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Recognition memory for social and non-social odors: Differential effects of neurotoxic lesions to the hippocampus and perirhinal cortex

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ABSTRACT

The contributions of the hippocampus (HC) and perirhinal cortex (PER) to recognition memory are currently topics of debate in neuroscience. Here we used a rapidly-learned (seconds) spontaneous novel odor recognition paradigm to assess the effects of pre-training *N*-methyl-D-aspartate lesions to the HC or PER on odor recognition memory. We tested memory for both social and non-social odor stimuli. Social odors were acquired from conspecifics, while non-social odors were household spices. Conspecific odor stimuli are ethologically-relevant and have a high degree of overlapping features compared to non-social household spices. Various retention intervals (5 min, 20 min, 1 h, 24 h, or 48 h) were used between study and test phases, each with a unique odor pair, to assess changes in novelty preference over time. Consistent with findings in other paradigms, modalities, and species, we found that HC lesions yielded no significant recognition memory deficits. In contrast, PER lesions caused significant deficits for social odor recognition memory at long retention intervals, demonstrating a critical role for PER in long-term memory for social odors. PER lesions had no effect on memory for non-social odors. The results are consistent with a general role for PER in long-term recognition memory for stimuli that have a high degree of overlapping features, which must be distinguished by conjunctive representations.

1. Introduction

Recognition memory is the ability to remember previously encountered items, a faculty essential to declarative memory. It is well established that the medial temporal lobe (MTL), which includes the hippocampus (HC) as well as the adjacent entorhinal cortex, perirhinal cortex (PER) and postrhinal cortex (parahippocampal cortex in primates), is critical to declarative memory (Eichenbaum, 2000; Suzuki & Eichenbaum, 2000; Squire, 2009; Teyler & Rudy, 2007). Damage encompassing these brain areas leads to deficits in declarative memory, including recognition, spatial, temporal order, episodic, and semantic memory (O'Keefe & Nadel, 1978; DeVito & Eichenbaum, 2011; Eichenbaum, Yonelinas, & Ranganath, 2007; Fortin, Agster, & Eichenbaum, 2002; Kesner, Raymond, Gilbert, & Barua, 2002; Squire, Stark, & Clark, 2004). However, the specific function of individual MTL structures, including the nature of their contribution to recognition memory, remains unclear.

One prominent theory proposes that item and context information are processed in segregated parallel streams through PER and postrhinal cortex, respectively, and converge onto the HC to contribute to the formation of episodic memories (Brown & Aggleton, 2001; Diana, Yonelinas, & Ranganath, 2007; Eichenbaum et al., 2007; Teyler & Rudy, 2007). This theory suggests that the HC is critical for episodic memory, but not for item recognition memory, a notion that remains a subject of debate (Albasser, Davies, Futter, & Aggleton, 2009; Broadbent, Gaskin, Squire, & Clark, 2010; Brown & Aggleton, 2001; Fortin, Wright, & Eichenbaum, 2004; Fortin et al., 2002; Winters, Saksida, & Bussey, 2008). The same model proposes that PER is crucial for item memory, in part based on observations that PER plays an important role in object recognition memory (Aggleton, Albasser, Aggleton, Poirier, & Pearce, 2010; Albasser et al., 2009; Brown & Aggleton, 2001; Eichenbaum et al., 2007; Winters et al., 2008). However, recent evidence suggests that the role of PER in item memory is more complex than originally thought. For instance, several studies have shown that PER is particularly necessary when objects contain a high degree of overlapping features (Albasser et al., 2009; Buckley, Booth, Rolls, & Gaffan, 2001; Bussey, Saksida, & Murray, 2002; Eacott, Machin, & Gaffan, 2001; Norman & Eacott, 2005; Wan, Aggelton, & Brown, 1999; for a review see Winters et al., 2008). PER-lesioned animals demonstrate greater levels of impairment as the degree of feature ambiguity increases (Bartko, Winters, Cowell, Saksida, & Bussy,

Abbreviations: MTL, medial temporal lobe; HC, hippocampus; vHC, ventral hippocampus; PER, perirhinal cortex; LEC, lateral entorhinal cortex; NMDA, *N*-methyl-D-aspartate; *DI*, unadjusted discrimination index; *DI'*, normalized discrimination index.

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2007; Buffalo, Bellgowan, & Martin, 2006; Bussey et al., 2002). PER lesions also cause impairments in distinguishing simultaneously-presented stimuli, suggesting that PER might mediate the perceptual disambiguation of overlapping stimulus representations, in addition to serving aspects of recognition memory (Baxter, 2009; Bussey, Saksida, & Murray, 2006; but see Suzuki, 2009, 2010). Notably, perceptual and mnemonic theories of the contributions of PER to recognition processes are largely based on studies using visual stimuli. The question of whether PER plays a generalized role in recognition memory and/or perception outside the visuotactile realm is not well understood.

The overall objective of this study was to extend these influential theories by directly comparing the roles of the HC and PER in odor recognition memory. Specifically, we used olfactory stimuli to determine whether these theories extend to another modality, and to directly compare the use of social and non-social stimuli. Rodents are capable of rapidly learning and remembering odors over long periods of time, and have particularly sensitive olfactory discrimination abilities (Linster, Johnson, Morse, Yue, & Leon, 2002; Schellinck, Price, & Wong, 2008). Here, we contrast the use of highly overlapping social odors and relatively distinctive non-social odors.

Olfaction is a critical modality for mammals, guiding numerous aspects of their daily lives including food preference, reproductive status, maternal bonding, and identification of conspecific allies and predators (Doty, 1986; Sanchez-Andrade & Kendrick, 2009; Schellinck et al., 2008). Furthermore, olfactory inputs are highly interconnected with numerous mnemonic structures. In particular, the olfactory bulbs have direct projections to a number of putative memory structures in the MTL (Brennan & Kendrick, 2006; Kay, 2008).

Importantly, social odors are processed differently and have a unique composition compared to non-social odors. The rodent olfactory system is comprised of two distinct pathways, the main olfactory pathway and the accessory (vomeronasal) olfactory pathway, which are thought to transmit differential information about volatile and non-volatile olfactory stimuli, respectively (Martinez-Marcos, 2009). Social odors from conspecifics are composed of a complex assortment of various molecules with components shared between individuals, conveying information about the age, sex, health status, and relatedness (Brennan & Kendrick, 2006). These social odors are processed through both olfactory pathways, while non-social odors are processed through the main olfactory pathway.

Here, we use odor-based stimuli in an adaptation of the spontaneous novel object recognition paradigm (Ennaceur & Delacour, 1988; Monaghan et al., 2010; O'Dell, Feinberg, & Marshall, 2011; Spinetta et al., 2008) to elucidate the effects of pre-training lesions to the HC and PER on odor recognition memory. Additionally, we tested five retention intervals (5 min, 20 min, 1 h, 24 h, or 48 h) because of known time-dependent contributions of the HC (Anderson, 2007; Rolls, 1996; Zola-Morgan & Squire, 1990) and PER (Mumby, Piterkin, Lecluse, & Lehmann, 2007; Sacchetti, Sacco, & Strata, 2007) to other memory tasks. In particular, these retention intervals allowed assessment of short- and long-term odor recognition memory.

We also tested the effects of HC and PER lesions on recognition memory for both conspecific social odors and non-social odors (household spices). Conspecific and household odor stimuli represent an ecological and arbitrary approach, respectively, to the study of odor recognition memory (Domjan, Cusato, & Krause, 2004). Considering that HC lesions have been shown to impair various aspects of social memory (Alvarez, Wendelken, & Eichenbaum, 2002; Kogan, Franklandand, & Silva, 2000), it is possible that the HC plays a general role in social odor memory. Also, because PER has been implicated in the learning of social stimuli (Furtak, Allen, & Brown, 2007; Kholodar-Smith, Allen, & Brown, 2008; Petrulis & Eichenbaum, 2003), we sought to investigate whether PER lesions differentially affect recognition memory for social versus non-social odors.

Overall, HC-lesioned rats showed normal recognition memory for social and non-social odors, whereas PER-lesioned rats were selectively impaired in the long-term recognition memory for social odors. These data demonstrate that the HC is not necessary for short- or long-term odor recognition memory, consistent with models of HC memory function (Diana et al., 2007; Eichenbaum et al., 2007). These findings also indicate that the PER is not always critical for "item" memory (Eichenbaum et al., 2007), but rather only necessary when the cues are more complex or have a high degree of overlapping features (Bussey et al., 2006; Suzuki, 2009). Importantly, PER-lesioned rats demonstrated normal recognition of social odors at short intervals (5 and 20 min), providing strong evidence that the impairment was not due to a perceptual deficit. Overall, these findings contribute to a growing body of knowledge about the roles of the HC and PER in recognition memory for both social and non-social stimuli. These data are consistent with the hypothesis that PER contributes to recognition memories that require long-term storage of conjunctive feature representations.

2. Methods and materials

2.1. Subjects

Seventy-four male Long-Evans rats were used in this study: 39 served as conspecific odor donors, and 35 underwent surgical and behavioral procedures (375–450 g at the time of surgery). Rats were individually housed in clear rectangular polycarbonate cages and maintained on a 12 h light–dark cycle (lights off at 8:00 am). Access to food and water was unrestricted before surgery. Following surgery, rats were mildly food restricted to maintain 85% of their free-feeding body weight with free access to water throughout testing. All surgical and behavioral methods were in compliance with the University of California Irvine Institutional Animal Care and Use Committee guidelines.

2.2. Surgeries

Rats were randomly assigned to treatment groups: bilateral HC Lesion ($n = 9$), HC Control ($n = 8$), PER Lesion ($n = 10$), or PER Control ($n = 8$). Lesions were induced by infusions of *N*-methyl-D-aspartate (NMDA; Sigma, St. Louis, MO), in order to produce excitotoxic neural damage. Rats received a pre-operative injection of buprenorphine (0.5 mg/kg, 0.2 mg/ml, i.p.) approximately 10 min prior to induction of anesthesia. During surgery, all rats were administered glycopyrrulate (0.2 mg/ml, 0.5 mg/kg, s.c.) to help prevent respiratory difficulties and 5 ml Ringer's solution with 5% dextrose (s.c.) for hydration. General anesthesia was induced (5%) and maintained by isoflurane (1–2.5%) mixed with oxygen (800 ml/min). Rats were placed into the stereotaxic apparatus (Stoelting Instruments, Wood Dale, IL) and the scalp was locally anesthetized with Marcaine® (7.5 mg/ml, 0.5 ml, s.c.). The skull was exposed following a midline incision and adjustments were made to ensure bregma, lambda, and sites ±0.2 mm lateral to the midline were level.

Following lesion procedures for the HC or PER (details below), skull fragments were replaced and anchored in position over the exposed cortex with bone wax. Incision sites were sutured and dressed with Neosporin®. Rats were returned to their home cages and monitored until they awoke from anesthesia. One day following surgery, rats were given an analgesic (Flunixin, 50 mg/ml, 2.5 mg/kg, s.c.) and Neosporin® was applied to the incision site.

Rats were allowed to recover from surgery for approximately 2 weeks before behavioral testing.

2.2.1. Hippocampus lesions

The bone overlying the HC infusion sites was resected bilaterally and remained hydrated in sterile saline during infusions. Infusions were performed using a 33-gauge 10 μl syringe (Hamilton Company, Reno, NV) driven by a motorized infusion pump (World Precision Instruments, Sarasota, FL) that was mounted onto a manipulator arm of the stereotax. The needle remained at the injection site for 3 min after drug infusion to allow for diffusion. HC sites were infused with 200–225 nl NMDA at 200–250 nl/min (coordinates for dorsal HC: anteroposterior (A/P) −2.2 mm, mediolateral (M/L) ±1.0 mm, dorsoventral (D/V) −3.0 mm; A/P −3.0 mm, M/L ±1.8 mm, D/V −2.8 mm; A/P −4.0 mm, M/L ±2.8 mm, D/V −2.6 mm; coordinates for ventral HC: A/P −4.8 mm, M/L ±4.8 mm, D/V −6.5 mm; A/P −4.8 mm, M/L ±4.5 mm, D/V −3.3 mm; A/P −5.7 mm, M/L ±4.9 mm, D/V −2.8 mm; A/P −5.7 mm, M/L ±5.1 mm, D/V −5.8 mm). Dorsoventral coordinates were measured from the *dura mater*. Sham-operated controls underwent the same surgical procedures as the lesion group, except no infusion was made.

2.2.2. Perirhinal cortex lesions

Two holes were drilled on each hemisphere of the dorsal skull (∼−4 and −7 mm A/P relative to bregma, ∼1 mm medial to the temporal ridge) for anchor screws to hold a tissue spreader (Allen, Furtak, & Brown, 2007; Kholodar-Smith et al., 2008). Temporal muscles were pulled away to expose the temporal and parietal plates of the skull until the zygomatic arch was visible. The tissue spreader was then secured between the anchor screws and the inner surface of the temporal muscles.

The bone overlaying the temporal cortex (∼2 mm × 5 mm) was resected and the fragment remained hydrated in sterile saline during infusions. A hypodermic non-coring needle (33-gauge) was positioned at a 45° angle from the vertical surface of the temporal cortex, with the needle eye facing ventral and posterior to direct flow of NMDA toward PER. The needle extended out from the swiveling arm of a manipulator and was connected to a 10 μl microsyringe via polyethylene tubing. PER-lesioned rats received NMDA infusions (340 mM; 50 mg/ml) at approximately 7–8 sites (0.08 μl per infusion; 0.07 μl/min; equally spaced at ∼0.5 mm) spanning the rostrocaudal extent of PER from −2.8 to −7.6 A/P relative to bregma (Burwell, 2001). Occasionally, only seven injections were made when a large blood vessel was present at an intended infusion site (Kholodar-Smith et al., 2008). The needle tip was inserted ∼1.5 mm into the cortex, measured from the *dura mater*. Sham-operated controls underwent the same surgical procedures as the lesion group, except no infusion was made.

2.3. Olfactory stimuli

All odor stimuli were presented on 1″ round wooden beads (Woodworks Ltd., Haltom City, TX). Experimental rats were familiarized with wooden beads prior to testing by placing a number of unscented beads in their cages (beads were removed before testing began; O'Dell et al., 2011; Spinetta et al., 2008). This general familiarity with wooden beads ensured that, during testing, animals focused their investigation on the social or non-social odor added to the experimental beads (see below). Importantly, the natural odor of the wooden beads served as a familiar background odor, which was consistent across both non-social and social odor stimuli.

2.3.1. Non-social odors

Non-social odors were presented to the rats on wooden beads, each scented with an individual household spice. Beads were scented by being placed in a container holding a mixture of playground sand and a single household spice (e.g., cumin) for 48 h. Sand was included to dilute odorants and served as a consistent background odor for all non-social odor beads. Although experimental rats had no prior experience with the specific experimental non-social odors, rats had a history with many non-social odors (e.g., latex gloves, wood, bedding, ointments).

2.3.2. Social odors

Social odors were presented to the rats on wooden beads, each scented with the odor of a single conspecific animal. Beads were scented by being placed in the cage of an individually-housed odor donor rat for 1 week (O'Dell et al., 2011; Spinetta et al., 2008). The conspecific odor donor rats were free to interact with the wooden beads during this period.

Odor donor conspecifics were healthy adult male Long-Evans rats completely segregated from experimental rats, which were housed in a separate vivarium space. Considerable effort was made to ensure that the experimental rats had no prior experience with the odor donor rats. Thus, the social odor beads contained a mixture of both familiar odors present in all rats' cages (e.g., bedding, food) and the unique combination of odors of an unfamiliar conspecific rat (e.g., saliva, urine, feces). Beads from conspecifics were "preference tested" using an independent cohort of naïve rats to help ensure equal levels of innate preference/aversion to individual odor donors. Additionally, upon arrival, experimental rats were individually-housed in cages with specialized filter tops to help isolate the experimental rats from the odors of neighboring experimental rats.

2.4. Behavioral procedures

Naïve rats were briefly handled for 3–5 days after initial arrival and throughout behavioral procedures. Behavioral testing started after a 2-week postsurgical recovery period. All test sessions took place during the dark phase (active period) of the light cycle under ambient red lighting conditions.

One hour prior to the study phase, food hoppers and water bottles were removed to acclimate rats to testing conditions. In the study phase, a single bead scented with a novel odor (Novel Odor 1; N1) was placed in the center of the front-most quadrant (most accessible to the experimenter) of the cage. Upon initiation of exploration (defined as sniffing and whisking within ∼1 cm of the bead), rats were given 1 min to investigate the bead. Exploration times were recorded on a laptop computer using ODLog software (www.macropodsoftware.com). Beads were discarded at the end of each presentation. The experimenter changed gloves each time a new bead was used to prevent cross contamination. All odors were counterbalanced between rats and retention delays.

Following a variable retention interval (5 min, 20 min, 1 h, 24 h or 48 h), each rat was presented with two beads: one bead scented with the odor presented in the study phase (N1), alongside one bead scented with a novel odor (Novel Odor 2; N2). During testing, novel odors were always paired with the same odor type (social or non-social) that the rat had sampled during the study phase. Test beads were placed in the same cage quadrant as the sample bead and were positioned approximately 3 cm apart. Upon initial exploration, rats were given 1 min to investigate the beads. Bead position (right or left) was counterbalanced for all rats and presentations. Exploration time for each bead was recorded in ODLog. See Fig. 1 for a diagrammatic representation and video still of the spontaneous odor recognition task. See Supplemental materials for a sample video of the task.

Rats were tested on the odor recognition task over many days. Odor type and retention interval were counterbalanced across rats (within a session) and across sessions so each rat was tested twice on each combination.

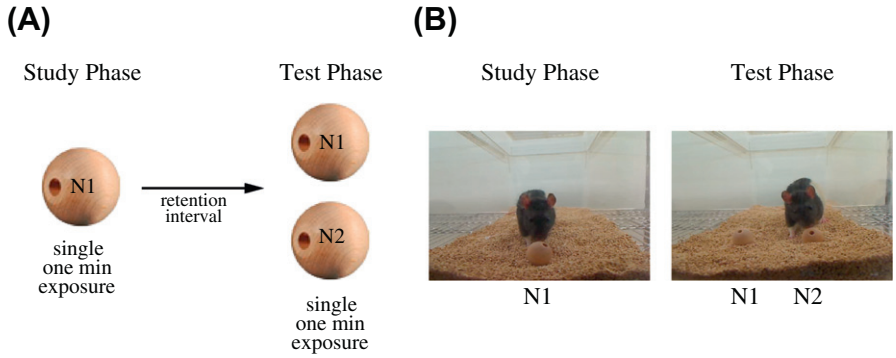

Fig. 1. Spontaneous novel odor recognition memory paradigm. During the study phase, a single wooden bead was presented (Novel Odor 1; N1) to a rat in his home cage, scented with either a non-social or social odor not previously encountered by the rat. Upon initiation of olfactory exploration (active sniffing, whisking, nose within 1 cm) toward the bead, the rat was given 1 min to investigate the odor. Following a retention interval (5 min, 20 min, 1 h, 24 h, or 48 h), the test phase occurred, in which two odor beads were presented simultaneously to the rat in his home cage. One bead was scented with the previously encountered odor from the study phase (N1) and the other bead was scented with a completely novel odor (Novel Odor 2; N2). Panel (A) shows a diagrammatic representation of the study and test phase. Preferential exploration toward N2 indicates recognition memory for N1. Panel (B) shows video stills of a rat during the study and test phases. A full video of the odor recognition task is included in Supplemental materials.

2.5. Data analysis

Two measures of discrimination were calculated from the exploration data (Aggleton et al., 2010; Ennaceur & Delacour, 1988). The difference in seconds of exploration toward the novel odor (N2) minus seconds of exploration toward the familiar odor (N1) in the test phase is the unadjusted discrimination score (*DI*).

$$DI = \mathrm{sec}_{N2} - \mathrm{sec}_{N1} \quad (1)$$

The second discrimination measure (*DI′*) was calculated by dividing *DI* by the total exploration time and multiplying that number by 100, providing a percent difference score between exploration toward the novel odor (N1) and the familiar odor (N1). The *DI′* values range from +100 to −100. Positive values correspond to a preference toward the novel odor (N2). Negative scores correspond to a preference toward the previously encountered odor (N1). A score of zero indicates no preference for either odor. *DI′* scores significantly higher than zero are interpreted as recognition memory for the previously encountered odor. Here we only observed novelty preferences in the positive direction, thus the *DI′* axis of graphs only displays positive values.

$$DI' = DI/(\mathrm{sec}_{N2} + \mathrm{sec}_{N1}) \times 100 \quad (2)$$

Each animal was tested twice on every retention interval for both social and non-social odor stimuli. Discrimination scores for the same retention interval and stimulus type were averaged for each rat.

Statistics were performed using SPSS 17 and custom-written MATLAB (R2009a) scripts. Group data were analyzed using analysis of variance (ANOVAs) and *t*-tests. Group data is expressed as the mean ± standard error. The family-wise α-error rate was maintained at 0.05. Significant trends are noted when $p \leqslant 0.10$, but >0.05. Lesion effect sizes (*d*) on discrimination was computed as (Cohen, 1988):

$$d = (\mathrm{mean}_{\mathrm{Controls}} - \mathrm{mean}_{\mathrm{Lesion}}) \Big/ \left(\sqrt{(\mathrm{SD}^2_{\mathrm{Control}} + \mathrm{SD}^2_{\mathrm{Lesion}})/\mathrm{N}}\right) \quad (3)$$

The numerator is the difference between the mean discrimination index of control and lesioned animals, and the denominator is the standard deviation of pooled estimates from control and lesioned animals.

2.6. Histology

Rats were administered an overdose of sodium pentobarbital (Euthasol, 390 mg/ml, 150 mg/kg, i.p.) and were transcardially perfused with 100 ml PBS followed by 200 ml of 4% paraformaldehyde (pH 7.4; Sigma–Aldrich, St. Louis, MO). Brains were post-fixed overnight in 4% paraformaldehyde and afterwards placed in a 30% sucrose solution for cryoprotection. Frozen brains were sectioned on a sliding microtome (60 μm; coronal orientation) into four sets of immediately-adjacent sections for a cell body-specific Cresyl Violet stain, a neuron-specific NeuN stain, a myelin-specific gold chloride stain and a spare set (see Supplemental Figs. 1–4 for samples of each stain in each experimental group). Exact methods for each stain are described in detail elsewhere (see Supplementary materials from Kholodar-Smith et al., 2008).

2.7. Lesion reconstructions

Using Image J software, the extent of neurotoxic damage to the HC and PER, as well as lateral entorhinal cortex, was estimated on the basis of serial NeuN-stained sections (Paxinos & Watson, 1998; PER localization based on Burwell, Witter, & Amaral, 1995). Gold-chloride sections were qualitatively assessed with a light microscope for damage to major fiber bundles.

3. Results

3.1. Histology

3.1.1. Control subjects

HC and PER Controls had no noticeable evidence of brain damage as assessed with Nissl, gold-chloride, and NeuN histological stains. Both HC and PER Controls are interpreted as having full and normal neural capabilities during all behavioral experiments, and were combined for subsequent analyses. See Fig. 2 for sample histology from a control subject.

3.1.2. Hippocampus lesioned subjects

HC-lesioned subjects had large and complete lesions to the entire HC while surrounding fibers were spared. There was a clear lack of HC tissue throughout the rostral-caudal extent of the brains that was evident in all three stains. Two-dimensional lesion area analysis was performed using the NeuN-stained sections. Overall, 90.3 ± 0.2% of the HC was lesioned. There was no difference in

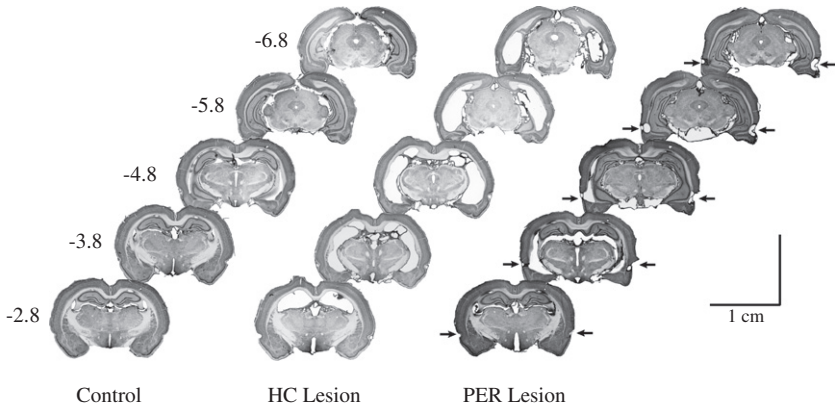

Fig. 2. Sample Control and Lesion brains. The photomicrographs show coronal histological sections covering the anterior–posterior extent from −2.8 to −6.8. Sections were stained for NeuN. A sample brain is presented from each of the three experimental groups (Controls, HC lesions, and PER lesions). The localized neurotoxic lesions were induced with multiple injections of NMDA, and resulted in neuronal loss and atrophy of the target regions. The arrows indicates the location of the PER lesions.

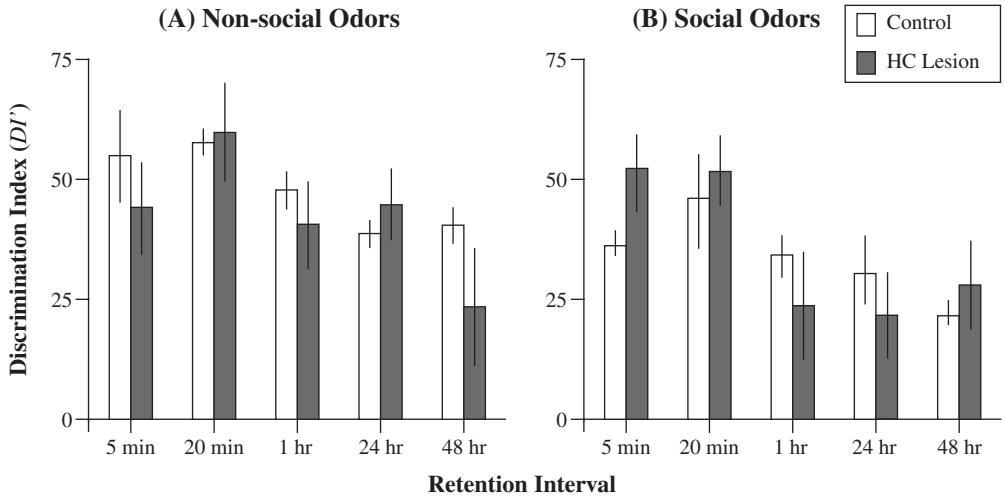

Fig. 3. Hippocampus lesions did not impair recognition memory for either non-social or social odor stimuli (see Sections 3.3.1 and 3.3.2). Performance (mean ± SEM) of Control and HC Lesion groups on non-social (panel A) and social (panel B) novel odor recognition tests at five retention intervals. HC-lesioned rats demonstrated normal recognition memory (significant preference for novel odor during test phase) at all retention intervals for both odor types.

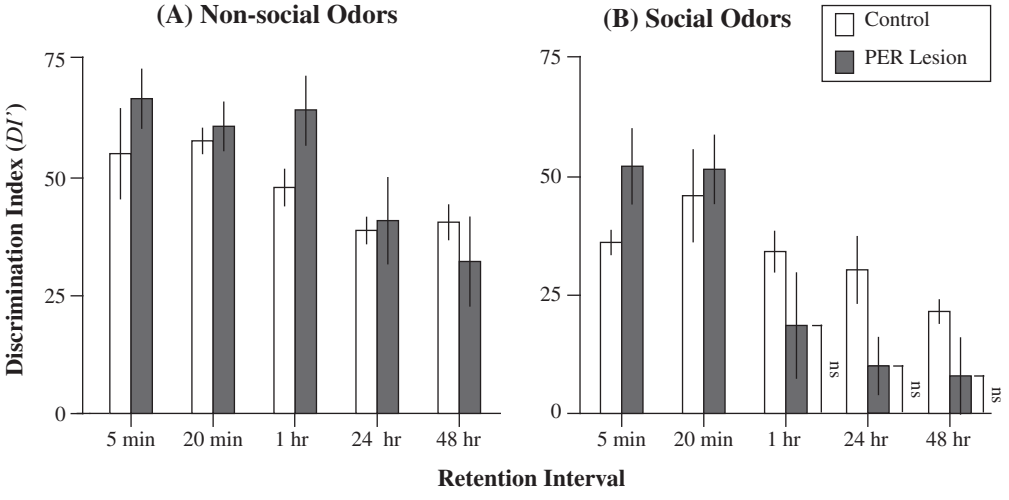

Fig. 4. Perirhinal cortex lesions significantly impaired recognition memory for social, but not non-social, odor stimuli (see Sections 3.3.4 and 3.3.5). Performance (mean ± SEM) of Control and PER Lesion groups on non-social (panel A) and social (panel B) novel odor recognition tests at various retention delays. (A) PER-lesioned rats demonstrated normal recognition memory (significant preference for novel odor during test phase) at all retention intervals for non-social odor stimuli. (B) PER-lesioned rats had a significant deficit for social odor recognition memory at 1 h, 24 h, and 48 h compared to Controls, suggesting specific long-term memory deficits for social odors. Abbreviation: ns, not significantly different from no odor preference ($DI' = 0$).

damage produced in the left hemisphere (90.0 ± 0.03%) compared to the right hemisphere (90.6 ± 0.01%) $t_{(8)} = -0.26$, $p = 0.799$. Using the gold-chloride stained sections, we visually confirmed that the major fiber bundles surrounding the HC, such as the corpus callosum, were intact. See Fig. 2 for an example of a HC-lesioned brain.

3.1.3. Perirhinal lesioned subjects

In PER-lesioned subjects, damage was centered in the cortical tissue surrounding the mid-posterior rhinal sulcus. These rats had large lesions to PER, and, to a lesser extent, a region of lateral entorhinal cortex (LEC) situated immediately ventral to area 35 of PER. There was very minor damage to the ventral HC (vHC).

PER, LEC and the vHC were included in a quantitative two-dimensional lesion area analysis. A Brain Region × Hemisphere repeated-measures ANOVA was run to examine differential damage to these regions and any potential laterality. There was a main effect of Brain Region, with PER being the most damaged (60.2 ± 0.4%), followed by LEC (23.8 ± 0.06%) and very little damage to vHC (1.4 ± 0.004%), $F_{(2,18)} = 95.15$, $p < 1 \times 10^{-5}$. The amount of damage is similar to what has been previously found with a similar lesion technique (Kholodar-Smith et al., 2008). Additional examination of gold-chloride sections showed that the major fiber bundles surrounding the lesion area, such as the external capsule, remained intact. See Fig. 2 for an example of a PER-lesioned brain.

3.2. Study phase odor exploration

During the study phase, rats were allowed to explore the odor bead for up to 60 s. Overall, rats spent 12.53 ± 0.48 s actively investigating the bead during the study phase. Exploration time during the study phase was compared between HC, PER, and Control rats using a Retention Interval × Odor Type × Lesion Group repeated-measures ANOVA to examine any potential differences in exploration times between conditions. Importantly, there were no main or interaction effects of the Lesion Group (HC, 12.98 ± 0.89 s; PER, 12.63 ± 0.85 s; and Controls, 11.99 ± 0.67 s), p's $\gg 0.10$. Thus neither the HC lesions, nor the PER lesions, significantly affected the exploration time of the rats during the study phase and cannot account for any differences in memory-based performance during test phases.

There were some differences in investigation time depending on the type of odor stimulus and the retention interval. Overall, rats investigated social odors (14.44 ± 0.71 s) more than non-social odors (10.63 ± 0.41 s), seen in a main effect of Odor Type, $F_{(1,32)} = 31.27$, $p < 0.001$. This 36.8% increase in exploration time for social odors likely reflects a real difference in spontaneous investigation of social odors compared to non-social odors, and suggests that rats may need to sample social odors longer to fully perceive and/or encode their multifaceted composition. There was also a significant main effect of Retention Interval, $F_{(4,128)} = 4.45$, $p < 0.05$. The difference in exploration time was relatively small, with the mean difference of 1.00 ± 0.18 s in exploration times between the groups, representing a modest 7.9% change from overall mean levels. The retention intervals were randomly assigned and the study phases were identically presented for each retention interval, and thus we do not make any strong interpretations from this effect.

3.3. Novel odor discrimination during the test phase

Exploration behavior during the spontaneous novel odor recognition task was quantified using a difference score in seconds (*DI*, Eq. (1)) and a discrimination index (*DI′*, Eq. (2)) as the measures of behavioral performance. Both measures yielded the same pattern of results. Here we are presenting the more commonly used *DI′* when reporting data from the memory-based test phase performance.

3.3.1. Control subjects: odor type and retention intervals

We found that control rats demonstrated odor recognition memory for both non-social and social odors at all retention intervals. Using one-sample *t*-tests tested against no odor preference (*DI′* = 0), we found that control rats showed a significant preference for the novel odor (N2) under all conditions (all p's < 0.001; see Fig. 3 and 4).

However, there were differences in performance levels based on the odor type and retention interval. A repeated-measures ANOVA was used to examine differences in Odor Type × Retention Interval. Control rats had larger *DI′* scores for non-social odors (47.92 ± 8.30) compared to social odors (33.80 ± 7.15), revealed by a main effect of Odor Type, $F_{(1,15)} = 7.87$, $p < 0.05$. This difference may reflect a need for longer investigation times to identify or recognize social odors, which would result in smaller *DI′* scores. This possibility is consistent with the longer search times found during the study phase for social odors compared to non-social odors. In addition, there was a main effect of Retention Interval in Controls ($F_{(4,60)} = 3.50$, $p < 0.05$), indicating a moderate decline in the *DI′* scores as retention intervals increased. There was no significant effect of Odor Type × Retention Interval, $F_{(4,60)} = 0.17$, $p = 0.95$.

3.3.2. Hippocampus-lesioned subjects versus control subjects: non-social odors

HC-lesioned rats showed no detectable impairments in recognition memory for non-social odors compared to Controls. There was no significant main effect of Group, $F_{(1,23)} = 0.87$, $p = 0.36$, nor a significant interaction effect of Retention Interval × Group, $F_{(4,92)} = 0.50$, $p = 0.73$. There was a significant trend for a main effect of the length of the Retention Interval, $F_{(4,92)} = 2.32$, $p = 0.06$. The *DI′* of HC rats are plotted against Control rats, for all five Retention Intervals (Fig. 3A).

3.3.3. Hippocampus-lesioned subjects versus control subjects: social odors

HC-lesioned rats showed no detectable impairments in recognition memory for social odors compared to Controls. There was no significant main effect of Group, $F_{(1,23)} = 0.37$, $p = 0.55$, nor a significant interaction effect of Retention Interval × Group, $F_{(4,92)} = 0.43$, $p = 0.79$. There was a significant trend for a main effect of the length of the Retention Interval, $F_{(4,92)} = 2.12$, $p = 0.08$. The *DI′* of HC rats are plotted against Control rats, for all five Retention Intervals (Fig. 3B).

3.3.4. Perirhinal-lesioned subjects versus control subjects: non-social odors

PER-lesioned rats showed no detectable impairments in recognition memory for non-social odors compared to Controls. There was no significant main effect of Group, $F_{(1,24)} = 0.86$, $p = 0.36$, nor a significant interaction effect of Retention Interval × Group, $F_{(4,96)} = 0.64$, $p = 0.64$. There was a main effect of the length of the Retention Interval, $F_{(4,92)} = 3.74$, $p < 0.01$. The *DI′* of PER rats are plotted against Control rats, for all five Retention Intervals (Fig. 4A).

3.3.5. Perirhinal-lesioned subjects versus control subjects: social odors

PER lesions significantly impaired the ability of rats to demonstrate recognition memory for social odors following long retention intervals ($\geqslant$1 h), but not after short retention intervals ($\leqslant$20 min). There was a significant interaction effect of Retention Interval × Group, $F_{(4,96)} = 2.68$, $p < 0.05$. Post-hoc one-sample *t*-tests were performed against no odor preference (*DI′* = 0) in order

to identify the specific retention intervals affected by PER lesions. The *t*-tests showed that PER-lesioned rats only showed significant preference for the novel odor in the 5 min condition ($t_{(9)} = 6.70$, $p < 0.001$) and the 20 min condition, ($t_{(9)} = 7.39$, $p < 0.001$). However, there was no significant preference for the novel odor (N2) in the 1 h condition ($t_{(9)} = 1.66$, $p = 0.13$), 24 h condition ($t_{(9)} = 1.72$, $p = 0.12$), or the 48 h condition ($t_{(9)} = 1.01$, $p = 0.34$; see Fig. 4B). Thus, given lesions to PER, rats fail to demonstrate significant long-term memory for social odors. These findings strongly suggest that the PER is critical for long-term, but not short-term, memory for social odors. Notably, the interaction effect rules out the possibility that PER is necessary to perceive social odors given the lack of effect at short retention intervals (5 and 20 min).

There was no main effect of Group, $F_{(1,24)} = 0.63$, $p = 0.44$, but there was a main effect of Retention Interval, $F_{(4,96)} = 10.11$, $p < 0.001$. The social odor DI' of PER-lesioned rats are plotted against Control rats for all five retention intervals (Fig. 4B).

The magnitude of the behavioral impairment was determined by calculating effect sizes (Cohen's *d*; Eq. (3)). By convention, there was a large effect of PER lesions on the 24 h retention interval ($d = 1.08$; large effect $\geqslant 0.8$), a medium effect at the 1 h time point ($d = 0.53$; medium effect $\geqslant 0.5$), and a small effect observed at the 48 h time point ($d = 0.44$; small effect $\geqslant 0.3$).

4. Discussion

4.1. Summary of main findings

The present study assessed recognition memory for olfactory stimuli in rats with neurotoxic NMDA lesions to either the HC or PER. Stimuli included both non-social and social odors. Non-social odors consisted of household spices that served as relatively distinct, minimally overlapping stimuli that are commonly used in olfactory-based tasks (DeVito & Eichenbaum, 2011; Fortin et al., 2002; Kesner et al., 2002). Social odors were obtained from individually-housed conspecifics and served as ethologically-relevant stimuli with highly overlapping olfactory features. The present experiments address three important issues pertaining to olfactory recognition memory: HC and/or PER dependence, effects of non-social versus social odors, and effects of short and long retention intervals.

We first sought to determine the necessity of the HC and PER in odor recognition memory. Despite complete focal lesions to the entire extent of the HC formation, there were no significant differences in discrimination indices for the HC-lesioned rats compared to Controls. In contrast, precisely-targeted PER lesions yielded interval-dependent and stimulus-type specific deficits.

Second, we wanted to investigate differences in behavior when using non-social versus social odors. Controls showed significant differences in their performance on non-social compared to social odors, spending more time investigating social odors in sample phases and having smaller, though significant, DI' scores in test phases. We found no effect of HC lesions on subsequent recognition memory for odors of either type (Fig. 3). PER lesions did not affect recognition memory for non-social odors, but was found to impair recognition memory of social odors at long retention intervals ($\geqslant 1$ h; Fig. 4).

Third, we tested rats at several retention intervals from study to test phase to investigate whether recognition performance decays over time following a single exposure to an odor. Rats demonstrated a significant decrease in preference indices over retention intervals from 5 min to 48 h. We did not find any effects of stimulus type on decay rate, as both non-social and social odors yielded similar gradients over time.

4.2. Spontaneous social odor recognition task

Intact item recognition memory is experimentally characterized by correct identification of a previously encountered stimulus. The behavioral paradigm employed in the present study was adapted from Spinetta et al. (2008), in which spontaneous novelty preference was used to assess social odor recognition memory in rats (O'Dell et al., 2011; Monaghan et al., 2010). In contrast to Spinetta et al. (2008) in which rats habituated to a novel odor over three 1 min exposures, rats here were given a single 1 min exposure. We demonstrate that a single encoding trial is sufficient to yield novelty preference during the subsequent test phase at similar preference levels. Using a rapid one-trial learning paradigm enabled us to investigate recognition memory for singly encountered, incidentally encoded events. Furthermore, study phases consisting of a single exposure trial afford the opportunity to assess recognition memory at shorter retention intervals from the initial encounter to test (e.g., 5 min). Albasser et al. (2009) demonstrated that PER lesion-induced deficits in novel object recognition are not reversed by extending the study duration, further justifying our use of a single-trial exposure.

Additionally, a single study trial decreases encoding time, which should result in a weaker memory trace that is more likely to decline over time, reducing ceiling and overtraining effects. Here, we observed a significant gradient of preference indices as the retention interval increased, suggesting a significant decrement in recognition memory over time. Alternatively, this may reflect a change in novelty preference over time.

We also expanded the stimulus set used by Spinetta and colleagues (2008) by using both social and non-social odors, in order to probe for differential recognition memory profiles as a result of odor type. Rats demonstrated greater exploration for social compared to non-social odors in the study phase. Given longer exploration times for the social odors during the study phase, one might expect greater familiarity for the social compared to non-social odors and thus larger DI' scores for the social odors during the test phase due to stronger memories. Interestingly, we observed smaller DI' scores for social compared to non-social odors during the test phase. It is possible that the multifaceted and overlapping composition of social odors results in the need for longer exploration times for full encoding and recognition, compared to more distinctive non-overlapping non-social odors. This latter possibility would predict both longer search times in the study phase and smaller DI' scores in the test phase for social odors, compared to non-social odors, as we observed.

4.3. Neural basis of social and non-social recognition memory

Rats with HC and PER lesions revealed differences in the neural processing of recognition memory for social and non-social odors. PER-lesioned rats had a recognition memory deficit for social odors at retention intervals greater than or equal to 1 h. By contrast, HC-lesioned rats showed no detectable recognition memory deficits.

The retention-interval dependent impairment in odor recognition memory following PER lesions is consistent with the literature on visual recognition memory in different species. It is important to note that, although the same pattern of findings is observed across studies, the specific length of the retention interval at which deficits are detected in PER-lesion subjects varies across studies. For example, in human patients with MTL damage that includes PER, Buffalo, Reber, and Squire (1998) reported normal visual recognition memory at short retention intervals (0–2 s), but found deficits at longer retention intervals (6–10 s), with the most severe impairments at the longest interval tested (25–40 s). In non-human primates, memory for visual stimuli in a delayed-nonmatching-to-sample task is impaired following

PER lesions at 30 s intervals, with the most severe impairments at 120 s intervals. In rats, performance on simultaneous feature-ambiguous visual object discrimination is unimpaired by PER lesions (Clark, Reinagel, Broadbent, Flister, & Squire, 2011). However, Clark et al. (2011) further demonstrated that the same PER lesions cause impairments in a novel object recognition memory version of the task at 24 h. Overall, these studies suggest the effects of PER lesions on recognition memory become evident and/or more severe as retention intervals increase (see also Bussey, Muir, & Aggleton, 1999; Mumby & Pinel, 1994; Mumby et al., 2007). These findings are consistent with our results, demonstrating that PER is necessary for long-term, but not short-term, social odor recognition memory.

Additionally, these data argue against the necessary role of the HC in recognition memory, consistent with several previous studies using olfactory (DeVito & Eichenbaum, 2011; Fortin et al., 2002; Kesner et al., 2002) and visual stimuli (Albasser, Poirier, & Aggleton, 2010; Brown & Aggleton, 2001; Mumby, 2001; Mumby et al., 2007; Murray & Mishkin, 1998). The inputs to, and recurrent circuitry within, the HC implies that it may be more involved with integrating information about items and contexts from upstream brain systems, generating representations that allow for declarative and episodic memory (Eichenbaum et al., 2007; O'Reilly and Rudy, 2001; Rudy & Sutherland, 1995; Squire, 1992; Teyler & DiScenna, 1986; Teyler & Rudy, 2007).

The findings that the PER lesion deficits are specific to the social odors used here highlights the qualitatively distinct nature of these stimuli. Not only are social odors comprised of greater numbers of overlapping features compared to non-social odors, they are processed through anatomically different systems and impart a great deal of ethologically-relevant information to the rat (Sanchez-Andrade & Kendrick, 2009). Additionally, rats may have had a different history with social and non-social odors, which could have led to differences in the neural representation of the two odor types before testing. However, there were several controls over the experiential history of the rats with the experimental odors, and both odor types would have been experienced during the life of the experimental rats (see Sections 2.3.1 and 2.3.2). Furthermore, PER lesions have been shown to impair recognition memory after subjects have had a vast experience with similar stimulus sets prior to receiving lesions (Clark et al., 2011; Eacott, Gaffan, & Murray, 1994; Meunier, Bachevalier, Mishkin, & Murray, 1993; Mumby & Pinel, 1994; Prusky, Douglas, Nelson, Shabanpoor, & Sutherland, 2004), and when subjects have had no experience with stimulus sets prior to receiving lesions (Bussey et al., 1999). Thus, it seems unlikely that any differences in experiential history between odor types is the major factor in the lesion effects observed here.

The experimental approach here was to use both "ethologically-relevant" and "neutral" stimuli (Domjan et al., 2004) to examine the role or the HC and PER in odor recognition memory. Notably, both HC and PER lesions have been shown to cause deficits in various social memory paradigms (Alvarez et al., 2002; Kholodar-Smith et al., 2008; Kogan et al., 2000; Petrulis & Eichenbaum, 2003), while the same lesions can be without effect when neutral odor stimuli are used (Fortin et al., 2002, 2004; Kholodar-Smith et al., 2008). The use of social and non-social odors in a single experimental design allowed us to assess whether ethologically-relevant stimuli rely upon the same neural processing as neutral stimuli. Indeed, we found differential effects of lesions on recognition memory for social versus non-social odors revealing different neural pathways in processing these different types of stimuli. Despite our findings that PER lesions uniquely caused significant memory deficits for long-term social odor memory, we cannot determine here whether these effects were due to the social nature of the stimuli, the degree of overlapping elements present in the stimuli, or the experiential history with the stimuli. Future experiments could specifically address these explanations by deconstructing and recombining the constituent features of social odors, mixing variable ratios of household spices and through parametric manipulations of the pre-exposure to odors.

4.4. Perirhinal cortex and the conjunctive stimulus hypothesis

The hypothesis that PER is necessary for visual object recognition memory is well established in humans, monkeys, and rodents (Eacott et al., 1994; Ennaceur & Aggleton, 1997; Norman & Eacott, 2005; for review see Baxter, 2009; Eichenbaum et al., 2007; Squire et al., 2004). Studies in animals have found that PER lesions induce recognition memory deficits specifically for objects with a high degree of overlapping features, but not for highly-distinct objects (Bussey & Saksida, 2002; Iordanova, Burnett, Aggleton, Good, & Honey, 2009; Murray, Bussey, Hampton, & Saksida, 2000; for review see Murray, Bussey, & Saksida, 2007). This led to the notion that PER serves as a "perceptual-mnemonic" structure, important not only for memory, but necessary for the ability to perceptually distinguish objects and visual representations with overlapping features (Baxter, 2009; Murray et al., 2007).

This hypothesis predicts that recognition memory will differ for olfactory stimuli containing low and high amounts of overlapping features, regardless of the retention-interval. Here, wooden beads absorbed the odor of either a household spice or conspecific rats through several days of direct exposure. In the case of non-social odorants, beads were scented over days by being immersed in a mixture of sand and a household spice. Social odor stimuli were generated by placing beads into home cages of individually-housed rats for 1 week. Each social odor obtained in this manner consists of a unique ratio of multiple odorants found in saliva, hair, urine, feces, including major urinary proteins (Mups), pheromones, sulfur-containing compounds produced by intestinal bacteria, hydrogen-sulfide, and odorous metabolic waste products. Thus, social odors are comprised of overlapping shared identifiers compared to non-social odors, which are differentiated by a single unique spice (Brennan & Kendrick, 2006; Linster et al., 2002; Schellinck et al., 2008).

PER-lesioned rats had a recognition memory deficit for social odors when tested at long retention delays ($\geqslant$1 h). This deficit cannot be accounted for by perceptual ability alone, as these rats showed normal memory for non-social odors at all retention intervals, and showed Control-level preference indices for social odors up to 20 min. Thus, these findings support the mnemonic role of PER in processing highly overlapping stimuli (Kholodar-Smith et al., 2008; Suzuki, 2009, 2010).

4.5. Conclusions

These data suggest that PER is critical to long-term recognition memory for odor-based object representations containing highly overlapping features, such as the social stimuli used here. Our data do not support the hypothesis that PER is necessary for perception of such odor stimuli, nor did we find PER to be necessary for recognition memory of odors that can be distinguished by a single odorant feature, such as the non-social odors used here. Additionally, our findings argue against the necessity of the HC for odor recognition memory. These findings motivate future experiments isolating the precise conditions that cause social odor stimuli to rely on an intact PER for long-term recognition memory.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nlm.2011.08.008.

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