Single Neuron Activity and Theta Modulation in Postrhinal Cortex during Visual Object Discrimination

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SUMMARY

Postrhinal cortex, rodent homolog of the primate parahippocampal cortex, processes spatial and contextual information. Our hypothesis of postrhinal function is that it serves to encode context, in part, by forming representations that link objects to places. To test this hypothesis, we recorded postrhinal neurons and local field potentials (LFPs) in rats trained on a two-choice, visual discrimination task. As predicted, many postrhinal neurons signaled object-location conjunctions. Another large proportion encoded egocentric motor responses. In addition, postrhinal LFPs exhibited strong oscillatory rhythms in the theta band, and many postrhinal neurons were phase locked to theta. Although correlated with running speed, theta power was lower than predicted by speed alone immediately before and after choice. However, theta power was significantly increased following incorrect decisions, suggesting a role in signaling error. These findings provide evidence that postrhinal cortex encodes representations that link objects to places and suggest postrhinal theta modulation extends to cognitive as well as spatial functions.

INTRODUCTION

The predominant view of medial temporal lobe function emphasizes that spatial and nonspatial information reach the hippocampus through segregated parahippocampal pathways (e.g., Eichenbaum et al., 2007; Knierim et al., 2006). Spatial and contextual information is conveyed by the postrhinal (POR) cortex (parahippocampal cortex [PHC] in the primate brain) and the medial entorhinal cortex (MEC), whereas nonspatial information is conveyed by the perirhinal cortex (PER) and the lateral entorhinal cortex (LEC). These two pathways, however, are not completely segregated. For example, intrinsic entorhinal connections span the LEC and MEC in both rats and monkeys (Chrobak and Amaral, 2007; Dolorfo and Amaral, 1998). In addition, in both species, the PER located in the nonspatial pathway is reciprocally connected with the POR/PHC in the spatial pathway (Burwell and Amaral, 1998b; Suzuki and Amaral, 1994b).

Given the anatomical evidence for nonspatial input to the spatial pathway, it is not surprising that the PHC is implicated in a variety of higher-order cognitive functions, some of which are not strictly limited to the spatial domain. These functions include visual scene processing (Epstein et al., 1999), processing of objects in large spaces (Maguire et al., 1998), binding of objects and contexts (Hayes et al., 2007), retrieval of spatial context (Burgess et al., 2001), object location processing (Böckler et al., 1998), and episodic memory (e.g., Gabrieli et al., 1997; Ranganath et al., 2004). Evidence from neuroimaging studies suggests that activity in the PHC increases when individuals are presented with objects that have strong contextual associations (Aminoff et al., 2007; Bar and Aminoff, 2003; Bar et al., 2008). In addition, Mullally and Maguire (2011) provided evidence that the PHC is more active in response to objects that are larger and stationary (spatially defining) as opposed to objects that are smaller and more portable (spatially ambiguous). Results of experimental lesion studies in rats also suggest that the POR processes information about objects, especially with respect to place or context (Gaffan et al., 2004; Norman and Eacott, 2005).

Based on the above review, a reasonable hypothesis is that the POR and PHC represent contexts and scenes, in part, by encoding the spatial layout of objects in the local environment. To test this hypothesis, we recorded from POR neurons during performance on a visual discrimination task in which rats learned object discriminations in multiple places (Figure 1). Stimuli were pairs of two-dimensional (2D) objects back-projected onto the floor of a bow-tie shaped testing area in a novel apparatus, the floor projection maze (Furtak et al., 2009). The location of stimulus presentation alternated by trial between the east and west sides of the maze. We predicted that POR neurons would signal the presence of conjunctions of objects and places as well as particular locations.

Consistent with our prediction, POR cells indeed signaled the conjunction of objects and locations. This finding argues against...
RESULTS

Animals were trained on two discrimination problems, each consisting of a pair of 2D visual stimuli (Figure 1D) back-projected onto the floor of the maze (Figure 1A). Object pairs were presented in two locations (east and west) to allow assessment of conjunctions of object-location selectivity. After a series of shaping steps (see Table S1 and Supplemental Text available online), rats were trained on the final task in which presentation of object pairs alternated from east to west by trial (Figures 1B and 1E). Each new trial was signaled by the onset of white noise when the rat was in the reward area on the side of the maze opposite the side on which stimuli would next be presented (Figure 1E). Stimuli were presented when the rat had remained still in the ready position for a variable time (500–700 ms). The rat made a choice by approaching one of the two stimuli. A correct choice was followed by chocolate milk reward delivered in the reward area at a location behind the correct stimulus. If the rat first approached the incorrect stimulus, the trial terminated and no reward was provided. Initially, the two problems were presented in blocks of 10 trials. Following surgery, implanted rats were retrained on the blocked-trial version of the task until performing at >70% accuracy. They were then placed on a random-trial version of the task. Single-unit and local field potential (LFP) recordings were obtained during daily sessions of 100 trials. All sessions in which the animal performed at or above 65% correct were analyzed. If performance dropped below 65%, rats were returned to blocked trials until accuracy improved. Preliminary analyses indicated no differences in selectivity between blocked and random sessions, so data from both session types were combined for all analyses. Stereotrodes were lowered at the end of daily recording sessions, and no attempt was made to hold single units across sessions.

For recorded sessions, percent correct ranged from 65%–79% (69% ± 1%). Mean latencies to choice were 3.67 ± 0.10 s for correct trials and 2.95 ± 0.17 s for incorrect trials. Median latencies were 2.45 s for correct trials and 0.89 s for incorrect trials. Once well-trained, rats exhibited highly stereotyped pathways when approaching the chosen object, and nearly always checked the other food port before returning to the ready position (Figure 1C, right).

Postrhinal Correlates of Object, Location, and Egocentric Response

We recorded 97 well-isolated cells from 31 stereotrodes implanted in the POR of five animals during 32 sessions (electrode tip locations; Figure 2A). The mean firing rate per session for all cells was 3.66 ± 0.29 Hz (range, 0.55–15.64 Hz). Firing rates were analyzed separately for three behaviorally relevant epochs of time (Figure 1E): the “stimulus” epoch, the 500 ms following stimulus presentation; the “selection” epoch, the 500 ms before stimulus choice; and the “reward” epoch, the 500 ms following stimulus choice during which reward was delivered. Behavioral correlates were determined by factorial analysis of variance (ANOVA) of correct trials (side × object × response). Analyses were restricted to correct trials because low numbers of incorrect trials resulted in low sampling of some trial types. Of the 97 cells isolated, 71 met an analysis criterion of at least three correct trials for each of the eight trial types and a minimum of 20 spikes in the epoch analyzed (stimulus, selection, or reward). Of those 71 cells, 14 cells were recorded on stereotrodes in which one wire was compromised. All cells including those 14 cells were determined by autocorrelation analysis and cluster separation to be well isolated (Figure 2C).

Of the 71 criterion cells, 55 (77%) displayed selectivity as demonstrated by main effects or interactions of object, side, and response in at least one epoch. For example, some cells showed selectivity for a side of the maze (west, Figure 3A, left), a particular object (object 1, Figure 3B, left), a particular object...
in a particular location (object 2 in the southeast, Figure 3B, right), or an egocentric response (right response, Figure 3C, right).

We predicted that POR cells would show patterns of activity consistent with representing conjunctions of 2D objects and places. As expected, a number of POR cells (25/71, 35%) showed selectivity for both object and location in at least one behavioral epoch. Numbers of such cells were roughly equal across epochs (Table 1). Object-location conjunction cells were of three types. The first type, cells with object 3 side interactions, fired more to an object depending on the side of the maze on which it was presented. For example, a cell might fire more to object 1 in the east and object 2 in the west. A second type of object-location conjunction cell, identified by an object 3 response interaction, was similar except that the location of firing for a particular pattern was located on the diagonal. For example, a cell would fire more to object 1 when it appeared on the right, regardless of the side of the maze (Figure 3B, center). We considered these cells to be object-location cells because they also fired more to one object than the other in specific locations (e.g., in the northwest and southeast). Finally, we observed cells that fired preferentially to a particular object only when it was in a single quadrant. These cells were identified by a significant object 3 side 3 response interaction. The example cell shown in Figure 3B (right) fired preferentially to object 2 only when it was located in one of the four quadrants.

Because the POR is implicated in the processing of spatial and contextual information, we also predicted neural correlates of specific locations. There were two types of location correlates identified by factorial ANOVA. Selectivity for side was indicated by increased firing on the east or the west side of the maze (Figure 2B, middle panels; Figure 3A, left). A conjunction of side and response could indicate selectivity for the north or south of the maze (Figure 2B, lower; Figure 3A, center) or for a single quadrant (Figure 3A, right). Overall, 41% of cells meeting criterion (29/71) exhibited a main effect of side or a side 3 response interaction in at least one epoch. During the stimulus epoch, when the rat was positioned near the center of the maze, five cells demonstrated such location correlates (Table 1). This is interesting because, at stimulus onset, the animal was in the center of the maze viewing the location in which the object had appeared, but was not physically in the location. During the selection and reward epochs, when the animals were approaching or were in the location of a stimulus, more cells showed selectivity for location—13 and 16 during selection and reward, respectively (Table 1). These results suggest that attending to a particular location from a distance does control activity of POR cells, but not as robustly as the animal’s physical location, at least in this task.

Four cells (6%) exhibited a main effect of object, in that firing rate was significantly higher to one of the two correct objects (Figure 3B, left). Two of those cells, however, also showed conjunctive selectivity in that they also exhibited a significant effect or interaction for some other aspect of the task (Table 1). Unexpectedly, a large proportion of POR cells showed selectivity for a left versus right motor response regardless of the identity of the correct object or the side of the maze on which it was presented (Figure 2B, upper; Figure 3C; Table 1). Of the 71 cells

**Figure 2. Histology and Examples of Neuronal Correlates**

(A) Location of stereotrode tips in coronal sections of POR (shown in gray) between −7.70 to −8.80 mm relative to bregma.

(B) Example behavioral correlates of postrhinal cells during the stimulus (left), selection (center), and reward (right) epochs. Raster plots and peri-event histograms are shown for representative examples of cells with task-related firing patterns. For the stimulus epoch, time 0 is stimulus onset. For the selection and reward epochs, time 0 is choice. The upper row shows cells that fired more during left or right responses for correct trials. Examples of cells with spatial selectivity are shown in the middle and bottom rows.

(C) Waveforms for the isolated cells shown in (B). For each row, the upper, middle, and lower waveforms correspond to the left, center, and right histograms and rastergrams. Time bins = 0.05 s, scale bar = 250 μs, 100 μV.
meeting criterion, 49% (35) exhibited a main effect of response in at least one epoch. The cell shown in Figure 3C fired more for right than left responses. The effect was marginally significant during the stimulus epoch and significant during the selection and reward epochs. It might be argued that the response cells were simply signaling two different spatial locations rather than the egocentric response. If that were the case, we would expect to see roughly equal numbers of cells that signal the two locations in the north (left east and right west) or the two locations in the south (east right and west left). We quantified the number of cells with a significant side response interaction that fired more in the north or the south and observed 0, 4, and 2 such cells during stimulus, selection, and reward epochs, respectively. In contrast, we observed 3, 10, and 15 response cells during the same epochs (Table 1), suggesting that these cells encode something other than location, most likely egocentric responses.

We were interested in whether differences in patterns of selectivity depended upon the laminar location of cells. Of our 71 criterion cells, 32 were in superficial layers, 18 were in deep layers and the layer of the remaining 21 cells could not be precisely determined. Whether or not a cell showed selectivity for egocentric responses, particular objects, or object-location conjunctions was not influenced by laminar location (Table S2). As might be predicted by connectivity, however, cells in deep layers were more likely to exhibit spatial selectivity ($\chi^2_{df} = 3.125, p < 0.039$). Deep layers are targeted by subicular input (Kloosterman et al., 2003). In addition, although the posterior parietal cortex projects to superficial and deep layers, the deep layers are preferentially targeted (Burwell and Amaral, 1998a).

In general, the proportion of cells showing some type of selectivity differed significantly across epochs ($\chi^2_{df} = 12.07, p < 0.002$), such that the numbers increased as the trial progressed.
Table 1. Response Patterns of POR Cells by Factorial ANOVA

<table>
<thead>
<tr>
<th>Significant Main Effects and Interactions</th>
<th>Cells by Epoch (n = 71)</th>
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<tbody>
<tr>
<td></td>
<td>Stimulus</td>
</tr>
<tr>
<td>Object-Location Conjunctions</td>
<td>7</td>
</tr>
<tr>
<td>Object*Side</td>
<td>1</td>
</tr>
<tr>
<td>Object*Response</td>
<td>1</td>
</tr>
<tr>
<td>Object<em>Side</em>Response</td>
<td>1</td>
</tr>
<tr>
<td>Multiple Object/Response</td>
<td>4</td>
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<tr>
<td>Multiple Object/Side/Response</td>
<td></td>
</tr>
<tr>
<td>Object Correlates</td>
<td>†</td>
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<tr>
<td>Location Correlates</td>
<td></td>
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<tr>
<td>Side</td>
<td>2</td>
</tr>
<tr>
<td>Side*Response</td>
<td>1</td>
</tr>
<tr>
<td>Multiple Side/Response</td>
<td>2</td>
</tr>
<tr>
<td>Response Correlates</td>
<td>3</td>
</tr>
<tr>
<td>Total Selective Cells (of 71)</td>
<td>15</td>
</tr>
</tbody>
</table>

Numbers of cells that exhibited significant main effects or interactions during each of the three behavioral epochs (Stimulus, Selection, and Reward). The 71 cells were analyzed separately for each epoch. Within each epoch, most cells exhibited a single main effect or interaction, but some cells exhibited multiple main effects and/or interactions (shown in italics). For example, two cells in the Multiple Side/Response category showed a main effect of response and a response*side interaction. † Under Object Correlates, one cell in the Stimulus and one in the Selection epochs showed a main effect of Object, but each of those cells had multiple main effects and/or interactions and are thus counted in one of the multiple categories. See Table S2 for laminar differences and Table S3 for stability across epochs.

from 15 cells during presentation of the stimulus to 34 and 41 during selection and reward, respectively (Table 1). Numbers of cells exhibiting object and object-location conjunctions were not significantly different across epochs (p = 0.60). Cells showing egocentric response correlates, however, increased significantly across epochs (χ² = 7.79, p < 0.02). Numbers of cells showing location correlates were marginally significantly different across epochs (χ² = 5.71, p < 0.06). Thus, location and response correlates were more evident in the selection and reward epochs.

To understand the stability of behavioral correlates, we examined patterns of selectivity across the three epochs. Of the 55 cells that were responsive in at least one epoch, 26 (47%) exhibited selectivity only in a single epoch and 29 (53%) exhibited selectivity in 2 or 3 epochs (Table S3). Of the 29 cells selective in more than one epoch, 11 exhibited similar patterns of selectivity across epochs. Of those, 6 were stable for location, 4 for response, and one for object-location selectivity. The remaining 18 of 29 cells changed patterns of selectivity across epochs. In all cases, the change was between stimulus/selection epochs and the reward epoch when the stimulus was no longer visible. One-third of these cells (6) changed from object-location to location selectivity and one third (6) changed from object-location to response selectivity. Three cells changed from some type of location selectivity to object-location selectivity in that the cell acquired selectivity for a particular object in the same location. Finally, three cells changed from selectivity for one location or response to selectivity for another location or response during the reward epoch. Thus, location and response cells tended to be stable across epochs, and cells that exhibited object location-selectivity changed between the stimulus/selection epochs and the reward epoch, when the stimulus was no longer visible.

Theta Modulation in POR

Because brain oscillations, particularly in the theta and gamma ranges, are thought to represent or encode various important aspects of memory and cognition, we conducted multitaper spectral analyses of the POR LFP signals, focusing on the theta and gamma bands. The power spectrum of 42 LFPs (21 sessions from five rats) was calculated over the entire session. Theta rhythms were defined as 6–12 Hz oscillations, low gamma as 30–50 Hz, and high gamma as 70–110 Hz.

For POR LFPs, power in the theta range was higher than that expected from a 1/f power spectrum for 37 of 42 LFPs (88%; Figure 4A). To examine whether there were any task-dependent variations in the theta-band LFP signal, we calculated the power spectrum for each of the four task epochs, averaging across all trials of a session. Theta power differed across epochs in 90% of the LFPs (38/42). Specifically, theta power was greater for the task-related epochs (ready position, stimulus, and selection) when the animal was waiting for or processing the visual stimulus, as compared to the non-task-related reward epoch, when the stimulus was no longer relevant (Figure 4B).

Theta oscillations in the hippocampus are strongly modulated by the speed at which an animal moves (reviewed in Buzsáki, 2005), so we next asked if there were systematic changes in running speed across epochs, and if POR theta oscillations were also modulated by speed. Figure 5A shows examples of event-triggered average running speed for three representative animals. Rats were required to be in the ready location for

![Figure 4. Increased Theta Power in POR](image-url)
500–700 ms prior to stimulus onset, so speed was low during that time (Figure 5A, upper panels). Immediately after presentation of the stimulus, animals began to move toward the choice point. Speed tended to be highest during the selection epoch, prior to choice, and lowest during the reward epoch when animals were checking the reward port for food (Figure 5A, lower panels). We found a strong correlation between running speed and the amplitude of theta oscillations (Figures 5B and 5C). Running speed was an excellent predictor of theta power in the nontask phase (reward to ready position), as well as the ready and stimulus periods of the task. However, theta power during both the selection and reward epochs was significantly lower than expected based on running speed alone (Figure 5C). Thus, theta power was additionally modulated (in this case, decreased) by the selection and reward epochs of the task.

An analysis of correct versus incorrect trials revealed that running speed was significantly slower for incorrect trials as compared to correct trials during the selection epoch (t = 17.40, n = 21 sessions, p < 0.0001), but not for any other epoch (Figure 6A). Interestingly, for the reward epoch, in which running speed was exactly the same for correct and incorrect trials (p = 0.58; Figure S1), theta power was significantly greater following incorrect choices (t = −3.88, n = 42 LFPs, p < 0.0004; Figure 6B).

Finally, using circular statistics, we examined whether the activity of individual cells was modulated by POR theta. Of 69 cells recorded during LFP sessions, 26 (38%) showed significant phase-locking (Figure 7). This proportion was similar whether phase locking was determined using a local (same stereotrode) or nonlocal field potential (different stereotrode, but also in POR). Figure 7A shows three examples of cells phase locked to theta. Although some cells were phase locked to the peak of theta (5/26; 19%), most were phase locked to the trough (21/26; 81%). We examined the possibility that the cells phase-locked to the peak of theta are interneurons by assessing a spike width parameter, the duration from trough to peak. Four of the 26 phase locked cells (15%) exhibited narrow spike widths, with a mean trough to peak duration of 135.2 ms and were thus classified as putative fast-spiking inhibitory interneurons. The remaining phase locked cells (22 of 26) had broad spike widths, with a mean trough to peak duration of 425 ms and were classified as putative excitatory cells. Three of the four putative interneurons were phase locked to the peak of theta oscillations. Twenty of twenty-two excitatory cells were phase locked to the trough of theta. These results are similar to observations of the distinct theta phases of identified pyramidal cells and fast-spiking parvalbumin-positive basket cells in the hippocampus (Klausberger and Somogyi, 2008).

The circular mean preferred phase of all cells was 197.05 ± 16.06° (17° or ~5 ms after the trough; Figure 7D). Interestingly, in some cases, POR cells recorded on the same stereotrode sometimes exhibited very different phase-locking preferences (Figure 7E). For 7 of 11 LFPs (64%) with simultaneously recorded cells, the preferred phase of significantly phase locked cells differed by 40° or less. For the remaining 4 (36%) LFPs, phase preferences differed by greater than 100°. In three of those cases, preferred phases were split between the trough and peak of theta. The broad distribution of phase preferences...
suggests that POR cells comprise different cell types that may play different roles in theta-based information processing.

**Gamma Modulation in POR**

Gamma power was not visually increased compared to the 1/f power log decay either for low gamma or high gamma. Because of a substantial level of 60 cycle noise, we were not able to conduct event related analyses of gamma power. We did, however, examine whether the activity of individual cells was modulated by low gamma or high gamma. Of 69 cells for which LFPs were available, 64% showed significant phase-locking to modulated by low gamma or high gamma. Of 69 cells for which LFPs were available, 64% showed significant phase-locking to low gamma (Figure S2A) and 93% showed significant phase-locking to high gamma (Figure S2B). In contrast to phase locking to theta, spiking of individual cells was phase locked only to the same-electrode LFP and not the nonlocal field potential (recorded on a different POR electrode than the cell). There were no task differences in phase locking to low or high gamma. Numbers of cells phase locked to gamma were similar across epochs, correct versus incorrect trials, and task versus nontask phases.

**DISCUSSION**

Anatomical, functional imaging, and experimental lesion evidence supports the hypothesis that the POR in the rodent brain and the PHC in the primate brain are involved in processing information about space, places, scenes, and contexts. There is little agreement, however, about the relevance of individual objects to representations of places and contexts. We used single-unit recording in rats performing a novel visual discrimination task to test the hypothesis that the POR encodes contextual information, in part, by combining spatial information with object information to form representations that link objects to places. We found that a substantial proportion of POR cells exhibited object-location conjunctive encoding. We also report that POR LFPs show increased power in the theta band, that the object from a distance, as if viewing a scene.

POR and Visuospatial Attention

In addition to linking objects to places, available evidence suggests that the POR contributes to processing information about context by modulating attention to changes in the environment. In rodents, POR lesions alter performance in attentional orienting (Bucci and Burwell, 2004). The human PHC is also implicated in attention; activity in the parahippocampal place area attenuates for repeated scenes, but only when the scenes were attended during initial and repeated presentations (Yi and Chun, 2005). In monkeys, neuronal activity in PHC is altered by changes in the context (Vidyasagar et al., 1991) and by changes to stimuli in the periphery (Sato and Nakamura, 2003), suggesting a role in bottom-up, stimulus-driven attention.

The observation of object-location correlates in the POR argues against a strict functional segregation of spatial and nonspatial input to the hippocampus. Our findings also provide evidence that the spatial layout of objects in local contexts may be encoded upstream of the hippocampus in the POR rather than configured in the hippocampus.
Both the POR in the rat and the PHC in the monkey are strongly interconnected with the posterior parietal cortex (Agster and Burwell, 2009; Burwell and Amaral, 1998a; Munoz and Insausti, 2005; Suzuki and Amaral, 1994a). Interestingly, in monkeys performing a delayed match to sample task, activity in the posterior parietal cortex increased before activity in the medial temporal lobe increased (Saalmann et al., 2007).

In the present study, the location selective cells in the POR exhibited selectivity for the locations in which objects appeared, regardless of the identity of the object. Some cells even signaled location when the animal was viewing the location from a distance. These findings are consistent with an interpretation that POR signals attention directed to particular locations. Taken together, the evidence suggests that the POR, based on posterior parietal input, monitors the environmental context for changes and deploys attention to locations in which changes are likely to occur.

**Egocentric Response Correlates in the POR**

A number of cells were selective for egocentric response to the left or to the right, regardless of the identity of the object or the side of the maze on which it was presented. The number of cells exhibiting this phenomenon was greater during the selection and reward epochs. During these epochs, the animal was either in motion performing the egocentric response or had just arrived at the final location targeted by the egocentric response, suggesting that neural activity correlated with egocentric motor responses. Consistent with our finding, POR damage in rats has been shown to cause deficits in egocentric responses (Gaffan et al., 2004), and PHC neurons in monkeys respond to egocentric views (Rolls and O’Mara, 1995). Functional neuroimaging and neuropsychological studies in humans during performance on a navigation task also provide evidence that PHC has a role in egocentric spatial learning (Weniger and Irle, 2006; Weniger et al., 2010). Correlates of egocentric responses and views in POR and PHC may reflect input from the posterior parietal cortex, which is implicated in the attentional encoding of salient locations and objects in order to guide perception and action (e.g., Gottlieb et al., 2009). Indeed, posterior parietal neurons in rats do show correlates of egocentric responses (McNaughton et al., 1994), and the posterior parietal-PHC pathway in primates and humans has been implicated in action-guiding visuospatial information processing and in visuomotor coordination (Kravitz et al., 2011; Tankus and Fried, 2012). Thus, it may be that the posterior parietal input to POR and PHC provides visual information that both supports attention to particular locations and guides actions in the local context.

**Theta Modulation in POR**

Theta oscillations are implicated in a number of cognitive and sensorimotor functions, but the most prevalent theories suggest theta is important for learning and memory (but see Kelemen et al., 2005; Ward, 2003). In our study, theta oscillations were prominent in the large majority of postrhinal LFPs, manifesting as clear ~8 Hz rhythms in the time domain and as prominent increases in 6–12 Hz power in the frequency domain. Similar to hippocampal and entorhinal theta, POR theta power was

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**Figure 7. Phase-Locking to Theta in POR**

(A) Spike phase-locking to extracellular theta oscillations was observed in 38% of the 69 cells recorded simultaneously with the 42 LFPs. Examples shown here are from the same three representative LFPs shown in Figure 4 and Figure 5A. Left panel: mean phase = 152°, kappa = 1.25, ln(Rayleigh’s Z) = 6.57; middle panel: mean phase = 211°, kappa = 0.43, ln(Rayleigh’s Z) = 5.88; right panel: mean phase = 23°, kappa = 0.39, ln(Rayleigh’s Z) = 4.36.

(B) Distribution of Rayleigh’s Z for all cells recorded with LFPs. The Z value distributions are shown in log form because some cells were strongly significant. The orange line is at ln(3) = 1.098 on the graph to indicate threshold for significance. Of the 69 cells, 26 showed significant phase locking to theta.

(C) Mean kappa score (orange line) and distribution for the 26 significantly phase-locked cells. All values of kappa > 1 are included in the rightmost bin.

(D) Mean phase (orange line) and distribution of the 26 significantly phase-locked cells.

(E) Two POR cells recorded on the same stereotrode are phase locked to different phases of theta oscillations showing quite different phase preferences.

For results of analyses of gamma see Figure S2 and Supplemental Text.
strongly correlated with running speed, providing evidence for POR’s role in spatial information processing. Importantly, theta oscillations during the selection and reward phases had lower power than expected based on the rat’s running speed during those epochs, suggesting a possible role of theta modulation in choice behavior (Womelsdorf et al., 2010b).

An analysis of correct versus incorrect trials indicated that theta power during the reward epoch was significantly increased following an incorrect choice. This difference was not due to differences in spatial behavior, as spatial behavior was well controlled in our study (Figure 1C, right, and Supplemental Text). In the absence of another explanation, our finding is consistent with a role for theta in cognition, e.g., in signaling prior error (Jacobs et al., 2006; Womelsdorf et al., 2010a), and suggests that theta oscillations in the POR are important for decision making and error processing, at least with respect to objects and locations.

A substantial proportion of POR neurons were phase-locked to theta, primarily at the trough of theta oscillations, which is when these cells are expected to be most depolarized by local theta-frequency synaptic inputs. A small subset of neurons fired close to the peak of theta oscillations. It is possible that the theta sinks in these cases are in layers distant from the location of the cell resulting in theta oscillation phase reversal as a function of cortical depth, as has been observed in the hippocampus (Buzsáki, 2002). Alternatively, this subset of cells could represent fast-spiking interneurons. Consistent with the latter possibility, we found that 3 out of 4 putative fast-spiking interneurons with narrow waveforms were phase locked to the peak of theta. Such opposite theta phase relationships for pyramidal cells and subsets of interneurons have been observed in the hippocampus (Klausberger and Somogyi, 2008). Indeed, we observed neurons recorded on the same electrode that had very different phase relationships (Figure 7E), an observation that cannot be explained by the phase reversal of theta as a function of cortical depth.

The robust theta modulation in the POR is interesting given that theta is proposed to coordinate activity across distant brain structures (Jutras and Buffalo, 2010; Klimesch et al., 2010). As an example, hippocampal theta rhythms are thought to coordinate activity between the hippocampus and associated regions in the service of episodic memory (Buzsáki, 2002, 2005; Jacobs et al., 2006). A recent relevant paper provided evidence that face-location associative learning was mediated by theta power in the parahippocampal gyrus (Atienza et al., 2011). As in the hippocampus, POR theta oscillations are probably dependent on theta-frequency inputs from multiple generators. Indeed, the POR is strongly interconnected with regions that show robust theta modulation, including the PER, entorhinal cortex, and hippocampus (Bilkey and Heinemann, 1999; Kerr et al., 2007; Lee et al., 1994; Naber et al., 1997). The POR, but not the PER, receives a strong input from the septum arising almost entirely from the medial septal nucleus (Deacon et al., 1983; Furtak et al., 2007). Taken together, the evidence suggests that POR theta, possibly generated by septal input, is in a position to modulate transmission of incoming nonspatial information from PER and spatial information from the posterior parietal cortex.

**POR and Context**

Visual information is certainly critical for representations of environmental context, and places in the real world comprise a variety of features. Real-world contexts contain large and small objects that may or may not remain in the same location, are often characterized by multimodal features, and demonstrate a variety of sizes and shapes. In addition, many places and objects are imbued with meaning based on personal experience and semantic knowledge. Notably, the POR is the target of heavy input from the PER in both rats and monkeys (Burwell and Amaral, 1998a; Suzuki and Amaral, 1994a). It should not be surprising that damage to either PER or POR causes deficits in contextual learning (e.g., Bucci et al., 2000, 2002; for PER see also Corodimas and LeDoux, 1995). The robust reciprocal connectivity with PER provides POR with access to information about individual objects, and connections with other medial temporal structures also provide links to mnemonic input. Moreover, the PER is anatomically and functionally integrated with the amygdala, which is involved in emotion processing and reward learning (LeDoux, 2000; Pitkänen et al., 2000). The POR also receives very strong input from retrosplenial cortex and appears to rely on this information for contextual learning (Keene and Bucci, 2008; Robinson et al., 2012). Thus, the POR is optimally situated to combine object and pattern information from the PER with incoming contextual and spatial information from retrosplenial and posterior parietal cortices to form complex representations of specific environmental contexts.

The hippocampus is also implicated in contextual learning, so one question of interest is how the processing of contextual information differs between POR and the hippocampus proper. Results of experimental lesion studies of contextual fear conditioning suggest context is processed differently by hippocampus and POR. For example, posttraining lesions of the hippocampus are ineffective 50–100 days after training (Anagnostaras et al., 1999; Maren et al., 1997). In contrast, posttraining PER or POR lesions are effective even 100 days after training (Burwell et al., 2004). Object-location correlates similar to those described here in POR have been observed in the hippocampus. Komorowski et al. (2009) reported that hippocampal cells signaled item-context conjunctions in a biconditional discrimination task in which the place determined which of two odor stimuli would be rewarded. In that study, item-location conjunctions developed over time as animals learned to associate items with reward. We have not examined the emergence of object-location conjunctions in the POR, but other work suggests that changes in the spatial layout of local stimuli result in immediate remapping in the POR (Burwell and Haftman, 2003). The evidence suggests that POR supports online processing of context and provides representations of the current context to the hippocampus for the purposes of associative learning and episodic memory. This is consistent with the idea that the hippocampus is located above the PER and POR in a hierarchy of associativity (Lavenex and Amaral, 2000).

We suggest that object information in the POR arrives by the well-documented direct PER to POR pathway. Alternatively, it could be that object information arrives at the POR by an indirect pathway that involves both the PER and the hippocampus. Indeed, the PER and POR each have reciprocal connections.
with the entorhinal cortex and CA1 of the hippocampus, and both project to the subiculum. This alternative view, however, does not account for the function of the direct PER–POR projections. Thus, a related idea is that the PER provides object information directly to the POR and the hippocampus, but for different purposes. Object information provided to the POR would be for the purpose of representing and updating the current context. Given that most of the object information in the POR is also associated with a place, the POR seems optimized for encoding the spatial layout of objects rather than detailed features of objects. At the same time, the PER provides detailed object information to the hippocampus for the purpose of associative learning and episodic memory.

Conclusions
We propose a view of posthinal and parahippocampal function that provides a reasonable account of the available data across species and approaches. By this view the POR combines object and feature information from the PER with spatial information from retrosplenial and posterior parietal cortices to form complex representations of the spatial layout of specific environmental contexts. Such representations would include the objects and physical features of the environment, as well as the locations of objects and features within the environment.

We further propose that the POR not only maintains a representation of the current context, but also monitors the context for changes, updating the representation of the current context when changes occur. This is consistent with hints from monkey electrophysiology and human imaging studies (Nakamura et al., 1994; Vidyasagar et al., 1991; Yi and Chun, 2005), the anatomy (Burwell et al., 1995), and evidence for a posthinal role in attentional orienting (Bucci and Burwell, 2004). It may be that the POR signals the PER when changes in features and objects have occurred and require further processing by the PER. In addition, the increased theta in POR may reflect states in which information can be transmitted between PER and POR, as suggested by Nerd and Bilkey (2005). The representation of context maintained in the POR could be referenced for a number of purposes, for example, the facilitation of recognition of an object in a scene or place (Gronau et al., 2008), the use of contextual associations to guide behavior (Badre and Wagner, 2007), or the formation of episodic memories (Eichenbaum et al., 2007).

Our findings, together with studies in rats, monkeys, and humans, suggest a model that could account for the neural basis of context representation. By this model, the parahippocampal cortex is necessary for encoding representations of specific contexts and for updating such representations when changes occur. More specifically, the posthinal/parahippocampal cortex (1) combines spatial information from posterior parietal and retrosplenial cortices with object information from perirhinal cortex to form representations that link objects to places, (2) collects those object-place associations into representations of a specific context including the spatial layout, (3) monitors the current context for changes, and (4) updates the representation of the current context with identified changes. The representation is made available to other regions for the binding of events with context to form episodes that are located in time, for guiding context-relevant behavior, and for recognizing objects in scenes and contexts.

EXPERIMENTAL PROCEDURES

Subjects
Subjects were five male Long-Evans rats (Charles Rivers Laboratories, Wilmington, MA). Rats were singly housed in a 12:12 hr light/dark cycle with ad libitum access to water. After arriving in the colony, animals were handled several days per week until the beginning of behavioral training. Prior to training, rats were placed on a feeding schedule to maintain body weight at 85–90% of free feeding weight. All procedures were in accordance with the appropriate institutional animal care and use committee and NIH guidelines for the care and use of animals in research.

Apparatus
The apparatus, the Floor Projection Maze (Figure 1A), consisted of an open field (81.3 × 81.3 cm) in which images were back-projected to the floor and the position of the animal was tracked from above (Furtak et al., 2009). Computer controlled pumps provided food reward (2% fat chocolate milk) to four reward ports. The maze was interfaced with integrated systems for location tracking, neuronal data acquisition, and behavioral control.

Behavioral Training
Rats were trained on a discrimination task in which pairs of 2D visual stimuli were presented on the floor of the exploratory maze. Stimuli were well within the limits of visual acuity for Long-Evans rats (Douglas et al., 2005; Furtak et al., 2009). In all phases of shaping and training in which two objects were presented, the left versus right location of the correct stimulus was counterbalanced.

Animals were shaped in a number of steps indicated in Table S1. The final stage of shaping was exactly the same as the final task and differed only in the stimuli and the number of problems. Once an animal reached criterion (8 out of 10 trials correct for two consecutive days), the animal was advanced to the object discrimination task.

Animals were trained on two discrimination problems, each consisting of a pair of stimuli. Each stimulus was a high contrast, circular pattern. For each problem, the two stimuli were matched for area of light and dark. The two problem pairs differed in contrast and the ratio of light area to dark area (Figure 1D). Animals began with 10-trial blocks alternating between the two problems for 100 trials. Once an animal reached criterion, 10 consecutive correct trials over 2 blocks, the electrode array was implanted. Following surgery, animals were retrained on blocked trials for 100 trials per day. Recording was initiated as soon as rats were reliably performing the task. When animals were performing at >75% correct, they were given 100 trials per day of randomly-interleaved presentations of the two problems. If performance dropped, rats were returned to blocked trials until accuracy improved. Recordings were obtained on both blocked and random presentation of stimuli.

Surgery and Histology
Under anesthesia electrodes were stereotaxically implanted in the POR as defined by Burwell (2001). The implanted microdrive assembly was produced in-house and consisted of 8 individually drivable stereotrodes (25 μm nichrome wires, A-M Systems, Inc., Carlsborg, WA). A 2.0 mm craniotomy was prepared at −0.1 mm anterior to and 5.0 mm lateral to lambda, allowing for visualization of the transverse sinus. The electrodes were inserted 300–500 μm anterior to the transverse sinus at a 22° angle along the mediolateral axis with tip pointed in the lateral direction. The electrodes were lowered 300 μm from the cortical surface and secured with dental cement, dental acrylic, and anchor screws. Rats were allowed 7 days to recover prior to behavioral training. At the end of the experiment, animals were given an overdose of Beuthanasia-D (100 mg/kg, i.p.), electrode tip placements were marked with a small lesion, the animals were perfused, and the brains were extracted and prepared for histology and subsequent localization of electrodes. The locations of electrode tips were reconstructed with a light microscope and localized in POR as...
defined by Burwell (2001). During recording, microdrivers were generally advanced about 1/6 turn or 55.5 μm. Total distance advanced ranged from 333 to 610.5 μm. Given this short distance and the trajectory of electrodes, we assumed that all cells recorded from a particular stereotrode were in the layer in which the tip was histologically located. See Supplemental Experimental Procedures for details.

Electrophysiology

Neuronal activity recorded from stereotropes (McNaughton et al., 1983) was multiplied by 20 with an operational amplifier at the head stage (HST8650-G20-GR, Plexon, Inc., Dallas, TX). Signals were then passed through a differential preamplifier with a gain of 50 (PBX2/16sp-r-G50, Plexon, Inc.). Also at this stage, single-unit activity was filtered between 154–8,800 Hz and LFPs were filtered between 0.7–170 Hz (PBX2/16sp-r-G50, Plexon, Inc.). The signal was then digitized at 40 kHz for single-unit activity and 1 kHz for LFP activity and further amplified for a total gain of 10,000 (MAP system, Plexon, Inc.). Waveforms with signal-to-noise ratios greater than ~3:1 were extracted by real-time thresholding (Sort Client, Plexon, Inc.) and stored along with time stamps of behaviorally relevant events for offline analysis.

Analysis

Spikes associated with putative individual cells were isolated offline for each session using a variety of manual and partially automated techniques for classification based on waveform characteristics (Offline Sorter, Plexon, Inc.). Separation of sorted spikes by at least 1 ms was verified by autocorrelograms. Sorted files were corrected for an identified issue of time alignment between spike data and field potential data using FPAAlignV1 (Plexon, Inc.) (Nelson et al., 2008). Timestamps for spikes and behaviorally relevant event markers were extracted from sorted files using Neuroexplorer (NEX, Plexon, Inc.).

For each isolated cell, neuronal activity (spikes/s) was analyzed during four, 500 ms epochs: the ready, stimulus, selection, and the reward epochs. We used factorial ANOVA to examine selectivity of cells for behavioral events during the stimulus, selection, and reward epochs of correct trials. Between-trial variables were object (correct stimulus in problem 1 versus problem 2), trial type (correct versus incorrect trials), and trial variable (object 1 versus object 2). Because of occasional side biases, the experimental design was altered such that event types were not counterbalanced for some sessions. For the factorial ANOVA only sessions in which there were at least 3 trials per event were included. In addition, only cells that met a criterion within an epoch of firing at least 20 spikes on at least half the trials were included in the analysis. A Pearson’s chi-square test was used to compare the overall distribution of postural cell selectivity across epochs.

All analyses were conducted using NEX, SPSS (IBM Corporation, Somers, NY), SAS (Version 9.1.3, SAS Institute Inc., Cary, NC), or Matlab (Mathworks, Natick, MA). Level of significance was p < 0.05. Results are reported as mean ± 1 standard error.

LFP Analysis

The Chronux toolbox for Matlab was used for the multitaper spectral analysis of the LFP. The spectrum of each of the 42 LFPs (21 sessions from 5 rats) recorded from the POR was calculated over the entire session. The spectrum was also calculated independently for the task-related epochs defined above. Normalized power in a given frequency band during a particular epoch was calculated by dividing the power during that epoch by the overall power in that frequency band during the entire session. This permitted comparisons of normalized power across sessions and across rats. Spike-LFP phase relationships were analyzed by first filtering each LFP between 6–12 Hz (for theta oscillations), 30–50 Hz (for low gamma) or 70–110 Hz (for high gamma) using a symmetric 4-pole butterworth filter. The filtered LFP was then Z-scored on its entirety, setting the mean of the entire signal at 0 and the standard deviation at 1. The peak and trough of each theta or gamma cycle were identified using an extrema-detection algorithm whose sensitivity was set to detect peak-to-trough or trough-to-peak amplitudes as small as 0.2 Z.

For spike assignments to theta or gamma cycles, the start time of the cycle was defined as the time of the peak. The next peak represented the end time of that cycle. Spikes occurring within the start and end peak were assigned to the cycle. Spike phase was expressed in degrees and calculated as follows: $\frac{(t_{\text{spike}} - t_{\text{start}})(t_{\text{end}} - t_{\text{start}})}{360}$, such that $t_{\text{start}}$ and $t_{\text{end}}$ are the start and end times of each theta or gamma cycle and $t_{\text{spike}}$ is the time of the spike.

Circular statistics were used to analyze the spike phase distributions during the various epochs of the task (Siapas et al., 2005; Zar, 1999). In particular, the description of circular distributions formalized by von Mises was used (Zar, 1999). von Mises distributions are characterized by a circular mean and circular concentration ($\kappa$) parameter. The higher the value of $\kappa$, the tighter the distribution is around the mean. $\kappa$ is analogous (but not equivalent) to the inverse of the standard deviation of a normal distribution. The value of $\kappa$ was thus the most appropriate estimate of the width of the spike phase distribution, and hence an appropriate estimate of the precision of spike times around the mean.

To determine the significance of phase locking to a particular frequency we used Rayleigh’s Z test. The null hypothesis for this Z test is that the circular distribution is uniform at all phases. The $p$ value for this test is approximated using the term $\exp^{-k}$ such that a $p$ value of 3 and above indicates significant phase locking (Siapas et al., 2005). For cells that showed significant phase-locking, we also calculated mean phase and $\kappa$. We did not calculate $\kappa$ for cells that were not significantly phase-locked because cells with a low number of spikes can exhibit an artificially large $\kappa$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three tables, two figures, and Supplemental Text and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2012.10.039.

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REFERENCES


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Supplemental Information

Single Neuron Activity and Theta Modulation in Postrhinal Cortex during Visual Object Discrimination

Sharon C. Furtak, Omar J. Ahmed, and Rebecca D. Burwell

Inventory of Supplemental Information

Supplemental Data

Table S1. Shaping procedure. Related to Figure 1C. Table shows the six levels of behavioral shaping completed prior to training on the final task.

Table S2. Laminar differences in patterns of selectivity. Related to Table 1. Cells in deep layers were more likely than cells in superficial layers to show spatial correlates as indicated by significant effects of side or side by response interactions.

Table S3. Stability of patterns of selectivity. Related to Table 1. Of the 29 cells responsive in multiple epochs, 11 were stable across epochs and 18 changed across epochs. In all cases the change was between stimulus/selection epochs when the stimulus was visible and the reward epoch when the stimulus was no longer visible.

Figure S1. Running speed during reward. Related to Figure 6. Theta power increases significantly after incorrect trials. This cannot be explained by running speed because running speed does not change for correct vs incorrect trials during the Reward epoch.

Figure S2. Phaselocking to gamma in POR. Related to Figure 7. This figure shows phase locking to low gamma and high local gamma. Unlike phase locking to theta, phase locking to gamma was not observed for non-local LFPs. Therefore, we elected to put these data into supplementary information.

Supplemental Text. We included additional explanation regarding behavioral results, latencies to choose a stimulus, the role of theta in error signaling, and gamma oscillations in the POR.

- Detailed behavioral results: Shaping and choice latencies. Related to Figures 5 and 6 and Table S1.
- The role of theta in signaling error: Spatial behavior. Related to Figures 5, 6, and 7.
- Gamma oscillations in POR, related to Figures 7 and S2.

Supplemental Experimental Procedures. We included additional details of the apparatus, behavioral training, surgery and histology here.

- Apparatus
- Behavioral Training
- Surgery and Histology
Supplemental Data

Table S1. Shaping procedure. Related to Figure 1C.

<table>
<thead>
<tr>
<th>Shaping Procedure</th>
<th>Training Days</th>
<th>Stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habituation</td>
<td>3</td>
<td>Pump noise</td>
</tr>
<tr>
<td>Stimulus and reward approach</td>
<td>2</td>
<td>Grey plus</td>
</tr>
<tr>
<td>East-west alternation</td>
<td>2-5</td>
<td>Grey plus</td>
</tr>
<tr>
<td>Luminance discrimination</td>
<td>2</td>
<td>Dark plus, light plus</td>
</tr>
<tr>
<td>Shape and luminance discrimination</td>
<td>12-33</td>
<td>Dark “Q”, light triangle</td>
</tr>
</tbody>
</table>

Rats completed all phases of shaping in three to six weeks. The mean number of trials to reach criterion in the final stage of shaping (dark “Q” vs. light triangle) was 484±185 trials.

Table S2. Laminar differences in patterns of selectivity. Related to Table 1.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Total Cells</th>
<th>Number</th>
<th>Type of Selectivity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Selective</td>
<td>Response</td>
<td>Location</td>
</tr>
<tr>
<td>Superficial</td>
<td>32</td>
<td>21 (66%)</td>
<td>4 (13%)</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>Deep</td>
<td>18</td>
<td>15 (83%)</td>
<td>3 (17%)</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>21</td>
<td>17 (81%)</td>
<td>4 (19%)</td>
<td>6 (29%)</td>
</tr>
</tbody>
</table>

Of the 71 criterion cells, patterns of selectivity were similar with the exception of location (spatial selectivity). Cells in deep layers were more likely than cells in superficial layers to show spatial correlates as indicated by significant effects of side or side by response interactions.

Table S3, related to Table 1. Stability of patterns of selectivity.

<table>
<thead>
<tr>
<th>Cell Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells meeting criterion and responsive in at least one epoch</td>
<td>55</td>
</tr>
<tr>
<td>Responsive in a single epoch</td>
<td>26</td>
</tr>
<tr>
<td>Responsive in multiple epochs</td>
<td>29</td>
</tr>
<tr>
<td>Stable across epochs</td>
<td>11</td>
</tr>
<tr>
<td>Stable for location</td>
<td>6</td>
</tr>
<tr>
<td>Stable for response</td>
<td>4</td>
</tr>
<tr>
<td>Stable for object-location</td>
<td>1</td>
</tr>
<tr>
<td>Changed across epochs</td>
<td>18</td>
</tr>
<tr>
<td>From object-location to location in reward epoch</td>
<td>6</td>
</tr>
<tr>
<td>From object-location to response in reward epoch</td>
<td>6</td>
</tr>
<tr>
<td>From location to object-location in reward epoch</td>
<td>*3</td>
</tr>
<tr>
<td>From one location or response to another in reward epoch</td>
<td>3</td>
</tr>
</tbody>
</table>

Of the 71 criterion cells, 55 were responsive in some epoch and 29 were responsive in multiple epochs. For this analysis, selectivity was characterized as described in Table 1 such that cells were categorized as having object-location, location, object, or response correlates in each epoch and then stability across epochs was assessed. Of the 29 cells responsive in multiple epochs, 11 were stable across epochs and 18 changed across epochs. In all cases the change was between stimulus/selection epochs when the stimulus was visible and the reward epoch when the stimulus was no longer visible. In two thirds of the cases an object-location cell converted to an object or response cell. *In three cases a cell that fired in certain location(s) during the selection epoch continued to fire in that location during reward, but with the addition of an object preference in that location.
Figure S1. Running speed during reward. Related to Figure 6.

Figure S1. Running speed for correct versus incorrect trials during the Reward epoch. Animals moved at about the same speed, though slowly, for correct and incorrect trials during the reward period (p=0.58), and there were no outliers. In contrast, theta was significantly higher after incorrect trials (Figure 6B, p < 0.0004).

Figure S2. Phase-locking to gamma in POR. Related to Figure 7.

Figure S2. Phase-locking to gamma in POR. A. Phase locking to low gamma (30-50 Hz) on a local LFP was observed in 64% of cells recording during the 42 LFPs. B. Phase locking to high local gamma (70-110 Hz) was observed in 93% of cells. Unlike phase locking to theta, phase locking to gamma was not observed for non-local LFPs. Left panels show the distribution of Rayleigh’s Z for all cells recorded with LFPs. The Z-value distributions are shown in log form because some cells were strongly significant. The colored line is at ln(3) = 1.098 on the graph to indicate threshold for significance. The middle panels show kappa scores (orange line) and distribution for the significantly phase-locked cells. The right panel shows the mean phase (orange line) and distribution of the significantly phase-locked cells.
Supplemental Text

Detailed behavioral results: Shaping and choice latencies. Related to Figures 5 and 6 and Table S1.

Following shaping animals were trained on the two-choice discrimination task. The two problems were first presented in alternating 10-trial blocks. The mean number of trials to criterion on the blocked-trial version of the object discrimination task prior to implantation was 617±72 trials. Once a criterion was reached, rats were implanted with the microdrive assembly. After recovery and reacquisition of the blocked-trial version, the mean number of trials to criterion (65% correct) when animals were switched to the random-trial version of the object discrimination task after implantation was 147±57 trials. For recorded sessions, performance on the visual discrimination task ranged from 65-79% with a mean of 69%±1% correct. Mean latencies to make a choice were 3.67±0.10 s for correct trials and 2.95±0.17 s for incorrect trials. Median latencies were 2.45 s for correct trials and 0.89 s for incorrect trials. It should be noted that latencies to choose a stimulus varied across trials with some latencies dropping below one second. The majority of latencies, however, were longer than one second. When there was overlap, the proportion of the trials in which epochs overlapped was most likely to be relatively small. Because of the potential for overlap for some trials, we may have underestimated the number of cells showing selectivity, but it is unlikely that numbers of cells showing selectivity is overestimated.

The role of theta in signaling error: Spatial behavior. Related to Figures 5, 6, and 7

An analysis of correct vs. incorrect trials indicated that theta power during the reward epoch was significantly increased following an incorrect choice. This difference was not due to differences in spatial behavior, as spatial behavior was well controlled in this study (Figure 1C, right). All rats exhibited stereotyped paths in that they always checked the port behind the choice, even on incorrect trials, and then reliably checked the port not chosen before proceeding to the ready position for the next trial. Differences in speed could cause changes in brain temperature that might be reflected in increased amplitude or frequency of theta, but there were no differences in speed during the reward epoch for correct vs. incorrect trials. There were, however, correct-incorrect differences in speed during the immediately preceding selection epoch, in that rats approached incorrect objects more slowly than correct objects. Thus, if speed during the selection epoch affected theta in the reward epoch through changes in brain temperature, brain temperature and theta parameters would be expected to decrease (Kelemen et al., 2005). In any case, in our task, brain temperature is unlikely to change as a function of speed differences in a 500 msec epoch prior to incorrect choice on trials interspersed among correct trials. In the absence of another explanation, our finding is consistent with a role for theta in cognition, e.g. in signaling prior error (Jacobs et al., 2006; Womelsdorf et al., 2010) and suggests that theta oscillations in the POR are important for decision making and error processing, at least with respect to objects and locations.

Gamma oscillations in POR, related to Figures 7 and S2

Gamma-band sychonization is proposed to signal attentional states and to increase the efficiency of identifying changes in sensory input (Womelsdorf and Fries, 2007). Based on the evidence for POR attentional functions in combination with anatomical connections with regions involved in visuospatial attention, we had hypothesized that gamma oscillations modulated POR activity. Although gamma power was not increased overall relative to the 1/f power function, POR neurons were phase locked to gamma. Phase locking was observed in the low and high gamma bands, but only on electrodes on which the LFP was recorded. We did not observe task-related gamma modulation of POR activity, though our analyses were hampered by intractable noise in the 60 Hz range. Additional work is needed to determine whether gamma activity is relevant to POR.
function. It may be that, in POR, gamma coherence with other frequency bands or other regions is relevant to its function.

Supplemental Experimental Procedures

Apparatus

The behavioral chamber, the Floor Projection Maze (Figure 1A), was previously described in detail (Furtak et al., 2009). Briefly, the maze was a square open field (81.3 x 81.3 cm) with opaque white Plexiglas walls (33 cm) and a clear Plexiglas subfloor (1.25 cm thick). Dual Vision Fabric (Da-Lite Screen Company, Warsaw, IN), a unity gain flexible fabric designed for rear screen projection, covered the subfloor. A sheet of 0.32 cm thick Plexiglas covered the fabric. Images were back-projected onto the unity gain fabric with an LCD projector (1200MP projector, Dell Inc.) directed to a mirror located below the maze floor at a 45° angle relative to the floor. Clear Plexiglas inserts (33 cm in height) were positioned in the maze during both training and testing procedures resulting in a bowtie-shaped test area (Figure 1B). Food reward (2% fat chocolate milk) was delivered by automated pumps (Med Associates, Inc, St. Albans, VT) to four custom stainless steel food ports located in the east and west walls of the maze (Figure 1B). Auditory stimuli were delivered through a speaker located above the maze and controlled by an automated auditory stimulus generator (ANL926, Med Associates, Inc.). For the duration of each session background masking noise was provided by a fan located above the maze.

The Floor Projection Maze was interfaced with three Windows XP-based systems, for location tracking, neuronal data acquisition, and behavioral control. For tracking subjects, we used a CinePlex V2 Digital Video Recording and Tracking System (Plexon, Inc., Dallas, TX) with an Imaging Source TM camera (640 x 480 resolution, 30 frames per sec). During shaping, subject location was tracked by contrast and the system recorded xy coordinates of the centroid of the body silhouette. After implantation, subject location was either tracked by contrast or by two light emitting diodes located on the headstage, yielding two pairs of xy coordinates. We used a Multichannel Acquisition Processor (MAP, Plexon Inc) and SortClient (Plexon, Inc) for real time acquisition of location, behavioral event timestamps, and neuronal activity. Custom Matlab programs (Mathworks Inc., Natick, MA) translated the x and y coordinates from CinePlex into behaviorally relevant positions and relayed them to the behavioral control system (Med Associates, Inc, St. Albans, VT). For reward delivery, stimulus presentation, and behavioral data collection, we used a DIG-700P2 PCI Interface Connection Card, a DIG-716P2Smart Control Output Module, and the MED PC IV software environment (Med Associates, Inc.). To interface between MED PC IV and the MAP system, we used a DIG-713A SuperPort TTL Input Module and a DIG-726SuperPort TTL Output module (Med-Associates, Inc.).

Behavioral Training

Animals underwent a number of shaping steps described in detail, here. For three consecutive days prior to shaping and training, animals were habituated to the behavior room, the sound of the food-delivery pumps, and chocolate milk. A rat in its home cage was left in the behavior room for 40 min during which time a pump was activated every few minutes. Several food pellets soaked in chocolate milk were provided in the home cage during habituation. On the third day, animals were also handled in the behavior room.

Once habituated to the behavioral room and reward, animals were shaped in a sequence of a 20-25 minute sessions (Supplemental Table 1). On the first two days of shaping, animals learned to approach a stimulus back-projected to the floor for a reward. On the first day, the procedure was 10 stimulus-reward presentations in the west side of the maze with a varying inter-trial interval (ITI; mean, 12.5 sec; range, 5-20 sec). At the beginning of the session and during the ITI, the floor was grey. Stimuli were presented on the same grey background. At the start of a trial, the shaping stimulus (a dark grey plus) appeared in front
of a reward port on the west wall at the same time a reward was delivered. When the animal approached the stimulus and reward port, a tone signaled availability of reward, and the stimulus disappeared. The same procedure was followed on the second day, but on the east side of the maze.

For the next 2-5 days, animals learned to alternate between east and west trials and to remain still in the center of the maze in the “ready position” prior to stimulus presentation for increasing durations up to 750 ms. There were no obvious features that distinguished the west and east side of the apparatus. White noise (70 db) signaled the beginning of each trial. When an animal entered the ready position the noise terminated. If the animal left the ready position prematurely, the white noise was again presented and the trial was restarted. If the animal remained in the ready position for the required duration, the shaping stimulus was presented. The trial was terminated if the animal failed to enter the ready position in 30 s or to approach the object in 45 s.

On the next two days, animals learned a two-choice luminance discrimination. Again, white noise signaled the start of the trial and trials alternated between east and west trials. When the animal advanced to the ready position, the white noise was terminated. When the animal remained stable in the ready position for the required duration, a pair of objects was presented, the familiar stimulus (a dark grey plus) and a new stimulus (a light grey plus). Approach to the familiar, correct, stimulus was reinforced by presentation of the tone (1.5 kHz, 70 db, 1.5s) and delivery of reward. Approach to the incorrect stimulus was followed by a burst of white noise (80 dB, 1s), but the animal was allowed to correct by approaching the reinforced stimulus. Again, the trial was terminated if the animal failed to enter the ready position in 30 s or to approach a stimulus in 45 s.

The final stage of shaping was the same as the prior stage except that the animals received a new discrimination problem and the trial terminated if the animal approached the incorrect stimulus. The new problem consisted of a dark grey “Q” (correct) and a light grey triangle. Once an animal reached criterion (8 out of 10 trials correct for two consecutive days), the animal was advanced to the object discrimination task.

The task was exactly the same as the final stage of shaping and differed only in the stimuli and the number of problems. Animals were trained for one session per day.

Surgery and Histology

Under isoflurane anesthesia rats were chronically implanted with a microdrive assembly produced in-house, which consisted of 8 individually drivable stereotrodes (25 µm nichrome wires, A-M Systems, Inc., Carlsborg, WA). Stereotrodes were two twisted wires similar to tetrodes (McNaughton et al., 1983). Stereotrodes are sufficient for isolating single units in neocortex where cells are less densely packed as compared to the hippocampus, for example. The microdrive assembly was secured within a shortened flexible plastic funnel for protection. Immediately before surgery, electrode tips were cut on an angle, 4 - 5 mm in length, and electroplated with chloroplatinic acid solution (cat# 262587; Sigma-Aldrich, St. Louis, MO). Impedances ranged from 200 to 250 kΩ measured at 1000 Hz (2900 Metal Electrode Meter, A-M Systems, Inc.). Microdrivers were lowered in increments of 1/6 turn (~52 µm). Stereotrodes were lowered at the end of each daily recording session.

Animals were pretreated with glycopyrrolate (0.5 mg/kg, s.c.), butorphanol tartrate (0.5 mg/kg, s.c.), carprofen (5 mg/kg, s.c.), and diazepam (2-5 mg/kg, i.p.) to counteract respiratory affects, to control pain and to decrease risk of seizures. Subjects were brought to a surgical level of anesthesia with isoflurane gas and secured in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) in the flat skull position (bregma and lambda level in the horizontal plane). Body temperature was maintained during surgery using a heating pad set to 37°C. Ophthalmic ointment was applied to the eyes. The skin above the
dorsal surface of the skull was cut along the midline and retracted to expose the skull. Five holes were drilled along the lateral ridges of the skull and threaded with self-tapping stainless steel anchor screws. A ground wire was attached to a sixth screw, placed on the dorsal surface of the skull. A 2.0 mm craniotomy was made in the left hemisphere at approximately -0.0 mm AP and 5.0 mm lateral to lambda, allowing for visualization of the transverse sinus. The electrodes were inserted 300-500 µm anterior to the transverse sinus at a 22° angle along the mediolateral axis with tip pointed in the lateral direction. The electrodes were lowered 300 µm from the cortical surface. Dental cement and dental acrylic were used to secure the electrode assembly to the dorsal surface of the skull. Following surgery, each stereotrode was advanced ~50µm. Rats were monitored until awake and then returned to the vivarium. Post-operatively rats received Rimadyl (120 mg/kg, g.d. for 2 days) for pain and Keflex as an antibiotic (60 mg/kg, g.d. for 7 days). Rats were allowed 7 days to recover from this procedure.

At the end of the experiment, animals were given an overdose of Beuthanasia-D (100 mg/kg, i.p.). Electrode tip placements were marked with a small lesion (1-15µA for 10 s). Rats were intracardially perfused with 0.1M phosphate-buffered saline followed by 4% formaldehyde. Brains were cryoprotected in 30% sucrose for 3-5 days prior to sectioning in the coronal plane. Sections (60 µm) were mounted on gelatin-coated slides, stained with thionin, and coverslipped with DPX Mountant (Sigma-Aldrich Corp., St. Louis, MO). The locations of electrode tips were reconstructed with a light microscope. Tip locations were determined to be located in POR as defined by Burwell (2001). Briefly, POR is located at the caudal end of the rhinal sulcus posterior to the PER and dorsal to the MEA.