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Time cells in the retrosplenial cortex

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Abstract

The retrosplenial cortex (RSC) is a key component of the brain's memory systems, with anatomical connections to the hippocampus, anterior thalamus, and entorhinal cortex. This circuit has been implicated in episodic memory and many of these structures have been shown to encode temporal information, which is critical for episodic memory. For example, hippocampal time cells reliably fire during specific segments of time during a delay period. Although RSC lesions are known to disrupt temporal memory, time cells have not been observed there. In this study, we reanalyzed archival RSC neuronal firing data during the intertrial delay period from two previous experiments involving different behavioral tasks, a blocked alternation task and a cued T-maze task. For the blocked alternation task, rats were required to approach the east or west arm of a plus maze for reward during different blocks of trials. Because the reward locations were not cued, the rat had to remember the goal location for each trial. In the cued T-maze task, the reward location was explicitly cued with a light and the rats simply had to approach the light for reward, so there was no requirement to hold a memory during the intertrial delay. Time cells were prevalent in the blocked alternation task, and most time cells clearly differentiated the east and west trials. We also found that RSC neurons could exhibit off-response time fields, periods of reliably inhibited firing. Time cells were also observed in the cued T-maze, but they were less prevalent and they did not differentiate left and right trials as well as in the blocked alternation task, suggesting that RSC time cells are sensitive to the memory demands of the task. These results suggest that temporal coding is a prominent feature of RSC firing patterns, consistent with an RSC role in episodic memory.

KEYWORDS
episodic memory, retrosplenial cortex, temporal coding, temporal context, time cells

1 | INTRODUCTION

The retrosplenial cortex (RSC) is a key component of the brain's memory system, with anatomical connections to the hippocampus, anterior thalamus, and entorhinal cortex (Sugar et al., 2011; Van Groen, 1993; Van Groen & Wyss, 2003; Wyss & Van Groen, 1992). This circuit has been implicated in episodic memory (Aggleton et al., 2023) and many of these structures have been shown to encode temporal information, which is critical for episodic memory. For example, time cells, which reliably fire during a specific epoch of time during a delay period in a manner analogous to the way that place cells fire in a specific part of the environment, have been found in CA1 (Gill et al., 2011; Kraus et al., 2013; MacDonald et al., 2011; Mau et al., 2018; Pastalkova et al., 2008), CA3 (Salz et al., 2016), the medial entorhinal cortex (Heys & Dombeck, 2018; Kraus et al., 2015), the prefrontal cortex (Ning et al., 2022; Tiganj et al., 2017), and the striatum (Akhlaghpour et al., 2016; Mello et al., 2015).

Time cells have not been reported in the RSC, although there is evidence that the RSC is involved in temporal memory. Amnesic patient T.R., who had an RSC lesion, was found to have striking deficit

in temporal memory which was not attributable to generally poor memory for nontemporal information (Bowers et al., 1988). Freeman et al. (1996) and Smith et al. (2004) proposed a theoretical account of how learning- and time-dependent changes in RSC firing patterns could encode the spatiotemporal context of a learning situation (for review, see Smith et al., 2018). RSC neurons have also been found to simulate future goal locations in rats (Miller et al., 2019). There is also circumstantial evidence in the form of sequence coding in the RSC, including sequential firing as subjects traverse a postcue "delay" segment of a virtual T-maze (Koay et al., 2022) and selective encoding of specific sequences of movements through space (Alexander & Nitz, 2015, 2017), and time cells have been predicted in theoretical accounts of the RSC (Alexander et al., 2020).

In this study, we re-examined archival neuronal firing data from the RSC recorded during two different behavioral tasks. Both tasks involve intertrial delay periods where time cells might be found, but they have differing memory requirements. One of these tasks involves spatial alternation across blocks of trials on a plus maze (Smith & Mizumori, 2006). In this task, rats must remember the reward location for the upcoming trial, and hippocampal neurons were previously found to exhibit time cell firing in this task (Gill et al., 2011). The second task is a beacon navigation task in which a salient light cue signaled the location of the reward on a T-maze and the rats simply had to approach the light for reward (Vedder et al., 2017).

2 | METHODS

2.1 | Subjects and surgical procedures

The subjects were 15 adult male Long-Evans rats with 10 of them used in the blocked alternation task and 5 of them used in the cued T-maze task. For this study, we analyzed data from previous studies which focused on spatial firing patterns of RSC neurons during the trials (Smith & Mizumori, 2006; Vedder et al., 2017). Here, we focus our analysis on the firing patterns during the intertrial delay period. All rats underwent stereotaxic surgery to implant recording electrodes targeting the granular b subregion of the RSC (Rgb), also referred to as the posterior cingulate cortex or Brodmann's Area 29c (3.0–6.0 mm posterior to bregma, 0.5 mm lateral and at least 1.0 mm ventral to the dorsal surface, Paxinos & Watson, 1998). Rats were given at least 1 week to recover from surgery before beginning training. All procedures complied with guidelines established by the Cornell University Animal Care and Use Committee.

2.2 | Behavioral procedure

The general procedures for the blocked alternation and cued T-maze tasks were similar. For both tasks, the maze was placed in the center of a dimly lit room, surrounded by black curtains that formed a circular area 3 m in diameter with high-contrast distal visual cues attached to the curtains. Correct choices were rewarded with chocolate milk (200 μL Nesquik, Nestle Inc.). For the blocked alternation task, rats were trained to retrieve rewards from the east arm of a plus maze during the first block of 15 trials and then switch to the west arm for the second block of 15 trials. This yielded 28 intertrial delay periods, 14 for each block. The start positions for each trial were randomly designated from among the three nonreward arms. The reward locations were not cued, so the rat was required to remember which arm was rewarded for each trial. For the cued T-maze task, rats were trained on a T-maze equipped with LED lights positioned 9 cm above each of the reward locations and the rats were trained to approach a flashing light cue for a reward. At the start of each trial, the rat was placed on the stem of the maze facing away from the choice point and one of the two light cues was turned on (flashing at 3 Hz) to indicate the reward location for that trial. The left or right reward positions were randomized across trials and importantly, this task did not have a spatial memory requirement since rats could simply approach the cued location on each trial. Each training session consisted of 40 trials, with 20 rewarded on the left and 20 on the right.

Analysis of the neuronal firing data was focused on the intertrial delay period, which began when the rat was placed on a platform (5.5 × 25 cm) adjacent to the maze (Figure 1) after the completion of each trial and ended when the rat was picked up to be placed on the maze for the start of the next trial. The duration of the intertrial delay varied as the experimenter rebaited the maze and prepared for the next trial, but typically lasted 19–28 s (mean = 24.14 ± 0.23 s). The experimenter took particular care to ensure that the procedures were identical for the east and west or left and right trials in order to avoid cueing the rats as to the upcoming reward location. The position of the platform relative to the maze was similar for the two tasks and was constant throughout training. The rats' behavior during the delay period of the two tasks was similar (velocity: alternation task = 12.15 ± 0.03 m/s; cued T-maze task = 13.51 ± 0.03 m/s; acceleration: alternation task = 22.07 ± 0.06 m/s^2, cued T-maze task = 25.32 ± 0.08 m/s^2). The rats did not orient themselves differently in the two tasks. The mean vector length (MVL) of the occupancy times for each

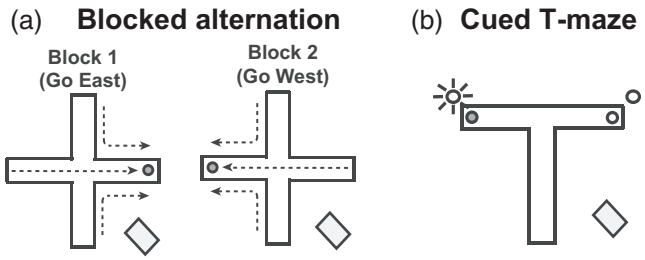

FIGURE 1 The blocked alternation and cued T-maze tasks. (a) The blocked alternation task, which involved two reward locations, on the east and west arms. The three nonrewarded arms served as start locations and were randomized. Between trials, the rats were placed on the platform adjacent to the maze. The reward locations were not cued. (b) The cued T-maze task. At the start of each trial, a flashing LED was presented at one of the two reward locations and the rats learned to approach the light for reward. Between trials, the rats were placed on the platform adjacent to the maze.

of 18 directional heading bins was near zero for both tasks (blocked alternation: MVL = 0.09, range 0.04–0.17; cued T-maze: MVL = 0.07, range 0.02–0.15), indicating that they sampled all directional headings approximately equally during the delay period in both tasks. These measures were taken from asymptotic performance sessions. Values were similar for early training sessions.

2.3 | Data collection and analysis

Neuronal spike data and video data were collected using the Cheetah Data Acquisition System (Neuralynx Inc., Bozeman, MT). Video data were used to establish the beginning and end of the intertrial delay period. Our main data set consisted of 383 neurons recorded during asymptotic performance of the 2 tasks, 180 neurons recorded from 10 rats in the blocked alternation task and 203 neurons recorded from 5 rats in the cued T-maze task. We classified putative pyramidal neurons and interneurons according to spike width (see Brennan et al., 2020). This yielded 237 pyramidal neurons (mean width = 0.358 ± 0.005 ms) and 146 interneurons (mean width = 0.142 ± 0.004 ms), all of which were included in our analyses. In order to determine whether responses developed as a function of learning, we also examined recordings taken during the first two training sessions (blocked alternation $N = 41$, cued T-maze $N = 60$). Behavioral performance during the early sessions was 54.07% ± 4.6% and 63.07% ± 3.3% correct for the blocked alternation and cued T-maze, respectively, compared with 84.07% ± 0.91% and 94.72% ± 1.02% correct at asymptote.

In the cued T-maze task, the right and left reward locations were randomized and the rat could not predict the upcoming trial type (left or right). However, firing during the delay period could reflect memory for the preceding trial, so we grouped the delay periods according to the preceding trial type for our main analysis. As described in the results, we also constructed a nonmnemonic control analysis of the T-maze data by examining the delay periods immediately before the left and right trials. Because this effectively randomized the delay periods with respect to the trial type, any firing during the delay in this condition could not be related to memory. A similar approach was not possible for the blocked alternation task because the east and west trials were presented in 15-trial blocks and each intertrial delay period was preceded and followed by the same kind of trial.

2.3.1 | Time cell classification and differential firing between conditions

We applied a linear–nonlinear (L-N) model (Hardcastle et al., 2017) to identify neurons that exhibited significant firing changes during discrete epochs during the intertrial delay period. Briefly, the model estimates the firing rate of an individual neuron as a function of time and determines whether the tuning is statistically significant. We binned the spikes into 20 ms bins and assessed the degree to which firing was significantly tuned to 500 ms epochs in either condition (east or west in the blocked alternation task, right or left in the cued T-maze task). We used a two-step process to first determine whether a time field was present for each neuron and then to determine whether the firing within the time field differed across the two conditions of the task. The L-N model approach is useful because it allows for independent assessment of how each variable or combination of variables influences neuronal firing. In the first step, the model determines whether firing is significantly tuned to temporal epoch within the delay period for one or both trial types (east/west or left/right). Neurons with significant tuning for at least one trial type were classified as time cells. Then, for each of the resulting time cells, we identified the 'time field' as any epoch where the firing rate diverged from the mean by at least two standard deviations. We then used a second L-N model that included a trial type variable (east/west or left/right) to determine whether the firing activity within the time fields was significantly different across the two trial types. For both the L-N model implementations, we used a 10-fold cross validation and significance was determined using a one-sided signed rank test with significance criteria of $p < .05$. This approach to classifying time cells yielded similar results to methods previously applied to hippocampal neurons (Gill et al., 2011; Pastalkova et al., 2008). However, the L-N model is better suited for RSC neurons, which have high baseline firing rates and responses to task variables can consist of increased or decreased firing (e.g., Miller et al., 2019; Subramanian et al., 2024; Vedder et al., 2017), and it yielded fewer apparent misclassifications than methods optimized for hippocampal neurons. Data for all the neurons and their classification are shown in Figure S1.

We also computed measures of time cell quality, including a reliability measure (the proportion of trials in which the firing rate during the time field was at least 2 SD above or below the mean) and a measure of contrast reflecting the in-field versus out-of-field firing rates (absolute value of (in-out)/(in+out)). Previous studies have shown that time fields can be aligned to the beginning or end of the intertrial delay period (MacDonald et al., 2011). However, our delay periods were variable, which could obscure time fields near the end of the delay, so we also analyzed the firing data of each neuron aligned to the end of the delay period.

3 | RESULTS

Many RSC neurons reliably exhibited discrete periods of elevated firing during the intertrial delay period (i.e., time fields, Figure 2a, plots 1–8, Figure S1), similar to those previously reported in the hippocampus (Gill et al., 2011; Pastalkova et al., 2008). In the blocked alternation task, 36% of neurons (65/180) exhibited significant time fields in at least one of the two blocks (east or west). Firing during the time fields was quite reliable, with firing >2 SD above the mean during the time field on 75.4% (± 1.45) of trials, and time fields were generally brief, circumscribed segments of the delay period (mean duration = 2.95 ± 0.22 s). These time fields were distributed throughout the intertrial delay period, but they were most prevalent during the first 5 s of the delay (Figure 2b). Some neurons had two

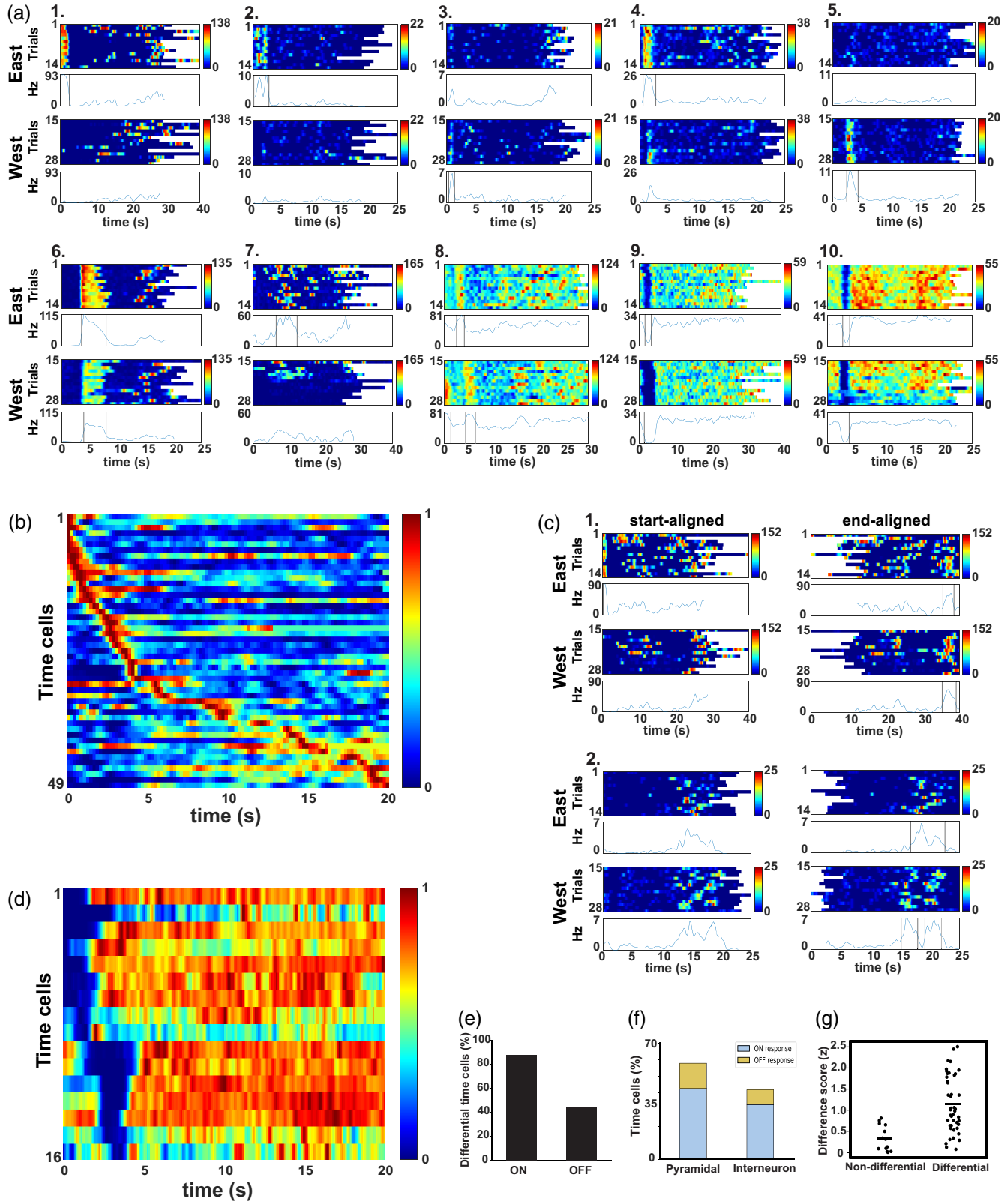

FIGURE 2 Legend on next page.

time fields ($n = 11$, e.g., Figure 2a, plot 8) and sometime fields were more closely aligned to the end of the delay period than the start ($n = 14$, Figure 2c).

Interestingly, we found that RSC neurons could also exhibit discrete periods of inhibited firing (Figure 2a, plots 9–10). These off-responses accounted for 25% (16/65) of the significant time cells seen

in the blocked alternation task. These off-response time fields were shorter than the on-responses in terms of their duration (on-responses: 2.95 ± 0.22 s, off-responses: 1.55 ± 0.12 s, $t(93) = 3.77$, $p < .001$), but interestingly, they were somewhat more reliable (on-responses: 0.75 ± 0.01, off-responses: 0.97 ± 0.02, $t(93) = 7.29$, $p < .001$) and their contrast between in-field and out-of-field firing was significantly higher (on-responses: 0.38 ± 0.02, off-responses: 0.72 ± 0.04, $t(93) = 7.11$, $p < .001$). However, off-responses were only observed during the first 5 s of the delay period (Figure 2d) and they were less likely to differentiate the east and west conditions (Figure 2e). On- and off-responses were not exclusively associated with pyramidal neurons or interneurons. Instead, on- and off-responses occurred in similar proportions within the pyramidal neurons and interneurons (Figure 2f).

Time cells frequently differentiated the "go east" and "go west" blocks of the alternation task. Overall, 80% of time cells (52/65) showed differential firing across the two conditions. Typically, the neurons had a clear time field in one block, while firing in the other block could range from a complete absence of firing during the same epoch in the other block (e.g., Figure 2a, plots 1–2) to a clear time field, but with reduced firing rate (e.g., plot 6). The range of differential responses during the east and west trials is illustrated in Figure 2g. In some cases, the neuron had time fields in both blocks, but during different epochs ($n = 9$, Figure 2a, plot 8). Differential firing was more prevalent for the on-response time cells (88%, 43/49) than for the off-response time cells (44%, 7/16).

Time cells were also observed in the cued T-maze task (Figure 3). However, they were not as prevalent, and they did not differentiate the left and right conditions as frequently as in the blocked alternation task. Only 15% (31/203) of neurons had significant time cell firing and among these, only seven neurons (23%) differentiated the left and right trials. The overall quality of the time fields also differed across the tasks. Compared with the blocked alternation task, the time fields observed in the cued T-maze were larger (cued T-maze duration = 3.17 ± 0.19 s, alternation = 2.58 ± 0.17 s, $t(160) = 2.25$, $p < .05$), less reliable (cued T-maze = 0.67 ± 0.02, alternation = 0.81 ± 0.02, $t[160] = 5.64$, $p < .001$), and had lower in-field to out-of-field contrast (cued T-maze = 0.30 ± 0.02, alternation = 0.47 ± 0.03, $t[160] = 4.99$, $p < .001$). Most of the time cells in the cued T-maze task had on-responses (94% of time cells, 29/31), while only two had off-responses. All seven of the cases of differential firing on left and right trials occurred in neurons with "on" responses. Time fields were seen in pyramidal ($n = 13$) and interneurons ($n = 18$). Only three time cells had a secondary time field and for two of those cells, the secondary time field was present in only one of the trial types while the third one had a secondary time field in both trial types.

For the above analyses, we compared the delay periods that occurred immediately *after* left and right trials. We reasoned that although there was no requirement that the subject remember the reward locations, firing during the delay period might nevertheless have reflected memory for the events of the previous trial. In contrast, the rats could not anticipate the upcoming trial because the left- and right-rewarded trials were randomized. This feature of the task allowed us to use delay periods that occurred *before* left and right trials as a nonmnemonic control. When we sorted left and right trials that way, we still found that 15% (30/203) of the neurons had time fields. Six of those 30 neurons differed for the left and right conditions. Since the rats could not know whether the upcoming trial was going to be rewarded on the left or right, these six cases presumably represent the random chance rate of falsely detecting a time field within a population of 203 neurons. In any case, the number of time cells seen in this nonmnemonic control suggests that even the relatively small percentage of time cells seen in our original analysis (above) are not related to memory for the trials.

The above-described results indicate that time cell firing patterns differed during asymptotic performance of the two tasks. We also examined data from the early (first two) training session in order to determine whether time cell firing emerged as a function of learning (Figure 4). In the blocked alternation task, the percentage of neurons with time fields doubled during learning and notably, the percentage of time cells that differentiated the east and west conditions tripled. In contrast, the proportion of differential time cells did not change with learning in the cued T-maze task and the overall number of time cells increased only modestly.

RSC neurons are known to exhibit spatial firing (Alexander & Nitz, 2015; Miller et al., 2019; Subramanian et al., 2024) and many of the time cells observed here also showed spatial firing during the trials (Figure 5). As in previous studies (e.g., Gill et al., 2011), time cell firing

FIGURE 2 Retrosplenial time cells in the blocked alternation task. (a) Examples of retrosplenial cortex (RSC) time cells observed in the blocked alternation task. The firing rate heatmaps illustrate trial-by-trial firing, shown separately for the "Go East" and "Go West" conditions. The line plot shows the average firing rate across trials with the two vertical black lines indicating the boundaries of the time field. Because the delay duration was variable, average firing rate was calculated only for time periods containing at least two thirds of the trials. Neurons could have more than one time field (e.g., plot 8). Plots 9 and 10 illustrate example neurons with off-response time fields, in which firing was reliably suppressed during a well-defined part of the delay. (b,d) The temporal firing of all the neurons with significant on-response (b) and off-response (d) time fields across the first 20 s of intertrial delay period of the west reward condition. Each row illustrates the normalized firing rate of a single neuron, averaged across all trials, and sorted according to the time of maximum (b) or minimum (d) firing. (c) Two example neurons with time fields aligned to the end of the delay period. For each neuron, the start-aligned firing is plotted on the left and the same data realigned to the end of the delay are plotted on the right. The neuron in plot 1 had a time field aligned to the start of the delay on the east trials, along with a time field aligned to the end of the delay for both trial types. (e) The percentage of time cells that showed differential firing for the on-response and off-response time cells. (f) The percentage of time cells observed in putative pyramidal neurons and interneurons, with on- and off-responses shown in blue and yellow, respectively. (g) The range of differential responses during the east and west trials. Many neurons had large firing rate differences, but even small firing rate differences could result in significantly different east/west firing if the trial-by-trial reliability was high.

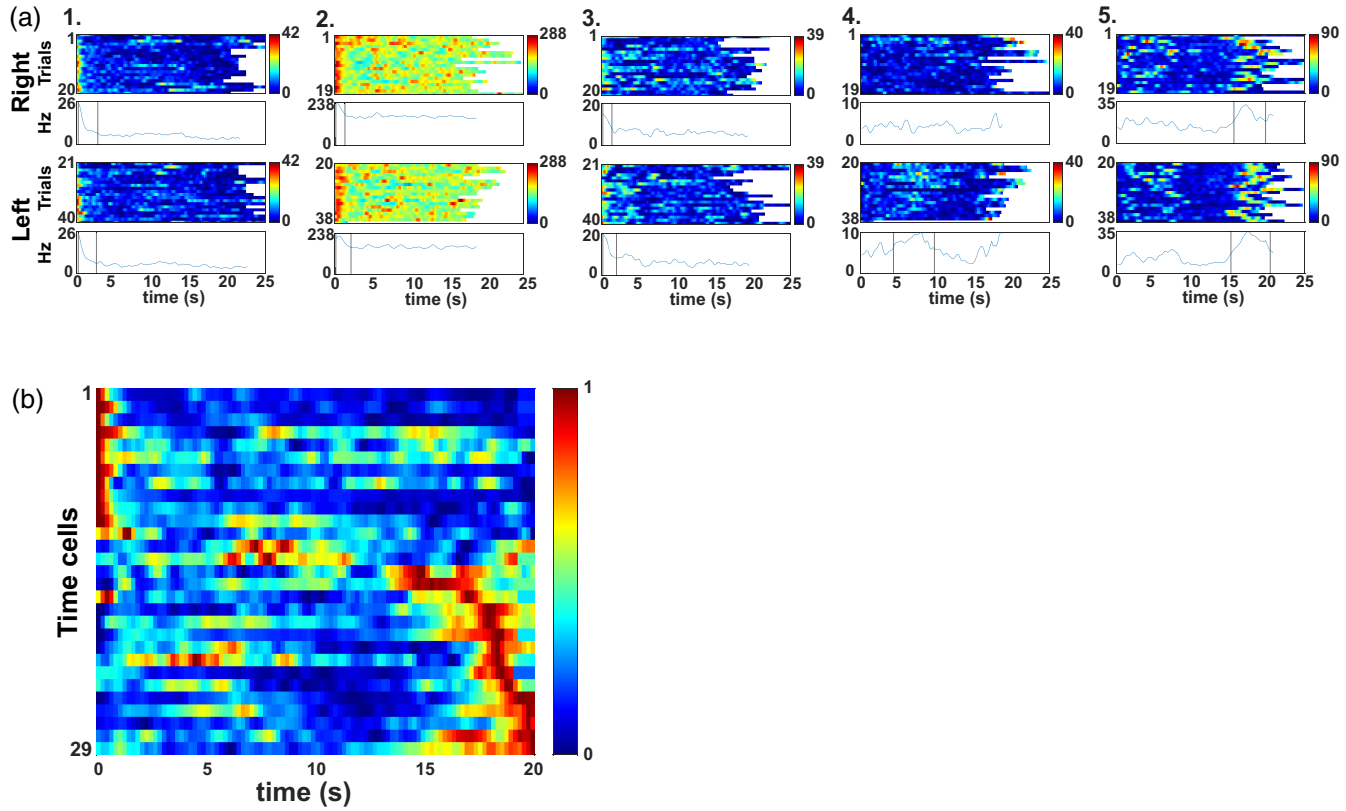

FIGURE 3 Retrosplenial time cells in the cued T-maze task. (a) Example time cells observed in the cued T-maze task. Firing rate heatmaps are illustrated as in Figure 2, with separate plots for right and left trials. (b) The temporal firing of all the neurons with significant on-response time fields for right-rewarded trials. Only two neurons were found to have off-response time fields in this task (not shown).

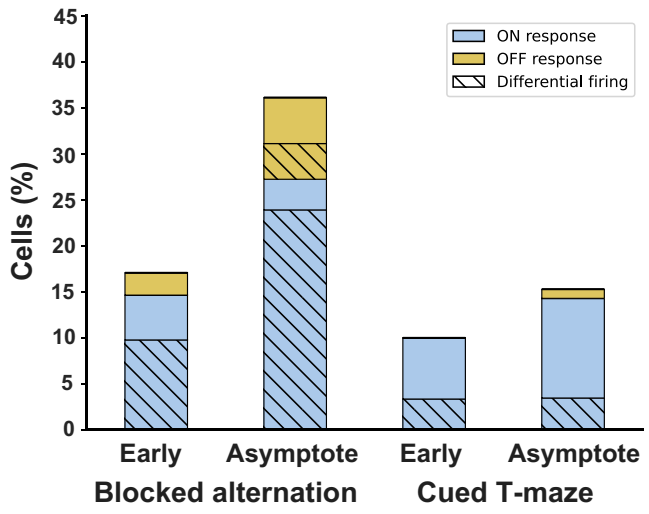

FIGURE 4 Time cell characteristics differ across behavioral tasks. The percentage of neurons with significant time cells for early and asymptotic sessions in each task are shown, with on- and off-response time cells shown in blue and yellow, respectively, and differential firing across the east/west or left/right conditions indicated by diagonal lines.

did not appear to be related to spatial firing during the trials. However, we examined this by binning the firing rates into 40 equal-sized spatial bins along the trajectory for each trial from the start to the reward and correlating them with 40 equal-sized time bins representing the duration of the delay period. Positive correlations would be expected, for example, if neurons that fired during the early (or late) part of the delay period also fired early (or late) in the trial run. We performed separate correlations for each of the possible trajectories in the two tasks. For example, spatial firing from blocked alternation trials that started from the north arm and ended on the east arm were compared with the time-binned firing for delay periods immediately following those trials. Similarly, left turn runs on the T-maze were correlated with time-binned firing during the delay period following those trials. All of these analyses produced very low correlations (alternation task: mean Pearson's $r = .0007 \pm .005$, range: $-.019$–$.019$; cued T-maze task: mean $r = -.0034 \pm .009$, range: $-.007$–$.0001$). This suggests that temporal firing during the delay period could not have been a recapitulation of spatial firing on the maze. This finding is not surprising, given that RSC neurons typically do not have compact place fields like those of the hippocampus (e.g., see Subramanian et al., 2024). Instead, they fire at reliably elevated rates across wide areas of the maze, so precise relationships between broad spatial firing and relatively brief temporal firing patterns are unlikely.

4 | DISCUSSION

Time cells were a prevalent feature of RSC representations, and their time fields were similar to those previously observed in the

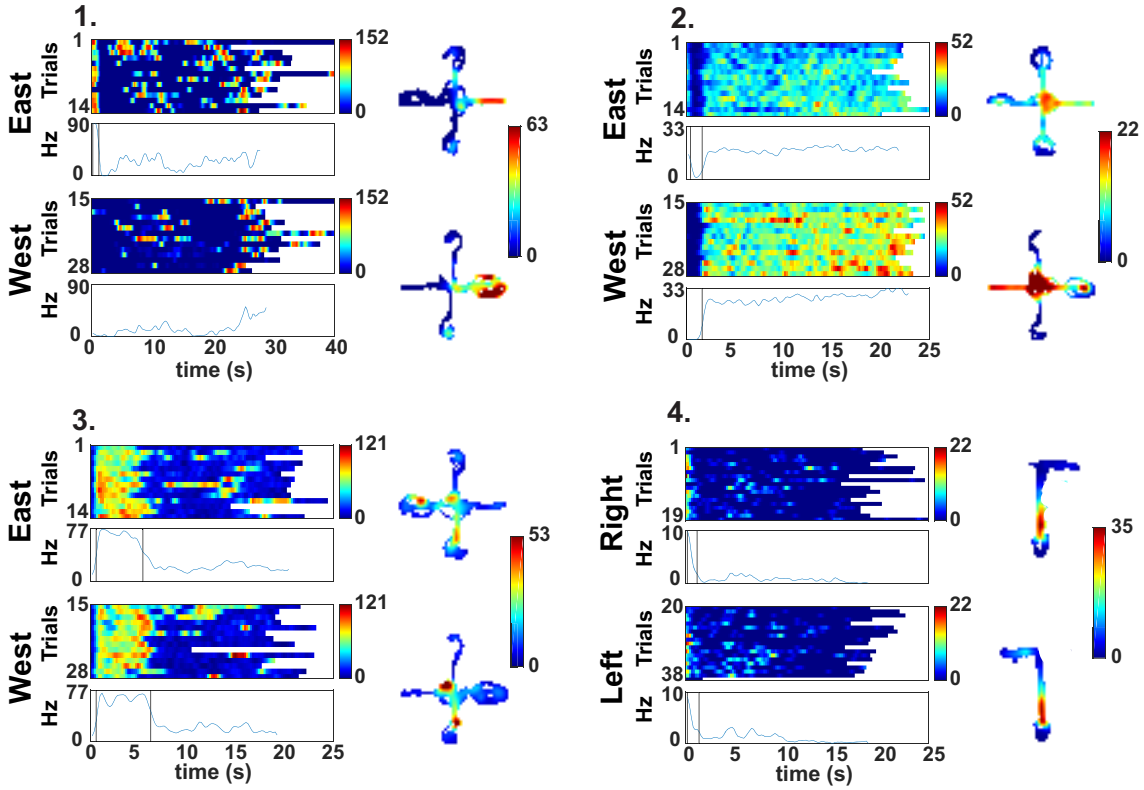

FIGURE 5 Spatial firing in RSC time cells. Spatial firing patterns observed during the trials are shown for four example neurons with time fields during the intertrial delay period. Plot 1 is the same neuron illustrated in Figure 2c1. Plot 4 is an example neuron from the cued T-maze task.

hippocampus (Gill et al., 2011; MacDonald et al., 2011; Pastalkova et al., 2008) and the medial entorhinal cortex (Heys & Dombeck, 2018; Kraus et al., 2015). These responses took the form of discrete periods of reliably elevated, or suppressed, firing with high contrast to the background firing rate. The on-response time fields covered the entire intertrial delay period and they differentiated the east and west conditions of the blocked alternation task, which is also similar to hippocampal time fields (Gill et al., 2011). Interestingly, more than one third of RSC neurons had time fields in the blocked alternation task, compared with about 20% of hippocampal neurons in the same task (Gill et al., 2011), suggesting that time cells may be more prevalent in the RSC.

Off-response time fields were unexpected, as they have not been reported before. These responses are possible in the RSC because, unlike the hippocampus, RSC neurons often have high baseline firing rates which allow responses to take the form of either increases or decreases from the baseline. Previous studies of the RSC have found reliable off-responses to critical stimuli and events (Smith et al., 2012; Vedder et al., 2017) and these responses likely contribute importantly to population-level representations of space and context (Miller et al., 2019, 2021). The off-response time fields were discrete and reliable, and they differentiated the east and west conditions of the blocked alternation task at a rate similar to our previous study of hippocampal time cells in this task (Gill et al., 2011). However, there were some potentially important differences between the on- and off-response time cells in the RSC. Differential firing was far less common in the off-response neurons (Figure 4) and off-response time fields were only seen during the first 5 s of the delay period (Figure 2d). However, the relative rarity of these responses in the population makes it difficult to determine whether longer latency time fields are completely absent or simply less common than early time fields.

A small number of RSC neurons exhibited asymmetric responses that had a sudden onset of firing followed by slow return to baseline (e.g., Figure 2a, plot 6, see Figure S1 for all time fields). A recent model of time cell firing suggests that these response patterns may occur in response to salient external stimuli, especially at the start of the delay period (Liu et al., 2019). We cannot rule out the possibility that some of our apparent time cell responses may have been driven by external stimuli, such as the actions of the experimenter as they prepared for the upcoming trial. However, we think this is unlikely to have been a significant factor in our results. We have found that RSC neurons do, in fact, respond to salient cues (for review see Smith et al., 2018), but such stimuli typically evoke time-locked responses from many neurons (e.g., Vedder et al., 2017). Here, the time fields were distributed throughout the delay period in the alternation task (Figure 2b). They did not cluster around one or a few time points where salient cues might have occurred. Additionally, the experimental procedures for the east and west delay periods of the alternation task were identical, but the east and west time fields differed for most neurons. Although most time fields probably were not purely stimulus-driven, rats are

typically attentive to the experimental procedures, and they may use this information to update their internal estimate of the passage of time, allowing them to anticipate the end of the delay period and the start of the upcoming trial. We speculate that this updating could underlie the end-aligned time fields observed here (Figure 2c, also see MacDonald et al., 2011). This may be analogous to how the path integration system can be updated on the fly to produce hippocampal place fields that are anchored to the end of an unpredictable and variable length trajectory (Gothard et al., 1996).

The task differences observed here are consistent with the idea that time cells are sensitive to the memory demands of the task. Time cell firing was both more prevalent and more likely to differentiate the east and west conditions in the blocked alternation task, as compared to the cued T-maze task. The blocked alternation task has a clear memory requirement insofar as the rats must hold two distinct goal memories in preparation for the "go east" and "go west" trials. The observation that these firing patterns developed as a function of learning suggests that they are related to the cognitive demands of the task. In contrast, the cued T-maze task simply requires the rat to approach the light cue for reward, a single procedural memory which likely does not engage the episodic memory system (Lee et al., 2008; McDonald & White, 1994). Consistent with this idea, our previous study (Vedder et al., 2017) found that RSC neurons respond to the light cue the same way, regardless of whether it was presented on the left or the right. Similarly, we found that differential time fields were rare in this task.

These task differences are notably similar to previous reports on the hippocampus, where time cells were found in tasks that require subjects to hold different memories during the delay period, but not in similar tasks which do not (Gill et al., 2011; Pastalkova et al., 2008; but see Sabariego et al., 2019). Interestingly, 80% of the RSC time cells differentiated the east and west trials, as compared to about half of hippocampal time cells recorded in the same task (Gill et al., 2011), suggesting that memory-related time cell coding might be more prevalent in the RSC.

Our results suggest that RSC time cell firing is related to the memory demands of the task. However, because we used data from two different experiments (Smith et al., 2012; Vedder et al., 2017), we cannot rule out the possibility that other variables may have played a role in our results. In addition to the differing memory requirements, the two tasks differed in the use of blocked VS randomized trials, different maze apparatus, as well as differing start locations and trajectories to the goal locations. The general procedures were similar for the two experiments (see Section 2) and we took particular care to use identical procedures for the east/west and left/right trial types in both experiments in order to avoid cueing the rats as to the upcoming reward location. Therefore, the greater prominence of differential firing patterns in the blocked alternation task is not likely due to methodological differences between the two experiments. Nevertheless, carefully controlled studies with formally similar memory and nonmemory trials in the same subjects will need to be carried out before these variables can be conclusively ruled out.

Our observation of time cell coding is consistent with an RSC role in episodic memory (Ranganath et al., 2005; Steinvorth et al., 2006). Previous authors have suggested that hippocampal time cells play a role in encoding the temporal context, which provides a mechanism for encoding the sequence of stimuli and events that define an episodic memory (Eichenbaum, 2014; Howard & Eichenbaum, 2013). Our results suggest that the RSC may play a key role in this process. This is consistent with findings from patient T.R. whose left-RSC lesion produced a remarkable impairment in temporal memory (Bowers et al., 1988). The deficit was a specific failure to remember which items came earlier or later within lists of to be remembered items, even though recognition of the individual items was unimpaired. Bowers et al. (1988) describe this impairment as a failure of time-tagging, which echoes the idea of temporal context as a mechanism for sequencing items in memory (Howard & Kahana, 2002). In a notably similar pattern of results, rats with RSC lesions were found to be impaired in memory for the temporal order of objects, but not for recognition of the objects themselves (Powell et al., 2017). Relatedly, damage to the anterior thalamic nuclei, a primary input to the RSC, in Korsakoff's amnesia also produces deficits in temporal memory (Hunkin & Parkin, 1993; Kopelman, 2015; Kopelman et al., 1997; also see Nelson, 2021). Finally, studies of instrumental discrimination learning have shown that RSC neurons exhibit systematic time-dependent changes in stimulus-evoked responses, which are thought to underlie a similar kind of temporal context (Freeman & Gabriel, 1999). Time cell firing was prominent in the RSC even though neither of our behavioral tasks had an explicit temporal memory requirement, as with other time cell studies, possibly suggesting that temporal firing patterns are automatically generated in manner analogous to the spatial firing seen in the hippocampus and elsewhere.

The finding of time cell coding in the RSC provides an additional point of functional similarity between the RSC and the hippocampus, along with spatial and contextual memory (Subramanian et al., 2024), which highlights the importance of understanding the roles of these structures as components of a coherent memory circuit (Smith et al., 2022). The source of RSC time cell information is not known, but hippocampal input is known to be critical for some kinds of RSC representations (Alexander et al., 2018; Cowansage et al., 2014; De Sousa et al., 2019; Kang & Gabriel, 1998; Katche et al., 2013; Mao et al., 2018; Milczarek et al., 2018). However, the above-mentioned model of time cell firing (Liu et al., 2019) suggests that sudden onset responses like those described above are characteristic of the early layer neurons that contribute to the emergence of time fields, suggesting that these RSC neurons may serve as inputs to time cells in other brain regions. In any case, findings of time cells in the entorhinal cortex, prefrontal cortex, and the striatum (Akhlaghpour et al., 2016; Heys & Dombeck, 2018; Kraus et al., 2015; Mello et al., 2015; Ning et al., 2022; Tiganj et al., 2017) suggest that, whatever the source of this signal, time coding is widespread in brain regions known to be involved in learning and memory.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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