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Representation of 3-Dimensional Objects by the Rat Perirhinal Cortex

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Abstract

The perirhinal cortex (PRC) is known to play an important role in object recognition. Little is known, however, regarding the activity of PRC neurons during the presentation of stimuli that are commonly used for recognition memory tasks in rodents, that is, 3-dimensional objects. Rats in the present study were exposed to 3-dimensional objects while they traversed a circular track for food reward. Under some behavioral conditions the track contained novel objects, familiar objects, or no objects. Approximately 38% of PRC neurons demonstrated ‘object fields’ (a selective increase in firing at the location of one or more objects). Although the rats spent more time exploring the objects when they were novel compared to familiar, indicating successful recognition memory, the proportion of object fields and the firing rates of PRC neurons were not affected by the rats’ previous experience with the objects. Together these data indicate that the activity of PRC cells is powerfully affected by the presence of objects while animals navigate through an environment, but under these conditions, the firing patterns are not altered by the relative novelty of objects during successful object recognition.

Keywords

object recognition; medial temporal lobe; novelty; object field; place field

Introduction

The ability to recognize whether or not a given stimulus is novel or familiar is critical for an animal’s survival and the perirhinal cortex (PRC) is integrally involved in this cognitive skill (Buffalo et al., 1999; Malkova et al., 2001; Winters and Bussey, 2005). It has been hypothesized that the PRC supports stimulus recognition by participating in the perception of complex stimuli, which is necessary for the discrimination of new stimuli from those that have been experienced (see Wise and Murray, 2012 this issue). The capacity in which that PRC serves perception may involve performing “stimulus unitization”, that is, the ability to treat two or more separate items or stimulus elements as a single entity (see Kent and Brown, 2012 this issue). Unitization could be particularly important for the perception of complex 3-dimensional objects since, in the rat, this involves integrating tactile and

olfactory information with several distinct visual features in order to identify the stimulus a whole single object.

In the humans (Kahn et al., 2008; Libby et al., 2012), monkeys (Suzuki and Amaral, 1994), and rats (Burwell and Amaral, 1998) the PRC receives polymodal input from olfactory, auditory, somatosensory and visual association cortices, indicating that the PRC is anatomically positioned to perform the polymodal unitization processes that would be necessary to recognize complex 3-dimensional objects. Little is known, however, regarding the activity patterns of PRC neurons during the presentation of objects.

A number of investigations have monitored PRC neuron activity during the presentation of 2-dimensional visual stimuli and these electrophysiological recordings have reported that neurons in this brain region have higher firing rates during the presentation of 2-dimensional novel stimuli, but during repeated presentations and as the stimuli become familiar, the firing rates decrease (e.g., Fahy et al., 1993; Miller et al., 1991; Zhu et al., 1995a). These data have lead to the hypothesis that the neural mechanism supporting stimulus recognition is a 'response decrement' in PRC neuron firing. Moreover, imaging studies that have used the immediate-early gene c-fos to label cell activity in the medial temporal lobe have reported higher levels of c-fos protein in the PRC after rats are exposed to novel 2-dimensional images on a computer monitor compared to when the images are familiar (Zhu et al., 1995b).

The response decrement hypothesis, however, cannot fully account for the PRC's role in perception and recognition memory for several reasons. First, under certain circumstances novelty-modulated changes in PRC neuron activity have been decoupled from successful stimulus recognition (Miller and Desimone, 1993; Zhu and Brown, 1995). In addition, more recent data from humans have shown that response decrements in the BOLD signal are not observed in cortical visual association regions when 3-dimensional objects are used as stimuli rather than 2-dimensional images (Snow et al., 2011). This suggests that PRC neuron novelty-modulation may not occur under more naturalistic conditions such as the active exploration of 3-dimensional objects.

Another factor that remains to be understood in terms of the response decrement hypothesis is that under conditions in which images are presented to a monkey for hundreds of trials, there is a slow emergence of increased PRC neuron firing rates to the highly familiar stimuli (Holscher et al., 2003). In line with these data, is the observation that, in rats undergoing a fear-conditioning paradigm, pairing a stimulus with a foot-shock increases the PRC responsiveness to the conditional stimulus (Furtak et al., 2007). Thus, the data are conflicting as to whether or not the mechanism for stimulus recognition is an increase or a decrease in the population activity of PRC neurons.

Finally, it has recently been shown that when rats move through an environment that contains familiar objects, the probability that a distal CA1 neuron (i.e., the area of CA1 closest to the subiculum) will express a place field increases compared to empty track conditions (Burke et al., 2011). This occurs in the region of CA1 that receives direct projections from the lateral entorhinal cortex and PRC (Amaral and Witter, 1995; Naber et al., 1999). Objects have also been shown to increase the information content of the firing of lateral entorhinal cortical neurons (Deshmukh and Knierim, 2011). Because the primary source of non-spatial information to the lateral entorhinal cortex and distal CA1 is the PRC (Burwell, 2000; Insausti et al., 1997), it is tempting to suggest that PRC afferent input is responsible for modulating object-induced changes in the activity patterns of cells in these regions. The activity of PRC neurons has never been monitored under conditions that have shown objects to affect the firing properties of CA1 and lateral entorhinal cortical cells,

however. The present study was designed to characterize the firing patterns of PRC neurons under conditions in which objects have been shown to modulate the firing properties of distal CA1 neurons, namely, while rats traversed a circular track containing either novel or familiar 3-dimensional objects.

Methods

Subjects and behavioral training

Electrophysiological studies were conducted on eight young (8–10 months old) Fisher-344 male rats. The rats were housed individually and maintained on a 12:12 light–dark cycle. During electrophysiological recordings, the animals were food deprived to about 85% of their *ad libitum* weight and trained to run on a circular track (~335 cm in circumference) in both the counterclockwise and clockwise directions for food reinforcement. The food reward was a mixture of rat food pellets made soft by soaking them in water, applesauce, and the diet supplement *Ensure*. All electrophysiological recordings took place during the dark phase of the rats' light–dark cycle. Food rewards were given in a small plastic food dish (4 cm × 4 cm) at two positions on the track. Both food dishes were located at the position on the track that marked the completion of one lap on opposite sides of a barrier, that is, where the rat was required to turn around and run in the opposite direction (Figure 1A). During all electrophysiological recording sessions, rats were required to run at least 20 laps (10 in the counterclockwise direction and 10 in the clockwise direction) during two distinct episodes of behavior. Each track running epoch was flanked by a rest period in which the rat was placed in a towel-lined pot located in a position that was central to the circumference of the track. Data from the initial rest (before epoch 1 of track running) and the final rest (after epoch 2 of track running) periods were used to assess firing stability across the entire recording session. Furthermore, only cells that showed comparable firing rates during rest 1 and rest 3 were included in the analyses, and there was no significant difference in cell activity between Rest 1 (pre-behavior) and Rest 3 (post-behavior) ($T_{[7]} = 0.97$, $p = 0.36$; paired-samples).

The behavioral procedures are summarized in Table 1 and described below. Eight rats participated in 2 different track running conditions. During the first behavioral condition (day 1), rats traversed an empty track for both epochs of behavior (**'no objects condition'**; Figure 1A). A 20 minute rest period occurred between epoch 1 and epoch 2. For the second procedure (day 2), during epoch 1, eight novel objects that varied in size, color and texture were placed at eight different locations along the track. The side of the track that the objects were placed on alternated between the left and the right side and the rat had to run past these objects in order to obtain the food reward. Following either a 20 min or a 2 hr rest period, during epoch 2, six of the same objects used in epoch 1 remained in the same location on the track and two objects that were on the track during epoch 1 were replaced with two novel objects (**'objects both epochs condition'**; Figure 1B). Two delay conditions (20 min versus 2 hours) were included to measure the stability of PRC responses across short (20 min) and long delays (2 hrs) for a comparison between young and aged rats. Patterns of PRC neuron activity between the two delays conditions, however, were statistically indistinguishable for all variables that were measured and these data were grouped together into a single behavioral condition.

Each rat completed both behavioral procedures on consecutive days, and this process was repeated a minimum of 2 times and a maximum of 6 times. In 2 rats the behavioral procedures for electrophysiological recordings included an additional **'novel objects both epochs condition'**. In this procedure, 6 novel objects were placed on the track during both epochs of behavior (**'novel objects, both epochs condition'**; Figure 1C). This was done to quantify the extent that PRC neuron activity was affected by object identity.

For all conditions in which objects were placed on the track, the objects were fixed in place using *Velcro*, thus, rats could actively explore, rear, and climb on the objects without displacing them. Additionally, during all rest periods the objects were removed from the track so that the rat could not see them during the intervening delay period.

Surgical Procedures

Surgery was conducted according to National Institutes of Health guidelines for rodents and protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Prior to surgery, the rats were administered penicillin G (30,000 units intramuscularly in each hind limb) to combat infection. The rats were implanted with a “hyperdrive” manipulator device that held an array of 14 separately moveable tetrode recording probes. During surgical implantation the rats were maintained under anesthesia with isoflurane administered at doses ranging from 0.5% to 2.5%. The hyperdrive recording device, implantation methods, and the parallel recording methods have been described in detail elsewhere (Gothard et al., 1996). Briefly, each hyperdrive consisted of 14 drive screws coupled by a nut to a guide cannula. Twelve of these cannulae contained tetrodes (McNaughton et al., 1983b; Recce and O’Keefe, 1989), four-channel electrodes constructed by twisting together four strands of insulated 13 μm nichrome wire (H. P. Reid, Inc., Neptune, NJ). Two additional tetrodes with their individual wires shorted together served as an indifferent reference and an electroencephalogram (EEG) recording probe. A full turn of the screw advanced the tetrode 318 μm and all tetrodes were lowered between 4.0 and 6.0 mm ventral to the surface of brain. In all rats, recordings were made from the middle to caudal PRC region (between 4.0 and 6.5 posterior, 6.0 lateral to bregma, and angled 14° towards the midline). Following experimental procedures, 20 μA of direct current was administered to each tetrode and the location of each recording probe was verified histologically. Only the units recorded from tetrodes in the PRC were used in the current analyses and neurons recorded from other brain regions (e.g. ventral CA1 or area TE of the inferotemporal cortex) were excluded.

The majority of tetrodes were located in area 36 of the perirhinal cortex, but in 2 rats 4 tetrodes reached dorsal area 35. In only 1 rat, however, did a tetrode record neurons from area 35 that met the criteria for analysis. In this rat (8670), a total of 63 cells were recorded from the superficial layers of area 35, and there were no quantitative differences in the properties of cells in this subregion of PRC relative to area 36. Neurons were also recorded from both layer V and layers II/III. Supplemental Figure 1 shows Nissl-stained coronal sections from 2 different rat brains with representative tetrode recording tracks and lesions.

The implant was cemented in place with dental acrylic anchored by small screws. Immediately after surgery, all tetrodes were lowered approximately 1 mm into the cortex, and rats were orally administered 26 mg of acetaminophen (Children’s Tylenol Elixir, McNeil, PA) for analgesia. Oral administration of acetaminophen was continued for 3–5 days after surgery. Additionally, all rats were given either 25 mg of ampicillin (Bicillin, Wyeth Laboratories, Madison, NJ) or a combination of 20 mg of sulfamethoxazole and 0.4 mg trimethoprim (Hi-Tech Pharmacal Co., Inc, Amityville, NY) on a 10 days on/10 days off regimen for the duration of the experiment.

Neurophysiology

After surgery, tetrodes were lowered into the PRC over several weeks. The neutral reference electrode was advanced with other tetrodes and when an area of cortex was reached that did not record any unit activity, it was not moved again. The four channels of each tetrode were attached to a 50-channel unity-gain head stage (Neuralynx, Inc., Bozeman, MT). A multi-wire cable connected the head stage to digitally programmable amplifiers (Neuralynx, Inc.).

The spike signals were amplified by a factor of 1,000 – 5,000, band pass-filtered between 600 Hz and 6 kHz, and transmitted to the Cheetah Data Acquisition system (Neuralynx, Inc.). Signals were digitized at 32 kHz, and events that reached a predetermined threshold were recorded for a duration of 1 ms. Spikes were sorted offline on the basis of the amplitude and principal components from the four tetrode channels by means of a semiautomatic clustering algorithm (KlustaKwik, author: K. D. Harris, Rutgers–Newark). The resulting classification was corrected and refined manually with custom-written software (MClust, author: A. D. Redish, University of Minnesota; updated by S. L. Cowen and D. R. Euston, University of Arizona), resulting in a spike-train time series for each of the well-isolated cells. No attempt was made to match cells from one daily session to the next. Therefore, the numbers of recorded cells reported does not take into account possible recordings from the same cells on consecutive days.

Putative principal neurons in the deep and superficial layers of the PRC were identified by means of their waveform characteristics (peak-to-trough distance) and autocorrelogram features (Bartho et al., 2004). Specifically, neocortical “bursting” cells tend to have autocorrelograms with peaks at 3–6 ms followed by an exponential decay. Regular-spiking principal cells have an auto-correlogram with an exponential rise from 1 to tens of milliseconds. In contrast, the autocorrelograms of putative interneurons are not as fast decaying or slow rising as those of principal neurons (Bartho et al., 2004). These features were quantified for all of the recorded PRC neurons and are shown in Supplemental Figure 2.

Several diodes were mounted on the head stage to allow position tracking. The position of the diode array was detected by a TV camera placed directly above the experimental apparatus and recorded with a sampling frequency of 60 Hz. The sampling resolution was such that a pixel was approximately 0.3 cm.

Analyses and Statistics

Spike activity diagrams were constructed by plotting the circular trajectories of the animals on a linearized, one-dimensional scale, using a linear interpolation (Maurer et al., 2005). The track was then divided into 161 ~4.1 cm bins in order to calculate the the maximum firing rate, mean firing rate, and spatial information content (bits/spike) of each cell. Maximum and mean firing rate were obtained after normalizing spike activity by the occupancy of the animal, where occupancy was the amount of time that the rat spent in each ~4.1 cm bin. Finally, information content was calculated from the 161 bins of ~4.1 cm with the formula:

$$\sum P_i(R_i/R) \log_2(R_i/R)$$

Where P_i was the probability of occupancy for a bin, R_i was the firing rate of the bin, and R was the mean firing rate of cell (Skaggs et al., 1993). Information content was used to examine the activity correlates of PRC neurons. Specifically, it was observed that many PRC neurons have increased firing rates at the location of objects (see Results). These patterns of activity were termed ‘object fields’, and a PRC neuron was considered to have an object field if it had a spatial information score greater than 0.5 bits/spike, and the occupancy normalized mean firing rate within a bin exceeded the mean firing rate for at least 4 consecutive bins. Finally, if the 4 consecutive bins of higher firing overlapped with the area of the track that contained an object, the neuron was considered to have an ‘object field’.

Results

Behavioral correlates of perirhinal cortical cell spiking activity

The activity of 1,000 PRC principal neurons was recorded in this experiment. All putative interneurons were excluded from the subsequent analyses. A summary of the database from which the current results were derived is given in Supplemental Table 1. The parentheses indicate the layer of the PRC that the principal cells were recorded from. The majority of neurons were recorded from area 36 of the PRC. In four young rats, 539 neurons were recorded from layers II/III (Supplemental Table 1). Table 2 shows the mean and maximum firing rates by condition for all rats. The firing rates for layers II/III and layer V are shown separately. In the two rats where neurons from the different cortical laminae were recorded simultaneously during 24 different recording sessions, there was not a significant difference in the firing rates between layers II/III and layer V ($T_{[23]} = 1.23$, $p = 0.23$, paired-samples T test). Overall, there was not a significant effect of epoch or condition on mean or maximum firing rate ($p > 0.05$ for all comparisons).

When rats traversed the track with objects on it, many PRC neurons in both layers II/III and layer V showed a selective increase in their firing rates at the locations of objects. This occurred when objects were novel, as well as when objects were familiar (during epoch 2 of the objects, both epochs conditions). This increase in spiking activity in the vicinity of objects will be referred to as “object fields”. Figure 2 shows a representative example of the activity of eight PRC neurons recorded when objects were on the track (Figure 2A), and when the rat traversed the track in a behavioral condition without objects (Figure 2B). Because the object positions alternated between the left and the right side of the track, the rats had to modify their trajectory in order to run around the objects. This also ensured that the rats had to briefly view the objects in order to avoid colliding with them.

In order to quantify the number of PRC neurons that increased their firing rate at locations containing objects (i.e., the proportion of PRC neurons expressing ‘object fields’) the spatial information score (Skaggs et al., 1993) was calculated for each neuron, and then neurons with an information score above 0.5 bits/spikes were considered to have an object field if their occupancy normalized firing rate histogram exceeded the mean firing rate for at least 4 consecutive 4.1 cm bins, and this area of higher firing rate overlapped with an area of the track that contained an object.

The area of the track within 7 bins of a food dish (28.7 cm) was excluded for the calculation of information score, because this activity could presumably be related to the barrier, food dish, and/or reward rather than to the objects. The regions within 28.7 cm of a food dish were selected for exclusion because when the rats were within this area of the track their running speed was either zero or it was changing rapidly as the rat was stopping to obtain reward or accelerating after eating (Supplemental Figure 3).

When the spatial information score was calculated for all cells that showed activity on the maze (a maximum firing rate greater than 2 spikes/bin occupancy), there was not a significant main effect of epoch 1 versus 2 on the mean information score across all behavioral conditions ($F_{[1,15]} = 1.08$, $p = 0.32$; repeated-measures ANOVA). Moreover, there was not a significant interaction effect between epoch and behavioral condition on information score ($F_{[2,15]} = 0.50$, $p = 0.62$; repeated-measures ANOVA). This indicates that the spatial information score did not change significantly between epochs for any of the behavioral conditions. Finally, for the 2 rats that had tetrodes in both the deep and the superficial layers of the PRC, the information score for neurons in layer V (0.61 bits/spike) versus neurons in layers II/III (0.53 bits/spike) was not significantly different ($T_{[5]} = 1.51$, $p = 0.19$; paired-samples T test).

In contrast to the lack of effect of epoch on the spatial information scores of PRC neuron activity, there was a significant effect of behavioral condition on the mean spatial information score ($F_{[3,15]} = 5.26$, $p < 0.05$; repeated-measures ANOVA). Specifically, the increase in activity of PRC neurons at the locations of objects, as observed in the present study, significantly increased the spatial information score to 0.82 bits/spike relative to 0.41 bits/spike in the no objects condition on the present track. This is consistent with a previous report showing that in an open arena without objects, the mean spatial information score of PRC neurons is low (0.19 bits/spike) relative to the hippocampus (1.05 bits/spike) and to the medial entorhinal cortex (0.61 bits/spike; Hargreaves et al., 2005). Figure 3A shows the mean information scores of PRC neurons recorded during epochs 1 and 2 for the different behavioral conditions.

The proportion of cells that expressed ‘object field’ activity was determined using the criteria described in the Methods section. Briefly, a PRC neuron was considered to have an object field if its information score was above 0.5 bits/spike, and there were at least four consecutive 4.1 cm bins where the neuron’s firing rate exceeded the mean firing rate in a region of the track that contained an object. Supplemental Figure 4 shows the firing rate histogram of a representative PRC neuron with a spatial information score of 0.51 bits/spike and multiple object fields. The horizontal red line indicates the mean firing rate, and the red circles indicate the boundaries of the object fields.

Figure 3B shows the proportion of PRC cells that expressed object fields during epochs 1 and 2 of the different behavioral conditions. Using the criteria for determining whether a cell had an object field (described earlier), the proportion of PRC neurons that met this criteria varied significantly between the different behavioral conditions ($F_{[2,15]} = 5.54$, $p < 0.05$; repeated-measures ANOVA). Post hoc analysis indicated that significantly more cells met the criteria for having an object field when the track contained objects compared to when it was empty ($p < 0.05$; Tukey). Moreover, for the 2 rats that had tetrodes in both layer V and layers II/III of the PRC there was not a significant difference in the proportion of cells with object fields between the different layers of cortex ($T_{[5]} = 0.24$, $p = 0.82$; paired-sample T test). Epoch 1 versus epoch 2 did not significantly affect the proportion of cells with object fields for any of the behavioral conditions ($F_{[2,15]} = 0.01$, $p = 0.96$; repeated-measures ANOVA). In fact, most cells that fired at an object during epoch 1 retained this field during epoch 2 (see below).

In order to investigate the specificity of object fields to a particular object, the activity pattern correlations were calculated between epochs 1 and 2 for the two rats that participated in all three behavioral conditions. The correlation coefficients between the occupancy normalized firing rate histograms (4.1 cm bins) of epoch 1 and epoch 2 were calculated for each cell that showed activity on the maze (max firing rate > 2.0 Hz during at least one epoch). Figure 4 shows the occupancy normalized firing rate histogram and associated lap raster plots during epoch 1 (top panels) and epoch 2 (bottom panels) for two PRC neurons that were recorded during the Novel objects both epochs condition. In this example, the neuron in panel (A) showed uncorrelated activity when the objects were changed between epochs, while the neuron in panel (B) showed stable activity even though the objects changed between epochs. In fact, a large portion of PRC principal cells retained their firing pattern between epochs even when the objects were swapped.

When the proportion of cells that showed stable activity between epochs ($r = 0.8$) was calculated for the three different behavioral conditions it was observed that when objects remained the same between epochs (Objects both epochs condition) 42.1% of cells showed high stability. In contrast, when the objects changed between epochs (Novel objects both epochs) 32.7% of cells showed stability. Finally, when the track did not contain objects

between epochs only 12.5% of cells had a correlation greater than 0.8 between epochs. Figure 5 shows the proportion normalized frequency histograms of the correlation values for all active cells in (A) Objects both epochs, (B) Novel objects both epochs, and (C) No objects conditions. It was not possible to test for statistical significance with rat number as the sample size ($N = 2$), therefore, statistics were calculated for individual cells. There was a significant effect of condition on mean correlation between epochs ($F_{[2,219]} = 20.38$, $p < 0.01$; ANOVA). Post hoc analysis indicated that the cells had significantly lower correlation values between epochs during the No objects condition relative to the two conditions with objects ($p < 0.05$ for both comparisons, Tukey). Although there was not a significant effect of having the same objects on the track between epochs versus changing the objects ($p = 0.91$) on correlation values, the observation that ~10% fewer neurons showed high correlation values when the objects changed suggests that at least a subset of neurons were sensitive to the identity of an object. The remaining neurons appeared to have an object field at a spatial location with an object regardless of the identity of the object.

Does 3-dimensional object novelty affect PRC neuron activity during track running?

Rats have a natural tendency to explore objects, or other stimuli, that are novel (Ennaceur and Delacour, 1988). Therefore, the running velocity during epochs with novel versus familiar objects was compared, the idea being that behavioral slowing would be an indication of recognition that the object was novel, and faster velocities would indicate that the rats recognized objects as familiar. Figure 6A shows the mean running velocities during epoch 1 (white) and epoch 2 (light grey) for the No objects (8 rats), Objects both epochs (8 rats), and Novel objects both epochs (2 rats) conditions. Overall, the rats had significantly faster running velocities during epoch 2 relative to epoch 1 ($F_{[1,15]} = 12.24$, $p < 0.01$; repeated-measures ANOVA). Behavioral condition, however, did not significantly affect the running speed of the rats ($F_{[2,15]} = 1.75$, $p = 0.21$; repeated-measures ANOVA), which suggests that placing the objects on the track did not impede that animals' ability to traverse the track.

To examine whether or not novel objects on the track lead to increased exploration and slower running speeds, the rats' velocities during laps 1–2 was compared to laps 19–20 for conditions in which the track contained novel objects (epoch 1 of the objects both epochs condition, and epochs 1 and 2 of the novel objects both epochs condition), familiar objects (epoch 2 of objects both epochs condition), or no objects (Figure 6B). When the mean running speed of the rats for the first 2 laps was compared to the velocity for laps 19–20, statistical analysis revealed that the rats ran significantly more slowly during laps 1–2 relative to laps 19–20 ($F_{[1,21]} = 27.33$, $p < 0.001$; repeated-measures ANOVA). There was also a significant effect of behavioral condition on the difference in running velocity between laps 1–2 and laps 19–20 ($F_{[2,21]} = 8.25$, $p < 0.01$; repeated-measures ANOVA). Post hoc analysis indicated that the difference in running speeds between the first laps and the last laps were greater when there were novel objects on the track compared to conditions when the track contained familiar objects or to no objects ($p < 0.01$ for both comparisons, Tukey HSD). Together these data suggest that the rats spent more time traversing the track during early laps when the objects were novel, presumably due to stopping to explore objects, but as the objects became familiar the rats increased their running velocity.

In the hippocampus, the firing rate of CA1 pyramidal neurons is modulated by velocity (e.g., Czurko et al., 1999; Maurer et al., 2005; McNaughton et al., 1983a), therefore, slower overall running speed could lead to lower mean firing rates in regions of the brain where firing rate increases as the rat moves faster. This could potentially interfere with a possible novelty-modulated change in firing rate. In order to explore this possibility, firing rate was examined as a function of velocity for velocities between 3 cm/sec to 57 cm/sec. Figure 7 shows the relationship between mean firing rate and velocity for epoch 1 (A) and epoch 2

(B) during the No objects (black; 8 rats), Objects both epochs (red; 8 rats), and Novel objects both epochs (blue; 2 rats) conditions. There were no significant correlation between running velocity and PRC neuron mean firing rate for any behavioral condition ($r_{[79]} < 0.1$, $p > 0.5$ for all comparisons; Pearson's correlation coefficient). These data suggest that, in contrast to neurons in the hippocampus (e.g., Czurko et al., 1999; Maurer et al., 2005; McNaughton et al., 1983a) and medial entorhinal cortex (Sargolini et al., 2006), PRC neurons do not show significant velocity modulation in their firing rates.

Previous studies have reported that in rats PRC neurons have lower firing rates for familiar versus novel stimuli, and that this decrease in PRC neuron activity to previously experienced stimuli may provide a 'familiarity signal' that supports recognition memory (Zhu and Brown, 1995; Zhu et al., 1995a). Thus, a major hypothesis examined in the current experiment was whether novelty and/or familiarity modulate the firing characteristics of PRC cells to 3-dimensional objects.

In order to examine the predicted ability of novelty to modulate PRC discharge, the firing rate was measured across laps for epochs 1 and 2 of the different behavioral conditions, and then was normalized within a cell by calculating the Z-score firing rate for each lap. Neuron activity within 28.7 cm of a food dish was excluded from this analysis because of possible contamination from the increased activity that occurred in this region. Finally, the counterclockwise (CC) and clockwise (clock) laps were analyzed separately. If the relative novelty of objects modulated the firing rate of PRC neurons, then the difference in normalized firing rate between laps 1 and 10 should be greater for conditions with novel objects (in which the objects become more familiar within an episode of track running) compared to conditions with familiar objects or no objects. Figure 8A shows the mean normalized firing change between laps 1 and 10. Contrary to the hypothesis, there was no significant effect of behavioral condition or epoch on the firing rate difference between the first laps and the last laps ($F_{[3,66]} = 1.04$, $p = 0.38$; repeated-measures ANOVA). Thus, these data do not allow the null hypothesis (that is, that novelty does not affect PRC neuron firing rates) to be rejected. Moreover, given that the mean firing rate change when no objects were on the track is -0.03 Hz and 0.02 Hz when novel objects were on the track, it is possible to use the pooled variance from these distributions to calculate the probability of making a type II error or the statistical power ($1 - \beta$). Where β is determined by subtracting the mean firing rate change that was observed when novel objects were on the track from the critical value that would have allowed the null to be rejected and dividing by the standard error of the mean ($(\bar{X}_{critical} - \bar{X}_{novel}) / (s \sqrt{n})$). This equation provides a z transformation that can be used to derive β and the probability of incorrectly failing to reject the null hypothesis. When power was calculated it was determined that in the current data there was only a 4% chance of obtaining a type II error. This suggests that, under the current experimental conditions, the firing rates of the population of PRC neurons recorded in this experiment are not significantly influenced by the relative novelty of 3-dimensional objects.

Although there was no systematic change in the normalized firing rate over laps during any of the behavioral conditions, it is conceivable that a subset of PRC neurons might show a response decrement as objects go from being novel to relatively familiar. It is possible that this was not detected when the data were collapsed across all active neurons. To examine this explanation, PRC neurons that had firing rates at least 2 standard deviations above mean firing rate during lap 1 were identified. The proportion of recorded PRC neurons that met this criterion were then compared between the different behavioral conditions and between epochs 1 and 2 (Figure 8B).

Statistical analysis revealed that there was a significant effect of behavioral condition on the proportion of neurons showing a response decrement ($F_{[3,66]} = 4.55$, $p < 0.01$; repeated-

measures ANOVA). Post hoc analysis indicated that the conditions with familiar objects (Epoch 2 of the objects both epochs conditions) and the conditions with novel objects, (Epoch 1 of the objects both epochs conditions, and the Novel objects both epochs condition) had significantly more cells that showed a response decrement compared to the conditions without objects ($p < 0.05$ for all comparisons; simple contrasts). Contrary to the hypothesis that the response decrement is due to novel objects becoming familiar there was not a significant difference between the conditions with novel objects and conditions with familiar objects in the proportion of neurons that showed decremental firing rates ($p = 0.75$; simple contrast). Therefore, these data are not consistent with the hypothesis that the activity patterns of PRC neurons decline systematically as objects go from being novel to familiar. Moreover, because the rats expressed behavior that was indicative of object recognition (Figure 7), the mnemonic mechanism that supports stimulus recognition is not likely to be changes in PRC neuron firing rate.

Discussion

Two major novel findings have emerged from the current experiment. First, the activity of a portion of perirhinal (PRC) neurons is increased in areas of the track in which objects were located (Figure 2). The areas of higher PRC activity are referred to as ‘object fields’ because this firing property was not observed in conditions in which the track did not have objects. Second, the firing rates of PRC neurons, and the proportions of these cells that express object fields is not modulated by the relative novelty or familiarity of 3-dimensional objects (Figure 7).

Perirhinal cortical object fields

The observation that PRC neurons show punctate increases in activity at areas of a track that contain objects suggests that this structure could be involved in relaying non-spatial information to the hippocampus. There is general agreement that the hippocampus is critical for recollection (Fortin et al., 2004) and episodic memory (e.g., Eichenbaum et al., 2007; Nadel and Hardt, 2004; Nadel et al., 1985). Both of these cognitive abilities require the association of ‘what’ stimulus was encountered with ‘where’ it was experienced. Thus, the primary afferents of the hippocampus must relay this information. Interestingly, there is evidence that the encoding of spatial and non-spatial information is, at least partially, dissociated within the medial temporal lobe between the medial and lateral entorhinal cortices (Deshmukh and Knierim, 2011; Hargreaves et al., 2005). Two lines of evidence reported in the current paper support the notion that the PRC participates in the encoding of non-spatial information. In addition to object-related activity, in contrast to neurons in the hippocampus (e.g., McNaughton et al., 1983a; Shen et al., 1997) and medial entorhinal cortex (Sargolini et al., 2006), which are sensitive to an animal’s self-motion, PRC neurons do not show a significant relationship between firing rate and running velocity (Figure 7).

This is illuminating considering that one hypothesis regarding the function of the PRC is that it contributes to perception by being at the highest level of the ventral ‘perceptual pathway’ (Bussey et al., 2002; Bussey et al., 2005; Murray et al., 2007; Wise and Murray, 2012). Moreover, as a structure within the medial temporal lobe, it also supports memory for objects and the contents of scenes (Murray et al., 2007). The observation that PRC cells were responsive to objects, but showed non-specific activity when objects were not present, and that in a portion of PRC neurons the objects-related activity remained consistent across delays of up to 2 hours, supports this view.

An interesting observation from the current experiment is that a proportion of PRC cells (~32%) showed very high correlation values between 2 distinct epochs of behavior when the objects from epoch 1 were replaced with new objects for epoch 2 (Novel objects, both

epochs; Figure 5). Although some cells (~10%) showed uncorrelated activity when the objects were changed (i.e., their increased activity was dependent on the object identity), these data indicate that the presence of objects could lead to the expression of an object field in a particular place, regardless of the stimulus characteristics. There are at least two potential explanations for this finding. First, it is possible that because the rats were not required to detect a change in objects in order to receive a reward with the current study design, the PRC did not show discriminative activity. In fact, unlike the first epoch with novel objects, the running velocity of the rats that performed the Novel objects, both epochs condition was fast during epoch 2 even for laps 1 and 2 (Figure 6A), which suggests that the rats did not behave as if they detected that the objects from epoch 1 had been replaced. Thus, the rats may have had a general awareness of objects without encoding specific details of object identity. Future experiments should examine whether or not a requirement to detect stimulus differences would lead to more orthogonal activity patterns. It is also conceivable that object identity is sparsely encoded in the PRC and that a 10% change in the population of active cells when the object stimuli change is sufficient to represent the new sensory input within this brain region.

An alternative explanation is that the PRC responds to a conjunctive of an object in a spatial location and that in some circumstances the location may have more influence on the activity than the stimulus characteristics. This has interesting implications regarding the recent discovery of boundary vector cells in the entorhinal cortex (Solstad et al., 2008), subiculum (Lever et al., 2009), and pre- and para-subiculum (Boccarda et al., 2010). One theory is that these neurons, which show activity near the boundaries of an environment, may be instrumental in planning trajectories and anchoring grid fields and place fields to a geometric reference frame. It is known that objects influence the firing characteristics of CA1 place fields (Burke et al., 2011). If objects are analogous to landmarks, it is possible that these object fields are performing a similar function as the boundary vector cells by supporting the rat's allocentric representation of the environment and providing the animal with information necessary to navigate around the track without colliding with an object. Moreover, because object-related activity was observed in both the deep and superficial layers of the PRC, it is also possible that this pattern of activity does contain some spatial information that is projected to the PRC from the hippocampus and subicular complex. Finally, the postrhinal cortex (analogous to parahippocampal cortex in the primate) projects strongly to the PRC and is also thought to be involved in the encoding of spatial context (Eacott and Gaffan, 2005) and is connected with other brain areas that are involved in spatial cognition (Burwell and Amaral, 1998; Libby et al., 2012; Suzuki and Amaral, 1994). Thus, it is unlikely that spatial and non-spatial information are isolated within the medial temporal lobes.

The lack of novelty modulation on perirhinal cortical neuron activity

The lack of a significant effect of novel versus familiar objects on PRC neuron activity, under these experiment conditions, suggests that a response decrement of PRC neuron firing rates as a stimulus becomes familiar may not be the physiological correlate of PRC-dependent object recognition for real world 3-dimensional stimuli. These results have implications regarding the type of neural coding scheme employed by the PRC. A long standing view is that the PRC encodes information about the relative familiarity and recency of a stimulus with a simple rate code. Specifically, neuron firing rates are higher if a stimulus is novel or if a stimulus has not been experienced recently (for review, see Brown and Aggleton, 2001). The current results do not support the idea that response decrements support stimulus recognition. Given that this type of rate code can be vulnerable to noise and spike failures, and the probability of synaptic transmission between the PRC and its cortical efferents is low (Pelletier et al., 2004), the signal-to-noise ratio for relaying a 'response

decrement' code might not be optimal. In light of the current findings, it is more plausible that the PRC utilizes a population code, which relies on the joint activities of a number of neurons and each neuron has a different distribution of responses over some set of inputs.

The question remains, however, why previous experiments have reported response decrements of PRC neuron activity (e.g., Miller et al., 1991; Xiang and Brown, 1998; Zhu et al., 1995a). There are several methodological explanations for the apparent discrepancies between the current experiment and previous ones. The current experiment used chronically implanted recording probes, while many previous experiments obtained recordings acutely. It is possible that during acute recordings there is a bias to record from higher firing rate cells, which are more likely to decrease their rate over time.

An alternative explanation is that this is the first experiment in which the animal was moving through space in order to be exposed to 3-dimensional objects. Typically, PRC neuron activity is monitored while an animal views 2-dimensional images and is fixed in place (e.g., Miller et al., 1991; Xiang and Brown, 1998; Zhu et al., 1995a). This condition may be more conducive to observing novelty-modulated response decrements. Finally, the previous PRC recordings reporting novelty-modulated firing rate changes have not isolated putative principal cells from interneurons. Recent data suggest that it may be the interneurons of the inferior temporal cortex that show reduced activity to familiar versus novel stimuli (Woloszyn and Sheinberg, 2012), and the current data excluded interneurons.

Summary

When rats traverse a track with 3-dimensional objects, the primary behavioral correlate of PRC single-unit activity was increased spiking at the location of objects. This pattern of activity was termed 'object fields'. Contrary to previous electrophysiological recordings from rats (Zhu and Brown, 1995; Zhu et al., 1995a), we did not observe a response decrement in PRC neurons as objects went from being novel to familiar. Because the rats that participated in the current experiment expressed behavior that was indicative of successful object recognition, the lack of novelty-modulated firing rate decreases, as reported in the current paper, suggests that a response decrement is not the neural correlate of a familiarity signal under these behavioral conditions. It is also notable that a significant portion of the object-related activity in the population of PRC neurons recorded during this experiment retained their firing fields even after the objects were replaced with unique objects. This suggests that although PRC cells require the presence of objects in order to show punctate increases in activity at specific locations, the stimulus characteristics may not be the only factor that influences PRC cellular activity. Rather it is possible that these cells respond to a conjunction of the landmark features of an environment and spatial location.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Behavioral procedures used for electrophysiological recordings

The track used for behavior during all electrophysiological recordings. Rats were required to run 20 laps bi-directionally (10 counterclockwise, 10 clockwise) for a food reward. **(A)** During the No objects condition the track was empty during both epochs of track running. Rewards were given in two food dishes located on opposite sides of a barrier (indicated by squares), at the position where the rat was required to turn around. The “X” indicates the location of the pot that the rat was placed in during rest episodes. **(B)** Examples of behavioral procedures where objects were placed on the track. Numbers indicate the approximate positions of the different objects. In the ‘objects both epochs condition’, 8 novel objects were placed at discrete locations around the track for the first epoch of behavior (top panel), and the rat had to run past the objects to obtain the food reward. During the second epoch of behavior (bottom panel), 6 of the 8 objects used in epoch 1 were placed on the track at the same location as in epoch 1, while 2 of the 8 objects were removed and substituted with 2 novel objects (in this case objects 3 and 5 were replaced with objects 9 and 10 as indicated by the grey boxes). **(C)** Two of the eight rats participated in an additional ‘novel objects both epochs condition’. For this behavioral procedure, 6 novel objects were placed around the track for the first epoch of track running (top panel; objects 1–6). During the second epoch the objects from epoch 1 were replaced with 6 new objects (bottom panel objects 7–12).

Figure 2. PRC neuron activity patterns

(A) The activity of four representative PRC neurons under conditions with objects on the track. In the top panels, the black trace indicates the path of the rats and the red spots indicate the locations of spikes. The blue numbers represent the locations of objects. The bottom panels show the occupancy-normalized firing rate maps of the cells shown in A. (B) The raw spike data of four PRC neurons recorded when the track did not contain objects (top panels), and the associated occupancy-normalized firing rate maps (bottom panels).

Figure 3. The effect of objects on PRC neuron spatial information content

(A) The mean spatial information score of the PRC neurons that showed activity during track running for epoch 1 (white) and epoch 2 (light grey) during the different behavioral conditions. (B) The mean proportion of the PRC neurons that met the criteria for having at least one object field during track running for epoch 1 (white) and epoch 2 (light grey) for the different behavioral conditions. Error bars represent ± 1 SEM.

Figure 4. Example PRC neuron activity patterns for the Novel objects both epochs condition
 The firing patterns for two representative PRC neurons during the condition in which the 6 novel objects on the track were changed between epoch 1 (top panels) and epoch two (bottom panels). The firing rate histogram by 'linearized' position during the first epoch (top panel) and the second epoch (bottom panel). The X axes are position on the track (cm) with zero indicating the position of the barrier. Positive numbers are for laps when the rat was running in the counterclockwise direction while negative numbers indicate the position when the rat was running in the clockwise direction. The Y axes are the occupancy normalized firing rates for each neuron. The associated raster plots by lap are also shown. Each horizontal line indicates a lap and blue lines are the laps in which the rat ran in the counterclockwise direction while red lines represent laps run in the clockwise direction. The black vertical lines indicate the position of the objects. (A) One neuron showed a low correlation value ($r < 0.4$) between the two epochs of track running while the other cell (B) had a similar firing field during both epochs even though the objects were different ($r > 0.8$).

Figure 5. Activity pattern correlations between epochs 1 and 2

Frequency distributions of activity correlations (r) between epoch 1 and epoch 2 for PRC neurons in the (A) Objects both epochs, (B) Novel objects both epochs, and (C) No objects conditions. Each distribution was normalized by the number of neurons recorded during a given condition. The vertical dashed line indicates a correlation value of 0.8.

Figure 6. Running velocity during the different behavioral conditions

(A) The mean running velocity during epoch 1 (white) and epoch 2 (light grey) for the different behavioral conditions. Rats had significantly faster running velocities during epoch 2 relative to epoch 1 ($F_{[1,15]} = 12.24$, $p < 0.01$; repeated-measures ANOVA). Behavioral condition, however, did not significantly affect the running speed of the rats ($F_{[2,15]} = 1.75$, $p = 0.21$; repeated-measures ANOVA). (B) The mean running velocity during laps 1–2 compared laps 19–20 when the track contained novel objects (grey), familiar objects (grey dashed), or no objects (black). Rats ran slower during laps 1–2 relative to laps 19–20 ($F_{[1,21]} = 27.33$, $p < 0.001$; repeated-measures ANOVA). Moreover, behavioral condition significantly affected the difference in running velocity between laps 1–2 and laps 19–20 ($F_{[2,21]} = 8.25$, $p < 0.01$; repeated-measures ANOVA), with the novel objects condition showing the greatest velocity change.

Figure 7. Firing rate by running velocity

Mean firing rate was not significantly modulated by velocity during (A) epoch 1 or (B) epoch 2 for any of the behavioral conditions. Error bars represent ± 1 SEM.

Figure 8. The effect of novelty on firing rate

(A) The difference in normalized firing rate between lap 1 and lap 10. There was no systematic change in firing rate between lap 1 and lap 10 during either epoch 1 or epoch 2. Moreover, there was no significant difference in normalized firing rate between lap 1 and lap 10 for the No objects (black), Objects both epochs (blue), or Novel objects both epochs conditions (red). (B) The proportion of cells that had a response decrement between of at least 2 standard deviations between laps 1 and 10 for the No objects (black), Objects both epochs (blue), and Novel objects both epochs conditions (red). Error bars represent ± 1 SEM.

Table 1

Behavioral procedures

Condition	Epoch 1	Delay	Epoch 2	# of rats
No objects	Empty track	20 min	Empty track	8
Objects both epochs	8 novel objects	20 min or 2 hr	6 familiar objects, 2 novel objects	8
Novel objects both epochs	6 novel objects	20 min	6 novel objects	2

Table 2

Mean and maximum firing rate

rat	No objects	Objects both epoch	Novel objects both epochs
8412	0.79/3.59 (V)	1.83/4.17 (V)	
8509	0.78/3.59 (V)	1.71/7.80 (V)	
8583	1.68/4.32 (II/III)	1.36/5.10 (II/III)	
8661	2.92 & 1.28/5.96 & 5.21 (II/III & V)	2.62 & 2.28/5.74 & 6.01 (II/III & V)	
8670	2.02 & 2.40/5.11 & 4.97 (II/III & V)	1.56 & 1.84/4.58 & 4.75 (II/III & V)	
8883	1.74/2.97 (V)	0.88/4.97 (V)	
8993	1.74/4.82 (V)	0.97/3.24 (V)	2.12/5.73 (V)
9095	0.66/2.47 (II/III)	4.25/8.00 (II/III)	1.46/4.25 (II/III)
Mean ± SEM	1.93 ± 0.38	1.59 ± 0.22	1.79 ± 0.33