Essential Role of the Perirhinal Cortex in Complex Tactual Discrimination Tasks in Rats

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We designed a battery of tactual discrimination tasks to study whether rats with perirhinal cortex (Prh) lesions had any deficit in resolving complex/ambiguous tactual tasks in the dark. Animals had to discriminate among 3 stimuli simultaneously exposed in 3 arms of a 4-arm plus-shaped maze. Rats with Prh lesions showed a profound impairment in a texture discrimination learning task when the stimuli had a high or intermediate degree of feature ambiguity (experiments 1a and 1b), but not when they had a low degree of feature ambiguity (experiment 1c). Hippocampal lesions, however, did not cause any impairment in task acquisition even when the stimuli had a high degree of feature ambiguity (experiment 2). Experiments 3a, 3b, and 4 showed that perirhinal and control rats performed the task similarly when the animals had to discriminate on the basis of simple/individual, nonoverlapping features of the stimuli (size) with different levels of difficulty. Finally, to isolate the task’s memory functions from its perceptual functions, a reversal learning task revealed a profound deficit in the initial learning phase, but unimpaired learning in the reversal phase with identical stimuli (experiment 5). The findings suggest that the Prh plays an essential role in somatosensory perceptual functions.

Keywords: discrimination learning, medial temporal lobe, perirhinal cortex, radial maze, rat

Introduction

Research involving rats, nonhuman primates, and humans has suggested that structures in the medial temporal lobe (MTL), including the hippocampus and its related cortices (entorhinal, perirhinal, and postrhinal), constitute a “MTL memory system” specialized in declarative/relational learning and memory (Mishkin 1978; Squire and Zola-Morgan 1991; Brown and Aggleton 2001; Eichenbaum et al. 2007). This view, known as the “mnemonic hypothesis” of the MTL function, posits that this region participates in the learning and memory process, but not in perception (Suzuki 2009; Suzuki and Baxter 2009; Suzuki 2010; Kim et al. 2011). Supporting this view, a considerable number of studies in rats and monkeys have demonstrated, using a variety of delayed recognition tasks, a delay-dependent deficit following perirhinal cortex (Prh) lesions (Zola-Morgan et al. 1989; Meunier et al. 1993; Mumby and Pinel 1994; Bussey et al. 1999; Winters et al. 2004, 2008; Brown et al. 2010).

Within the last 18 years or so, a growing body of reports has challenged such a conception, suggesting that the MTL might also play an essential role in certain forms of high-level perception, in addition to its originally accepted role in relational learning and memory. This view is known as the “perceptual-mnemonic hypothesis” of the MTL (Bussey and Saksida 2002; Bussey et al. 2002; Buckley and Gaffan 1998; Buckley et al. 2001; Bussey et al. 2003). A few studies using rats have focused on the perceptive functions of the Prh, but most of them support the perceptual-mnemonic hypothesis in the visual (Eacott et al. 2001; Norman and Eacott 2004; Bartko et al. 2007a; but see, Aggleton et al. 2010; Clark et al. 2011), auditory (Lindquist et al. 2004), and olfactory modalities (Feinberg et al. 2012). These data are in concordance with anatomical studies showing that the higher-order cortical areas of each sensory system project to the Prh (Suzuki and Amaral 1994; Burwell and Amaral 1998; Furtak et al. 2007; Kearly and Commins 2011), suggesting that the Prh might represent a common mechanism for perceptual disambiguation of the complex representations of different sensory modalities. That the Prh has a functional role in processing tactual information is suggested by experiments showing a deficit in tactile-to-visual crossmodal recognition in rats with Prh lesions (Winters and Reid 2010; see also Goulet and Murray 2001; Taylor et al. 2006; Holdstock et al. 2009).

Up to now, however, it has not been demonstrated that the Prh is necessary for representing ambiguous/complex somatosensory/tactual information in the rat. The aim of the present study was to investigate the effect of Prh lesions on a series of tactual discrimination tasks that differed in the nature and complexity of the stimuli or stimulus configurations. The results suggest that the role of the Prh is determined not only by the difficulty of the task, but rather by the complexity of the stimuli used.

Materials and Methods

Subjects

The subjects were 120 male Wistar rats from Harlan Laboratories (Barcelona, Spain). Specifically, the number of animals per experiment was (n lesioned vs. n controls): Experiment 1a = 8 versus 7; experiment 1b = 7 versus 7; experiment 1c = 8 versus 10; experiment 2 = 8 versus 7; experiment 3a = 7 versus 8; experiment 3b = 7 versus 8; experiment 4 = 7 versus 8; experiment 5 = 7 versus 6. The rats, initially weighing between 270 and 300 g, were individually housed in single cages and maintained on a 12:12 h light:dark cycle at a constant temperature of 22 ± 1°C. Behavioral testing was carried out in the morning, during the light phase of the cycle; however, during this period all the lights of the experimental room were turned off, and the only light source was a red-tinted light bulb of 25 W. The bulb was hanging from the ceiling 1.6 m above the center of the testing apparatus. All experimental procedures were performed in conformity with European (86/609/EEC) and Spanish (BOE 252, 2005) legislation and
were approved by the Ethics Committee for Animal Research of the University of Granada.

**Surgery**

**Perirhinal Lesions**

Under the effects of sodium pentobarbital anesthesia (50 mg/kg, intraperitoneally, i.p., Sigma Chemical, St. Louis, MO, USA), the rats were placed in a David Kopf stereotaxic apparatus (mod. 900, David Kopf Instruments, Tujunga, CA, USA) with the incisor bar adjusted, so that lambda and bregma were level. Rats were randomly assigned to either an experimental group or to a control group. The lesioned subjects received bilateral injections of N-methyl-D-aspartic acid [NMDA, Sigma Chemical, phosphate-buffered saline (PBS), pH 7.4, 0.07 M] through the insertion of a 30-gauge stainless steel cannula in 8 sites of the Prh. The cannula was oriented laterally at 26° from the vertical. The coordinates were derived from the atlas of Paxinos and Watson (1998) and based on the anatomical location of the Prh, as delineated by Burwell and colleagues (Burwell et al. 1995; Burwell and Amaral 1998; Furtak et al. 2007). The anteroposterior (AP) stereotaxic coordinates were calculated relative to the bregma, the lateral (L) relative to the midline, and the dorsoventral (V) relative to the top of the skull: AP = −2.5, L = ±2.4, V = 9.8; AP = −3.6, L = ±2.9, V = 9.8; AP = −4.8, L = ±3.3, V = 9.8; AP = −5.8, L = ±2.8, V = 9.8. NMDA was administered in a 0.4-µL volume at each site through the cannula that was attached to a 5-µL Hamilton microsyringe (Teknokroma, Barcelona, Spain). Delivery of the solution was carried out with a Harvard Apparatus pump set (model 22, Panlab-Harvard Apparatus, Barcelona, Spain) at an infusion rate of 0.1 µL/min. The cannula was left in situ for an additional 5 min before being withdrawn. The control groups received identical surgical procedures with the exception that equivalent volumes of PBS were infused into the Prh.

**Hippocampal Lesions**

The initial procedure was the same as that used for the perirhinal lesions. The dorsal and ventral hippocampi were damaged at 7 different anteroposterior sites in relation to the interaural zero point (Paxinos and Watson 1998): AP = ±5.9, L = ±1.6, V = ±6.5; AP = ±4.8, L = ±2.5, V = ±6.5; AP = ±4.2, L = ±5.2, V = ±3.4; AP = ±3.8, L = ±3.2, V = ±6.5; AP = ±3.8, L = ±5.0, V = ±4.2; AP = ±3.0, L = ±4.0, V = ±5.4, and AP = ±3.0, L = ±5.0, V = ±4.0. The procedure for the bilateral injections of NMDA (Sigma Chemical, PBS, pH 7.4, 0.07 M) was the same as that used for the perirhinal lesions.

**Apparatus**

A 4-arm plus-shaped maze, built by the University of Granada Technical Services Department, was used. Each arm of the maze measured 60 cm in length × 10 cm in width and was connected to an octagonal central platform 35 cm in diameter. The walls of the central platform were made of transparent Plexiglas and were 15 cm in height. The walls of each arm were made of wood and measured 5 cm in height. The maze was 60 cm from the floor.

**Behavioral Procedure**

**Experiments 1a, 1b, and 1c**

**Acquisition of Tactual Discriminations with Varying Degrees of Feature Ambiguity: High, Intermediate, and Low.** To investigate whether the Prh is required to disambiguate somatosensory representations with feature ambiguity, we developed a discrimination learning task in which rats had to discriminate among 3 grades of sandpaper of different texture, simultaneously exposed in a 4-arm plus-shaped maze. One of the arms of the maze was used as the starting arm, while in the other 3 arms sandpaper of different textures was placed all along the floor. In this and the following experiments, the sandpaper or other floor insert placed in the arms of the maze extended 5 cm into the central platform of the maze, which allowed for simultaneous exposure to the 3 stimuli. In experiments 1a, 1b, and 1c, the degree of feature overlap among the discriminanda was determined by the average particle diameter of the sandpaper and by the density of grain in an area measuring 0.5 cm². Textures defined by these 2 variables share a number of features, making them complex tactual stimuli that are difficult to discriminate (Johnson 2001; Maricich et al. 2012; Wu et al. 2013). In experiment 1a, the average particle diameter and the mean grain density were very similar in all 3 textures, and the discrimination thus presented a high degree of feature ambiguity. In experiments 1b and 1c, the values were further apart from each other, so the tasks presented an intermediate and a low degree of feature ambiguity, respectively. In each of these experiments, we evaluated the magnitude of the impairment following Prh lesions. In these and the following experiments, the dependent variable used was mainly the percentage of correct responses during each day of the training. Experiments 1a, 1b, and 1c, and the rest of the experiments of the current series, were performed in the dark, with the sole source of illumination being a red-tinted light bulb. Based on the previous data obtained in the visual modality (Bussey et al. 2002, 2003; Murray et al. 2007; Bartko et al. 2007a; Saksida and Bussey 2010; see also Burwell and Witter 2002), we hypothesized that Prh-lesioned animals would present the higher levels of impairment the greater the degree of feature ambiguity.

**Intramaze Stimuli.** The intramaze stimuli used in these experiments were 3 pieces of aluminum oxide sandpaper that differed in roughness. The roughness of the texture was determined by the grain density and by the average particle diameter of the sandpaper. In experiment 1a, the 3 stimuli were very similar to one another. The mean grain density was calculated by counting, under the microscope (Olympus SZ40, Técnicas Médicas MAB, Barcelona, Spain) the number of grains per 0.5 cm². This operation was carried out 4 times on each grade of sandpaper, in different randomly selected areas. To facilitate the counting, we used a coordinate grid upon which we had marked columns of 0.5 × 0.1 cm². The microslide was placed over the different areas in which the counting was done. The results indicated an average grain density of 147.2 ± 5.6, 222.2 ± 4.1, and 294.0 ± 9.1 per 0.5 cm². The average particle diameter of each grade of sandpaper, according to information provided by the manufacturer (http://www.sgabrasivos.es), was 156.0, 97.0, and 78.0 µm, respectively. The sandpaper was Debray or Norton brand and was supplied by A. Debray S. en C. (Barcelona, Spain) and by Saint-Gobain Abrasivos S.A. (Navarra, Spain); the reference numbers of the grades were P100, P150, and P180, respectively. These 3 stimuli, classified as having an apparently high degree of feature ambiguity, were used in experiments 1a and 2. In experiment 1b also, 3 grades of sandpaper were used but with an average density per 0.5 cm² of 106.5 ± 5.3, 603.0 ± 9.9, and 1194.2 ± 18.2 grains, respectively. The average particle diameter was 326.0, 58.5, and 27.75 µm, respectively (reference numbers of the grades: P50, P240, and P600, respectively). These 3 stimuli were classified as having an apparently intermediate degree of complexity. Finally, in experiment 1c, the 3 grades of sandpaper used had an average density per 0.5 cm² of 66.8 ± 5.9, 886.4 ± 16.3, and 1771.7 ± 44.1 grains, respectively. The average particle diameter was 412.0, 40.5, and 15.3 µm, respectively (reference numbers of the grades: P40, P360, and P1200, respectively). These 3 stimuli were classified as having an apparently low degree of complexity.

In all the experiments, one of these intramaze stimuli was associated with the reward in 50% of the animals, while a different stimulus was associated with the reward in the remaining 50%.

**General Training Procedure.** In all the experiments conducted in the current series, the rats were given 10–12 days to recovery from the surgery. Following this period, all subjects were placed on a food-deprivation schedule to maintain them at 90% of their free-feeding body weight. Beginning on the same day as the deprivation program, all rats were handled on 7 successive days for 10 min each. On the following day, training began for the various discrimination learning tasks. Rats received 8 trials per session, 1 session per day, until they reached learning criterion. It was...
considered that an animal had reached the learning criterion when its
performance on 2 consecutive days was 87%. At the beginning of a
trial, the 4 guillotine doors separating the arms from the central
platform were raised, and the rat was placed at the end of the starting
arm, with its back to the central platform. The reward, two 45-mg
food pellets (P.J. Noyes Company, Inc., Lancaster, NH, USA), was
placed in the food cup located at the end of the goal arm.
Identification of the goal arm by smell was prevented by placing 5
inaccessible 45-mg food pellets under each of the 4 arms. The pellets
were placed at the end of each arm, under the food cup, using
adhesive tape. The pellets were replaced by fresh ones every 2 days.
The order in which each of the 4 arms was used as starting or goal
arm was randomized from trial to trial. The rat was considered to
have made a choice when, having entered an arm, it crossed the
halfway point with all 4 limbs. After a choice was made, the guillotine
doors were lowered and the animal was left in the chosen arm for
10–12 s. The rat was then picked up and confined in a box for an
intertrial interval of 30 s. When the animals showed signs of a certain
amount of learning, specifically when in the previous day’s training
session they had reached a performance of 62% (5 correct trials of 8),
the intramaze stimuli on the floor of the arms were replaced, halfway
through each animal’s daily session, by new ones or by ones that had
been used by other animals in order to control the use of olfactory
cues by the rats.

Experiment 2
High Feature Ambiguity Tactual Discrimination in
Hippocampus-Lesioned Rats. To better understand the
heterogeneity of functions within the MTL, experiment 2 studied
whether hippocampal lesions produced a deficit in discrimination
among textures, using the hard problem previously used in
experiment 1a. Based on the previous data obtained in the visual
modality, we hypothesized that there would be no deficit in
hippocampal-lesioned rats (Saksida et al. 2006, 2007).

Intramaze Stimuli. They were the same ones used in
experiment 1a.

Experiments 3a and 3b
Hard and Easy Tactual Discrimination of Size. Since
experiments 1a, 1b, and 1c did not have an explicit control for
difficulty, it could be argued that the cause of the deficit is the
difficulty of the task per se and not the feature ambiguity.
Experiments 3a and 3b were conducted to dissociate between a
perceptual deficit caused by the complex nature of the stimuli and
one caused by the task’s difficulty per se. In previous experiments
performed on the visual modality, controls for difficulty have used
visual discrimination tasks between items with simple features (i.e.
size, shape, color, etc.). In these experiments animals with Prh
lesions showed impairment when the discriminations involved
complex stimuli that required conjunctive representation, but no
impairment when the discrimination involved simple/individual
features, even though the discriminanda were very similar and the
task was very difficult (Buckley et al. 1997, 2001; Bussey et al.
To demonstrate that the Prh is necessary only for tactual discriminations
when the discriminanda share many features and have a certain
degree of complexity, regardless of task difficulty, in these
experiments perirhinal and control rats performed a size
discrimination learning task designed in our lab. On this occasion, 3
pieces of wood of different heights/sizes were placed along the floor
of 3 arms of the 4-arm plus-shaped maze, with the wood extending 5
cm into the central platform of the maze. Thus, once the animals
reached the central platform, they had to discriminate between the
size of the 3 “steps” simultaneously exposed. In experiment 3a, the
steps were very similar to one another in size, and the discrimination
was thus very difficult. Once the animals of experiment 3a had
learned this task, they began a second experimental phase in which
the rats were subjected to a high feature ambiguity discrimination
task identical to the one used in experiment 1a. In experiment 3b,
other groups of rats learned an easy task requiring tactual
discrimination of size. On this occasion, the range of values between
the stimuli was greater than in experiment 3a.

Intramaze Stimuli. The intramaze stimuli used in these
experiments were 3 planks of wood measuring 10 × 65 cm. The
difference among the 3 stimuli was their height. In experiment 3a, the
discrimination was very difficult as the heights of the 3 planks were
8, 12, and 16 cm. In experiment 3b, however, the discrimination
learning task was easy (heights of 8, 16, and 24 cm).

Experiment 4
Hard Tactual Feature-Based Discrimination (Wire Mesh). In
an effort to further dissociate between a perceptual deficit caused by
the complex nature of the stimuli and one caused by the task’s
difficulty per se, in the present experiment we increased the difficulty
of the task. Now, the animals had to discriminate among 3 pieces of
wire mesh placed along 3 of the 4 arms of the maze. The pieces of
wire mesh differed from one another in the size of the squares. A
previous pilot experiment in neurologically intact rats in our lab had
shown that the task was very difficult, so in the present study the rats
were trained for 14 consecutive days. After this initial training, a
second phase of the experiment began, in which the same rats were
trained in an intermediate feature ambiguity tactual discrimination
task, identical to the one used in experiment 1b.

Intramaze Stimuli. The intramaze stimuli used were 3 pieces of
wire mesh measuring 10 × 65 cm. The wire had a diameter of 1 mm in
all 3 pieces, and the difference between the pieces of mesh was the
size of the squares. Specifically, the squares measured 0.6 × 0.6,
1.2 × 1.2, and 1.8 × 1.8 cm.

Experiment 5
Reversal Learning of a High Feature Ambiguity Tactual
Discrimination. Since in the previous experiments the
discrimination learning tasks involved reward and presentation of the
same stimuli over multiple trials, deficits in these paradigms may be
interpreted as impairments in learning and memory processes and
not as a perceptual deficit (Shrager et al. 2006; Squire et al. 2006;
Suzuki 2009; Clark et al. 2011; Kim et al. 2011). To adequately isolate
the perceptual demands of the task from its learning and memory
demands, in the present experiment rats that learned a 3-choice high
feature ambiguity task (as in experiment 1a) were subjected
immediately after reaching criterion to a reversal learning paradigm.
During this training, the texture that was initially rewarded
was not rewarded, and a different texture was rewarded instead.
This way, when the rats start the reversal learning task they have already
demonstrated their ability to discriminate among the 3 textures,
having achieved perceptual mastery of the problem. Therefore,
during reversal learning, the only new demand required of the
animals is to learn and retain a new association. Thus, the reversal
learning provides a measure of the animal’s mnemonic capacity
without new demands on the perceptual or representational process
(Gaffan and Harrison 1986; Hampton and Murray 2002; Hampton

According to the considerations set forth above, if the deficit ob-
served in the Prh-lesioned rats of experiments 1a and 1b was due to a
mnemonic failure, the Prh-lesioned rats should fail during the initial
learning and reversal learning phases of the present experiment.
However, if the impairment were due mainly to a perceptual/rep-
sentational failure, then the Prh-lesioned rats should fail only
during the learning, but not during the reversal learning.

In this experiment, animals were trained in the same task used in
experiment 1a. Each rat ended the first phase of the experiment (the
learning phase) when it reached criterion. The next day, the second
experimental phase (reversal learning) began. This phase ended
when all the animals reached learning criterion again. During the first
phase, in half of the animals, the reward was associated with the
finest sandpaper (147.2 grains) and in the other half it was associated
with the roughest (294.0 grains). During the reversal learning task, the reinforcement contingencies were reversed.

**Intramaze Stimuli.** They were the same as the ones used in experiment 1a.

**Histology**

After each experiment, when the behavioral testing was completed, the rats were deeply anesthetized with sodium pentobarbital (90 mg/kg, i.p.) and perfused intercardially with 0.9% saline, followed by 10% formalin. After extraction from the skull, the brains were post-fixed in 10% formalin for several days and subsequently in 10% formalin–30% sucrose until sectioning. Coronal sections (50 µm) were cut on a cryostat (Leica CM 1850, Leica Microsystems, Germany) and stained with cresyl violet, a Nissl stain.

To quantify the extension of the damage in each lesioned rat, regions of cell loss and gliosis identified microscopically were plotted on drawings of coronal sections from the atlas of Paxinos and Watson (1998). For each perirhinal-lesioned rat, the reconstruction of the lesion was made based on 8 coronal sections (AP levels from the bregma: −3.3, −3.8, −4.3, −4.8, −5.2, −5.6, −6.0, and −6.3). Each coronal section was digitized and the lesioned area was measured in square millimeters by a computer program (Autocad, version 2004). The anatomical limits of the perirhinal, entorhinal, and postrhinal cortices were defined using works by Burwell and associates (Burwell et al. 1995; Burwell and Amaral 1998; Burwell 2001; Furtak et al. 2007). The volume of damage was expressed as a percentage of normal volume.

**Results**

**Experiments 1a, 1b, and 1c**

**Histological Findings**

Tissue damage was microscopically identified by pronounced thinning of the cortex, necrosis, or missing tissue. An 1-way analysis of variance (ANOVA) indicated that the percentage of perirhinal damage among the Prh-lesioned rats of experiments 1a, 1b, and 1c was similar (F < 1). The lesions, aimed at areas 35 and 36, were generally limited to the target area, creating a longitudinal groove on both sides of the rhinal fissure (Figs 1 and 2 and Table 1). In general, the lesion affected the 6 layers of the Prh and only reached the rostral portion of the post-rhinal cortex in 2 animals used in experiment 1a, 1 animal used in experiment 1b and 1 animal used in experiment 1c. The Cornu Ammonis 1 (CA1) field in the ventral hippocampus was minimally affected in 1 rat in the experimental groups of experiments 1a and 1b and in 2 rats of experiment 1c. The lateral entorhinal cortex and the ventral temporal association areas were affected to varying degrees in 3 and 3 (experiment 1a), 4 and 3 (experiment 1b), and 2 and 3 rats (experiment 1c), respectively, always unilaterally. Area 35 was slightly more affected than area 36 in all experimental rats (Table 1).

**Behavioral Findings**

Figure 3A shows the performance of the Prh and control groups during the 10 days of training in the high feature ambiguity discrimination task (experiment 1a). A 2-way ANOVA (lesion × days) revealed a significant effect of lesion (F<sub>1,13</sub> = 15.00, P < 0.001), days (F<sub>9,117</sub> = 33.67, P < 0.0001), and interaction (F<sub>9,117</sub> = 3.58, P < 0.001). Post hoc Newman–Keuls comparisons indicated that no significant differences between the Prh and the control groups were present in either the early or the final days of training, but they were present during the intermediate days (day 5, P < 0.005; day 6, P < 0.0003; day 7, P < 0.001; and day 8, P < 0.01). Finally, an 1-way ANOVA detected significant differences in relation to the number of incorrect trials to criterion (F<sub>1,13</sub> = 16.72, P < 0.001; mean Prh group 39.9 ± 5.1 vs. mean control group 25.8 ± 6.4).

Figure 3B represents the performance of the Prh and control groups on the intermediate feature ambiguity discrimination task (experiment 1b). A 2-way ANOVA (lesion × days) indicated a significant effect of lesion (F<sub>1,12</sub> = 13.04, P < 0.01), days (F<sub>9,108</sub> = 99.57, P < 0.0001), and interaction (F<sub>9,108</sub> = 5.42, P < 0.001). The analysis of the interaction revealed that there were significant differences between groups
Figure 2. Coronal sections showing the largest (gray) and smallest (central white area) perirhinal (experiments 1a, 1b, 1c, 3a, 3b, 4, and 5) and hippocampal (experiment 2) lesions. AP coordinates are shown in relation to the bregma (Paxinos and Watson 1998).
only on day 6 of the training (Newman–Keuls, *P* < 0.02). On the other hand, an 1-way ANOVA showed that the number of incorrect trials before reaching criterion was significantly higher in the Prh than in the controls (*F*_{1,12} = 18.69, *P* < 0.001; mean Prh group 30.9 ± 4.1 vs. mean control group 22.1 ± 3.9).

Figure 3C illustrates the performance of the Prh and control groups on the low feature ambiguity discrimination task (experiment 1c). A 2-way ANOVA indicated that perirhinal and control rats discriminated similarly, with no significant differences being detected except for the “days” factor (*F*_{1,16} = 1.56, *P* = 0.22; *F*_{5,144} = 73.95, *P* < 0.0001; and *F*_{5,144} interaction = 1.20, *P* = 0.29). One-way ANOVA also revealed that perirhinal and control animals did not differ significantly in the number of errors to criterion (*F*_{1,16} = 2.98, *P* = 0.10; mean Prh group 20.9 ± 3.7 vs. mean control group 17.6 ± 3.1).

To compare the performance of lesioned and control rats in the 3 tasks with different degrees of feature ambiguity, we performed a general analysis of the data obtained in experiments 1a, 1b, and 1c. On this occasion, the number of errors to criterion was used to evaluate the performance of the subjects. The dependent variable (Fig. 3D). A factorial 2 × 3 ANOVA (lesion × level of feature ambiguity) showed a significant effect of lesion (*F*_{1,141} = 36.33, *P* < 0.0001), level of feature ambiguity (*F*_{2,141} = 28.20, *P* < 0.0001), and interaction (*F*_{4,282} = 4.72, *P* < 0.01). Post hoc Newman–Keuls comparisons indicated a significantly poorer performance by Prh-lesioned rats in the groups learning tasks with high and intermediate levels of feature ambiguity, when compared with their controls (*P* < 0.0001 and <0.002, respectively). However, no significant differences were detected between the Prh-lesioned and control groups in the task with the low degree of feature ambiguity (*P* = 0.20). With respect to the 3 groups with Prh lesions, the performance of the lesioned group in a task with an intermediate degree of feature ambiguity differed significantly from that observed in the lesioned groups learning tasks with high (*P* < 0.0001) and low (*P* < 0.0002) degrees of feature ambiguity. As regards the 3 control groups, the control group faced with a task having an intermediate degree of ambiguity did not differ significantly from the high ambiguity group (*P* = 0.14) or from the low ambiguity (*P* = 0.27) group. However, significant differences were observed when comparing the performance of the high degree of feature ambiguity control group with that of the low degree of feature ambiguity control group (*P* < 0.02). Overall, these data suggest that, within the same stimulus class, perirhinal rats are more impaired when the shared features of the stimuli present a smaller range of values, presumably creating more “ambiguity” or a higher degree of complexity among the stimuli.

### Table 1
Percent damage following Prh lesion

<table>
<thead>
<tr>
<th></th>
<th>Area 36</th>
<th>Area 35</th>
<th>LE</th>
<th>ME</th>
<th>PoC</th>
<th>TeA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1a</strong></td>
<td>8</td>
<td>61.9 ± 4.4</td>
<td>65.3 ± 6.8</td>
<td>5.2 ± 0.7</td>
<td>0</td>
<td>7.8 ± 1.1</td>
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<tr>
<td><strong>Experiment 1b</strong></td>
<td>7</td>
<td>56.7 ± 4.3</td>
<td>64.1 ± 5.8</td>
<td>4.6 ± 0.6</td>
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<td>8.0 ± 0.0</td>
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<tr>
<td><strong>Experiment 1c</strong></td>
<td>8</td>
<td>62.3 ± 5.5</td>
<td>68.8 ± 6.1</td>
<td>3.9 ± 0.5</td>
<td>0</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td><strong>Experiment 3a</strong></td>
<td>7</td>
<td>55.8 ± 4.7</td>
<td>64.2 ± 5.1</td>
<td>6.1 ± 0.6</td>
<td>0</td>
<td>9.6 ± 1.4</td>
</tr>
<tr>
<td><strong>Experiment 3b</strong></td>
<td>7</td>
<td>59.1 ± 5.0</td>
<td>61.8 ± 4.3</td>
<td>4.1 ± 0.4</td>
<td>0</td>
<td>6.7 ± 0.6</td>
</tr>
<tr>
<td><strong>Experiment 4</strong></td>
<td>7</td>
<td>61.1 ± 6.7</td>
<td>69.6 ± 7.2</td>
<td>6.9 ± 0.9</td>
<td>0</td>
<td>10.3 ± 1.7</td>
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<tr>
<td><strong>Experiment 5</strong></td>
<td>7</td>
<td>63.7 ± 7.4</td>
<td>67.2 ± 7.0</td>
<td>5.8 ± 0.8</td>
<td>0</td>
<td>8.1 ± 0.6</td>
</tr>
</tbody>
</table>

Note: Data represent mean ± SEM. The lesions were intended to encompass both areas 35 and 36.

### Experiment 2

#### Histological Findings

As shown in Figure 2, in most of the animals, the lesions began in the anterior pole of the hippocampus, at the most caudal level of the paraventricular nucleus. At this level, the lateral two-thirds appeared to be lesioned in all the rats, but the most medial portion was intact in most of the subjects. At more posterior levels, specifically at the level of the ventromedial nucleus of the hypothalamus and the mamillary nuclei, the lesion had the same configuration, with extensive regions showing necrosis or missing tissue in the hippocampal CA1, CA2, and CA3 fields. At these levels, the dentate gyrus was affected in most of the cases, but its most medial region appeared intact to varying degrees. An average of 77% of the CA1–CA3 fields of the ventral hippocampus was lesioned (range between 59% and 91%), with no damage being observed in the nearby perirhinal–entorhinal region. In general, lesions ended between 3.2 and 2.8 mm posterior to the interaural point according to the atlas of Paxinos and Watson (1998). At this level, the dorsal hippocampal CA1 field and the dentate gyrus were partially affected in most of the rats.

#### Behavioral Findings

Figure 3E shows the performance of the hippocampal and control groups throughout the 10 days of training. A 2-way ANOVA (lesion × days) showed that lesioned and control rats discriminated exactly the same, with no significant differences being detected except in the factor “days” (*F*_{1,13} = 0.29, *P* = 0.61; *F*_{9,117} = 59.99, *P* < 0.0001; and *F*_{9,117} interaction = 1.5, *P* = 0.33). Finally, a 1-way ANOVA revealed that hippocampal and control rats did not differ significantly in the number of errors to criterion (*F*_{1,13} = 0.31, *P* = 0.58; mean hippocampal group 27.8 ± 4.9 vs. mean control group 26.3 ± 4.7).

#### Experiments 3a and 3b

##### Histological Findings

A schematic reconstruction of the Prh lesions appears in Figure 2. The extent of the perirhinal damage was very similar to that observed in previous experiments. Thus, four 1-way ANOVAs indicated similar percentages of perirhinal damage between lesioned animals of experiments 3a and 1a (*F* < 1), experiments 3b and 1a (*F* < 1), experiments 3a and 1b (*F* < 1), and experiments 3b and 1b (*F* < 1).

#### Behavioral Findings

Figure 4A shows the performance of the Prh and control groups during the 14 consecutive days of training in the difficult tactual discrimination of size task (experiment 3a). A 2-way ANOVA (lesion × days) indicated that perirhinal and control subjects discriminated similarly, with no significant differences being found except in the factor “days” (*F*_{1,13} = 2.42, *P* = 0.14; *F*_{13,169} = 31.10, *P* < 0.0001; and *F*_{13,169} interaction = 0.83). Additionally, a 1-way ANOVA revealed that lesioned and control rats did not differ significantly in the number of errors before reaching criterion (*F*_{1,13} = 2.47, *P* = 0.14; mean Prh group 54.9 ± 6.1 vs mean control group 47.1 ± 5.4).

Figure 4B depicts the performance of the same rats as in experiment 3a, but on this occasion during 10 days of training in the high feature ambiguity tactual discrimination task.
2-way ANOVA (lesion × days) showed a significant effect of lesion ($F_{1,13} = 4.79$, $P < 0.04$) and days ($F_{9,117} = 18.14$, $P < 0.0001$), but not of interaction ($F_{9,117} = 0.90$, $P = 0.52$).

When the number of incorrect trials to criterion was employed as a dependent variable, a 1-way ANOVA indicated that Prh rats committed significantly more errors before
reaching the criterion than the control rats did ($F_{1,13} = 4.88$, $P<0.04$; mean Prh group 29.8 ± 7.3 vs. mean control group 20.4 ± 6.9).

Figure 4C shows the performance of the Prh and control subjects during the 12 consecutive days of training in the easy tactual discrimination of size task (experiment 3b). A 2-way ANOVA (lesion × days) revealed that lesioned and control groups discriminated similarly, with no significant differences being found except in the factor “days” ($F_{11,143}$ lesion = 37.02, $P<0.0001$; and $F_{11,143}$ interaction = 1.54, $P=0.12$). In addition, a 1-way ANOVA indicated that Prh and control animals did not differ significantly in the number of errors to criterion ($F_{1,13} = 0.03$, $P=0.84$; mean Prh group 35.5 ± 5.7 vs. mean control group 36.1 ± 5.2).

**Experiment 4**

**Histological Findings**

A schematic reconstruction of the perirhinal lesions appears in Figure 2. The extent of the perirhinal damage was very similar to that observed in previous experiments. One-way ANOVA indicated similar Prh damage in the lesioned animals of this experiment and in the perirhinal-lesioned rats of experiment 1a ($F<1$).

**Behavioral Findings**

Figure 5A illustrates the performance of both groups in the tactual discrimination task involving the wire mesh. A 2-way ANOVA (lesion × days) indicated that perirhinal and control rats discriminated similarly, with no significant differences being detected, except for the “days” factor ($F_{1,13}$ lesion = 0.35, $P=0.56$; $F_{13,169}$ days = 26.75, $P<0.0001$; and $F_{13,169}$ interaction = 1.26, $P=0.24$). One-way ANOVA revealed that perirhinal and control rats did not differ significantly in the number of errors to criterion ($F_{1,13} = 0.30$, $P=0.58$; mean Prh group 52.7 ± 6.1 vs. mean control group 50.7 ± 5.8). During the second phase of the experiment, 2-way ANOVA (lesion × days) revealed that Prh subjects performed the intermediate feature ambiguity tactual discrimination task significantly worse than controls (Fig. 5B, $F_{1,13}$ lesion = 7.01, $P<0.02$; $F_{9,117}$ days = 42.87, $P<0.0001$; and $F_{9,117}$ interaction = 1.91, $P<0.05$). An 1-way ANOVA indicated that the Prh group committed significantly more errors to reach criterion than controls ($F_{1,13} = 9.94$, $P<0.01$; mean Prh group 24.9 ± 4.1 vs. mean control group 16.2 ± 4.0).

In an attempt to compare the difficulty of the various tactual 3-choice discrimination tasks employed so far, we compared the mean number of errors to criterion committed by Prh-lesioned and control subjects in experiments 1a, 1b, 1c, 3a, 3b, and 4. A factorial 2 × 6 ANOVA (lesion × type of stimuli) showed a significant effect of lesion ($F_{1,80} = 18.45$, $P<0.0001$), type of stimuli ($F_{5,80} = 57.60$, $P<0.0001$), and interaction ($F_{5,80} = 2.48$, $P<0.03$; Fig. 5C depicts these results). The interaction analysis with post hoc Newman–Keuls comparisons showed that the Prh-lesioned groups that performed the high and intermediate feature ambiguity discrimination tasks committed significantly more errors to criterion than their respective controls (high feature ambiguity, $P<0.001$ and intermediate feature ambiguity, $P<0.02$). For all other perirhinal-lesioned groups and their controls, no
significant differences were observed (low feature ambiguity, $P=0.33$; size easy, $P=0.84$; size difficult, $P=0.10$; and wire mesh, $P=0.55$). An important result for ruling out a difficulty-related interpretation of the deficit observed with textures (experiments 1a and 1b) is that perirhinal and control rats committed significantly more errors to criterion when they performed difficult size discrimination tasks (experiment 3a) than when they performed the discrimination task with a high degree of ambiguity (Prh groups, $P<0.0001$ and control groups, $P<0.0001$). Finally, Prh and control rats of experiment 4, which performed a feature-based discrimination task, committed significantly more errors than the animals of experiment 1a (Prh groups, $P<0.002$ and control groups, $P<0.0001$) and experiment 1b (Prh groups, $P<0.0001$ and control groups, $P<0.0001$). Together, these data suggest that it is not the difficulty of the task that makes the Prh-lesioned animals of experiments 1a and 1b perform poorly, but rather the type of stimuli used in the task.

Experiment 5

Histological Findings

As illustrated in Figure 2, the extent of the Prh lesions was very similar to that observed in previous experiments of this series. One-way ANOVA found no significant differences between the percentage of damage observed in this experiment and that of experiment 1a ($F<1$).

Behavioral Findings

Figure 6A shows the mean number of errors to criterion for the Prh and control groups during the initial phase of training. An 1-way ANOVA indicated that the Prh rats committed significantly more errors to criterion than control subjects ($F_{1,11}=8.57$, $P<0.01$). Consequently, the mean number of days required to reach criterion was also significantly higher in the Prh group ($F_{1,11}=9.58$, $P<0.01$; mean Prh group 9.9 ± 1.8 vs. mean control group 6.7 ± 1.6).

One day after reaching criterion in the training phase, rats began the reversal learning phase. Figure 6B shows the performance of both groups until all the animals reached the acquisition criterion again. A 2-way ANOVA (lesion × days) revealed that lesioned and control rats performed the reversal learning similarly, with no significant differences except the factor “days” ($F_{15,165}$ lesion = 1.72, $P=0.22$; $F_{15,165}$ days = 57.23, $P<0.0001$; and $F_{15,165}$ interaction = 0.49, $P=0.94$). In addition, during the reversal learning, the performance of the lesioned animals and the controls did not differ significantly in the number of errors before reaching criterion (Fig. 6C, $F_{1,11}=0.89$, $P=0.36$) or in the number of days to criterion ($F_{1,11}=0.01$, $P=0.91$; mean Prh group 9.8 ± 3.2 vs. mean control group 9.7 ± 3.0).

Discussion

The main findings of the present study indicate that rats with Prh lesions have a profound impairment in resolving texture discrimination learning tasks when the stimuli present high or intermediate complexity (experiments 1a and 1b). However, within the same stimulus class, no differences were detected when the discriminationanda had a low degree of feature ambiguity (experiment 1c). This deficit is perirhinal-dependent since hippocampal lesions did not cause any impairment in the acquisition of the task with high feature ambiguity (experiment 2). The nature of the stimuli seems to be a critical factor in the occurrence of the above deficit. In support of this, experiments 3a, 3b, and 4 show that, when the rats performed a task that was similar but based on simple/individual features of the stimuli, Prh-lesioned and control subjects performed with the same degree of mastery.
implies a high-level perceptual/representational function for the Prh in tasks with tactual stimuli, a function that must be added to its recognized function in memory.

Given that the dependent variable in the current series of experiments was based on the performance of discrimination learning tasks over multiple trials, impairment in associative learning as well as long-term memory could have contributed to the deficit observed in experiments 1a and 1b (Hampton 2005; Suzuki 2009; Baxter 2009). However, the data obtained in experiment 5 indicate that this criticism cannot be easily accepted. In fact, during reversal learning, the perceptual demands of the task are drastically reduced, while the mnemonic demands of the task remain unchanged. Therefore, since during the reversal learning phase Prh and control rats performed similarly, the deficits observed in experiments 1a, 1b, and 5 (the learning phase) probably cannot be attributed to impaired learning or memory per se. Therefore, our data point to a primary deficit in perceptual functions, but not in memory.

Another possibility is that the impairment observed in the texture discrimination tasks (experiments 1a and 1b) was not only due to a perceptual deficit, regarding complex stimuli, but also to task difficulty per se. If this view were correct then the performance of any difficult tactual discrimination task might be compromised after Prh damage. However, data from experiments 3a and 4 in the current series show no significant differences between Prh and control animals despite the difficulty of both tasks. Importantly, in the second part of these experiments, when the same rats had to perform a discrimination learning task involving textures with a high or intermediate degree of feature ambiguity, a profound deficit was observed. Our data thus suggest that the complexity of the stimuli, rather than the difficulty of the discrimination, is what determines the presence of the deficit.

These data agree with previous studies performed on monkeys using complex visual discrimination and oddity tasks (Buckley et al. 1997, 2001; Bussey et al. 2002, 2003; Hampton and Murray 2002; Baxter 2009). In the above reports, mainly the size, color, or shape of objects were used to control for difficulty in monkeys. Results showed that simply the increment in task difficulty was not enough to produce a deficit in animals with Prh lesions. In contrast, in these studies, and in agreement with our data, the key factors determining the deficit in discrimination in the Prh-lesioned animals were the attributes of the stimuli. Thus, impairment was only observed when discriminations contained a certain degree of feature ambiguity (Bussey and Saksida 2005; Bussey et al. 2006; Murray et al. 2007). In conclusion, our data suggest that the Prh does play an essential role, but not in any type of perceptually difficult discrimination. Thus, the role of the Prh may be essential only in complex discriminations in which feature ambiguity is the critical factor that generates the difficulty.

The central finding of the present study is the profound deficit observed in rats with Prh lesions when they performed a tactual discrimination learning task with a high or intermediate degree of feature ambiguity (experiments 1a, 1b, and 5). Although previous studies have shown that the Prh is critical for visual recognition memory (Mumby and Pinel 1994; Brown and Aggleton 2001; Winters and Bussey 2005; Winters et al. 2008) and for disambiguating visual discrimination tasks with complex stimuli (Eacott et al. 2001; Saksida and Bussey

Despite the difficulty of these tasks, the Prh-lesioned rats were only impaired when the stimuli shared certain characteristics that made them very similar perceptually. Under these conditions, the rats, in our opinion, had to use conjunctive representations to facilitate the identification of the stimuli. Finally, to dissociate between a perceptual deficit and a deficit in learning or memory abilities, experiment 5 showed a profound impairment in Prh rats during the phase of discrimination learning with complex stimuli, but the absence of deficit during the reversal learning phase using the same set of stimuli. Taken together, these data imply a high-level perceptual/representational function for the Prh in tasks with tactual stimuli, a function that must be added to its recognized function in memory.
differences were detected between Prh-lesioned and control animals. In contrast, when the stimuli had a low level of feature ambiguity (experiment 1c), the degree of task interference decreased and no differences were detected between Prh-lesioned and control rats in the performance of the task. The results therefore suggest that pattern separation for somatosensory information is affected by stimulus interference in rats with Prh damage (Hunsaker and Kesner 2013).

Besides, the results presented in the current series, to our knowledge, only a very few studies have shown in rats an essential involvement of the Prh in complex discriminations using nonvisual information. In the auditory modality, Lindquist et al. (2004) showed that Prh lesions blocked the acquisition of fear delay conditioning when complex auditory stimuli are employed as conditioned stimuli (ultrasonic vocalization), but not to simple tones as conditioned stimuli (see also Campolattaro and Freeman 2006a, 2006b). Also in the olfactory modality, Feinberg et al. (2012) recently showed, using an odor recognition memory paradigm with different delay intervals, that the Prh is only essential when the stimuli have high/intermediate complexity. Specifically, in the choice phase, Prh-lesioned rats only showed deficit for social odors (which have high/moderate complexity), but not for nonsocial odors (low complexity) at long retention intervals. Thus, the results of the above studies are consistent with a general role for Prh in the processing of stimuli that have a high/moderate degree of complexity. Our study extends these conclusions to the somatosensory modality.

In summary, based on the studies reviewed above and in the data of the current series, it could be suggested that the critical factor in the operations performed by the Prh is the degree of complexity of the stimuli, with the sensorial modality being less critical (Gilbert and Kesner 2003; Lindquist et al. 2004; Norman and Eacott 2004; Bartko et al. 2007a; Feinberg et al. 2012). These operations are necessary for the functions of object representation, especially when a stimulus must be precisely represented (Eacott et al. 2001; Murray et al. 2007; Saksida and Bussey 2010). Some authors have hypothesized that the Prh houses these representations, which are essential for memory and perceptual purposes (Buckley and Gaffan 1998; Bussey and Saksida 2002; Cowell et al. 2006). Thus, lesions to the Prh in rats produce deficits in both memory and discrimination tasks with complex stimuli (Norman and Eacott 2004; Bartko et al. 2007a, 2007b; Feinberg et al. 2012). In line with the aforementioned ideas, our data suggest, for the first time, that the Prh is essentially involved in the representation of tactual stimuli with a high or intermediate degree of ambiguity.

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References