phase. Furthermore, PPC-lesioned rats were impaired in the transition from AF- to RF-based navigation. However, we failed to find an impairment following the opposite transition (RF to AF), and also found that PPC-lesioned rats were impaired in RF reversal. These findings imply a more specific role for the PPC in the navigation in the Carousel maze then we had anticipated. Our results indicate that PPC conveys behavioral flexibility for RF-, but not for AF-based navigation. We even observed an insignificant trend for a slight improvement of performance in the AF-based navigation in PPC-lesioned rats.

Although our data clearly indicate differential effects on behavioral flexibility in RF- and AF-based avoidance on the Carousel, we need to take some caution before generalizing these findings as differential roles of the PPC in the flexibility in allocentric and egocentric navigation. This is due to certain limitations that are inherently contained in our study design. First, while AF-based avoidance is primarily egocentric, the animals also have local scent marks and tactile cues to their disposal. Stuchlik et al. [28] demonstrated that these cues are used by rats in AF-based place avoidance to regularly update their egocentric position. As egocentric navigation using self-motion cues accumulates error over time, these intra-maze cues are necessary to maintain accurate egocentric position over longer distances [28]. It is possible that animals use these local cues to construct allocentric representation of the intra-maze (AF) environment. Second, compared to goal-directed behavior, spatial avoidance is in principle less demanding in terms of spatial precision. This is because animals can avoid a larger area

than the actual footshock-zone on the Carousel, but this is not analogously possible in tasks where localization of a specific goal area is required. Furthermore, the to-be-avoided area on the Carousel comprise approximately 17% of arena surface compared to less than 1% of the water surface covering platform location in the Morris water maze. The role of the PPC in egocentric navigation has been established using tasks requiring accurate egocentric pointing to a goal [11,29,30]. Therefore, there is a possibility that the differential effects of PPC lesion on RF to AF and AF to RF transition might be attributed to a differential level of spatial precision required, or task difficulty. However, the difficulties of transitions from RF- to AF-based navigation and vice versa should be more alike than the difficulties of AF- to RF-transition and RF reversal. Since we observed PPC lesion-induced deficits in AF to RF transition and RF reversal, but not in RF to AF transition, the possibility that the task complexity alone drives the differences in the effects of PPC lesions on the respective strategy shifts seems unlikely. Third, the spatial cues determining the RF and AF were largely conveyed by different modalities. While visual cues vastly prevailed in the RF, AF provided a host of self-motion, olfactory and tactile cues. Hence, it is conceivable that the modality of spatial cues involved plays a critical role and that the PPC preferentially conveys flexibility in processing visuospatial information. Although more research is needed to draw a definitive conclusion, we believe this explanation is plausible as it would account for the discrepancy between our results and the results of groups who observed PPC-lesion related impairment in navigation using intra-maze but not extra-maze cues, such as in Save and Poucet [15]. In this study, authors found that PPC-lesion affected navigation using proximal, but not distal visuospatial cues. We replicated the study and obtained results similar to those of Save and Poucet, confirming that the discrepancy was not caused by other factors, such as differences in genetic background of rats, animal care and handling, PPC lesion technique, and diet. Since in both our and Save and Poucet's designs the PPC-lesioned rats are impaired in tasks requiring processing of visuospatial cues, it is likely that the modality of spatial information is important. PPC lesions may therefore interfere with processing of visuospatial, but not self-motion and olfactory information.

However, it is important to note, that the observed deficit was not in the ability to utilize visuospatial cues for navigation, but rather, to utilize them flexibly. Although we report a significant deficit in the flexibility in the RF-based avoidance, the initial acquisition of RF-based avoidance remained unaffected. This result is consistent with our previous work [21] and might indicate a deficit in attentional processing. PPC has been implicated in attentional processing in both spatial and non-spatial domains. The effect of a PPC lesion on reversal in a non-spatial domain was examined in a study by Fox et al. [23] employing a simple attentional discrimination task [31] in which rats learnt to discriminate a rewarded stimulus from a pair of presented stimuli. In contrast to our results, the reversal of the rewarded stimuli remained intact after PPC lesion [23]. Thus, it appears that a deficit in reversal occurs specifically when the task requires handling allocentric spatial information. Furthermore, Fox et al. [23] found an effect of PPC lesion on set-shifting, i.e. attending to previously disregarded stimuli, which together with reversal form two main classes of cognitive flexibility [32]. On the Carousel, intramaze and extra-maze spatial cues must be disregarded in RF-based and AF-based avoidance tasks, respectively. Hence, according to this definition, a transition from AF- to RF-based avoidance (or vice versa) is a set-shifting task. However, our results are not reciprocal, i.e. we found a deficit only shifting from AF to RF, but not vice versa. Thus, our data cannot be interpreted in terms of deficit of attentional network underlying set shifting, which includes the medial prefrontal cortex [32], a structure densely interconnected with the PPC [33]. For the same reason, the impairment in the ability to transition from

AF- to RF-based navigation strategies as well as the impaired reversal cannot be interpreted as an inability to inhibit a previously learned strategy.

Our results thus indicate that PPC-lesion renders a specific inability to flexibly process allocentric visuospatial information. Since recent anatomical examinations underscored dense interconnection of the PPC and the retrosplenial cortex (RSC) [34], we can speculate that RF based information reaches the PPC from the RSC [35] which coordinates allocentric RF information in conjunction with the hippocampus [36,37]. Our replication of the modified water maze task of Save and Poucet [15] provided further validation of our results against the only other known study evaluating involvement of the PPC in intra-maze and extra-maze cue processing. For the sake of consistency and comparability with literary data, we chose to employ thermocoagulation lesion technique, although more novel neurotoxic methods are available which might provide a greater level of anatomic precision. In fact, the results of Save and Poucet [15] were nearly replicated emphasizing the greater effect of PPC lesion upon navigation based on proximal visual cues than distal ones. Although this result seems to contradict the outcomes of the Carousel experiments, we believe that the discrepancy is due to a difference in patterns of available cues and different navigational demands. The set of cues defining the extra-maze environment is highly comparable between the water maze and the Carousel. However, in the standard water maze task, the rats perform an approaching maneuver, using distal cues in the absence of intra-maze cues. On the other hand, the distal or RF variant of the Carousel requires rats to use distal visual cues to actively move away from a punishment sector while disregarding all available arena surface based cues, including egocentric navigation. Thus, it is likely that the cognitive load is greater in case of the Carousel. PPC lesion affected performance in proximal cues navigation in the water maze but not the arena frame avoidance on the Carousel. Save and Poucet [15] hypothesized that the deficit they observed was due to a high cognitive demand of the proximal cues version of water maze. In comparison with more distant extra-maze cues, the azimuthal position of the intra-maze cues change much more rapidly, resulting in the need of a more frequent updating of one's egocentric location relative to the allocentric representation in the case of intra-maze cues variant. Accordingly, the RF-based avoidance is likely to present a higher cognitive load due to the conflict between proximal and distal landmarks as well as the necessity to disregard egocentric self-motion cues.

However, since the ability to learn RF and AF tasks is not impaired in PPC-lesioned rats [21], the high cognitive load of the spatial task alone is not the limiting factor causing the deficit of PPC-lesioned rats on a Carousel. Rather, it is the ability to flexibly change behavioral strategies under high cognitive load for allocentric processing of visuospatial cues.

Acknowledgements

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