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Research report

Excitotoxic lesion of the perirhinal cortex impairs spatial working memory in a delayed-alternation task

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ABSTRACT

The perirhinal cortex (PRh) is strategically located between the neocortex and memory-related structures such as the entorhinal cortex and the hippocampal formation. The pattern of strong reciprocal connections between these areas, together with experimental evidence that PRh damage induces specific memory deficits, has placed this cortical region at the center of a growing interest for its role in learning and memory mechanisms.

The aim of the present study is to clarify the involvement of PRh in learning and retention in a novel experimental model of spatial working memory, the water T-maze. The data show that pre-acquisition neurotoxic PRh lesions caused task-learning deficits. This impairment was observed during the acquisition phase as well as the retrieval phase. On the other hand, a post-acquisition PRh neurotoxic lesion failed to impair the acquisition and the retrieval of the water T-maze task performed 32 day after lesion. These results suggest a possible key role of PRh in the acquisition but not in the retention of a working memory task. Furthermore, these results show that the water T-maze may be a suitable learning paradigm to study different components of learning and memory.

1. Introduction

Experimental research has shown that the integrity of the medial temporal lobe (MTL) is critical for learning and memory functions [1–5]. The main MTL components (hippocampus, entorhinal cortex and perirhinal/postrhinal cortex) contribute to learning and memory in different ways [6]. In particular, the perirhinal cortex (PRh) is strategically located within the MTL as a crucial relay for the flow of sensory information from the neocortex to the hippocampus. In the rodent brain, the PRh is located along the rhinal sulcus and it is composed of two distinct fields, Brodmann's areas 36 (dorsal) and 35 (ventral) [7]. It has been shown that a complex network of cortical afferents (arising from the cingulate, parietal, frontal, piriform, insular, pre and infralimbic, visual and auditory cortex) topographically targets the PRh. This supports its role as polymodal associational cortex [8–11]. PRh receives and integrates both unimodal sensory input as well as input from other associational regions. Such integration is supported by a cascade of intrinsic synaptic connections, from dorsal area 36 to ventral area 35 [12,13]. In turn, all PRh fields send strong projections to CA1 and subiculum, both directly and through the entorhinal cortex [14–16]. This pattern of connectivity is reciprocated by upstream projections tracing back the cascade of synaptic interactions from the hippocampus to the neocortex [12]. While the strong reciprocal connections between the PRh and the hippocampus imply an interaction in mnemonic functions [17], evidence points to distinct roles for the two regions [18]. The hippocampus is especially important in the processing of spatial information [2], while the PRh seems to be more involved in object recognition memory. In this respect, many studies have demonstrated that neurotoxic lesions in the hippocampus cause deficits in spatial memory tasks [19–21]. In contrast, PRh lesions induce recognition memory deficits in both spontaneous recognition and object recognition tasks [3]. This requires that information have to be maintained over a short period of time. Immediate early gene imaging studies have shown that increased level of c-Fos in the hippocampus or in the PRh strongly supports the functional dissociation between spatial learning tasks (hippocampus-dependent) and recognition tasks (PRh-dependent) [22,23]. Furthermore, the rhinal complex plays an important role in information storage and the ablation of this complex induces an impairment in learning and retention of new discrimination problems [24].

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It has been reported that MTL lesions are associated with impairment on traditional working memory tasks, such as delayed alternation [25,26]. Rats with PRh lesions displayed a significant delay-dependent deficit in the T-maze [18] and in the radial arm maze [6]. Davachi and Goldman-Rakic showed that the PRh in primates is activated during the performance of both object and spatial working memory tasks (delayed spatial alternation, delayed object alternation and delayed match-to-sample) [27]. Studies carried out on humans have illustrated that PRh is involved in verbal working memory [28] and episodic memory [29]. Therefore, the PRh seems to play a pivotal role in remembering information over a brief time period, in specific working memory processes [2,6,27,30,31]. Due to the close reciprocal connections with the hippocampal formation, it has been suggested that PRh may contribute directly to the hippocampal-dependent consolidation process of contextual fear conditioning [5].

The aim of the present work was to investigate the contribution of the PRh to the neural mechanisms involved in the acquisition and retention of working memory information. PRh lesions were induced by local injection of ibotenic acid (IBO), an excitotoxin analogue of kainic acid. IBO injections are known to induce relatively selective lesions, characterized by a low diffusion rate and affecting local cell bodies while sparing "en passage" fibers [32,33]. To clarify the involvement of the PRh in the acquisition of the working memory, we examined the effect of the PRh lesions 32 day before and after acquisition of a delayed alternation task in the water escape T-maze.

2. Materials and methods

2.1. Subjects

Thirty-nine male Sprague–Dawley rats weighing 200–250 g were included in the experiments (Charles River, Italy). Rats were housed in groups of three under controlled light (lights on from 07.00 to 19.00 h), temperature (22 ± 2 °C) and humidity (65%) conditions. Rats were allowed free access to standard laboratory diet and tap water. Animals were tested during the light period (between 09.00 and 15.00 h). All experimental procedures using animals were carried out under the National Animal Welfare Act and were approved by the local ethical committee (Veterinary Service of the University of Bologna). Efforts were made to minimize suffering and the experiment was designed to keep the number of animals used to a minimum.

2.2. Experimental design

The experimental design was divided in experiment 1 (lesion after the acquisition phase) and experiment 2 (lesion before the acquisition phase), as shown in Table 1.

In experiment 1, during the acquisition phase rats were trained in the water escape T-maze. Rats who reached the criterion of the behavioral test (>70% correct responses) were randomly divided into two groups and subjected to either sham lesions (sham post) or IBO lesions (PRh post). After a 32-days recovery period, both sham post and PRh post groups were subjected to the retention test.

In experiment 2, lesions were made before the acquisition phase in the water escape T-maze. Animals were randomly divided in two groups: one group was subjected to sham lesions (sham pre) and the other to IBO lesions (PRh pre). After surgery, rats were allowed to recover for 32 days and then trained in the delayed-alternation task (acquisition phase). After 32-days, both sham pre and PRh pre groups were subjected to the retention test.

In both experiments 1 and 2, the retention test was performed according to the same procedure described below.

Table 1
Experimental design.

Experiment	*N*	Surgery	Time of surgery
1	8	Sham lesion	After training
1	11	IBO lesion	After training
2	8	Sham lesion	Before training
2	12	IBO lesion	Before training

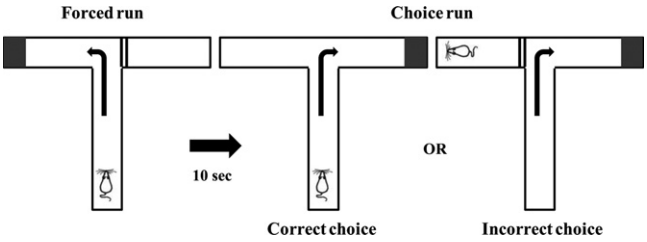

Fig. 1. Water T-maze. The delayed alternation task consists of a forced run and a choice run. During the forced run the rat is forced to turn to the left (as illustrated) or to the right depending on the position of the platform. During the choice run, the platform is located in the opposite arm (the arm not visited during the forced choice) and its finding is considered a correct choice. If the animal enters the wrong arm of the maze (incorrect choice), the door will be closed behind the rat, forcing it to swim in the arm without access to the platform. Acquisition phase consisted of 10 trials (30 s inter trial interval) a day, for 10 consecutive days.

2.3. Surgery

Animals from the two experimental groups (described above) were randomly assigned to either obtain a PRh-lesion or sham lesion. Rats were first anesthetized with equitesine (sodiopenthobarbital/chlorale hydrate) 0.4 ml/100 g and their heads were secured in a Kopf stereotaxic frame. Under aseptic conditions, the skull was exposed and a series of three holes was drilled above the intended lesion side. Bilateral neurotoxic lesions of the PRh were performed via injection of IBO (10 μg/μl; Sigma, St. Louis, MO) dissolved in 0.1 M sodium phosphate buffer (PB). Three injections for each side (0.1 μl) were made at the following coordinates: 3.3, 4.3 and 5.3 mm posterior to bregma; 6.4, 6.5 and 6.6 mm lateral to the midline; 5.5, 5.5, and 5.3 mm ventral from the skull surface respectively [34]. Rats were allowed to recover for a 32-day period before behavioral training and memory retrieval test. Sham-lesioned animals were anesthetized and holes were drilled as above, but no injections were made.

2.4. Water escape T-maze

The water escape T-maze was used as previously described [35]. The apparatus consisted of a black plexiglas T-shaped pool, 40 cm deep, filled with water (23 ± 1 °C). The main alley (100 cm long, 20 cm wide) was connected to two lateral arms (right and left, 45 cm long, 20 cm wide) by two manually operated sliding doors. A submerged (2 cm below the surface of the water) rectangular platform (plexiglas, 15 cm × 18 cm) was located at the end of one of the two lateral arms (Fig. 1).

All the extra maze visual cues were eliminated. The aim of the task was to swim down the main alley and to reach the platform.

The delayed alternation task consisted of a forced run and a choice run, as shown in Fig. 1. During the forced run, one of the two doors was closed. The animal swam to the open arm and was allowed to rest 10 s after reaching the platform. After a further 10 s retention delay (intra-trial interval), the rat was placed back in the main alley to perform the choice run. During the choice run, both sliding doors were kept open, and the platform was placed in the arm that was closed during the forced run. In this way, the animal learned to remember the previously explored arm and to choose the opposite one in order to reach the platform. If the correct arm was chosen, the animal was allowed to rest on the platform for 10 s, and taken out of the maze for a 30 s inter-trial interval. If the wrong arm was chosen, the sliding door was closed behind the rat, forcing the animal to swim around for 10 s. Afterwards, the door was re-opened, and the animal was allowed to reach the platform located at the end of the opposite arm, where it rested for another 10 s, and then taken out of the maze for a 30 s inter-trial interval. During the intra-trial and inter-trial intervals, animals were placed in a plastic holding cage (27 cm × 27 cm × 23 cm) adjacent to the maze. A 3-day preliminary training preceded the acquisition phase: on day 1 (habituation day), animals were gently introduced in the water T-maze and allowed to swim for 1 min, without access to the platform, and on days 2 and 3, the animals were subjected to 10 trials of forced-forced alternating runs, i.e. trials in which both runs were forced, with the second run always giving access to the arm opposite to the first. The acquisition phase consisted of daily sessions of 10 forced and 10 choice trials, using each day a different pseudorandom sequence of forced runs (for example L-R-L-L-R-L-R-R-L-R). The animals were trained during 10 days, and acquisition of the task was assessed by the percentage of correctly performed choice runs (for example seven correct choice runs out of ten = 70% correct responses). The group of animals was considered trained when they reached the threshold of 70% correct responses for three consecutive days.

2.5. Histology

Histological verifications of lesions were performed after the completion of all behavioral experiments. Rats were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline, followed by a