NEURONS IN THE POSTRHINAL CORTEX ENCODE NONSPATIAL CONTEXT DURING VISUAL BICONDITIONAL DISCRIMINATION

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Summary

Representation of the context in which events occur is essential for episodic memory. Yet context is also important for other cognitive processes including, for example, making decisions, sequencing behavior, and associative learning. The rodent postrhinal cortex and its primate homolog, the parahippocampal cortex, are thought to preferentially process visuospatial information in order to represent the spatial features of contexts and scenes. An open question, however, is whether the postrhinal cortex is also involved in representing non-spatial contexts. Here, we used electrophysiology in behaving rats to show that postrhinal contextual representations extend to the non-spatial domain. Based on the pattern of results, we suggest that postrhinal representations of context, whether spatial or nonspatial, behave like occasion setters by modulating the meaning of other items in the environment.

Keywords: parahippocampal cortex, medial temporal lobe, bi-conditional discrimination, floor projection maze, occasion setting

Introduction

Representations of the context are critical for episodic memory, yet context is also important for other cognitive processes including, for example, making decisions, sequencing behavior, and associative learning. One view of hippocampal information processing is that spatial and nonspatial information arrives to the hippocampus by separate processing streams (Eichenbaum et al., 2012). The rodent postrhinal cortex (POR) and its primate homolog, the parahippocampal cortex (PHC), preferentially process visuospatial information and provide such information to the hippocampus; whereas, the perirhinal cortex (PER) provides nonspatial object or item information to the hippocampus. The hippocampus binds spatial and non-spatial information in the service of context guided memory and associative learning. It is increasingly clear, however, that these pathways are not entirely segregated and may not be processing spatial and nonspatial information in parallel. Although the POR and the PHC do project strongly to the medial entorhinal cortex, they are also robustly interconnected with the PER and lateral entorhinal cortex, regions associated with the so called nonspatial pathway (Burwell and Amaral, 1998, Suzuki and Amaral, 1994, Doan et al., 2019). Disconnection of the POR and the perirhinal cortex impairs recognition of objects in particular contexts (Heimer-McGinn et al., 2017). Thus, the POR appears to have access to nonspatial object and item information from the PER. We have suggested that the PER provides object and pattern information to the hippocampus for associative learning and episodic memory and to the POR for representing the local physical context (Heimer-McGinn et al., 2017). Such information could be used to represent the local physical context (Estela-Pro and Burwell, 2022, Ho and Burwell, 2014, Peng and Burwell, 2021). POR representations of context are available to other brain regions for context-guided behavior and cognition, for example, the prefrontal cortex (Hwang et al., 2018).

There is substantial evidence in humans, monkeys, and rodents that the POR/PHC plays an important role in processing information related to spatial context including the geometry of spaces, landmark configurations, spatial layout of objects, and processing spatially

object characteristics such as size and portability (Mullally and Maguire, 2011, Martin et al., 2013, LaChance et al., 2022, Bonner and Epstein, 2021, Bachevalier et al., 2015). For example, fMRI studies in humans show that the PHC is activated in response to scenes, landmarks, and objects in spatial context (Aminoff et al., 2013, Bar and Aminoff, 2003, Bonner and Epstein, 2021), and PHC damage impairs landmark identification, as well as navigation and spatial memory (Aguirre and D'Esposito, 1999, Ploner et al., 2000). Similarly, PHC lesions in monkeys impair both object-location and object-in-context recognition (Malkova and Mishkin, 2003, Bachevalier et al., 2015). In rodents, POR lesions impair context discrimination (Bucci et al., 2002), scene discrimination (Gaffan et al., 2004), object-in-context recognition (Norman and Eacott, 2005), and contextual fear conditioning (Bucci et al., 2000, Burwell et al., 2004a). POR lesions also impair contextually driven spatial choices (Park et al., 2017). Moreover, disconnection of the POR and PER results in object-in-context impairment, suggesting that POR relies on object and pattern information transmitted directly from PER to encode representations of context (Heimer-McGinn et al., 2017). Interestingly, the POR is also responsive to objects in motion (Nishio et al., 2018) and to changes in the local environment (Burwell and Hafeman, 2003). Finally, POR responds to both objects and locations, and encodes object-location conjunctions (Furtak et al., 2012). Taken together, the evidence suggests that the POR is important for representing spatial context including the geometry of the space and the spatial layout of objects and patterns, object and pattern information likely arrives to the POR directly from PER, and the POR also signals changes to the current spatial context.

The present study was designed to address two open questions. The first question is whether the POR has a role in representing non-spatial contexts. There are some hints of this possibility in the literature. Diana (2017) reported that the PHC is activated by context retrieval in a source memory paradigm that minimized spatial processing by crafting encoding questions that drew upon lexical characteristics of the words to be remembered. Additionally, fMRI studies in humans show that the PHC responds to objects with strong contextual associations, e.g. the

target word “microscope” is strongly associated with laboratories (Bar and Aminoff, 2003). The second open question is how POR representations of context might modulate behavior. One theory is that context operates as an occasion setter (Fraser and Holland, 2019). Occasion setting refers to the capability of a stimulus to modulate the meaning of other cues, for example, the association of another stimulus with a reward. Thus, stimuli can be directly associated with one another as in stimulus-stimulus associations, or a stimulus can modulate the meaning of another stimulus by acting as an occasion setter. Stimuli tend to operate as occasion setters when separated from other stimuli by longer intervals and when the occasion setter differs from other stimuli in character or modality. This perspective is particularly useful in understanding how nonspatial contexts might guide behavior.

To address our two open questions, we developed a nonspatial contextual biconditional discrimination (nscBCD) task in which a non-spatial context determined which of two objects was correct. The task was designed to favor occasion setting in two ways. The non-spatial context differed from the objects in character, and the presentation of the non-spatial context was separated in time from the presentation of the objects. In the nscBCD task, rats learned to discriminate which of two 2-dimensional (2D) objects presented on the floor would be rewarded based on context, which was operationalized as the pattern on the floor. The task was conducted in a bow-tie shaped maze, such that presentation of pairs of objects alternated from one side of the maze to the other. One of two floor patterns was randomly selected at the beginning of each trial. Because objects were presented in two locations, we were able to dissociate neural correlates of non-spatial context from neural correlates of location. Neural recordings collected during task performance were analyzed to show neural correlates of context onset as well as object stimulus onset. We also identified selectivity for conjunctions of objects, locations and contexts during task relevant epochs. To determine how objects, locations and contexts were represented by the ensemble of recorded single units, we used population similarity analyses. Our results provide evidence for the hypothesis that the POR

represents non-spatial contexts and that those representations are used to guide behavior in a manner consistent with occasion setting.

Results

Subjects were shaped and trained on the novel nscBCD task in which two 2D object stimuli were back-projected onto the floor of a bowtie-shaped maze in an automated manner (Figures 1A, 1B). The pattern on the floor determined which of a pair of objects was correct. There were two pairs of objects and two different floor patterns (Figure 1C). For each pair of objects, one was correct on the solid floor and the other was correct on the striped floor regardless of allocentric location (east or west) or egocentric location (left or right). One of two background patterns was randomly chosen for the next trial. Thus, sometimes the floor was the same as the prior trial, and sometimes the floor was changed. At the beginning of each trial, the onset of white noise signaled the rat to enter the 'ready position' in the center of the maze (Figure 1D). Trials alternated between east and west. Stimuli were presented when rats remained in the ready position for a variable fixation period (0.8 - 1.8 sec). A choice was made when the rat approached one of the two objects. A correct choice was rewarded, whereas an incorrect choice terminated the trial and no reward was delivered. Correct choices were counterbalanced for egocentric location (left/right) such that the correct choice could be presented in one of four possible locations (east left, east right, west left, and west right). Overall, there were 32 trial types (4 locations, 4 objects, and 2 floor patterns). Rewards were given in the form of medial forebrain intracranial stimulation (ICS) consisting of bipolar electrical pulses delivered to the medial forebrain bundle. The 100 Hz ICS pulses were 500 μsec in length, separated by 500 μsec, and sustained for 500ms. Amplitude varied depending on the sensitivity of the subject.

Task Performance

Rats were first trained on a series of shaping steps (Supplemental Table S1). Upon

reaching a criterion of 10 correct choices in 12 consecutive trials, rats progressed to the next shaping step. The mean number of sessions required for each stage was as follows: 5 days of hand shaping, 5 days of auto shaping, 3.5 days of simple discrimination, 6.5 days of biconditional discrimination training with the first pair, 3.5 days for biconditional discrimination with the second pair, and 1.5 days on the full nscBCD task. Once trained, rats performed daily sessions of 150-200 trials, and sessions were analyzed when performance was above 55% accuracy.

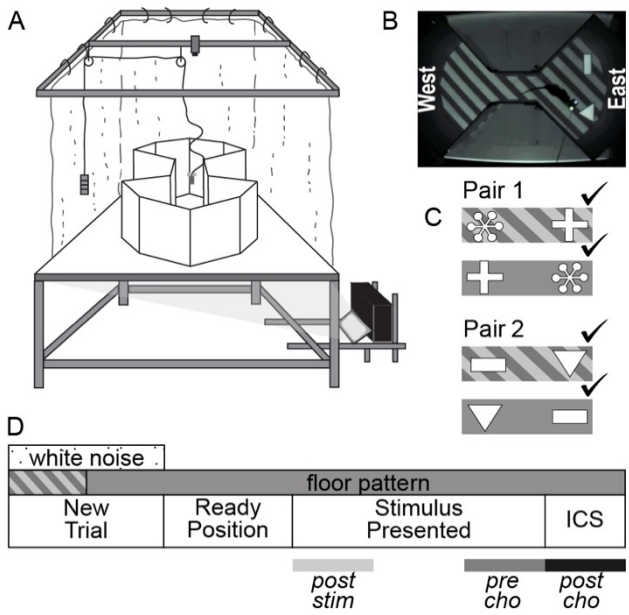

Figure 1. Behavioral Task. Overview of the behavioral task used in this study. (A) Schematic of the Floor Projection Maze. (B) Overhead view of a rat in the maze. (C) For each of the four possible discrimination combinations, the correct object is indicated by a checkmark. Each combination could appear on either the west or east of the maze and the correct object could be on egocentric left or right. This yielded 16 trial types. (D) At the start of each trial, the context was randomly assigned. White noise signaled the rat to go to the ready position in the center of the maze and maintain the ready position for a variable time (800-1800 ms) after which the object pair was presented. The rat made a choice by approaching an object. The choice was recorded and both stimuli disappeared. Correct choices were rewarded with intracranial stimulation (ICS) to the medial forebrain bundle. Analysis epochs (500 ms) are represented by gray boxes at the bottom of the panel: post-stim = *stimulus* epoch; pre cho = *pre-choice* epoch; post cho = *post-choice* epoch.

Histology and Unit Isolation

Stereotrodes were implanted in the POR of five animals. We recorded and isolated 120 cells from 21 stereotrodes across 54 sessions. Recordings spanned the POR along the rostro-caudal axis from −8.40mm to –9.48 from Bregma (Figure 2). Stereotrodes were lowered ~20 μm after each daily session, and thus new neurons were recorded in each session. Recorded units were sorted using a variety of manual and partially automated techniques for isolation based on waveform characteristics in Offline Sorter (Plexon, Inc. Dallas, TX). Only well-isolated units with a signal-to-noise ratio greater than 2:1, and spike separation of at least 1 ms were retained for

further analysis. At the end of training, electrode location was marked by electrolytic lesion, rats were euthanized, and the brains were processed to identify the location of electrode tips.

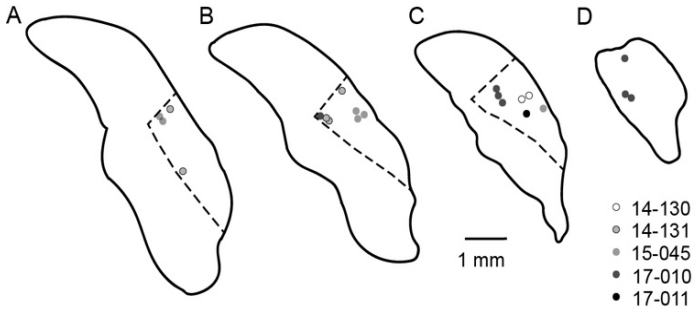

Figure 2. Histology. Schematic drawings show the lesions for the POR by animal. Contours are displayed on standard coronal sections at −8.40 (A), −8.72 (B), −9.24 (C) and –9.48(D) mm from Bregma. Dotted lines indicate POR boundaries.

POR neurons signal changes in non-spatial context

To determine whether the POR signals a change in non-spatial context, we analyzed firing rate (FR) before and after context (floor pattern) changes. The goal of this analysis was to determine whether POR cells were selective for the onset of a new floor or the onset of a particular floor. For the 120 isolated cells, the mean baseline FR was 1.95 Hz and ranged from 0.2 to 12.94 Hz. A total of 104 cells met criterion for this analysis: at least 20 trials in which the floor changed from gray to stripe or vice versa, and at least 20 spikes in the 500 ms before (*prefloor)* and after (*postfloor)* floor onset. For each cell, we conducted a 2 X 2 repeated measures analysis of variance (rANOVA) with 'Onset' as the within-trial factor (*prefloor* vs *postfloor*), 'Context' as the between-trial factor (gray floor or striped floor), and FR as the dependent variable. We predicted that POR cells would change their FR in response to a change in floor pattern (main effect of 'Onset') and that the magnitude and/or direction of that change may vary depending on the identity of the floor change, either stripe-to-gray or gray-to-stripe ('Onset x Context' interaction). When cells were selective for both the onset of the floor and the onset of a particular floor, we interpreted the interaction.

Table 1. POR neurons signal context change

Significant main effects and interactions	Number of cells	Percent
Onset	27	26
Onset*Context	25	24
No response	52	50
TOTAL	104	100

Note: Eleven Onset*Context cells also had a main effect of Onset

Based on our hypothesis, we would expect the POR to signal a change in context. We found that 50% of POR cells signaled a change in context, i.e. were selective for the onset of a different floor (Table 1). Twenty-six percent of cells signaled the onset of a different context showing a main effect of Onset. These cells signaled a change in context, irrespective of which floor appeared (Figure 3A). Of particular interest were the 24% of POR cells that signaled the onset of a specific context, i.e. the onset of a particular floor pattern. For example, one cell increased its FR when the gray floor was switched to the striped floor (Figure 3B) but not on striped to gray changes (Figure 3A). These cells were identified by significant interactions of 'Onset x Context' (p<0.01). The remaining 50% of cells (52 out of 104) did not show selectivity for floor onset. Since floor pattern provides a non-spatial context in this task, our results indicate that POR neurons are responsive to changes in non-spatial context as well as to specific non-spatial contexts.

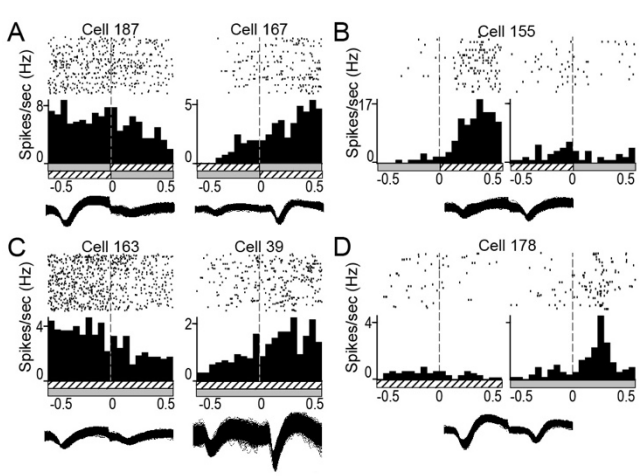

Figure 3. POR neurons signal changes in context and onset of 2D objects. Postrhinal cells exhibit neuronal correlates during both floor change (A-B) and stimulus onset (C-D). Raster plots and perievent histograms are shown for representative cells displaying behavioral correlates. (A) Examples of cells that increase or decrease firing rate (FR) in response to a change in floor pattern, irrespective of the identity of the floor. (B) An example of a cell that increases FR when the floor changes from gray to striped but not when it changes from striped to gray. (C) Examples of cells that increase or decrease FR when object stimuli appear, irrespective of object or floor identity. (D) An example of a cell that increases FR in response to object onset, but only when the context is a gray floor. Time bins = 25ms. Dashed lines represent floor or stimulus onset.

POR neurons signal onset of 2D object stimuli

We also predicted that the POR would signal the presentation of objects on the floor. To determine the extent to which POR cells signaled the onset of object pairs, we analyzed the change in FRs before and after the onset of 2D stimuli. Of the 120 cells twe recorded and isolated, 114 met criteria for this analysis. The criteria were at least twenty trials for each of the context-stimulus pairs (striped-pair1, striped-pair2, gray-pair1, and gray-pair2) and at least 20 spikes in the 500 ms before and after object stimulus onset. For each cell, a repeated measures ANOVA was carried out with stimulus 'Stimulus Onset' as the within-trial variable (*500 ms* before *vs 500 ms* after). Between-trial variables were 'Context' (gray or striped) and 'Pair' (object pair 1 or 2). We considered main effects to represent simple, non-conjunctive coding or selectivity and interactions to represent conjunctive coding or selectivity. We predicted that POR cells would change their FR in response to the onset of the object stimuli, and that this might in some cases depend on either the Context or the Pair.

We found that 44 of 114 cells (38.6%) signaled the onset of object stimuli either by a main effect of Stimulus Onset, an interaction with Stimulus Onset, or multiple main effects and/or interactions (Table 2). Some POR cells (25 of 114, 22%) responded only to the object stimulus onset by either increasing or decreasing FRs as demonstrated by a main effect of Stimulus Onset (Figure 3C). A total of 7 cells exhibited one or more interactions with Stimulus Onset in the absence of a main effect. Interestingly, of the cells exhibiting interactions, 16 (14%) interacted with Context, whereas only 7 (6%) interacted with Pair. Figure 3D shows an example of a cell that increased firing when objects appeared but only when the context was the grey floor. Thus, a surprising number of POR neurons respond to object stimuli onset and some of those neurons showed conjunctive coding of stimulus onset with Context and/or Object Pair.

Table 2. POR neurons signal stimuli onset

Main effects and interactions by cell	Number	Percent
StimOn	25	21.9
StimOn*Context	3	2.6
StimOn*Pair	2	1.8
StimOn*Context*Pair	1	0.9
StimOn & StimOn*Context	9	7.9
StimOn & StimOn*Pair	1	0.9
StimOn*Context & StimOn * Pair	1	0.9
StimOn & StimOn*Context*Pair	2	1.8
Signaled Onset	44	38.6
Did not Signal Onset	70	61.4
TOTAL	114	100

Given that the context changed well before the object pairs are presented, we were also interested in the extent to which responses to stimulus onset were modulated by particular contexts as compared to the extent that responses to stimulus onset were modulated by particular object pairs. In other words, how does conjunctive coding with particular Contexts compare with conjunctive coding of particular object Pairs. To address this question, we conducted a frequency analysis based on the total numbers of main effects (Table S2). Of the 44 cells that showed main effects and interactions with stimulus onset, there were 57 total main effects and interactions. The distribution of main effects and interactions were different for context and object pair. There were 16 significant interactions with context (StimOn*Context or StimOn*Context*Pair), whereas there were only seven interactions with Object Pair (StimOn*Pair or StimOn*Context*Pair). Interactions with Context were significantly greater than interactions with object Pair (($X^2(1)=4.62$, $p<0.032$). The fact that context modulates responses to stimulus onset, even when context changes earlier in time, is consistent with context operating as an occasion setter.

POR units display conjunctive and non-conjunctive selectivity in task relevant epochs

In addition to the onset of floor contexts and the onset of object stimuli, we were interested in neuronal responses during task relevant behavioral epochs. We were especially interested in correlates of Context and Location given that Context was predictive of correct choices, but

Location was not. For these analyses, we took into account which object in which location had been chosen by the animal irrespective of whether the choice was correct or not. We separately analyzed selectivity in the *stimulus*, *pre-choice*, and *post-choice* epochs (Figure 1D). For each cell and each epoch, we conducted a 2 X 4 X 4 analysis of variance (ANOVA) with Context, Location, and Object as the between-trial factors and firing rate as the dependent variable. For example, one condition might be the striped floor as Context, the animal chooses Object 1, and the chosen object is in the north-east Location.

Selectivity was demonstrated by main effects and interactions among 'Context', 'Location', and 'Object'. As for the onset of stimulus pairs, we considered main effects to represent simple, non-conjunctive coding or selectivity and interactions to represent conjunctive coding or selectivity. When cells had an overlapping main effect and interaction, we counted only the interaction in Table 3A. For example, if a cell had a main effect of Context and a Context by Object interaction, this cell was counted under Interactions and was considered to exhibit conjunctive coding. However, if a cell had non-overlapping main effects and interactions, it would have been noted under Multiple Effects and Interactions in Table 3A, e.g. a cell with a main effect of Location and a Context by Object interaction would be considered to exhibit both conjunctive and non-conjunctive selectivity.

Of the 120 cells we recorded and isolated, 111 cells met criteria in at least one epoch, and 86 met criteria in all three epochs. In the *stimulus* epoch, 104 cells met criteria and 63 (61%) displayed selectivity (Table 3A). In the *pre-choice* epoch, 93 cells met criteria and 70 (75%) displayed selectivity. In the *post-choice* epoch, 97 cells met criteria and 79 (81%) displayed selectivity. Of the 111 cells that met criteria, 102 (92%) displayed selectivity in at least one epoch. Most cells had a single main effect or interaction: 55, 59, and 58, for the *stimulus*, *pre-choice*, and *post-choice* epochs, respectively. Fewer cells had multiple main effects and/or interactions: 8, 10, and 19, for the *stimulus*, *pre-choice* and *post-choice* epochs, respectively.

Table 3A. POR neurons show selectivity in three behavioral epochs

Significant Main Effects and Interactions	Stimulus Num(%)	PreChoice Num(%)	PostChoice Num(%)
MAIN EFFECTS			
Context	4(4)	1(1)	4(4)
Location	22(21)	36(39)	27(28)
Object	1(1)	0(0)	1(1)
INTERACTIONS			
Object*Context	9(9)	0(0)	5(5)
Object*Location	5(5)	4(4)	2(2)
Context*Location	6(6)	8(9)	5(5)
Context*Object*Location	8(8)	10(11)	14(14)
MULTIPLE EFFECTS and INTERACTIONS			
Context & Location	4(4)	1(1)	5(5)
Location & Object	2(2)	0(0)	1(1)
Context & Location & Object	2(2)	2(2)	0(0)
Context*Object & Location	0(0)	5(5)	10(10)
Context*Location & Context*Object	0(0)	1(1)	0(0)
Context*Object & Location*Object	0(0)	2(2)	2(2)
Context*Object & Location*Object & Context*Location	0(0)	0(0)	3(3)
Total selective cells	63(61)	70(75)	79(81)
Total non-selective cells	41(39)	23(25)	18(19)
TOTAL	104	93	97

A central question of the present study was the extent to which the POR represents nonspatial context and whether representations involving context differed from those involving location. In the nscBCD task, Context determined which Object was correct and would be rewarded, but Location was not predictive. For that reason, we were particularly interested in the distributions of main effects (non-conjunctive coding) of Location and Context vs interactions (conjunctive coding) involving Location and Context (Table 3B). These differences were examined a by 2 x 2 Chi Square analyses for each epoch in which the groups were main effects or interactions and the outcomes were selective vs non-selective (Table S3).

Even though the location of objects was not predictive of reward in this task, the location in which objects appeared was robustly represented with cells showing non-conjunctive (main effects) as well as conjunctive coding (interactions). Taken together, cells showed more main effects of location than interactions with location; 21 to 27 percent of the total effects included a main effect of Location, whereas 13 to 16 percent showed interactions with Location (Table 3B). These differences were examined a by 2 x 2 Chi Square analyses for each epoch in which the

groups were main effects vs interactions with Location and the outcomes were selective vs non-selective (Table 3B and Table S3). (Table S3 p-values ranges from 0.004 to 0.00001). Interestingly, quite a few cells showed selectivity for Location in the stimulus epoch when the rat was physically located near the middle of the maze. We observed three types of location selectivity including allocentric (east, west, north, and/or south), egocentric (left/right), and single locations. For some cells, preference for a single location was sustained across epochs (Figure 4A). Other cells showed interactions (Figure 4E (right) and Figure 4F).

Table 3B. POR main effects and interactions with Context vs Location

Main Effects and Interactions		Stimulus Num(%)	PreChoice Num(%)	PostChoice Num(%)
CONTEXT		*	****	****
	Main Effects	10(7)	3(2)	9(5)
	Interactions	23(16)	27(17)	38(20)
LOCATION		#	*	*
	Main Effects	30(21)	43(27)	43(22)
	Interactions	19(13)	25(16)	25(13)
ALL MAIN EFFECTS		**	****	****
	Context	10(7)	3(2)	9(5)
	Location	30(21)	43(27)	43(22)
ALL INTERACTIONS				#
	Context	23(16)	27(17)	38(20)
	Location	19(13)	25(16)	25(13)
INTERACTIONS WITH OBJECT		#	#	*
	Context	17(12)	18(11)	38(20)
	Location	13(9)	16(10)	25(13)
TOTAL EFFECTS & INTERACTIONS		146	160	194

p<0.10; * p<0.02; ** p<0.001; *** p<0.0001; **** p<0.00001

Context showed the opposite pattern from Location with respect to conjunctive and non-conjunctive coding. Context exhibited significantly more conjunctive (interactions) than non-conjunctive coding (main effects) for all three epochs (Table 3B and Table S3). Proportions of 22 to 38 percent of cells showed interactions with Context, whereas only 3 to 10 percent of cells showed main effects of Context. Again, these differences were confirmed by 2 x 2 Chi Square analyses in which the groups were main effects vs interactions with Location and the outcomes were selective vs non selective (Table S3, p-values = 0.00001). Figure 4B shows an example

cell with a main effect of Context. Figures 4C and 4E (left, center) show example cells with Context interactions.

There were also significant differences confirmed by 2 x 2 Chi Square analyses in which the groups were Context and Location and the outcomes were selectivity (Yes or No). Again, analyses were conducted separately for each epoch. Briefly, there were more main effects of Location than main effects with Context and POR cells showed significantly more non-conjunctive selectivity for Location than non-conjunctive selectivity for Context (Table 3B, main effects), and this was the case for all three epochs (p's<0.00016). Location main effects ranged from 29 to 46 percent compared with 3 to 10 percent for Context. Chi square analysis showed significant distributions for all three epochs (all p-values less than 0.0004; see Table S3 for contingency tables and statistical results). In contrast, Context was more robustly represented by cells that showed conjunctive coding (Table 3B). Numerically there were more interactions with Context overall, and this was significant in the *postchoice* epoch ($\chi^2(1)=4.04117$ $p<0.0444$) in which 38% of cells showed interactions with Context compared with 25% for Location (Table 3B and Table S3). Likewise, more cells showed Context*Object interactions (22%, 28%, 38%) than Location*Object interactions (13%, 17%, 20%) for *poststim*, *prechoice*, and *postchoice* epochs, respectively. These distributions were marginally significant for the *poststim* ($\chi^2(1)=3.35917$ $p<0.06683$) and *prechoice* ($\chi^2(1)=3.0754$ $p<0.07949$) epochs and significant for the *postchoice* epoch ($\chi^2(1)=4.04117$ $p<0.0444$).

Figure 4C shows examples of Object*Context conjunctive coding. One Context*Object cell showed conjunctive coding in the stimulus epoch, firing preferentially to objects 1 and 3 when the floor was striped and to object 2 when the floor was solid (Figure 4C, *left panel*). Another Context*Object cell fired preferentially to objects 1 and 3 when the floor was striped and to object 2 and 4 when the floor was solid during the pre-choice epoch (Figure 4C, *left panel*). In the post-choice phase, another Context*Object cell fired preferentially to objects 1 and 3 when the floor was striped and to object 2 and 4 when the floor was solid (Figure 4C, *left panel*). This

could be interpreted as a reward cell. However, these analyses are based on the chosen object and include correct and incorrect choices.

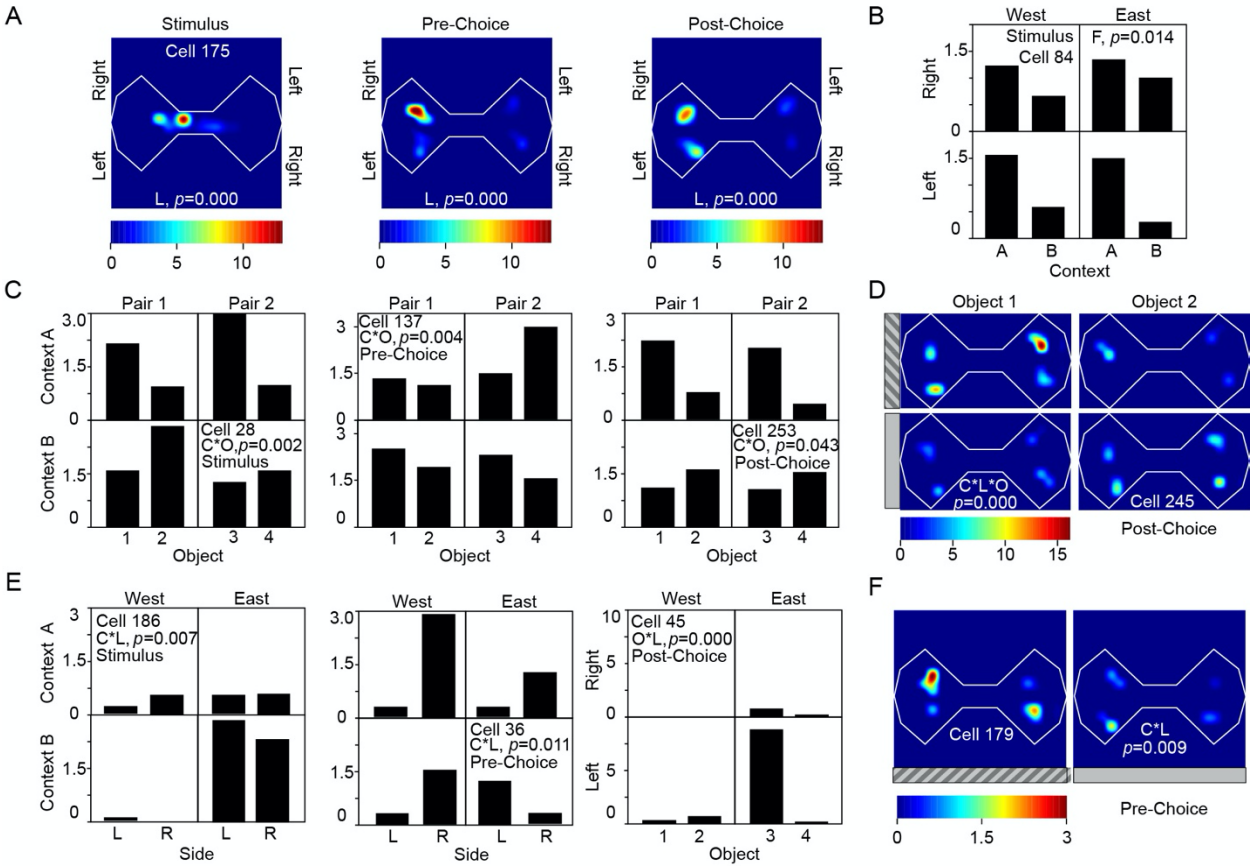

Figure 4. POR cells show conjunctive and non-conjunctive, task relevant coding for context, location, and object. Shown are representative cells in the POR that showed non-conjunctive main effects or conjunctive interactions among location (east-left, east-right, west-left, west-right), object (1,2,3,4), and context (A = striped, B = gray). Firing rates (FR) are denoted by heatmaps for spatial firing maps and are shown on the Y axis for histograms. (A) Spatial FR maps show an example of a cell with selectivity for a particular location sustained across the *stimulus*, *pre-choice,* and *post-choice* epochs. (B) Histogram for a cell showing non-conjunctive 'Context' selectivity as confirmed by a main effect of Context. Mean FR was higher for Context A (striped floor) regardless of location or stimuli. (C) Histograms for examples of 'context x object' (C*O) conjunctive coding. Cells in the left, center, and right histograms are from the *stimulus*, *pre-choice*, and *post-choice* epochs, respectively. For example, the cell in the left panel fired more for objects 1 and 3 in Context A and more for object 2 in Context B. (D) Spatial map for a cell with 'context x location x object' (F*L*O) conjunction. This cell responded preferentially when object #1 was presented in Context A on the egocentric left. (E) Histograms for examples of 'context x location' (C*L) conjunctions (left and middle) and an object by location (O*L) conjunction (right). Cells in the left, center, and right histograms are from the *stimulus*, *pre-choice*, and *post-choice* epochs, respectively. (F) Spatial FR maps for a cell with a C*L response.

Some cells showed Context*Location*Object interactions. For example, one cell fired equally for objects #1 and #2 in any location when the floor was gray. However, when the floor was striped, it fired preferentially to object #1 but only when it appeared on the egocentric left side (Figure 4D, *right panel*). Cells with a three-way interaction were observed in the *stimulus* (8

out of 104), *pre-choice* (10 out of 93), and *post-choice* epochs (14 out of 97). Taken together, these data are consistent with the hypothesis that POR cells encode the spatial layout of objects and patterns in the local physical context, even when location of objects is not relevant to the task.

POR ensembles code for nonspatial context and object locations

Finally, we examined whether the ensemble coded for context, location, and object identity by conducting similarity analyses (McKenzie et al. 2014). Firing rates from recorded neurons were z-scored using the mean and standard deviation from the firing rates recorded from that neuron. The Pearson's correlation coefficient was then calculated for every trial epoch for each neuron pair. For each component of the trial epoch examined (context, object pair, and choice location) the magnitude of the effect of each component was measured by comparing the mean correlation within a condition versus between conditions for that component (for example: comparing the mean correlation between trials with the same floor and the mean correlation between trials with different floors). To estimate significance, a bootstrapped dataset was created using shuffled epoch assignments repeated 10,000 times and the correlations described above were recomputed for each shuffled set.

We found that the population activity showed significant correlation, defined as the difference of the average correlations across trials with the same value and trials with different values is greater than the 95th percentile of the distribution of differences of the bootstrapped datasets, with the Context (floor) during the Post-Context period and the Post-Stim period, and Location during all epochs (Figure 5A). In the Pre-Context and Post-Context period, while the correlations between same-location trials were significantly different from different-location trials, this appears to be due to a higher anti-correlation between the different-location trials.

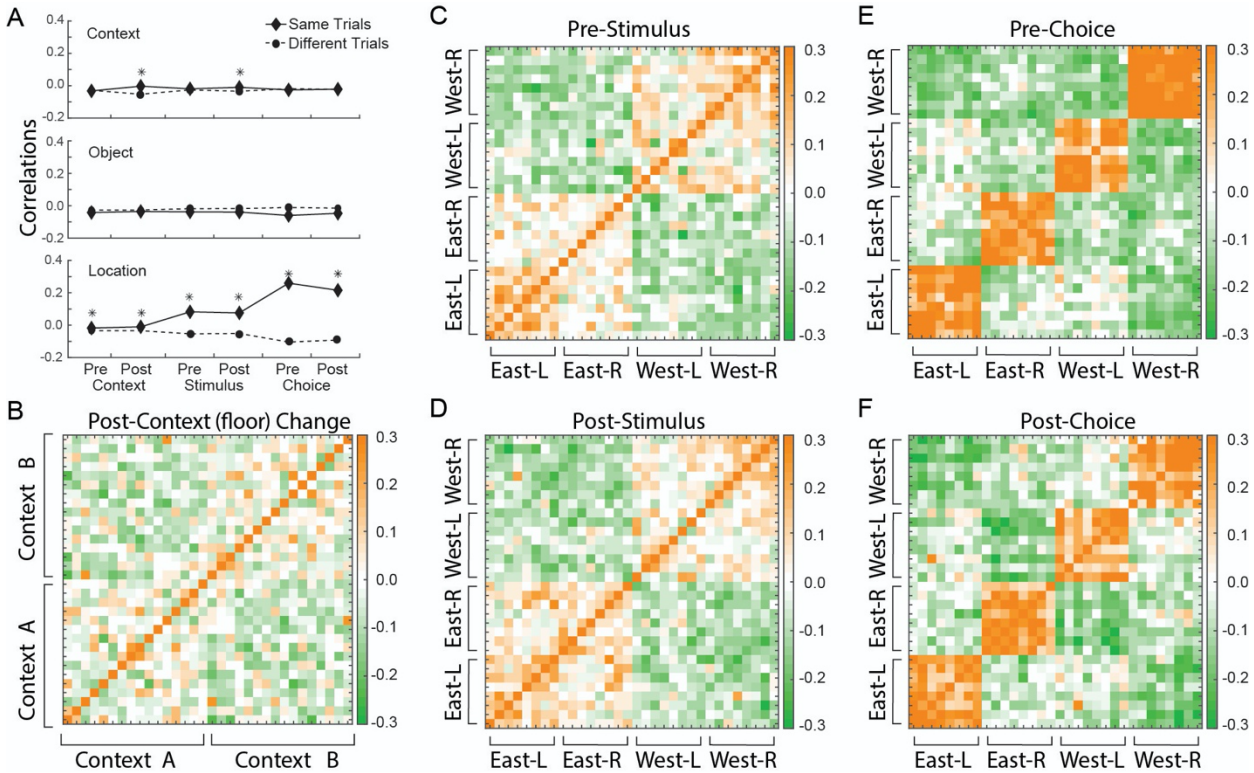

Figure 5. Postrhinal ensembles code for context and location in different epochs. A. Mean correlation coefficients for same-condition comparisons (solid line) and different-condition comparisons for the floor (top), object (middle), and object location (bottom). * indicates the difference between the solid and dotted line was greater than the 95th percentile of the same measure from bootstrapped distributions. B. A correlation matrix using all cells across all sessions showing correlation coefficients by color code (right scale) for the 500 msec after the floor changed indicating a new context. The animal could be anywhere in the maze and the time to stimulus onset varies considerably. Context A and B are anticorrelated. C-D. Correlation matrices for pre-stimulus onset (C) and post-stimulus onset (D). The animal is in the center of the maze facing east or west. East and west are anticorrelated. E-F. Correlation matrices for pre-choice onset and post-choice onset. The animal is in one of the object location areas. The strongest anticorrelations are for combined opposite egocentric and allocentric locations. For example, West-Right is strongly anticorrelated with East-Left.

Using similarity matrices organized first by context (Figure 5B) we saw that during the Post-Context period there is correlation between similar contexts and anti-correlation between different contexts. Looking at similarity matrices organized by object location (Figure 5C-F and Figure S1), we can see that during the *pre*- and *post-stimulus* periods the broader allocentric locations of the east or west side of the maze were represented (Figure 5C, D), while the individual locations were represented more strongly during the pre- and *post-choice* periods (Figure 5E, F). Interestingly, during the *post-choice* period there was a strong anti-correlation with the location that was the furthest away in the combination allocentric and egocentric space, such as between the west-left location and the east-right location.

Discussion

We have proposed that the POR represents environmental contexts including the spatial layout of objects and patterns, that the POR automatically monitors the current context for changes and updates context representations when changes occur, and that object and pattern information arrives to the POR directly from the PER (Estela-Pro and Burwell, 2022, Furtak et al., 2012, Ho and Burwell, 2014, Peng and Burwell, 2021, Burwell and Hafeman, 2003). Such representations are available to multiple brain regions, for example, the hippocampus for associative learning (Agster and Burwell, 2013) and prefrontal cortex for context guided behavior and cognition (Hwang et al., 2018). We addressed two open questions about POR function. The first question was whether the POR represents nonspatial contexts by which we mean contexts that are not specifically tied to locations. The second question was whether POR representations of context modulate other cues in a manner consistent with occasion setting. To address these open questions, we recorded POR neurons as rats performed a nonspatial biconditional discrimination task in which the pattern on the floor, or the context, determined which of two objects was correct. The location of objects was not relevant to the discrimination. Thus, the experimental design allowed dissociation of location from non-spatial context (i.e. floor pattern).

POR context representations operate like occasion setters

In our nscBCD task, we were able to compare neural correlates of context with neural correlates of location in three task relevant epochs – stimulus, pre-choice, and post-choice. We would expect POR neurons to exhibit both object-context conjunctive coding and object-location conjunctive coding. However, if POR representations of context operate as occasion setters, we would expect object-context conjunctive coding to be more frequent than object-location conjunctive because locations are not predictive of which object will be rewarded. For context, we found more conjunctive coding (interactions) than non-conjunctive coding (main effects),

whereas for location, we found more non-conjunctive than conjunctive coding. Additionally, location was represented in more main effects than context was. Although location, is certainly a component of the context, in this task the floor pattern interacts with other cues in a way that is more consistent with how contexts have been suggested to work in that modulate the meanings of other cues in an environment (Fraser and Holland, 2019). The patterns of conjunctive and non-conjunctive coding we observed for the predictive context and the nonpredictive location are consistent with two interpretations. First POR does appear to represent the nonspatial context operationalized as a pattern on the floor, and the data are consistent with an occasion setting interpretation of how POR representations of context interact with other features of the environment.

POR object-context coding is not related to learning

In prior work we established that the POR has a role in processing contextual information (Bucci et al., 2021, Bucci et al., 2002, Burwell et al., 2004a, Burwell et al., 2004b, Heimer-McGinn et al., 2017). We also reported that the POR exhibits object-location conjunctive coding (Furtak et al., 2012), which can be viewed as a signature of context representations. In that study, in which location was also not predictive, 10-15% of cells showed object-location coding depending on the epoch. In the present study, 13-20% showed object location conjunctive coding across the same three epochs. The number of cells with non-conjunctive location correlates was also similar with the Furtak et al. (2012) study reporting 11-44% depending on the epoch, and the present study reporting 29-46%. Thus, our findings regarding POR object location correlates when location does no predict outcome was largely replicated.

It is also useful to compare our findings with those of a study in which location was predictive. McKenzie et al. (2014) recording hippocampal neurons in a similar context biconditional discrimination task, except that the two contexts differed in location as well as other cues. On the east side of the maze, one odor of a pair was correct and on the west side of

the maze the other odor was correct. That study reported that 40.7% of isolated cells were influenced by context (east vs west), 34.0% were influenced by egocentric location of the samples (left vs right), 13.5% coded for odor identity, and 42.7% showed conjunctive coding of objects with context or location. We found that, when taking into consideration all three task relevant epochs, 42.6% of isolated cells were influenced by context (floor pattern), 78.7% were influenced by egocentric and/or allocentric location, 40.7% coded for object identity conjunctively or non-conjunctively, and 36.1% showed conjunctive coding of objects with context or location. In our study, a higher number of cells coded for object identity, but the majority was conjunctive coding, and only 9.3% of isolated cells showed non-conjunctive coding in the form of main effects of object identity, which may be more comparable to the McKenzie et al. (2014) study. We also observed a higher proportion of cells influenced by location. In our study, however, location cells included egocentric and allocentric correlates, whereas location correlates in the McKenzie and colleagues study included only the left vs right position of item stimuli. If we consider that perhaps half of our location correlates signalled egocentric locations, then the numbers are roughly comparable. Importantly, McKenzie et al. (2014) reported that 40.7 percent of recorded hippocampal cells were influenced by spatial context, whereas we found that 42.7% of recorded POR cells were influenced by non-spatial context.

One interesting question about object-context coding in the POR and item-location coding in the hippocampus is the timing of the emergence of such codes. Using the same task as McKenzie and colleagues, Komorwski et al. (2009). reported that item-location coding emerged as rats learned the task. In that study, the proportion of active cells with item-location correlates increased during learning from 6.4% in the first 30 trial block to 25% in the second block to 31.3% in the third 30 trial block. Over the same blocks, percent correct increased from about 45.5% to 79.4% to 84.8%. Thus, the proportions of item-location selective cells predicted accuracy. In our study, rats performed at 55-65% correct throughout recording and proportions of object-context cells were stable across sessions at about 32.6% of active cells and up to

42.4% if we include object-location cells. This suggests that object-context coding in the POR does not depend on learning or predict accuracy. These findings are consistent with the hypothesis that the POR is broadly and automatically representing both spatial and nonspatial contexts, and making those representation available to other brain regions, including the hippocampus. The hippocampus, then, uses these representations to form context-guided object associations (Komorowski et al., 2009, McKenzie et al., 2014).

Interestingly, although location was not predictive of reward, the locations in which objects appeared were robustly represented by POR neurons. When animals were in the middle of the maze just after the onset of objects, east and west were strongly anticorrelated. In subsequent epochs, all four locations were represented. Interestingly the most different locations east-left vs west-right and east-right vs west-left in that they were the most anticorrelated in the ensemble analyses. Thus, the POR appears to be mapping task-relevant locations in the context.

POR monitors the current context for changes

Our study also provides evidence that the POR monitors the current context for changes. We found that about half of POR neurons signaled when the floor changed, and about half of those cells signaled the appearance of particular floors. We also found that POR neurons signaled the presentation or onset of the object pairs to be discriminated. Notably, when the objects appeared, cells were more likely to show conjunctive coding involving context as opposed to conjunctive coding involving the identity of the object pairs. This is surprising given that the context would have changed well before the objects were presented and that object presentation was a highly salient event. These findings are consistent with the idea that the POR monitors the current context for changes and updates the representation of context when changes occur.

Spatial and non-spatial components of context

A growing body of evidence indicates that visual context includes both spatial and non-spatial elements, and that the POR/PHC is involved in processing both of these streams (Mullally and Maguire, 2011). In rodent and non-human primate work, for instance, it is common to examine context as defined by background images (Bachevalier et al., 2015), patterns on the floor (Heimer-McGinn et al., 2017), or patterns on the wall (Norman and Eacott, 2005, Park et al., 2017). In these studies, lesions to the POR impair context-guided behaviors in the absence of location cues. Beyond simple visual cues, human studies have defined context as a movement contingency (stable vs in motion) (Karanian and Slotnick, 2014),and as a specific written question (question defines trial type) in a lexical task (Diana, 2017). In these studies, PHC is preferentially activated during contextual processing. Other studies go even further, showing that POR/PHC is involved in contextual processing as defined by motivational state (hungry vs satiated) (Burgess et al., 2016, LaBar et al., 2001). Taken together, these studies provide strong evidence that processing of context in POR/PHC goes beyond location and spatial layout. Our study is the first to investigate the neural correlates of this non-spatial contextual processing, suggesting that POR neurons bind together streams of spatial and non-spatial contextual information.

Cortical integration of object and spatial information

For some years, now there has been a focus on segregated spatial and nonspatial processing streams that provide information to the hippocampus. More specifically, the PER provides nonspatial input to the lateral entorhinal cortex and the POR provides spatial input to the medial entorhinal cortex. In fact, available data argues against such a segregation. For example, there is considerable evidence that the PER processes spatial and contextual information (e.g. Bucci et al., 2021, Heimer-McGinn et al., 2017, Lim et al., 2022). Anatomically,

we have known for some time that, although the PER is preferentially connected with the lateral entorhinal cortex, the postrhinal cortex is robustly interconnected with both the lateral and the medial entorhinal cortices (Burwell and Amaral, 1998, Doan et al., 2019). Moreover, the rodent POR and the primate PHC are robustly and reciprocally connected with the PER (Burwell and Amaral, 1998, Suzuki and Amaral, 1994), which could explain the presence of object information in the POR.

In addition to the anatomy, other lines of evidence indicate that object information is processed in the POR. Although the POR/PHC is not necessary for object discrimination (Norman and Eacott, 2005), object-in-place and object-in-context discriminations do rely on POR/PHC, as indicated by lesion studies in rodents and primates (Norman and Eacott, 2005, Park et al., 2017, Malkova and Mishkin, 2003, Bachevalier et al., 2015). These findings are also supported by human imaging studies showing PHC activation during similar discriminations (Karanian and Slotnick, 2017, Karanian and Slotnick, 2014, Yeung et al., 2019, Faivre et al., 2019, Stevenson et al., 2020). Importantly, disconnection of the POR and PER impairs object-in-context discrimination, suggesting that POR may require object input from the PER in order to perform this task (Heimer-McGinn et al., 2017). Moreover, molecular studies reveal arc activation in POR during both spatial and non-spatial tasks (Beer et al., 2013), and demonstrate that delivery of protein kinase C to POR enhances shape discrimination (Zhang et al., 2019, Zhang et al., 2017). Finally, a handful of studies report directly on the neural correlates of object processing in POR. These studies discuss the presence of POR correlates for object-location conjunctions (Furtak et al., 2012) and for object movement (Nishio et al., 2018, Beltramo and Scanziani, 2019). Taken together, these studies support the idea that information about objects is bound to context in the POR.

POR monitors the layout of scenes and contexts

Our results indicate that cells in the POR respond to changes in the visual layout of a

scene and that this response may depend on salience of the change. An appropriate analogy might be a familiar empty room with gray walls. If patterned wallpaper was added, or if two large pieces of furniture were added, the room would feel quite different. Our data demonstrates that POR cells responded to these types of highly salient changes, specifically, changes to floor pattern and object onset. In addition, 25% of cells were tuned to floor changes by floor identity, meaning they responded only to gray-to-stripe changes or to stripe-to-gray. This makes sense considering that rats were highly familiar with both of these contexts. In fact, there is some data to suggest that POR representations may be stable within testing sessions (Furtak et al., 2012) and possibly as long as 2-10 days (Burgess et al., 2016). This may account for the ability of POR cells to discriminate the *direction* of the floor change between two highly familiar contexts. POR cells also discriminated between more subtle changes, such as object onset in a specific context (10%; new furniture in gray room vs newly painted room), onset of particular objects (3%; new red vs blue armchairs of similar size/shape), and onset of particular objects in a specific context (3%; red armchairs in the gray room only). In each of these analogous situations, the differences between layouts are more subtle than gray vs wallpaper (floor change) or empty vs furnished (object onset). It makes sense, then, that less cells would respond to these less pronounced changes. It very well could be that the number of responsive cells corresponded to how salient the change or difference between layouts was. Thus, our results support the hypothesis that the POR monitors the current layout of a scene, including objects and non-spatial context, and that it is responsible for signaling when the layout changes.

Conclusion

Our study shows that cells in the POR process non-spatial context and that POR context representations can be used to process the meaning of other available cues. The nonspatial context, in this case, was the pattern on the floor that predicted which of two objects would be rewarded. Although location was part of our nscBCD task in that objects did appear in particular

locations, location was not predictive of reward. We observed significantly more object-context conjunctive coding compared with object-location conjunctive coding. These observations are consistent with the idea that context representational are coded in the context operates as an occasion setter. Object-context conjunctive coding was not predictive of learning or accuracy as has been observed in the hippocampus. POR and made available to other brain regions for context-guided behavior and cognition. We also observed that the POR signals the onset of context changes and the onset of objects. This finding is consistent with the idea that the POR is monitoring the current environment for changes and updating the current representation of context. This study provides further evidence that the object information is processed by the POR, but primarily in the service of representing the spatial layout of objects and patterns that characterize the current context.

Our study is one of only a handful that report on firing patterns of single POR cells (e.g.Furtak et al., 2012, Nishio et al., 2018, Beltramo and Scanziani, 2019, Gofman et al., 2019, LaChance et al., 2019, Bucci et al., 2021, Heimer-McGinn et al., 2017, Lim et al., 2022). The importance of this understanding how and where context is processed and how representations of context guide learning, memory, and other cognitive processes is underscored by recent research highlighting the clinical relevance of the PHC in memory processes related to Alzheimer's disease (Zhu et al., 2020, Qi et al., 2019), normal aging (Dalton et al., 2019, Qi et al., 2019, Yeung et al., 2019, Rémy et al., 2020), and stress (Shields et al., 2019).

Detailed Methods

Contact for reagent and resource sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Rebecca D. Burwell (rebecca_burwell@brown.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Experimental model and subject details

Subjects were five adult Long Evans rats (Charles River Laboratories, Wilmington, MA), singly housed in diurnal conditions (12 h light/dark cycle) with *ad libitum* access to food and water. Before surgery rats were handled at least three times per week and maintained at 85–90% body weight. At the time of surgery, all subjects were 3–5 months old and weighed 250–300 g, and had no involvement in previous procedures. After surgery, rats were allowed to recover and then handled daily. Rats were group housed before surgery (2-3 per cage) and single housed after surgery to prevent damage to the implanted devices. Supervised play time with previous cage mates was allowed for at least 1 hr per day. All procedures were in accordance with NIH guidelines for the care and use of rats in research. The protocol covering these experiments was approved by the Brown University Institutional Animal Care and Use Committee.

Method details

Apparatus

Testing was performed in a bowtie-shaped arena (147 x 111 cm) made of white matte acrylic and placed over a Floor Projection Maze (Jacobson et al., 2014, Furtak et al., 2009, Furtak et al., 2012), in which images are back-projected to the floor. The apparatus was housed in an isolated testing room monitored by an overhead video camera and interfaced with integrated systems for neuronal data acquisition, location tracking, and behavioral control. Reward was delivered by inter-cranial stimulation (ICS) in the form of bipolar electrical pulses to

the medial forebrain bundle. ICS pulses were 500 μsec in length, separated by 500 μsec, and sustained for 500ms. Pulse frequency was fixed at 100 Hz. Amplitude ranged 15-35 μA depending on the sensitivity of the subject. The stimuli were pairs of 2D objects measuring 12.5 cm in diameter spaced 24 cm apart and presented on the floor, which is ideal considering the visual acuity limits for Long Evans rats (Furtak et al., 2009). All pairs of objects were luminance-matched to ensure that shape was the salient cue. Context was defined visually by floor pattern and was different on each trial; floor A was striped grey and floor B was solid gray. The behavioral program, including white noise, floor image displays, and ICS delivery, were controlled by DIG-716P2 Smart Control Output Interface (Med Associates Inc, St. Albans, VT) using a custom-written script in Med-PC IV software. Trial information stored in Med-PC output files was extracted using custom MATLAB scripts (Mathworks).

Stimuli

The visual stimuli consisted of two floor patterns and three pairs of objects. All pairs of objects were luminance-matched to ensure that shape was the salient cue. Existing literature on rats' visual acuity indicates that pigmented rats can discriminate approximately 1 cycle per degree (cpd), or 30 arc minutes (Lashley, 1930, Prusky et al., 2002). The stimuli were 12.5 cm in diameter and were presented at a distance of 24 cm, occupying >1700' of the visual field. The smallest feature of the shapes was the dark borderof the shapes, which is 0.42 cm at the proscribed viewing distance of 24 cm. The angular size corresponds to 51 arc-minutes, well within the 30 arc-minute threshold.

Previous literature and data from our lab indicate that pigmented Long-Evans rats can detect differences in grey with contrast differences as low as 12% on a linear brightness scale such that white is 0% and black is 100% (Furtak et al., 2009, Powers and Green, 1978). In order to ensure that subjects in this task were able to distinguish between the two different floor patterns, the stripes of the striped floor were designed to be 27% grey and 61% grey, whereas

the solid floor was designed to be 44% grey. To provide the greatest possible level of contrast, the interior of each of the 2D objects was white (0%) with outlines that were 91% grey.

Surgery

Rats were anesthetized with isoflurane and implanted with custom-designed microdrives built in-house and containing up to 32 individually drivable stereotrodes (25 μm nichrome wires, A-M Systems, Inc., Carlsborg, WA). The incisor bar was adjusted such that the bregma and lambda were in the same horizontal plane (±0.2 mm). A 2 mm craniotomy was made in the left or right hemisphere at -0.5 mm anterior and 5.0 mm lateral to lambda, allowing for visualization of the sagittal sinus. The electrodes were inserted at a 16° angle along the ML axis with tip pointed in the lateral direction and lowered 3.5 mm relative to the cortical surface just anterior to the sinus. Two ICS electrodes were implanted -2.5 mm posterior and ±2.0 mm lateral to bregma and lowered 8.5 mm relative to skull. Dental cement and anchor screws were used to secure the electrode assembly to the dorsal surface of the skull. Following surgery, rats were allowed 3 days to recover and then each stereotrode was advanced daily in increments of 1/8 turn (~20 μm).

Behavioral Training

In this study we present a contextually-guided bi-conditional discrimination task (nscBCD), in which location and context can be dissociated. Two pairs of 2D objects, pair 1 and pair 2, were randomly intermixed in every session. In each trial, the rats were presented with one of two pairs of objects on one of two different floor pattern in a bowtie shaped maze. The floor patterns determined which object was correct within each pair. The pairs of objects were presented on either side of the bowtie maze, and the animals were rewarded for approaching the correct object. The location of the objects was counterbalanced in both egocentric (left vs right) and allocentric (east vs west) space. Thus, correct choices in this task required associating the floor pattern with the rewarded object, but location did not modify the likelihood of *post-choice*. An incorrect choice was followed by a correction trial with the same parameters

(e.g. pair 1, floor A, correct on left) on the opposite side of the bowtie maze. The number of consecutive correction trials was limited in order to prevent spatial biases.

Before surgery, rats were habituated to the room and apparatus. After surgery, rats were shaped to ICS reward, to stop in “ready” position, and to approach the stimulus. After shaping, rats were sequentially trained on simple and context discrimination (using training objects), and finally on pairs 1 and 2. For each stage of training, rats were expected to meet trials to criterion (TTC) for two days in a row before moving on to the next stage. TTC was defined as 10 ten correct choices in 12 consecutive trials. In all phases of shaping and training in which two items were presented, the left versus right location of the correct stimulus was counterbalanced. At each stage, the number of trials per session was increased gradually until the animals were able to complete 200 trials per session. Once training was complete, rats received one daily session of 200 trials with pairs 1 & 2 and floors A & B.

Electrophysiology

Neuronal signals were recorded from stereotrodes, amplified with a gain of 20 at the headstage (HST/32V-G20, Plexon, Inc., Dallas, TX), and passed through a differential pre-amplifier with a gain of 50 (PBX2, Plexon, Inc.). Single unit activity was filtered between 154-8,800 Hz and digitized at 40 kHz. LFPs were filtered between 0.7–170 Hz and digitized at 1 kHz. Signals were further amplified for a total gain of 10,000 (MAP system, Plexon, Inc.). Two rats were recorded using the Plexon OmniPlex® system. Signals were collected through a 64-channel digital headstage (HST/64D, Plexon, Inc., Dallas, TX). Single units were filtered between 0.77–6000 Hz and upsampled at 40 kHz through a digital headstage processor (DPH). LFPs were filtered at 0.7-170 Hz and digitized at 1 kHz. For all rats, waveforms were extracted by real-time thresholding (Sort Client, Plexon, Inc.).

Histology

After recording was concluded, animals were given an overdose of Beuthanasia-D (100 mg/kg, intraperitoneal) and a small marking lesion (1-15 uA current for 15 sec) was made at the

end of each stereotrode. Rats were pericardially perfused with 0.1M phosphate-buffered saline (PBS) followed by 10% formalin. Brains were cryoprotected in 30% sucrose for 3-5 days prior to sectioning. Brains were cut in the coronal plane (60 μm), mounted on charged slides, stained with thionin, and coverslipped with DPX. The locations of electrode tips were reconstructed with a light microscope.

Quantification and statistical analysis

Unit Isolation

Recorded units were sorted using a variety of manual and partially automated techniques for isolation based on waveform characteristics in Offline Sorter (Plexon, Inc). Only cells recorded during sessions with at least 55% accuracy on the behavioral task were considered. Furthermore, only well-isolated units with a signal-to-noise ratio greater than 2:1, and spike separation of at least 1 ms were retained for further analysis. Overall, we isolated 120 cells from 5 animals across 54 sessions. Time alignment was corrected based on a known issue between spike data and field potential data using FPAlign (Plexon, Inc) (Nelson et al., 2008). Spike timestamps were extracted from sorted files using NeuroExplorer and used to compute FR across key behavioral events (NEX; Plexon, Inc).

Factorial Analyses of Neural Correlates

In order to assess the neuronal correlates of behaviorally-relevant event markers we ran a between-trials factorial ANOVA for each cell during three epochs (Table 3A; Figure 4). For each cell, neuronal activity (spikes/sec) was analyzed during three epochs: *stimulus* (500 ms after onset of object stimuli), *pre-choice*, and *post-choice* (500 ms before and after rat makes a choice). Between-trial variables were ‘object pair’ (pair 1 or pair 2), ‘Context’ pattern (stripe or solid), ‘Location’ (east-left, east-right, west-left, and west-right). Analyses were performed in SPSS Version 24. Main effects and interactions were considered significant at p-value < 0.05, and cells were included for analysis if they fired at least 30 times within a given epoch.

Floor Change Analysis

The floor change analysis was conducted to examine the effects of floor change on FR (Figure 3). For each cell, we compared the FR 500 ms before (*prefloor)* to 500 ms after (*postfloor)* a change in floor. A repeated measures ANOVA was carried out with 'epoch' as the within-trial factor (*prefloor* vs *postfloor*) and 'Context' as the between-subject factor (striped or gray). Analyses were performed in SPSS Version 24 and main effects and interactions were considered significant at a p-value < 0.05. Cells were included if they fired at least 20 times within a given epoch.

Stimulus Onset Analysis

The object onset analysis examined whether FR changed in response to onset of a 2D object. Similarly to the floor change analysis, FRs were compared across epochs that occurred 500 ms before (*prestim)* and after (*poststim)* stimulus onset. For each cell, a repeated measures ANOVA was carried out with 'epoch' as the within-trial variable (*prestim* vs *poststim*), and 'Context' (gray or striped) and 'pair' (object pair 1 or 2) as the between-trial variables. Analyses were performed in SPSS Version 24 and main effects and interactions were considered significant at a p-value < 0.05. Cells were included if they fired at least 20 times within a given epoch. Out of the 120 isolated cells, 114 met analysis criterion for this analysis.

Similarity Analyses

The similarity matrices were calculated in the same manner as in (McKenzie et al. 2014). Firing rates from recorded neurons were z-scored using the mean and standard deviation from the firing rates recorded from that neuron. The Pearson's correlation coefficient was then calculated for every trial epoch for each neuron pair. For each component of the trial epoch examined (floor, object, location of the correct object) the magnitude of the effect of each component was measured by subtracting the average of the correlations between trial epochs where the component was different from the average of the correlations between trial epochs where the components were the same. To estimate significance, 10,000 bootstrapped datasets were created using shuffled epoch assignments and the correlations described above were

recomputed for each shuffled set. The population firing rate is said to be significantly correlated with a trial component when the magnitude of the effect is greater than the 95th percentile of the distribution of effect magnitudes from the bootstrapped datasets.

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