Title
Active Navigation: Transformation of Spatial Representations to Planned Motor Action in the Freely Behaving Rat

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University of California, San Diego
2017
DEDICATION

This dissertation is dedicated to the many family, friends, and the cognitive science faculty who offered constant encouragement and consistently voiced their beliefs that this document would come to fruition. Thank you for your enduring support.
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LIST OF ABBREVIATIONS

CA1: Cornu Ammonis 1
SUB: Subiculum
EC: Entorhinal cortex
RSP: Retrosplenial cortex
PPC: Posterior parietal cortex
MPC: Medial precentral cortex
LTP: Long term potentiation
LFP: Local field potential
SWR: Sharp wave ripple
MEC: Medial entorhinal cortex
LEC: Lateral entorhinal cortex
BVC: Boundary vector cell
EM: Estimation-maximization
SSE: Sum squared error
RCTX: Retrosplenial cortex
DG: Dentate gyrus
CP: Choice Probability
CDF: Cumulative density function
M1: Primary motor cortex
ACC: Anterior cingulate cortex
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ABSTRACT OF THE DISSERTATION

Active Navigation: Transformation of Spatial Representations to Planned Motor Action in the Freely Behaving Rat

by

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Episodic memories are inherently spatial experiences. This is reflected in the neural implementation of space and memory in mammals, where the hippocampus is a cortical structure necessary for successful spatial navigation and encoding of episodic memories. While the spatial encoding properties in this region are well studied in rodents, the neural pathway that allows these representations to utilize this information and shape navigation behavior is not well understood. This dissertation proposes a dorsal navigation
circuit from subregion CA1 of the hippocampus through to primary motor cortex. This cortical circuit passes through subiculum (SUB) and entorhinal cortex (EC), the two main output structures of hippocampus, before proceeding through retrosplenial (RSP) and posterior parietal (PPC) cortices. Both of these regions reciprocally connect to the premotor medial precentral cortex (MPC), a premotor cortex of the rat that projects directly to primary motor cortex.

Evidence of the navigational utility of these regions is substantial. Lesion studies show that RSP, SUB, and EC are all necessary for full performance of hippocampus-dependent spatial navigation. PPC shows deficits more consistent with spatially driven action planning, while MPC lesions cause laterality bias in choices. Neural representations support the proposed circuit with unique spatial representations in EC, RSP, and PPC. Spatial representations in SUB and MPC, however, are not well studied in during navigation.

To address the knowledge deficiencies in the proposed circuit, this dissertation contains electrophysiology studies of SUB and MPC during a complex navigation task. In SUB, a novel form of spatial encoding encoded the axis of travel. This neural population shows temporal firing properties consistent with an integrated role with hippocampus. In MPC, action encoding dominated neural representations across spatial and choice contexts. A widespread spatial signal was also present in the region.

This dissertation describes the neural correlates of two cortical regions essential to a navigational control circuit. We discovered a novel spatial representation in the hippocampal formation with important navigational implications and show its link to CA1 activity through its temporal firing dynamics. Together, these studies provide further support for the existence of a dorsal cortical circuit controlling navigation in the rat.
CHAPTER 1: Memory, Space and a Route to Planned Action

The purpose of cognition, no matter how complex the animal, is to effect action. Like any evolutionary adaptation, to persist, a trait must not impair fitness of the organism. For cognition, the ability to improve fitness lies in the control of motor output. In an environment where resources are abundant, the simplest biological organisms evolved mechanisms for propulsion that increased access to resources. As competition proliferated, sensory systems developed that could guide actions away from harm and toward rewards. While the complexity of the cognitive processes erected upon sensory systems increased, the immediacy of their impact to control motor output diminished, often modulating internal states that are less direct in their motor role. Nevertheless, even in humans, cognition may be well summarized as the process of optimizing motor outputs to avoid harm and gain rewards. Given this overall function, investigations framed around motor output utility may reveal functions for otherwise puzzling aspects of neural circuitry and mechanisms underlying these complex cognitive processes.

Perhaps the most extensively studied cognitive processes in systems neuroscience are learning and memory. This proliferation of research can be traced back to the influence of three classic findings. In 1957, Scoville and Milner reported the case study of patient H.M., an anterograde amnesiac who could no longer form declarative memories after undergoing a bilateral lesion of the hippocampus and cortex of the medial temporal lobe.¹ In 1973, long term potentiation (LTP) was discovered in the hippocampus of rabbits.² LTP is a Hebbian³ cellular mechanism for learning implemented by the synaptic adaptation to temporally proximal neural activity. Finally, in 1976, John O’Keefe described ‘place cells’ in the hippocampus of rats.⁴ These place cells fire action potentials only when the animal occupies specific locations in the environment, termed a place field. O’Keefe
and Nadel\textsuperscript{5} later synthesized these findings into a hypothesis of place cells as the basis of a spatial and cognitive map. Their hypothesis posited that place cells provide the spatial framework on which experience is encoded.

An explosion of research into hippocampus' role in space and memory followed, primarily undertaken in rats as an animal model. Lesion studies verified that navigation performance requiring recall of recent experience was impaired. Hippocampus-lesioned rats attempting to find a previously-found submerged platform in a tank of opaque water show profound impairments.\textsuperscript{6} Hippocampus-lesioned animals also make errors and run at speeds on multi-arm mazes that indicate a lack of memory of preceding traversals.\textsuperscript{7,8} Overall, evidence strongly supports that hippocampus is required for typical performance in episodic memory based navigation tasks.

The encoding of place cells also underwent scrutiny. Place cells maintain their relationship to landmark cues when an animal is replaced into a symmetric enclosure that has been rotated.\textsuperscript{9} This trait is consistent with a reading of place cells as an abstract representation and not as a result of a local sensory cue. Further supporting this interpretation, place cells maintain their firing in complete dark.\textsuperscript{10–12} Despite this robust encoding in the identical environment, changes to the environment, the landmark cues, or even the task can all lead place cells to fire in different locations or not at all.\textsuperscript{13,14} This property, referred to as remapping, can be compared to the need to differentiate different episodes in the same or similar space.

Additional evidence points to a representation following the properties of episodic memories. When navigating a track, place cells often only fire in their place field during running in one direction.\textsuperscript{15} When recorded on tracks with the availability of multiple trajectories, place cells often are differentially active in their place field depending on their arrival path (retrospective encoding) or upcoming path choice\textsuperscript{16–18} (prospective encoding).
Other contextual properties such as odor or the presence of novel objects can also modulate place cell firing.\textsuperscript{19,20} Hippocampal neurons even show ‘time field’ firing at a specific time into a running epoch on an exercise wheel.\textsuperscript{21}

Altogether, the spatial and contextual firing patterns of hippocampal place cells strongly support an interpretation as feasible building blocks to episodic memories. But to encode an episodic memory, place cells must somehow be linked during the experience. LTP is a cellular learning mechanism capable of creating a long-lasting strengthening of synapses, but the neurons must be active in a precise temporal window and order.\textsuperscript{2} The temporal dynamics of the hippocampus place cells also appear to meet this requirement.

The local field potential (LFP) of the hippocampus in rodents exhibits rhythms at multiple frequency ranges. The most prominent of these is the 4-10 Hz theta rhythm that has its greatest power while the animal is active.\textsuperscript{22} This relationship to behavior led to many early conjectures of function, especially upon observation of spatial defects when interrupting the input to hippocampus that generates the rhythm.\textsuperscript{23} A direct relationship to neural firing was not found until it was discovered that place cells fire earlier with respect to the hippocampal LFP theta phase as the animal progresses through the neuron’s firing field.\textsuperscript{24,25} This precise relationship between location and LFP theta phase of neural firing, termed phase precession, has been hypothesized as the mechanism linking place cells and the basis for encoding of episodic memories.

While phase precession as defined is a trait of individual neurons, the alignment to the local LFP synchronizes the phenomenon across place cells. At any time and location, as an animal moves through space they concurrently occupy many place fields. Some of those fields the animal has just entered, but in others, they are about to leave. Due to the nature of phase precession, within each theta cycle, the neurons will sequentially fire with respect to how far the animal has traveled through the field. This has
the effect of aligning the firing of the neurons in the order of experience and in a time window small enough for LTP effectiveness. Through this, phase precession could be the mechanism allowing the linking of these place field building blocks into an episodic memory. When sufficient neurons are recorded simultaneously, a phase precession-esque ordering of spike timings as described above can be seen in the population during one theta cycle.

Multiple traits of phase precession make it viable as a precise timing learning mechanism. Phase precession occurs in nearly all place cells in all environment types.\textsuperscript{24,25} It is not an artifact of an analysis method of mean phases over many trials as hippocampal place cells precess within the context of individual traversals through firing fields.\textsuperscript{26} It even occurs in all of the contexts of exhibited place fields including during time fields in wheel running and virtual reality.\textsuperscript{21,27,28}

As an episodic memory system, mechanisms for both encoding and retrieval should exist. A complimentary proposed function of phase precession is to retrieve memories of sequences through the reactivation of experienced sequences. Once a set of place fields is activated corresponding with an event, the learned connections to upcoming locations activate neurons in the order of the remembered experience.\textsuperscript{29}

Related phenomena that also appear important for both encoding and retrieval have also been discovered, coordinated by other aspects of the hippocampal LFP. When assessing simultaneously recorded place cells with overlapping fields, the temporal coordination of the ordering of these neurons within each individual theta is beyond that expected by individual cell phase precession alone.\textsuperscript{30} While phase precession may be the initial mechanism, it instead suggests their connectivity drives coordination at a higher, 10-30 ms window in the gamma frequency within the theta sequence firing.\textsuperscript{31}
Unlike when the animal is active, the hippocampal LFP when the animal is inactive or asleep consists of irregular activity marked by occasional sharp wave/ripple (SWR) events.\textsuperscript{5,32} When in slow wave sleep, it was initially reported that the animal’s place cells fire in activity bursts during these SWR events that replay recently experienced sequences.\textsuperscript{33} This compacted sequential firing can occur moving forward as ‘preplay’ as the animal prepares for a traversal of the space or moving backward as ‘replay’.\textsuperscript{34–36} Preplay has been shown to predict the upcoming movements in a two-dimensional space.\textsuperscript{37} When SWRs and preplay are interrupted when they occur while an animal completes a maze task, performance is impaired and continues to be so even after interruption stops, suggesting an encoding impact.\textsuperscript{38}

An incredible amount has been learned regarding hippocampus’ role in learning and memory. Evidence strongly implicates the region as the nexus of encoding and recall of recent episodic memories. Given the complexity and abstraction of place cell activity from individual sensory cues, definitive evidence for how these representations arise remains elusive. Researchers therefore began studying the inputs to the hippocampus in similar spatial experiments for clues. This pursuit has uncovered many cell types with spatial representations that are hypothesized to support the hippocampus in its memory and spatial mapping role.

Chronologically, the first major spatial cell type discovered outside hippocampus was head direction cells in postsubiculum.\textsuperscript{39,40} These neurons fire only when the animal is facing in a preferred orientation but do in any environment and regardless of spatial location. This tuning is maintained by the vestibular system input\textsuperscript{41} but adapts its anchoring to prominent cues in a manner consistent with hippocampal place cells. In fact, it is this input signal, primarily arriving at hippocampus through entorhinal cortex (EC), which appears to be anchoring place cell orientation mappings.\textsuperscript{42,43} These neurons have
gone on to be described in many subcortical and cortical regions and appear to be a widespread signal anchoring spatial representations across the rodent brain (for a review, see Winter & Taube\textsuperscript{44}).

Forming the first synapse onto dentate gyrus in the trisynaptic loop and receiving a substantial output back from hippocampus CA1 and subiculum (SUB), the EC is anatomically positioned for a highly related role to hippocampus.\textsuperscript{45} Findings are consistent with this anatomically derived theory as multiple different spatially defined cell types have been discovered there. Grid cells are a cell type showing periodic firing fields aligned to a tessellating triangle pattern.\textsuperscript{46,47} They are found in layers II and III of medial entorhinal cortex (MEC), the same layers projecting to hippocampus. Boundary cells, a population that fires when encountering any boundary in a certain environmental direction, have also been reported in the region,\textsuperscript{48,49} along with head direction and speed sensitive populations.\textsuperscript{50,51} Lateral entorhinal cortex (LEC) does not show spatial locations but instead may be encoding object representations.\textsuperscript{52,53} Based on these many representations, the entorhinal cortices appear to separately encode spatial and object aspects that may be combined in the more episodic place cell population.

The question remains as to what mechanisms and circuitry allows this abstract spatial and episode encoding system to actually effect behavior. Fundamentally, important aspects to any episode are where it occurred and whether it was a rewarding or aversive experience. The hippocampus has a pronounced organizational structure that suggests a dissociation of function between the poles of the longitudinal (dorsal-ventral, or septal-temporal) axis. Anatomical outputs change along the longitudinal axis. Ventral hippocampus is primarily connected to prefrontal cortex, the amygdala, and ventral striatum, whereas dorsal hippocampus is preferentially connected to retrosplenial cortex\textsuperscript{45} (RSP). Place field size grows larger and less specific as the longitudinal axis is traversed.
from dorsal to ventral hippocampus.\textsuperscript{54,55} Lesion studies show dorsal hippocampus to be more involved in spatial localization tasks, whereas ventral hippocampus is necessary for contextual and emotional tasks.\textsuperscript{56,57} In full, this leads to the conclusion that there are two broad connectivity streams between hippocampus and motor cortices. One is a ventral pathway carrying a value signal, and the other is a dorsal cortical circuit carrying spatial information for navigational purposes.

The targets of ventral hippocampus have been studied in spatial behavioral contexts and shown to represent values related to goal or aversive locations. The amygdala, while encoding a variety of associative memory contexts, is shown to have a dissociation from the more spatial encodings of hippocampus\textsuperscript{58} (for a recent review of amygdala function, see Janak & Tye\textsuperscript{59}). Ventral striatum studies suggest an area important for linking goal representations to locations. The connectivity between ventral striatum and hippocampus has been shown to be necessary in a conditional place preference task.\textsuperscript{60} Representations in spatial tasks often involve ramping of firing to reward locations\textsuperscript{61} that phase precess to hippocampal theta.\textsuperscript{62} Finally, prelimbic and infralimbic cortices of medial prefrontal cortex have spatial representations reminiscent of place cells but their distribution over-represents goal locations.\textsuperscript{63} There are many neurons encoding value and reward signals in the regions, but few representing an action signal.\textsuperscript{64} Together, these results suggest that these ventral projections form networks that apply hippocampal episodic memory information to modulate motor outputs through choice valuations and spatial selection but not navigation.

This dissertation proposes a dorsal navigational cortical circuit. This circuit would afford spatial episodic memory to effect motor output. The anatomical and functional connectivity of a dorsal cortical pathway from hippocampus to effect motor output is both less direct and less complete than ventral projections and has not previously been fully
hypothesized. SUB and EC are the main outputs of dorsal hippocampus and both receive large inputs from CA1. Both SUB and EC are connected reciprocally to RSP. Lesion studies in each of these regions implicate them in successful spatial navigation in hippocampus-dependent tasks. RSP is highly interconnected with the neighboring associative cortex, PPC. Finally, no direct project exists from RSP and PPC to motor cortex, but both are reciprocally connected with medial precentral cortex (MPC), a premotor region projecting to both the spinal cord and primary motor cortex that we propose is the final step in the dorsal cortical pathway.

EC receives a large projection from CA1 and is one of two main output regions of dorsal hippocampus. As discussed previously, MEC contains many neurons with spatially specific outputs. Furthermore, lesion studies have established the importance of MEC to hippocampal-dependent spatial memory tasks. In this study, while MEC lesions alone induced impairment in the Morris water maze comparable to HPC lesions, lesions of both areas increased the effect over either region alone. This result suggests that another output structure such as SUB is capable of leveraging hippocampal activity in the absence of MEC, albeit incompletely. MEC lesions did not result in deficits in object recognition tasks whereas LEC lesions did, dissociating their complimentary roles.

Characterization of dorsal SUB has been more elusive. In the Morris water tank, SUB lesions impair navigation as seriously as hippocampal lesions. The behavior, however, is different, with rats exhibiting random exploration instead of the circling resulting from hippocampal lesions. Like MEC, when both SUB and hippocampus are lesioned, impairments are even more severe, suggesting another route such as MEC can also facilitate the use of hippocampal representations even in the absence of SUB. Electrophysiology experiments recording from dorsal SUB have been conducted in open
field and track environments. Open field results are less conclusive regarding a different function, with place-like activity with larger fields.\textsuperscript{78,79} Additionally, SUB neurons stretch their fields to varyingly-sized environments of the same shape and are less prone to remapping across similar environments than CA1 place cells.\textsuperscript{80,81} These results have been interpreted as the same but more generalized signal of CA1. Additionally, boundary cells have been described in SUB.\textsuperscript{82} On a track, similar results of larger fields was reported along with a transition of firing activity from sparse near CA1 to more dense when adjacent to RSP or presubiculum.\textsuperscript{83} Overall this lack of differentiation in representations does not match the more pronounced differences observed in lesion studies and suggests more research is necessary to uncover the representations underlying the role of SUB in navigation and episodic memory more generally.

RSP is an associative cortex with strong connectivity to visual sensory cortices, anterior thalamic nuclei, postsubiculum, SUB, EC, and CA1 of the hippocampus.\textsuperscript{86–69,73} This anatomy suggests an important spatial orienting role considering the aforementioned inputs from visual, head direction, and spatial inputs. Complete lesions of RSP result in deficits in memory performance on multi-arm mazes and in the Morris water maze.\textsuperscript{72} Navigation in the dark is also particularly reliant on RSP as inactivations lead to increased errors on a multi-arm maze task.\textsuperscript{84} RSP has head direction neurons aligned to the rest of the head direction cell network as well as head direction neurons sensitive to contextual and visual contexts.\textsuperscript{85,86} Electrophysiology recordings from a multi-turn track maze detail a region with individual neurons sensitive to one or more of three frames of reference simultaneously: location with respect to distal landmarks, location with respect to route, and action.\textsuperscript{87} Here, route refers to a neural firing pattern consistent for a given pattern of actions, a representation previously described in PPC.\textsuperscript{88} Overall, substantial evidence across anatomical, lesion, and individual neural activity methods is consistent in
describing RSP as a region mediating and transforming information between space and action.

Just lateral to RSP in the rat is PPC. The neighboring cortices are highly interconnected but differentiated by their anatomy. Unlike RSP, PPC is interconnected with primary somatosensory and auditory cortices in addition to visual cortices. It also has connectivity with perirhinal and postrhinal cortices. Furthermore, the thalamic inputs are from lateral dorsal and lateral posterior thalamic nuclei. Lesion studies of PPC during hippocampal-dependent spatial navigation tasks are less conclusive in their impact, often depending on the extent of the lesion. It can be said they were always less than the effect of hippocampus for these tasks. However, while parietal cortex does not cause severe impairments to spatial memory, it does seem to impair tasks requiring execution of a motor plan or procedure to reach a location. There was not an impairment when moving to a visible goal, suggesting a function of constructing motor plans beyond the immediate target. Single cell recordings from the region support this interpretation with neurons that consistently map out the space of route even if the individual segments of the route scale or change location in the room. In addition, egocentric action encoding neurons have been reported.

The final link in the proposed dorsal navigational circuit is MPC. MPC is the most medial and dorsal subregion of rodent prefrontal cortex and boasts strong reciprocal connectivity with PPC and RSP. It projects both to primary motor cortex and the spinal cord. Anatomically, the MPC subregion of PFC is ideally suited to transform knowledge of current position into a plan for specific motor actions. To my knowledge, this region has not been studied with the same lesion studies of hippocampal-dependent tasks. Instead it has primarily been studied in a choice context. Unilateral lesions cause contralateral neglect and reaction delays in choice tasks that can be extinguished through contralateral
reward only. This suggests a role necessary for choice rather than motor execution. Individual neurons in the region correlate with upcoming choices and actions earlier than any other frontal regions previously studied.

**Organization of the Dissertation**

This dissertation proposes a dorsal cortical circuit transforming spatial episodic information into planned motor outcomes. In this proposed pathway, two regions lack strong single cell data supporting their proposed roles. First, only two studies exist investigating neural activity of the dorsal SUB while an animal is navigating. In each, representations are difficult to distinguish from neighboring CA1, inconsistent with lesion results. Second, no analysis of spatial aspects during navigation has occurred in MPC. This dissertation contains electrophysiology recordings investigating the neural activity patterns of each of these regions during navigation of a complex multi ‘T’ maze. This maze was chosen to separate actions and rewards over multiple aspects of space to enable separation of these confounding variables. An additional analysis of SUB data provides evidence for the connectivity organizing the representations identified in the region during navigation.

In Chapter 2, we record from the dorsal SUB of rats navigating a triple ‘T’ maze. We report the presence of neurons representing the axis of travel on the apparatus. The head direction tuning maintained alignment with the distal landmark cues of the room upon rotation of the track apparatus, but disappeared in an open arena under in the same environment. This result describes a new spatial cell type sensitive to context and encoding a variable that may be applicable in navigational uses.

Chapter 3 explores the inputs driving the axis-tuned neurons from Chapter 2 through the investigation of their relationship to hippocampal theta. The axis-tuned neurons robustly phase precess in a manner very similar to other hippocampal pyramidal
cells. The findings link their existence to place cells of the hippocampus in addition to the clear orientation influences.

In Chapter 4, we record from MPC on the same triple ‘T’ maze. MPC neurons prominently encode upcoming and ongoing action. Concurrently they discriminate the spatial and choice contexts during the navigation. We interpret these results as evidence to the regions role in the transformation of spatial information to planned motor action and as evidence of completion of a proposed spatial navigation circuit.

The dissertation concludes with a summary of the novel findings of this dissertation and their implications for the spatial navigation circuit of the rat.

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CHAPTER 2: Subiculum Neurons Map the Current Axis of Travel

Abstract

Flexible navigation demands knowledge of boundaries, routes, and their relationships. Within a multi-path environment, a subpopulation of subiculum neurons robustly encoded the axis of travel. Axis-tuned neurons' firing peaked bimodally at head orientations 180 degrees apart. Environmental manipulations showed these neurons are anchored to environmental boundaries but lack axis-tuning in an open arena. Axis-tuned neurons thus provide a powerful mechanism for mapping relationships between routes and the larger environmental context.

Main Text

Hippocampal CA1 neurons are known for the location specificity of their action potential firing during free-foraging within an arena.\(^1\) Such location-specific firing is modulated by constraints governing running behavior and available trajectories.\(^2\)\(^-\)\(^5\) In this way, CA1 can and does encode multiple navigationally relevant spatial relationships.

As an efferent target of CA1, the dorsal subiculum (SUB) may be primed to encode more complex spatial relationships.\(^6\)\(^,\)\(^7\) SUB neurons sometimes exhibit place-specific firing akin to that of CA1,\(^8\)\(^,\)\(^9\) yet reported differences include increased generalization of place fields across environments, scaling of firing fields to match arena size, and increased numbers and sizes of fields.\(^9\)\(^,\)\(^10\)\(^,\)\(^11\) Some SUB neurons, termed boundary vector cells (BVC), exhibit spatial tuning reflecting proximity and orientation to arena borders.\(^12\)\(^,\)\(^13\)

Multiple pathway environments greatly increase the prevalence of task-relevant spatial relationships, but whether SUB encodes such spatial features is unknown. We therefore obtained single neuron recordings in rats performing a navigational task (Figure
2.1A, Supplemental Figures 2.1-2) wherein the layout of six interconnected routes ensured that each track section had a characteristic direction and axis of travel (Figure 2.1B).

Immediately apparent from firing rate maps and directional tuning plots is a distinctive neuron subpopulation firing strongly whenever the animal ran in either of two opposing directions (Figure 2.1C). Such firing is largely independent of room location. Put another way, such neurons fire when the animal travels in either direction along a single axis.

Since neurons with axis-tuned activity were not reported in work utilizing open-field foraging paradigms, we considered the possibility that axis-specific firing emerges during route-running. Therefore, we also examined spiking activity during free-foraging in an arena centered just atop the track environment with clear view to the same distal landmarks (Figure 1A). Neurons with axis tuning on the track exhibited little evidence of axis-tuned firing in the arena (Figure 2.1C). As previously observed some SUB cells exhibited spatially-specific firing (Supplemental Figures 2.3-4) akin to ‘place cells’ in the CA1 (~29%) and BVCs in SUB (~21%).

To assess the prevalence of SUB axis-tuned neurons, quantification of their characteristics is required. Such neurons should exhibit bimodal directional tuning peaks separated by 180 degrees, and firing should be independent of environmental location (Figure 2.2A). Therefore, we fit von Mises mixture models of multiple orders to each neuron's firing data from half the recorded track traversals (Supplemental Figure 2.5; Supplemental Methods). Comparison of fits across multiple model orders allowed objective estimation of the prominent modes in the neural data. Cross-validation was applied to the remaining half of the data. The lowest-order model with a strong fit (>50% less error versus a 0th-order circular model) and substantial improvement over the
Figure 2.1 Axis-tuned firing of SUB neurons. A. Schematic of route-running and open-field foraging tasks. Left panel depicts the 160 cm X 125 cm track apparatus. Left: Animals made multiple runs along each of four partially overlapping routes (dashed white lines) leading from a start site (green circle) to any of four goal sites (red circles). From each goal site, the animal returned to the start via either of two return paths (dashed yellow lines). Right: Recordings were also obtained as animals foraged in a circular, 60 cm diameter arena. Animals had clear view to the surrounding environment at all times. B. Left: Color-mapped mean directions of travel superimposed on representative tracking data. Right panel: Color-mapped mean axes of travel (same recording). C. Three example axis-tuned SUB neurons. Each panel depicts firing rate color-mapped as a function of track position. White arrows mark directions/positions with highest firing. Each panel also depicts firing rate-maps for the arena foraging session. Polar plots depict mean firing rate against head orientation.
preceding model order (a further 20% error reduction) was chosen as the ‘best’ model. A higher proportion of SUB neurons were categorized as bimodal (i.e., 2nd-order) than any other model order (Supplemental Figure 2.6) within a wide range of model improvement criteria (10-22.5%). This bimodality bias existed in track-running but not arena free-foraging data. For neurons fit by a 2nd-order model, thresholding for 2X greater firing at model maxima relative to minima removed weakly tuned neurons.

We also assessed the positional independence of bidirectional firing to quantify reliability in axis-tuned firing. Over track locations associated with either of the neuron’s preferred tuning directions, we determined whether firing rate exceeded at least 50% of the mean rate for those same directions. Neurons were considered spatially independent if the majority of locations met this criterion.

The 47 neurons (of 542 tested) meeting criteria were strongly tuned to a specific axis of travel on the track. Orientation separations between each neuron’s two peaks overwhelmingly clustered near 180 degrees (Figure 2.2B). Tuning specificity was high, having a mean circular variance of 0.27 radians (+/- 0.08 STD). Firing rates at model-identified tuning peaks were, on average, 4.65X those at tuning minima (+/- 2.23 STD, outlier value of 90.4 removed; Figure 2.2C). Thus, the actual strength in bimodal tuning was well above the applied 2X criterion. Mean spatial independence of axis-tuned firing was also well above criterion (73.9% +/- 12.5 STD). Across this subpopulation, the preferred tuning directions of firing were evenly distributed (Hodges-Ajne uniformity test, N=94, p=0.9182; Figure 2.2D).

Applying the same methodology and criteria, no neurons with strong axis-tuned firing were found in arena recordings (Figures 2.2E, 2.2F, Supplemental Figures 2.6-7). For a few neurons (N=8), a 1st-order model identified a more moderate bias to a single direction both on the track and the arena. For the 47 neurons identified as axis-tuned on
Figure 2.2 Quantification of axis-tuned firing. A. The same directional tuning plot as for the neuron in Figure 1C (left panel) is depicted again to describe axis-tuning metrics. A mixture model using two von Mises distributions (red ellipses) provided a good fit to the neuron’s directional tuning plot. The model yields two tuning maxima at 100 and 260 degrees from room north (dashed yellow lines; circular variances shown as yellow lines at the margin). Robustness and bias in tuning are given by maxima:minima and maxima:maxima ratios, respectively. B. For all 47 axis-tuned neurons, the directional tuning plots were aligned to the larger model peak of each neuron (black line). Blue lines depict the relative orientations of these neurons’ opposite, smaller tuning peaks. Gold lines depict the second-peak orientations for Figure 1C neurons. C. Mean of the max-normalized directional tuning plots for the 47 strongly axis-tuned neurons (plots aligned by the highest firing rate bin). D. Orientations of all primary and secondary peaks relative to the space of the surrounding environment (Figure 1C neurons in gold). E. Hashed bars depict the number of SUB neurons (of 542) for which the 2nd-order von Mises mixture model produced the best fits. Dark blue and light blue bars depict the number of neurons that also met criteria for ratio of directional tuning maxima versus minima and spatial independence (N=0 neurons for the arena session). F. Peak to minima ratios for axis-tuned neurons were significantly higher (left panel) for the track versus the arena recording epochs, whereas circular variance was lower (middle panel). Spatial independence of directional tuning was statistically similar (right panel).
the track, model maxima to minima ratios for the arena session were significantly reduced 
(N=47, \( p=1.79 \times 10^{-11} \), Wilcoxon rank sum test) and circular variance in peak tuning was 
significantly increased (N=94, \( p=2.35 \times 10^{-18} \), Wilcoxon rank sum test). Spatial 
independence was unchanged (N=94, \( p=0.07 \), Wilcoxon rank sum test), consistent with 
axis-tuned firing on the track and the broad spatial tuning typical of SUB arena data^8.

The preceding results indicate that the context of track-running and/or the 
constraints on available trajectories are critical in generating axis-tuned firing of SUB 
neurons. As the track allowed clear view to distal visual cues along recording room walls, 
axis-tuned neurons could, in principle, anchor to either the recording room or the track 
structure. To determine which frame of reference served to anchor axis-tuned firing, a 
partially overlapping subset of neurons (N=170) were also recorded following 90-degree 
track rotations (Figure 2.3A). In all cases (N=26), axis-tuning was found to be anchored to 
the spatial frame of reference given by the recording room. Highly similar directional tuning 
plots were observed for the standard and rotated conditions when plots are aligned to the 
room frame of reference (Figure 2.3B). In this case, correlations between tuning curves 
for the two track configurations were high (mean 0.76 +/- 0.16 STD). When the rotated 
track session’s tuning curve was rotated to maintain the relationship to the track, mean 
correlations were far weaker (mean -0.41 +/- 0.22 STD; N=26, \( p=6.55 \times 10^{-10} \), Wilcoxon rank 
sum test; Figure 2.3C).

Taken together, the present analyses of directional and spatial tuning properties 
of SUB neurons reveal a previously unknown form of orientation encoding, the animal’s 
current axis of travel. Axis-tuned firing of SUB neurons is recognized and quantified from 
directional tuning plots characterized by two distinct peaks in firing rate, separated by 
approximately 180 degrees. Such axis-tuned firing: 1) can exhibit near complete 
independence from environmental location; 2) is expressed primarily in the context of
Figure 2.3 Spatial frame of reference for axis tuning. A. Schematic depicting the normal (left panel) and rotated (right panel) track placements. B. Color-mapped firing rates for axis-tuned neurons in the normal and rotated track configurations alongside the associated directional tuning plots. The neuron in the right panel is repeated from Figure 1C (upper left panel). Because tuning orientations persist, track positions yielding maximal firing differ substantially for the normal and rotated track orientations. C. Comparison of orientation tuning across track orientations. Top left panel: Alignments of tuning peaks during the rotated track session (blue lines, N=26) relative to those for the same neurons during the normal track orientation session (the latter are all aligned to 90-degrees). Bottom left panel: Correlations between all directional firing rate values for the normal (blue) versus rotated (red) track conditions were compared to correlations between the same following 90-degree rotation of the values for the rotated track data (dotted red). Right panel: Mean (N=26) correlations between directional firing rate values for the normal and rotated track conditions (dark blue) and for the normal and rotated track conditions subsequent to 90-degree rotation of the rotated track data (matching track rotation; light blue).
route-running along tracks as opposed to unconstrained movement in an open arena; and 3) carries the environmental boundaries (a.k.a., allocentric space) as its spatial frame of reference. In addition, axis tuning persisted in darkness (Supplemental Figure 2.8) suggesting that it is not strictly dependent on visual information and can be updated idiothetically. Notably, SUB axis-tuned neurons are clearly distinguishable from head direction neurons\textsuperscript{15} whose activity maps only a single orientation and whose tuning is present during foraging in open arenas. Axis-tuning is also distinguishable from boundary vector tuning since only a minority (7, or 14.9\%) of the 47 axis-tuned neurons exhibited firing in the arena consistent with boundary vector encoding (Supplemental Figure 2.4).

The context dependency of axis-tuned SUB activity parallels that of several other forms of spatial representation. For hippocampal neurons, route-running induces directional dependence in place-specific firing,\textsuperscript{2,3} and the emergence of trajectory-specific place fields.\textsuperscript{4,5} The presence of high walls defining a ‘switchback’ path yields resetting of MEC grid cell alignment across repeating route subspaces.\textsuperscript{16} Finally, the action correlates of most posterior parietal cortex neurons in free-foraging are replaced by route-position-dependent firing on tracks.\textsuperscript{17,18} Thus, route-running induces qualitative changes in spatial mapping in several brain structures and such changes yield conjunctive information concerning relationships between paths and the space of the larger environment. The neural mechanism gating the influence of movement directions in track-based versus arena environments remains a mystery.

The expression of axis-tuned SUB activity in an environment having multiple interconnected paths is perhaps an initial clue to its functional significance. Axis-tuned activity encodes the track segment orientations relative to environmental boundaries. In humans, such route-boundary relationships can powerfully impact memory for the spatial layout of environmental landmarks.\textsuperscript{19} In this sense, encoding of axis of travel could be
particularly relevant in real-world environments where boundaries can be difficult to define. For example, a commonly used orientation tool in human navigation is to align oneself to a city’s street grid based on a prominent, well-known, linear landmark such as a coastline, river, or well-recognized street. In this respect, layering representation of movement axes atop the cognitive map provided by place and grid cells enhances its behavioral relevancy and therefore yields a more functionally complete mapping of space.

**Supplemental Methods**

**Subjects**

All subjects were adult male Sprague-Dawley rats (N=3). From these rats, a total of 542 subiculum neurons were recorded (81, 321, and 140 from each – see Supplemental Figure 1). Rats were housed individually and kept on a 12-hour light/dark cycle. Prior to experimentation, animals were habituated to the colony room and handled for 1-2 weeks. During training and experimentation, rats were food restricted and weights were maintained at 85%-95% of free-fed weight with water available continuously. Rats were required to reach a minimum weight of 350 g (5-10 months of age) prior to surgery and subsequent experimentation. All experimental protocols adhered to AALAC guidelines and were approved by IACUC and the UCSD Animal Care Program.

**Statistical tests**

The Hodges-Ajne Uniformity test is employed to consider potential bias in the distribution of directional tuning among the full population of neurons (Figure 2.2D). The Wilcoxon Rank Sum test is employed to compare metrics for directional tuning of neural activity in the track-running versus arena foraging sessions (Figure 2.2F); this test carries no assumption of normality in distribution of the data.

No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications.¹⁷
Data collection was not randomized, nor was analysis performed blind to the conditions of the experiment.

We here report exclusion of one value in calculating the mean for maxima to minima ratios across cells. Although no prior criterion was defined, the value is fully 19X the mean and so clearly an outlier. Notably, this outlier was in the direction favoring a finding of significance in the statistical test applied and so its exclusion reflects a more conservative approach.

**Apparatus**

Behavioral tasks were conducted using both a circular wall-less arena and a triple 'T' track maze. The track (Figure 2.1A, left panel, 8cm-wide pathways, overall 1.6m x 1.25m in length and width, painted black) stood 20 cm high in the middle of a large recording room and was visually open to prominent distal cues. The track edges were only 2cm in height, allowing for an unobstructed view of the environment. The arena (Figure 2.1A, right panel, 60 cm in diameter) was placed 20 cm above the center of the track. The arena was also visually open to the same prominent distal cues as well as the track below. For the first recording of rat NS14 (N=17 neurons), a high-walled pot (30 cm in diameter, 22 cm walls) was used in place of the arena.

**Behavior**

Rats were habituated to the maze during two 30-minute periods of free exploration. Animals were then trained to run ballistically from the midpoint of one of the long edges of the maze into the center of the apparatus and continue until reaching the long edge opposite the start point (Figure 2.1A, white dashed lines). This consisted of straight sections interleaved with three left or right turns for a complete path run. The total path lengths were 140cm, with turns at 51 cm, 87 cm, and 118 cm. Reward (1/2 piece of Cheerio's cereal) was made available at the four reward sites. Over 1-2 weeks, animals
were trained by approximation to make route traversals between food reward sites. Over at least 2 additional weeks, animals were trained by simple trial and error to a criterion of 80% for ballistic (uninterrupted) path traversal. Once animals met criterion, they were trained 2-3 times on the track in the normal orientation, immediately followed by training on the track in the 90-degree rotated orientation. This established familiarity with the rotated track, but the rats were not extensively trained in this orientation. Animals were surgically implanted only after this level of task performance had been achieved.

Multiple reward paradigms were used across the set of animals. In an all-but-repeats paradigm, used for animal NS14, the animal was rewarded at any of the four locations except when the animal repeated the same location as the previous run. In a visit-all paradigm used for NS15 and NS16, the animal was rewarded at all locations, but needed to visit all locations before rewards were reset at all paths.

In the arena, two different behavioral epochs were used, each for approximately half of the time in the arena (~5 minutes each epoch). For the first half of the time, the animal was cued to make trajectories across the full arena for a 1/4 Cheerio's cereal reward at the track edge. The trajectory orientation was varied in order to obtain adequate sampling of the full arena surface. This pattern produced running activity similar to the track apparatus. The second half of each arena session was free-foraging for small pieces of Cheerio's reward dispersed randomly in the arena. Data was analyzed together in all portions of the paper to maximize sampling.

**Surgery**

Rats were surgically implanted with tetrode arrays (twisted sets of four 12.5-µm nichrome wires) inserted into custom-built microdrives (4-8 tetrodes per microdrive). Rats were implanted bilaterally with 2-3 microdrives into dorsal subiculum. Rats were anesthetized with isoflurane and positioned in a stereotaxic device (Kopf Instruments).
Following craniotomy and resection of dura mater, microdrives were implanted relative to bregma (A/P -5.6 – -6.6 mm, M/L ±1.6 – ±2.7 mm, D/V -1.5 – -2.2 mm).

**Recordings**

After recovery from surgery, animals were retrained for at least one week before beginning recordings to ensure adequate behavior and running ability with the new weight of the implant. Because of this procedure, all recordings were from animals that were well trained on the task. Electrodes were moved ventrally in 40 µm increments between recordings to maximize the amount of distinct units collected. Each microdrive had 1-2 electrical interface boards (EIB-16, Neuralynx) connected to a single, amplifying headstage (20X, Triangle Biosystems). A tether led to a set of preamplifiers (50X) and a high pass filter (>150Hz). Signals then fed into 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 in Plexon OfflineSorter software. Waveform parameters used were peak height, peak-valley, energy, full-width half-maximum, and principal components. Waveform clusters appearing to overlap with the amplitude threshold set for collection were discarded to avoid collection of neurons with partial spiking data. Waveform amplitudes were monitored to ensure systematic fluctuation did not produce confounds in isolating single units.

After completing unit isolation, a modified isolation distance value was calculated for each unit to assess cluster quality. Introduced by Harris et al.\textsuperscript{20}, isolation distance measures the separation of clusters by finding the Mahalanobis distance, reported in units of cluster variance, of the $n$th closest non-cluster spike, where $n$ is the number of spikes in the cluster. Put another way, isolation is the size from the center of the cluster to the circle that includes double the number of spikes as actually classified in the cluster. Accordingly, this measure is undefined when the number of the spikes in the cluster
exceeds the number of spikes out of the cluster, and starts to lose intuitive meaning as a measure of distance to the nearest cluster as this limit is approached. Due to the propensity of high firing SUB neurons, we have adapted this measure to be the minimum of the isolation distance as defined by Harris et al. and the distance to the non-cluster spike 20% into the non-cluster spike distance distribution. This modification to isolation distance serves to define the value for all neurons and to reduce the isolation distance for clusters with many spikes. While only reducing our neurons’ scores, we believe this conservative adjustment more accurately represents cluster quality in these situations.

Animals’ position was tracked using a camera set 8.5 ft above the recording room floor. Plexon CinePlex Studio software was used to detect red and blue LED lights placed on the animal’s surgical implant, centered on the animal’s head and separated by approximately 5 cm. Position location of the lights was captured at 60 Hz. The animal's position and orientation was determined by averaging the location of the two lights and calculating the orientation of the vector between the lights. Using the fact that the track apparatus was squared to the room, we averaged the orientation of all time periods with >3 cm/second running and positions on the middle half of the return arms of the track. This angle was defined as 0 degrees, or "room north," for the recording and was used to align the animal's heading to the room. Recordings lasted approximately 45 minutes for arena and track recordings and 1 hour 15 minutes for recording sessions with track rotation data. The animal would run in the arena for 3-10 minutes and then on the track for approximately 80 rewarded runs (Figure 2.1A). For track rotation recordings, the animal had access to water for 5 minutes after completing the first session while the track was wiped down and rotated and then ran for another 80 rewarded runs (Figure 2.3A). For our dark recording, we began by running the animal on the typical arena and track sessions. Then, the same protocol for a track rotation recording was carried out except the track
was not rotated and only a red LED was used on the animal’s implant. All other light sources in the room were turned off or covered. All other recording details were identical to other recordings.

We recorded a total of 542 SUB neurons across three rats (N=81/321/140). For the first animal, all 81 neurons were recorded from the right hemisphere. For the second animal, 127 neurons were recorded from left SUB and 194 from right SUB. For the third animal, 42 and 98 neurons, respectively, were obtained from medial and lateral tetrode bundles in the right hemisphere. No neurons were excluded from analysis, even if activity was minimal.

**Histology**

Animals were perfused with 4% paraformaldehyde (vol/vol) under deep anesthesia. Brains were removed and sliced into 50-μm sections and Nissl-stained to reveal the final depth of electrode wires in SUB. Microdrive depth monitored across recordings and final electrode depth as observed in histology were compatible in all cases.

**Directional Tuning Vectors**

Head direction tuning vectors were calculated using the same sample of running data as the positional firing rate maps (i.e., using the same velocity thresholds). Head orientations were binned into 36 10-degree bins. The total number of spikes per bin was divided by the total time in each bin to calculate the mean directional firing rate.

**Orientation Maps**

Orientation maps (Figure 2.1B) were created by calculating the mean circular direction of all samples at each spatial location.

**Positional Firing Rate Maps**

To characterize the firing activity of the SUB neurons, we calculated individual neurons’ positional firing rates by dividing the total number of spikes of each neuron at
each location by the total occupancy time at each location. To include only data where the animal is running, we excluded all samples with less than 3 cm/second velocity or greater than 20 radians/second angular velocity. The latter threshold was used to exclude cases of rapid head turning in the absence of locomotion. Positional firing maps were smoothed using a 2D convolution with a Gaussian filter with s.d. of 2 cm that also accounts for bins with no occupancy. Raw, unsmoothed data were downsampled to 2 cm x 2 cm bins for analysis of spatial independence of directional firing. For Supplemental Figure 2.3, 2.4, and 2.7, data is downsampled to 2 cm x 2 cm bins and smoothed using a 2D convolution with a Gaussian filter with s.d. of 4 cm and the same occupancy adjustment as above.

**Burst Index for Spiking Activity**

To assess burstiness in SUB spiking activity, we applied the method outlined and utilized by Kim et al. (2012). We did this for two reasons. First, the method developed by Kim et al. is not confounded by firing rate differences, a key factor considering the diverse firing rates of SUB neurons. Second, using the same method allowed direct comparisons of our results to previous findings. The burst index is computed by integrating the spike autocorrelogram from 1-6 ms and dividing the result by the integrated power from 1-20 ms. The measure allowed us to demonstrate that the basic firing properties of SUB neurons recorded in our work are in line with those observed previously.

**Arena Spatial Measures**

Arena spatial firing was described using three classic spatial measures: spatial information, spatial information per spike, and spatial coherence. All analyses were implemented as described in the respective sources on arena data downsampled to 2 cm by 2cm bins. Only those neurons with at least 250 spikes in the arena (N = 354/542) were analyzed. Briefly, spatial information is the number of bits of information per second the neuron communicates while the animal is on the arena. Spatial information per spike is
this same value but now as a rate of bits per spike of the neuron. This reframing is useful for neural populations with low variability in firing rates, but greatly skews values against high firing neurons in more variable populations. For this reason, we primarily utilize spatial information in this paper. We do include information per spike for comparison’s sake to other work. Finally, spatial coherence is the Pearson correlation of all locations on the arena to the mean of their surrounding locations. Coherence is a measure of smoothness of firing activity across space and is high for spatially reliable neurons provided binning is adequately small.

**Spatial Correlates (BVC and Place) Model Comparison**

Both place cell and boundary vector cell (BVC) spatial representations have been previously described in dorsal subiculum\(^8\). To better describe the prevalence and form of strong spatial tuning in the open arena, we devised a brute-force template-matching procedure that compares the 2D spatial firing rate map of each neuron to a set of all possible ideal place and BVC firing maps.

The 2D firing rate map templates for BVCs were formed by following the BVC-defining model equations provided in Hartley et al.\(^{25}\). As stated in Hartley et al.\(^{25}\), equation 1, the receptive field \(g(r, \theta)\) of a BVC tuned to a boundary distance \(d\) and bearing \(\phi\) from the rat is

\[
g(r, \theta) \propto \frac{1}{\sqrt{2\pi \sigma_{rad}^2(d)}} \times \frac{1}{\sqrt{2\pi \sigma_{ang}^2}} e^{-\frac{(r-d)^2}{2\sigma_{rad}^2(d)}} e^{-\frac{(\theta-\phi)^2}{2\sigma_{ang}^2}}
\]

where \(r\) is the distance and \(\theta\) is the bearing to that boundary. By utilizing Hartley et al.’s\(^{25}\), equation 2, the firing rate of a BVC at any Euclidian location \(f(x, y)\) is calculated
by summing the contribution of all boundaries:

\[ f(x, y) = \sum_b g(r_b, \theta_b) \times \Delta \theta \]

where \( b \) is one instance from the set of all boundaries. We calculated the distance and orientation to the edge of the arena at 5° increments (\( \Delta \theta = 5^\circ \)) for each location and used this as our set of boundary distances and orientations. We defined \( \sigma_{ang}^2 = 0.2 \) radians to mimic the settings of Hartley et al.\textsuperscript{25}, although it is worth noting any reasonably small value gives similar results. Hartley et al.\textsuperscript{25} define \( \sigma_{rad}^2 \) as

\[ \sigma_{rad} = \frac{d}{\beta + 1} \sigma_0 \]

so that \( \sigma_{rad}^2 \) varies linearly with distance. We utilized \( \beta = 10 \) and \( \sigma_0 \) at 4 values, \( \sigma_0 = [0.25, 0.5, 0.75, 1] \), to allow the model a variety of distance tolerances and remain as agnostic as possible about predicted parameter values. The final free parameter, \( d \), is the BVC’s preferred distance to the border. Allowing for any reasonable parameter values as was done with \( \sigma_0 \), we chose \( d \) values from distance 0 - the edge of the arena - to the arena radius (30 cm) at 4 cm intervals, resulting in 9 distances. Finally, creating BVCs by combining all possible orientations (72, 5° increments), \( \sigma_0 \) values (4), and feasible distances (9), we created \( \sim 2500 \) ideal BVC firing rate maps per neuron.

The 2D firing rate map templates for place cells was a similar procedure. A 2D Gaussian distribution was utilized to create templates with centers at each 2 cm by 2 cm bin location. Like the BVC models, we used 4 standard deviations for each location: \( \sigma = \)
5, 7, 9, and 11 cm. This resulted in ~2800 \((\pi r^2 \times 4, \text{where } r \text{ is in bins})\) place cell templates per neuron.

To assess the fit of a neuron to a place cell or BVC model, we ran a Pearson correlation on all bins with occupancy and their corresponding values in all BVC and place cell templates. We then selected the template with the highest \(r\) value for each of the BVC and place cell template sets and used this as our estimate of the quality of fit of each cell type to our data. We did this for all 354 neurons with at least 250 spikes on the arena. As nearly all neurons displayed significance as compared to a bootstrap method creating 1000 shuffles of the same neuron’s firing rates before correlating with the templates, we arbitrarily chose a higher Pearson \(r = 0.4\) cutoff value to determine spatial model correlation. This value was chosen by visual inspection as a threshold where neurons with higher \(r\) values did often fit the templates reasonably well.

**Fitting of Von Mises Mixture Models**

To fit direction tuning data, we utilized von Mises distributions, a periodic generalization of the Gaussian distribution. For a von Mises distribution evaluated at angle \(x\), centered at mean angle \(\mu\) and with concentration \(\kappa\), the probability density function \(f_{vm}(x \mid \mu, \kappa)\) is

\[
f_{vm}(x \mid \mu, \kappa) = \frac{e^{\kappa \cos(x - \mu)}}{2\pi I_0(\kappa)}
\]

where \(I_0(\kappa)\) is the modified Bessel function of the first kind of order 0. The dispersion, \(1/\kappa\), is analogous to variance. If \(\kappa = 0\), the distribution is uniform, and as \(\kappa\) increases, the distribution approaches the normal distribution with mean \(\mu\) and variance \(1/\kappa\). Because our data potentially displayed multiple peaks, a mixture of von Mises with
multiple means and variances were fit and compared. The von Mises mixture model for orientation $x$ is

$$f_{vm}(x | \mu, \kappa, \pi) = \sum_{j=1}^{M} \pi_j f_{vm}(x | \mu_j, \kappa_j),$$

a weighted sum of multiple von Mises distributions of varying means and dispersions. We define $\pi_j$ as the weight of the $j$th model in the mixture model of order $M$. Bold denotes a vector of all of the values for the corresponding von Mises models. The order of the von Mises mixture model is the number of combined von Mises distributions. The 0th-order model is equivalent to a uniform circular distribution whose values, like those of the normalized rate data, sum to 1. Orders 1 to order 8 models employ 1-8 von Mises distributions, respectively. Model orders up to 8 were included so that all reasonably feasible multimodal possibilities given the empirical width of tuning peaks were evaluated.

For each condition (arena, track standard, and track rotated) and neuron, a directional tuning vector was calculated from a randomly-selected half of the data as described in the directional tuning vector section of the Supplemental Methods. Mean firing rates were converted to a proportional number of data samples in the mean direction for each bin. Then, separately for each order model from 1-8, we implemented the estimation-maximization (EM) algorithm$^{26}$ to find a model fit to the data. The EM algorithm for a von Mises mixture model has already been applied and used in audio source research$^{27}$ and can be described in four steps:

Initialize parameters for the each of the von Mises distributions in the model. We initialized the means by separating the means as far as possible on the circle, given the
order of the model being used. So for a 4th order model, means were initially located 90° apart. Concentration values scaled linearly with the number of distributions. This was done to keep distribution overlap at initialization relatively similar across the model orders. Finally, the mixing parameter was always set to equal probabilities for all models. To account for the possibility that mean initialization orientations effected the outcome, we offset our equally spaced distributions at 10 equal offsets between the original model locations and ran the algorithm with each initialization. The model with the lowest log likelihood was the one used for further analysis.

**E Step:** Evaluate the responsibility $\gamma$ each von Mises distribution model has in the prediction of each of $N$ data points

$$y_{ij} = \frac{\pi_j f_{vm}(x_i \mid \mu_j, \kappa_j)}{\sum_{m=1}^{M} \pi_m f_{vm}(x_i \mid \mu_m, \kappa_m)},$$

where $y_{ij}$ is the responsibility of the $j$th von Mises distribution in the model for the $i$th data point, $x_i$.

**M Step:** Re-estimate the parameters $\mu$, $\kappa$, and $\pi$ using the current responsibilities $\gamma$. Specifically, this is done by solving the following equations for each of the means

$$\mu_j = \tan^{-1} \frac{\sum_{i=1}^{N} y_{ij} \sin(x_i)}{\sum_{i=1}^{N} y_{ij} \cos(x_i)},$$

the concentrations

$$A(\kappa_j) = \frac{I_1(\kappa_i)}{I_0(\kappa_i)} = \frac{\sum_{i=1}^{N} y_{ij} \cos(x_i - \mu_j)}{\sum_{i=1}^{N} y_{ij}},$$
and the mixture parameters

\[ \pi_j = \frac{1}{N} \sum_{i=1}^{N} y_{ij} \]

for each von Mises distribution in the model. Please note that \( k_j \) is best solved by numerically inverting the defined function \( A(k_j) \).

Check for convergence of the log likelihood of the data given the model. We quit when the log likelihood improved by less than 0.01%. If convergence is not reached, update the parameter values using the newly calculated parameters and repeat steps 2 and 3.

**Evaluation of Von Mises Mixture Model Fit**

For each neuron, each model was cross-validated by calculating the sum-squared-error (SSE) between model values and actual direction tuning values for the remaining half of the data. The ratio of SSE of each order mixture model to SSE of the circular (0th-order) model for that same neuron was used to determine overall goodness of fit. Taking into account the trade-off between model fit and model complexity, we defined the 'best' model as the model yielding a 50% improvement in fit over the circular model and a 20% improvement over the next simplest model. Similar results were obtained using thresholds of 40-60% improvement over the circular model and 10-22.5% improvement over the next simplest model (Supplemental Figures 2.5-6)

**Spatial Independence**

The spatial independence criterion was used to identify neurons that were consistently active when the animal was facing a preferred orientation regardless of spatial location on the track. For all 2 cm by 2 cm spatial bin locations with at least 10 samples in a peak 10-degree bin orientation, the neuron was considered active if its mean rate at that
position and orientation was at least 50% of its overall mean rate for that orientation. If more than 50% of the viable locations for both peaks of a neuron were active, the neuron was considered to meet this criterion.

**Maxima and Minima Orientations and Ratios**

Peak orientations are the von Mises mixture model orientation parameters. Large and small peaks are determined by the mixture parameters of the model. Maxima and minima values for ratio calculations are determined by actual data values from the orientation bins containing the model maxima and minima. Peak to minimum ratios are the mean of the peak values divided by the mean of the minima values. Peak to peak ratios are simply the larger peak value divided by the smaller peak value.

**Correlation Across Track Positions**

Pearson correlations for the track rotation experiment were calculated between the directional tuning vectors of the track data in the normal orientation and directional tuning data from the 90-degree rotated track session for each individual neuron. Pearson correlations were also calculated between the normal track orientation data and the 90-degree rotated track data shifted 90 degrees. A Wilcoxon ranked sum test was run between the two populations for the axis-tuned subpopulation.

**Alignment of Peaks**

Peak alignments for the track rotation experiment are calculated using the angle difference between the larger peak on the non-rotated track and the closest peak on the rotated track.

**Data and Code Availability**

The data that support the findings of this study and code used for analysis are available from the corresponding author upon request.

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Supplemental Figure 1: Summary of recording site histological data. Recordings of subiculum neurons (N=542) were obtained from a total of five four-tetrode bundles in three animals. Numbers of total recorded neurons and numbers of neurons with axis-tuned firing are included above each figure. Red arrows depict tracks left by the bundles and their approximate endpoints. Three of the recording sites were restricted to the subiculum, and two (NS15-left and the lateral bundle in NS16-right) were in a transition zone bordering the CA1 subregion. Abbreviations: RCTX (retrosplenial cortex), DG (dentate gyrus), SUB (subiculum).
**Supplemental Figure 2:** Waveform discrimination. **A.** Histogram of isolation distance values, binned in log space, for all 542 neurons in the dataset (blue). Overlaid in orange is the histogram of isolation distance scores for the model-defined axis-tuned neurons. **B.** Waveform discrimination for five axis-tuned neurons recorded on the same tetrode from one recording of rat NS14. **Center:** Color-coded clusters of individual waveforms in a 3D plot of peak-valley voltage on wires 2, 3, and 4 (wire 1 not recorded). For each cluster, the color-coded waveform plots of all waveforms were included. Isolation distance values are reported for each cluster here as well as in C and D. **Surrounding polar plots:** Adjacent polar plots to each waveform plot show the directional tuning of that neuron on the track. **C.** Waveform discrimination of one axis-tuned neuron (red) with all other waveforms recorded (black points) shown from rat NS15. The same three plots are included here as in panel A, but the peak-valley voltage plot is only of wires 1 and 2. Wires 3 and 4 were not recorded on this tetrode. **D.** Waveform discrimination for three neurons recorded on a single tetrode from rat NS16. Here, two are axis-tuned and one is not.
Supplemental Figure 2 – Waveform Discrimination

A

Isolation Distance Scores

- All Subiculum Neurons
- Axis-Tuned Neurons

B

10 Hz
Iso Distance = 120.9

15 Hz
Iso Distance = 50.4

15 Hz
Iso Distance = 78.8

20 Hz
Iso Distance = 98.3

40 Hz

D

20 Hz
Iso Distance = 214.6

15 Hz
Iso Distance = 63.2

20 Hz
Iso Distance = 45.4

60 Hz
Iso Distance = 149.4
Supplemental Figure 3: Spike characterization and open arena spatial specificity

**A.** Neurons (N=542) plotted based on spike width versus burst index. The distribution is consistent with prior work (Kim et al., 2012).

**B.** Histogram of spatial information scores for the entire dataset. Similar to previous studies, the degree of spatially-specific activity among the population varied greatly.

**C.** Shown are example neurons whose positional firing rate maps reflect the observed range of high to low specificity as measured by spatial information (Skaggs et al., 1993). Selected neurons are the median representatives from each decile of the spatial information value distribution. This is done to provide a holistic representation of the range of informativeness of SUB neurons.
**Supplemental Figure 4:** Open arena spatial correlates A. Four sample neurons with strong correlations to ideal place cell templates. 2D arena firing rate maps (larger images) are shown using the same color mapping as in Supplemental Figure 3. Max firing rate listed above and to the right of the rate map. Smaller images are the best fitting BVC (top) and place cell (bottom) templates. Pearson r values for each is overlaid on the corresponding template. In all four neurons, the place cell template r value greatly exceeds that of the BVC template, capturing the patterns seen in the actual rate map. B. Four sample neurons with strong correlations to ideal BVC templates, presented identically to those in A. C. **Bottom panels:** Histograms of Pearson r values for all 354 neurons with greater than 250 spikes on the arena (blue) and of just the axis-tuned neurons (orange). The left histogram shows the place cell template values while the right histogram presents the BVC template values. The red lines show the cutoff value of 0.4 chosen to characterize a neuron as well fit by the template. Middle histogram: Histogram of the difference between the best BVC and place cell templates for all neurons with either best template Pearson r value over the 0.4 cutoff (blue N = 114/354) and the axis-tuned neurons meeting the same criteria (N =12/47, orange). This orders neurons having spatially specific firing from more place-like on the left to more BVC-like on the right. Top panel: 2D arena firing rate maps from an arbitrarily-selected bin where the superior template between the place cell and BVC templates are visually identified to be inconclusive. At this juncture, approximately 40 neurons (3 axis-tuned) are more BVC-like and 75 (9 axis-tuned) are more place-like.
Supplemental Figure 4 – Open Arena Spatial Correlates

A
17 Hz
r = 0.43
r = 0.72
Rat NS 15 - Neuron 87

37 Hz
r = 0.31
r = 0.47
Rat NS 14 - Neuron 46

11 Hz
r = 0.43
r = 0.69
Rat NS 15 - Neuron 237

10 Hz
r = 0.40
r = 0.56
Rat NS 16 - Neuron 110

B
20 Hz
r = 0.65
r = 0.49
Rat NS 16 - Neuron 77

29 Hz
r = 0.67
r = 0.48
Rat NS 15 - Neuron 47

16 Hz
r = 0.56
r = 0.41
Rat NS 15 - Neuron 136

17 Hz
r = 0.51
r = 0.32
Rat NS 16 - Neuron 138

C
More Place-Like

More BVC-Like

Best Place Template R Values

Neuron Count

Best BVC Template R Values

BVC – Place R Value Difference

Pearson r

All Subiculum Neurons
Axis-Tuned Neurons
**Supplemental Figure 5:** Model schematic. **A.** Example neuron training data and model fits. For all plots, directional tuning data is shown from a randomly-selected half of the track data used for training (blue rose plots). Von Mises mixture model fits of each order used (0-8) are overlaid (red ellipses). **B.** Directional tuning plots from the remaining half of data were used for cross-validation. Sum-squared-error (SSE) between the cross-validation data and each order model (red, same models as in A) is printed above each figure along with its SSE Ratio, the remaining error normalized by the amount of SSE of the naïve circular model (order 0). For a model to be considered a ‘best fit’, the SSE Ratio must be below 0.5 (<50% error of naïve circular model). Among model orders with SSE Ratios below 0.5, the difference between the model and preceding order model must be greater than 0.2 (20% improvement; difference value printed above plots, see also Supplemental Figure 5). For this neuron, the criteria lead to selection of the 2nd-order model (red box). **C.** To be considered as strongly axis-tuned, two more criteria must be met. First, the ratio of mean actual data firing rate at model maxima to mean actual data firing rate at model minima must exceed 2. Mean peak (maxima) values for this neuron (green lines) were 8.2X mean minima values (black lines). Finally, the spatial independence of each model’s maxima must both exceed 50%. For all track locations associated with movement in either of the two preferred directions, the neuron was considered ‘active’ if its mean rate at that position and orientation was at least 50% of the overall mean rate for that direction. In the case of this neuron, most of the points in the light green orientation lie along the light green arrows on the right panel (see also Figure 1B – right panel) and the points in the dark green orientation lie along the dark green arrows. Neurons were considered spatially independent if the majority of associated locations met this criterion. For this sample neuron, the larger, light green peak had 88% spatial independence and the smaller, dark green peak had 58% spatial independence. Because it met all three criteria, this neuron is in the axis-tuned subpopulation.
Supplemental Figure 5 – Model Schematic

A  Training Data and Von Mises Mixture Model Fit

B  Cross-Validation Data and Von Mises Mixture Model Fits

C  Rat NS14 - Neuron 28  Big Peak  Small Peak
Peak Rates (green)  22 Hz  11 Hz
Min Rates (black)  2 Hz  1 Hz
Spatial Independence  88.7%  57.1%
Ratio Peaks:Mins  8.2 : 1
Supplemental Figure 6 – Model Parameter Flexibility

Supplemental Figure 6: Model parameter flexibility. To select the best order for the von Mises mixture model for each neuron, we trade off model complexity with fit improvement by selecting the most complicated model that yields 20% improvement in sum squared error over the preceding order model. The criterion is arbitrary but qualitatively consistent across a wide range of values. Here we plot the number of neurons in the population categorized into each order type based on a range of criterion values. Criterion values ranged from 2.5-40% by increments of 2.5% (blue lines, dark colors – light colors = low to high criterion values). We selected the 20% value for our criteria (red lines). Track model fitting data is given in the left panel. From 10-22.5%, more neurons are classified as 2nd-order than any other order. This demonstrates that this is a property of the population and not the model parameters, especially when compared to arena data (right panel), where no model parameter results in a population bias to 2nd-order (bimodal) mixture models.
Supplemental Figure 7: Track axis-tuned neurons in the open arena. For each neuron meeting criteria for strong axis-tuned firing during the track running session, the positional firing map for the same neuron is shown for the arena foraging session. Peak (arena max) firing rates are given above each, utilizing the same color map from Figures 1 and 3 and listed in the legend in the top left.
Supplemental Figure 8 – Axis-Tuned Neurons in Light vs Dark

Supplemental Figure 8: Axis-tuned neurons in light versus dark. One day of both light and dark recording was obtained which included 3 model-defined axis-tuned neurons. Each panel contains an individual axis-tuned neuron’s firing rate color-mapped as a function of track position for all time periods associated with travel >3 cm/second for both the light and dark conditions, highlighting the similarity in firing without the presence of prominent visual cues.
Works Cited


CHAPTER 3: Phase Precession of Subiculum Axis Cells

Abstract

Subiculum is a primary cortical output structure in the hippocampus and a link to the associative retrosplenial and parietal cortices. However, what information it contributes to these regions’ spatial representations is largely unknown. Recently we described a population of axis-tuned neurons that fire when the animal runs in either of two opposing directions on a complex maze. This head-direction-constrained signal was only present on the track, showing a contextual specificity consistent with place cells of the hippocampus. Here, we report that this population of neurons robustly phase precesses throughout its firing fields. There are, however, prominent differences in field shape, length, and variability as compared hippocampal CA1 place cells. This result suggests a close link between these populations while also providing evidence to the importance of the difference in input and network properties to phase precession. We therefore propose that the region may also be an important new system for the investigation into phase precession mechanisms.

Introduction

Ever since Scoville and Milner’s seminal paper detailing the cognitive impairments of H.M.,¹ the hippocampal formation has been considered the nexus of memory in the mammalian brain. The groundbreaking discovery²,³ and subsequent description (e.g. McNaughton, Barnes, & O’Keefe, 1983; Muller, Kubie, & Ranck, 1987) of neurons in rats with spatially specific firing fields area, termed place cells, added spatial navigation to the fundamental processes of the hippocampus. It has been proposed that these place cells are the foundation of a cognitive map of both space and general memories, linking the two cognitive domains⁶,⁷. The activation of a sequence of place cells while an animal traverses
a path can be thought of as a potential episodic memory but requires a mechanism for
linking the individual neurons and encoding the experience. The hippocampal theta
rhythm, a prominent 4-10 Hz rhythm in the hippocampal local field potential (LFP) during
active behavior of mammals \cite{8-10} (for a review, see Winson \cite{11}), had long been posited to be
important for behavior. In 1993 it was discovered that place cells fire earlier with respect
to the hippocampal theta as the rat progresses through the neuron's firing field. \cite{12}
Termed phase precession, this relationship has been hypothesized as the mechanism linking the
place cells through experience and the mechanism for forming episodic memories.

Phase precession is a robust phenomenon that has been extensively studied in
the hippocampus (for a recent review, see Jaramillo & Kempter \cite{13}). Briefly, nearly all
hippocampal subfield CA1 principal cells phase precess when animals run through place
fields in either two dimensional or track environments. \cite{12,14} Typically analyzed as trial
averages, hippocampal pyramidal cells do precess during individual traversals of firing
fields, although the trial averaged analysis are slightly different from individual traversal
analysis results. \cite{15} It even exists when neurons fire in REM sleep, during running on a
wheel and in navigation of virtual reality setups. \cite{16-19} Phase precession starts at
approximately 120 degrees before maximal peak firing of CA1 pyramidal cells and
precesses anywhere from 120 to nearly 360 degrees through the theta cycle. \cite{12,14}
Phase/space coupling is stronger at the beginning of the firing field. \cite{14}

The reliability and widespread nature of phase precession has led to hypotheses
of multiple different functions supporting learning and memory. Phase precession
produces a phase code similar to that of a rate code for spatial location. \cite{12,20}
Firing phase of a given neuron can be more predictive than firing rate for spatial location. \cite{21}
As mentioned in the opening, phase precession may be a mechanism for the encoding of
experienced sequences. \cite{14} As an animal traverses space, it is simultaneously in the place
fields of many neurons – some early, some late. Due to the precession of spike timing through the phase of the theta rhythm, in individual theta cycles, the neurons fire first for the field first entered (that the animal is currently leaving) and then continue through to fields it is just beginning to encounter. This leads to a tight sequencing of action potentials from consecutive place cells in the time windows necessary for the Hebbian learning rules of long term potentiation and depression the components to asymmetric spike-timing-dependent plasticity rules.\textsuperscript{22–24} A complementary function has been proposed of retrieving memories of sequences and predictions of upcoming actions. This is hypothesized to occur through the network interactions of these learned sequences due to phase precession timing.\textsuperscript{25}

The existence of phase precession is not limited to CA1 in the hippocampus. Phase precession has been reported in all other hippocampal subfields\textsuperscript{14,26,27} and in grid cells of entorhinal cortex.\textsuperscript{28} Most recently, the remaining hippocampal subregion, the subiculum, was reported to show phase precession.\textsuperscript{29} It has even been described in limited populations in two ventral outputs from hippocampus – medial prefrontal cortex and ventral striatum.\textsuperscript{30,31} This extension to output regions constrains potential mechanistic models for phase precession generally. At the same time, it hints at the manner of connectivity with hippocampus to these output regions.

In Chapter 2,\textsuperscript{32} we reported on the existence of axis-tuned neurons in the dorsal subiculum. Given the vital hypothesized function of phase precession and previous reports in hippocampal output structures including subiculum, we investigated whether axis-tuned neurons also exhibit phase precession. Here we report that phase precession is robust in nearly all subiculum axis-tuned neurons. The shape of firing fields and precession resembles that of CA1, with notable exceptions to field size and shape. Finally, positive correlation diagonal striping of firing inhibition in the phase/space firing plots suggests an
additional rhythmic element occurring in high firing neurons. These findings identify a new population of neurons exhibiting phase precession and inform current mechanistic models.

**Methods**

**Subjects**

All subjects were adult male Sprague-Dawley rats ($N = 3$). As described previously, 32 rats were housed individually and kept on a 12-h light/dark cycle. Prior to experimentation, animals were habituated to the colony room and handled for 1–2 weeks. During training and experimentation, rats were food restricted and weights were maintained at 85–95% of free-fed weight with water available continuously. Rats were required to reach a minimum weight of 350 g (5–10 months of age) before surgery and subsequent experimentation. All experimental protocols adhered to AALAC guidelines and were approved by the IACUC and the UCSD Animal Care Program.

**Apparatus**

Behavioral tasks were conducted using a triple ‘T’ track maze. The track (Figure 3.1A; 8-cm-wide pathways, overall 1.6 m x 1.25 m in length and width, painted black) stood 20 cm high in the middle of a large recording room and was visually open to prominent distal cues. The track edges were only 2 cm in height, allowing an unobstructed view of the environment.

**Behavior**

Rats were habituated to the maze during two 30-min periods of free exploration. Animals were then trained, as described previously, to run ballistically from the midpoint of one of the long edges of the maze into the center of the apparatus and continue until reaching the long edge opposite the start point (Figure 3.1A, black dashed lines). This consisted of straight sections interleaved with three left or right turns for a complete route run. The total route lengths were 140 cm, with turns at 51 cm, 87 cm and 118 cm. Reward
(a half piece of Cheerios cereal) was made available at the four reward sites. Over 1–2 weeks, animals were trained by approximation to make route traversals between food reward sites. Over at least 2 additional weeks, animals were trained by simple trial and error to a criterion of 80% for ballistic (uninterrupted) route traversal. Once animals met criterion, they were trained two or three times on the track in the normal orientation, immediately followed by training on the track in the 90° rotated orientation. This established familiarity with the rotated track, but the rats were not extensively trained in this orientation. Animals were surgically implanted only after this level of task performance had been achieved.

Multiple reward tasks were used across the set of animals. In an all-but-repeats task, used for animal NS14, the animal was rewarded at any of the four locations except when the animal repeated the same location as the previous run. In a visit-all task used for NS15 and NS16, the animal was rewarded at all locations, but needed to visit all locations before rewards were reset at all routes.

**Surgery**

Rats were surgically implanted with tetrode arrays (twisted sets of four 12.5-μm nichrome wires) inserted into custom-built microdrives (four to eight tetrodes per microdrive). Rats were implanted bilaterally with two or three microdrives into dorsal subiculum. Rats were anesthetized with isoflurane and positioned in a stereotaxic device (Kopf Instruments). Following craniotomy and resection of dura mater, microdrives were implanted relative to bregma (A/P −5.6 to −6.6 mm, M/L ±1.6 to ±2.7 mm, D/V −1.5 to −2.2 mm).

**Recordings**

After recovery from surgery, animals were retrained for at least 1 week before beginning recordings to ensure adequate behavior and running ability with the new weight.
of the implant. Because of this procedure, all recordings were from animals that were well trained on the task. As described previously,\textsuperscript{32} electrodes were moved ventrally in 40-μm increments between recordings to maximize the number of distinct units collected. Each microdrive had one or two electrical interface boards (EIB-16, Neuralynx, Bozemon, MT) connected to a single amplifying headstage (20×, Triangle Biosystems, Durham, NC). A tether led to a set of preamplifiers (50×) and a high pass filter (>150 Hz). Signals are amplified, filtered, digitized, and written to disk using the Plexon ‘MAP’ system (Plexon, Dallas, TX). Signals then fed into the acquisition computer running Plexon SortClient software and were filtered at 0.45–9 kHz; further amplified 1–15× (to reach a total of 1,000–15,000×), and digitized at 40 kHz. Local field potentials are obtained from alternate filtering of unit recording wire signals using a 0.7 – 300Hz bandpass. Single units were isolated in Plexon Offline Sorter software. Waveform parameters used were peak height, peak valley, energy, full width at half maximum, and principal components. Waveform clusters appearing to overlap with the amplitude threshold set for collection were discarded to avoid collection of neurons with partial spiking data. Waveform amplitudes were monitored to ensure systematic fluctuation did not produce confounds in isolating single units.

After completing unit isolation, a modified isolation distance value was calculated for each unit to assess cluster quality. For a complete procedural description, see Olson, Tongprasearth, and Nitz.\textsuperscript{32}

Animals’ position was tracked using a camera set 2.6 m above the recording room floor. Plexon CinePlex Studio software was used to detect red and blue LED lights placed on the animal’s surgical implant, centered on the animal’s head and separated by approximately 5 cm. Position location of the lights was captured at 60 Hz. The animal’s position and orientation was determined by averaging the location of the two lights and
calculating the orientation of the vector between the lights. Using the fact that the track apparatus was squared to the room, we averaged the orientation of all time periods with >3 cm/s running and positions on the middle half of the return arms of the track. This angle was defined as 0°, or ‘room north’, for the recording and was used to align the animal’s heading to the room. Recordings lasted 45-75 min. The animal would run in an arena for 3–10 min and then on the track for approximately 80 rewarded runs (Figure 3.1A, top panel). For track rotation recordings, the animal had access to water for 5 min after completing the first session while the track was wiped down and rotated and then ran for another 80 rewarded runs (Figure 3.1A, bottom panel).

We recorded a total of 542 subiculum neurons across three rats (Supplemental Figure 3.1). In this paper, we only analyze those neurons identified as axis-tuned neurons. For the first animal, 22/81 neurons recorded from the right hemisphere were axis-tuned. For the second animal, 6/127 left hemisphere and 1/194 right subiculum neurons met axis-tuned criteria. For the third animal, 4/42 and 14/98 neurons, respectively, were axis-tuned from medial and lateral tetrode bundles in the right hemisphere. No axis-tuned neurons were excluded from analysis, and all identified neurons are included in the above neuron counts, even if activity is minimal.

**Histology**

Animals were perfused with 4% paraformaldehyde (vol/vol) under deep anesthesia. Brains were removed and sliced into 50-μm sections and Nissl-stained to reveal the final depth of electrode wires in subiculum. Microdrive depth monitored across recordings and final electrode depth as observed in histology were compatible in all cases.

**Identification of Ballistic Route Traversals**

To identify clean runs for individual routes, a multistep process using custom MATLAB graphical user interfaces is used. All runs through user-defined starting and
ending gates that sustained 3 cm/s or faster running throughout was labeled as a clean run for the given route. Human verification occurs afterwards to ensure no deviations from the route. The results of this process can be seen in Figure 3.1B. By this method, stalled track traversals, reward periods, and other position data captured between runs are not conflated with data with controlled action and spatial values.

**Identification of Axis-Tuned Neurons**

Detailed procedures for identifying axis-tuned neurons are written in Olson, Tonprasearth, and Nitz. Briefly, von Mises mixture models were used to fit direction turning vectors. The order model with the best fit, measured by quality overall fit and substantial improvement over the model of one order less, was considered the order of the neuron. Axis-tuned neurons were those neurons with order two models, spatially independent firing in both model-defined peak orientations, and well-defined directional tuning peaks as compared to minima.

**Positional Firing Rate Maps**

To characterize the firing activity of the subiculum neurons, we calculated individual neurons’ positional firing rates by dividing the total number of spikes of each neuron at each location by the total occupancy time at each location. To include only data where the animal was running, we excluded all samples with less than 3 cm/s velocity or greater than 20 radians/s angular velocity. For maps analyzing only clean runs, only data within the run time markers was included. The latter threshold was used to exclude cases of rapid head turning in the absence of locomotion. Positional firing maps were smoothed using a 2D convolution with a Gaussian filter with s.d. of 2 cm that also accounts for bins with no occupancy. Raw, unsmoothed data were downsamped to 2 cm × 2 cm bins for analysis of spatial independence of directional firing.

**Directional Tuning Vectors**
Head direction tuning vectors used in the polar plots of Figures 3.2, 3.3, and 3.4 were calculated using the same sample of running data as the positional firing rate maps (i.e., using the same velocity thresholds). Head orientations were binned into 36 10° bins. The total number of spikes per bin was divided by the total time in each bin to calculate the mean directional firing rate.

**Track Linearization Firing Rate Calculation**

For each recording, custom MATLAB software is utilized to generate a spatial template matching the average movement of the animal through 2D along each route. While highly similar, the recording by recording approach ensures the best possible match to animal behavior due to slight shifts between animals, LED placements, etc. The beginnings and ends of each straight run and turn period are marked and equal space bins are interpolated between marked locations. After establishing the template, each tracking position sample is mapped to the nearest bin. Firing rates for each traversal are calculated by summing the number of spikes in each template bin and dividing by the total amount of time the bin was occupied. A Gaussian filter with s.d. of 2 cm is used to smooth each traversal. Mean firing rates are calculated by summing mean rates at each location and dividing by the number of runs. Only the clean run data identified previously is used (see Identification of Ballistic Route Traversals).

**Local Field Potential Theta Filtering and Selection**

Local field potentials (LFPs) used for theta phase calculation were chosen from all LFPs of wires in the same drive bundle (3-8 tetrodes). Each signal was forward-backward filtered using a 5-10 Hz band pass filter. This filtering approach achieves no phase distortion – an important aspect of the filter for our purposes. Mean power over the 5-10 Hz window was calculated and the LFP for each recording bundle recording was selected as the wire with the highest mean power. This was chosen as a proxy for signal quality. A
Hilbert transformation was used to calculate the phase angle at each spike location for each neuron on the recording. We defined 0° to be the positive peak of the theta oscillation.

**Position-Phase Plots**

We use two types of position by phase firing activity visualizations in Figures 3.3 and 3.4. The middle portion of each figure bundle is a linearized position by phase spike raster plot. Each spike for the neuron is represented by a dot and the appropriate location in linearized route space and phase where it occurred. Phase is repeated over 720° to assist in visualization of patterns over any 360° window, so each spike is plotted twice on the figure. The bottom plots are spike density maps. These are binned and smoothed color-mapped versions of the spike raster plots. Bin sizes are 2 cm × 5° and smoothing is a Gaussian filter with s.d. of 1.5 cm and 7°. Visualizing rate changes improves on these plots as compared to spike raster plots, especially for high firing neurons. The figure axis are also the linearized route space by 720° of phase, identical to the spike raster plots.

**Identifying Position-Phase Defined Firing Fields**

In order to assess phase precession and firing field properties, firing fields must be defined. Traditionally, firing fields are assessed purely by firing rate over position, but because we see in our data that fields often overlap in position but not position by phase, we chose to identify fields in position by phase. To achieve this, we have adapted the approach of Kim, Ganguli, and Frank, who also identify fields in this manner. Briefly, we find all peaks in the position-phase spike density plots with greater than 15 spikes/sec and over 35% of the global peak value for the individual spike density plot. We then define the field as all adjacent pixels around the peak with values above the 35% of global peak value threshold. We then sort by highest peak to lowest for all fields and discard any fields that are subsets of other fields with higher firing rates.
Figure 3.1: Behavior and histology. A) Schematic of triple ‘T’ maze and route-running task. Animals made runs along each of up to eight partially overlapping internal routes (dashed black lines) on the 160 cm × 125 cm track apparatus, leading from a start site (green circle) to any of four goal sites (red ‘x’). From each goal site, the animal returned to the start via either of two return paths (dashed yellow lines). The behavior was run with the track in standard (top panel) and 90 degree rotated (bottom panel) orientations with respect to environmental rooms and prominent distal cues. B) Behavioral tracking of identified clean runs. Shown are all clean runs from one recording session in the visit-all-8 reward setup. Each color shows a separate route. Routes are minimally translated and stretched for visualization purposes. Routes shown in separate colors. C) Electrode placement and recording ranges in dorsal subiculum (three rats). Filled circles indicate final electrode placement and lines track dorsal-ventral recording depths across recordings.
Results

Rats were trained on the triple ‘T’ track maze to navigate through the center of the maze to the end-of-route reward locations in both standard and rotated track orientations relative to the room (Figure 3.1A). Animals moved freely in all areas except on the inside routes where backtracking was prevented during training. Animals were extensively trained before any recordings occurred. Animals’ locations were tracked using an overhead camera and head-mounted LEDs. To control for behavioral variation and ensure smooth traversal through spaces for analysis, we defined routes and subsequently scored the animal tracking data for smooth, ballistic running of entire routes. This gives us data with stereotypical movements through any section of a route. The defined routes are either internal routes towards rewards or external routes leading to the internal entrance (Figure 3.1B). Henceforth we will refer to these ballistic routes traversals as clean runs. Each clean run was linearized by mapping tracking locations to the nearest 1 cm bin of a template of the traversed routes. To assess the representations present in subiculum, single unit activity was recorded from 194 left hemisphere and 348 right hemisphere subiculum neurons (542 total) of three rats under the aforementioned conditions. Electrode tracks and endpoints are depicted in Figure 1c with complete histological data in Supplemental Figure 3.1.

As previously reported,32 many neurons in dorsal subiculum were active when the animal ran in either of two opposing directions on the track (Figure 3.2A). We referred to this as axis tuning and quantified this activity to consist of two robust peaks in direction firing that were independent across space. This resulted in ~10% (n = 47/542) of neurons classifying as axis-tuned. As seen in the example in Figure 3.2, these “axis cells” maintained their orientation to the distal cues of the room, consistent with head direction neurons.34 They do not, however, maintain the firing when in other environments (Figure
Figure 3.2: Axis-tuned neuron in dorsal subiculum. An example axis-tuned subiculum neuron. 

A) Each panel (left) depicts firing rate color-mapped as a function of track position in either the standard (top) or rotated (bottom) track configuration. Polar plots (right) depict mean firing rate against head orientation of the corresponding data on the left. Rate displayed is the value at the magnitude of the outside circle. NS15 was the designation of the rat. 

B) Positional firing rate map (left) and directional firing polar plot (right) of the same neuron on an open arena environment.
3.2B). These properties could be described as a mixture of both head direction and place cell attributes. Since head direction cells do not phase precess but place cells do, we examined the position by LFP theta phase to assess whether axis cells phase precess.

Apparent by visual inspection of linearized position by theta phase spike raster and spike density plots, phase precession is prevalent and striking in robustness in axis cells (Figures 3.3, 3.4). A characteristic of axis cells is multiple firing fields on the apparatus. All strong fields for both neurons in Figures 3.3 and 3.4 show the characteristic progression of earlier phase firing as the animal traverses the field. Like hippocampal CA1 neurons, the phase that precession begins at is approximately 120° before the local theta peak and consistent across the neurons and fields shown. Often the precession extends for nearly 360°. Low firing locations consistent with the neuron’s tuning directions only appear to have phase locked firing at this same frequency but without the strong precession, potentially hinting at differences in circuitry driving activity at separate locations.

CA1 and subiculum reciprocal connectivity exists at corresponding levels of the transverse axis of the hippocampus. The place fields in the input areas of CA1 that project to the area of dorsal subiculum that we are recording are approximately 30 cm. Axis neuron precession field size may be linked and comparable to CA1. This was not strictly the case. Field length often mapped to the size of the track where the preferred direction of travel was ongoing. For example, fields aligned with directions only abruptly experienced on turns were limited to 15 cm bouts of firing and phase precession (Figure 3.4, right neuron), whereas some fields stretched the entire 125 cm of the return route straightaways (Figure 3.4, left neuron). Importantly, this was not always the case. When routes of sustained preferred-direction running were larger than a typical dorsal CA1 field, multiple fields sometimes occurred, perhaps showing an intermediate form of place cell influence on axis tuning.
Figure 3.3: Phase precession of axis-tuned subiculum neurons. A) For one neuron, firing rate maps from the standard (top) and rotated (bottom) alignment recording sessions. In each graph cluster, the two dimensional positional firing rate (top right of cluster) for data from identified clean runs is color-mapped as a function of track position. The polar plots inset in the two dimensional positional firing rate maps depicts mean firing rate against head orientation of the corresponding data in the same style as Figure 3.2. For each route (six total per session/cluster, four internal, two external), three representations of firing activity with respect to linearized route position are shown next to an icon of the corresponding run: linearized mean firing rate maps (top), position by phase spike raster plots (middle), and color-mapped position by phase spike density plots (bottom). Data is organized so that animal progression is from left to right. For linearized position by phase plots, the full phase cycle is repeated twice to allow full period patterns to be visualized without interruption. B) Identical arrangement of firing rate maps from another example neuron. Note that for this neuron, the animal made no clean runs for one of the internal runs in the rotated session.
Field size did appear to determine many phase precession traits of axis cells. CA1 phase precession fields often have a downward bend in the slope of precession and an increase in variance of spiking as the animal progresses through the field.14 Both of these traits are not as clear in subiculum axis cells and appear to depend on field length. For small fields, i.e. those of similar length or shorter to CA1 fields, some downward bend of the precession may occur but it is not extremely pronounced. The variance further in the field stays comparable to initial values for small fields instead of growing, suggesting a difference in traits for the mechanism determining variance of firing between the two populations. On the other hand, during longer fields of axis cells, downward bend is not seen but variance becomes very large, encompassing nearly all phases, as can be seen on the long return routes of the left neuron of Figure 3.3.

We adapted a computational approach previously used to identify firing fields of subiculum neurons.29 We chose this method because of the use of position by phase firing maps to find firing fields. As can be seen in multiple locations such as the first return route of the rotated dataset for the left neuron in Figure 3.3, firing rate may not show differences in fields that phase by space does. Field lengths identified are shown in Figure 3.5A. Field lengths are clearly often much longer than typically seen in adjacent dorsal CA1. A hypothesis of many phase precession models suggests that firing rate modulates precession rate. We see no relationship between phase precession field length and firing rate Figure 3.5B.

Finally, an additional rhythmic phenomenon appears to exist in the long firing fields of some high firing neurons. Positively sloped position by phase stripes of inhibition exist in these firing fields. This is highly visible in the return firing fields of the left neuron of Figure 3.4. The slope suggests an additional inhibitory influence at a lower frequency than the LFP theta.
Figure 3.4: Additional examples of phase precession of axis-tuned subiculum neurons. For two additional neurons, firing rate maps from the standard alignment recording sessions, shown in the same style and layout as Figure 3.3. In each graph cluster, the two dimensional positional firing rate (top right of cluster) for data from identified clean runs is color-mapped as a function of track position. The polar plots inset in the two dimension positional firing rate maps depicts mean firing rate against head orientation of the corresponding data in the same style as Figure 3.2. For each route (six total per session/cluster, four internal, two external), three representations of firing activity with respect to linearized position are shown next to an icon of the corresponding run: linearized mean firing rate maps (top), position by phase spike raster plots (middle), and color-mapped position by phase spike density plots (bottom). Data is organized so that animal progression is from left to right. For position by phase plots, the full phase cycle is repeated twice to allow full period patterns to be visualized without interruption. Linearized firing rate scale is consisted across paths within a neuron and the max is listed in the top left. All spike density plots are normalized to max firing of that individual plot.
Figure 3.5: Phase precession field size properties. A) A histogram showing the distribution of identified phase precession field lengths on the triple ‘T’ maze. B) Maximum spike density, a proxy for firing rate, as a function of the length of the phase precession field. Each dot represents one phase precession field.
**Discussion**

Axis cells of dorsal subiculum phase precess with respect to the local LFP theta rhythm. The phase that fields begin firing at is consistent with CA1 neurons. Precession typically extends over a longer range of phases, traversing nearly the entire cycle. Spatial firing fields for individual axis cells phase precess over drastically different distances, showing a precession rate flexibility within individual neurons not previously reported. Fields often extended to the entire length of the segment in the preferred firing head direction but was more often to segment into fields with extended distance. Phase/space coupling is at least as strong as CA1 neurons for short firing fields, but the majority of elongated fields have much weaker phase/space coupling. Finally, a slower inhibitory rhythm appeared in spike density plots of particularly high firing and elongated fields.

An open question remained after the initial reporting of axis cells as to the role of spatial inputs versus head direction inputs to produce the firing properties of this class of neurons. Axis cells clearly show contextual differences in firing across paradigms that is more consistent with the spatial specificity and remapping of place cells. Unlike place cells, however, they maintained orientation to the room and not the track. Strong phase precession strengthens the tie of input importance to the hippocampal spatial system. Concurrently, the propensity for the fields to stretch the entire length of segments at a length well beyond place cells in the region separates axis tuned neurons from place cells. CA1 place cells have a typical size that is fairly robust\(^{36,37}\) (but see also Nitz\(^{38}\)). Subiculum axis cells do appear to only segment at lengths beyond that of individual place fields. A conjunction of head direction and place cell input could be required for EPSPs sufficient for robust firing. Incomplete spatial coverage of place cell inputs to that individual axis neuron may potentially explain segmenting of fields.
Many different mechanistic computational models have been proposed to account for phase precession in the hippocampal formation. Broadly speaking, phase precession models fall into two categories. In one camp are models focusing on intracellular mechanisms for phase precession generation. In these models, the internal somatic oscillation at the LFP theta frequency interacts with either a dendritic oscillation due to excitatory EPSPs\textsuperscript{12} or potentially a ramping of excitatory activity.\textsuperscript{39} The alternative group predicts the phenomenon arises from the interactions of a feedforward network of place cells as the animal moves through the sequential fields.\textsuperscript{21} Evidence for each has been mixed (see Burgess & O'Keefe\textsuperscript{40} and Maurer & McNaughton\textsuperscript{41} for a review of models and evidence), and a combination of current explanations is a real possibility.

Testing these models is particularly difficult due the difficulty of isolating and disturbing coordination phase precession without creating drastic differences in firing activity of the neurons or the theta oscillation. Unfortunately, the limited capability of dissociating phase precession from firing rates and the theta rhythm has slowed definitive determinations of both phase precession mechanism and function. There have been a few creative successes, such as the findings that cannabinoids impair hippocampus-dependent memory and have been shown to disrupt sequences on a theta time scale.\textsuperscript{42} Additionally, new technologies like intracellular recordings in head-fixed animals during virtual reality paradigms are used to investigate intracellular activity patterns during phase precession.\textsuperscript{43} Overall, new access to data could be extremely valuable in constraining models and determining the underlying mechanism.

Axis cells could prove to be an ideal test bed for investigations into phase precession mechanisms. Many aspects of axis neuron phase precession are different from that in CA1 and would constrain models considerably. Axis neuron firing fields vary in their length of fields, and exhibit increased firing variance for elongated fields, an aspect
nonexistent in CA1. The majority of these long fields more closely reflect the latter stages of CA1 place fields, posing an additional constraint on models. Many current models propose faster precession during higher firing rates, a result contradictory to axis neuron’s phase precession data. Another intriguing aspect of the data is the shape of the firing fields and their precession. CA1 place fields have been shown to be largely negatively skewed so that firing increases as the animal progresses through the field,\textsuperscript{44} and this was posited to be best explained by ramp models of precession.\textsuperscript{39} Observationally, many of the firing fields in the linear rate maps reach maximums early in the field with a long slow decrease in firing. Investigating what network or intracellular differences exist between the classes of neurons and if the current model could also account for these firing field shapes would considerably strengthen the evidence for its validity. Others suggest that phase precession seen in hippocampal outputs such as subiculum, medial prefrontal cortex, and ventral striatum may just be a propagation of the effect from upstream CA1 neurons.\textsuperscript{45} While this remains to be tested, axis cells’ unique characteristics should allow for creative behavioral paradigms to tease apart predictions of these hypotheses.

Overall, the unique aspects of axis neuron phase precession add to the complexity of a unique cell type. These neurons’ activity is clearly modulated both contextually and directionally, providing an output that links route spaces with analogous utility. The dorsal subiculum is positioned as a hippocampal output to spatial cortices such as retrosplenial cortex that are known to transform spatial information in multiple different spatial frames of reference.\textsuperscript{46} These results show that this transformation is already occurring in subiculum in a population temporally coordinated with the entirety of hippocampus, and emphasizing the integration of a subregion understudied for its role in navigation and memory.
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The authors declare no competing financial interest.
Supplementary Figure 3.1: Summary of recording site histological data. Reprinted from Olson, Tongprasearth, & Nitz, 2017. Recordings of subiculum neurons (N=542) were obtained from a total of five four-tetrode bundles in three animals. Numbers of total recorded neurons and numbers of neurons with axis-tuned firing are included above each figure. Red arrows depict tracks left by the bundles and their approximate endpoints. Three of the recording sites were restricted to the subiculum, and two (NS15-left and the lateral bundle in NS16-right) were in a transition zone bordering the CA1 subregion. Abbreviations: RCTX (retrosplenial cortex), DG (dentate gyrus), SUB (subiculum).
**Supplementary Figure 3.2: Model schematic.** Adapted from Olson, Tongprasearth, & Nitz, 2017.

**A)** Example neuron training data and model fits. For all plots, directional tuning data is shown from a randomly-selected half of the track data used for training (blue rose plots). Von Mises mixture model fits of each order used (0-8) are overlaid (red ellipses).

**B)** Directional tuning plots from the remaining half of data were used for cross-validation. Sum-squared-error (SSE) between the cross-validation data and each order model (red, same models as in (a)) is printed above each figure along with its SSE Ratio, the remaining error normalized by the amount of SSE of the naïve circular model (order 0). For a model to be considered a ‘best fit’, the SSE Ratio must be below 0.5 (<50% error of naïve circular model), SSE Difference greater than 0.2 (20% improvement). For this neuron, the criteria lead to selection of the 2nd-order model (red box).

**C)** To be considered as strongly axis-tuned, two more criteria must be met. First, the ratio of mean actual data firing rate at model maxima to mean actual data firing rate at model minima must exceed 2. Mean peak (maxima) values for this neuron (green lines) were 8.2X mean minima values (black lines). Finally, the spatial independence of each model’s maxima must both exceed 50%. For all track locations associated with movement in either of the two preferred directions, the neuron was considered ‘active’ if its mean rate at that position and orientation was at least 50% of the overall mean rate for that direction. In the case of this neuron, most of the points in the light green orientation lie along the light green arrows on the right panel (see also figure 1B – right panel) and the points in the dark green orientation lie along the dark green arrows. Neurons were considered spatially independent if the majority of associated locations met this criterion. For this sample neuron, the larger, light green peak had 88% spatial independence and the smaller, dark green peak had 58% spatial independence. Because it met all three criteria, this neuron is in the axis-tuned subpopulation.
Works Cited


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CHAPTER 4: Medial Precentral Cortex Transforms Spatial Information into Planned Action in the Navigating Rat

**Abstract**

Fluid navigation requires constant updating of planned movements to adapt to the current location relative to obstacles and goals. A neural substrate would need spatial and sensory information as inputs and motor targets as outputs to adapt and manipulate the action plan. Medial precentral cortex, a frontal association cortex of the rat, is a prime candidate for this role due to its interconnectivity with spatial- and sensory-encoding associative cortices and its projection to primary motor cortex. Here we conduct recordings of individual neurons in medial precentral cortex of rats while they navigate a complex multi-route maze. We report predictive and concurrent activity encoding actions that is robust to spatial or decision context. Concurrently differentiation between contexts for identical actions exists, demonstrating influences of context on the firing activity. Overall, the time course of action encoding and presence of spatial influences lead us to conclude medial precentral cortex is a region critical to the online updating of the ongoing motor plan during navigation.

**Introduction**

Goal-directed navigation is a fundamental cognitive process that necessitates sensing and utilizing information about the environment to execute actions. To achieve fluid goal-oriented movement, upcoming actions must be planned in advance of their execution. And as most environments are filled with obstacles, barriers, and possibly even moving goals (i.e. an animal’s herd or prey), this process must also be capable of being dynamically updated during navigation.
Studies of navigation in rodents have identified multiple different neural representations of space. Place cells of the hippocampus\(^1\) and grid cells of the medial entorhinal cortex\(^2,3\) are hypothesized to form the foundation of a cognitive map of space,\(^4\) while head direction cells anchor to the external cues and maintain an animal's orientation.\(^5,6\) Understanding how and where this spatial information is transformed into choices and actions is still evolving, however.

Medial precentral cortex (MPC), the most medial and dorsal subregion of rodent prefrontal cortex, has been proposed as a region where this transformation may occur.\(^7–9\) This subregion, also referred to in the literature as the shoulder region, M2, Fr2, medial agranular cortex, dorsomedial prefrontal cortex, or frontal orienting field, boasts reciprocal connectivity to many sensory and associative cortices.\(^7,10,11\) Of particular interest is the extensive reciprocal connectivity between MPC, posterior parietal cortex (PPC), and retrosplenial cortex (RSP). Large populations of neurons in both PPC and RSP have spiking activity that maps animals' positions within the space of complex routes as well as those that correlate with turning behaviors.\(^12–18\) Completing the hypothesized sensory to motor pathway, MPC has direct projections to primary motor cortex as well as along the corticospinal tract to the spinal cord.\(^10,19\) Anatomically, the MPC subregion of prefrontal cortex is ideally suited to transform knowledge of current position into a plan for specific motor actions.

Existing studies of MPC function describe a region involved in decision making during orienting behaviors. Unilateral lesions lead to contralateral neglect and reaction delays in choice tasks that cease if only the contralateral action is rewarded, suggesting a role necessary for choice rather than motor execution.\(^20–22\) This bias increases if a working memory delay is added\(^22\) or cue-response mappings change\(^23\) but disappears if the task is a simple stimulus-response pairing.\(^24\) Individual neurons in the region correlate
with upcoming choices and actions earlier than any other frontal regions previously studied.\textsuperscript{22,25} Still at issue is the separation of action planning and execution versus decision making, with evidence for MPC as accumulator/integrator\textsuperscript{26} or only a categorical output.\textsuperscript{24,27}

Thus, in the present work, we examined MPC neural activity patterns in the context of a large multiple ‘T’ maze. The chosen environment and task structure spatially and temporally distanced individual actions from surrounding actions and goal locations. MPC neurons discriminated turns reliably across all turns during the maze traversal and do so well before the action is executed. Simultaneously the region also represented multiple spatial features as well as the presence or absence of choice, suggesting a more involved role in the action preparation process than just that of the decision of a left or right categorical choice. Together these findings further elucidate MPC’s role in this complex cortical circuit during choice and navigation.

\textbf{Materials and Methods}

\textbf{Subjects.} Subjects were 8 adult male Sprague-Dawley rats and 1 male Long Evans rat. From these rats, a total of 331 neurons were recorded (73 left, 258 right, for per animal counts, see Supplemental Table 1). Rats were housed individually and kept on a 12-h light/dark cycle. Upon receipt and prior to experimentation, animals were habituated to the colony room and handled for 1–2 weeks. During training and experimentation, rats were food restricted with weights maintained at 85–95\% of free-fed weight. Water was available continuously. Rats were required to reach a minimum weight of 350 g (5–10 months of age) before surgery and subsequent experimentation. All experimental protocols adhered to AALAC guidelines and were approved by the IACUC and the UCSD Animal Care Program or Scripps Research Institute.

\textbf{Apparatus}
Behavioral tasks were conducted using a triple ‘T’ track maze. The track (Figure 4.1A, left panel; 8-cm-wide pathways, overall 1.6 m × 1.25 m in length and width, painted black) stood 20 cm high in the middle of a large recording room and was visually open to prominent distal cues. The track edges were only 2 cm in height, allowing an unobstructed view of the environment.

**Behavior**

Rats were habituated to the triple ‘T’ maze during two 30 minute periods of free exploration. Animals were then trained to run ballistically from the midpoint of one of the long edges of the maze into the center of the apparatus and continue until reaching the outside of the maze (Figure 4.1A, yellow lines). This consisted of straight sections interleaved with three left or right turns for a complete route run. The maze design creates eight equally long internal routes from the single internal maze entry location through a three turn sequence to one of the reward sites. The animal must then navigate around to the entrance to repeat, choosing a route depending on its current location. The total internal route lengths were 140 cm, with turns at 51 cm, 87 cm and 118 cm. External routes were 197 cm with turns at 15 and 127 cm. Reward (a half piece of Cheerios cereal) was made available at the reward sites. Over 1–2 weeks, animals were trained by approximation to make route traversals between food reward sites. Over at least 2 additional weeks, animals were trained by simple trial and error to a criterion of 80% for ballistic (uninterrupted) routes traversal. Animals were surgically implanted only after this level of task performance had been achieved.

**Reward Schedules**

Multiple reward schedules were used across the set of animals. For the visit-all-8 reward paradigm, the animal was rewarded at all locations, but needed to visit all locations before rewards were reset at all reward locations. For the visit-all-4 reward paradigm, only
the far four routes and reward locations were included. For these animals, the other four routes were blocked and the animals never had access to that track space. In the high-low task two locations out of the eight were randomly chosen to be rewarded for each recording. One location contained one half cheerios and the other one quarter. After 20 minutes, two more randomly selected locations were chosen to be rewarded in the same high-low fashion and the recording continued for 20 more minutes.

**Surgery**

Rats were surgically implanted with stereotrode or tetrode arrays (twisted sets of two or four 12.5-µm nichrome wires) inserted into custom-built microdrives (four to eight arrays per microdrive). Rats were implanted unilaterally or bilaterally with one microdrives per hemisphere into medial precentral cortex. Rats were anesthetized with isoflurane and positioned in a stereotaxic device (Kopf Instruments). Following craniotomy and resection of dura mater, microdrives were implanted relative to bregma, centered at (A/P 2.5 mm, M/L ±1.2 mm, D/V -0.5 mm).

**Recordings**

After recovery from surgery, animals were retrained for at least 1 week before beginning recordings to ensure adequate behavior and running ability with the new weight of the implant. All recordings were from animals that were well trained on the task. Electrodes were moved ventrally in 40-µm increments between recordings to maximize the number of distinct units collected. Omnetics connectors were connected to the microdrives for all animals. One of two recording systems was used for data collection. In the first, utilized for five animals, each Omnetics connector was connected to a single amplifying headstage (1× gain, NB Labs). A tether led to an amplifier (Lynx=8 amplifiers, Neurolynx) and then fed into an acquisition computer running the AD system (courtesy of Loren Frank and Matt Wilson, MIT) and filtered at 0.600 to 6 kHZ. Data was digitized at
32 kHz. For the other for animals (N = 4), each microdrive had one or two electrical interface boards connected to a single amplifying headstage (20×, Triangle Biosystems). A tether led to a set of preamplifiers (50×) and a high pass filter (>150 Hz). Signals then fed into the acquisition computer running Plexon SortClient software and were filtered at 0.45–9 kHz, further amplified 1–15×, and digitized at 40 kHz. Total amplification regardless of system was a total of 1,000–20,000×. Single units were isolated in either XClust (courtesy of Loren Frank and Matt Wilson, MIT) or Plexon OfflineSorter software. Waveform parameters used were peak height, peak valley, energy, and principal components. Waveform clusters appearing to overlap with the amplitude threshold set for collection were discarded to avoid collection of neurons with partial spiking data. Waveform amplitudes were monitored to ensure systematic fluctuation did not produce confounds in isolating single units.

Animals’ position was tracked using a camera attached to the ceiling above the recording room floor. Either Dragon Tracker or Plexon CinePlex Studio software was used to detect LED lights placed on the animal’s surgical implant. Position location of the lights was captured at 60 Hz. Recordings lasted approximately 30-60 min. The animal would run on the track for approximately 80 rewarded runs. No neurons were excluded from analysis, even if activity was minimal.

Histology

Animals were perfused with 4% paraformaldehyde (vol/vol) under deep anesthesia. Brains were removed and sliced into 50-μm sections and Nissl-stained to reveal the final depth of electrode wires in MPC. Microdrive depth monitored across recordings and final electrode depth as observed in histology were compatible in all cases.

**Identification of Ballistic Route Traversals**
To identify clean runs for individual routes, a multistep process using custom MATLAB graphical user interfaces is used. First, a user defines starting and ending gates for each route. Then the program finds all runs crossing these locations with sustained running speeds of 3 cm/s throughout and labels them as clean. Finally, a researcher uses the interface and verifies that all runs included did not diverge from a clean traversal of the route. The results of this process can be seen in Figure 4.1B. By this method, stalled track traversals, reward periods, and other position data captured between runs are not conflated with data with controlled action and spatial values.

**Positional Firing Rate Maps**

To characterize the firing activity of the MPC neurons, we calculated individual neurons' positional firing rates by dividing the total number of spikes of each neuron at each location by the total occupancy time at each location. We included only data when the animal was performing one of the identified clean runs. Positional firing maps were smoothed using a 2D convolution with a Gaussian filter with s.d. of 2 cm that also accounts for bins with no occupancy.28 Each route was calculated separately.

**Track Linearization Firing Rate Calculation**

For each recording, custom MATLAB software is utilized to generate a spatial template matching the average movement of the animal through 2D along each route. While highly similar, the recording by recording approach ensures the best possible match to animal behavior due to slight shifts between animals, LED placements, etc. The beginnings and ends of each straight run and turn period are marked and equal space bins are interpolated between marked locations. After establishing the template, each tracking position sample is mapped to the nearest bin. Firing rates for each traversal are calculated by summing the number of spikes in each template bin and dividing by the total amount of time the bin was occupied. A Gaussian filter with s.d. of 2 cm is used to smooth
each traversal. Mean firing rates are calculated by summing mean rates at each location and dividing by the number of runs.

**Perievent Mean Firing Rates**

Perievent mean firing rates were used to calculate choice probabilities at turns. For the defined linear space around the event, firing rates for all occupied bins of the linearized firing rate are averaged for each trial.

**Choice Probability**

Choice probability (CP) is a metric coined by Britten et al.\textsuperscript{29} It is the probability that an observer can correctly identify an outcome given a single sample from one of two distributions. It is equivalent to the area under the curve of the receiver operator characteristic.\textsuperscript{30,31} It can be calculated from the U statistic of the Mann Whitney U test of two distributions,

\[
\text{choice probability}_{U_1} = \frac{U_1}{n_1n_2}
\]

where \(n_1\) and \(n_2\) are the number of samples in distributions 1 and 2, respectively. The difference of the value of this metric from chance (50\%) is symmetric but depends on the order of the magnitudes of the medians of the two distributions. For our purposes, the higher magnitude of the distributions was not important, only the separation. Because of this, the maximum choice probability

\[
\text{choice probability} = \max\{\text{choice probability}_{U_1}, \text{choice probability}_{U_2}\}
\]

was always selected.

For all perievent action choice probabilities, perievent mean firing rates were used from the distributions of routes left and right actions. For the time course analysis, choice probabilities were computed from firing rates of a particular bin instead of mean values. To control for the effect of noisier data at this fine granularity and multiple comparisons,
the significance level for the time course test was established by a bootstrapping procedure. We shuffled the left/right action identities of the same runs analyzed for each bin and neuron to create one thousand shuffled left/right datasets for each bin of each neuron. Choice probabilities from this shuffled distribution were calculated and used to establish a p < 0.05% criteria for choice probability values.

For contextual choice probabilities, we always controlled for action (left/right turn) by comparing only events (turns) with the same action being executed. We always controlled for action and only compared left (right) turn firing rates to other left (right) turn firing rates. For many contextual factors, more than two conditions needed to be examined for discriminability. To preserve the ease of interpretation, we decided upon pairwise choice probability calculations for each combination of distributions. For progress within a route, this consisted of 12 comparisons: 1st, 2nd, and 3rd turns for both lefts and rights. For location, we controlled for route progress and therefore had up to 26 comparisons: between four 3rd turn locations (6 combinations), and between two 2nd turn locations (1 combination) for each of left and right turns. For route, we controlled for location and progress by analyzing at only the 1st progress turn. We therefore had 12 comparisons: four left routes and 4 right routes. For orientation we had 12 comparisons: four directions for lefts and rights. Finally, for choice, we only had two comparisons, forced versus choice for each of lefts and rights. We present minimum, mean, and maximum values from these comparisons to aid in the comprehension and comparison due to the necessary differences in analyses.

**Statistical tests**

The Mann Whitney U test was used to evaluate significance of perievent mean firing rates between left and right actions. This test carries no assumption of normality in distribution
Figure 4.1: Behavior and histology. A) Schematic of triple ‘T’ maze and route-running task. Animals made runs along each of up to eight partially overlapping internal routes (solid yellow lines) on the 160 cm × 125 cm track apparatus, leading from a start site (green circle) to any of four goal sites (red ‘x’). Numbers indicate the index of the turn in the progress of an internal route. From each goal site, the animal returned to the start via either of two return paths (dashed yellow lines). For some task setups, only the routes leading to the top four goal locations were used. B) Behavioral tracking of identified clean runs. Shown are all clean runs from one recording session in the visit-all-8 reward setup. Each color shows a separate route. Routes are minimally translated and stretched for visualization purposes. Routes shown in separate colors. C) Electrode placement and recording ranges in MPC (nine rats). Filled circles indicate final electrode placement and lines track dorsal-ventral recording depths across recordings.
of the data. Fisher’s exact test was used to assess the quantities of significant neurons of
the first turn and all pooled turns. The binomial test was used to assess deviation from
chance (50%) for laterality preference of MPC neurons. The Kruskal-Wallis test was
utilized to evaluate if action choice probabilities from separate locations were from different
distributions. This test carries no assumption of normality in distribution of the data. The
Kolmogorov-Smirnov test was used to assess if pairwise distributions were significantly
different for CP distributions. No assumption of normality is necessary for this
nonparametric test. No statistical methods were used to predetermine sample sizes, but
our sample sizes are similar to those reported in previous publications. Data collection
was not randomized, nor was analysis performed blind to the conditions of the experiment.

Data and code availability

The data that support the findings of this study and code used for analysis are
available from the corresponding author upon reasonable request.

Results

Rats were trained on a triple ‘T’ track maze (Figure 4.1A) to navigate through the
center of the maze to end-of-path reward locations. Animals were allowed to move freely
in all areas except on the inside paths where backtracking was prevented. This was rarely
necessary during recordings as animals were extensively trained before any recordings
occurred. This maze design forced animals to traverse multiple straight runs and left/right
turns between reward locations, separating out action and reward influences. Data was
pooled across three separate reward schedules - high-low, visit-all-8, or visit-all-4 (see
methods for more details).

Animals’ locations were tracked using an overhead camera and head-mounted
LEDs. Accurately identifying and defining actions of a freely navigating animal for analysis
can be difficult due to the wide variation of movements. To control for behavioral variation
Figure 4.2: MPC robustly encodes action across space. A) Example neuron positional firing rate maps. Shown are the firing rates color-mapped as a function of route and track position. As in Figure 4.1B, routes are minimally translated and stretched from the actual track location to separate each map for visualization purposes. Under each rate map is the choice probability (CP) for left and right actions, pooled across all locations. B) Linear perievent firing rate maps. Mean (line) and s.d. (shaded) of the individual linear firing rate values surrounding each of left and right turns, shown in blue and red, respectively, for the corresponding neurons in a). C) Percentage of MPC neurons significantly discriminating left/right actions for the first internal turn and all turns pooled. D) Cumulative density functions (CDFs) of the entire MPC populations’ action CPs for one turn location. Each color is a different spatial location on the maze. E) CDFs of pooled left/right turn CPs (orange), mean action CPs of individual locations, and shuffled action CPs. F) Probability density function of the action CPs for all turns pooled across locations.
during analysis, we defined routes and subsequently scored the animal tracking data for smooth, ballistic running of entire routes. This gives us data with stereotypical movements through any section of a route. The defined routes are either internal routes towards rewards or external routes leading to the internal entrance (Figure 4.1B). The animals were well trained with an average of 63% of route traversals per recording session categorized as smooth, ballistic running. Henceforth we will refer to these ballistic routes traversals as clean runs. Animals averaged a velocity of 42 cm/s during clean runs. Each clean run was linearized by mapping tracking locations to the nearest 1 cm bin of a template of the traversed routes.

To assess the representations present in MPC, single unit activity was recorded from 73 left hemisphere and 258 right hemisphere MPC neurons (331 total) from nine rats under the aforementioned conditions. Electrode tracks and endpoints are depicted in Figure 1c. The distribution of firing activity found is skewed towards lower rates and is consistent with a log normal distribution as described in other cortical regions\(^{32-34}\) (Supplemental Figure 4.1).

**MPC Robustly Encodes Actions Across Contexts**

Action encoding was prevalent in MPC across all turn locations and contexts. Firing rate maps of clean run traversals from three sample neurons are shown in Figure 4.2A. To quantify these findings, we defined a turn space around each track turn from 20 cm before to 10 cm after the turn apex (Figure 4.2B). This space was the maximal possible that prevented overlap with adjacent turn spaces. We required at least 8 clean runs through each turn grouping analyzed. Fifty-five percent of all MPC neurons had significantly different mean firing rates for the first internal path turn (Figure 4.1A, turn labeled 1, Mann-Whitney U test, \(p < 0.05, n = 320\)). Action encoding was extremely
**Figure 4.3: Temporal dynamics of MPC action encoding.**

**A)** Colors code for choice probabilities of individual neurons (rows) across space bins (columns) in the perievent window surrounding the first left/right turn on the internal routes (Figure 4.1A, labeled 1). Neurons sorted by the location of their max choice probability. Black line indicates turn apex.

**B)** MPC action encoding strength as a function of time. Shown are percentages of neurons encoding the action at each spatial distance from the turn apex. Blue trace is the percentage above a 5% criteria of a bootstrapped distribution. The dotted black line would then be the expected chance value. Orange and green traces are percentages of neurons at the chosen thresholds of 66% and 90%, respectively.
consistent across turn locations and contexts as 48% of all MPC neurons significantly discriminated turn direction even when data is pooled across all turn locations (Mann-Whitney U test, $p < 0.05$, $n = 331$, sample sizes matched to first turn sample sizes). This population value is not significantly reduced from the single turn discriminability (Figure 4.2C, Fisher's exact test, $p = 0.12$). The MPC neural population as a whole showed no laterality preference with 49% and 51% of turn encoding neurons displaying higher mean firing rates for ipsilateral and contralateral turns, respectively (binomial test, $p = 0.94$, $n = 160$).

To assess the quality of MPC neurons’ turn discrimination, we adopted choice probability\textsuperscript{22,29} as a measure of effect size. Choice probability (CP) is simply the probability that an observer can correctly identify an outcome given a single sample from one of two distributions. It is equivalent to the area under the curve of the receiver operator characteristic.\textsuperscript{30,31} Due to the structure of our maze, sample sizes varied widely across turn locations. This measure is invariant to sample size and therefore is a particularly useful method for comparing our results.

CP confirms MPC neurons’ encoding of turn directions is both widespread and reliable. To summarize population discrimination quality, we have adopted easily interpreted benchmarks of two-thirds (66%) CP for general discrimination and 90% for a high discrimination threshold. The neurons shown in Figure 4.2A-B have choice probabilities thresholds as examples. On average, half of the MPC neurons have choice probabilities exceeding 66% at any given left/right turn location, including 17% of neurons classifying at a rate exceeding 90%. There is no effect elevating or suppressing turn discriminability at certain locations as all of these locations show statistically indistinguishable distributions of choice probabilities (Figure 4.2D, Kruskal-Wallis test, $p = 0.11$). Pooling turn data across all turns, the choice probabilities of the distribution are
significantly lower (Kolmogorov-Smirnov test, p < 0.0001) but remain comparable to the location specific values, with the population counts of neurons exceeding 66% and 90% discriminability dropping from 50% and 17% to 38% and 10% of the population, respectively. For comparison, shuffling turn directions of the same data results in no neurons classifying above 66% (Figure 4.2E-F, max 61%). While action encoding in MPC is strong and reliable irrespective of turn location, differences between turn contexts hint that action outcome is not the sole variable represented in MPC neural activity during navigation and that perhaps more is represented in this region than just action output.

**The Time Course of MPC Action Discrimination**

To further delve into the time course of action discrimination we applied the CP method to each 1 cm bin surrounding the first internal 'T' (Figure 4.1A, turn labeled 1). This particular turn has a long approach straightaway before the turn and allows for an extended examination into left/right action discrimination. A space from 40 cm before the turn apex to 15 cm afterwards was analyzed.

Individual MPC neurons had high peak CP values throughout the investigated epoch (Figure 4.3A). As a population, action direction was discernible by the CP measure for a significant portion of MPC neurons during the entire epoch, (Figure 4.3B, bootstrap, p < 0.05). A minimum of 20% of MPC neurons significantly differentiate the turn outcome at any time point. This number increases and peaks at the turn apex with over 50% of individual MPC neurons significantly discriminating the action. The count then decreases through the end of the turn. In spite of the complete temporal coverage as a population, CP values for individual neurons do not stay high for the entire period. Instead, peak CP values typically extend for a limited span of less than 20 cm for any given neuron (Supplemental Figure 4.2). High reliability (>90% discrimination) neurons followed this same pattern with counts ramping to the turn apex and decreasing afterwards.
Figure 4.4: Spatial representation in MPC. A) Graphs of cumulative density functions (CDFs) for the entire MPC populations’ CPs under different spatial conditions. Also included for comparison are CDFs of action CPs from individual locations and shuffled action CPs. Separate graphs are the mean pairwise CPs (top left), minimum pairwise CPs (bottom left), and maximum pairwise CPs (bottom right). Top right is a bar graph percentages of MPC neurons with mean pairwise CPs above our threshold for each condition. Color labels are consistent across all of Figure 4.4. B) Choice probability correlation table. Listed are the Pearson R correlation coefficients for the mean pairwise CPs of each pair of conditions graphed in A. C) Example neuron positional firing rate maps highlighting different encodings of spatial contexts. The white arrow on the left example highlights an example of route encoding. Shown are the firing rates color-mapped as a function of route and track position. As in Figure 4.1B, routes are minimally translated and stretched from the actual track location to separate each map for visualization purposes. D) CPs from each of the three plots in A for the corresponding example neurons shown above. E) Linear perievent rate maps. Perievent plots showing the mean firing rates for the conditions from the highlighted spatial contexts of the example neurons shown above. These are split by action since CPs controlled for action and would only compare the condition within each graph.
**Widespread Representations of Spatial Context in MPC**

Considering the extensive reciprocal connectivity to PPC and RSP, two associative cortices with known spatial representations, MPC neurons may be encoding multiple spatial aspects of the environmental context. We have selected four navigationally-relevant spatial features to investigate - location, orientation, route, and progression. Different locations and orientations call for different preferences in turn behavior and therefore the site or direction of the turn in environment space may be encoded. MPC activity may also reflect the eventual goal location or action sequence planned to get there, which would be a representation of route. Finally, if using a route representation of the environment, the animal would need to know their progress traversing the chosen route to determine the appropriate action to choose.

To investigate the effect of these spatial features we employed the same CP analysis as used for turn discriminability. One benefit of CP as a measure of discriminability is that we can use it to assess the separation of any two distributions. In this setting, we controlled for action (i.e. left or right turn) and instead measured the discriminability of the same action across different samples of the variable in question. For example, we can calculate the CP between the left turn firing rates of any two left turns to evaluate the effect of location. To maintain the intuitive meaning and ease of comparison of the measure we were limited to two distributions at a time. We therefore made pairwise comparisons for variables with more than two conditions and used the mean CP to summarize discriminability of that spatial variable. When possible, we controlled for other spatial factors in any given analysis. We controlled for route progress during location analyses by only comparing turns at the same progress position. When analyzing route, we only analyzed the first turn of the internal routes which controlled for space, progress, and orientation.
Encoding of all four spatial features is widespread but not highly reliable in MPC. The distributions of choice probabilities for the spatial factors in MPC are all significantly higher than chance but significantly lower than the pooled turn discrimination (one-sided Kolmogorov-Smirnov test, $p < 0.0001$ for all tests). The variability within individual pairwise tests can be examined in Figure 4.4A. Cumulative distribution functions (CDFs) of the mean, minimum, and maximum pairwise CPs for each neuron are shown for each spatial factor. This can be contrasted with the CDFs of the mean, minimum, and maximum pairwise action discrimination CPs. The variability between action discrimination CDFs is considerably reduced compared to the wide variability in the spatial factor CDFs for the minimum and maximum pairwise CP scores. Even the minimum action discrimination CDF includes many high CPs, highlighting the robustness of the action signal. It is worth noting that the spatial factors analyzed are inherently more complex than the two alternative left/right action. Orientation is assessed as one of four directions, location may include up to four locations, route labels four routes per action, and progress has three levels. If neural representations are continuous for these factors, comparable mean CPs could exist. However, if the encoding is such that the neuron is active for one value of the factor (i.e. ‘East’) but not the others, a maximum pairwise CP would be high but the overall mean CP would be low due to all of the comparisons of unrepresented values (i.e. ‘North’, ‘South’, ‘West’). For both the mean and max values, however, CDFs of spatial factor CPs do not reach the level of the CDFs of action CPs. When using our performance thresholds, the prevalence of MPC neurons distinguishing spatial factors at the general performance threshold of 66% is reduced at mean values but comparable at a maximums to action categorization, but 10% fewer of the neurons do so at a 90% discriminability rate for either distribution. These results indicate that as a whole, while these neurons do encode spatial
information, it is not as robust of an output as action for individual neurons in the MPC population.

Given the widespread occurrence of different forms of spatial information, we considered that the distribution of types of information and its co-occurrence with action discrimination may be important. Correlations of neurons’ CP values across factors is shown in Figure 4.4B. Correlations between action encoding and each spatial factor are positive but relatively weak. The same is true for correlations between spatial factors except for the correlation between progress and orientation which is quite high. Position rate maps of the routes taken are shown for two example neurons in Figure 4.4C, highlighting example of conjunctive encodings. Figure 4D shows the calculated mean CPs for the neurons and Figure 4E highlights the mean individual firing rate vectors highlight the separation of firing rate activity for different conditions. These neurons are typical examples of neurons with complex firing patterns that encode multiple factors to varying degrees.

**A Widespread But Limited Effect of Choice in MPC Action Representations**

The current literature converges on a role for MPC in orienting decisions. If the primary function of this region is as a decision making output, a difference in action encoding may exist in situations where the animal may proceed in multiple directions (e.g. a ‘T’ turn) versus a forced turn with no alternatives (an ‘L’ turn). We therefore sought to evaluate the activity in the region under the context of a forced action (no alternative path, ‘L’) versus an action at a choice point (‘T’). Specifically, we hypothesize firing discrimination of action may dissipate in the forced action context where no active decision is necessary.

To analyze this situation, we grouped all turn locations based on action (left/right turn) and choice context (forced vs choice). We then evaluated action discrimination at
Figure 4.5: Choice context does not affect action encoding discrimination. A) Graphs of cumulative density functions (CDFs) for the entire MPC populations’ action CPs under choice (gold) and forced (purple) turn contexts. Also included for comparison are CDFs of action CPs from individual locations (orange) and shuffled action CPs (blue). B) Choice context discrimination. Top: CDF of mean pairwise CPs of choice context (maroon) with comparison CDFs of individual location action CPs (orange) and shuffled action CPs (blue). Bottom: Threshold values of choice context as compared to the spatial contexts. C) Example neuron positional firing rate map highlighting encoding of choice context. Shown are the firing rates color-mapped as a function of route and track position. As in Figure 4.1B, routes are minimally translated and stretched from the actual track location to separate each map for visualization purposes. D) CPs from each of the three plots in Figure 4.4A for the example neuron shown above. E) Linear perievent rate maps. Perievent plots showing the mean firing rates for the action values (top) and the choice contexts (bottom) of the example neuron shown above. All comparisons for CPs were done on data being presented in the same plot.
choice turns and forced turns separately. There is no apparent effect on action discrimination CPs due to choice context (Figure 4.5A). The CP distributions for forced and choice contexts did not significantly differ from each other or the pooled turn action CP distribution (Kolmogorov-Smirnov test, all p > 0.15). This result contradicts our hypothesis of lower firing discrimination during forced contexts and throws doubt on choice output as the primary function of the region.

Encoding of choice context is discernible as CPs do discriminate between forced and choice contexts of a given action. The discriminability is similar to that of spatial factors (Figure 4.5B). The position rate maps for an example action discriminating neuron with very strong modulation to choice context is highlighted in Figure 4.5C. This neuron has high CPs for many factors but choice and action are by far the most robust (Figure 4.5D-E).

**Discussion**

We trained animals to traverse a large multiple ‘T’ maze with dispersed reward locations (Figure 4.1). In previous studies of MPC in behaving animals, tasks typically consisted of one choice point. By increasing the number of choice points, this design allows for the assessment of an action discrimination signal while marginalizing over other contextual variables. Simultaneously, encoding of those contextual variables can be examined while controlling for action executed. While multiple studies have shown action and orienting correlates in two-alternative forced choice settings, to our knowledge, no current studies have investigated the role of MPC during navigation of a complex environment.

The strongest neural signal in MPC was the encoding of upcoming and ongoing actions. Approximately half of all MPC neurons robustly discriminate left/right turning actions during the approach and execution of a turn. If this signal is causally preparing
and executing a given action, the representation must be discriminable to downstream neurons regardless of any contextual variation at the time and place of the action. Firing rates during left and right turning was consistent and discriminable between the two actions across locations for many MPC neurons (Figure 4.2). In fact, no significant drop in the population count of discriminating neurons occurred when data was pooled across space. We believe this is the first reported evidence that encoding of action in MPC generalizes to different spatial contexts.

Current theories of MPC function posit a causal role in action preparation. Previous studies have reported MPC correlates that correlate with trial-by-trial fluctuations in firing rates with upcoming motor behavior.\textsuperscript{22} Predictive activity has been reported to lead behavior by as much as 500 ms.\textsuperscript{25} A fine-grained look at the temporal dynamics on a straightaway starting 40 cm before a left/right turn revealed over 20\% of the neurons discriminated the upcoming action outcome for the entire window, a distance that took the animals ~1 s to traverse (Figure 4.3). Our findings support a premotor function of MPC and, as far as we are aware, report the earliest action predictive signal identified in the rodent brain.

In addition to known action correlates, we reported that MPC had widespread activity encoding spatial context (Figure 4.4). While not as dissociative between conditions as action correlates, many neurons showed conjunctive representations of multiple variables. Considering the extensive reciprocal connectivity with RSP and PPC,\textsuperscript{7,11} two regions very prominently encoding environmental location, orientation, and route progress,\textsuperscript{14,17,35} we consider this evidence of MPC as a hub in the dispersed multi-region cortical network actively translating between sensory, spatial, and motor frames of reference.
The precise role of MPC in decision making is still very much in debate. While lesion studies firmly establish the importance of the cortical region for decision making performance,\textsuperscript{21-23} modeling results have suggested both an accumulator/integrator\textsuperscript{26} or only a categorical output function.\textsuperscript{24,27} In our study, we contrasted activity between forced (one action possibility) and choice (two action possibility) turns. Individual neuron action discrimination between the two contexts was indistinguishable across the population (Figure 4.5). This suggests that while the region is necessary during decision making tasks, it doesn’t fundamentally change its activity in a non-decision context.

Further evidence for this consistency in function emerges in the action predictive representations. Early predicting neurons often encoded near peak levels of reliability for a short continuous distance, typically less than 20 cm, as opposed to the entire pre-turn epoch (Supplemental Figure 4.2). These results are compatible with Murakami and colleagues’ finding of neurons with activity at specific times before movement initialization in a waiting task.\textsuperscript{26} They interpreted these firing patterns as a timing-based adaption of ‘evidence’ as part of an integration to a decision threshold, and suggested an adapted use of the general integration to bound circuitry in non-decision contexts. However, this firing pattern is also seen in primary motor cortex and may be a general encoding property of motor cortices\textsuperscript{36}.

Based on this evidence, we propose that the process occurring in MPC is better understood as one of motor preparation as opposed to active decision making. Here, we use the term decision making to refer to active value assessment and selection of potential action plans, whereas the term motor preparation describes the execution of an action plan. Given the ballistic running of the animals in the analyzed portion of our task, behavioral indications of decision making are not apparent in the time window. Coupled with a clear neural discernibility of upcoming action over the entire period, we find the
results most consistent with a motor preparation role carrying out a largely predetermined decision. This is consistent with previous results and models,\textsuperscript{22,24,26,27} as integration to bound models do not necessitate alternative options. From an ecological perspective, a motor preparation role for a premotor cortex seems likely as motor plans to fulfill decisions are more nebulous choices of timing and execution. This shift in interpretation does however shift what paradigms may be most useful for future research to those investigating precise timing and execution of actions.

We conclude that MPC’s role is best understood as a part of a sensory and spatial associative cortex network that outputs preparatory motor actions. This view aligns closely with that posited by many before us\textsuperscript{8,9,22} but emphasizes MPC’s situated place in the PPC and RSP cortical network. This shift is supported by our results showing the existence and characteristics of only previously hypothesized spatial influences in MPC. Taken together, these findings suggest an active role in navigational action preparation. Many future experiments will be required to investigate how each of these regions’ contributions differ during active navigation.

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The authors declare no competing financial interest.
**Supplemental Table 4.1: Neuron counts.** Listed in each row is the identifier index and neuron counts by hemisphere for each rat in the study.

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Supplemental Figure 4.1: Firing rate distributions. Mean firing rate histograms for the 331 recorded medial precentral neurons in both linear (left) and log-linear scales (right).
Supplemental Figure 4.2: Length of peak choice probabilities. Plotted is the number of spatial bins within 10% of the maximum choice probability for each individual neuron during the time course analysis (Figure 4.3). Each dot represents one neuron.
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CHAPTER 5: A Viable Dorsal Cortical Circuit of Navigation

This dissertation proposed a dorsal cortical network in the rat that could transform spatial episodic information from the hippocampus into motor output. This network consists of medial entorhinal cortex (EC) and subiculum (SUB) of the hippocampal formation and three interconnected associative cortices: retrosplenial cortex (RSP), posterior parietal cortex (PPC), and medial precentral cortex (MPC). Information flow would separate into SUB and EC before converging back onto RSP. While RSP is directly connected to MPC, the widespread evidence for PPC in spatial and orienting behaviors and its connectivity with both regions suggests an important role in the transformation. 1–3 Finally, MPC is the proposed output node of the spatial network due to its connections to both primary motor cortex and the spinal cord.4,5

Considerable evidence exists through lesion studies establishing the necessity of most of these cortical regions in hippocampal-dependent spatial memory tasks.6–9 The proposed network involves both of the dorsal cortical outputs of CA1 in SUB and EC. Both MEC and SUB have been shown to severely hamper navigation while hippocampus and the other region remain viable.6,9 This strongly supports the importance of both SUB and MEC for propagating spatial information in the navigation circuitry and opens the question of what role each region is playing. LEC lesions have instead caused deficits for object recognition.8 As such, it may play an important role in anchoring spatial information to objects. Considering the status of EC as prominent inputs to hippocampus, an alternative explanation is that they do not significantly propagate navigational information but instead cause deficits due to deteriorated hippocampal representations. When hippocampus and SUB were lesioned, the impairment was greater than SUB alone.6 This suggests that another route does exist, casting doubt on this explanation. Regardless, this could be
determined by directed lesions to only RSP-projecting EC projections, leaving hippocampal function intact.

If both MEC and SUB project spatial information to RSP for navigational purposes, the neural representations in these regions may elucidate these roles. EC neurons display a host of different spatial representations.\textsuperscript{10–14} The representations remain relatively static across unrelated environments that cause hippocampal remapping,\textsuperscript{15} also suggesting independence from hippocampal outputs. If this information is reaching RSP, it may provide a general spatial scaffolding necessary for planned movements in the environment.

SUB representations have largely been reported to be very similar to CA1 with the addition of larger fields with greater generalization and additional boundary fields.\textsuperscript{16–20} This result is peculiar given the markedly different navigation behavior following separate lesions of the two regions.\textsuperscript{6} Chapter 2 explored the representations of dorsal SUB while rats navigated a complex track maze. We report the existence of a novel spatial cell type that is active when the animal is running in either of two opposing directions. These ‘axis cells’ take the frame of reference of the larger allocentric environment when the track is rotated and persist in the dark. However, when exploring and running on an open platform in the same location, direction tuning ceases, a property unlike that of head direction cells whose firing is robust regardless of spatial context.\textsuperscript{21}

Chapter 3 then explored clues to the inputs of these neurons through investigation into their temporal firing characteristics. While the directional tuning was reminiscent of head direction cells, the contextual component hints at an important role of place cell input from CA1. We report robust phase precession in the axis neuron population. The onset of the fields also occurs at the same theta phase as CA1 phase precession. This result
strengthens the connection to hippocampus as head direction cells do not exhibit phase precession.

Multiple phase precession traits exhibited in axis cells are unique to this population and may constrain viable mechanisms to give rise to the phenomenon. Phase precession fields covered a breadth of distances within one neuron, and, as such, precess at varying rates. There is not a clear correlation with firing rate and precession rate, even within one neuron. These features restrict interpretations relying on speed of field traversal to determine precession rate, or at the very least, need to suggest how the input strengths could be adapted to account for this. Furthermore, it is a clear example with a very different input and local network than CA1, hampering network interpretations as a valid mechanism. It is possible this signal occurs from the same CA1 neuron population as for CA1 precession, but that would need to be verified and account for the different field sizes. The field size also appeared to modulate the level of phase variability in the firing field. This suggests the fields longer than the inputting CA1 fields are fundamentally different in the mechanism for their firing. Finally, an inhibitory positively correlated phase/firing relationship is seen within some of the firing fields. It is unclear as to what is creating this particular relationship. Further research and modeling is needed to answer all of these novel questions. As such, and given the possibility of designing environments to create specific situations and field sizes, axis neurons may be an ideal population for the continued study of the general characteristics of phase precession.

The function of axis cells in the context of spatial navigation also has more questions than answers. A potential utility of such a representation is to link analogous spaces. These neurons are active on all of the parallel avenues of the maze. Thus, if one of the avenues is impeded, a mechanism such as preplay of the hippocampus\textsuperscript{22–25} may activate these neurons which could in turn activate the representations of other paths
equivalent to the currently traveled path. A future experiment inspecting sharp wave ripple activity across CA1 and SUB during a navigation task could assess the presence of such a mechanism. An alternative and perhaps more straightforward value of axis neurons is to link the direction of movement to the direction of origin. This could help update location along a route as well as provide quick access to an escape route. Another related possibility is that the neurons are actively linking possible movement directions. This could explain the lack of firing in the open field context. However, no evidence was seen for three directional tuning despite the presence of many ‘T’s on our maze. For these two hypotheses, a circle track would result in bidirectional neurons with a shift from 180° in their peaks.

Taking into account axis neurons along with previously reported the spatial representations of SUB, we conclude that the region creates functional generalizations across space. Axis neurons generalize across track segments that are identical in their utility for movement (e.g. they all allow for east/west movement). Many other SUB neurons exhibited place fields that also were in functionally similar locations. For example, a neuron may have fields at the same locations on the two return paths, or all four internal paths (unpublished observations). When observing the generalization of fields in open arenas, it is worth noting that the general task structure of exploration was the same. From a functional perspective, the space was not differentiable; hence the large, unspecific fields. SUB’s proposed encoding of spatial task function complements that of MEC, which encodes metric space, and LEC, which encodes objects.

In the proposed circuit, SUB, MEC, and LEC all send these various spatial signals through their projections to RSP. Consistent with this input, RSP neurons often encode many frames of reference, and the region has been proposed to mediate transformations of spatial information. RSP lesions severely impair spatial navigation, further supporting
its role as a critical hub for integration and transformation to more action driven frames of reference.\textsuperscript{7} This also meshes with PPC lesions causing impairments to motor and spatial planning to a greater extent than to spatial memory related navigation.\textsuperscript{27,28} In this proposed circuit, both RSP and MPC can drive motor output through MPC. PPC is not necessary for crude spatial navigation; however, its function is to create and monitor goal-directed navigation, an interpretation consistent with lesions and neural representations.\textsuperscript{28–30}

The PPC and RSP projections to MPC complete the dorsal navigation circuit. Previous studies have established a role in orienting and decision making,\textsuperscript{31,32} but no experiments have investigated its role in spatial navigation. Chapter 4 consisted of recordings from rats on the same complex track maze as used in Chapter 2. We reported action correlates that discriminated turn activity regardless of spatial location. Over 20\% of the neurons did so during any point in the \textasciitilde1 s approach to the turn, highlighting the premotor role of MPC. In addition, many different individual neurons differentiated the turns for short temporal windows to reach this population code as opposed to a small fraction of neurons encoding the upcoming turn for the entire period. This pattern is also seen in motor cortex.\textsuperscript{33} In addition to this premotor function, a strong signal of multiple different spatial variables was concurrently encoded. These results highlight a spatial input to these neurons and supports the regions role in determining navigation action plans.

Recent reports have attempted to dissociate the roles of PPC and MPC in orienting decision-making.\textsuperscript{34,35} Neural activity suggested a role for PPC in the decision making process,\textsuperscript{34} whereas inactivations of PPC had minimal effects on decision outcomes.\textsuperscript{35} Here we are proposing MPC is part of an association cortex circuit with RSP and PPC that forms and prepares action plans. These lesion results are then consistent with our theorized circuit and previous lesions studies, as PPC is involved and representing motor
plans but can be compensated for by RSP and MPC, especially when a complex sequence of actions is not necessary.

Taken together, there is mounting evidence that a dorsal cortical circuit is responsible for spatial navigation in the rat. This dissertation has outlined the evidence for this circuit, showing that MPC, a region previously unstudied in relation to space, represents location using multiple frames of reference while performing a premotor function. In addition, a novel spatial representation, axis-tuning, was discovered in SUB. This discovery enhances the field’s understanding of this region and provides an additional representation in an intermediary region to investigate the transformation of spatial information between frames of reference. Finally, the temporal dynamics of axis cells link them to CA1 place cells and provide a novel opportunity for analysis of the mechanisms into the encoding of episodic memories and their transformation into motor output.

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