

Spatially Periodic Activation Patterns of Retrosplenial Cortex

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INTRODUCTION

The retrosplenial cortex (RSC) has emerged as a crucial brain region that supports a wide range of cognitive functions, particularly those related to spatial navigation, episodic memory, and contextual associations.

In rodents, RSC is located in the midline of the posterior cortex and is divided into two main subregions: the granular (RSCg) and dysgranular (RSCd) cortices (van Groen & Wyss, 2003). These subregions exhibit distinct connectivity patterns, with RSCg interconnected with the hippocampus, subiculum, and anterior thalamic nuclei while RSCd is primarily connecting with visual and visuospatial areas (van Groen & Wyss, 2003).

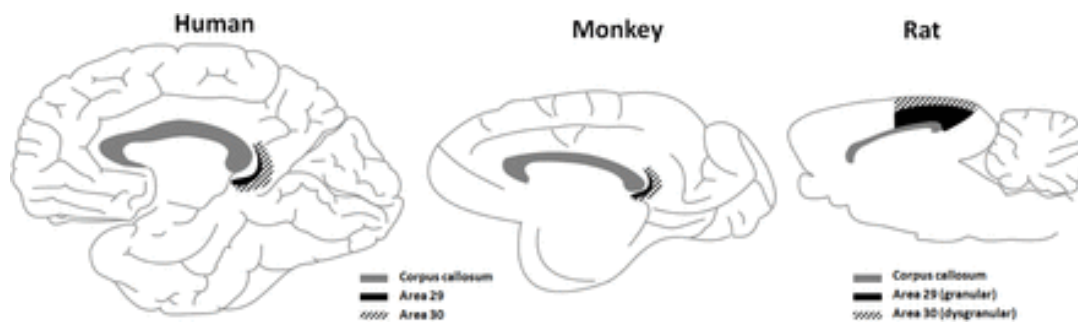


Figure 1: Anatomy of RSC

Schematic of the RSC as seen in midsagittal section and located just posterior to the corpus callosum, in humans, rhesus monkeys and rats. Figure by Jeffery, 2017

One of the most striking features of RSC is its heterogeneous spatial representations. RSC neurons exhibit conjunctive encoding of egocentric and allocentric reference frames, which allows for the transformation and integration of spatial information across different perspectives (Alexander et al., 2020). Moreover, RSC contains neurons sensitive to head direction (Jacob et al., 2017) and spatial position (Mao et al., 2017), as well as cells that encode route trajectories (Alexander & Nitz, 2015). These multifaceted spatial codes endow RSC with the capacity to support complex spatial cognition, including navigation, spatial memory, and context-dependent behavior (Vedder et al., 2017).

In addition to its rich spatial representations, RSC exhibits intricate temporal dynamics that are tightly coordinated with activity patterns in the hippocampal formation. RSC neurons show phase-locking to hippocampal theta oscillations and are modulated by sharp-wave ripples (Alexander et al., 2018), which suggests a role in the temporal

organization of spatial and episodic memories. Drawing on these findings, Alexander et al. (2023) propose two overarching functions of RSC: 1) the ability to flexibly shift between spatial reference frames and perspectives, and 2) the capacity to generate predictions about sensory and contextual states and detect mismatches between these predictions and actual experience.

Building on this comprehensive understanding of RSC function, the current project aims to further investigate a specific aspect of RSC spatial dynamics: the periodicity of RSC neural activity. Previous work by Alexander and Nitz (2017) demonstrated that RSC neurons exhibit periodic firing patterns during traversals of repetitive route spaces, with the periodicity of individual neurons encoding specific spatial scales. This periodic encoding was maintained even in track configurations (ring-shaped and irregular tracks) that are devoid of repeating action sequences, indicating independence from motor patterns. These findings suggest that RSC periodicity may play a crucial role in representing and organizing spatial information during navigation.

The present study seeks to extend the findings of Alexander and Nitz (2017) by examining RSC periodicity across a broader range of environmental geometries and spatial scales. We will investigate RSC activity in rats navigating through a variety of path shapes including a ring-shaped track, a plus-shaped track, a large and small hexagonal track, and an irregularly shaped track. By comparing RSC periodicity across these diverse environments, we aim to elucidate how RSC neural dynamics adapt to different spatial contexts and how this adaptation supports flexible spatial cognition.

METHODS

Three adult male Sprague Dawley rats were used in this study. Rats were housed individually on a 12-hour light/dark cycle and food-restricted to 85-90% of their free-feeding weight. Water was available ad libitum.

Rats were surgically implanted with microdrives housing 12-16 independently movable tetrodes targeting the retrosplenial cortex (RSC). Rats were anesthetized with isoflurane and positioned in a stereotaxic frame. Microdrives were implanted relative to bregma. Tetrodes were constructed from 17 μm platinum-iridium wire.

Rats were trained to traverse five different track configurations for food reward: a ring-shaped track, a large hexagonal track, a small hexagonal track, a plus-shaped track, and an irregularly shaped track. The edges of the tracks were 1 cm in height to allow the rat an unobstructed view of the recording room, which had fixed distal cues. Rats traversed each track in the clockwise direction. On each track, the rat was required to stop at a fixed goal location before receiving a reward (1/4 honey nut cheerio) placed on the track. The rat had to stop on its own volition, without any cue from the experimenter. If the rat stopped at the correct location, the reward was given; otherwise, no reward was provided. The track was cleaned between sessions to minimize local olfactory

cues. Training occurred over several weeks until the rat could consistently complete at least 12 uninterrupted laps on each track within a 30-minute session.

Electrical signals were amplified and digitized using a 48-channel MAP acquisition system (Plexon). Tetrodes were slowly advanced over days until reaching RSC. Spike data was sampled at 40 kHz. The rat's position was tracked at 60 Hz using red and green LEDs attached to the headstage. Recordings were made as the rat traversed each of the five tracks. The track type was varied pseudorandomly across days.

DATA ANALYSIS

For each well-isolated unit, firing rate vectors were constructed by first linearizing the rat's position on the track and dividing it into 3.5 cm spatial bins. The total number of spikes occurring in each bin was divided by the occupancy time in that bin to compute the firing rate. Raw rate vectors were smoothed with a Gaussian kernel (5 cm SD) to generate the final spatial tuning curves.

To quantify the degree of spatial periodicity in RSC firing patterns, we employed a generalized linear model (GLM) approach similar to that used by Alexander & Nitz (2017). Twelve spatial predictors were constructed using pairs of sine and cosine functions with periodicities corresponding to the full track length and smaller sub-spaces (e.g., halves, thirds, quarters). The complete GLM (cGLM) included all twelve spatial predictors, while partial GLMs (pGLM) were constructed by systematically dropping each predictor in turn. Model fits were quantified using the normalized mean squared error (NMSE) between the predicted and actual firing rate vectors. A lower NMSE indicates a better model fit.

To determine whether an RSC neuron exhibited significant spatial periodicity, we first fit the cGLM and pGLMs to the firing rate vector of each individual traversal. This yielded a distribution of NMSE values across traversals for the cGLM and each pGLM. If the NMSE distribution for any pGLM was significantly higher than the cGLM NMSE distribution (Kruskal-Wallis test, $p < 0.05$), the neuron was considered to be significantly modulated by the spatial periodicity represented by the dropped predictor. Neurons could be sensitive to multiple periodic predictors.

All data analysis was performed using custom MATLAB scripts (MathWorks). Statistical tests were deemed significant at $p < 0.05$.

RESULTS

Our analysis identified a total of 321 neurons recorded from the retrosplenial cortex (RSC) of three adult male rats during clockwise traversals of five different track configurations. Of these, 265 neurons exhibited firing rates greater than 3 Hz at least at one position along the track and were not classified as place cells.

To quantify the spatial periodicity in RSC firing patterns, we fit generalized linear models (GLMs) to the firing rate vectors of each neuron. The complete GLM (cGLM) included twelve spatial predictors corresponding to the full track length and smaller sub-spaces, while partial GLMs (pGLMs) were constructed by systematically dropping each predictor. Figure 2 shows representative examples of RSC neurons exhibiting dominant periodicities in different track shapes, as revealed by the impact of dropping the best spatial predictor from the model. These examples illustrate how the firing patterns of individual RSC neurons are strongly modulated by specific spatial scales that match the geometric structure of the environment.

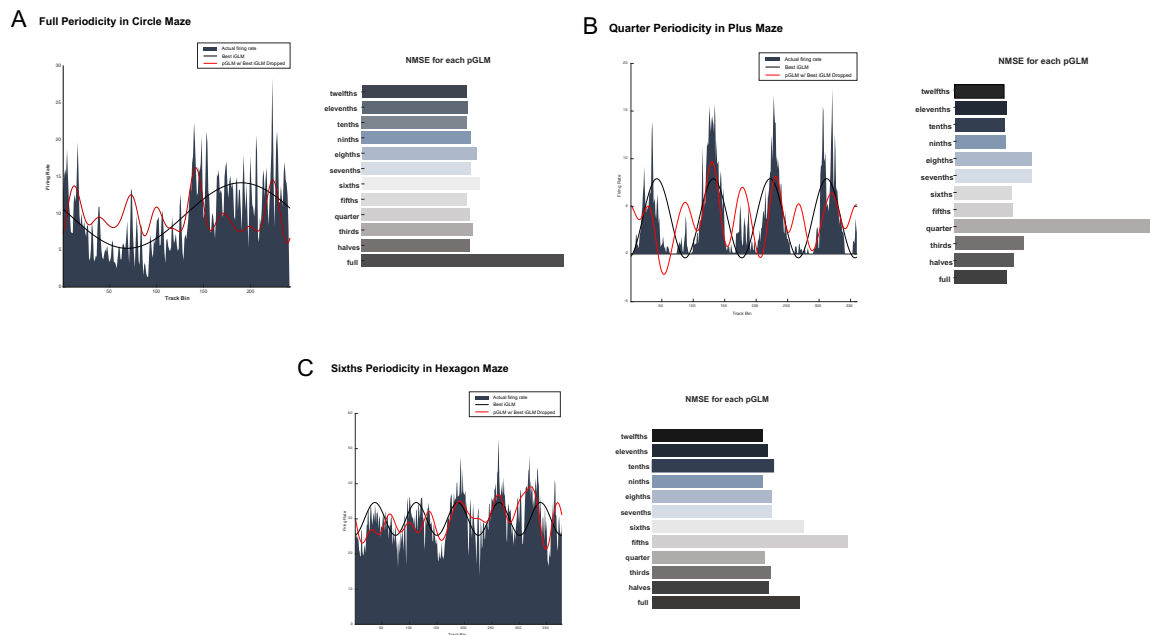


Figure 2: Dominant Periodicity in Different Track Shapes

For each plot, the model fit generated by the best individual spatial predictor (iGLM) is shown in black. In red, the partial GLM (pGLM) when the best predictor is dropped from the model shows the impact of that spatial periodicity in accounting for the firing pattern of the neuron. To the right of each figure is normalized mean squared error (NMSE) for all pGLMs for the neuron.

- (A) Full periodicity in ring-shaped track
- (B) Quarter periodicity in plus-shaped track
- (C) Sixths periodicity in hexagon track

The percentage of RSC neurons showing significant spatial periodicity varied considerably across track shapes (Figure 3). In the ring-shaped track, a remarkable 44.4% of neurons were significantly modulated by the full track periodicity, indicating that the circular symmetry of the environment was strongly reflected in RSC activity. The plus-shaped track elicited a high proportion of neurons sensitive to quarter (32.4%) and half (36.0%) periodicities, which suggests that RSC encoded the repeating structure of the four arms and the bilateral symmetry of the track. Interestingly, both the large and small hexagonal tracks induced significant sixths periodicity in more than 35%

of RSC neurons, which demonstrates that the six-fold symmetry of these environments was captured by the spatial firing patterns of RSC. In contrast, the irregularly shaped track showed a more distributed pattern of periodicity sensitivity, with no single periodicity dominating the population response.

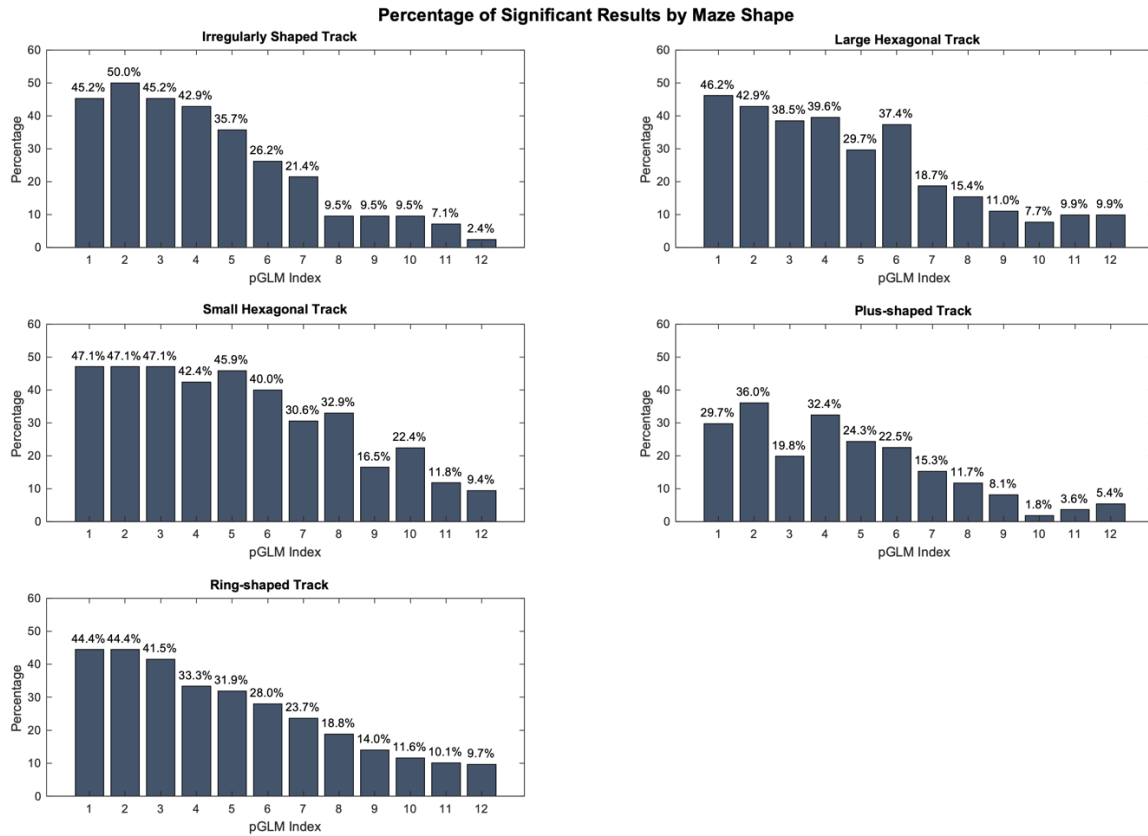


Figure 3: Percentage of Significant Results by Track Shape

Figure 4 presents the percentage of RSC neurons for which each spatial periodicity yielded the lowest p-value (i.e., the strongest modulation) in the different track shapes. This analysis contributes to a more comprehensive understanding of into the relative importance of different spatial scales in shaping RSC activity. Consistent with the findings in Figure 3, full periodicity was dominant in the ring-shaped track, while quarter and half periodicities prevailed in the plus-shaped track, and sixths periodicity was most prominent in the hexagonal tracks. The irregularly shaped track exhibited a mix of periodicities, with full, halves, and quarter periodicities being the most common. This diversity of dominant periodicities in the irregular track suggests that RSC neurons can flexibly combine multiple spatial frequencies to represent complex environments that lack regular geometric structure.

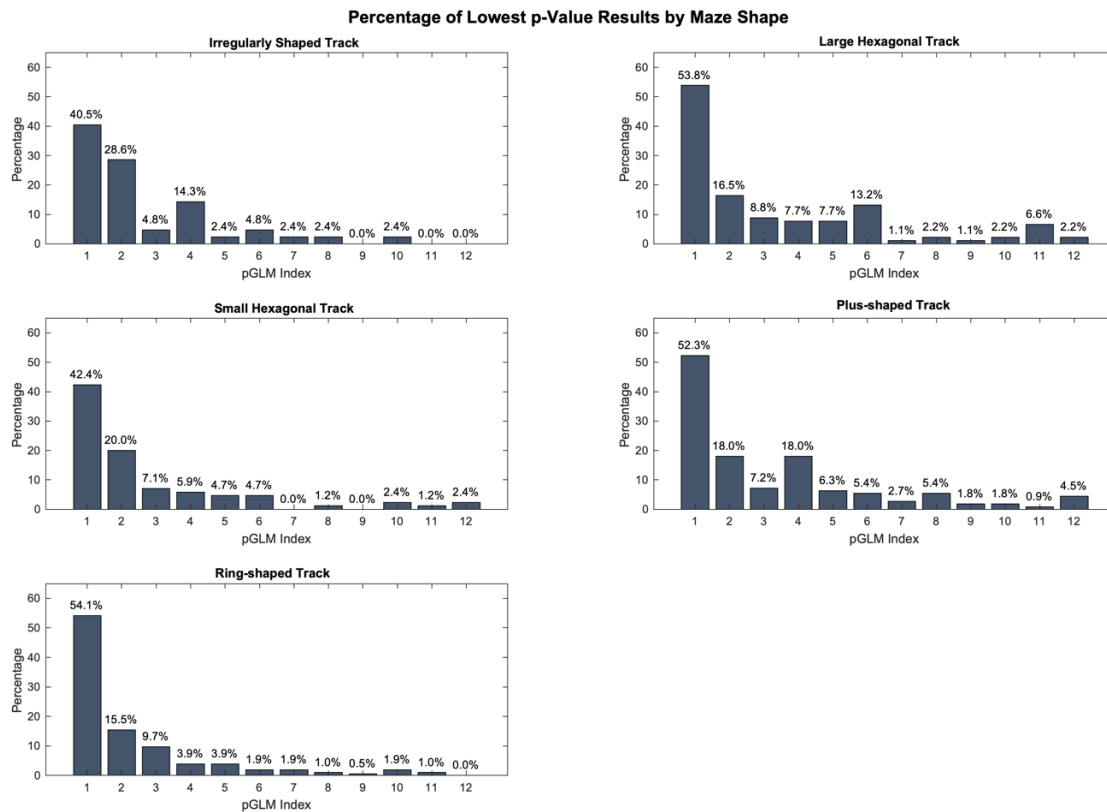


Figure 4: Percentage of Lowest p-Value Results by Track Shape

Taken together, these results provide compelling evidence that RSC neurons exhibit spatially periodic firing patterns that adapt to the geometry of the environment. The dominant periodicities in RSC activity align closely with the symmetries and sub-structures of each track shape, which indicates that RSC encodes spatial information at multiple scales that are relevant to the navigational context. The high proportion of neurons showing significant periodicity in the ring, plus, and hexagonal tracks demonstrates that RSC is highly sensitive to the regular structure of these environments. In contrast, the more distributed pattern of periodicity sensitivity in the irregular track highlights the flexibility of RSC spatial representations, suggesting that RSC can dynamically combine multiple spatial frequencies to capture the complexity of unstructured environments. These findings deepen our understanding of the role of RSC in integrating spatial information across different scales and provide a framework for understanding how RSC contributes to spatial cognition and navigation in diverse environmental contexts.

DISCUSSIONS

The present study investigated the spatial periodicity of neural activity in the rat retrosplenial cortex (RSC) during navigation in environments with varying geometric structures. Our results demonstrate that RSC neurons exhibit spatially periodic firing

patterns that adapt to the shape of the environment, with dominant periodicities aligning with the symmetries and sub-structures of each track. In the ring-shaped track, a high proportion of neurons showed significant modulation by the full track periodicity, while the plus-shaped and hexagonal tracks elicited strong quarter, half, and sixth periodicities, respectively.

These findings extend the work of Alexander and Nitz (2017), who first reported spatially periodic activity in RSC neurons during traversals of repeating route spaces. Our study builds upon their findings by demonstrating that RSC periodicity adapts to a broader range of environmental geometries and spatial scales. The high proportion of neurons exhibiting significant periodicity in the ring, plus, and hexagonal tracks suggests that RSC is particularly sensitive to the regular structure of these environments. Moreover, the diverse mix of periodicities observed in the irregular track highlights the flexibility of RSC spatial representations, indicating that RSC can dynamically combine multiple spatial frequencies to capture the complexity of unstructured environments.

The spatially periodic activity patterns observed in RSC may play a crucial role in supporting spatial cognition and navigation. By encoding spatial information at multiple scales relevant to the environmental context, RSC periodicity could facilitate the integration of local and global spatial cues, allowing animals to maintain a coherent representation of their position and orientation within the environment. The ability of RSC neurons to flexibly combine multiple spatial frequencies may be particularly important for navigation in complex, unstructured environments where local cues may be ambiguous or unreliable.

The adaptation of RSC periodicity to environmental geometry may also have implications for the role of RSC in spatial learning and memory consolidation. As animals explore novel environments, the spatial periodicity of RSC activity could evolve to capture the relevant spatial structure of the environment, which supports the formation of stable spatial representations. Over time, these periodic representations may be consolidated and integrated with existing spatial knowledge, which allows animals to develop a comprehensive cognitive map of their surroundings.

In conclusion, our findings reveal that RSC neurons exhibit spatially periodic firing patterns that adapt to the geometric structure of the environment, with dominant periodicities aligning with the symmetries and sub-structures of each navigational context. This adaptive periodicity may play a crucial role in supporting spatial cognition, enabling animals to integrate spatial information across multiple scales and consolidate spatial memories. Future studies could investigate the development of RSC periodicity over the course of spatial learning, as well as the interactions between RSC and other brain regions involved in spatial processing, such as the hippocampus and entorhinal cortex.

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