Title
Extraction and encoding of spatial relationships in the retrosplenial cortex

Permalink
https://escholarship.org/uc/item/6k32451f

Author
Alexander, Andrew

Publication Date
2017

Peer reviewed|Thesis/dissertation
Extraction and encoding of spatial relationships in the retrosplenial cortex

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy

in

Cognitive Science

by

Andrew Spencer Alexander

Committee in charge:

Professor Douglas A. Nitz, Chair
Professor Andrea A. Chiba
Professor Virginia R. De Sa
Professor Stefan Leutgeb
Professor Lara M. Rangel

2017
The Dissertation of Andrew Spencer Alexander is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2017
DEDICATION

This dissertation is dedicated to Jessica Priestley and my parents, Liz and Dean. Thank you for your support.
# TABLE OF CONTENTS

SIGNATURE PAGE........................................................................................................ iii
DEDICATION................................................................................................................ iv
TABLE OF CONTENTS.................................................................................................... v
LIST OF FIGURES......................................................................................................... vi
LIST OF TABLES........................................................................................................... vii
LIST OF ABBREVIATIONS........................................................................................... ix
ACKNOWLEDGEMENTS............................................................................................... x
VITA................................................................................................................................. xi
ABSTRACT OF THE DISSERTATION........................................................................... xiii

CHAPTER 1: Neural spatial representations, transformations, and the role of the retrosplenial cortex .................................................................................................................. 1

CHAPTER 2: Retrosplenial cortex maps the conjunction of internal and external spaces................................................................................................................................. 17

CHAPTER 3: Spatially periodic activation patterns of the retrosplenial cortex encode route sub-spaces and track distance ................................................................. 67

CHAPTER 4: Neurophysiological signatures of interaction between retrosplenial cortex and the hippocampal formation................................................................. 107

CHAPTER 5: Spatial computations of the retrosplenial cortex................................. 136
LIST OF FIGURES

Figure 2.1: Framework for assessing the contribution of distinct spatial frames of reference

Figure 2.2: RSC neurons map position in egocentric space

Figure 2.3: RSC neurons map position in route-centered space

Figure 2.4: RSC activity is sensitive to allocentric positioning of the track

Figure 2.5: Sensitivity to distinct reference frames in simultaneously recorded RSC neurons

Figure 2.6: RSC ensemble activity patterns are sensitive to allocentric positioning of the track

Figure S2.1: Recording room setup and track layout

Figure S2.2: Histology and tetrode placements

Figure S2.3: Sort quality metrics and quantification

Figure S2.4: Reliability in turn-related activity

Figure S2.5: Trial-by-trial reliability of RSC neuron activation is significantly greater than chance

Figure S2.6: RSC firing rate activity is not sensitive to trial-by-trial fluctuation in angular velocity

Figure S2.7: RSC firing rate activity is not sensitive to trial-by-trial fluctuation in linear velocity

Figure S2.8: Schematic of ensemble correlative reconstruction method

Figure S2.9: RSC neurons exhibit head-direction sensitivity

Figure S2.10: RSC neurons exhibit conjunctive encoding of position in multiple reference frames

Figure S2.11: Reconstruction of route position across track placements for RLR route

Figure 3.1: RSC encodes progression through a recurrent closed track

Figure 3.2: Spatial GLM shows RSC neurons exhibit spatial periodicity on track traversals
Figure 3.3: RSC neurons exhibit spatial periodicity biased to fourth, half, and full spatial scales.

Figure 3.4: RSC activation patterns are anchored to allocentric space.

Figure 3.5: RSC neurons exhibit periodic activation patterns on ring shaped track.

Figure 3.6: Full route spatial periodicity yields metric of distance traveled from a point within the full trajectory.

Figure S3.1: Representative histology, sort quality, and head-direction assessment.

Figure S3.2: Description of movement variables utilized in egocentric GLM during plus track running.

Figure S3.3: RSC neurons exhibit conjunctive sensitivity to multiple sub-route and full route spatial oscillations.

Figure S3.4: Description of movement variables utilized in egocentric GLM during ring track running.

Figure 4.1. Behavioral paradigms, electrode placements, and identification of relevant oscillatory signals in local field potentials.

Figure 4.2. RSC neurons phase lock to HPC and RSC theta oscillations independent of intrinsic theta rhythmicity.

Figure 4.3. RSC neurons are phase locked to high frequency oscillations in RSC local field potential but not in HPC local field potential.

Figure 4.4. HPC SWR and RSC high frequency oscillations co-occur.

Figure 4.5. HPC SWR modulates RSC single unit activity.
LIST OF TABLES

Table S2.1: Breakdown of recorded RSC neurons by sub-region, rat, and sensitivity to spatial frames of reference

66
LIST OF ABBREVIATIONS

RSC: Retrosplenial cortex
HPC: Hippocampus
CA1: Cornu Ammonis 1
PPC: Posterior parietal cortex
MEC: Medial entorhinal cortex
PoS: postsubiculum
LFP: Local field potential
SWR: Sharp wave ripple
cGLM: complete generalized linear model
pGLM: partial generalized linear model
eGLM: egocentric generalized linear model
SP: Symmetry point
ACKNOWLEDGEMENTS

Many thanks to Dr. Doug Nitz for being such an incredible advisor to me. He has been absolutely instrumental to all of the work composing this dissertation and my development as a scientist. Thank you also to Dr. Andrea Chiba for her guidance for nearly the last decade. Dr. Chiba invited me into her lab as an undergraduate and has nurtured my scientific maturation since day one. I would also like to thank several members of my scientific family for their friendship and advice, including: Dr. Lara Rangel, Dr. Laleh Quinn, Dr. Victor Minces, Dr. Anita Disney, Dr. Janet Wiles, and Dr. Jeffrey Krichmar. Thank you also to committee members Dr. Virginia de Sa and Dr. Stefan Leutgeb. Finally, thanks to my labmates Laura Shelley, Jake Olson, David Tingley, Belinda La, as well as a number of brilliant and motivated research assistants.

Chapter 2, in full, is a reprint of the material in the following published manuscript: Alexander, A.S., & Nitz D.A., (2015). *Retrosplenial cortex maps the conjunction of internal and external spaces*. Nature Neuroscience., 18(8), 1143-1151. The dissertation author was the primary investigator and author of this paper.

Chapter 3, in full, is a reprint of the material as it appears in the following manuscript that is currently being prepared for submission for publication: Alexander, A.S., & Nitz, D.A. *Retrosplenial cortex periodic activation patterns encode route subspace relationships and yield a metric of distance*. The dissertation author was the primary investigator and author of this paper.

Chapter 4, in full, is a reprint of the material as it appears in the following manuscript that is currently being prepared for submission for publication: Alexander, A.S., & Nitz, D.A. *Neurophysiological signatures of interaction between retrosplenial cortex and the hippocampal formation*. The dissertation author was the primary investigator and author of this paper.
VITA

2009 Bachelor of Science, Cognitive Science, University of California, San Diego

2009-2011 Research Associate, University of California, San Diego

2011-2016 Teaching Assistant, Department of Cognitive Science, University of California, San Diego

2011-2016 National Science Foundation Graduate Research Fellow

2014-2015 Kavli Institute for Brain and Mind Innovative Research Grant Principal Investigator

2015-2016 Instructor, Department of Cognitive Science, University of California, San Diego

2017 Doctor of Philosophy, Cognitive Science, University of California San Diego

PUBLICATIONS


ABSTRACT OF THE DISSERTATION

Extraction and encoding of spatial relationships in the retrosplenial cortex

by

Andrew Spencer Alexander

Doctor of Philosophy in Cognitive Science

University of California, San Diego, 2017

Professor Douglas A. Nitz, Chair

Complex behavior demands interactions between an animal and its immediate environment. Spatial relationships are a critical component of nearly all such interactions. Accordingly, there exist distinct neural systems that construct and store representations of space. To effectively generate and utilize these spatial mappings, coordination must occur between cortical regions important for sensory processing and sub-cortical spatial systems, such as the hippocampus (HPC). The retrosplenial cortex
(RSC) is a candidate structure to facilitate this coordination process, as it is anatomically positioned between the HPC formation and neocortex.

To investigate the role of RSC in spatial cognition, in vivo electrophysiological recordings were performed in rats during spatial navigation tasks. First, the spatial reference frames that influence RSC neuron firing were assessed in rats running routes placed at different environmental locations. RSC neurons encoded conjunctions between the animal’s position in the environment, position within a trajectory, and action state. Conjunctive sensitivity to multiple spatial variables suggested the region could interrelate representations encoded in distinct coordinate systems. In a second experiment, rats traversed a route with recurrent structure. Individual RSC neurons exhibited periodic activation patterns on the route that repeated across analogous route segments. Simultaneously, RSC ensembles defined a framework for the encoding of route segment positions relative to the whole. The nature of the firing activity provided a novel metric of distance from each route position to all others. Finally, neurophysiological signatures of interaction between RSC and HPC were identified. RSC neurons exhibited rhythmic spiking activity at temporal frequencies observed in HPC. These neurons, as well as those without rhythmic firing, could be temporally synchronized with synaptic inputs to the HPC. Additional interregional population interactions were observed during transient excitatory events in the HPC.

This work elucidates unique functions of RSC in transforming between spatial coordinate systems and parcellating complex space into sub-components. The experiments also identify the existence of novel symmetrical spatial activation patterns that construct a distributed distance code of the animal’s position from fixed points within a linear environment. Collectively, results from these studies indicate that RSC is a
powerful hippocampal-cortical conduit capable of important computations relevant to spatial cognition.
CHAPTER 1: Neural spatial representations, transformations, and the role of the Retrosplenial Cortex

Introduction to neural spatial representations

Virtually all behavior involves a complex interaction between an agent and the immediate environment. For most animals, survival depends on successful execution of goal-driven action plans that are dependent on either internal (e.g. hunger) or external (e.g. escape a predator) forces. The most evolutionarily fit agents learn to optimize their behavior as a function of the contingencies present within a given context and environment. Higher-level cognitive operations, such as attention, learning, and memory, emerged as a consequence of this optimization, as nature developed neural systems with the computational capability of associating locations and encounters in the external world with behaviorally-relevant outcomes\(^1\).

From this perspective, it is of perhaps no surprise that the brain possesses multiple neural systems that function to construct and store spatial representations of known environments. At the single cell level, these mappings take the form of neurons that have spatial receptive fields, wherein the cell exhibits action potential discharge when some combination of spatial relationships between the animal and environment are satisfied. Populations of neurons with distinct spatial receptive fields spanning the entire input space construct a distributed code of all locations, and thus, a spatial map.

These maps are thought to form the basis for intelligent spatial awareness while simultaneously constructing a framework in which behaviorally significant memories can be seated. Since the discovery of the first stored neural cognitive map in the early 1970s\(^2\), much research has been devoted to elucidating the modes by which these representations are constructed and the spatial and behavioral variables that modulate their structure. This field of research has elucidated much about the nature of spatial...
representation in neural systems, yet significant questions remain regarding the mechanisms for manipulation, interrelation, and cognitive organization of spatial mappings for flexible use and updating.

*Neural representations of space are encoded in distinct coordinate systems*

When examining space and corresponding neural representations of space, it is critical to consider the coordinate system or spatial frame of reference by which location is defined. There are multiple brain regions that map an animal's location relative to properties of the external world. This coordinate system is often referred to as the allocentric spatial reference frame. In the HPC, pyramidal cells termed ‘place cells’ typically exhibit activation when the animal occupies a distinct coordinate relative to fixed landmarks in the environment. Grid cells of the medial entorhinal cortex (MEC) form a second allocentric representation, in that this cell type has repeated activation at fixed intervals spanning the entirety of an environment. MEC also possesses neurons whose spatial receptive fields fall along the borders of an environment. An additional fundamental form of allocentric mapping is of the animal's head-orientation in an environment. Single neurons found in multiple regions, including the postsubiculum (PoS), anterodorsal thalamic nucleus (ADN), and lateral mammillary nucleus (LMN), exhibit increased activation when the animals head orientation occupies a specific range of angles relative to the constellation of distal cues. Experimentally, these representations are shown to be anchored to the external world by demonstrating that the spatial maps rotate proportionally with the rotation of external cues.

The brain also represents location in a route-centered frame of reference, in which locations are mapped within known routes through the environment. In the rodent brain, route encoding is primarily found in the posterior parietal cortex (PPC). PPC neurons exhibit complex, but reliable, patterns of activation during track running in
familiar environments. Under these conditions, PPC neurons generate a distinct population code for each position within the trajectory and the animal's progression through the route can be accurately decoded on the order of centimeters. Route mappings in PPC will remain unchanged when the same route is rotated or translated in allocentric space, demonstrating that the representation is indeed anchored to positions defined by the shape of the trajectory itself. The shape of the route is a key component to this representation type, and the nature of route-encoding shares components with object-centered spatial representations observed in the parietal cortex of primates.

Critically, all information utilized to construct representations of allocentric or route-centered spaces enters the brain via sensory systems. All sensory systems possess mechanisms for transducing the properties of physical stimuli in the world into distinct firing rate codes. The location of the external stimulus relative to the animal is encoded by the positioning of activated sensory receptors. For example, activation of photoreceptors near the fovea would typically correspond to the presence of a visual stimulus directly in front of the animal, whereas activation on the periphery of the retina might indicate a visual object to the side of the animal. This form of spatial information is encoded in egocentric coordinates, or as locations of stimuli in the external world relative to the animal's own body. As a consequence, the aforementioned allocentric and route-centric maps of the external world are in actuality abstractions of sequences of sensory information.

Unresolved spatial computations: Neural mechanisms for map transformations and interrelationships

Although much is known about different forms of internal and external spatial representations, there remain prominent gaps in knowledge of spatial systems.
Specifically, it is unclear which brain regions and underlying neural mechanisms are responsible for: 1) spatial transformations for the interrelation of positions both across and within spatial maps anchored in distinct reference frames, 2) modes of compartmentalization of linear or two-dimensional environments, 3) detection of spatial hierarchies for the organization and relation of distinct spatial maps, and 4) the continuous integration of distance traveled as a function of position in the environment.

Little is known as to how spatial representations of the external environment are generated from information that enters the brain in an egocentric coordinate system. This process would involve a computation that transforms spatial information encoded in one coordinate system (e.g. egocentric) into positions relative to another coordinate system (e.g. allocentric). For example, visual information about salient landmarks that define allocentric space is first processed in the brain via retinotopic coordinates, yet transformed into an abstract spatial representation of the locations of visual objects relative to each other. Several studies have examined a potential function of parietal cortex sub-regions in transforming sensory information (i.e. visual, auditory, tactile information) into motor actions\textsuperscript{15-17}, but there has been little exploration of the neural mechanisms important for converting the same types of information into stored spatial representations.

A second unresolved question is how the brain parses space into fragmentations that are useful for motor-planning, organizing spatial relationships, and orienting spaces relative to each other\textsuperscript{18}. In many cases, animals are able to break space into sub-components on the basis of features of the environment. In San Diego for example, the segmentation of the Hillcrest and North Park neighborhoods is defined by Florida Canyon, which runs north to south through the region. However, landmarks that define logical separations of space are not always available, yet abstract fragmentations of
space can still be generated quite readily. For instance, environments can be broken down into quadrants or other roughly proportional fragmentations. This process can apply to even more abstract spaces, like routes between locations. During movement between starting and ending points, humans often track distance from their goal in fragments (e.g. “halfway there”). Further, complex navigational problems are parceled into simpler sequences of sub-routes. In fact, the common act of providing directions always involves the fragmentation of a complex route into a sequence of sub-routes. Activation patterns from neurons in the MEC and HPC have been shown to fragment complex spaces and routes\textsuperscript{19,20}, but the neural mechanisms underlying this spatial computation are still being hashed out.

The modes by which the brain combines or interrelates multiple external spatial representations to construct coherent maps of the environment are also not well understood. Ground dwelling animals are afforded limited viewpoints at any moment, but still are capable of generating rich and complex understandings of large spatial environments. Several computational theories have been proposed to compute map relationships and integrations, yet no neural mechanism has been discovered at this time. One major component of the process might be the identification of spatial hierarchies\textsuperscript{21,22}. For example, a sufficient understanding of the structure of a city requires knowledge that groupings of city blocks compose individual neighborhoods and that the collective arrangement of neighborhoods compose the city. These types of known spatial hierarchies can serve as a useful organizational tool for mapping spatial relationships, yet the neural systems capable of detecting and storing patterns relevant to hierarchical relationships are not well understood.

Finally, the construction of coherent spatial representations requires an accurate mapping of the geometric structure of an environment. Knowledge of the distances
between boundaries, landmarks, and other salient features of the environment is of particular importance to this process. Neurons receiving input from MEC grid cells could potentially track the animal’s distance from fixed points in the world by integrating activation across time\textsuperscript{23}, but recent work has demonstrated that grid maps are distorted by alterations of environmental boundaries\textsuperscript{24,25}. This distortion would subsequently disrupt the reliability of distance information present within this circuit. Thus, it remains to be seen if there are alternative mechanisms to track distance within the brain.

Many of these spatial computations require access to sensory information encoded in cortex, allocentric spatial representations encoded by sub-cortical regions, and higher level cognitive processes often relegated to frontal cortices. As such, flexible use of spatial representations involves a distributed system of structures that encode vastly different forms of information. This network likely requires coordination for effective use. A candidate structure in this coordination process would likely be reciprocally connected to sensory and frontal cortices, as well as the hippocampal formation and associated structures. A consideration of these properties suggests that the retrosplenial cortex (RSC) is potentially best situated to transform, combine, and fragment spatial representations and direct information flow between the cortex and HPC.

*Retrosplenial cortex as a hippocampal-cortical processing hub*

In primates and rats, RSC is anatomically positioned as a centralized processing hub between cortical regions important for executive function and sensory processing, and sub-cortical spatial systems. The rat RSC is often subdivided into four highly interconnected sub-regions: dysgranular (Rd), granular c (Rgc), granular b (Rgb), and granular a (Rga)\textsuperscript{26}. The structure is vast, spanning several millimeters along the rostro-caudal axis and lies along the midline of the brain bounded laterally by parietal cortices.
Rat RSC is positioned along the dorsal surface of the brain and is ventrally bordered by subiculum and superior colliculus.

RSC is reciprocally connected with a diversity of cortical regions. Multiple RSC sub-regions receive prominent inputs from the rat cortical equivalent to visual region V2 in humans\textsuperscript{27-30}. All RSC sub-regions have direct and indirect projections with parietal cortex, which likely transmits multiple forms of sensory information including visual, motor efference, optic flow, and proprioceptive signals\textsuperscript{27-31}. The structure also forms afferent and efferent pathways with dorsolateral prefrontal cortex and anterior cingulate cortices known to be critical for executive function\textsuperscript{27-30}.

RSC has a unique pattern of connectivity with the HPC formation. RSC is reciprocally connected with thalamic structures that also directly innervate the HPC\textsuperscript{32}. As such, there are indirect projections by which RSC may modulate thalamic inputs to the HPC. RSC efferents reach both lateral (LEC) and medial entorhinal cortices which together form the primary input to all HPC sub-regions\textsuperscript{27-32}. Of particular interest, caudal RSC sub-regions form a prominent excitatory projection onto layer V of MEC wherein grid and head-direction modulated grid cells have been observed\textsuperscript{33}. RSC is also strongly interconnected with the primary output structures of the HPC including: subiculum, presubiculum, and postsubiculum\textsuperscript{27-32}. These regions, in addition to thalamic nuclei, form a strong head direction signal into RSC. Given these projection patterns, RSC forms a potentially significant feedback loop with the HPC formation. This system might provide the framework by which cortical sensory and executive processes are combined with spatial and mnemonic functions supported by the HPC.

\textit{Known RSC involvement in spatial cognition}

Despite this interesting anatomy, few studies have examined potential spatial correlates of RSC activation in rats. The most well described subset of RSC neurons
exhibit allocentric head-direction sensitivity. Cho and Sharp (2001) also described a subset of RSC neurons that were slightly modulated by angular velocity and an additional population of neurons that exhibited “place field” like responses that were head-direction sensitive. Interestingly, it has recently been reported that dysgranular RSC neurons exhibit bimodal head direction tuning in an environment with sparse visual cues that are rotationally symmetric. This finding may indicate a sub-population of neurons that are modulated by the animal’s heading orientation with respect to local cues rather than the full allocentric space.

In 2012, Smith and colleagues described RSC neurons that exhibited spatial response properties similar to place fields found in the HPC. These fields were commonly found at track locations in which the animal was navigating a turn, and thus, were potentially conflated with movements in egocentric space rather than locations with respect to the allocentric position of the animal. The same experiment reported that RSC neurons developed context-specific reward responses in which the neuron fired for reward at a particular location on the maze. Recent work by Vedder et al. utilized a cued navigation task and observed that neurons within the region distinguished cue and reward locations and exhibited prospective encoding of the animal’s future trajectory.

Damage to RSC further supports its significant contribution to spatial cognition.

Most notably, RSC lesions produce impairments in navigation. Humans with damage to the region cannot identify the spatial relationships between known distal landmarks adequately enough to construct routes between them. The profile of detriment following damage to the region is often referred to as ‘topological disorientation’ or ‘heading disorientation,’ and indicates that RSC could be important for integrating across spatial coordinate systems to anchor navigation plans within allocentric space. Damage to RSC in humans is also associated with disorders of mnemonic function. Accordingly,
RSC is one of the first regions to degrade in Alzheimer’s disease which is commonly associated with spatial disorientation and severe memory impairments.\textsuperscript{42,43}

In rats, lesion and inactivation work further supports that RSC plays a significant role in processing distal cues that define allocentric space for use in spatial learning.\textsuperscript{44-49} During RSC inactivation or lesion, many experiments report that rats switch to an egocentric-based strategy wherein a set sequence of actions are executed as a heuristic for the animal to adequately perform the spatial task. However, this strategy is severely decremented when the task arena is rotated to disrupt the structure of local cues, confounding claims that this strategy is solely based on intact egocentric processing following RSC inactivation. Instead, these results may indicate a role for RSC in relating egocentric information to spaces defined by both local and distal cues. In accordance with these behavioral impairments, a decreased reliability of spatial tuning is observed for both place cells and head direction cells following RSC inactivation.\textsuperscript{50,51}

Further, a number of experiments indicate that RSC is important for a navigational strategy called “path integration,” in which animals integrate self-motion information across time to estimate their position within the environment. This hypothesis is supported by the fact that RSC lesioned animals can navigate to spatial locations in the light, with access to visual cue information, but cannot do so in the dark in which reliance on integrated self-motion information would be required in the absence of other navigational cues.\textsuperscript{44,52,53} Disrupted path integration following RSC inactivation reveals that the region may track egocentric self-motion information, such as actions and distance traveled.

Several fMRI experiments support a role for RSC in encoding locations of objects or visual landmarks with respect to the external environment, a potentially crucial piece of information for the construction of allocentric maps. RSC BOLD activity is increased
when subjects reported changes in the known location of a target with respect to the boundaries of a virtual environment\textsuperscript{54}. Auger and Maguire also found that intensity of RSC BOLD activity in "good navigators" could be utilized to discriminate the presence of permanent spatial landmarks in an array of visual objects\textsuperscript{55}.

Human imaging work also implicates the region in various navigation processes. Sherrill et al. found increased RSC recruitment when subjects navigated a virtual map from a first person (egocentric) perspective\textsuperscript{56}. The same study found increased RSC activation when individuals surveyed a map and then successfully navigated between goal locations\textsuperscript{56}. This result supports a potential function of the region in encoding the allocentric spatial relationship between two locations (start and endpoints) and translating that information into a series of egocentric actions for future use. In related work, Chrastil et al. demonstrated that RSC activity could be utilized to accurately track a subject's Euclidian distance within a trajectory\textsuperscript{57}. Together, these findings implicate RSC in both coordinate transformations for generation of spatial mappings and encoding of self-motion necessary for path integration processes.

Human RSC also encodes allocentric heading direction and local viewpoints in humans. Shine et al. utilized fMRI as humans navigated a virtual environment and demonstrated fMRI correlates that tracked the allocentric heading direction of the subject\textsuperscript{58}. In related work from the Epstein lab, several clever virtual navigation paradigms have demonstrated that human RSC computes heading direction in local environments in a manner invariant to heading in the broader allocentric space\textsuperscript{59}. These findings are related to the aforementioned bimodal tuning properties reported in rats traversing rotationally symmetric environments. Consequently, RSC has the requisite activation to detect potential offsets between current heading direction and local distal cues to potentially align downstream spatial representations.
Organization of the dissertation

RSC projection patterns and spatial responsivity suggest it is primed to perform unique computations on spatial representations. The dissertation work includes three experiments designed to address potential functions of RSC in transforming spatial representations across reference frames, fragmenting complex spaces into organized sub-components, and tracking distance information for path integration and allocentric map construction. The dissertation also provides evidence of functional connectivity between the RSC and HPC.

Chapter 2 presents an experiment designed to systematically manipulate and register the animal’s position within multiple spatial coordinate systems simultaneously. The results indicate that RSC possesses a population of neurons having spatially-specific activity conjunctively sensitive to allocentric, route-centric, and egocentric frames of reference. The findings identify the RSC as a key structure in mediating transformations across distinct spatial coordinate systems.

Chapter 3 further tests the regions spatial functionality by examining activation patterns of RSC neurons while rats traverse a route with recurrent structure and embedded spatial hierarchies. Here, firing properties of RSC neurons demonstrate a capability of the region in identifying and organizing sub-route relationships. Additionally, the spatial structure of RSC patterns could be decoded to track the animals route distance from fixed points within the track.

To assess the functional connectivity between RSC and downstream spatial representations, Chapter 4 examines potential physiological signatures of RSC-HPC interaction by examining single unit responses with respect to synchronous synaptic currents recorded in the local field potential. Synchronization between the regions was manifested as phase-dependent firing of RSC neurons to the HPC theta oscillation, an
intrinsic rhythm reflecting synaptic inputs within HPC. With respect to HPC output, this work revealed that a sub-population of RSC units are modulated during HPC sharp wave ripple (SWR) events, short duration excitatory field potential oscillations thought to reflect cortico-hippocampal communication.

References


Chapter 2: Retrosplenial cortex maps the conjunction of internal and external spaces

Abstract

Intelligent behavior demands not only multiple forms of spatial representation, but also coordination among the brain regions mediating those representations. Retrosplenial cortex is densely interconnected with the majority of cortical and subcortical brain structures that register an animal's position in multiple internal and external spatial frames of reference. This unique anatomy suggests that it functions to integrate distinct forms of spatial information and provides an interface for transformations between them. Evidence for this was found in rats traversing two different routes placed at different environmental locations. Retrosplenial ensembles robustly encoded conjunctions of progress through the current route, position within the larger environment, and the left versus right turning behavior of the animal. Thus, the retrosplenial cortex has the requisite dynamics to serve as an intermediary between brain regions generating different forms of spatial mapping, a result consistent with navigational and episodic memory impairments following damage to this region in humans.

Introduction

Animals navigating through space simultaneously occupy positions with respect to at least two external reference frames: 1) the boundaries of the broader environment, often referred to as the allocentric space; and 2) positions within planned routes, the spaces of which are defined by sequences of actions and the distances between them. The animal must also process spatial information registered within internal reference frames (also referred to as egocentric frames), defined, in the context of navigation, by combinations of motor and sensory correlates associated with specific actions.
The brain registers the animal’s position both in the world (allocentric) and in the space of any route taken through the world (route-centered). In the hippocampus (HPC), environmental locations are encoded by ‘place cells’, which exhibit action potential discharge across specific regions relative to distal cues defining the environment’s boundaries\(^1,2\). A second form of allocentric mapping exists in the medial entorhinal cortex (MEC) where ‘grid cells’ are activated whenever an animal occupies a vertex within a repeating grid of tessellated triangles that spans the entirety of the environment\(^3\). Both regions are considered core structures for mapping space within an allocentric reference frame, and are strongly implicated in effective navigation and spatial memory.

Neurons with route-centered reference frames are found in the posterior parietal cortex (PPC) of rats\(^4-6\). Critically, PPC activity patterns discriminate route positions sharing the same actions and headings and, in addition, are ‘portable’, in that they persist when the route is moved to different locations in allocentric space\(^7\). In addition, PPC ‘route-cell’ firing patterns are scale-independent, compressing or expanding in size as any given path shape is contracted or expanded\(^4,5\). Finally, both primate and rodent studies have demonstrated that PPC can register position in multiple egocentric and external spaces simultaneously\(^8,5,9\).

The internal, or egocentric reference frame, is also registered by a subset of PPC neurons whose activity peaks are closely tied to specific left-turning, right-turning, and straight-running behaviors\(^10,4,6\). It is presently unknown whether such firing correlates represent responses to turn-related dynamics in optic flow, vestibular responses, proprioceptive responses, or motor efference copy.

Thus, a great deal is known concerning how positions in distinct reference frames are mapped by specific neural activity patterns. However, multiple forms of
spatial representation are relevant to the problem of navigation and a neural substrate capable of unifying them has yet to be identified. Several lines of research indicate that retrosplenial cortex (RSC) is a prime candidate for mediation of such a process. First, RSC has reciprocal connections to multiple brain regions that map space in different reference frames and/or by different forms including: PPC, HPC, MEC, subiculum, anterior cingulate, and anterior thalamic nuclei. Second, humans with RSC damage cannot identify the spatial relationships between distal landmarks adequately enough to construct routes between them. Third, lesion and inactivation work in rats indicates that RSC plays a significant role in processing distal cues that define allocentric space and suggests that RSC serves to integrate self-motion information across time (i.e. participates in path integration processes). Finally, computational modeling efforts predict a role for RSC in relating egocentrically-based views of the world to the allocentric positions and head orientations with which they are associated.

The known properties of RSC neuron firing dynamics in behaving animals are also consistent with this contention. For example, a subpopulation of RSC neurons exhibits head-direction (HD) tuning preferences. Other RSC cells appear to be modulated by the spatial location of reward obtainment or locomotor variables such as angular and linear velocity.

Nevertheless, no study has, thus far, taken on the problem of defining the reference frame, or frames, to which RSC activity is anchored. This gap in knowledge precludes an organized assessment of RSC’s potential role in interfacing distinct forms of spatial representation. Addressing this issue is critical at this time, as few unit-recording studies have been conducted within the region despite its significant connectivity to nearly all structures considered to map position in the world for the purposes of navigation and memory.
Thus, the present experiments were designed to systematically manipulate and register the animal’s position within multiple frames of reference. In contrast to the HPC, we find that the RSC is unique in possessing a population of neurons having spatially-specific activity simultaneously sensitive to allocentric, route-centric, and egocentric frames of reference. The findings identify the RSC as a key structure mediating relationships among the multiple forms of spatial information relevant to fluid, intelligent movement through the environment.

Results

To systematically explore the relationship between RSC activity and two distinct external spatial frames of reference (allocentric and route-centric), animals were tracked as they traversed two routes along a track that, for different trial blocks, was placed at one of two different positions (termed location α and location ß) within the space of the environment (Figure 2.1A, upper left). Fixed and easily observable distal visual cues (track walls < 2cm, Fig. S2.1) provided consistent spatial relationships that defined the boundaries of the recording room, here termed the allocentric space. Within this allocentric frame of reference, all positions along the track, at both the α and ß locations, occupy distinct spaces (Figure 2.1A, lower right).

Shuttling between reward locations at the track ends required the animal to fluidly execute left-right-left (LRL) and right-left-right (RLR) turn sequences. The turns and the straight running sections between them define distinct routes through the environment. In these route-based frames of reference, positions along both the LRL and RLR routes remain fixed relative to each other, regardless of the location of the track in the broader environment (i.e. regardless of the allocentric space). As depicted in Figure 2.1A (lower left), the first and second left turns of the LRL route are similarly spaced at both track location α and ß.
The track shape forced the animal to make a total of three leftward or rightward changes from its current heading. Leftward versus rightward heading changes are, of course, associated with distinct patterns of muscle activity, opposing responses of the vestibular system, opposing directions of optic flow, and different views to the local geometry of the track (e.g., presence of a track corner in the left versus right field of view). Each of these sensory and motor correlates differing between turn types are registered relative to the animal itself. Thus, left versus right turning, irrespective of the position in the route or broader environment, differs with respect to egocentric, or internal, frames of reference (Figure 2.1A, upper right).

To examine all three frames of reference as determinants of RSC activity, it was important to always know not only where the animal was, but also what behavior (i.e., action) was being executed. For this reason, analyses focused on trials wherein route traversals were made in an uninterrupted fashion (Figure 2.1B). Under these conditions, we recorded 133 and 95 RSC neurons (228 total) from the left and right hemispheres, respectively, across 6 rats.

Neurons were primarily recorded within the dysgranular, granular c, and granular b sub-regions of RSC. Supplemental table S2.1 presents, for each of these RSC sub-regions, the associated number of neurons having the specific spatial firing properties we consider in the results sections that follow. In brief, all forms of RSC firing that we describe were relatively distributed. For comparison, we also recorded 109 HPC sub-field CA1 neurons in the same animals. Endpoints of each RSC wire bundle (sets of 4 tetrodes) are shown in Figure 2.1C, with each line reflecting the dorsal to ventral space from which the neurons were recorded (complete histological data and example spike waveform data are given in Fig. S2.2 and S2.3, respectively).
Figure 2.1. Framework for assessing the contribution of distinct spatial frames of reference. A. Track-running task and definitions of spatial reference frames. Upper left panel: Animals executed left-right-left (LRL) and right-left-right (RLR) trajectories on a “W” shaped track at two distinct locations (α and β) in the recording room. The four panels describe the three frames of reference associated with track running along with depiction of hypothetical firing patterns for neurons sensitive to each frame. Upper right: In egocentric frames of reference, left or right turning actions produce contrasts in egocentric information. Consistent firing responses across most or all instances (green circles for LRL path) of left or right turning indicate sensitivity to egocentric, or internal, reference frames. Lower left: The route-based frame of reference. Here, the ordering of left and right turns and the spaces between them define a route-based (or trajectory-based) frame of reference. Track locations associated with left turns on the LRL trajectory (blue and yellow circles), occupy different positions in this frame and these relationships are preserved regardless of track location in the room. Firing activity will discriminate such positions. Lower right: the allocentric frame of reference. Track locations α and β occupy different positions within the allocentric space defined by environmental boundaries. All left turns (blue, yellow, purple, and green circles) occupy different positions and neurons with sensitivity to this frame will discriminate them. B. Tracking data (black traces) for all runs at one track position. Example interrupted (red arrow indicates stall) and uninterrupted, or ballistic, traversals (thick black and grey traces) are also shown. Only ballistic traversals were analyzed (examples in light blue). Positions along these (light blue traces) were fit to templates (dashed black line) to create positional firing rate profiles. Bottom trace: Example mean linearized firing rate profile (± SE). C. Electrode placement and recording ranges in RSC for six rats (KB3-8). Filled circles indicate final electrode placement and lines depict dorsal-ventral recording depths across recordings.
Figure 2.2. RSC neurons map position within egocentric space. **A.** For both α and β track locations, mean firing rate profiles (thick blue and black traces) and single-trial rate profiles (light blue and black traces) are shown for a neuron with rate increases at left versus right turns. **B.** A neuron that exhibited significantly greater activity at right versus left turns. **C.** Left and middle panels: Average firing rates across each of the six different left turn (1st six columns) and right turn spaces (2nd six columns). The two panels correspond to the neurons of 2A,2B. Dashed red lines depict mean overall left-turn and right-turn rates. The ratio of the larger to the smaller and coefficient of variation for the larger are given below. Right panels: Ratios for all RSC neurons having significant left versus right turn rate differences. Blue traces are percentage of RSC neurons (Y axes) having left versus right firing ratios of different magnitudes (X axes). Red traces depict mean ratios (± SD) for 25 instances of left/right turn identity randomization.
RSC neurons map position within egocentric space

Consistent with RSC neuron sensitivity to egocentric, or internal, frames of reference, a large proportion of RSC neurons exhibited significant differences in firing when comparing activity at all possible left versus right turns without regard to turn locations. An example firing rate pattern for a left-turn sensitive neuron is given in Figure 2.2A. Turn-related bias was quantified by comparing integrated mean firing activity at all left versus all right turn sites. Figure 2.2B depicts the positional firing rate profile for a right-turn sensitive neuron on the RLR route for both track locations. The integration window was chosen to approximate the typical length of an adult rat (without tail) on either side of each turn’s apex such that neurons having activity peaks as the animal begins or completes a turn would be detected. Mean firing rates across trials for each of the possible left and right turns for the neurons of Figures 2.2A and 2.2B are given in Figure 2.2C (left and middle panels) alongside the ratio between mean rate for the preferred turn type and the non-preferred type. Across the full population, 22% of RSC cells had turn-bias ratios greater than 2, and 45.6% of RSC neurons exhibited significant differences in overall left-turn versus right-turn firing rates (Wilcoxon rank-sum test, n=104/228 at p<0.05; see Figure 2.2C-right panel). Of this subset, 75% (n=78/104) also had turn-bias ratios significantly greater than expected by chance as determined by multiple randomizations of left/right turn identities (one-tailed Wilcoxon sign rank test with α at 0.05). The full distribution of turn-bias ratios for all turn-related RSC cells was significantly greater than the distribution of ratios calculated by randomizing turn identity (Figure 2.2C, right panel; 25/25 iterations, one-tailed Kolmogorov-Smirnoff, n = 104, D > 0.4, p<0.0001). For the same subset, the coefficient of variation (CV) of mean firing activity across all six instances of the preferred turn type was low (Fig. S2.4, top panels, n = 104, mean = 0.52 ± 0.33) and statistically lower than CVs calculated from
randomizations of the same left/right turn firing rate data (n = 104, mean = 0.73 ± 0.24, one-tailed Wilcoxon rank-sum test, p = 6.55x10^{-11}). Furthermore, 21 of the 104 neurons exhibiting left turn versus right turn rate differences exhibited no statistically significant differences in firing across the 6 spatially-distributed instances of the preferred turn type (Kruskal-Wallis test; 54 < n_{turn} < 162; 0.75 < \chi^2(5) < 10.86; 0.05 < p < 0.98) suggesting that a subset of RSC neurons may encode egocentric space rather exclusively. Together, the findings evidence high sensitivity and reliability of rate differences associated with left versus right turning behavior in a sub-population of RSC neurons. Therefore, RSC neurons robustly encode the current actions associated with movement through the environment.

**RSC neurons map position in route-centered space**

We next examined the possibility that RSC neurons could simultaneously map position in route-centered space. Neural activity mapping this frame of reference has been demonstrated in PPC where it takes two main forms: 1) neurons exhibiting complex but reliable changes in activity across the space of a route; and, 2) neurons with action related activity correlates that exhibit significant differences in firing intensity at different action sites (e.g., two right turns) within the route. Neurons with either of these firing characteristics have been used as the primary evidence of route specific firing for PPC cells\textsuperscript{4,7}.

We first defined the population of neurons that did not exhibit action specificity (i.e., left versus right turn rate differences), but that did maintain odd versus even trial consistency in activation pattern along the space of the route. This first step was intended to exclude the contribution of turn-selective neurons whose similarity in firing patterns across track placements might be conflated with their action correlate.
Figure 2.3. RSC neurons map position in route-centered space. A. A sub-population of RSC neurons did not exhibit left versus right turn rate differences (gray, see also 3B). RSC neurons that did exhibit left/right turn rate differences could be split into two groups: 1) those that also exhibit rate differences for the preferred turn type depending on route position (dark blue, see also 3C), and, 2) those that exhibit similar rates for preferred turns at different route positions (light blue, see also 3D). B. LRL and RLR mean rate (± SE) profiles for an RSC neuron without left/right turn rate differences, yet reliably encoding route position. Rate profiles are very similar at the α (upper panel) and β (lower panel) track positions as evidenced by high Pearson correlations for LRL-α versus LRL-β and RLR-α versus RLR-β rate profiles. Bottom: two-dimensional rate colormaps for the same neuron. Bottom left schematic depicts relative positions of α and β track locations. C. An RSC neuron with right turn rate increases significantly modulated by route position. Both mean rates (dark blue and black) and individual trial rates (light traces) are shown for the α and β track locations. Peak rates across turns are significantly different at both track locations, indicating sensitivity to the route-centered frame of reference. D. An example neuron significantly modulated by left versus right turning behavior, but not by the positions of turns in a route. E. Reconstruction of position in route-centered space. Correlation matrices computed from odd and even trial ensemble rate vectors (n = 228) for both routes and both track positions. Each row corresponds to the correlation of the odd-trial ensemble rate vector at that route position with the even-trial ensemble rate vectors at that and all other route positions. Across odd-trial positions, the highest correlation is marked in black and reflects the reconstructed position of the animal. The degree of deviation from a perfect diagonal across all positions (white dashed line) reflects the error in route position reconstruction. Pattern differences across track positions, even those associated with the same turn type, yield enough information to allow precise reconstruction.
54.3% of RSC neurons (n=124/228, Wilcoxon rank-sum test, p ≥ 0.05) did not have significant left turn versus right turn firing rate differences (Figure 2.3A, 2.3B). Of this group, 52.4% (n=65/124) of the cells nevertheless exhibited strong correlations (Pearson’s r > 0.4) between mean positional firing rate profiles for odd and even-numbered trial runs (for each route individually), thereby providing evidence of reliability in their rather complex spatial firing patterns (see also Fig. S2.5 for an extensive assessment of pattern reliability). More directly pertinent to the issue of mapping within route space, 21.7% (n=27/124) of the turn-insensitive sub-group exhibited firing rate profile correlations across track locations α and β greater than r = 0.4 (Figure 3B). As a control, α/β correlations were recalculated 50 separate times following randomization of neuron identities. Across all these randomizations, a mean of only 4.32% (± 1.7% SD) of all neurons showed α/β rate profile correlations greater than r=0.4. Thus, sub-populations of RSC neurons reliably mapped the space along RLR and/or LRL trajectories regardless of allocentric position.

Next we determined whether neurons that did exhibit action correlates also exhibited modulation of their action-specific activity according to route position. Evidence of such modulation would suggest route position information in RSC could take the form of changes in the ‘gain’ on action-related firing according to the position of actions in a sequence. Of the neurons (45.6% of all, n=104/228) exhibiting differential activation across all left versus right turns, 43.3% (n=45 neurons, or 19.7% of all neurons, Wilcoxon rank-sum test, p < 0.05) also exhibited significantly different rates depending on the location of the preferred turn type within a route (e.g., differential firing for the first versus second right turn of the RLR route). Such differences in turn-related firing were not secondary to differences in the angular or linear velocities associated with turning behavior given the poor correlation between the two measures (Fig. S2.6 and
Thus, this sub-group encoded spatial information concerning movement types as well as the specific location of those movements within routes (Figure 2.3A, 2.3C). In contrast, a separate 26% of all neurons had left/right turn activity rates that were insensitive to the route positioning of turns (Figure 2.3D, see also Figure 2.2A, 2.2B).

The preceding analyses identified two forms by which RSC neurons, like PPC neurons, can map route position and suggest that the animal’s position within a route can be discerned from positional firing rate profiles for the full ensemble of RSC neurons. To determine whether this is indeed the case, we utilized a simple correlative reconstruction process. First, the ensemble firing rate vectors for every route position for all even-numbered trials were correlated with the ensemble firing rate vectors for all route positions for all odd-numbered trials (see Fig. S2.8). Such cross-positional correlations in ensemble firing patterns are color-mapped for each route and for each track location separately in Figure 2.3E. Second, for each row, the column associated with the highest correlation value was determined. To the extent that ensemble rate vectors are unique and reliably observed for each route position, we expect the points of highest correlation between odd and even-trial data to vary minimally along a ‘perfect prediction’ line that moves from upper left to lower right. As indicated by the high correlations along these diagonals, the even-trial ensemble activity at all route positions is most strongly correlated with odd-trial ensemble activity for the same or nearby positions. Although track positions sharing the same behaviors (e.g., right or left turning) yield high correlations as well (appearing as off-diagonal red patches), the patterns of activation are distinct enough to enable a very accurate prediction of the animal’s location, evidencing strong mapping of route position by RSC neurons.
Figure 2.4. RSC activity is sensitive to allocentric positioning of the track. A. Mean (± SD) Pearson correlation values for rate profiles at track locations α and β and α and α' (a second run at α) are depicted for HPC and RSC neuron populations. B. Rate profile correlations for α/α' (x-axis) and α/β (y-axis) are plotted for all RSC neurons. 70% of all RSC neurons exhibit higher correlations for α/α' versus α/β. C. An RSC neuron (mean rate ± SE) whose firing peaks at right turns along the RLR route are route position-dependent at the β, but not at the α track location. At position β (middle), the neuron exhibited activity peaks of significantly different magnitudes for the two right turns (Wilcoxon rank-sum test, n = 46, z = 3.82, p=0.0001). Dark blue shading is aligned with mean firing activity at the first right turn to aid visualization of rate differences. D. An RSC neuron (mean and individual trial rates) with higher rates across the 1st versus 2nd right turns of the RLR route at both track locations. Across the LRL route, much stronger activity is seen across the sole right turn when the track is at the β as compared to the α location indicating sensitivity to the allocentric position of the track. E. Mean rate profiles (± SE) for both routes are overlaid for all track positions. At the α location, this RSC neuron displayed increased activation just after the center turn, irrespective of route identity. Different (uncorrelated) patterns emerged at the β location indicating alteration of the route-based pattern by allocentric position. F. Mean and individual trial rate profiles for an RSC neuron (LRL route only). At location α/α', firing gradually increases leading up to the second left turn. The pattern was disrupted at track location β. G. Mean and trial firing rate profiles for an RSC neuron (LRL route only). At location α/α', rates peak along straight segments following left turns. This pattern was disrupted at track location β. H. Mean and trial rate profiles for an HPC ‘place cell’ on (LRL route only). This neuron’s single firing field was specific to the α/α' running sessions.
RSC neurons map track location in allocentric space

We also sought to examine whether RSC firing patterns, like HPC neurons (Figure 2.4A, 2.4H) can reflect the position of the track in the allocentric frame of reference. To our knowledge, such a finding would represent the first evidence of a single brain region exhibiting conjunctive encoding of position within the three reference frames most relevant to fluid, efficient navigation within an environment. Some evidence for sensitivity to the allocentric frame of reference came in the form of a small contingent of neurons (6% of all) sensitive to head orientation relative to allocentric space (Figure S2.9), a result consistent with prior findings\(^{26,28}\).

More directly consistent with the hypothesis that RSC neuron firing is sensitive to the track’s position in the environment (i.e., the allocentric space), the mean correlation across all RSC neurons for positional rate profiles taken from track locations α and β was low but statistically different than what was observed in the HPC ($\mu_{RSC}=0.16$, $n_{RSC}=374$ (both routes); $\mu_{HPC}=0.07$, $n_{HPC}=218$; Kruskal-Wallis with post-hoc Scheffe test; $p=0.0009$, Figure 2.4A). This difference between RSC and HPC likely reflects the sub-population of RSC neurons that were turn-sensitive and/or reliably encoded route progression, irrespective of allocentric track location. In contrast, the correlation for RSC firing rate profiles taken from the first and second sessions at track location α was significantly higher ($\mu=0.34$; Kruskal-Wallis with post-hoc Scheffe test; $n_{RSC}=374$ for both $\alpha/\alpha'$ and $\alpha/\beta$ correlations, $p=3.44\times10^{-12}$), indicating that RSC neurons are reliably sensitive to the allocentric position of the animal. In fact, $\alpha/\alpha'$ spatial profile correlations were greater than $\alpha/\beta$ correlations for 70% of RSC neurons (Figure 2.4B). RSC correlations for the first and second sessions at track location α were as strong, statistically, as those for the HPC neurons recorded ($\mu=0.40$; Kruskal-Wallis with post-hoc Scheffe test; $n_{RSC}=374$ (both routes), $n_{HPC}=218$; $p=0.60$).
Despite the foregoing evidence for RSC encoding of allocentric position, there was little evidence for single, isolated firing fields of the type typically observed for HPC neurons (a.k.a., ‘place cells’, see, for example, Figure 2.4H). Instead, in RSC, the animal’s position in allocentric space is mapped as a conjunction among allocentric, route-centric, and egocentric reference frames. Examples of this phenomenon are given in Figures 2.4C – 2.4G. The RSC neuron of Figure 2.4C exhibited stronger activation at the second right turn compared to the first right turn at track location β (Wilcoxon rank-sum test, n_β = 46, z = 3.82, p = 0.0001). This difference disappears at track location α and is also absent for the α’ epoch (Wilcoxon rank-sum test, n_α = 22, p_α = 0.15; n_α’ = 28, p_α’ = 0.07). In Figure 2.4D, a right turn sensitive RSC neuron exhibited stronger activation for the first right turn across all three track running sessions, regardless of allocentric position. However, in contrast to a purely egocentric turn sensitive neuron, this RSC cell had minimal discharge for the right turn on the LRL route, except when the route occupied track location β. Thus, for these neurons, the modulation of turn-related activity that yields information as to position in the route is, in turn, dependent on the allocentric position of the track. All three reference frames impact the firing of these neurons in a reliable fashion. The RSC neuron in Figure 2.4E exhibits a more complex pattern in which there are strong increases in activation immediately preceding the middle turn on both the LRL and RLR routes. Thus, this neuron was sensitive to progression through route irrespective of the heading direction or action sequence that composed the trajectory. More importantly, these activity correlates are reliably observed only at track location α. Figure 2.4F depicts a RSC cell with complex route-related activation that gradually increased as the animal progressed through the LRL route, but exclusively exhibited this tendency when the track was placed at location α/α’.
Figure 2.5. Sensitivity to distinct reference frames in simultaneously recorded RSC neurons. A-D. Mean (thick traces) and individual trial (thin traces) rate profiles (both α and β locations) for pairs of simultaneously recorded RSC neurons. For all neurons, α/β correlations and turn-ratios are also given. A. Top two plots: RSC neuron having similar route-position-dependent activity patterns at locations α and β. Bottom two plots: An RSC neuron tuned to head direction in the allocentric frame of reference. Polar plots of directional tuning for each track location are shown to the left. B. Top two plots: Complex firing patterns during RLR route traversals for an individual RSC neuron differ across track locations α and β indicating sensitivity to allocentric space. Bottom two plots: An RSC neuron sensitive to the egocentric frame of reference with activity peaks consistently accompanying right turns at both track locations. C. Top two plots: Complex firing patterns for an individual RSC neuron differentiate allocentric track locations α and β. Bottom two plots: Simultaneously, another RSC neuron exhibits highly similar activation patterns for RLR routes at track locations α and β indicating sensitivity to the route-centered frame of reference. D. Top two plots: An RSC neuron exhibits highly similar activation patterns for RLR routes despite their differential placement in allocentric space. Bottom two plots: Simultaneously, the complex firing patterns for another RSC neuron differ across allocentric space.
Similarly, the RSC neuron in Figure 2.4G exhibited robust discharge following both left turns on the LRL route, but only in conjunction with track placement at location \( \alpha \). For comparison, the HPC cell in 2.4H, as expected, shows a robust firing field (i.e., a ‘place field’) at track position \( \alpha' \), but not at position \( \beta \). A more extensive set of examples of multifactorial reference frame encoding are given in Figure S2.10.

Sensitivity of RSC neurons to multiple reference frames was also observed in simultaneously recorded populations (Figure 2.5). Figure 2.5A depicts firing rate profiles for two concurrently observed RSC neurons that exhibited complex route-referenced activation \((r = 0.42, \text{top plots})\) and allocentric head-direction encoding (bottom plots). The two simultaneously recorded RSC neurons in Figure 2.5B collectively represented the allocentric position of the track \((r = -0.02, \text{top plots})\) and the execution of right turns (bottom plots) in the egocentric reference frame. Figures 2.5C and 2.5D show groups of distinct RSC neurons that, across both neurons, encoded the animal’s position in the RLR route and the location of the track in the environment at the same time.

Finally, to determine if the allocentric modulation of spatial firing patterns seen for these example neurons was present at the level of RSC and HPC neuronal ensembles, we repeated the correlative reconstruction analysis, this time correlating the ensemble firing rate for each route position recorded at room location \( \alpha \) against all ensemble firing rate vectors obtained at room location \( \beta \) (Figure 2.6A, upper panels, again separately for LRL and RLR routes). The lower panels of Figure 2.6A depict the same analysis with neuronal ensembles from the two sessions at track location \( \alpha \). For both RSC and HPC neuron populations, reconstruction of route position at track location \( \beta \) based on track location \( \alpha \) ensemble rate vectors was poor \((n = 200 \text{ spatial positions, } \mu \text{ error RSC } \alpha/\beta = 51.32\text{cm}; \mu \text{ error HPC } \alpha/\beta = 63.45\text{cm})\). This would be expected if ensemble firing
patterns differ appreciably according to the track’s position in the room. In contrast, reconstruction error was minimal for both brain regions when using \( \alpha \) population vectors to predict route position for the \( \alpha' \) session (\( n = 200 \) spatial positions, \( \mu \) error RSC \( \alpha/\alpha' = 4.51\)cm; \( \mu \) error HPC \( \alpha/\alpha' = 7.82\)cm). Figure 2.6B depicts the mean error in reconstruction for each of the analyses shown in Figure 2.3E, 2.6A, as well as those of Fig. S2.11 where data for the RLR route is depicted. Reconstruction error in RSC is minimal, except when attempting to reconstruct position at track location \( \beta \) from population vectors taken at track location \( \alpha \).

The same overall pattern is observed in the HPC. For both structures, a Kruskal-Wallis (\( \chi^2(7)_{RSC} = 537.5, p = 6.9 \times 10^{-112} \); \( \chi^2(7)_{HPC} = 581.1, p = 2.9 \times 10^{-121} \)) and post-hoc Tukey-Kramer test revealed significant differences between \( \alpha/\beta \) reconstruction error (for both RLR and LRL routes) and error in all other within-position reconstructions (odd/even or \( \alpha/\alpha' \), \( p < 0.0001 \)). No significant differences in reconstruction error were found among any within-position reconstructions in RSC. Therefore, RSC neuron ensembles effectively discriminate, on par with HPC ensembles, all analogous positions for any individual route traversed through different sub-regions of allocentric space.

**Discussion**

The foregoing analyses of RSC neuron firing patterns during route-running indicate that RSC ensembles robustly map position within two distinct, external spatial frames of reference while simultaneously mapping the animal’s left versus right turning behavior. Left versus right-turning behavior is, as stated previously, associated with a host of distinct differences in sensory and motor variables (e.g., optic flow, geometric visual appearance of the track) which vary within egocentrically-defined (or internally-defined) spatial frames of reference. Thus, RSC neurons map positions within both external and internal frames of reference.
Figure 2.6. RSC ensemble activity patterns are sensitive to allocentric positioning of the track. A. Upper panels depict color-mapped correlation matrices computed by Pearson correlation of the ensemble rate vector at a given LRL route position for track position $\beta$ with the ensemble rate vectors for the same and all other route positions obtained for track location $\alpha$. Lower panels depict the same but for the $\alpha$ and $\alpha'$ running sessions. The column with the maximum value of each row is the route location for track location $\beta$ or $\alpha'$ yielding the greatest similarity among all track location $\alpha$ rate vectors. These points are marked in black. A perfect fit of positional ensemble patterns across conditions yields the white dashed line in the upper left panel. Deviations from this diagonal reflect error in reconstruction across allocentric track positions. Large deviations were observed for $\alpha/\beta$ reconstructions. In contrast, $\alpha/\alpha'$ reconstruction is associated with minimal error indicating recovery of $\alpha$ ensemble patterns during the $\alpha'$ session.

B. Summary of route reconstruction error (mean ± SD) under different conditions. Left and right bar graphs depict RSC and HPC data separately. The "within position error" groups refer to error in odd versus even trials as depicted in Figure 3E. The "$\alpha/\beta$ error" groups refer to error across track locations $\alpha$ and $\beta$ (as in upper panels of 2.6A). Mean error for $\alpha/\beta$ reconstructions (bars 5 and 6) was significantly higher than that for all other reconstructions (Kruskal-Wallis; $\chi^2(7)_{\text{RSC}}=537.5$, $p=6.9 \times 10^{-12}$; $\chi^2(7)_{\text{HPC}}=581.1$, $p=2.9 \times 10^{-12}$) including $\alpha/\alpha'$ reconstructions ("$\alpha/\alpha'$ error" group – bars 7, 8) which did not differ from the within position error group (bars 7 and 8, $\mu$ error LRL = 4.32 cm, $\mu$ error RLR = 4.70 cm). A similar pattern was seen for HPC neuron ensembles indicating that firing patterns in both structures generate reliable differences in firing patterns across track locations (i.e., according to the allocentric frame of reference).
Approximately 1/2 of all RSC neurons exhibit a significant firing rate bias for changes in heading associated with leftward versus rightward movements. Slightly more than half of this sub-population exhibited relatively stable, position-independent firing rate increases for like turns at different positions within a route. In contrast, slightly less than half of the same sub-population exhibited reliable differences in turn-related activity according to the position of turns within the space of a route. This latter group was complemented by another sub-population that was turn-insensitive, yet exhibited complex positional firing rate profiles specific to individual LRL or RLR routes. Because of this, the position of the animal in any route could be discerned from the ensemble positional rate vectors, thereby indicating encoding of route space in RSC activity comparable to PPC neuronal ensembles. The information source or sources responsible for encoding of route position are, as for hippocampal encoding of allocentric position, yet to be completely understood. Such encoding conceivably reflects the geometric appearance of the track as it changes across a route traversal\textsuperscript{29}. Given that PPC route position encoding persists in the dark\textsuperscript{4}, it may also reflect some form of integration of the full action sequence associated with route traversal. Irrespective of the source, it is clear that route position encoding by RSC neurons is, in turn, impacted by allocentric positioning of the route. Movement of the track to different positions in the environment altered the route-based firing patterns of RSC neurons to a considerable degree such that the position of the animal in the larger space of the full environment could be determined from ensemble firing patterns.

Despite strong sensitivity to allocentric position, RSC spatial firing patterns differ from HPC patterns in three significant ways: 1) RSC firing fields rarely manifest as single, isolated ‘place-specific’ firing fields against a background of near-zero out-of-field
firing; 2) some RSC neurons robustly map the incidence of specific locomotor behaviors irrespective of their positioning; 3) a sub-population of RSC neurons maps position in route irrespective of the route’s environmental location. RSC firing patterns also differ from PPC spatially-specific patterns in that the latter appear to be relatively insensitive to position of the track and animal in allocentric space\textsuperscript{4,7}. Thus, the mapping of multiple forms of spatial information observed in the present work appears to be unique to the RSC and perhaps a product of the conjunction between the complementary forms of spatial mapping observed for two of its primary inputs, the HPC and PPC. An alternative, non-exclusive explanation for the observed spatially-specific firing of RSC neurons is that it arises as a product of a unique form of sensory and motor integration within RSC itself. Such a view is consistent with the interconnectivity of RSC with cortical regions that encode visual and motor information\textsuperscript{30}.

Given the observed RSC neuron firing properties, any structure downstream of RSC may be in position to extract information as to the animal’s current action, position in a route, position in the environment, or any combination of these variables. Thus, RSC provides exceptionally rich contextual information that could be critical to a number of cognitive processes. Consistent with this, RSC has been implicated as a critical component of multimodal associative learning and spatial memory\textsuperscript{31-33}.

The complex spatial representations registered in RSC are also well suited to mediate transformations between spatial representations registered in different reference frames. The spatial firing patterns of most RSC neurons reflected conjunctions between the animal’s position in two or three frames of reference. In this sense, RSC neurons could be described as multifactorial in their responsiveness to variables relevant to spatial mapping and navigation. Furthermore, those RSC afferents providing sensory information (e.g., visual areas 17 and 18b)\textsuperscript{14} may provide a means by which specific
sensory features of an environment can be integrated with and even define space and its impact on RSC firing patterns. Such multifactorial sensitivity, similar to the concept of category-free information encoding\textsuperscript{34}, is the hallmark of a system that operates as a basis function network capable of approximating non-linear transformations among two or more forms of spatial representation\textsuperscript{35,36}. For example, in tasks requiring hand-eye coordination, basis function units may manifest as ‘gain fields’ wherein the intensity of responses to a target’s position in one reference frame (e.g., the eye) is modulated in a systematic fashion by the position of the same target in another reference frame (e.g., relative to hand position)\textsuperscript{37,38}. Such hybrid responses (i.e., sensitivity to multiple reference frames) were observed in the present work for three reference frames with clear relevance to the problem of navigation. For some neurons, hybrid signaling appeared to reflect changes in gain on the responses of turn-sensitive neurons according to route position and track location. The existence of RSC neurons encoding conjunctions of spatial information is consistent with theoretical work considering brain mechanisms by which animal’s may generate trajectories toward a target position\textsuperscript{39} as well as work attempting to explain how egocentrically-based views of the world can be imagined given knowledge of allocentric position in an environment\textsuperscript{25}.

Ensemble activity of the type observed in RSC could also serve to anchor, in target structures, representations of external space. RSC projections reach, for example, the medial entorhinal cortex (MEC) which contains grid cells and head-direction cells whose spatially-specific activity, in an open arena, is anchored to the space defined by distal visual cues\textsuperscript{3,40}. Here RSC neurons may be capable of detecting offsets between the expected positions of distal visual cues based on self-motion integration and the actual positions of those cues relative to the animal\textsuperscript{17}. Such offsets could form the basis for ongoing re-anchoring of the grid cell maps to the space of the
environment through known excitatory projections into layer V of MEC\textsuperscript{17}. Although RSC responses to visual cues were not examined in the present work, this concept is at least consistent with the fact that PPC, a primary input to RSC, contains neurons that map the position of a distal visual target relative to the animal\textsuperscript{9}. Finally, under track-running conditions where movement through the environment is constrained to specific, repeating routes, HPC place cell and MEC grid cell firing patterns may reset at track sites associated with turning behavior and often exhibit directional sensitivity\textsuperscript{29,41-43}. The present data demonstrates that RSC population activity registers turning behavior, route-progression, and current heading direction and is, in principle, capable of directing resets within the grid and/or place cell networks and, perhaps, defining the conditions under which resets occur.

A role for conjunctive RSC neurons in mediating transformations of spatial information is consistent with RSC function in humans. RSC BOLD signal increases are observed when subjects: learn spatial relationships between landmarks from a first person perspective, plan novel routes to match current environmental constraints, identify permanent spatial landmarks important for mapping allocentric space, and register egocentric viewpoint change relative to environmental cues\textsuperscript{44-48}.

Finally, our findings are also compatible with observed impairments following RSC damage. The rich contextual information provided by RSC neuron ensembles may explain why RSC damage is often associated with episodic memory deficits and/or early onset Alzheimer’s disease\textsuperscript{49}. Further, individuals with RSC lesions also exhibit significant spatial disorientation, often manifested as an inability to select appropriate routes and directions of travel to navigate between known goal locations in allocentric space\textsuperscript{18,50}. Thus, the presence of hybrid representations in RSC may be critical for relating
particular routes, orientations, and/or action sequences to the current environmental location.

**Methods**

**Subjects**

Male Long-Evans rats (n = 6) served as behavioral subjects. Rats were housed individually and kept on a 12-h light/dark cycle. Prior to experimentation the animals were habituated to the colony room and handled daily for a period of 1-2 weeks. After this period, animals were placed on food restriction until they reached 85-90% free-fed weight. Water was available continuously. All experimental protocols adhered to AALAC guidelines and were approved by IACUC and the UCSD Animal Care Program.

**Shaping/Behavior**

Animals were trained to navigate along a “W” shaped track for reward (Figure 2.1A, top left). The track edges were only 2 cm in height, allowing for an unobstructed view of the full environment. Outbound and inbound traversals required the animal to negotiate three total turns in alternating directions. For outbound runs, the route was composed of a sequence of left-right-left turns (LRL route). For inbound runs, the route traversal required navigation of right-left-right turns (RLR route). Total route lengths were 215 cm with turns located at 60 cm, 110 cm, and 170 cm. For the initial 2 weeks, rats were habituated to the track and rewarded at the two ends freely. After the animal was rapidly running both routes, rats were required to traverse the track when set in two distinct positions (here called α and β) in the recording room before the track was returned to the initial position (α’) for a third and final session. Room locations for the α and β track locations were chosen randomly. Fixed spatial cues on the walls ensured consistent spatial relationships that defined the boundaries of the recording room across days.
Surgery

Rats were surgically implanted with tetrode arrays (twisted sets of four 17 micrometer platinum-iridium wires) fitted to custom-fabricated microdrives that allowed movement in 40µm increments. Each microdrive contained 4 tetrodes. Rats were implanted with 3 microdrives (2-3 bilateral RSC, 1 HPC, depending on the specific animal). Rats were anesthetized with isoflurane and positioned in a stereotaxic device (Kopf Instruments). Following craniotomy and resection of dura mater overlying the retrosplenial cortex, microdrives were implanted relative to bregma (A/P -5.8 mm, M/L ± 1.3 mm, D/V -0.5 mm, 10-12° medial/lateral angle). 4 of the 6 animals received a unilateral HPC microdrive targeted to the CA1 sub-region (target coordinates relative to bregma, A/P -3.8mm and M/L ± 2.3mm, D/V -0.5mm).

Recordings

Electrodes were moved ventrally (40µm) between recordings to maximize the amount of distinct units collected for each animal. Each microdrive had an individual electrical interface board (EIB-16, Neuralynx) that was connected to a single, amplifying headstage (20X, Triangle Biosystems). A tether led to a set of preamplifiers (50X) and a high pass filter (150 Hz). Signals were then led to the acquisition computer running Plexon SortClient software, filtered at 0.45-9 kHz, further amplified 1-15X (to reach a total of 1,000-15,000X), and digitized at 40 kHz. Single-units were isolated using Plexon OfflineSorter software. Waveform parameters utilized were peak height, peak-valley, energy, and principal components. Waveform clusters appearing to overlap with the amplitude threshold used in collection were discarded to avoid examination of neurons for which a full complement of spiking data could not be obtained. Waveform amplitudes across the time period of the full recording session were always examined to ensure that systematic variation did not produce confounds in determination of positional firing rates.
Animal position was tracked using a camera set 10ft above the recording room floor. Plexon’s CinePlex Studio software was utilized to separately detect blue and green LED lights. Lights sat approximately 4.5 cm apart and were positioned perpendicular to the length of the animal’s head. Recordings lasted approximately 45 minutes, the amount of time needed for the animal to complete an absolute minimum of 5 ballistic runs for LRL and RLR routes at all track positions. For 5 minutes prior to and following track running sessions, the animal was placed in a circular environment to generate a non track-running epoch to enable verification of directional tuning preferences (Fig. S2.3). We recorded a total of 243 RSC neurons and 109 HPC neurons across the 6 rats. Although we did not conduct an explicit power analysis, this sample size was deemed consistent with those found in previous publications within the field\(^{4,26,29}\). RSC units that did not exhibit peak activation of at least 3Hz for a single track bin on either route at any track location were excluded from analyses. HPC neurons were excluded if they had mean activity across all track sessions greater than 8Hz (i.e. fast-firing, putative interneurons), no bins exhibiting a peak firing rate amplitude above 3Hz, or less than 150 spikes total across the entire recording session. Further, only HPC neurons that exhibited place specific activation (i.e. a place field) during track running sessions were included for analysis. Defined place fields had peak activation greater than or equal to 5Hz and a minimum length of 20 adjacent template bins (approximately 24cm total).

**Histology**

Animals were perfused with 4% paraformaldehyde under deep anesthesia. Brains were removed and sliced into 50µm sections and Nissl-stained to reveal the final depth of electrode wires in RSC and HPC. RSC was defined at the dorsolateral edge at 1-2 mm lateral to the lip of the cingulate bundle (lateral extent depends on
anterior/posterior level). This definition was determined to be consistent with the Paxinos and Watson atlas, the Zilles atlas, and previous recording studies in RSC of the rat. The ventral lateral edge was defined by the transition from retrosplenial cortex to the post-subiculum, which is easily observable in Nissl-stained tissue. All tetrodes were determined to be within the bounds of RSC. Documented micro-drive depth across recordings and final electrode depth observed in histology were compared and found to be compatible in all cases.

**Data Analysis**

*Identification of ballistic track traversals*

Position tracking data was pulled into a custom MATLAB guided user interface. From trial-to-trial, runs in which the animal moved uninterruptedly across the track were identified as ‘clean’ traversals and pulled out for subsequent analysis. By this method, stalled track crossings, reward periods, and other position data captured between runs were not conflated with spatial or behaviorally related activity. A minimum of 5 uninterrupted traversals of both the LRL and RLR routes and for all track positions (α, β, α’) was required for a recording session to be included in subsequent analyses, though attaining only 5 ballistic runs was rare. Across all routes and track locations, the mean number of clean ballistic runs in each was 16.

*Track linearization and firing rate calculation*

The space of the track was linearized to analyze spatial and behavioral dependency of neural activity. Custom MATLAB software was utilized to generate a spatial template match for the average movement of the animal through pixel space along the track. All LRL/RLR trials were plotted independently and the coordinates of track start, end, and apex of all three turns for each route were identified (in pixel space) based on each animal’s unique and stereotyped movement through the track. Between
behavioral epochs (start, end, and turn apices) a series of template bins was generated with a spacing of 3 pixels (~1.2 cm). The tracking data for a particular session was linearized by fitting each trial to template space and positional data was smoothed using a narrow Gaussian filter with a standard deviation of 2 pixels. Firing rates were calculated by summing the number of spikes in each template bin and dividing by the total amount of time in which the bin was occupied.

Across recordings, templates varied in overall number of bins for two reasons: 1) slight distortion of positional data dependent on the placement of the track with respect to the view of the camera and, 2) differences in the precise trajectories taken by animals. In order to look at the population of neural activity across LRL/RLR runs and recording sessions, firing rate profiles from different recordings were interpolated or extrapolated (MATLAB 'interp1' function using 'nearest neighbor'). The procedure yields firing rate profiles of the same length for all routes across all recordings as is necessary to permit comparison of ensemble firing rate patterns between different route positions. The final template length (200 bins), across all animals, recordings, and routes, was determined by averaging the length of templates across all recordings.

**Trial-by-trial firing rate reliability**

To assess the reliability of spatial firing patterns, rate profiles for individual trials were correlated with corresponding mean firing rate profiles (fig. S2.5, A-C). A one-tailed Wilcoxon rank-sum test determined whether the distribution of all trial-to-mean correlation values for a RSC neuron was significantly greater than two ‘chance’ distributions of correlation values computed from: 1) a ‘random’ distribution of trial-to-mean profile correlation values generated by correlating randomly selected trials taken from any route or track location among the entire population of recorded RSC neurons and, 2) a ‘spatial shuffle’ distribution of correlation values generated by shuffling the
spatial position of individual firing rate values within each trial and correlating to the corresponding mean rate profile.

**Directional tuning sensitivity**

Head direction was calculated as a perpendicular vector originating from the midpoint of the two tracking LEDs placed laterally on the rats head, equidistant from the center. Three periods of directional tuning were of particular interest: pre-track running exploration of a circular environment, track position ρ, and track position β. Non-running times (i.e. stalled runs, periods between track traversals, and reward periods) were included in this analysis to ensure an adequate sampling of all head-directions. Directional tuning was calculated in 5° bins. To assess the magnitude of directionality, the mean length of the resultant vector was calculated for each behavioral session using the circular statistics toolbox for MATLAB55. Similarly, the mean direction of tuning (direction of resultant vector) was determined for each of the track running and baseline sessions, independently. RSC cells that had a consistent mean directional tuning (<20° difference) and large resultant lengths (>0.5) across both ρ and β track locations were determined to be head-direction neurons (see Fig. S2.9).

**Identification of neurons sensitive to turning behavior**

An analysis of activation at right and left turns across trials was carried out for all RSC neurons. The typical rodent (without tail) was determined to be approximately 15 template bins in length. An integration window of 25 bins centered on each turn apex was utilized to summate firing activity across turns for all trials (Figure 2.2A). For each neuron, the distributions of integrated firing rate activity at all left turns versus all right turns was subjected to a Wilcoxon rank-sum test to determine whether or not the neuron exhibited preferential activation for changes in heading to the left versus the right. To assess whether turn-related neurons were active at all instances of the preferred turn
type, the coefficient of variation (CV) of the mean firing activity at all six preferred turns was calculated and compared to CVs obtained from a series of randomizations of left/right firing rate data. (Fig. S2.4). The same analysis was applied to HPC neurons that exhibited significantly different activation for left versus right movements along the route.

**Angular and linear velocity correlation analyses**

To determine whether turn-related sensitivity was dependent on angular or linear velocity we examined the relationship between trial-by-trial firing rate activity and both velocity measures. The right versus left bias was utilized to determine what type of turn to analyze with respect to angular or linear velocity. All instances of the preferred turn, across both routes and track locations (6 total preferred turns), were included for analysis. The firing activity and absolute angular and linear velocity for every trial, at each preferred turn, was summated across the same integration window utilized for firing rates in the turn-related analysis described above (see Fig. S2.6 and S2.7, A-C). Firing rate and angular or linear velocity profile relationships were then assessed using Pearson’s $r$. To generate a distribution of velocity correlation values that would be expected by chance, trial order was randomized for each cell and a second correlation value was determined for each neuron (Figs. S2.6D and S2.7D, red). Differences between the actual and randomized velocity correlation distributions were assessed with a Wilcoxon rank-sum test.

**Identification of neurons with route-dependent activation**

**Analysis of spatial reliability within a route**

Neurons exhibiting firing patterns not related to actions in many cases exhibited complex, yet spatially reliable, firing patterns. To demonstrate this, separate mean firing rate profiles were calculated for each neuron utilizing odd-trials and even-trials separately. These non-overlapping mean firing rate profiles were then correlated for
each trajectory (Pearson’s $r$) to determine the reliability of the spatial firing pattern along the space of the track. High correlation values ($r>0.4$) evidenced consistent activation patterns and anchoring of firing to route space.

**Analysis of spatial reliability across track locations**

Route-centered activation patterns are sustained regardless of the position of the route within the broader allocentric environment\(^7\). In order to test whether RSC neurons exhibit these firing properties, rate profiles taken from track positions $\alpha$ and $\beta$ were tested for correlation. Neurons that had no action correlates, yet a high correlation value ($r>0.4$) exhibited a consistent mapping of the route space regardless of position in the recording room. Correlations between firing rate profiles taken from track sessions at $\alpha$ and $\alpha'$ were also calculated. The same analysis was conducted on the recorded HPC population. A one-way Kruskal-Wallis test with Scheffe post-hoc pairwise comparisons was implemented to determine if statistical differences existed amongst the four correlation distributions ($\alpha/\beta$ and $\alpha'/\beta'$ for both HPC and RSC).

**Analysis of route modulation of turn-related firing activation and potential behavioral explanation**

Neurons having pure action correlates would fire in an invariant manner for the preferred action regardless of turn locations. Such neurons will have strong correlations across track locations, but may not encode information about position in a route. To test if turn-sensitive RSC neurons were modulated by route position of turns, each neuron’s preferred action type (i.e., left versus right) was identified and the route in which two instances of the preferred turn type occurred was pulled for analysis (e.g., right turn neuron and the RLR route). Integrated activity across each preferred turn was summated for every trial. Wilcoxon rank-sum tests were utilized to determine if the activation for the two right or two left turns were significantly different across all trials.
This process was repeated for each track location, independently. Turn-related RSC neurons that exhibited significant differences in activation intensity at track sites associated with the same turning behavior were considered to exhibit sensitivity to the route-centered spatial frame of reference. Neurons that did not reach significance were determined to be pure turn neurons (i.e., primarily egocentric).

RSC neurons that exhibited route modulation of turn-related activation could result from systematic differences in the animal's turning behavior at particular turn sites. Thus, the relationship between firing activity at preferred turns and angular velocity was again assessed. For each trial, the difference in integrated firing rate at the two preferred turn locations was computed by subtracting the integrated activation at turn site 2 from turn site 1. Similarly, the difference in angular velocity at the two preferred turn locations was found for each corresponding trial. Relationships between angular velocity differences and firing rate differences across trials were then assessed using Pearson correlations (Fig. S2.6 and S2.7, A-C, iii).

**Correlation matrix and route position reconstruction**

To determine the extent to which route positions were encoded across the entire RSC ensemble, a correlative route position reconstruction was conducted\(^{56}\), both within and across track locations. Only RSC neurons that were recorded when the animal ran all sessions (\(\alpha, \beta, \alpha'\)) were included (n=187 of 228).

For reconstruction of route position for a single track placement, individual mean firing rate profiles for all track locations and routes were determined using data collected on odd and even trials separately. Four correlation matrices were generated using these odd-trial and even-trial mean rate profiles for both routes (LRL and RLR) at each track position (\(\alpha\) and \(\beta\)) in the recording room. Each row of a correlation matrix corresponds to the correlation of the odd-trial ensemble rate vector for that position with the even-trial
ensemble rate vectors at that and all other track positions (e.g., row 10 is track bin 10; for schematic, see Fig. S2.8).

To assess route encoding across different track locations, ensemble mean firing rate vectors taken from track locations α, β, and α’ were utilized to reconstruct the rat’s position along corresponding routes taken from different track running epochs (i.e., α predicts β or α predicts β’). Four correlation matrices were generated by correlating the track location α ensemble rate vectors with track location β and α’ ensemble rate vectors for both routes. Each row of a correlation matrix corresponds to the correlation of the track location α at the corresponding position along the route (i.e. row 10 is linearized track bin 10) with the track location β or track location α’ ensemble rate vector at that position along the route and all outer route locations in track template bin space.

For each row of a correlation matrix, the column with the maximum value has the greatest similarity to the ensemble activity at that route position and is the ‘predicted’ location of the rat. These values are shown in black in Figure 2.3E and 2.6A. If the reconstruction were perfect, each row would have a maximum correlation at the same-numbered column. Therefore, perfect predictions form a straight line from upper left to lower right. Any absolute deviation from the ‘perfect prediction’ line (white dashed in upper left of Figure 2.3E and 2.6A) reflects route reconstruction error. A one-way Kruskal-Wallis test, with a post-hoc Tukey-Kramer test was utilized to determine if reconstruction error differed significantly between conditions.

Author Contributions: A.S.A. and D.A.N. each contributed significantly to all components of the work (design, experimentation, analysis, writing).

Acknowledgements: Thanks to several colleagues for their review of the manuscript: Stephen Cowen, Laleh Quinn, Lara Rangel, Jake Olson, and Laura Shelley. Special
thanks to Andrea Chiba, Belinda La, and Sean Kolbu for help with design and implementation of experiments and analyses.


The dissertation author was the primary investigator and author of this paper.

References


*Online Methods references*


Supplemental Figures

Figure S2.1. Recording room setup and track layout. **Left panels:** photographs of recording room from opposing viewpoints. **Upper right panel:** Diagram of recording room layout. Blue circles indicate the two viewpoints from which the photographs in the left panels were taken. The recording room is 13.5’ x 13.5’. Fixed and salient distal cues are schematized. The numbered squares in the bottom portion of the room correspond to a desk with the recording computer (1), and a storage/utility desk (2). **Bottom right panel,** photograph of ‘W’ track with rat for scale. White numbers indicate the distances of each of the turns and full distance of the track.
Figure S2.2. Histology depicting final tetrode placements for bilateral RSC implants in all rats (n=6, KB3 had no left hemisphere implant). Tetrode trajectories are schematized in upper left panel as in Figure 1. 4 tetrodes were typically bundled into a single microdrive cannula. Black triangles depict final electrode depth of each bundle. RSD, retrosplenial dysgranular; RSGc, retrosplenial granular c; RSGb, retrosplenial granular b; RSGa, retrosplenial granular a; ml, midline; V2, secondary visual; cg, cingulum; DS, dorsal subiculum.
Figure S2.3. Sort quality metrics and quantification. A-B. Top panels: Representative RSC cluster identification for two tetrodes in different animals. Different color clusters represent individually isolated neurons. Grey points correspond to unsorted spikes. Below, Mean waveform shape and standard deviation for neurons of top graphs in corresponding colors. Associated patterns of firing activity across each route and track location are shown beneath each waveform plot. C. For data from all recording datasets, plot of the peak amplitude for the mean waveform of sorted neurons versus the peak amplitude of the unsorted waveforms recorded on the same tetrode. The average peak amplitude for sorted waveforms was 5.5 (± 3.6 SD) times greater than that for corresponding unsorted waveforms. D. Additional mean waveform for an RSC neuron with corresponding firing rate profile. The waveform schematizes the method for calculating the coefficient of variation (CVs) at the peak, in which the standard deviation at the peak is divided by the mean value across all spike waveforms at the peak. Mean peak-amplitude CVs for waveforms grouped as individual (sorted) neurons were 0.16 ± 0.13, a value significantly lower than that for the remaining, unsorted waveforms of each tetrode (0.36 ± 0.17, one-tailed Wilcoxon rank-sum test, n = 243, z = -16.7, p = 9.52x10^{-63}).
Figure S2.4. Reliability in turn-related activity. A. Left panel: the mean firing activity, at each of the 12 turns (6 left, 6 right across both routes and allocentric track locations), for individual RSC neurons that exhibited a significant bias for left or right turning (n = 104). Each row represents the mean of the max-normalized firing rate discharge of a single RSC neuron across all trials for each of the 12 turns. Middle panel: same data as left, with each row max-normalized. For both left and middle panels, neurons are sorted from lowest coefficient of variation on left turns (CVL), at top, to lowest coefficient of variation on right turns (CVR), at the bottom. Right panel: mean coefficient of variation (and SD) for all turn-sensitive neurons was 0.52 ± 0.33, which was significantly less than a distribution of CV values generated by randomizing the entire matrix and recalculating (0.73 ± 0.24, one-tailed Wilcoxon rank-sum test, n = 104, p=6.55x10^{-11}). B. Same figures as above, but for HPC neurons that exhibited statistically significant biases for left versus right turning (n = 39). Although these HPC neurons exhibited a significant egocentric bias, the CV for the preferred turn type was nearly 3 times greater than for RSC neurons (μ±SD = 1.50 ± 0.53) and was not significantly different than CV values calculated after randomization of the same data (μ±SD = 1.57 ± 0.42, Wilcoxon rank-sum test, n = 39, p = 0.24). Thus, HPC neurons with statistically significant left versus right turn firing rates do not exhibit reliable turn-related activity across route and allocentric positions. We conclude that the HPC neurons in this subset largely exhibited unidirectional place fields that happened to overlap with a turn site.
Figure S2.5. Trial-by-trial spatial reliability of RSC neuron activation is significantly greater than chance. A-C, Mean activation patterns across track space for three RSC neurons (top trace in each). Bottom three traces in each depict, in corresponding colors (RLR blue, LRL black), the firing rate profiles for three randomly selected route traversals from the same track running session (trials 4, 8, and 12 in each). To assess spatial reliability across individual trials, the firing rate profiles for every trial was correlated (Pearson’s r) with the mean firing rate profile for the same route and same track location. Correlation values for the representative trials are given (r). D. i-iIII correspond to the RSC neurons depicted in A-C, respectively. Here, distributions of actual trial-to-mean correlation values (black) are shown in conjunction with two ‘chance’ distributions; First, a ‘random’ distribution of trial-to-mean profile correlation values generated by correlating randomly selected trials taken from any route or track location among the entire population of recorded RSC neurons (red); Second, a ‘spatial shuffle’ distribution of correlation values generated by randomizing the spatial position of individual firing rate values within a given trial and correlating to the corresponding mean firing rate profile (purple). Mean correlation values for each distribution are represented by filled circles of the corresponding color. iv.) Full distribution of all correlation values, (actual, black; random, red; spatial shuffle, purple) across all neurons, track locations, and routes. Means and standard deviations are depicted as dashed lines and shaded boundaries of the corresponding color. The actual trial-to-mean profile correlation distribution was significantly greater than both the random and spatial shuffle correlation distributions for the full population (one-tailed Wilcoxon rank-sum test, z=121.3 and z=135.6 for ‘random’ and ‘spatial shuffle,’ p=0 for both). 100% of RSC neurons had significantly greater actual correlation distributions than both of the ‘chance’ correlation distributions (one-tailed Wilcoxon rank-sum test, p<0.01).
Figure S2.6. RSC firing rate activity is not sensitive to trial-by-trial fluctuation in angular velocity. A-C. i.) Max-normalized mean firing rate and mean absolute angular velocity for a single neuron along the RLR or LRL route (as indicated). ii.) Max-normalized firing rate and absolute angular velocity for representative individual trials. iii.) Route position modulation of turn-related activity (e.g., greater mean firing rate at the first right turn versus the second right turn) could potentially be explained by systematic angular velocity differences across the two similar turns within a given route. To determine if this was the case, the difference in firing rate between the first and second like turns (rights on RLR route and lefts on LRL route) was calculated for each trial (y-axis). This data was then correlated with the difference in angular velocity at the same turn sites for corresponding trials (x-axis, example trials in red). iv.) Based on the preferred turn type (higher firing for left versus right turns) of the neuron, activity in the relevant turn window (horizontal green bars in ii.) was integrated for every trial for both routes and for both track locations (x-axis), then correlated with the integrated absolute angular velocity for the same route positions across corresponding trials (y-axis). D. Upper left panel: distribution of trial firing rate and angular velocity correlations across the entire recorded RSC population (black, μ=0.01). For each cell, the trial order was randomized and correlations were recalculated to generate a second distribution approximating the angular velocity correlation values expected by chance (shown in red, μ = 0.00). The actual angular velocity correlation distribution was not significantly greater than the randomized distribution (one-tailed Wilcoxon rank-sum test, z = 1.02, n = 228, p = 0.15). Upper right panel: ratios of mean rates across all left versus all right turns for each RSC neuron (x-axis, truncated at 6.5) are plotted against each neuron’s corresponding absolute angular velocity correlation (y-axis) demonstrating that there is no relationship between the magnitude of turn preference and angular velocity correlation. Lower panels: Same as above two panels but shown solely for the population of RSC neurons that exhibited significant turn-related activation (μ_actual = 0.01, μ_rand = -0.01, one-tailed Wilcoxon rank-sum test, n = 104, z = 0.66, p = 0.25).
Figure S2.7. RSC firing rate activity is not sensitive to trial-by-trial fluctuation in linear velocity. A-C. i.) Max-normalized mean firing rate and mean linear velocity for a single neuron along the RLR or LRL route (as indicated). ii.) Max-normalized firing rate and linear velocity for representative individual trials. iii.) Route position modulation of turn-related activity (e.g., greater mean firing rate at the first right turn versus the second right turn) could potentially be explained by systematic linear velocity differences across the two similar turns within a given route. To determine if this was the case, the difference in firing rate between the first and second like turns (rights on RLR route and lefts on LRL route) was calculated for each trial (y-axis). This data was then correlated with the difference in linear velocity at the same turn sites for corresponding trials (x-axis, example trials in red). iv.) Based on the preferred turn type (higher firing for left versus right turns) of the neuron, activity in the relevant turn window (horizontal green bars in ii.) was integrated for every trial for both routes and for both track locations (x-axis), then correlated with the integrated linear velocity for the same route positions across corresponding trials (y-axis). D. Upper left panel: Distribution of trial firing rate and linear velocity correlations across the entire recorded RSC population (black, $\mu = -0.02$). For each cell, the trial order was randomized and correlations were recalculated to generate a second distribution approximating the linear velocity correlation values expected by chance (shown in red, $\mu = 0.01$). The actual linear velocity correlation distribution was not significantly greater than the randomized distribution (one-tailed Wilcoxon rank-sum test, $n = 228$, $z = -2.01$, $p = 0.98$). Upper right panel: Ratios of mean rates across all left versus all right turns for each RSC neuron (x-axis) are plotted against each neuron’s corresponding linear velocity correlation (y-axis) demonstrating that there is no relationship between the magnitude of turn preference and linear velocity correlation. Lower panels: Same as above two panels but shown solely for the population of RSC neurons that exhibited significant turn-related activation ($\mu_{\text{actual}} = -0.04$, $\mu_{\text{rand}} = 0.004$, one-tailed Wilcoxon rank-sum test, $n = 104$, $z = -2.56$, $p = 0.99$).
**Figure S2.8. Schematic of ensemble correlative reconstruction method.**

*Top two panels:* Two separate mean spatial firing rate profiles for each neuron were calculated using odd and even trials. For all RSC neurons, odd-trial mean firing rate profiles (left figure) and even-trial mean firing rate profiles (right figure) are shown for a single-track location ($\alpha$) and route (LRL). White vertical lines indicate turn sites and the space of the track is shown relative to the firing rate vector in blue. *Middle panels:* The mean RSC ensemble activity at each individual route position (bin 10 shown outlined in red) across odd trials was correlated with the mean RSC ensemble activity at every route position bin from even trials (e.g. even-bin number 20 shown outlined in black). Thus, for any individual route bin for odd trials, a 200-bin vector of correlation values was generated reflecting the similarity of the ensemble activity at that bin to the ensemble activity at all route positions taken from even trials (color mapped horizontal bar). *Bottom panel:* The full population of correlation values for each bin forms a correlation matrix. The column with the largest correlation value for each row represents the even-trial ensemble rate vector bin with the greatest similarity to the current odd-trial ensemble rate vector. Therefore, a reconstruction of the animal’s position on even trials can be estimated from the greatest correlation value across each row of the correlation matrix (see Figures 3E, 6A).
Figure S2.9. RSC neurons exhibit head-direction sensitivity. *Left column, top and middle:* 2D firing rate maps for a single neuron at track locations α and β (LRL and RLR route-running periods are shown adjacent to each other (approximate actual locations given by white (RLR) and red arrows (LRL)). The example RSC neuron exhibited increased activation when the animal was oriented towards the bottom-left of the camera’s view as it traversed two different route segments during RLR runs (white arrow). *Left column, bottom:* Gray trace is the animal’s position during a baseline session in a circular holding container. In accordance with the preferred directional tuning on the track, the cell spikes at locations (in blue) in which the animal is perched and facing towards the bottom-left of the camera’s view. *Middle column:* Polar mean firing rate tuning plots for the same RSC neuron. Direction and length of the resultant vector (in red) indicate the mean and magnitude of directional tuning for the neuron across track locations α and β, and in the holding container. *Right column:* top, For each RSC neuron, mean directional tuning at track location α is plotted against mean directional tuning preference at track location β. If the preferred tuning direction was the same at both track locations for a given neuron, its data point would fall along a diagonal moving from lower-left to upper-right (center dashed red line). Neurons with mean tuning preferences within 0.36 radians (~20 degree differences) across the two track locations were considered potential head-direction neurons. *Right column, bottom:* For each RSC neuron, the mean resultant length (tuning magnitude) at track location α is plotted against mean resultant computed at track location β. RSC neurons that had high resultants (> 0.5, red dashed line) were strongly directionally tuned across both track locations and considered potential head-direction neurons. Neurons that had both strong (resultants > 0.5) and consistent mean directional tuning across both track positions (within 0.36 radians) were determined to be allocentrically-referenced RSC head-direction neurons (6%, n=15/243, indicated by black data points in both plots; example neuron of left column shown in green).
Figure S2.10. RSC neurons exhibit conjunctive encoding of position in multiple reference frames. Mean positional firing patterns of 10 RSC neurons across individual routes (LRL in blue, RLR in black, dark lines means, light lines trials), for $\alpha$ (top), $\beta$ (middle), and $\alpha'$ (bottom) track running epochs. The 4 RSC neurons of columns 1 and 2 exemplify single reference frame encoding. The 6 neurons of columns 3-5 illustrate a variety of conjunctive patterns observed across the full RSC population, wherein highly complex spatial firing patterns vary according to the combinations of specific reference frames.
Figure S2.11. Reconstruction of route position across track placements for RLR route. Left and right panels depict RSC and HPC RLR route reconstructions, respectively. Ensemble firing rate vectors taken from track locations $\alpha$, $\beta$, and $\alpha'$ were utilized to reconstruct the animals' position along the RLR route across allocentric track locations. Four correlation matrices were generated by correlating all track location $\alpha$ ensemble rate vectors with track location $\beta$ and $\alpha'$ ensemble rate vectors. Predicted location of the animal is the highest correlation in each row, here shown by the black line. Error of reconstruction for RLR routes across both regions is depicted in Figure 6B.
Table S2.1. Breakdown of recorded RSC neurons by sub-region, rat, and sensitivity to spatial frames of reference. A total of 243 RSC neurons were recorded across 6 rats. Neurons recorded in each sub-region (sR) of RSC from each rat (rD, retrosplenial dysgranular; rgC, retrosplenial granular C; rgB, retrosplenial granular B; rgA, retrosplenial granular A; columns repeat through the rest of the table). The ‘allocentric’ category refers to neurons that had minimal correlations between track locations α and ß (r < 0.4). The ‘non-allocentric’ category refers to RSC neurons that had strong correlations between track locations α and ß (r > 0.4). ‘headDir’ are RSC neurons that were determined to be head-direction sensitive. The ‘egoTurn’ category is composed of RSC neurons (‘headDir’ neurons excluded) that exhibited significant right vs. left turning sensitivity, but did not exhibit differential modulation of activation at the preferred turn type dependent on turn position within a route. 36% (n = 21, indicated in parentheses on table) of turn-sensitive neurons have non-significant firing rate differences across the 6 instances of the preferred turns, Kruskal-Wallis, p ≥ 0.05 and thus can be consistent with encoding of purely egocentric spatial information. The ‘routeTurn’ category is composed of RSC neurons that exhibited significant right vs. left turning sensitivity and exhibited differential modulation of activation at the preferred turn type dependent on turn position within a route or the location of the track in the room. The ‘nonTurn’ category consists of RSC neurons that did not exhibit significant turning activity. Within this sub-group, the ‘odd/even; r > 0.4’ category of RSC neurons refers to cells that had complex but spatially reliable patterns of activity within a single allocentric track location. The ‘αβ; r > 0.4’ subset of non-action-specific cells (‘nonTurn’) exhibited strong correlations across both allocentric track locations (i.e. route-referenced activation patterns) for at least one of the two routes. In the ‘all animals’ column, neuron counts for each of the aforementioned categories are collapsed across rats and depicted by RSC sub-region.

<table>
<thead>
<tr>
<th>sub-regions (sR)</th>
<th>KB3</th>
<th>KB4</th>
<th>KB5</th>
<th>KB6</th>
<th>KB7</th>
<th>KB8</th>
<th>all animals</th>
<th>totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>total neurons</td>
<td>11</td>
<td>8</td>
<td>119</td>
<td>61</td>
<td>13</td>
<td>31</td>
<td>243</td>
<td>6</td>
</tr>
<tr>
<td>all totals</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>non-allocentric</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>allocentric</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>headDir</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>egoNonRoute</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>9</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>routeTurn</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>nonTurn</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>odd/even; r &gt; 0.4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>
Chapter 3: Spatially periodic activation patterns of the Retrosplenial cortex encode route sub-spaces and track distance

Abstract

Complex navigational problems, such as traversing a difficult route, are often solved by breaking the navigational domain or trajectory into simpler sub-components. Neural mechanisms for compartmentalizing spaces and routes into useful sub-components remain to be elucidated. Retrosplenial cortex (RSC) is potentially implicated in this process, as neurons within RSC have activation patterns that encode position within routes and other spatial coordinate systems simultaneously. This feature is compatible with sub-route extraction and encoding. To assess the role of RSC in representing sub-components of trajectories, rats traversed a track with recurrent structure. Individual RSC neurons exhibited periodic activation patterns along the route that repeated across analogous route segments. Concurrently, RSC ensembles defined a framework for encoding sub-route positions relative to the whole. Further, the structure of activation patterns provided a novel metric of distance from each route position to all others, consistent with research implicating the region in path integration.

Introduction

For most animals, including humans, movement through an environment is frequently constrained to routes along fixed, interconnected pathways. Fluid, efficient navigation under such circumstances demands knowledge of specific routes as well as their locations and orientations within the broader environment. Operationally, a route can often be defined as a series of turns separated by straight-run sections of varying length. As a consequence of the spatial structure associated with such action sequences, a route can also be defined and recognized as a unique space in its own
right (i.e. a shape). Notably, identically or similarly shaped routes may exist in many places within the broader environment, potentially at different scales.

The forms of neurophysiological representation associated with route knowledge are only beginning to be understood. Different routes through single environmental positions often yield modulation of in-field firing rates for hippocampal neurons mapping current environmental location\(^1,2\). Current position within a route itself is represented in the posterior parietal cortex (PPC) and retrosplenial cortex\(^3\)\(^-\)\(^6\). However, PPC and RSC route representations diverge. PPC maps position within a route in a manner largely independent of that route’s position in the broader environment, while RSC route encoding is highly sensitive to route position relative to distal cues defining environment boundaries (often referred to as the ‘allocentric’ frame of reference). While neural activity in these regions is also predictive of specific left- and right-turning actions\(^7\)\(^-\)\(^10\), the encoding of progression within a route lies at the scale of the full trajectory since clearly discriminant patterns of activity are generated for different route positions sharing the same action\(^3\)\(^,\)\(^11\).

Routes of extensive length and complexity are often described according to their recognizable sub-components. For example, the first leg of a route may be a straight section followed by a second leg that has an ‘S’ shape. This suggests that there may be regions of the brain, even in rodents, that are capable of representing route sub-components and their relationships to each other. An examination of this possibility at the level of individual neuron spiking dynamics might reveal the form by which encoding of such information is achieved and the rules that govern it.

Among the many structures known to exhibit spatially-specific firing, RSC may be primed to generate the functional dynamics necessary to identify sub-spaces within a complex route and encode their locations within the environment. RSC is positioned as
an anatomical intermediary between the complementary PPC and HPC representations of position. Further, recent work has demonstrated that RSC neuronal ensembles simultaneously encode an animal’s position in multiple spatial frames of reference, including route and allocentric position\(^5\).

To address the role RSC in sub-route encoding, we trained rats to traverse a route having recursive properties at multiple spatial scales and recorded single-units in RSC. RSC populations generated unique patterns for all route positions. A subset exhibited periodic activation patterns across the full route that oscillated at the scale of specific sub-routes, primarily when they shared the same shape. Further, some RSC neurons oscillated a single time across the full route space, thereby encoding a metric of the animal’s distance from specific locations within the route. Thus, we identify RSC as a region with neurophysiological dynamics that support both the identification and interrelation of sub-spaces within a complex route and provide evidence that RSC can robustly encode distances between all route locations.

**Results**

*RSC ensembles generate a unique encoding of all route positions*

315 neurons from 5 adult male rats were recorded across all RSC sub-regions during clockwise traversals of a plus-shaped track (Figure 3.1A, 3.1B left; for histology and sort quality see Figure S3.1A-E). Of these, 229 exhibited a peak in firing rate greater than 3Hz for at least one track position and were not head-direction neurons (Figure S3.1F). Uninterrupted traversals (Figure 3.1B, right) were identified and positional firing rate vectors were generated. Consistent with prior work\(^5\), maximal firing rates for the full population of neurons were distributed evenly across the full route space (Figure 3.1C, left).
First, to determine whether RSC ensembles generate unique and reliable patterns for every route position, we split route traversals into odd and even trials, generated separate positional rate vectors for each, and created a correlation matrix which assessed the degree of similarity of ensemble patterns for each route position against all others (Figure 3.1C, right). The extent to which maximal similarity between odd and even trials lies along the upper-left to lower-right diagonal of such matrices provides a measure of the extent to which unique, reliable patterns are formed at each location. Such ‘reconstruction’ of the animal’s progression along the route was accurate to $1.9 \pm 2.7$ centimeters based on RSC populations, a value which compared favorably to the accuracy seen for a population of 141 hippocampal CA1 ‘place cells’ recorded in 3 animals (Figures 3.1C, 3.1D; CA1 accuracy = $5.2 \pm 6.7$ centimeters).

Positional correlation patterns for RSC and CA1 neurons were distinct, however, in the off-diagonal correlations that reflect similarity between non-adjacent track locations. Unlike matrices for the CA1 ensemble, off-diagonal correlations for RSC ensembles exhibited repeating patterns across the track. The spatial distribution of such off-diagonal correlations suggests, in particular, the presence of a sub-population of RSC neurons having repeating patterns over quarter and/or half sections of the full route.

**RSC neurons exhibit spatial periodicity at full route and sub-route spatial scales**

As stated, spatially periodic activation patterns in RSC ensembles could reflect concurrent encoding of route sub-spaces by individual neurons. To examine this question directly, we applied generalized linear models (GLM) to determine whether individual RSC cells exhibit activity peaks that follow the recurrence of sub-spaces at multiple scales inherent to the spatial structure of the route.
**Figure 3.1. RSC encodes progression through a recurrent closed track.**

A. Electrode placement and recording ranges across 5 rats in caudal RSC. Lines indicate recording depths included for analysis. 

B. Left, schematic of 'plus' track. Track is closed, in that the animal starts and ends at the same location and is rewarded at the arrow point. Shape of track creates recurrent action sequences that can occur at multiple spatial scales. Right, tracking of ballistic traversals of an individual animal in a single session. 

C. Max normalized firing rate profiles across all RSC neurons (n = 229), sorted by position along track with maximal firing rate. High firing along the diagonal indicates that a unique population of neurons exhibited peaks in activation for every position along the track. Right, for the same population as on the left, a correlation matrix of population vector similarity computed from odd and even numbered trials. Strong correlation along the diagonal indicates that across non-overlapping trials, the maximal similarity between population vectors occurred at the same position through the route. White line, peak correlation for each row showing that the animals position along the track can be accurately decoded to within 1.9 ± 2.7 cms. Off diagonal red indicates repetition of activation patterns for different positions along the track and thus, potential presence of spatial periodicity. For both plots, dashed vertical lines show position of left turns, non-dashed vertical lines show position of right turns. 

D. Same as plots in C but for HPC pyramidal neurons (n = 141).
The GLM analysis was designed to fit the positional firing rate profiles of individual neurons. We constructed multiple predictors (Figure 3.2A) composed of paired sin and cosine functions with different pairs having periods corresponding to full route or sub-route spaces. Each pair acts together as a single predictor having a distinct set of values for each position in the full route space or having repeating sets of values across same-sized sub-spaces. The latter are used to detect recurrence in firing patterns for analogous sub-spaces. We chose the predictors to reflect the logical fractionations of the space into twelfths, corresponding to the smallest track segments, through sixths, fourths, thirds, halves, and the full space of the track.

To quantify model fits to the actual positional rate vectors, we used the normalized mean squared error (NMSE) between the rate vector of any given neuron and a model that used all of the spatial predictors (the ‘complete model’, or cGLM; Figure 3.2B). To assess the contribution of each spatial predictor to model accuracy, we then removed each spatial predictor in isolation. We refer to these as partial GLMs (pGLMs). The increment in NMSE for pGLMs versus cGLMs corresponds to the decrement in fit and measures the strength of the removed predictor in improving cGLM fits. To complement this approach to measuring the influence of the full route or sub-route spaces on the firing pattern of each neuron, we also calculated NMSEs for models generated using only individual spatial predictors (iGLMs).
Figure 3.2. Discrete spatial GLM shows RSC neurons exhibit spatial periodicity on track traversals. A. Left column, schematic of fragmentation of plus track into multiples of twelve using oscillating spatial predictors constructed from sine and cosine functions. Track space is split into twelfths, sixths, fourths, thirds, halves, and full fragments. For each fragmentation, cosine and sine functions were constructed that cycled at the scale of the full track space of the corresponding spatial scale. Spatial predictors are shown in the plots to the right of the schematic for each fragmentation. B. Example of complete GLM (cGLM) model fit, using all spatial predictors, generated for the mean firing rate vector (± s.e.) for an individual RSC neuron. The black line is the complete GLM fit using all predictors and the fit (normalized mean squared error - NMSE, 1 is a perfect fit) between actual and predicted vectors is indicated above the plot. C. Four mean firing rate vectors for individual RSC neurons showing different scales of spatial periodicity. For each plot, the model fit generated by the best individual spatial predictor (iGLM) is shown in black, and the NMSE is indicated above the plot. In red, the partial GLM (pGLM) when the best predictor is dropped from the model showing the impact of that spatial periodicity in accounting for the firing pattern of the neuron. Below each plot, spike trains for individual trials. To the right of each figure is the corresponding two dimensional ratemap with a max threshold indicated in red (zero firing rate is blue). D. Population statistics of model fit. Across all RSC neurons, the mean (± std. error) fit of the cGLM (black bar) compared to all pGLMs (gray and blue bars). All pGLMs had significantly lower fits on average with the exception of the pGLM that fragmented the plus into individual segments (‘singles’, Kruskal-Wallis, $X^2(6) = 250.5$, $p = 3.2 \times 10^{-51}$, post hoc Bonferroni correction). E. Spatial versus egocentric predictors. Left, spatial cGLM and spatial pGLMs using the three best spatial predictors from 3.2D. Right, complete eGLM and partial eGLMs constructed with linear velocity, acceleration, and angular velocity as predictors. All eGLM predictors significantly impact the model. Spatial GLM model is significantly better at fitting RSC neural data than models generated with egocentric data as predictors. F. For each neuron and trial, a cGLM and eGLM was generated. All pGLMs (both spatial and egocentric) were then generated for that trial and NMSE was computed. This process was repeated across all trials for an individual neuron to generate a distribution of trial-by-trial pGLM values that were then significance tested against the same distribution for trial-by-trial cGLM values. Shown here, percentage of RSC neurons significantly impacted by each spatial fragmentation or egocentric-based movement variable.
**Figure 3.2C** shows the positional rate vectors of four individual RSC neurons with the best single-predictor fit (best iGLM model fit) overlaid in black and the pGLM model fit with that best single-predictor removed (overlay in red). Applying this same methodology to the full RSC population, the fit between the best individual predictor (based on iGLM models) was found to be $2.3 \pm 2$ times larger on average than to the second best, indicating that most RSC neuron firing patterns are dominated by a single full route or sub-space periodicity.

Similarly, examining the fit decrements in pGLMs relative to their associated cGLMs across the full RSC population, we found that every predictor significantly impacted the complete model fit (**Figure 3.2D**, Kruskal-Wallis, $X^2(6) = 250.5, p = 3.2 \times 10^{-51}$, *post hoc* Bonferonni correction). The only exception to this was the ‘singles’ predictor corresponding to the single segment spatial scale.

To statistically test the impact of each full and sub-route periodicity on the firing of individual neurons, we calculated fit correlations for pGLMs and cGLMs for the positional rate vectors associated with individual trials. In this analysis, an assessment of each predictor’s contribution to strength of fit is calculated for each run through the full route. The distributions of fit values for pGLMs versus cGLMs are then compared. The percentage of RSC neurons significantly impacted by each predictor is shown in **Figure 3.2F** (individual neuron Kruskal-Wallis across trials, *post hoc* Bonferonni, $p<0.05$).

Consistent with the results of the aforementioned GLM approaches and with the structure of correlation matrix given in **Figure 3.1**, 63.7% of RSC neurons ($n = 146/229$) exhibited periodicity in firing according to at least one full-route or sub-route space. Specifically, 47.2% ($n = 108/229$) of the neurons were modulated by sub-routes as manifested by significant spatial periodicity at scales subordinate to the full route. Of
RSC neurons that were sub-route modulated, the most prominent encoding fell at quarters (21%, n = 48/229) and halves (20.5%, n = 47/229). 38.9% (n = 89/229) of RSC cells had an activation pattern that cycled a single time across the full space of the trajectory. Example neurons with periodicity in firing rate patterns over quarter and half sections of the full route and with periodicity over the full route space are given in Figure 3.3. Populations of sub-route encoding neurons and those that exhibited ‘full’ oscillatory behavior were not mutually exclusive, as 48.3% of ‘full’ neurons (n = 43/89) were also conjunctively sensitive to one or more sub-routes. Of all neurons, 26.6% (n = 61/229) were conjunctively sensitive to multiple spatial scales simultaneously (Figure 3.2C bottom, Figure S3.3). This latter finding illustrates a neural framework by which sub-route relationships can be hierarchically mapped to other sub-routes or the full trajectory.

RSC neurons are capable of generating reliable responses to specific actions such as left or right turns, though such responses are not correlated to variation in angular velocity on a trial by trial basis. For the plus-shaped route utilized here, quarter sections of the full route represent the smallest sub-space over which such specific

Figure 3.3. RSC neurons exhibit spatial periodicity biased to fourth, half, and full spatial scales. A. Mean linear firing rate vector (± s.e.) of two example RSC neurons exhibiting significant modulation that oscillates four times across the full route. For each plot, the black line is the iGLM fit using the best predictor. In red, cGLM fit with best predictor dropped showing decrement in fit when best periodicity is no longer included. Below, spike trains for individual trials showing reliability of firing patterns across track space. Right, corresponding 2D ratemap showing where the activation occurs in the full space of the room. Red number indicates peak firing rate in Hz for these plots (blue is zero firing). NMSE, normalized mean squared error of best model (prefixes - q = quarter, h = halve, f = full). B and C, same as A, but for RSC neurons exhibiting periodicity that oscillates across half of the track and the full track space, respectively. *Quarter section periodicity is not accounted for by locomotor actions.*
actions repeat. Consistent with this feature of the route, movement profiles were well described by spatial predictors that oscillated at the scale of quarters across the full trajectory (Figure S3.2). For these reasons, it was important to determine whether some or all of the sub-space periodicity in firing at the level of quarter sections in particular could be explained by correlation of firing rates to specific locomotor variables such as linear and angular velocity and linear acceleration. Full-route and half-route periodicity in firing is unlikely to be explained by locomotor action correlates because those correlates repeat over quarter sections. Nevertheless, we considered locomotor variables for their potential contribution to the firing of all RSC neurons and for all full and sub-route spaces.

To do so, we generated GLM fits for each neuron’s positional rate vector using predictors based on the linear and angular velocities and linear acceleration profiles observed during track-running. We refer to these as eGLMs with ‘e’ itself denoting the egocentric nature of these movement variables. Overall, eGLM fits indicated that such movement variable predictors produced poorer fits than the previously-described spatial predictors, even when matched for total number of predictors in the model (eGLM; Figure 3.2E, Figure S3.2, Kruskal-Wallis, $X^2(6) = 905.2$, $p = 3.6 \times 10^{-191}$, post hoc Bonferonni correction). Thus, moment-to-moment fluctuations in RSC firing are not well described by moment-to-moment fluctuations in movement variables.

The proportion of individual RSC neurons significantly impacted by fluctuations in the aforementioned movement variables was also assessed with a trial-by-trial eGLM analysis. Here, firing rate predictors for each trial were constructed using the corresponding trials linear acceleration, velocity, and angular velocity vectors. Only 7.4% (n = 17/229) and 14.4% (n = 33/229) of RSC neurons were significantly impacted by linear acceleration and velocity, respectively. Less than 1% of trial-by-trial eGLM model
fits to RSC neurons were significantly decremented by the loss of angular velocity predictors (Figure 3.2F).

Finally, despite the overlay of spatial and movement variable periodicity at the level of route quarter sections, just 39.5% (n = 19/48) of neurons that exhibited quarter-scale spatial periodicity also exhibited significant sensitivity to any of the movement variables as assessed with this trial-based eGLM approach. These findings indicate that the presence of quarter periodicity is, in general, not directly reflective of recurrence in movement variables.

**RSC activation is spatially-anchored and invariant to distance or temporal integration**

Sub-route encoding in the form of periodic activation could potentially be explained by factors other than spatial relationships within route or allocentric space. Instead, RSC activation patterns could reflect path integration anchored to locations associated with actions or trajectory start points as observed in other structures. For example, grid cells of the medial entorhinal cortex (mEC) exhibit repeating activation patterns during track running and such patterns can be anchored to the start points of track segments\(^{12,13}\). Further, grid cells exhibit increased error in spatial firing as animals move farther from boundaries\(^{14}\) (i.e. start points). A second possible explanation for RSC periodicity could be firing related to passage of time during constant locomotion as observed in the hippocampus (HPC) and mEC\(^{15,16}\).

To test whether periodic activation of RSC neurons was related to distance from starting location, time from start of trial, or a combination of route and allocentric spatial position, we trained two rats to run randomly interspersed half and full routes within the same plus track environment (Figure 3.4A). The half trajectory was positioned over the middle portion of the full route which displaced the start point in allocentric space. Rats were required to utilize distal cue information to identify the route type of each trial, and
without cuing, either stop or continue through the position on the plus track associated with the endpoint of the half route. 101 neurons from bilateral RSC were recorded under these conditions.

Despite surrounding the shared cross-route spatial locations with different retrospective and prospective journeys, movement across the overlapping track segments was remarkably similar between half and full trajectories (Figure 3.4B). Within each recording, movement through most shared segments did not statistically diverge between the two routes (Figure 3.4B, right; n = 40 recordings, Kruskal-Wallis, post hoc Tukey-Kramer, p > 0.05). The final segment did have statistically different angular velocity (77.5%, n = 31/40) and linear acceleration (60%, n = 24/40) in a majority of recording sessions as the animal either stopped or continued through this segment depending on trial type (Kruskal-Wallis, post hoc Tukey Kramer, p<0.05). However, the overall similarity of movement indicated that any RSC activation differences found between full and half routes were not conflated with differences associated with the rat’s movement.
Figure 3.4. RSC activation patterns are anchored to allocentric space. A. Rats (n =2) were trained to run randomly interspersed full and half traversal routes, starting and ending at two different locations within the same plus track. Top row left, schematic of full route trajectories. Top row middle, schematic of half route. Top row right, schematic of route overlap. Red circles depict boundaries of first half of full route. Bottom, example tracking data from a single animal during a single recording session showing ballistic traversals for both routes. B. Mean (± s.d.) of linear velocity, rectified angular velocity, and linear acceleration movement variables across all rats and recording sessions for the overlapping portions of the track for each route. Blue and black vectors represent half and full route traversals, respectively. Overlapping segments are numbered. To the right of each figure is the percentage of recordings (n=40) with statistically significant behavior between half and full routes for each segment (Kruskal-wallis test, post hoc Tukey-Kramer, p<0.05). As expected, most significant differences occur on the first or last (1 and 6) segments, where the animal is either beginning, stopping or continuing through the segment depending on current route. C. Mean (± s.e.) firing rate profiles for 4 individual RSC neurons during traversals of half and full routes (blue and black profiles). For each neuron, the correlation between activation profiles in overlapping space are shown at the top right. Despite complex firing rates, a large portion of neurons exhibited strong spatial correlations in firing rate across the two routes, indicating that the activation pattern was not anchored to the distance from the beginning of the route, nor to time. Instead, spatial firing patterns are anchored to the allocentric position in the environment. Segments are numbered to match 3.4B. D. Distribution of half to full correlations for all neurons recorded under these conditions (n =110). The distribution is strongly biased to positive correlation values. In red, distribution of half correlations to the first half of full runs rather than overlapping track segments.
We next examined the relationship between RSC firing rate profiles between half and full trajectories. If RSC activation was related to path integration as a function of either distance or time from the start point, we expected the pattern on the half route to closely match the first half of the full route (red circles Figure 3.4A, top right). In contrast, if activation was anchored to space, we expected the overlapping portions of the half and full routes to exhibit strongly correlated profiles. This latter hypothesis, in which activation was anchored to the external environment, was the overwhelming trend.

Figure 3.4C depicts four example RSC neurons with activation on full and half trajectories shown in overlapping gray and blue mean firing rate vectors, respectively. Correlation values for the four neurons shown reveal strong similarity in patterning between the overlapping portions of the half and full route traversals. Further, correlations between half and full routes in overlapping space were statistically greater than those computed between the half route and the first half of full route traversals (Figure 3.4D, n = 101, μOverlap = 0.45, μFirstHalf = 0.09, Wilcoxon rank-sum test, z = 6.74, p = 1.60 x 10^{-11}). As such, we conclude that the RSC firing profiles described here are spatially anchored and not the product of distance or temporal integration from the start of trials.

Recurrent behavior is not required for RSC spatial periodicity

A striking feature of the spatially-anchored periodic activation found in RSC was the apparent bias to quarter and half sub-routes. These sub-route representations segmented the full trajectory into equivalent action sequences that collectively constructed the full route. Although the eGLM results demonstrated that the spatial periodicity could not be explained by a direct relationship between firing rate and behavioral correlates, the question remained whether this form of spatial representation would persist in environments that did not have recurrent structure that could explicitly
define boundaries of route fragments. To examine this question, rats (n = 3) were trained to run a ring shaped track in the clockwise direction. The ring track lacked action sequences and animals held consistent angular and linear velocity profiles for the majority of ring traversals (Figure S3.4). 90 RSC neurons were recorded under these conditions.

As observed in RSC activation patterns during plus traversals, the spatial cGLM analysis revealed sub-route representations in the form of spatially periodic activation patterns during ring track running. All spatial predictors significantly impacted the complete model fit with the exception of those that segmented the ring track into sixths and twelfths (Figure 3.5A, left, \(X^2(6) = 180.2, p = 3 \times 10^{-36}\), post hoc Bonferonni correction). 86.7% (n=78/90) of RSC neurons were significantly modulated by at least one oscillating spatial predictor when analyzed across trials (Figure 3.5B, gray and blue bars). Surprisingly, sensitivity to a particular sub-route did not generalize between the plus and ring, as relatively few of the neurons recorded on both tracks (n = 78/90) were statistically sensitive to the same predictor in the two route running conditions (Figure 3.5B, black bars). Proportions of neurons whose firing patterns were significantly predicted by linear acceleration, speed, and angular velocity (eGLM) were low (<15%) and compatible with proportions observed on the plus (Figure 3.5B, green bars) For neurons that exhibited significant modulation by full, half, or quarter spatial predictors, individual predictor model fits were as good on the ring as those for neurons exhibiting the aforementioned forms of spatial periodicity on the plus (Figure 3.5C, ‘Full’, \(\mu_{\text{Ring}} = 0.41, n = 63, \mu_{\text{Plus}} = 0.35, n = 89\) Wilcoxon rank-sum test, \(z = 1.32, p = 0.19\); ‘Half’, \(\mu_{\text{Ring}} = 0.27, n = 40, \mu_{\text{Plus}} = 0.29, n = 47, z = -0.95, p = 0.34\); ‘Quarter’, \(\mu_{\text{Ring}} = 0.36, n = 12, \mu_{\text{Plus}} = 0.33, n = 48, z = 0.49, p = 0.62\).
However, the proportions of RSC neurons that were significantly modulated by individual predictors was inconsistent across the plus and ring tracks, with spatial periodicity on the ring being biased to larger scale oscillations (Figure 3.5B). Figures 3.5D-F show the activation profiles, for both ring (bottom) and plus (top) sessions, of RSC neurons that exhibited significant spatially periodicity on the ring. Although RSC neurons exhibited quarter periodicity on the ring, the frequency of quarter sub-route encoding (13.3%) was diminished compared to the plus track (21%, Figure 3.5D). In contrast, the proportion of neurons that were modulated at the scale of half of the route increased between the ring (44.4%) and plus (20.5%, Figure 3.5E). Finally, 70% (n = 63/90) of RSC neurons exhibited periodic patterns on the track that oscillated at the scale of the full ring traversal (Figure 3.5F), in contrast to 38.9% during plus track traversals. These differences suggest that, although sub-route extraction is an inherent function of the RSC network, the proportion of neurons sensitive to specific sub-routes is adaptable and reflects the constraints of the current environment.

*RSC sub-population with full route periodicity yield a metric of distance*

Spatial periodicity of the form observed in RSC provides a neural mechanism for extracting analogous positions within repeating sub-trajectories. This encoding is manifested as a distinct firing rate code and pattern across locations within a sub-route, that then repeats for each iteration of the sub-route within the full trajectory. Many RSC neurons exhibited sensitivity to repeating sub-routes in the form of periodic activation patterns, yet an equally large number exhibited oscillatory patterns that scaled the full space of the route. The spatial representation for these ‘full’ neurons is unique relative to other spatial mappings currently reported. Here, activation is spatially reliable, continuous (rarely exhibits zero-firing), cyclic, and exhibits symmetrical firing activation across two complete halves of the track.
Figure 3.5. RSC neurons exhibit spatially periodic activation patterns on a ring shaped track. **A.** Across all RSC neurons recorded on the ring track, the mean (± std. error) NMSE of cGLMs and pGLMs. With the exception of 'single' and 'sixths' spatial periodicity, all other spatial predictors significantly impacted the complete model ($\chi^2(6) = 180.2, p = 3 \times 10^{-36}$, post hoc Bonferroni correction). **B.** For each neuron and trial, a cGLM and eGLM was generated. All pGLMs (both spatial and egocentric) were then generated for that trial and NMSE was computed. This process was repeated across all trials for an individual neuron to generate a distribution of trial-by-trial pGLM values that were then significance tested against the same distribution for trial-by-trial cGLM values. Shown here, percentage of RSC neurons significantly impacted by each spatial fragmentation or egocentric-based movement variable on the ring track. Black bars, percentage of all neurons recorded on the ring ($n = 90$), that were significantly modulated by the same spatial predictor for both ring and plus track sessions. Blue lines, percentage of RSC neurons recorded during plus track running that were significantly modulated by each spatial predictor (same as bars in Figure 3.2F). **C.** Mean (± s.e.) of fit to individual predictors for all neurons that exhibited significant modulation by full, halve, or quarter spatial predictors in either track running condition (blue, plus; black, ring). Fit was not statistically different between track types, indicating that the spatial periodicity was equally strong across conditions (‘Full’, $\mu_{\text{Ring}} = 0.41, n = 63$, $\mu_{\text{Plus}} = 0.35, n = 89$; Wilcoxon rank-sum test, $z = 1.32, p = 0.19$; ‘Half’, $\mu_{\text{Ring}} = 0.27, n = 40$, $\mu_{\text{Plus}} = 0.29, n = 47$, $z = -0.95, p = 0.34$; ‘Quarter’, $\mu_{\text{Ring}} = 0.36, n = 12$, $\mu_{\text{Plus}} = 0.33, n = 48$, $z = 0.49, p = 0.62$). **D.** Mean activation profiles for a single RSC neuron that exhibited significant modulation at the quarter scale during ring traversals (bottom, ring; top, plus). Right, corresponding 2D ratemaps. **E and F.** Same as **4D**, but for two RSC neurons that exhibited significant modulation at half and full scales during ring track running, respectively.
This symmetry is in stark contrast to that observed for HPC place cells, as the scale of symmetry covers the entire route space, not just locations within a field of firing (Figure 3.6A-B).

This novel form of spatial encoding, in turn, generates a novel form of spatial information. Specifically, symmetrical firing patterns anchored to route space provide information about the animal’s distance from a fixed point. To assess the presence of a distance code, all RSC firing rate profiles collected on the plus track were rotated to find the best point of symmetry (Figure 3.6A, bottom). Best symmetry points (SP) were those that reduced the error (again, NMSE) between the reflected halves of the track. Examination of rotated firing rate profiles sorted by max firing rate revealed the presence of a secondary firing peak at positions approximately equidistant from the SP (Figure 3.6C, left). For HPC pyramidal cells, the same process revealed symmetry by splitting place fields in half and leaving large portions of the rotated track space lacking representation (Figure 3.6C, right).

For the RSC ensemble, an autocorrelation of these linear firing rate profiles revealed a strong off-diagonal correlation that ran perpendicular to the point of symmetry (Figure 3.6D, left). This finding indicated that there was strong population vector similarity for track positions located at similar distances from the SP. A similar pattern emerged for the HPC, but the correlations were much lower and generally less reliable for positions between the start, end, and the SP (Figure 3.6D, right). Any semblance of HPC distance representation was afforded by a sub-population of neurons that exhibited multiple, distinct, firing fields across the track (highlighted by the vertical red bar running to the right of the second graph in Figure 3.6C).
Figure 3.6. RSC full route spatial periodicity yields metric of distance traveled from a point within the full trajectory. A. Top row, mean firing rate profiles for two RSC neurons on the plus track. Red line depicts ‘symmetry point’ where rotation of the vector yields maximal symmetry as shown in second row plots. Second row, rotated versions of top row plots that yield greatest reflective symmetry. Red dashed line shows that the symmetry point is now in the center of the firing rate vector. Rotated vectors have been slightly smoothed (see methods) to account for potential sharp jumps between starting and ending track locations in top row. B. Same plots as in 5A, but for a HPC place cell. C. Left, symmetrical max-normalized firing rate profiles for all RSC neurons. Right, symmetrical max-normalized firing rate profiles for all HPC neurons. Red bar two right of symmetrical HPC profiles indicates population of HPC neurons with multiple place fields. D. Left, linear spatial autocorrelation across full population of symmetrical RSC profiles from 5C. Strong correlation running perpendicular to the central point of the matrix (point where SP meets from both axes), indicates similar population vector pattern for similar distances from the point of symmetry. Right, linear spatial autocorrelation across full population of symmetrical HPC profiles reveals less strong correlations for points equidistant from the point of symmetry. E. Distribution of symmetry points along the plus track. F. Symmetrical firing rate profiles for all RSC neurons that were only significantly modulated by the full spatial oscillation along the plus track (n = 46). The first half of this matrix, from the blue line to the red line in the left graph, was extracted and reflected (right graph, notice flipped positions of red and blue lines). Population vectors from the reflection of the first half were then correlated with population vectors taken from the second half of the left matrix (from the black to the purple line). G. Distance correlation matrices, for both RSC and HPC, generated from the process described in 5F. Distance correlation matrices reflect the similarity of RSC population activity as a function of distance from the point of symmetry. Strong correlations along the diagonal, from upper left to lower right, demonstrate strong similarity for positions equidistant to the symmetry point. A distance estimate of the animal’s position from the symmetry point is computed by finding the maximal correlation in each row. This value is depicted by the white line. A perfect reconstruction of the animal’s distance would run diagonally from upper left to lower right, as shown by the red line. The error in distance estimate is computed by finding the absolute value of the difference between each point along the white line and the corresponding point on the red line. H. Mean (± s.e.) distance reconstruction error between RSC neurons exhibiting full periodicity and spatially-responsive HPC neurons (place cells). RSC distance estimates are significantly lower than those observed in HPC (µRSC = 5.09cm, µHPC = 13.0cm, Wilcoxon rank-sum test, n = 72, z = -4.46, p = 8.36 x 10^-6).
Symmetry points were uniformly distributed across the route space for RSC and HPC (Figure 3.6E, Rayleigh test, RSC, n = 229, z = 0.73, p = 0.48; HPC, n = 141, z = 1.01, p = 0.37). As such, the alignment of symmetrical vectors (as in Figure 3.6C) to examine the presence of a distance code is an artificial construct. Given constraints on the total possible number of simultaneously recorded neurons, it is not implausible to assume that a large number of neurons in reality have anchor points at the same location on the track. Several track locations in our dataset possess multiple neurons with anchor points in close proximity (Figure 3.6E).

The autocorrelations in Figure 5D demonstrated that there was a distributed distance code within the RSC population. However, the aforementioned analysis included all neurons, including those that lacked oscillatory firing patterns across the full space of the route. To test whether the animal’s distance could be accurately predicted as a function of distance from the SP we examined the neurons with ‘Full’ sensitivity as revealed from the trial-by-trial spatial GLM analysis from Figure 3.2F. The symmetrical vectors for all RSC neurons that exhibited this form of spatial tuning were halved at the point of symmetry and the first half of the firing rate profile was reflected as shown in Figure 3.6F. Distance reconstruction was computed by correlating the RSC population vectors for each position moving away from the SP with each reflected position moving towards the SP. The correlation matrix produced from this process is shown in Figure 3.6G for the RSC and HPC. Strong correlation along the diagonal reflects the presence of a similar population vector for positions equidistant, but occurring on opposing sides, of the SP. The animal’s predicted distance from the SP was simply the maximal correlation for each row (shown in white in Figure 3.6G) and the error in distance reconstruction is the absolute difference between each position on the white line and a
perfect distance prediction (shown in red in Figure 3.6G). The mean distance reconstruction error for the RSC was 5.09 centimeters and significantly lower than that observed in HPC (Figure 3.6H, $\mu_{HPC} = 13.0\text{cm}$, Wilcoxon rank-sum test, $n = 72$, $z = -4.46$, $p = 8.36 \times 10^{-6}$). This finding demonstrates a unique capability of RSC ensembles to encode distance from uniformly distributed points of symmetry present in known routes.

Discussion

The current analysis of RSC activation during route-based navigation further defines properties of spatially related activation within the region. We again demonstrate the presence of RSC neurons with spatial activation patterns anchored within multiple spatial frames of reference, including both route and allocentric coordinate systems. RSC ensembles generated reliable and distinct population vectors for every position within the plus track. These patterns could be utilized to accurately predict the animal's progression through the route. Given the recurrent structure of the trajectory utilized, this property of RSC spatial activation reflects the presence of a spatial code robust to repetition of actions and spatial sequences.

Novel to the current work, we report a subset of RSC neurons that encode sub-routes within complex trajectories. More than half of RSC neurons recorded under the current conditions exhibited activation profiles that cycled multiple times across the full trajectory. Periodic activation patterns of this nature could be utilized to assess position within fragments of the full route, as analogous locations across sub-routes had similar firing rate codes. Further, the presence of distinct population vectors for every position within the route provided an encoding framework against which sub-space spatial features of the environment could be mapped. We also report the presence of RSC neurons that simultaneously encode multiple sub-routes, via conjunctive periodicity.
These responses could potentially reflect neural mechanisms underlying hierarchical encoding and organization of spatial relationships\textsuperscript{17-19}.

The periodic activation patterns most frequently observed segmented the full trajectory into quarters or halves. For all animals, traversals along the track produced recurrent patterns in behavioral variables such as angular and linear velocity and thus, it was possible that sub-route encoding could be epiphenomenal to the animal’s behavior during navigation. Upon further consideration, several pieces of evidence were in conflict with this hypothesis. First, fragmentation or sub-route encoding at the scale of halves was inconsistent with this interpretation, as no behavioral measure oscillated twice along the full track space. Secondly, a GLM analysis using behavioral predictors yielded significantly poorer fits than those models constructed using spatial predictors. Further, many RSC neurons exhibited sub-route activation patterns when the animal traversed a ring shaped track that lacked repeating action sequences.

This latter finding further demonstrated that patterned motor efference, optic flow, or local visual information associated with recurrent behavior was not required for periodic firing patterns in RSC. Instead periodicity on the ring suggests a more general tendency of the network to fragment spaces into sub-components. Although the fitness of the spatial GLM was statistically the same across plus and ring tracks, the proportions of neurons that had oscillations at any given spatial scale shifted between the two routes. This fact suggested that, though RSC spatial fragmentation/sub-route encoding can exist without explicit sub-region demarcations (e.g. action sequences), the system can potentially be entrained to detect logical parsing of the environment when these cues are available.

The observed RSC sub-route representations should be considered with respect to novel forms of spatial representation recently reported in RSC. Specifically, cyclic
activation patterns during route running on tracks could potentially reflect the position of the animal relative to local features of the track. For instance, an RSC neuron with quarter periodicity may fire on each straight track segment in which there are two turns to the right of the animal (see Figure 3.4C, top). Recent fMRI work in humans has demonstrated that multivoxel patterns in RSC are most similar for virtual locations that share the same locally-referenced position and heading direction\textsuperscript{20}. In related work, a sub-population of dysgranular RSC neurons exhibited directional tuning that was seemingly referenced to local visual cues in a multiple compartment environment\textsuperscript{21}. Thus, it is possible that the sub-route representations reported in the current work are the manifestation of local position encoding within route space. Accordingly, RSC may be capable of detecting offsets between an animal’s position relative to both local and distal cues, which would be a significant computation for the alignment of spatial representations downstream of the region\textsuperscript{22}.

Periodic spatial firing has been reported in several other structures, most notably in the form of grid cells of the MEC or repeating place fields of the HPC\textsuperscript{23-28}. RSC spatial periodicity emerges during route running wherein there are consistent relationships between allocentric location and behavior. In this manner, RSC sub-space encoding is perhaps most similar to the observation of repetitive place fields in repeated action sequences\textsuperscript{29,30}, grid cell fragmentation observed during route running\textsuperscript{12}, or spatially repetitive activation of human MEC neurons in virtual route traversal\textsuperscript{31}. RSC projections reach the HPC\textsuperscript{32} and MEC\textsuperscript{33} via direct and indirect routes, and thus, the relationship between sub-route encoding in RSC and periodic activation patterns present in these spatial systems should be examined.

Another structure in which sub-route representations have been found is the PPC\textsuperscript{11}. PPC neurons exhibit repeating patterns along segments of nested routes that
share similar actions and heading sequences, but differ in allocentric placement. RSC sub-route encoding diverges from the form found in PPC in two major ways. First, RSC can detect sub-route components that do not share the same heading orientation. In contrast, PPC sub-route activation patterns are often not correlated across different heading directions. The same is true for the aforementioned sub-route representations found in HPC or MEC. Secondly, RSC sub-route activation is periodic in nature, exhibiting an activation profile that is cyclic as it moves from the beginning to ending of a sub-route. PPC sub-route representations instead exhibit increasing or decreasing linear ramps in firing rate that encode progression through a sub-route. A final key difference between periodic RSC patterns and those found in PPC, MEC, or HPC, is the larger spatial scale of the excitatory portion of the field, which, in conjunction with the cyclic nature of the response enables sub-route encoding and yields a metric of distance discussed next.

The current work reveals that ensembles of RSC neurons with periodic activation patterns collectively yield a distributed code of the animal’s distance from all locations within the route. The existence of a RSC distance code is consistent with detriments to path integration following damage to the region. Further, recent fMRI work has demonstrated strong RSC correlates with virtual distance integration.

The RSC distance metric relies on a sub-population of neurons that exhibit continuous, sinusoidal, mean activation profiles across the full track space. These novel representations of the full route rarely have stretches of track in which there is no firing. As a consequence of their sinusoidal shape, these spatial firing rate profiles possess a track location, here called the symmetry point, which segments the track into two mirrored, symmetrical, halves. We demonstrate that RSC neurons possessing this
property will exhibit highly similar firing rates for all locations approximately equidistant to the symmetry point location.

Our data show that a small population of neurons with symmetrical vectors anchored at the same track location will provide a distributed representation that can be used to accurately predict the rat’s distance from the symmetry point independent of whether it is approaching or leaving the location. Along similar lines, RSC neurons with spatial periodicity at shorter spatial scales (e.g. quarters or halves) can also exhibit symmetry and thus provide information about distance within sub-routes. It is plausible that the RSC distance code could be the product of integration of grid cell, head direction, or place cell signals. Consequently, we identify RSC as a unique spatial processing hub capable of unique computations relevant to efficient spatial representation and navigation.

**Methods**

**Subjects**

Male Long-Evans rats (n = 5) served as behavioral subjects and were housed individually and kept on a 12-h light/dark cycle. Animals were habituated to the colony room and handled daily for a period of 1-2 weeks prior to shaping. Rats were food restricted to approximately 85-90% of their free-fed weight. Water was available continuously. All experimental protocols adhered to AALAC guidelines and were approved by IACUC and the UCSD Animal Care Program.

**Behavior**

All animals were trained to navigate around plus-shaped tracks for reward. The track edges were 2 cm in height, which allowed the animal an unobstructed view of the full environment which had fixed distal cues across recording days. Animals ran the plus track in the clockwise direction.
Two rats were trained to run half traversals in addition to the full traversals that all rats ran. On half route trials the rat started at the quarter point and stopped a quarter from the endpoint. The animal was required to stop at the correct endpoint on its own volition, without cuing from the experimenter. If the animal stopped correctly a reward (1/4 honey nut cheerio) was placed on the track near the animal for consumption, otherwise no reward was given. Half trials were randomly interspersed during all plus track sessions. For both full and half route traversals, animals were picked up between trials and carried in random trajectories to the trial initiation point.

A subset of rats (n=3) were also trained to traverse a ring-shaped track, in addition to the plus-shaped track, for reward. The ring track was placed in the same general allocentric position as the plus-shaped track relative to the recording room. Track edges on the ring were 4cm in height. Rats ran the ring track and plus tracks in the same session, with the two tracks existing in overlapping positions within the room. Ordering of track conditions across days was counterbalanced to account for any potential sequence effects. Animals were trained to traverse the ring track in both directions, however clockwise data was solely analyzed as it allowed a more direct comparison to plus traversals given prominent head direction inputs to the RSC and observed directionality of spatial firing on linear environments. Fixed spatial cues on the walls ensured consistent spatial relationships that defined the boundaries of the recording room across days.

Surgery

Rats were surgically implanted with tetrode arrays (twisted sets of four 12 micrometer tungsten wires or 17 micrometer platinum-iridium wires) fitted to custom-fabricated microdrives that allowed movement in 40µm increments. Each microdrive contained 4 tetrodes. Rats were implanted with 3 microdrives (2-3 bilateral RSC, 1 HPC,
depending on animal). Rats were anesthetized with isoflurane and positioned in a stereotaxic device (Kopf Instruments). Following craniotomy and resection of dura mater overlying the retrosplenial cortex, microdrives were implanted relative to bregma (A/P - 5.8 mm, M/L ± 0.7-1.2 mm, D/V -0.5 mm, 10-12° medial/lateral angle). 3 animals received a unilateral HPC microdrive targeted to the CA1 sub-region (target coordinates relative to bregma, A/P -3.8mm and M/L ± 2.3mm, D/V -0.5mm).

**Recordings**

Each microdrive had one or two electrical interface boards (EIB-16, Neuralynx) individually connected to amplifying headstages (20X, Triangle Biosystems). Signals were initially amplified and filtered (50x, 150Hz) on the way to an acquisition computer running Plexon SortClient software. Here the signal was digitized at 40kHz, filtered at 0.45-9 kHz and amplified 1-15X (to reach a total of 1,000-15,000X). Electrodes were moved ventrally (40µm) between recordings to maximize the amount of distinct units collected for each animal.

Animal position was tracked using a camera set 10ft above the recording room floor. Plexon’s CinePlex Studio software was utilized to separately detect blue and red LED lights. Lights sat approximately 4.5 cm apart and were positioned perpendicular to the length of the animal’s head. Recordings lasted approximately 45 minutes, the amount of time needed for the animal to complete an absolute minimum of 5 ballistic runs for plus and/or ring track conditions.

**Unit isolation and sort quality**

Single-units were identified using Plexon OfflineSorter software. Primary waveform parameters utilized were peak height, peak-valley, energy, and principal components. To assess sort quality, custom MATLAB software was developed to compute isolation distance and L-Ratio metrics for each unit. No units were excluded.
on the basis of their cluster quality scores. Instead, we show the entire distribution of L-ratio and Isolation Distance values to demonstrate that neurons exhibiting spatial periodicity and distance encoding had a range of cluster quality scores, from average to extremely well isolated (Figure S3.1).

The only RSC units that were excluded from analyses on the tracks were those that did not exhibit peak activation of at least 3Hz for a single track bin and those that were statistically identified as head direction neurons. HPC neurons were excluded if they had mean activity across all track sessions greater than 8Hz (i.e. fast-firing, putative interneurons), no bins in which the firing rate dropped to 0Hz (i.e. fast-firing, putative interneurons), and no bins exhibiting a peak firing rate amplitude above 3Hz.

**Histology**

Animals were perfused with 4% paraformaldehyde under deep anesthesia. Brains were removed and sliced into 50µm sections and Nissl-stained to identify the trajectory and depth of electrode wires in RSC and HPC. RSC was defined in accordance with our previous work\(^5\) in the region as well as the Paxinos and Watson\(^{41}\) and Zilles\(^{42}\) atlases. Dorsally, the lateral boundary of RSC was considered to be 1-2 mm lateral to the lip of the cingulate bundle. Ventrally, the lateral edge was defined by the transition from retrosplenial cortex to the subiculum. All tetrodes were determined to be within the bounds of RSC. Documented micro-drive depth across recordings and final electrode depth observed in histology were compared and found to be compatible in all cases.

**Data Analysis**

**Identification of ballistic track traversals**

Position tracking data was pulled into a custom MATLAB guided user interface. From trial-to-trial, runs in which the animal moved uninterruptedly across the track were
identified as ‘clean’ traversals and pulled out for subsequent analysis. A minimum of 5 uninterrupted traversals was required for a recording session to be included in subsequent analyses. The average number of traversals on the plus and ring tracks were 14 and 13, respectively.

*Track linearization and firing rate calculation*

The space of the track was linearized to analyze spatial dependency of neural activity. Custom MATLAB software was utilized to generate a spatial template match for the average movement of the animal through pixel space along the track. All full, half, and ring trials were plotted independently and the coordinates of track start, end, and apex of all turns for each route were identified (in pixel space) based on each animal’s unique and stereotyped movement through the track. Between behavioral epochs (start, end, and turn apices) a series of template bins was generated with a spacing of 10 pixels (approx. 3.5 cm). Each session was linearized by fitting both position and neural data acquired on each trial to template space. Firing rates were then computed for each template bin by dividing the total number of spikes by the time of occupation. Activity patterns were smoothed via convolution with a narrow Gaussian filter (approx. 7 cm s.d.).

Like the linear firing ratemaps, two dimensional firing rate maps were constructed for each track running session individually and during ballistic track traversals only. Tracking data was binned into 3.5cm x 3.5cm spatial bins and the animal’s occupancy in seconds for each was determined as well as the corresponding number of spikes. Firing rates were computed by dividing the number of spikes in each bin by the total time in seconds that each bin was occupied. Raw two dimensional ratemaps were smoothed via convolution with a Gaussian kernel (7 cm standard deviation).

*Directional tuning sensitivity*
Head direction (HD) was calculated as a function of the angle of two tracking LEDs placed laterally on the rat's head. For each neuron, the firing rate for each heading direction, discretized to 5° bins, was calculated as the total number of spikes divided by the total time in seconds that the heading bin was occupied. Two head direction tuning curves were computed for each neuron, for the first and second half of the recording separately. Neurons with strong directional tuning that was reliable across these two head-direction tuning vectors were eligible to be classified as HD neurons. The length and mean direction of the resultant vector, as well as a Rayleigh test for non-uniformity were calculated for both tuning curves using the Circular Statistics toolbox for MATLAB. RSC cells that were statistically non-uniform in their firing rate as a function of heading direction, exhibited consistent mean directional tuning, and had large resultant lengths (>0.5) across both halves of the recording were determined to be head-direction neurons (see Fig. S3.1). Head direction neurons were removed from all analyses on the track.

**Correlative route position and distance reconstructions**

To determine the extent to which route positions were encoded across the entire RSC ensemble, a correlative route position reconstruction was conducted for plus track traversals. Individual mean firing rate profiles were computed using data collected on odd and even trials separately. Correlation matrices were constructed with these spatial activation profiles to estimate the animal's position on even trials from data collected on odd trials. In the correlation matrices of Figure 3.1C and Figure 3.1D, each row corresponds to the correlation of the odd-trial ensemble vector at a single track location (i.e. a template bin) with the even-trial ensemble vector at every track location (i.e. all template bins). For each row of the matrix, the column with the maximal correlation is the predicted position of the rat on even trials, because it is the position that yielded the
greatest similarity to the odd-trial ensemble rate vector for the track bin corresponding to that row of the matrix.

A similar correlative reconstruction process was computed for the distance reconstructions shown in Figure 3.6G. Here, instead of correlating ensemble rate vectors taken from odd and even minutes we correlate ensemble rate vectors taken from the first half of the symmetrical rate vectors (see Figure 3.1C, 3.1F) against those taken from the second half. Our prediction is now of the rat’s distance from the point of symmetry rather than overall position in the route, but is still quantitatively the maximum correlation in each row.

Spatial and Behavioral Generalized Linear Models

A series of spatial GLMs were implemented to assess the presence of spatial periodicity in the activation patterns of RSC neurons on the plus and ring tracks. To begin, a total of 6 spatial predictors were constructed using paired sine and cosine functions. Each spatial predictor had a specific number of complete periods for the sinusoidal functions depending on the scale of the oscillation being tested. Spatial predictors were generated to split the full space of the plus into multiples of 12, as there were 12 total segments on the plus. As such, for a given predictor the total number of complete cycles of the sine and cosine functions would take on discrete values: twelve, six, four, three, two, or one. The total number of complete cycles within the predictor would correspond to fragmentation of the space into single segments, sixths, fourths, thirds, halves, and fulls (Figure 3.2A).

All fragmentations were anchored relative to the start of the track and the paired cosine and sine functions were scaled to cycle across the appropriate template distance for the given spatial predictor. Using this method, corresponding track positions within each fragment (i.e. the first bin of the first half of the track and the first bin of the second
half of the track) have the same pair of cosine and sine values as the combination
defined the unique predictor value for that spatial position within the fragmentation. The
same spatial predictors that fragmented the plus track into multiples of 12 were
implemented in creating model fits to firing rate profiles taken from ring traversals.

Following construction of spatial predictors, a complete generalized model fit
(cGLM) was constructed for the max-normalized mean firing rate profile in linearized
track space for each neuron using all spatial predictors (‘glmfit’ in MATLAB). Regression
coefficients were evaluated (‘glmval’ in MATLAB) to construct the predicted firing rate
profile for the neuron and the fit between the actual and predicted firing rate profiles was
assessed via normalized mean squared error (NMSE, ‘goodnessOfFit’ function in
MATLAB). To test the impact of each spatial predictor this process was repeated with
each predictor dropped individually and the fitness reassessed (pGLM). Kruskal-Wallis
tests with post-hoc Bonferonni corrections were conducted on distributions of fitness
values for the pGLMs relative to the cGLM. To test the significance of individual
predictors for individual neurons, cGLMs and pGLMs were constructed for trial firing rate
vectors individually. Thus, for a single neuron, a distribution of trial fitness values was
generated for cGLMs and all pGLMs which could then be tested for significance.
Neurons were considered conjunctive in their periodic structure if they had significant
detriments to cGLM fits for multiple pGLMs.

cGLMs and pGLMs were also generated using behavioral (egocentric) predictors
in place of the spatial predictors described above. Behavioral predictors utilized were
linear speed, linear acceleration, and angular velocity profiles. Again, these models were
assessed using the mean firing rate profiles of individual neurons and mean behavioral
predictors, or individually for each trial. This method was applied to both plus and ring
track traversals.
Identification of symmetry points in spatial firing rate profiles

Points of symmetry (SP) within individual spatial firing rate profiles were identified for analysis of distance encoding in RSC ensembles. Identifying SPs was an iterative process wherein the spatial firing rate profile for each neuron was circularly rotated (‘circshift’ in MATLAB) a single position and the rotated vector was smoothed with a box car filter spanning 5 template bins (17.5 cm). The vectors were smoothed following rotations in an effort to reduce any drastic jumps in firing rate that may occur between the edges (first and final template bins) of the firing rate profile. After smoothing, the rotated vector was reflected from the center point and the NMSE was computed between the two halves and stored. This process continued until the vector had been rotated a full 360° and the fit between the two halves stored. The rotation that produced the maximal fit between the reflected halves was determined to be the SP. The rotated vector that corresponded to the SP was stored for further analysis.

Acknowledgements

Chapter 3, in full, is a reprint of the material as it appears in the following manuscript that is currently being prepared for submission for publication: Alexander, A.S., & Nitz, D.A. Retrosplenial cortex periodic activation patterns encode route subspace relationships and yield a metric of distance. The dissertation author was the primary investigator and author of this paper.

References


16. Kraus, B. J., Brandon, M. P., Robinson, R. J., Connerney, M. A., Hasselmo, M. E., & Eichenbaum, H. During running in place, grid cells integrate elapsed time


**Methods References**


Figure S3.1. Representative histology, sort quality, and head-direction assessment. A. Final placement of electrode tracts in RSC for example rats (n = 4). B and C. Left, example unit isolation and waveforms for a single tetrode. Right, corresponding spatial firing rate profiles for each neuron. Above each graph, scores for cluster quality metrics isolation distance and L-ratio. D. Cumulative distribution of sort quality metrics. Left, isolation distance. Right, L-ratio. For each plot, the cumulative percentage of neurons with sort quality values less than each value on the x-axis is shown in colored lines. In red, cumulative density of neurons that did not exhibit sensitivity to spatially periodic predictors. In black, cumulative density of neurons that exhibited sensitivity to a single spatial predictor. In blue, cumulative density of neurons that exhibited sensitivity to multiple spatial predictors. E. Mean (± std. error) isolation distance and L-ratio for neurons that were sensitive to full, half, and quarter spatial periodicities. F. Method for assessment of significant directional tuning. For each neuron, the mean directional tuning vector and resultant for the first and second half of the recording session were computed individually. Between the two blocks, RSC neurons that had mean tuning differences less than 0.35 radians, resultants greater than 0.2, and exhibited significantly non-uniform firing rate distributions as a function of heading (Rayleigh test, p<0.05) were identified as head-direction neurons and removed from further analyses. Two example neurons are shown in the bottom row of the graph with the tuning curves from the two halves of the recording session shown in blue and black.
Figure S3.2. Description of movement variables utilized in egocentric GLM during plus track running. A. Mean (± s.e.) linear movement variables across all recording sessions and animals (n = 91) for the full plus track. Top, linear velocity; Middle, absolute angular velocity; Bottom, linear acceleration. B. Mean (± s.e.) linear movement variables within a single recording session. Top three graphs: linear velocity, absolute angular velocity, and linear acceleration, respectively. Bottom plot, mean spatial firing rate profile (± s.e.) for a neuron recording during the corresponding session. In black, complete model fit, using all three movement variables, to the activation profile of the neuron.
Figure S3.3. RSC neurons exhibit conjunctive sensitivity to multiple sub-route and full route spatial oscillations. Mean spatial firing rate profiles (± s.e.) for three example neurons. Overlayed in black, iGLM fits for individual spatial predictors that significantly modulated each neuron (as assessed with tests of trial-by-trial pGLMs versus cGLMs). Above each plot, sort quality metrics for each neuron demonstrate that conjunctive sub-route sensitivity was not the product of poor unit isolation. Below each plot, corresponding spike trains across trials. Right, corresponding two dimensional ratemap with max firing rate indicated (blue indicates zero firing).
Figure S3.4. Description of movement variables utilized in egocentric GLM during ring track running. **A.** Top, schematic of ring track. Bottom two plots, example positional tracking from two rats and two ring track sessions. **B.** Mean (± s.e.) linear movement variables across all recording sessions and animals (n = 91) for the full plus track. Top, linear velocity; Middle, absolute angular velocity; Bottom, linear acceleration. Bottom plot, mean spatial firing rate profile (± s.e.) for a neuron during a single recording session. In black, complete model fit to the activation profile of the neuron using all three movement variables from the corresponding recording of the neuron.
Chapter 4: Neurophysiological signatures of interaction between retrosplenial cortex and the hippocampal formation

Abstract

Retrosplenial cortex (RSC) is a functionally significant processing hub between hippocampus (HPC) and the neocortex. RSC forms a feedback loop with the HPC while simultaneously communicating with a multitude of cortical regions important for complex behavior and sensory processing. As such, RSC is a likely coordinator of information transfer between the HPC and cortex for the purposes of spatial navigation and memory. Surprisingly, few studies have examined neurophysiological signatures of interaction between the two regions. The current work sought to characterize RSC-HPC communication by simultaneously recording single-units and local field potentials in the two regions while rats performed a variety of spatial navigation paradigms. We report that a sub-group of RSC neurons exhibited rhythmic spiking activity at the same frequency observed for neurons in the HPC. These neurons, as well as those without intrinsically rhythmic spiking, could be phase locked to theta oscillations reflecting synchronous synaptic activity within the HPC. Further interregional population interactions were observed during sharp wave ripples (SWRs), transient excitatory events in the HPC thought to coordinate offline processing for trajectory planning, memory consolidation, and memory retrieval.

Introduction

Several lines of research indicate that the hippocampus (HPC) is crucial for spatial cognition, learning, and memory\textsuperscript{1-3}. The retrosplenial cortex (RSC) has particularly interesting connectivity with the HPC and is also implicated in spatial and mnemonic processing. RSC serves as an anatomical intermediary between the HPC and cortex and likely influences HPC processing via excitatory projections into the
entorhinal cortex (EC). RSC receives HPC output through subiculum. As such, RSC-HPC circuitry may form an important feedback loop for both spatial and mnemonic processing. Despite this fact, little research has examined coordination between the regions for the purposes of spatial representation or memory function.

Single neurons in both HPC and RSC exhibit spatial responsivity, in that cells are discretely active in response to a particular spatial variable. In the HPC, this property is manifested as the ‘place cell,’ a functional cell type present in all sub-regions of the HPC that is activated when an animal occupies a particular position in the environment relative to distal cues. RSC spatial representations are more rich, in that they can represent the animal’s position in the environment, within a route, or the position of space relative to the animal (i.e. egocentric). In RSC spatial representations can also be conjunctive, suggesting a function of the circuit in transforming spatial information encoded in one coordinate system into positions in another.

There are several reasons to believe that the unique spatial encoding properties of RSC are likely critical for HPC representations. First, HPC place fields are modulated by spatial variables encoded by the RSC circuit, such as trajectory shape, heading direction, and actions. Secondly, individual place fields drift after prolonged periods in the dark, illustrating that the HPC requires sensory information updates to remain accurately anchored to space. RSC receives prominent visual inputs from early visual cortical areas and inactivation of RSC decreases the spatial reliability of place cell firing. The latter finding supports a function of RSC in updating and anchoring spatial representations of the HPC.

One potential mode of communication between RSC and HPC would be through synchronization of network oscillations. More specifically, the rat HPC local field potential reflects the presence of rhythmic synchronous modulation of HPC activity by the theta
rhythm (7-12Hz)\(^{20}\). Among many other things, the theta rhythm temporally organizes HPC spiking activity and has been proposed to gate information flow\(^{21-24}\). If RSC were to efficiently transmit information to the HPC, we might expect to see entrainment of individual RSC neurons to the HPC rhythm. There have been preliminary reports of RSC neurons exhibiting theta spiking activity for some time\(^{25}\), but no study has yet examined the phenomena in detail nor looked for RSC phase coupling to the HPC theta rhythm.

In rats and humans, damage to these regions leads to disorders of spatial navigation, as well as dysfunction of contextual, spatial, associative, and sequential learning and memory\(^{26-29}\). Perhaps unsurprisingly, both HPC and RSC exhibit atrophy and hypometabolism early in the progression of Alzheimer’s disease and related pathologies\(^{30}\) (e.g. mild cognitive impairment). Activation of cell assemblies in both regions is required for learning and recall, and there is evidence to suggest that memories can be offloaded to the RSC after initial encoding in HPC\(^{31-33}\).

A proposed neurophysiological mechanism for cortico-hippocampal communication underlying both spatial and memory processing is co-modulation during HPC sharp waves\(^{34}\). Sharp waves are irregular, large amplitude, excitatory oscillations that manifest in the apical dendrite layer of HPC sub-region CA1 and are accompanied by synchronous high frequency oscillations termed ‘ripples.’ The combination of the sharp wave and ripple has been denoted the sharp wave ripple or SWR. The events occur during awake immobility.

During SWRs, HPC pyramidal neurons exhibit sequential firing that can be decoded to predict the animal’s previous\(^{35}\) or future trajectories\(^{36}\) through the environment. Thus, the SWR is thought of as an offline processing state, in that activity of neurons during the oscillation is decoupled from their spatial correlates. The temporal window of single-unit activity during SWRs is consistent with spike timing dependent
plasticity\textsuperscript{34}, and thus, co-modulation during these events could strengthen cell assemblies. Critically, cortical ensembles in some regions exhibit alterations in activation during awake SWRs\textsuperscript{37}. This latter finding indicates that SWRs are important temporal windows of communication between HPC and cortex. Given this fact, it is surprising that the RSC, which receives HPC output via the subiculum, has not been examined for SWR modulation.

To test modes of RSC-HPC interaction we recorded units and local field potentials from both RSC and HPC in rats performing navigation paradigms. We report that a population of RSC neurons exhibit theta rhythmic spiking activity and are phase locked to the HPC theta rhythm. Further, we report the existence of RSC neurons without theta spiking that nonetheless exhibit significant locking to HPC theta oscillations. We also report the presence of SWR-like events in RSC as well as RSC neurons modulated by HPC SWRs.

Results

256 RSC neurons were recorded across 5 rats from RSC as animals performed various track running tasks, free foraged, or pursued a moving target in an open arena (Figure 4.1A). For the forgoing analyses, single-unit activity was recorded and collapsed across all RSC sub-regions (Figure 4.1B, top). In all animals, simultaneous single unit recordings were made in CA1 (n = 444) of the HPC (Figure 4.1B, bottom). HPC recording electrodes were also used to monitor the local field potential for theta rhythmicity or SWR events (Figure 4.1C). A majority of RSC neurons were recorded from the opposite hemisphere as the HPC local field potential.
Figure 4.1. Behavioral paradigms, electrode placements, and identification of relevant oscillatory signals in local field potentials. **A.** Electrophysiological data was collected from an assortment of tasks including plus track running (top, taken from Chapter 3 data), w-track running (middle, taken from Chapter 2; Alexander and Nitz, 2015), and ring-track running (bottom, taken from Chapter 3 data). In blue on right, actual tracking data from a single recording session on each task. Black circles are positions of the rat when SWR events occurred. Data was also taken from recordings in which the rat freely foraged or pursued a target in a circular arena the same diameter as the ring-track (not depicted). **B.** Top row, placement of electrode wires across all animals in RSC collapsed across hemispheres. Right, example electrode tract in tissue. Bottom row, same as top, but for placement of electrodes in dorsal HPC. **C.** First row, 2 seconds of raw local field potential with identified SWR in center of trace. Second row, corresponding linear speed of the animal. Third row, corresponding rectified value of SWR filtered (150-250Hz) LFP in blue. In black, slow modulation (filtered 0.75-6Hz), of SWR filtered (blue trace) LFP signal used to identify peaks in SWR power. Fourth row, corresponding theta filtered (7-11Hz, black trace) LFP with theta phase depicted in gray.
RSC neurons exhibit two forms of synchronization with HPC synaptic inputs

To assess potential synchronization of RSC and HPC unit activity we first examined the intrinsic rhythmicity of RSC single units through autocorrelations of spike trains. It has been widely reported that a majority of HPC principal cells and interneurons exhibit theta rhythmicity in their spiking activity. Thus, we hypothesized that RSC neurons could potentially interact with HPC circuitry by synchronizing their activation with this oscillation. To test this possibility, we computed the power spectral density (PSD) of each neuron's spike train autocorrelation and then examined the magnitude of power in the theta frequency range (7-12Hz) relative to the entire spectrum. If theta power was 90% greater than the average power for the entire PSD the neuron was classified as theta rhythmic. Using this method, 22.6% (n = 58/256) of RSC neurons had theta rhythmicity in their spike trains (Figure 4.2A, left). The proportion of theta rhythmic RSC neurons was lower than that observed in CA1 of the HPC (87.6%, n = 389/444). This finding indicates that a sub-population of individual RSC neurons fire in synchrony with HPC cells.

RSC signals may also coordinate with HPC synaptic inputs via spike-phase coupling to the theta rhythm recorded in the HPC local field potential. This possibility was examined by bandpass filtering the LFP recorded in dorsal CA1 of the HPC for theta frequency and utilizing a Hilbert transform to estimate phase (Figure 4.1C, bottom row). Spike phase firing rates were then computed as a function of theta phase (Figure 4.2A, bottom row). 29.7% of RSC neurons (n = 76/256) exhibited significant coupling to specific phases within a single HPC theta period (Figure 4.2B, left; Rayleigh test for non-uniformity, p<0.01).

A prominent LFP theta oscillation was also observed in RSC. High-amplitude, low frequency oscillations like the HPC theta rhythm can be observed in multiple non-
generative regions via volume conduction. However, it was possible that RSC theta oscillations were local to the cortical region for two reasons. First, RSC neurons can be theta rhythmic and thus oscillatory generators through intrinsic circuitry. Secondly, RSC receives inputs from both the medial septum (MS) and MEC which are critical for theta rhythmic oscillations observed in HPC. As such, we examined whether RSC neurons were phase modulated by the theta phase observed in RSC. We found that roughly the same proportion of neurons were phase coupled to RSC theta (31.6%, n = 81/256; Figure 4.2B, right) and that a majority of the neurons were modulated by theta phase in both regions (88.8%, n = 72/81). For neurons that were theta locked to both RSC and HPC LFP, the mean preferred phase and tuning strength (length of resultant vector) were highly similar (Figure 4.2C, left and middle). These results indicate that the RSC theta rhythm is not independent of theta oscillations observed in the HPC but further analysis is needed to assess the directionality of interaction or possibility of a common oscillatory source(s).

Of neurons with significant HPC theta phase modulation, 50% (n = 38/76) also had intrinsic theta rhythmic spiking activity as assessed via analysis of their spike train autocorrelations (Figure 4.2A, left column). However, not all neurons with HPC theta phase coupling were intrinsically theta rhythmic in their spiking activity (Figure 4.2A, right column). 50% (n = 38/76) of neurons that were coupled to theta phase in HPC did not have significant theta rhythmicity in their spike trains. Roughly similar proportions of theta-rhythmic and non-theta-rhythmic RSC neurons were found to be entrained to the theta rhythm recorded in RSC (47% and 53%, respectively). For neurons that were coupled to both RSC and HPC theta phase, theta rhythmic neurons had statistically stronger mean tuning than non-theta-rhythmic neurons that were similarly phase modulated (Wilcoxon rank-sum test, $\mu_{\text{Theta}} = 0.16$, $\mu_{\text{NotTheta}} = 0.11$, $z = 3.20$, $p = 0.001$;
Figure 4.2C, right). These findings indicate that there are potentially distinct subpopulations, rhythmic and non-rhythmic RSC neurons, capable of coordinating their activation with HPC synaptic oscillations.

**Figure 4.2.** RSC neurons phase lock to HPC and RSC theta oscillations independent of intrinsic theta rhythmicity. A. Top row, autocorrelations of theta rhythmic and non-theta rhythmic spike trains for two RSC neurons. Bottom, for each neuron above, the corresponding phase coupling to HPC (in blue) and RSC theta (in black). B. Peak sorted phase tuning curves for all RSC neurons with significant theta phase coupling to HPC (left) or RSC (right) theta phase. C. Left, for RSC neurons significantly tuned to both RSC and HPC theta oscillations, the mean of the phase tuning curve for RSC versus HPC. Red line depicts where points would fall if the mean phase was identical for the two simultaneously recorded oscillations. Middle, for RSC neurons significantly tuned to both RSC and HPC theta oscillations, the strength of theta phase tuning (length of resultant vector) for RSC versus HPC. Red line depicts where points would fall if the tuning strength was identical for the two simultaneously recorded oscillations. Right, mean strength of theta phase tuning is significantly greater for theta rhythmic RSC neurons versus RSC neurons with non-theta rhythmic spike train autocorrelations.
RSC single units and local field potential are modulated by gamma and SWR frequencies

With respect to both the HPC and RSC LFP, the total number of theta modulated RSC neurons was greater than the distribution of expected numbers of theta phase modulated neurons computed after circularly jittering the LFP phase 25 times (Figure 4.3A). However, this analysis revealed a divergence between HPC and RSC synaptic oscillations when considering phase-coupling to higher frequency bands. Specifically, RSC neurons were phase locked to high gamma (hGamma) frequencies (80-140Hz) and SWR frequencies (150-250Hz) observed in the RSC LFP but did not exhibit modulation to these frequency bands from HPC LFP (Figure 4.3A). Figure 4.3B depicts four RSC neurons with phase-coupling to these frequency bands in the RSC LFP but not HPC. 16% (n = 40/256) of neurons had significant phase coupling for hGamma (n = 31/40) and/or SWR frequency bands (n = 31/40; Figure 4.3C). Of those, 55% (n=22/40) were modulated by both bands. Critically, many of these neurons were simultaneously modulated by theta oscillations, either in their spiking activity or in theta phase coupling (50%, n = 20/40). This latter fact, in which single RSC neurons are simultaneously synchronized with distinct HPC and RSC oscillatory frequencies, highlights a mechanism by which local neural activity in RSC can be integrated with HPC-related synaptic activity.
Figure 4.3. RSC neurons are phase locked to high frequency oscillations in RSC local field potential but not in HPC local field potential. A. Proportion of neurons exhibiting significant phase locking to theta (θ), beta (β), low gamma (γ), high gamma (γ′), and SWR frequency bands when tested in both HPC and RSC local field potentials. Red dots and error bars represent the mean and standard error of proportions with significant phase locking following circular jitters of LFP phase relative to spike times (25 iterations). B. For four RSC neurons, phase coupling to high gamma (top row) and SWR frequencies (bottom row) is observed for RSC LFP (in black) but not HPC LFP (in blue). C. Peak sorted phase tuning curves for all RSC neurons with significant high gamma phase coupling (left) or SWR phase coupling (right).
RSC local field potential oscillations are modulated by HPC sharp wave ripples

The observation of RSC gamma phase coupling was not wholly unsurprising, given that experiments have indicated that power in higher gamma frequencies is correlated with spiking activity in local circuits. However, the presence of spike-phase coupling to SWR frequencies was interesting for several reasons. SWRs have been shown to initiate in HPC CA3, but there is little evidence for generation of SWR-like events occurring outside of the HPC. Further, low-amplitude, high-frequency oscillations do not volume conduct between structures in a reliable manner.

Thus, there were at least two possible explanations for local SWR modulation of RSC neurons. First, RSC may possess intrinsic circuitry capable of independently initiating SWR-like events. If this were the case, we might expect to observe isolated SWRs in RSC. Secondly, it is possible that the slower frequency excitatory component of HPC SWRs, called the sharp wave, potentially reached RSC where it could initiate a local ripple event that modulated RSC neurons. Both hypotheses were supported by the fact that RSC neurons were phase-locked to RSC SWR frequencies but not HPC, which implied that the SWR band was distinct between the two structures in some manner.

Constraints on the current recording setup, such as the lack of a laminar probe in RSC, cross-hemisphere recordings, and variability in HPC electrode locations, made answering these questions difficult with the current dataset. Regardless, the observation of RSC SWR phase coupling indicated that other features of this form of HPC-RSC communication could be characterized at this time.
We began by identifying SWR events in both RSC and dorsal HPC CA1 during moments in which the animal was awake but immobile (speed < 10cm/s; **Figure 4.4A**; **see also Figure 4.1C, top three plots**). To determine whether SWR events in HPC modulated synchronous synaptic potentials in RSC, a HPC-SWR triggered spectrogram was computed for the LFP recorded in RSC (**Figure 4.4B, top**). From both individual SWR-triggered spectrograms and the average across all such events (**Figure 4.4B, bottom**), we observed that there were RSC power increases in both the SWR frequency range (150-250Hz) and high gamma frequency ranges (approx. 80-140Hz).

In some cases, the power in SWR and high gamma frequency ranges increased prior to SWR detection in the HPC LFP (**Figure 4.4B**). If this were the case, it could...
indicate a bi-directional interaction between RSC and HPC SWR-like events. To explore this possibility, we looked for the co-occurrence of SWR events in RSC within 250ms of HPC SWRs. RSC SWRs often occurred in close temporal proximity to SWRs detected in HPC (Figure 4.4C). In some cases, RSC SWRs appeared to precede those observed in the HPC.

There are several issues to consider when assessing this finding. Mainly, there are multiple factors that make determining the actual onset of a HPC SWR difficult. These factors primarily include the observation that SWRs can be generated locally within the HPC formation\(^42\), are also observed in RSC-HPC intermediary subiculum\(^42,43\), and that SWR events can move through HPC as a traveling wave\(^34\). Collectively, these findings make an assessment of the direction of SWR ‘flow’ complex. Nevertheless, the temporal relationship between SWR activity in HPC and RSC is consistent with previous literature examining SWR modulation in cortical regions connected to the HPC.

**RSC single units are modulated by HPC sharp wave ripples (SWRs)**

If HPC SWRs reach RSC and impact the circuit, we hypothesized that there would be SWR-triggered firing rate alterations to RSC single-units as observed in HPC (Figure 4.5A) and other cortical structures\(^37\). To assess this possibility, we restricted the following analyses to those recordings in which HPC electrodes were known to lie in or just above the pyramidal cell layer of HPC CA1. 19% of RSC neurons (\(n = 39/205\)) recorded under these conditions exhibited significant modulation during SWR events recorded in HPC (Figure 4.5B). Of RSC neurons transiently modulated, roughly equal proportions were either excited (56%, \(n = 22/39\)) or inhibited (44%, \(n = 17/39\)) in response to awake SWRs recorded in HPC (Figure 4.5C).
An examination of the time-course of SWR modulation revealed that both SWR-excited (eRSC) and SWR-inhibited (iRSC) RSC populations reached peak modulation after the mean peak in modulation observed in the HPC (Figure 4.5D). The latency to peak modulation did not differ between the eRSC or iRSC groups (Wilcoxon rank-sum test, $\mu_{eRSC} = 113.4$ms, $\mu_{iRSC} = 106.2$ms, $z = -0.13$, $p = 0.89$; Figure 4.5E, left). The mean firing rate of eRSC and iRSC sub-populations appeared to flip in proximity to SWR events, with the eRSC group moving from lower baseline firing rates to higher firing rates and the iRSC group exhibiting the inverse pattern (Figure 4.5D). However, this
qualitative observation was not reflected in mean firing rates of the two groups, which were not significantly different (Wilcoxon rank-sum test, $\mu_{eRSC} = 7.46\text{Hz}, \mu_{iRSC} = 8.15\text{Hz}, z = 0.78, p = 0.44$; Figure 4.5E).

**Discussion**

The current work identifies and characterizes several forms of communication between the HPC and RSC. Specifically, we identify a population of RSC neurons that have intrinsic theta rhythmicity in their spiking activity, which can be synchronized to HPC synaptic potentials via phase coupling to theta oscillations within the region. We also report a population of RSC neurons that are phase coupled to HPC theta oscillations in the HPC, despite the neurons themselves not exhibiting significant intrinsic theta rhythmicity. Cortical theta phase coupling has been reported in other regions but not in RSC at this time$^{37,44}$. Future work is needed to assess whether this latter population exhibits theta rhythmic firing solely at specific moments, thus enabling local field potential theta coupling in the absence of significant theta rhythmic spiking across the entire spike train. The data currently indicates that there are potentially two distinct populations of RSC neurons capable of HPC synchronization in this form.

We also report the presence of RSC neurons that are phase locked to higher frequency oscillations observed in RSC local field potentials. Of particular interest, RSC neurons are phase modulated by SWR frequencies recorded in RSC, but not those same frequencies observed in HPC. Despite the divergence in SWR-coupling between RSC and HPC field potentials, independent detection of the sharp wave component of SWRs in RSC and HPC revealed that the presence of this oscillation in the two regions often occurred in close temporal proximity (usually $<10\text{ms}$)$^{43}$. In this manner, there is an interdependence between RSC SWR phase coupling and the HPC. This relationship could take many forms, including the possibility that HPC sharp waves reach RSC and
influence local cortical circuitry to instantiate ripple like activation. A second possibility is that high frequency components of RSC SWR events are more strongly correlated to those observed in the subiculum\textsuperscript{42}, which functions as the output of the HPC to RSC.

Interestingly, sub-populations of individual RSC neurons were either excited or inhibited by the onset of SWRs in the HPC despite the fact that RSC neurons were not phase modulated by ripple frequencies observed in the region. This finding suggests that RSC neurons are modulated by the aperiodic sharp wave component of HPC SWRs but not the higher frequency portion. Thus, RSC neurons are influenced by HPC SWRs at a lower temporal resolution than that observed for neurons within HPC. This fact may indicate that HPC SWRs cause more general network alterations to RSC rather than sequentially organizing cell assemblies as reported in HPC. If this were the case, the presence of RSC neurons that are phase-coupled to local SWR frequencies could indicate parallel circuitry for coordinating RSC cell assemblies locally following HPC interaction. An examination of the temporal properties of neural co-activation, both within RSC and between RSC and HPC, may reveal interesting dynamics regarding these issues\textsuperscript{45-47}.

Although not specifically tested here, it is likely that synchronization between these regions is bidirectional. As associative cortex, RSC is positioned to integrate a barrage of sensory and top-down inputs from posterior and frontal cortices\textsuperscript{4-8,18}. Combination of these signals with HPC mnemonic functionality could be critical for memory formation and retrieval\textsuperscript{48}. The current work provides the first evidence of this coordination at the level of single units and identifies novel forms of oscillatory network synchronization.

From a spatial cognition standpoint, efficient transfer of sensory information to HPC is extremely important for updating, and even initially constructing, spatial
representations in the HPC and associated structures. However, RSC also serves as a conduit in the opposing direction, by integrating HPC computations with processing in cortical regions. Spatial representations of the HPC formation are critical for complex behavior in the environment, as lesions or inactivation of the region decrement goal-directed actions, learning, and navigation. Further, offline HPC activation patterns correlate with movement planning. From this perspective, coordination of RSC-HPC circuitry could be significant for utilizing HPC spatial and trajectory planning output to modulate cortical activity for the purposes of guiding movement behavior or other higher-level cognitive operations.

**Methods**

**Subjects**

Male Long-Evans rats (n = 5) served as behavioral subjects and were housed individually and kept on a 12-h light/dark cycle. Animals were habituated to the colony room and handled daily for a period of 1-2 weeks prior to training on either track running, free foraging, or target chasing tasks. Rats were food restricted to approximately 85-90% of their free-fed weight. Water was available continuously. All experimental protocols adhered to AALAC guidelines and were approved by IACUC and the UCSD Animal Care Program.

**Behavior**

**Plus track running paradigm**

Three rats were trained to navigate around plus-shaped tracks for reward. The track edges were 2 cm in height, which allowed the animal an unobstructed view of the full environment. Animals ran the plus track in the clockwise direction.

Two rats were trained to run half traversals in addition to the full traversals that all rats ran. On half route trials the rat started at the quarter point and stopped a quarter
from the endpoint. The animal was required to stop at the correct endpoint on its own volition, without cuing from the experimenter. If the animal stopped correctly, a reward (¼ honey nut cheerio) was placed on the track near the animal for consumption, otherwise no reward was given. Half trials were randomly interspersed during all plus track sessions. For both full and half route traversals, animals were picked up between trials and carried in random trajectories to the trial initiation point.

*Ring track running paradigm*

One rat in the current dataset traversed a ring-shaped track, in addition to the plus-shaped track, for reward. The ring track was placed in the same general allocentric position as the plus-shaped track relative to the recording room. Track edges on the ring were 4cm in height. Rats ran the ring track and plus tracks in the same session, with the two tracks existing is overlapping positions within the room. Ordering of track conditions across days was counterbalanced to account for any potential sequence effects. Animals were trained to traverse the ring track in both directions. Fixed spatial cues on the walls ensured consistent spatial relationships that defined the boundaries of the recording room across days.

*Target chasing paradigm*

Two rats were trained to pursue a moving light stimulus in a 4ft diameter circular arena for reward. The light stimulus was an approximately 1 cm dot from a bright green laser pointer controlled by the experimenter. The arena was placed on a table 3ft off of the ground. The boundaries of the arena were approximately 2.5cm in height and there were fixed distal cues on the walls outside of the arena across all sessions.

Animals were shaped to pursue the light stimulus target in a series of steps. First, animals were habituated to the arena for one week by randomly placing ¼ honey nut cheerio pieces around the arena and allowing the animal to free forage for 20 minutes
per day. After the animal was freely moving around and spending significant time in the center portion of the arena the light stimulus was introduced. For a 20-minute period per day, between 5-10 cheerio pieces would be present on the arena at any given time and the light stimulus would hover on top of cheerios that the animal was about to ingest. After the animal acquired a given cheerio piece that the light stimulus was positioned on, the light stimulus would shut off. This began to create an association between light stimulus and reward.

After approximately one week of this process the next phase of shaping began. Here, a single cheerio piece was tossed to a random position in the arena at a time. The light stimulus hovered over the reward and shut off when the animal retrieved it. This step repeated continually for a 20-30-minute session for approximately one week or until the animal was readily running to the light stimulus/cheerio position.

In the final shaping phase, the onset of the light stimulus would occur in the absence of a reward in the arena. Because the light stimulus was associated with reward, the animal would approach it. Prior to the rat reaching the stimulus, the light would begin to move smoothly away from the animal and the animal would pursue. If the animal occluded the stimulus by ‘catching’ it, the light stimulus would shut off and a reward would be tossed into a random location within the arena. The light stimulus would then turn back on and hover over the rewards location, and would shut off after the animal retrieved the reward. Following reward consumption, the next trial would begin by turning the light stimulus on in a random location within the arena and the aforementioned process would repeat. Over time, the length of pursuit could be extended without causing the animal to lose interest or become frustrated. This training phase could last between 2 weeks to 1.5 months depending on the animal.
After shaping, rats would chase the light stimulus for approximately 20-minutes per day during concurrent in vivo electrophysiological recordings. The temporal length of individual pursuits lasted anywhere between 0.5-5s on average. The number of pseudorandom pursuits within a session was typically between 40 and 80. Further, characteristic laser patterns were instantiated, in which the moving target would execute a stereotypical path through the arena that began and ended at the same approximate allocentric locations within the arena and recording room. These characteristic paths occurred less frequently (< 20 times) than random pursuit trajectories and were randomly interspersed throughout the target chasing block. For most sessions, the target chasing block occurred between free foraging blocks (approximately 10 minutes each).

**Surgery**

Rats were surgically implanted with tetrode arrays (twisted sets of four 12 micrometer tungsten wires or 17 micrometer platinum-iridium wires) fitted to custom-fabricated microdrives that allowed movement in 40µm increments. Each microdrive contained 4 tetrodes. Rats were implanted with 3 total microdrives across RSC and HPC. Rats were anesthetized with isoflurane and positioned in a stereotaxic device (Kopf Instruments). Following craniotomy and resection of dura mater overlying the retrosplenial cortex, microdrives were implanted relative to bregma (A/P -5.8 mm, M/L ± 0.7-1.2 mm, D/V -0.5 mm, 10-12° medial/lateral angle). 3 animals received a unilateral HPC microdrive targeted to the CA1 sub-region (target coordinates relative to bregma, A/P -3.8mm and M/L ± 2.3mm, D/V -0.5mm).

**Recordings**

Each microdrive had one or two electrical interface boards (EIB-16, Neuralynx) individually connected to amplifying headstages (20X, Triangle Biosystems). Signals were initially amplified and filtered (50x, 150Hz) on the way to an acquisition computer.
running Plexon SortClient software. Here the signal was digitized at 40kHz, filtered at 0.45-9 kHz and amplified 1-15X (to reach a total of 1,000-15,000X). Electrodes were moved ventrally (40µm) between recordings to maximize the amount of distinct units collected for each animal.

Animal position was tracked using a camera set 10ft above the recording room floor. Plexon’s CinePlex Studio software was utilized to separately detect blue and red LED lights. Lights sat approximately 4.5 cm apart and were positioned perpendicular to the length of the animal’s head. During the target chasing paradigm, the green visual stimulus was simultaneously tracked using the same software.

For track running, recordings lasted approximately 45 minutes, the amount of time needed for the animal to complete an absolute minimum of 5 ballistic runs for plus and/or ring track conditions. For target chasing, recordings were on average 45 minutes, the amount of time needed to acquire suitable quantities of target chasing trials as well as free foraging blocks with adequate spatial coverage.

*Unit isolation and sort quality*

Single-units were identified using Plexon OfflineSorter software. Primary waveform parameters utilized were peak height, peak-valley, energy, and principal components. The only RSC units that were excluded from analyses on the tracks were those that did not exhibit peak activation of at least 3Hz for a single track bin and those that were statistically identified as head direction neurons. HPC neurons were excluded if they had mean activity across all track sessions greater than 8Hz (i.e. fast-firing, putative interneurons), no bins in which the firing rate dropped to 0Hz (i.e. fast-firing, putative interneurons), and no bins exhibiting a peak firing rate amplitude above 3Hz.
Histology

Animals were perfused with 4% paraformaldehyde under deep anesthesia. Brains were removed and sliced into 50µm sections and Nissl-stained to identify the trajectory and depth of electrode wires in RSC and HPC. RSC was defined in accordance with our previous work(ref) in the region as well as the Paxinos and Watson and Zilles atlases. Dorsally, the lateral boundary of RSC was considered to be 1-2 mm lateral to the lip of the cingulate bundle. Ventrally, the lateral edge was defined by the transition from retrosplenial cortex to the subiculum. All tetrodes were determined to be within the bounds of RSC. Documented micro-drive depth across recordings and final electrode depth observed in histology were compared and found to be compatible in all cases.

Data analysis
Spike train autocorrelations and test of intrinsic theta rhythmicity

For each recorded neuron an autocorrelation of the spike train was computed using the ‘xcorr’ function in MATLAB. The temporal resolution of the spike train was 1000Hz to match sampling of the simultaneously recorded local field potential. A power spectral density estimate (‘pwelch’ in MATLAB) was computed for the first 0.5s of the autocorrelation. The peak within the theta frequency range (7-11Hz) was identified and the average power was computed within 1Hz of said peak. The ratio between this value and the mean of the entire power spectrum (1-500Hz) was assessed. If the mean power within the theta frequency range was 90% greater than the mean power in the entirety of the spectrum the neuron was identified as having intrinsic theta rhythmicity in its spiking activity.
Local field potential phase coupling

For each recording, 16 total local field potentials (LFP) were recorded from wires placed in either RSC or HPC. Each LFP was examined individually for noise artifact and overall quality and the best LFP from each region and hemisphere (if possible) was identified and stored for that recording. LFPs were then band-pass filtered in MATLAB for theta (6-12 Hz), beta (20-35Hz), low gamma (45-65Hz), high gamma (80-140Hz) and SWR frequencies (150-250Hz). For the entire filtered LFP, the phase of each frequency band was estimated using a Hilbert transform (‘hilbert’ in MATLAB).

For each RSC neuron, the firing rate as a function of phase within each of the aforementioned bands was calculated. Phases were discretized into 5 degree bins and the number of spikes and total occupations for each phase bin were calculated for each neuron. For each neuron, the firing rate tuning curves as a function of phase were calculated by dividing the total number of spikes in each phase bin by the total amount of time that the phase bin was occupied in seconds. Tuning curves were smoothed via convolution with a moving low pass filter spanning 100 degrees to remove high frequency fluctuations created from using relatively small phase bins. For ease of visualization, all phase tuning curves are normalized via z-scoring, but all calculations of mean phase and strength of tuning (i.e. resultant vector lengths) were computed on peak normalized tuning curves using the Circular Statistics toolbox for MATLAB(Berens).

To assess whether a neuron had non-uniform phase related firing a Rayleigh test for non-uniformity was calculated using the same toolbox. Neurons with p-values less than 0.01 were determined to have significant tuning.

To test whether the proportion of neurons with significant phase tuning within the population was greater than expected by chance we decoupled phase and spike time relationships. To do this, we circularly jittered the LFP phase a random amount relative
to spike times, computed phase tuning curves, and tested for non-uniformity (significant Rayleigh tests). This process was repeated 25 times to acquire a distribution of the proportion of RSC neurons expected to be phase coupled by chance, which could then be statistically tested against the actual number of neurons exhibiting phase locking to non-jittered data.

Strength of phase coupling comparisons between theta rhythmic and non-theta rhythmic neurons were assessed by averaging the mean phase and mean tuning strength across both RSC and LFP theta phase tuning curves. The distributions were then tested using Wilcoxon ranksum.

**SWR detection**

Detection of SWRs was performed using custom MATLAB software developed specifically for this work. HPC and RSC LFP were initially filtered within the 150-250Hz (SWR) range. This filtered signal was then rectified and filtered again in the 0.75-6Hz range. The slow frequency filter of the SWR range filtered signal was intended to detect large power changes to the SWR frequency band that would be correlated with the sharp wave component of the SWR. Peaks in the slow filtered signal were identified, and those that were 3 standard deviations greater than the average power in the entire signal were extracted as potential SWR events. Next, any potential SWR events that occurred while the animal was moving at speeds greater than 10 cm/s were removed, as SWRs are well known to occur during awake immobility. Finally, any SWRs that occurred within 1s of each other were removed from the potential SWR pool, in an effort to accurately assign spikes to single ripples as well as remove any potential chew artifact erroneously classified as SWRs.
**SWR modulation**

Modulation of RSC neurons by SWRs was assessed using a modified version of the method previously described by Jadhav et al., 2015. We restricted this analysis to recordings made in or slightly above the HPC CA1 pyramidal cell layer where SWR detection is most accurate. For each neuron, we extracted the SWR-aligned spike rasters for 1 second centered on all SWRs within the recording. From this data, a SWR-aligned firing rate vector was computed. The same process was repeated 2000 times, each time for data in which the spike train was circularly rotated a random fixed amount relative to SWR onset times.

SWR modulation of the neuron was calculated by finding the mean squared error between activation in the 0-200ms window after SWR onset in the real SWR-aligned firing rate vector and activation in the same time window averaged across all 2000 randomizations. To attain a distribution of SWR modulation values for each neuron, the mean squared error between activation in the post-SWR window for each randomization versus the mean of all randomizations was also calculated. Neurons with real SWR modulation values that exceeded the 95th percentile of randomized SWR modulation values had significant SWR-aligned activation changes. To determine whether RSC neurons with significant SWR modulation were excited or inhibited, the SWR-aligned firing rate in the 0-200ms window following SWR onset was compared to the window -500 to -100 ms preceding SWR onset. If the post-SWR window had greater firing than the pre-SWR window the neuron was classified as excited, and vice versa for the inhibited population. The only neurons excluded from the above analysis were those that had less the 25 total spikes across all SWR-aligned rasters.
Time course of SWR Modulation

The peak or trough of SWR modulated neurons was found as the difference in milliseconds between SWR onset and the max modulation (either excitatory or inhibitory) in the SWR-aligned mean firing rate. Comparisons of onset times were tested with a Wilcoxon rank-sum test.

Acknowledgements

Chapter 4, in full, is a reprint of the material as it appears in the following manuscript that is currently being prepared for submission for publication: Alexander, A.S., & Nitz, D.A. Neurophysiological signatures of interaction between retrosplenial cortex and the hippocampal formation. The dissertation author was the primary investigator and author of this paper.

References


33. Czajkowski, R., Jayaprakash, B., Wiltgen, B., Rogerson, T., Guzman-Karlsson, M.C., Barth, A.L., Trachtenberg, J.T. and Silva, A.J. Encoding and storage of


Chapter 5: Spatial computations of the Retrosplenial cortex

The dissertation work has examined the retrosplenial cortex with the goal of identifying its potentially unique contribution to spatial cognition. Until recently, neurophysiological properties of RSC have been relatively unexplored\textsuperscript{1-5}, especially when considering its position within the classic Papez circuit\textsuperscript{6}. The data presented here characterizes several novel spatial representations of the RSC, and posits modes by which these mappings may contribute to or reflect spatial computations and transformations important for all neural spatial systems. First and foremost, this work was guided by the unique anatomy of RSC, in that the region is reciprocally interconnected to virtually all structures known to support spatial cognition\textsuperscript{7-13}.

Given the centralized nature of RSC within this circuitry, we hypothesized that neural dynamics within the structure may function to subserve several unclear components of spatial representation related to the transformation, interrelation, and organization of spatial relationships across or within coordinate systems. Thus, the experiments were designed to address these issues, which were outlined in Chapter 1 of the dissertation and reiterated here. Briefly, these studies hypothesized and/or elucidated that RSC is an important structure for: 1) neural transformations of locations across and within internal and external spatial reference frames, 2) identifying and extracting sub-components of complex spaces, 3) detecting spatial hierarchies useful for organizing and interrelating spatial maps, and 4) tracking distance traveled as a function of position in the environment.

The characterization of RSC spatial firing patterns reported in Chapter 2 provided significant evidence that the region is involved in transformations across distinct spatial coordinate systems. This data provided the first evidence that sub-populations of RSC neurons exhibit activity patterns anchored to the animal’s position in the environment,
but also the position of the environment relative to the animal. Thus, we identify RSC as a unique structure in that it possesses neurons whose spatial receptive fields are anchored in distinct spatial reference frames.

Even more pertinent to the issue of spatial transformation, individual RSC neurons exhibited conjunctive responses to multiple spatial reference frames simultaneously. Put another way, a single RSC neuron became activated when spatial parameters in multiple coordinate systems were simultaneously satisfied. This form of conjunctive encoding had been previously reported within egocentric systems useful for converting visual information into motor output\textsuperscript{14}, but no such result had been shown for more abstract spatial representations related to the animal’s position in the external world. Computational modeling efforts have demonstrated that networks of neurons with conjunctive responses can transform spatial positions across reference frames\textsuperscript{15}. Thus, this work demonstrates for the first time that RSC single units and ensembles subserv spatial transformations across egocentric, route-centric, and allocentric spatial reference frames.

After showing that RSC was capable of forming complex spatial representations that were sensitive to multiple spaces simultaneously, it was a natural next step to examine other modes by which this encoding property may manifest. To this end, the experiment of Chapter 3 was designed to determine if RSC neurons were capable of extracting and encoding analogous sub-routes within complex routes. If observed, this data would support the hypothesis that RSC ensembles can also compute spatial relationships within individual coordinate systems (e.g. route-centric reference frames). Accordingly, we observed that individual RSC neurons exhibited periodic firing patterns, at multiple spatial scales, along a track with recurrent spatial structure. These firing rate profiles repeated in a manner such that analogous positions across multiple repeats of
the same sub-route had similar firing rates. At the same time, RSC ensembles distinguished every position along the track, thus providing an encoding framework by which sub-routes could be organized relative the whole route.

This fact was further supported by individual RSC neurons that conjunctively encoded multiple sub-routes simultaneously through embedded periodic activation patterns. Often, these conjunctive sub-route representations were composed of a repeating pattern subordinate to the full route space in combination with a firing pattern that oscillated a single time between the start and end points of the track. Thus, in addition to recognizing analogous positions within sub-components of the route, an individual neuron of this type could hierarchically arrange sub-spaces relative to the full space. In this manner, these results demonstrate a neural circuit capable of fragmenting a complex space into useful sub-components while concurrently maintaining a representation of the sub-components relative to each other and the whole space\textsuperscript{16,17}.

Chapter 3 also reports a mechanism by which RSC neural ensembles track the animal’s distance from fixed points in route space. RSC neurons, especially those that exhibited firing rate profiles that had a single cycle of activation around the track, were discovered to have points of symmetry in their spatial representations. By aligning these symmetry points across multiple neurons, it was possible to decode the rat’s distance from the symmetry point. This finding indicated that RSC, and perhaps other structures with similar spatial activation profiles, could track the position of an animal from every point along the route. If true, it might indicate that RSC can integrate spatial representations of the MEC or HPC for the purposes of path integration.

Finally, modes by which RSC interacts with other spatial systems were identified for the first time. Chapter 4 describes entrainment of individual RSC neurons to synaptic oscillations recorded in the HPC. Further, these analyses describe modulation of RSC,
at the level of both individual neurons and network oscillations, by HPC initiated oscillatory events thought to reflect ‘offline’ processing\textsuperscript{18}. Consequently, we identify two modes by which RSC may synchronize the output of its unique spatial computations with downstream structures critical for spatial cognition and memory. Recently, use of paired in vivo electrophysiological recordings with virtual reality experimental paradigms has highlighted the importance of sensory influences on spatial representations in the HPC and connected structures\textsuperscript{19}. In light of this work, there is renewed interest in the modes by which cortical information may be relayed to the HPC via RSC.

This collection of data point to RSC as a critical processing hub for the integration of neocortical sensory processing with representations observed in sub-cortical systems necessary for learning and memory. Although the current work has focused on the function of RSC in spatial cognition, it is important to note that the region and neural dynamics described are likely adaptable to other domains. As with all association cortex, it is important to consider that the responsivity observed is potentially category-free\textsuperscript{20}, which would optimize the efficiency of cortical processing\textsuperscript{21}. RSC is implicated in a number of non-spatial learning and memory tasks. The ability of the network to translate between spatial coordinate systems or extract sub-components of a route could also be flexibly recruited to associate and generalize cross-modal stimuli in learning\textsuperscript{22,23} or encode non-spatial sequences\textsuperscript{24,25}, respectively.

Returning to spatial cognition, this work leads to the hypothesis that a primary function of RSC is to associate the HPC mapping of the animal’s current spatial location with the corresponding sensory information available to the animal from that viewpoint\textsuperscript{26}. These associations are potentially critical for higher-level cognitive operations such as intelligent goal-directed movement\textsuperscript{27}, allocation of attention, or even the encoding of memories\textsuperscript{28,29}. It also follows that RSC could detect error or mismatches between the
HPC representation of the animal’s current position and current sensory inputs, which would be critical for the construction, calibration, or anchoring of spatial representations in downstream structures. Consequently, the dissertation work elucidates a profound function of RSC in spatial cognition and provides theoretical structure for future investigation of the region.

References


