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Equivalence in Equivalent Places: Considering Similar Spaces in Neural Representations and Behavior

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In

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by

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The dissertation of Alexander Brian Johnson is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATION

This dissertation is dedicated to my wife, Laura. You have consistently been by my side through this journey. Your unending encouragement and unconditional love has been critical for my success.

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LIST OF ABBREVIATIONS

HPC: Hippocampus

CA1: Cornu Ammonis 1 subregion of the hippocampus

EC: Entorhinal cortex

LEC: Lateral entorhinal cortex

LFP: Local field potential

SUB: Subiculum

RSC: Retrosplenial cortex

PPC: Posterior parietal cortex

M2: Secondary motor cortex

M1: Primary motor cortex

HD: Head direction

LV: Linear velocity

AV: Angular velocity

AB: Alternation behavior

SAB: Spontaneous alternation behavior

GLM: Generalized linear model

NMSE: Normalized mean square error

HMMGLM: Hidden Markov model – Generalized linear model

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ABSTRACT OF THE DISSERTATION

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The study behavioral phenomena and the brain states which accompany these behaviors has a long history. The fields of neuroscience and behavioral psychology have, for over 100 years, utilized structured mazes in their work in order to probe how animals choose to navigate them, and how neuron activity reflects navigational features of the environment. This dissertation proposes that appreciating similar places within an environment requires a structurally complex environment to appreciate.

Evidence that structural complexity ought to elicit novel neural and behavioral phenomena is substantial. There is evidence that subiculum (SUB) neurons respond in a spatial manner similarly across different environments that share general structural complexity, and present unique directional tuning only on structured mazes. There is also evidence that less explicit structure in an open environment can drastically modulate activity in posterior parietal cortex (PPC) neurons. Both of these brain regions lay along an anatomical circuit connecting the hippocampal formation (HPC) to motor-output regions of the brain. Furthermore it is known that alternation behavior (AB), while reliable, is readily modulated by environmental factors in simple structures.

To address deficiencies in investigating these systems this dissertation employs both a structured maze, the triple-T, alongside a working memory task which allows for the animal to self-organize a strategy; thus providing structure in two forms, one explicit with the maze, and one implicit with the reward contingencies. Unique representations of place-analogies, and structural route-similarity are reported from SUB and PPC respectively. These reports alongside a report of AB being organized by spatial location provide a functional framework for the utility of appreciating similar places in complex spaces.

This dissertation describes the neural correlates of brain regions anatomically crucial to a space-to-action circuit while animals are navigating on a structurally complex environment. We discovered novel spatial representations in neuron populations linking HPC to motor-associated regions of the brain. We also discovered novel behavioral organization elaborating on well-known AB studies. Altogether these data show the importance of probing these systems with sufficiently complex structure in order to get a deeper understanding of these systems.

CHAPTER 1: The Utility of Space: Physical, Mental, and Neural Spaces

Space is a uniquely important concept for animals to understand. For all animals, the ability to perceive salient features in space affords them the ability to utilize those features in a reliable fashion. These features, such as the length of a pathway, would be critical to understand in order to navigate to where one wants to go most effectively. One set of features are those that can be considered ‘low-level’, such as a border or some impassable obstacle in our environment. The perception of where all these borders are in the environment can inform what could be defined as ‘high-level’ features such as the shape of a pathway as defined by the junction of borders and passable spaces. In order to understand the environment in a sufficient manner an accurate perception of and understanding of the environment at many levels is required

Perceiving the environment at different levels would mean that a parallel perception of lower-level and higher-level features is always occurring, and how they interact with one another defines the structure of the environment more generally. The higher-level features are poised to inform gross decision making processes such as which route to take home, considering each possibilities’ connectivity profile with the larger environment. Concurrently the lower-level features guide behavioral output at a more local level such as positioning the body relative to each specific boundary to traverse the pathway more optimally. Studies of how an animal’s spatial understanding shapes behavior across these various levels, or frames of reference as it can also be described, has a long history. These studies have had a focus on the relationship between decision making processes and the structure of the environment and task (Montgomery, 1951; Miller, 1960; Uster et al., 1976). From these studies the importance of spatial perception to behavior becomes quite clear, and the nervous system then becomes a critical focus of study for trying to better elucidate how this spatial understanding exists as a physical property of animals’ biology.

All animals with a nervous system, and particularly animals belonging to the class of mammals have the ability to integrate sensory information to plan and enact intelligent behaviors. Critical to these animals' navigation is an accurate mental representation of their environment; or the structure of the space they are in (Wolbers & Hergarty, 2010). Adapted for this task, these animals' nervous systems have the ability to store and represent information in the form of neuronal spiking activity (DeCharms, 2000). Important to note however, the nervous system is not equipped with any *one* sensory modality with which to perceive space with. Instead space and spatial information are inherently multi-modal unlike, for example, the perception of music which rests primarily on a single sense. This is due to the fact we interact and move through space using essentially every sensory organ.

Just as the ability to have an accurate understanding of the environment as a whole requires many forms of sensory information, there exist many different frames of reference in which to anchor this understanding. For example people have the ability to perceive two bus stops along the same route at once as different with regard to the literal Euclidean spatial frame of reference- they are located in different places, but also as the same kinds of place where the same kinds of experiences may be had at each space. Thus, the two bus stops are the same place in some functional frames of reference. This is one of many examples of how these different frames of reference coexist with neither being objectively correct. Furthermore, both are relevant for guiding behavior depending on the behavioral goal. Depending on the specific frame of reference being considered different information may be required to inform them. We may only be able to get where we need to go from one of the bus-stops, or it may not matter. This would suggest that information, as a neural code, should reflect all these various considerations of space. For as many relevant frames of reference which behavioral decisions can be made along, so to must the neural code represent space along the same frames of reference.

An important question to consider then is: what representations are important enough to consider at any time? In many real world examples the potential frames of reference are limited only by one's imagination. In order to begin answering the question one can look to the structure of the environment itself. The structure provides the animal with regularities in space around which the animal navigates around. During navigation of physical structure within the environment certain features of space such as the junctions of pathways present themselves as natural points at which behaviors are structured and at which experience traversing them directly relate to future navigational ability (Brunec et al., 2022). Structures such as junctions become more or less relevant depending on the specific task or motivational state of the animal (Tolman, 1930). The nervous system then is tasked with establishing relevant representations of spatial features that can be utilized in a variety of contexts and behavioral demands. Investigations into what spatial representations the nervous system actually creates is a specific branch of neuroscience kickstarted by the provocative neurophysiology work performed by O'Keefe and Dostrovsky (O'Keefe & Dostrovsky, 1971). Since then the past five decades have given a wealth of understanding into how brain dynamics change during navigation through various environments (Winson, 1978; Markus et al., 1995; Sharp, 1997; Derdikman et al., 2009; Dabghian et al., 2014). Parallel to investigations into the neurophysiological basis of spatial representations is over 100 years of investigation into how spatial structures of different forms elicit particular behaviors from animals (Carr, 1917; Montgomery, 1951; Douglas et al.,1972; Uster et al., 1976; Doeller & Burgess, 2010). Together these fields of study provide an answer to which spatial features are crucial to be encoded for navigation, and how those features reliably guide behavior.

As mentioned the nervous system, in a general sense, forms a coherent mapping of the spatial features around the animal through a distributed network of interconnected brain regions that is relatively stereotyped in mammals (Ekstrom et al,. 2017; Grieves & Jeffery, 2017). The

neurons found in these brain regions change their activity profile at the detection of particular spatial features, navigational behaviors, or both. Due to the distributed and interconnected nature of the brain regions defining this spatial navigation system it is not very surprising to learn that deficits in any one sensory system do not unilaterally prevent navigational phenomena (Poucet et al., 2000; Rossier & Schenk, 2003), and suggests that most if not all individual sensory systems contribute to the functioning of the spatial navigation system. Beyond sensory systems many regions of association cortex and subcortical structures such as posterior parietal cortex (PPC), retrosplenial cortex (RSC), entorhinal cortex (EC), subiculum (SUB), hippocampus (HPC), as well as several thalamic nuclei such as nucleus reuniens (NR) and anterior thalamus (ATN) are considered crucial to the spatial navigation system in that lesions to these regions produce profound deficits in navigation (Morris et al., 1982; Kolb & Whishaw, 1983; Kolb et al., 1994; Whishaw et al., 2001; Harker & Whishaw, 2004; Parron et al., 2004). Neurons in these brain regions also modulate their activity strongly based on observable spatial features (O'Keefe, 1976; Hafting et al., 2005; Nitz, 2006; Alexander & Nitz, 2015). Because several regions of this system exhibit projections into regions of the brain encoding motor output the anatomical positioning evidences the importance this system ought to have on mediating behavioral decisions (Donaghue & Parham, 1983; Petrides & Pandya, 1984; Jurgens 1984; Reep et al., 1990; Shibata et al., 2003; Yamawaki et al., 2016).

HPC appears unique in its importance within this spatial navigation system. This is evidenced by patients with lesions to HPC having difficulty identifying where they are in a new location (Smith & Milner, 1981; Cave & Squire, 1991; Rosenbaum et al., 2000), and animals with lesions to HPC having difficulty wayfinding and establishing spatial associations (Morris et al., 1982; Richmond et al., 1999; Clark et al., 2005). More evidence to this point comes from the activity of the neurons themselves. The individual neurons of the hippocampus are very often selectively active only in specific location on an environment dubbed 'place cells' (O'Keefe &

Dostrovsky, 1971; O'Keefe, 1976). As outlined in the seminal book by Nadel and O'Keefe (1984), a population of place cells has at least one neuron that becomes active at any given location, and together as a population appear to encode where an animal is within an environment. Altogether, these findings suggest that HPC is crucial within this spatial cognition system in its role in responding to navigation from an objective, world-centered frame of reference.

Since their discovery in 1971, place cells have captivated the attention of neuroscientists (O'Keefe & Dostrovsky 1971, O'Keefe 1976). Originally recorded in the hippocampi of rats as they wandered around an arena collecting food, these place cells only reliably become active when the rat occupied a particular location on the arena. The area defined by a neuron's activity, when spatially restricted, is referred to as the place field of that neuron. The fact that these neurons are responding to the environment in a manner that extends beyond the animal's immediate sensory experience - in an 'allocentric' frame of reference - is very intriguing. It demonstrates explicitly that the nervous system establishes an allocentric neural-representation of the world despite being bounded to sensory inputs, which are inherently anchored to the animal itself - what could be described as an 'egocentric' frame of reference. These place cells have since been shown to be incredibly context dependent. For example place cells modulate their activity based on the direction of approach to a place field (McNaughton et al., 1984; Wood et al., 2000), and even differentiate the same location across several trajectories that each run through the place field (Ferbinteaneu & Shapiro, 2003). This evidence challenges our understanding of how space is considered in the nervous system. Studies that link these neural phenomena to behavioral predictability directly link these representations of space to specific decision making processes (Johnson & Redish, 2007; Pfeiffer & Foster, 2013).

Place representation has also been seen to be modulated by shape, orientation, and context of the environment where the animal is located (Muller & Kubie, 1987; Cressent et al.,

2002; Lever et al., 2002; Anderson & Jeffery, 2003; Leutgeb et al., 2005), a sequence of events or time (Pastalkova et al., 2008; MacDonald et al., 2013; Terada et al., 2017), the task the animal is performing (Markus et al., 1995; Aranov et al., 2017), and recurrence present in the task environment (Nitz, 2011). The extensive heterogeneity seen in the activity patterns of place cells demonstrates that these neurons not only encode position in a literal spatial frame of reference, but also encode position in an abstract sense along frames of reference specific to the animal's context. When discussing HPC activity, this idea of 'place' extending beyond the literal Euclidean dimension can be understood better through the framework of HPC operating alongside a cognitive map. This cognitive map would represent the each experience within a situation as a cognitive place. These cognitive places are defined by the physical and contextual frames of reference within which the animal is able to navigate and problem solve. In this way the neural activity of place cells reflects much more than a literal representation of space.

Beyond the spiking discharge of neurons being largely organized by location as animals navigate around an environment, the larger electrophysical context of the HPC changes with regard to the local field potential (Vanderwolf ,1969). That is when animals are actively navigating the recorded LFP around place cells oscillates at a regular 6-10Hz known as the theta-oscillation (Whishaw & Vanderwolf ,1973). Perhaps to no surprise this dramatic change in LFP exerts a strong influence over the spiking activity of neurons in HPC including place cells (O'Keefe & Reece, 1993). One way to describe the relationship of spike activity to the cooccurring theta oscillation is seen in phase-precession (O'Keefe & Reece, 1993). The specific relationship HPC spiking activity has to the underlying theta oscillation can be summarized as: "Late in the field, early in phase. Early in the field, late in phase." That is to say that as an animal just enters a space encoded for by a single place cell, that place cell will respond preferentially at the late-phases of the underlying theta oscillation. As the animal traverses through the middle of the field the place cell will discharge action potentials at its maximum rate in the middle phase

of the underlying theta oscillation. Finally, as the animal exits the place field the place cell is encoding that place will discharge action potentials preferentially at the earlier phases of the underlying theta oscillation. This phase-precession phenomena serves as evidence that integration of space across time occurs naturally in a way that suggests that HPC neurons encoding position have access to information regarding what the past positions were, and what the future positions will be.

HPC activity patterns encode information regarding the temporal sequence of events which is expected. Thinking about HPC in this way in addition to the aforementioned contextual idea of place beyond literal space, it becomes natural to think of HPC as also the focus of memory systems. Historically HPC lesioned patients were remarkable in their acquired amnesia for events following their surgery (Scoville & Milner, 1957). Since then studies have described these patients as more specifically lacking the ability to form new episodic memories (Cohen & Squire, 1980; Hamman & Squire, 1995; Squire & Zola, 1998). On the one hand there is extensive research done in humans on the role of HPC for the creation of episodic memory. On the other hand there is extensive research done in animals demonstrating a crucial role for HPC in spatial processing. Researchers have sought to bring together these lines of research for decades, historically by demonstrating spatial deficits in human patients with HPC lesions (Cave & Squire, 1991), or through demonstrating memory processing in HPC activity (Redondo et al., 2014). These studies more often than not show how intertwined the neural processing for spatial cognition is to neural processing of episodic memories. This interplay between space and memory is further evidenced by looking at how place-associations are strong drivers of behavior (Brown et al., 1995, Lu et al., 2003), and how deficits in memory often coincide with deficits in navigation in dementia involving HPC (Kolb & Whishaw, 1996). Volumes of text have been written speculating on the specific link between spatial cognition and memory systems (Aggleton & Pierce 2002; Burgess et al., 2002; Rolls et al., 2002). Studying the neural circuits

underlying spatial cognition is one of several avenues one could take in studying the neural circuits underlying memory. The observed representations in the spatial navigation system can only be explained in part by space alone, and what are typically considered spatially responsive cells, such as place cells, may be the ideal substrate upon which the episodic memory system is built.

Regardless of what motivation one has for studying activity of HPC circuits a valid question that has been asked throughout the years is, at a mechanistic level, how do the specific responses to space and context observed in HPC come to be? This question is particularly enticing because as previously mentioned, the nervous system is only privy to sensory information that is anchored to the frame of reference of the animal itself in an egocentric manner. It is thought that the association of sensory inputs within the nervous system, across modalities and across time could establish the building blocks necessary for the activity patterns observed in HPC. Association cortices such as PPC are known for neuron activity patterns which seemingly respond to similar spaces across frames of reference anchored to a particular effector limb (Cohen & Andersen, 2002) or route shape within an environment (Nitz, 2009). These extrahippocampal representations of space become an important source of motivation for not restricting study to HPC itself, but also to include other brain regions where neurons activity exhibits different forms of spatial representations.

The major immediate input to the hippocampal system is EC (Wyss, 1981) which itself receives input from other association regions such as insula, piriform cortex, PPC, and RSC (Burwell & Amaral, 1998; Lee et al., 2020). With regards to sensory processing EC in many ways represents the terminus of parallel ‘what’ and ‘where’ pathways of information processing (Suzuki et al., 1997; Deshmukh et al., 2012; Nilssen et al., 2019). Based on its anatomy relative to HPC, as well ad the highly integrative and multimodal responses seen in the neurons of EC, it is likely that EC contributes directly to the establishment of place representation in HPC neuron

activity. This idea was bolstered in 2005 with the discovery of grid cells a type of neuron in EC defined by its unique activity profile in a spatial frame of reference (Hafting et al., 2005). Grid cells have their activity selectively increased at many repeating ‘nodes’ throughout an environment. For any one grid cell these nodes are arranged within the environment at an equal distance and angle apart from one another and they extend out in this repeating pattern throughout the accessible space. These grid cells have been thought to be a universal map from which place activity may be derived (Solstad et al., 2006). Inactivation studies of show that inactivating EC populations causes a remapping of individual HPC place cells , and biases which neurons within the population will be active (Rueckemann et al., 2015; Miao et al., 2015). These disruptions only seem to qualitatively disrupt HPC neurons exhibition of place representation in circumstances of extreme sensory deprivation (Hales et al., 2014; Jacob et al., 2020). It becomes clear that while EC plays a role in establishment of HPC activity EC activity is not the sole association cortex of interest.

Other association cortices have been studied in a similar manner to EC, such as RSC (Cooper & Mizumori, 2001) and PPC (Save et al., 2005), with similar findings in that place fields remap their preferred place, or change their shape in a quantitative manner. These association cortices are intriguing in this respect when considering their anatomical positioning. In addition to being components of the dorsal stream ‘space-to-action’ circuit which connects HPC to motor regions of the brain (Vann & Aggleton, 2002; Yamawaki et al., 2016; Olson et al., 2019) there is a reverse stream of connections from PPC to RSC (Reep et al., 1994; Kobayashi & Amaral, 2003) and from RSC to EC by way of the presubiculum (PrS) (Insausti et al., 1998; Kobayashi & Amaral, 2007). Other RSC efferent targets include regions such as the anterior thalamic nuclei (ATN) (Mathiasen et al., 2017), which overlaps with functional inputs to SUB (Frost et al., 2021). This directly functionally links PPC and RSC to SUB which traditionally is considered the output structure of HPC neurons. However, more recent studies are revealing that SUB also serves as

an input structure to HPC and facilitates spatial associative learning (Xu et al., 2016; Sun et al., 2019). This provocative view of extrahippocampal brain circuitry presents PPC, RSC, and SUB, as equally relevant input structures to HPC as the more often studied EC. However by contrast to EC, the study of spatial representations in association cortices such as PPC is sparse, and the literature on SUB activity profiles is orders of magnitude smaller than HPC. Considering the reverse of the space-to-action circuit in an 'action-in-space' framework provides new motivation to study often-overlooked regions such as SUB - a region where the neurons' activity patterns are not well described in the literature - with as much attention placed on HPC and EC.

When investigated SUB neurons have been studied in a similar fashion to HPC place cells they demonstrate less specificity within the place fields and a tendency toward generalization across environments that HPC place cells consider distinct (Sharp & Green, 1994; Sharp, 1997). The less specific firing properties of SUB neurons were also speculated to facilitate greater information transfer at the population level (Kim et al., 2012). SUB neurons have perhaps been better characterized by their responses to boundaries (Lever et al., 2009), and to the orientation of an animal (Olson et al., 2016). SUB neurons generalize their orientation by tuning for one heading direction and the exact opposite one, forming a representation the axis of travel when the animal was on a structured path-network. These response profiles are similar to head direction cells of ATN (Taube & Muller, 1998), RSC (Cho & Sharp, 2001), and postsubiculum PoS (Taube et al., 1990) all of which SUB is anatomically connected to, but differ in the contextual nature of them and the generalization across multiple directions . Other studies have shown a unique role in SUB neuron activity for the memory-trace of environmental structures (Poulter et al., 2021). Much like responses to boundaries but with the additional feature of persisting in their response for some time after the boundary is removed. These studies showing axis tuning, and the recorded activity patterns around the presence or remembered location of border structures both suggest that SUB responses may be impacted

by the structure to a degree greater than HPC. However, due to the largely understudied nature of SUB neurons the extent to which SUB spatial responses are a function of the environmental structure remain to be seen.

With respect to the bidirectional circuit connecting HPC and PPC, the anatomical positioning of SUB is suggestive. Connections to and from HPC suggest a role as both the beginning of the aforementioned space-to-action pathway and the penultimate node in the action-in-space pathway. This positions SUB as a unique neural-hub in the brain where representations across many frames of reference at many levels should coexist and influence the expression of one another. This raises an important question as to how activity from these brain regions ought to be analyzed and interpreted considering the level of complexity within the activity that can be expected.

Traditionally neurons throughout the spatial navigation system of the brain have had their activity profiles described with regard to explicit, observable, and manipulatable features of the environment. These features can be environmental ones like borders (Lever et al., 2009) or landmarks (Wang et al., 2020), but also can include navigational features such as the orientation of the animal (Taube et al., 1990) and what kind of movements the animal is making (McNaughton et al., 1994; Keshavarzi et al., 2022). The collection of ‘cell-types’ found throughout the spatial navigation system is one way scientists have tried to make sense of the general neural computations being performed. Increasingly ,however, it is being noticed that many of these ‘cell-types’ exhibit junctions of responses (Sargolini et al., 2006) across many of the already known response profiles. More intriguingly are neurons which exhibit responses that demonstrate a conjunction across egocentric and allocentric frames of references (Alexander & Nitz, 2015; Alexander et al., 2020). Studying and describing these neuron responses has given a lot of insight into what representations are relevant during navigation. However it is often the case that cells exhibiting the same or very similar activity profiles can be found throughout many

regions of the nervous system perhaps suggesting the importance of signal redundancy in the brain, or suggesting that some of these studies classify neurons in a relatively simple manner.

This collection of various cell types and their responses has provided the field of spatial navigation many metrics to analyze new cell populations with, and has undoubtably advanced a general understanding for what aspects of space are important for spatial cognition (e.g. orientation and the perception of boundaries). However, many of these features are relatively obvious ones which exist in almost every environment. Every environment which can be constructed has an inherent orientation to other spaces, however, not every environment will have decision points leading to different locations. The problem which arises for neuroscientists studying the spatial cognition system is that neurons are sensitive to particular structural features also respond differently to them as their behavior around them changes (Moore et al., 2021).

More tools are being developed to study the influences of high-level spatial features with regard to their impact on neural activity throughout the brain's spatial navigation system. These tools come in several forms, such as sophisticated analytical methods that employ neural networks to predict the navigational features around the animal. These decoding techniques can identify regions of space which the neural data being investigated has more or less utility in discriminating across (Bassett et al., 2018; Glaser et al., 2020). These studies have the promise of elucidating subtle influences on neural activity patterns, but are often difficult to utilize and still rely on relatively simple feature spaces in their analyses. Another branch of techniques which advance the sophistication of experimental design comes from looking at the structure of the experimental design itself. Studies which employ highly structured environments allow for probing high-level features of space such as the topology of connected spaces. For instance maze structures that have overlapping segments (Miller et al., 2019), consist of multiple choice points (Ainge et al., 2007), and involve sequential recurrence (Scmitzer-Torbert & Redish, 2003)

have all been used to demonstrate that many different spatial frames of reference are important for explaining neural activity along the regions of the spatial navigation system. Other environments which have been used allow for the animal to engage directly with designed structures within the environments, and have yielded fascinating results in how topology is encoded (Dabaghian et al., 2014), how the brain breaks down spaces into subspaces (Alexander & Nitz, 2017), how the memory associated to places carries into the representation of place (Keniath et al., 2019), and how frames of reference influence spatial representations (Alexander & Nitz, 2015; Wang et al., 2019).

A salient example of neurons needing certain features to have their activity patterns appreciated comes from lateral entorhinal cortex (LEC) and the class of neurons known as landmark anchored neurons. This cell population necessitates having a landmark which to anchor to, without landmarks these cells could mistakenly be considered to only encode head direction (Wang et al., 2020). Capturing phenomena such as this in experimental design is challenging, because to truly grasp the nature of the representations seen many very specific environments could be required. Experiments are inherently bounded as well with respect to what features of space can be used as explanatory. It would be entirely possible to miss specific neural responses to spatial features if not directly looking for them. Put another way, if the features of space which modulate activity within a brain region are not present the system is not tasked with perceiving those features and thus any neurons recorded cannot give a full demonstration for their representative power. Fully incorporating structurally rich environments is something which is still nascent in neuroscience research, presumably due to a desire for tightly controlled subject experiences and the problem of exponentially increasing considerations which must be made for each feature analyzed. One has to wonder how many representational phenomena within past studies on the spatial navigation system have been

dismissed or overlooked due to the experimental environment not being suitable for them, or not having said hypothetical phenomena specifically targeted for investigation.

Outlined, thus far, has been an account of studying spatial representations in the brain. First has been evidence has been presented which details the exquisitely specific place-representation of HPC and the many frames of reference which must be considered to get an appreciation for what 'place' is to HPC neurons. Second has been evidence presented which illustrates the many spatial representations extrahippocampal regions, particularly along the space-to-action circuit. Finally has been a detailed walkthrough of fundamental constraints when attempting to study the spatial navigation system, outlining how historic and contemporary environmental designs only occasionally lend themselves to unexpected findings, and even then they are often described along the same lines as previously defined 'cell-types'.

Careful attention clearly needs to be placed when analyzing data from brain structures within the spatial navigation system. Considering the structure of the space being used and how the animal is using that space prevents our interpretations of data from being overly simplistic. For example the nature of experiments performed across several different species has led to different conclusions of the representations in the association region, PPC. In human patients who have suffered damage to PPC there is a debilitating condition called neglect which can occur (Pierce & Buxbaum, 2002). In neglect, these patients seem unable to perceive an entire 'side' of what it is they are paying attention to. For instance the patient may be able to draw a circle on one side of a piece of paper, but when asked to draw something within the circle, it is as if one side of the circle vanishes from their perception. Another phenomena, topological amnesia can also occur in patients with similar brain damage. These patients, in spaces they have already been through before, have difficulty navigating – specifically the patients are unable to form routes to fluidly navigate around the space (DeRenzi et al., 1977). In both cases

PPC functioning in humans appears to be critically necessary for spatial cognition and navigation.

In non-human primates PPC neuron activity has been studied directly for its role in various cognitive functions. PPC neurons appear critical for transforming spatial coordinates across sensory modalities to motor effectors which guides sensory-motor behavior (Colby & Goldberg, 1999). PPC neurons are also seen to exhibit modulations with regard to attentional processes known as gain-fields (Pouget & Sejnowski, 1997). Gain-fields occur for certain spatial locations within a sensory or motor frame of reference and increase the firing of downstream neurons. PPC neurons have been described with regard to motor planning, and in particular the spatial component underlying the intention on where to move (Andersen et al., 1997). Lastly PPC neurons are also very well studied with regard to their role in working memory processes, and in particular working memory for particular sensory or motor locations along various frames of reference (Crowe et al., 2008). In general PPC in non-human primates appears to similarly be highly tuned to spatial frames of reference and often analyzed with regard to their role in active navigation (Arbib, 1997). The many cognitive processes PPC appears to be associated with speaks not only to the highly integrative nature of PPC, but also to the variability in task demands across the different studies.

The vast heterogeneity of cognitive processes associated with PPC has, in some ways, distracted from understanding the exact representational qualities of PPC neurons. Rat PPC studies have more directly attempted to answer what the representational qualities are for PPC neurons. However, the caveat for rat studies is that many of them have been performed with significantly deprived task requirements. Rat studies of PPC activity has revealed an extraordinarily strong correlation with self-motion (Whitlock et al., 2012). Alongside studies of self-motion PPC activity is also seen to reflect the specific body posture of the animal (Mimica et al., 2018). These studies which explicitly view PPC activity as being egocentrically anchored

could be missing some aspects to PPC representations. There are a few studies into how PPC neurons react similarly across the same shaped pathways, and how PPC neurons respond at several frames of reference within a single environment (Nitz, 2006; Nitz, 2009). There are also studies of PPC neurons showing that activity responses to self-motion modulate as the task the rat is performing becomes more predictable and structured (Alexander et al., 2020). This trend of imagining PPC activity in the rat as being more divorced from spatial representations as compared to their primate counterparts is surprising - especially because M2, a major efferent of PPC has some neurons that are heavily modulated by spatial position (Olson et al., 2019). It is possible that this difference is how to view and study PPC across animals is a direct cause of the choice of behavioral tasks and experimental environments being used.

Neural codes from regions of the brain along the space-to-action circuit will ultimately have their activity patterns dictated by the animal's perceived position as defined across many frames of reference. Much like how the neurons of the space-to-action circuit can have their activity altered by increasing the richness of the environment (Battaglia et al., 2003) it should be expected too that the neurons along this circuit would have their activity altered by behavioral richness as well. One frame of reference which is implicit when discussing these spatial navigation studies is the behavioral task itself the animal is undertaking. As seen with the phenomena of trajectory dependance (Wood et al., 2000) the spatial navigation system can distinguish across different behavioral trajectories at the same location even though the general behavior is the same. The same studies if performed without a divergent area on the maze would miss this phenomena because the set of behaviors needed would not be great enough to demonstrate it. Therefore designing experiments that allow investigation into what the animal is doing at several behavioral scales is critical to understanding the spatial cognition system as well. However to get an appreciation for what types of tasks one ought to employ to see

sufficiently rich behavior one must look back at the literature of how animal navigation has been studied.

Mazes and structured environments of many forms have been used by scientists studying animal navigation for a long time (Carr, 1917). In many ways the contemporary field of behavioral science still utilizes the same techniques established long ago. These experiments involve designing structured environments and placing animals of varying naivete on the environment to mark changes in behavior over time. In order to study interesting behaviors it necessitates scientist both are able to design structures that provoke interesting behaviors, and that scientists have the ability to identify discrete behaviors when they are being performed. Without both of these aspects of experimental design being considered there are fewer metrics which can be derived for analyses. In order to get animals to generate interesting discrete behaviors certain stereotyped structures have been used, such as the T-maze. The T-maze is a simple maze consisting of three linear corridors in the shape of the letter 'T'. Animals walk through a corridor which, at its end bifurcates into two choice arms the animal can choose to continue walking down. Using a T-maze structure scientists quickly observed spontaneous alternation behavior (SAB) where animals in subsequent trials will reliably choose the opposite decision as the prior trial (Carr ,1917; Tolman, 1925; Wingfield & Dennis, 1934; Deacon & Rawlins, 2006). It was speculated by Hull in 1943 that this phenomena was best explained by the animal's natural tendency to avoid performing the same action as it had previously done (Hull, 1943). This idea came under scrutiny by several researchers who investigated SAB under many different experimental conditions and claimed other explanations were better suited to explaining SAB. Many researcher saw SAB as an exploratory behavior suited to optimize exploration of the space as opposed to a simple inhibition to the behavioral reaction (Walker et al., 1955; Dember & Earl, 1957; Sherrick et al., 1979). What is surprising is that after decades of pushing this line of research forward through the employment of different types of structured

mazes (Douglas, 1972; Wathen & Roberts, 1994) there has been very little attention placed on how is organized in environments that extend beyond single decision points. That is to say that the structure of these experiments remains fundamentally unchanged in over a hundred years.

The incredibly robust and reliable presentation of SAB, along with the uncertain nature of it is perhaps why, for over a hundred years, behavioral science has studied SAB in the way that remains fundamentally unchanged. Studies have adjusted reward contingencies (Bryant & Church, 1974; Gaffan & Davies, 1982), orientations (Dember et al., 1966; Potegal et al., 1977), prior experiences (Sherrick et al., 1979), variable lengths of temporal and spatial inter-trial-phases (Dennis, 1939). There are also a host of inactivation studies using lesions or pharmacological techniques to inactivate regions of the brain (Baettig, 1963; Ellen & DeLoache, 1968; Egger et al., 1972; Parent et al., 1997; Barga et al., 2005; Nelson et al., 2020) . Still SAB remains a siren's song calling researchers to be stuck on its stubborn persistence using the same methodology as before. Many findings have been made into what factors influence the expression of SAB, but we are not much further along in understanding the nature of SAB. Behavior studies additionally, have not attempted to put animals in a scenario where SAB extends beyond a single decision point to investigate the reliability beyond a single choice.

Attempts have been made throughout the years to look for insights into behavioral strategies more generally. Typically these attempts have been made through employing structured environments in novel and exciting ways in behavioral studies such as sequential turns (Hunter & Hall, 1941; Michel et al., 1978; Schmitzer-Torbert & Redish, 2003), geometric layout of routes (Douglas et al., 1972; Bak et al., 2017), or incorporating shortcuts into the environment (Grieves & Dudchenko, 2013). These studies have put forward many useful insights into the cognitive processes underlying navigation, but the reach of these claims is only able to extend so far under still relatively confined circumstances. Even on environments which would afford themselves to richer data, the tasks being asked of the subject rarely require the

subject to fully understand or utilize the topology of the environment in a meaningful manner. Instead the subjects are often guided or cued in some fashion that does not demand the utilization of spatial understanding (Euston & McNaughton, 2006; Ainge et al., 2007). This oversimplification of task-design certainly would occlude functions of the spatial navigation system as planning, attention, and memory are all tightly interconnected with spatial cognition processes. Thus this constrained nature of the data not only damages the ability to draw meaningful conclusions from behavior itself, but neurophysiological studies into nearly every cognitive process are certain to be deprived in some fashion.

One challenge to applying a greater appreciation for both spatial and behavioral complexity in neuroscience is seen in the constraints necessary to generating meaningful hypotheses in the face of increasing degrees of freedom (Krakauer, 2017). This is not a new consternation however, David Olton's 1979 review discussing the need to "do hard things", as it involves designing meaningful experimental environments still holds true (Olten, 1979). There is a natural interplay between data, with fewer degrees of freedom, which can be easily interpreted and analyzed in the same manner as its predecessors; and data which has too many degrees of freedom to make any meaningful predictions or conclusions from. The latter still pushes our understanding further still by offering new and reliable clues into the functioning of the nervous system.

One structural feature which has become incorporated in the field of artificial intelligence navigation already (Soltoggio & Jones, 2009; Yaman et al., 2019), and only recently in the study of insect (Pasquier & Grüter, 2016; Okada & Norikuni, 2022) and human behavior (Rothacher et al., 2020) is the employment of sequential decision-making locations in a meaningful manner. These structures incorporate two binary decision points which lead to four, or three binary decision points which lead to eight end sites and allow for a multitude of tasks to be performed on them. Surprisingly behavioral studies on rats navigating such an environment are non-

existent despite the hundred years prior of studying the only slightly more simple single T-maze. This presents a problem in neuroscience that fully developing a framework of the spatial navigation system on richer behavioral tasks, and thus a more complete understanding of it in general, requires new foundational work to be done. This foundational work needs to set expectations not only for how regions of the spatial navigation system react to more complex environments, but also needs to establish a set of baseline behaviors for the field to measure in such experiments. These studies will contribute to fields beyond neural and behavioral science though. By establishing baseline behavioral metrics roboticists, which already employ such tasks, will be given a set of naturalistic observations to compare their automata to.

This dissertation serves as a response to that problem in neuroscience, and addresses it through the implementation of the triple-T environment which rats readily learn to perform a simple find-all working memory task very quickly. This environment coupled with the find-all working memory navigation task allow for the rat to naturally organize its behavior in reliable and unique ways while maintaining excellent performance. Additionally the structural and structural richness of the triple-T allow for SUB and PPC brain regions to exhibit novel, never before reported, encodings of structural similarity.

Organization of Dissertation

Overall, this dissertation underpins the importance of utilizing a complex structured environment when studying spatial representations throughout the nervous system. To emphasize this novel spatial representations encoded in regions of the brain (SUB & PPC) are presented along with a novel behavioral findings. All of these studies emphasize the importance of affording the animal (and thus their nervous system) a structurally complex environment. First through the discovery of novel spatial representations in the neuron activity of SUB neurons as compared to their primary afferent - CA1. Second through the discovery of embedded structural coding in a population of PPC neurons, adding to the corpus of work specifically investigating

the spatial properties of these neurons. Lastly through the discovery of a novel, spatially organized, behavior in rats performing ‘triple-T’ working memory tasks.

Chapter 2 presents neurophysiological data from SUB neurons as well as from CA1 neurons as rats were performing the ‘triple-T’ find-all-4 task. We report novel spatial responses seen exclusively in the SUB population. These neurons are highly selective for a specific location on the maze (though not any one specific location across the population) much like their CA1 place cell counterparts. However, unique to the SUB population of neurons these spatially responsive cells also respond to locations which share the same position across routes that share structural similarity with one another. This finding alongside finding that SUB neuron population organize these responses around structural features like corners are interpreted to mean that, one synapse away from the population of highly-selective CA1 place cells, exists a mapping of structure. This result presents a novel cell-type which suggest that understudied brain regions may not have their functions understood on environments lacking in sufficient structural complexity.

Chapter 3 presents neurophysiological data from PPC neurons as rats were performing the ‘triple-T’ find-all-4 working memory task. We report on the existence of a population of PPC neurons whose activity patterns were highly correlated across routes which shared general spatial structure yet exhibited exact opposite action sequences required to traverse them. We interpret these results to suggest that in addition to known frames of reference that PPC neurons respond to, PPC neurons exhibit the necessary activity patterns to encode general structure of a route. This result adds to our understanding of how different route-spaces can be used as frames of reference for the nervous system. This finding also presents a challenge to PPC studies in rats to consider space in addition to self-motion when analyzing neuron activity data.

Chapter 4 specifically presents the behavioral data from all five rats who were trained in a stereotypical fashion on the 'triple-T' find-all-4 task, as well as three rats who made the transition to the more advanced find-all-8 condition. We report that animals performing the find-all-4 condition of the 'triple-T' organize their decision making along the heuristic of alternation, and organize this heuristic spatially. Animals on the find-all-4 task utilize external routes of the triple-T in a stereotypical fashion as well. Additionally animals, when learning the find-all-8 condition of the 'triple-T' maze, incorporate alternation behavior at the additional decision point without any decrement to other alternation behavior. We present these results to demonstrate how overlapping sequential decisions are made in the rat. This suggests that allowing the animal to self-organize their behavior in an environment with appreciable structure allows for investigation into many robust spontaneous behaviors yet to be fully investigated by behavioral psychology. This result presents a novel behavior self-organized by rats which could be used to investigate further questions in neuroscience.

This dissertation concludes with a detailed explanation of the importance of complex spatial structures which elicit sophisticated self-organized behaviors when studying neuroscience.

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CHAPTER 2: Complementary Maps for Location and Environmental Structure in CA1 and Subiculum

Abstract

The dorsal subiculum lies among a network of interconnected brain regions that collectively map multiple spatial and orientational relationships between an organism and the boundaries and pathways composing its environment. A unique role of the subiculum in spatial information processing has yet to be defined despite reports of small neuron subpopulations that encode relationships to specific boundaries, axes of travel, or locations. We examined the activity patterns among populations of subiculum neurons during performance of a spatial working memory task performed within a complex network of interconnected pathways. Compared to neurons in hippocampal sub-region CA1, a major source of its afferents, subiculum neurons were far more likely to exhibit multiple firing fields at locations that were analogous with respect to path structure and function. Subiculum neuron populations were also found to exhibit a greater dynamic range in scale of spatial representation and for persistent patterns of spiking activity to be aligned to transitions between maze segments. Together, the findings indicate that the subiculum plays a unique role in spatial mapping, one that complements the location-specific firing of CA1 neurons with the encoding of emergent and recurring structural features of a complex path network.

Main Text

INTRODUCTION

The dorsal subiculum is situated within a distributed system of brain regions forming a 'cognitive map' that encodes an organism's spatial relationship to its environment (Andersen et al., 1973; Hjorth-Simonsen, 1973; Amaral & Witter, 1989; Amaral et al., 1991; Witter, 2006). The few studies that have examined the impact of damage selective to subiculum (SUB) support a

role for this structure in the two most prominent tests for spatial navigation, the Morris water tank and T-maze spatial alternation tasks (Morris et al., 1990; Galani et al., 1998; Frost et al., 2020). Despite this, relatively few studies have addressed the form or forms by which subiculum neurons represent spatial and orientational relationships to the environment.

Clues to a unique role for SUB as a component of a distributed cognitive mapping system come from its major sources of afferents (Witter, 1990; Witter et al., 2000; Naber et al., 2001; Cembrowski 2018). A prominent input from the anterior thalamus and moderate input from the presubiculum suggests a strong influence of orientation or 'head direction' tuning on SUB function (Goodridge & Taube, 1997; Winter et al., 2015; Viejo & Peyrache, 2019; O'Mara & Aggleton, 2019). Indeed, sensitivity to head orientation relative to environmental boundaries was observed in early studies examining the firing of SUB neurons during random foraging in open arenas (Barnes et al., 1990; Sharp & Green, 1994; Sharp, 1999) and, more recently, in a track based environment (Olson et al., 2017). Major inputs from both medial and lateral entorhinal cortex and hippocampal sub-region CA1 indicate a strong influence of tuning by location within an allocentric, 'world-centered', space defined by environmental boundaries (Muller & Kubie, 1987; Hafting et al., 2005). Accordingly, evidence for 'place-specific' firing (Sharp & Green, 1994; O'Mara, 2005; Kim et al., 2012; Olson et al., 2017; Lee et al., 2018) in SUB has been observed in a small percentage of neurons as has encoding of position and orientation relative to boundaries and barriers (Lever, 2009; Stewart et al., 2014; Stensola et al., 2015; Olson et al., 2017; Viejo & Peyrache, 2019; Poulter et al., 2020). Finally, SUB appears to play a role in the encoding of objects and landmarks and their relationships to environmental boundaries (Sun et al., 2019; Poulter et al., 2020).

Several studies examining the spatial firing properties of SUB neurons compared them to neurons of the CA1 sub-region of hippocampus, a region well-known for the presence of 'place cells' (O'Keefe & Dostrovsky, 1971). From such work, it is known that SUB neurons are

less focused in their tuning to environmental location, tend to exhibit a greater number of individual firing fields, and have a greater tendency to show similar spatial firing patterns across two arena environments that are the same but for differences in shape (circle versus square arena) or size (Sharp & Green, 1994; Sharp, 1997; Sharp, 1999 (2); Kim et al., 2012; Olson et al., 2017). These quantitative differences in tuning may function to permit generalization across similar environments or to maximize the efficiency of information output to efferent targets (Sharp, 1999; Kim et al., 2012). Nevertheless, it remains to be determined whether spatial tuning in SUB and CA1 can be viewed as part of a continuum or whether substantive differences in tuning evidence different contributions of each region to components of a distributed cognitive map.

To draw out potential differences between SUB and CA1, we examined the spatial firing properties of neuron populations in these regions during performance of a complex spatial working memory navigational task set within a complex network of pathways. The combined structure of the path network and set of task rules allowed us to determine to what extent rats exhibited behavior consistent with having knowledge of the overall layout of individual path segments and their relation to the full path network. The routes utilized to meet task demand bore structural similarity in having the same total number of turns and distances between them. This allowed us to detect spatial tuning of neurons that reflect emergent properties of the combined task and maze structure. Partial overlap between routes allowed us to determine whether trajectory-dependence (Frank et al., 2000; Wood et al., 2000; Ferbinteanu & Shapiro, 2003; Ainge et al., 2007) differs between CA1 and SUB and whether its presence or absence is a dynamic property of either system. Finally, the maze structure contained many intersections and path segments of different lengths, allowing us to compare the spatial scale of representation for CA1 versus SUB as well as the alignment of their population firing patterns to maze structure.

During sustained, highly accurate performance of the task, we find that individual neurons of the CA1 region primarily encode the animal's location and the trajectory taken through that location. In contrast, just one synapse beyond CA1, SUB neurons were often active for "kinds" of places that were analogous with respect to maze structure and with respect to multiple spatial variables such as head direction, axis of travel, and progression through a route. We also find that the spatial scale of representation is greater for SUB, and that it varies dynamically in both structures. Furthermore, persistence in CA1 and SUB population activity patterns across track positions segmented space in distinct ways, suggesting that spatial representation in these structures can follow qualitatively different rules. These findings identify a unique role for SUB in spatial cognition and suggest that this region is critical to encoding the fundamental structure of pathways through complex environments.

RESULTS

Robust navigation within a complex environment

We trained 6 rats on a variation of the "Triple-T" maze (Figure 1A, Olson et al., 2017; Olson et al., 2020). The task demands that animals learn the functional relationships among a set of interconnected pathways and provides a means by which to assess the impact of location, orientation and trajectory on the spiking activity of recorded neurons. Briefly, on each trial, the animal must move through 3 sequential left or right turns to arrive at 1 of 4 goal locations. The rat must then return to the starting location via external pathways that surround the internal pathways. Rewards at each goal location were 1/4 Cheerio piece and distributed on a "visit-all" schedule where reward access reset after all locations were visited. During post-implantation recording sessions, rats ran on average 148 trials per session.

The nature of this task and our training regimen purposely leads to highly stereotyped running behavior. The rats became proficient in the procedural aspect of the task, only moving in one direction at any given location (Figure 1B) and doing so at high speeds (Fig 1C). For analysis purposes, we selected out behavioral epochs where the animal ran uninterrupted from the start point to one of four reward locations (outbound routes) or from the reward locations back to the starting point (return routes, Fig 1D). This results in analyzing only the behavior during locomotion and controls over the range of head orientations and actions associated with any specific location. The rats met the uninterrupted running criteria on 85% of route traversals and averaged a velocity of 57 cm/sec during the accepted runs.

Rats also became proficient at the working memory aspect of the visit-all task on the Triple-T maze, receiving rewards on 81% of trials in recordings post-surgery (Figure 1E, mean 0.81 ± 0.15 s.d.; N = 95, P < 0.0001, one-sided Mann-Whitney U test; chance = 48%). The animals exhibited an understanding of the track space, taking the shorter return pathway on 92% of trials (Figure 1F, mean 0.92 ± 0.11 s.d.; N = 95, P < 0.0001, one-sided Mann-Whitney U test; chance = 50%). Animals even ran perfect blocks – a series of visiting all 4 locations without mistake – on 56% of trials (Figure 1G, mean 0.56 ± 0.21 s.d.; N = 95, P < 0.0001, one-sided Mann-Whitney U test; chance = 9%). Performance at this level may suggest formation of a habit with respect to the order of internal routes taken over a block, but a closer look at animal tendencies disagrees. The most common pattern used by an individual animal over an individual recording session accounted for only 48% of correct blocks (Fig 1H, mean 0.48 ± 0.20 s.d.; N = 94, P < 0.0001, one-sided Mann-Whitney U test; chance = 19%), and 26% ± 14% of total blocks. However, animals clearly used alternation at the first turn location as a mnemonic strategy, doing so on 91% of trials (Fig 1I, mean 0.91 ± 0.09 s.d.; N = 95, P < 0.0001, one-sided Mann-Whitney U test; chance = 50%).

Individual subiculum neurons are active across analogous maze locations

To compare neural activity in SUB and CA1, we recorded from the same 6 animals during task performance using microdrives outfitted with tetrodes. 573 neurons were recorded from dorsal SUB (4 SUB animals), with the majority being from the proximal half of SUB, and 401 neurons were recorded from dorsal CA1 (3 CA1 animals) (Figure 2B, Supplemental Figure 1). Maximum and minimum firing rate thresholds were used to exclude inactive cells and putative interneurons, leaving a final dataset of 480 SUB and 298 CA1 neurons.

Commonly used measures for spatially tuned CA1 and SUB spike firing were largely consistent with previous literature. SUB neurons fire more (CA1 mean 0.89 ± 1.43 s.d. N = 298; SUB mean 3.62 ± 4.23 s.d. N = 480, $P < 0.0001$, one-sided Mann-Whitney U test) and exhibit lower spatial information per spike (CA1 mean 2.97 ± 1.18 s.d. N = 298; SUB mean 1.55 ± 1.21 s.d. N = 480, $P < 0.0001$, one-sided Mann-Whitney U test) (Kim et al., 2012) as well as lower spatial selectivity (CA1 mean 63.9 ± 50.8 s.d. N = 298; SUB mean 31.1 ± 38.8 s.d. N = 480, $P < 0.0001$, one-sided Mann-Whitney U test) (Kim et al., 2012, Lee et al., 2018). There was no difference between CA1 and SUB in spatial coherence on the maze (CA1 mean 0.49 ± 0.12 s.d. N = 298; SUB mean 0.49 ± 0.14 s.d. N = 480, $P = 0.82$, Mann-Whitney U test), unlike previous reports (Sharp & Green, 1993, Lee et al., 2018).

The differences in general spatial properties were qualitatively apparent in CA1 and SUB activity on the Triple-T maze. Individual SUB neurons often exhibited larger or more firing fields than their CA1 counterparts. Beyond these previously-described differences in spatial tuning, we observed that many SUB neurons displayed another differentiating trait: increased structural relationships between the firing fields of individual neurons. While some SUB neurons did show single fields on the track, activity of individual SUB neurons more often occurred in multiple locations that often shared spatial or functional features (Figure 2D). For simplicity, we will refer to this as representation of structural or functional analogy between maze locations.

To quantify this propensity for multiple firing fields of individual neurons to distribute across analogous maze locations, we created linearized positional firing rate maps for each individual route. We then correlated individual neuron's firing activity for non-overlapping portions of all combinations of outbound runs as well as between the two return runs. For neurons with fields at analogous locations along two or more routes, correlations should be high, whereas correlations will be low or negative if the locations of firing fields across analogous portions of two routes are very different (Figure 2E). Rate vector correlations are mathematically undefined in cases where a neuron does not fire at all along one of two routes, but we note that, practically speaking, this can be considered a low or zero correlation result. We found that SUB neurons were much more likely to exhibit high correlations between routes. SUB neurons had higher maximum correlations between routes (CA1 mean 0.29 ± 0.34 s.d. N = 252; SUB mean 0.46 ± 0.30 s.d. N = 468, one sided KS test, $P < 0.0001$, KS test stat 0.29), as 47% of SUB neurons had either internal or external routes with correlations exceeding 0.5, as compared to 26% of CA1 neurons (Figure 2F). Thus, our simple measure to detect similarity in positional firing rates for analogous but spatially segregated locations along same-length routes provides a strong indication of a qualitatively different organization of spatial tuning for SUB versus CA1 neurons.

Analogous responses in subiculum include decreased trajectory dependence

Since individual SUB neurons exhibit much greater similarity in firing rates across analogous spaces of two routes than CA1 neurons, we next determined to what extent this is also seen in the two regions' population activity patterns. We first present data for the long straight segments of the return routes that have functional and directional similarity but are separated by nearly 2 meters (Figure 3A, labeled B). Pearson correlations of population mean firing vectors between the two segments show higher correlations for SUB than CA1 (Figure 3B, CA1 mean 0.28 ± 0.09 s.d. N = 110; SUB mean 0.60 ± 0.08 s.d. N = 110, $P < 0.0001$, one-sided

Mann-Whitney U test). We observed low correlation in population activity patterns as compared to the correlations for odd versus even trials of the same location (All control comparisons, N = 110, P <0.0001, one-sided Mann-Whitney U test). The final straight segments on the return routes maintain functional similarity but lack directional equivalence (Figure 3A, labeled C). Yet, SUB again had higher correlations than CA1 (Figure 3C, CA1 mean 0.25 ± 0.07 s.d. N = 59; SUB mean 0.64 ± 0.07 s.d. N = 59, P < 0.0001, one-sided Mann-Whitney U test; all control comparisons, P <0.0001, one-sided Mann-Whitney U test), and the segments' correlations for SUB were not measurably lower than the directionally consistent segments (P > 0.9984, one-sided Mann-Whitney U test).

We also used the population rate vector correlation technique to contrast pattern recurrence for SUB versus CA1 populations over overlapping portions of outbound routes. One well-described example of differentiation in CA1 is trajectory-dependent coding, wherein CA1 in-field firing rates vary according to the trajectories taken through the field (Frank et al., 2000; Wood et al., 2000; Ferbinteanu & Shapiro 2003, Ainge et al. 2007; Grieves et al., 2016). In the Triple T maze, the center stem (Figure 3A, labeled D) is a segment common to all four of the outbound routes. Correlation of mean firing rates between pre-left-turn and pre-right-turn activity show strong trajectory dependence in the CA1 population. CA1 population activity correlations were low as compared to the correlations for odd versus even trials of the same trajectory (Figure 3D, CA1 mean 0.24 ± 0.07 s.d. N = 51; both P < 0.0001, one-sided Mann-Whitney U test). The SUB population exhibits far less trajectory-dependence than the CA1 population (SUB mean 0.66 ± 0.06 s.d. N = 51; P < 0.0001, one-sided Mann-Whitney U test; all control comparisons, P < 0.0001, one-sided Mann-Whitney U test). That is, the SUB population activity patterns largely generalize in the representation of this space despite the very different trajectories. We note that trajectory dependence is nonexistent in both CA1 and SUB for the space where the animals approached the final pre-reward choice point (Figure 3A, labeled E,

Figure 3E, CA1 mean 0.92 ± 0.02 s.d. N = 67; SUB mean 0.94 ± 0.03 s.d. N = 67, all control comparisons, P > 0.9999, one-sided Mann-Whitney U test). This indicates that trajectory-dependence is dynamic in its expression for CA1 and for SUB, but much lower overall for SUB.

Finally, we examined encoding similarity as the animal approaches the goal locations (Figure 3A, labeled F). We hypothesized that SUB may generalize across the approaches to these four separate locations. Transitioning to the final segment, spatial locations diverge. However, correlations across the routes increased for both CA1 and SUB (Figure 3F, CA1 pre-final segment mean 0.13 ± 0.07 s.d. N = 67, CA1 final segment mean 0.30 ± 0.04 s.d. N = 21, P > 0.0001, one-sided Mann-Whitney U test; SUB pre-final segment mean 0.45 ± 0.14 s.d. N = 67, SUB final segment mean 0.61 ± 0.03 s.d. N = 21, P > 0.0001, one-sided Mann-Whitney U test), with SUB again generalizing more (N = 21, P > 0.0001, one-sided Mann-Whitney U test).

Subiculum and CA1 population firing patterns chunk epochs differentially relative to task phase and maze structure

Given the striking differences between SUB and CA1 encoding of multiple task phases, we looked deeper into how population firing patterns shift relative to task phase and maze structure. For each region, we assembled ensemble firing rate vectors of the even and the odd trials separately, using all recorded neurons at each position along each route. We then concatenated these route-based positional rate vectors and calculated Pearson correlations to assess pattern similarity for each route location relative to all others (Figure 4AB, Cowen & Nitz, 2012). The resulting correlation matrix can be used to compare how SUB and CA1 population activity patterns persist over contiguous locations and whether shifts in patterning are related to specific task phases or the beginnings and endings of maze segments.

Both SUB and CA1 populations reliably encode individual locations at similar levels. Values along the diagonal of the correlation matrix correspond to even versus odd trial

autocorrelations for the same location. If activity is consistent across trials at each location, correlations should be high (Cowen et al., 2014). This was the case for both SUB and CA1, as the median diagonal R values for both exceeded 0.7 (SUB = 0.76, CA1 = 0.74). The distribution of values for SUB was actually higher than that of CA1 (Figure 4C, one sided KS test, $P < 0.0001$, KS test stat = 0.17). Thus, both SUB and CA1 population activity patterns are reliable across trials at any single location, consistent with an encoding of location.

Representational scale

While both SUB and CA1 reliably encoded individual locations, the patterns in the correlation matrices suggest qualitative differences in spatial encoding. One feature of interest is the scale with which the two populations encode spaces. Consistent with historical precedent (Maurer et al., 2005), we operationalized the representational scale as the space surrounding a given location that is associated with population activity patterns that correlate at 0.5 or better to that location (Figure 4DE). This is determined by iteratively moving outward in both directions from any given location and finding the first position point at which the correlation value drops below 0.5. Notably, because task performance is associated with single directions of travel for all locations, drop-off points and their distance from any given location can be determined for the spaces visited both before and after the location of interest. The sum of these distances is the representational scale. Importantly, representational scale patterns were consistent for a wide array of correlation cutoff values (Supplemental Figure 2).

Representational scale of SUB was larger than CA1 (Figure 4F, one sided KS test, $P > 0.0001$, KS test stat = 0.37) with a median scale of 27 cm for SUB and 20 cm for CA1. This scale value was not constant across locations, however (Figure 4G). Both SUB and CA1 had larger scales on the return runs (Figure 4H, SUB: one sided KS test, $P < 0.0001$, KS test stat = 0.83; SUB: one sided KS test, $P < 0.0001$, KS test stat = 0.47). As a control to assess the validity of this dynamic change in scale, we created a bootstrapped distribution by randomly

shifting the mean odd-trial and even-trial positional mean firing rate vectors together for each neuron independently. This procedure preserves field integrity and spatial information on a single neuron level but randomizes relationships between field locations and the environment. SUB representational scale on the return paths was often larger than predicted by the shuffled population, showing that the representational scale is dynamic in response to the environment features, even within one environment (Figure 4G, 117/197 positions of return outside 99th percentile of shifted population bootstrap). CA1 is on the edge of expected representational scale for return runs, showing a similar but muted scale dynamic (54/197 positions of return outside 99th percentile of shifted population bootstrap). The two regions scale together (Figure 4I, correlation of scatter, r = 0.49), suggesting a common underlying mechanism.

Representational alignment and segmentation to maze structure and task phase

Another stark feature of the correlation matrices was the presence of square regions of high correlations around the diagonals for both the CA1 and SUB correlation matrices (Figure 4A,DE). These regions are areas where the coding of the path is consistent, whereas their corners on the diagonal suggest locations that are spatially close but representationally more distant. To put it another way, the square regions indicate track regions that are chunked together and represented highly similarly.

To examine these regions of high correlation in quantitative detail, we found the difference in distances at which correlations drop off forward (to the right on the correlation matrix main diagonal) versus backward (to the left on the correlation matrix) at each location on the matrix diagonal and termed it the representational alignment (Figure 4DE). Positive values indicate forward-facing representational alignment relative to a specific location along a route in that a longer length of space ahead of the animal will exhibit similar population representation than the length of space behind the animal. Negative values indicate a backward-facing representational alignment in that a longer length of space leading up to a given track location

will exhibit similar patterning than the spaces traversed subsequent to that location. A uniform alignment value would indicate a constant balance and a random distribution of place fields, whereas, alignments oscillating between positive and negative values indicate segmenting of space. Both SUB and CA1 dynamically changed their representational alignments as the animals navigated the maze (Figure 4J, CA1: 26% (89/337) of positions outside 99th percentile of shifted population bootstrap; SUB: 54% (182/337) of positions outside 99th percentile of shifted population bootstrap). Further, the variance of representational alignment for both regions was far greater than the shuffled population bootstrap (Figure 4K), suggesting there may be representational structure causing the large variations.

We therefore hypothesized that the dynamics of representational alignment followed the maze and route structure. Transition points at which the sign of the representational alignment flip from negative to positive indicate the end of one similarly represented section and the beginning of another. These transition points are the peaks of the derivative of alignment (Figure 4L). To ensure these variations are beyond expected random fluctuations if the place field distributions were random, only peaks that surpassed the 99th percentile of the shuffled population bootstrap were considered. Here, again, a major dissociation in between SUB and CA1 populations was observed. Transition points for the SUB population clustered at turns (Figure 4M, SUB circular median = 10.8°, P = 0.3877, circular median test, H0 = 0°). As turn locations can define the structure of the path network itself, this indicates that in SUB, population rate vectors exhibit persistent patterning over individual straight path segments. To our surprise, CA1 also transitions in forward-facing versus backward-facing representational alignment in CA1 population activity patterns. Unlike SUB, CA1 transition points were not near the apexes of turns, but instead surrounded the turns (CA1 circular median = 126.0°, P = 0.0023, circular median test, H0 = 0°). This distinction between SUB and CA1 shows a large-scale population difference in SUB and CA1 organization, and indicates that spatial

representation in SUB cannot be considered as merely quantitatively different from CA1. Instead SUB and CA1 population activity patterns are organized differently relative to maze structure and task phase.

DISCUSSION

We compared spatial and directional tuning of CA1 and SUB neuronal populations during performance of a spatial working memory task within a complex, multipath environment. While a multitude of studies have documented the role of CA1 in location mapping and navigation, it has not been determined whether SUB functions as secondary output of the hippocampal formation with slight differences in the tuning properties or if SUB tuning to location and orientation can be considered a qualitatively different form of representation. That is, can SUB be regarded as appendage of the hippocampus or a unique node among a distributed set of brain structures encoding different types of organism-environment relationships. Here, using a multipath network that allowed us to compare many spatial and movement variables, we discovered that in three main ways, SUB exhibits striking differences from CA1. First, the spatially isolated firing fields for individual SUB neurons often occupied analogous locations across the environment, whereas CA1 neurons did not bear fields at locations with structural similarity. Second, while both SUB and CA1 population activity patterns are dynamic with respect to scale of representation, SUB scaling is considerably greater. Finally, SUB and CA1 populations differentially shifted in how they encoded contiguous locations behind versus in front of the animal's current location. The recursion of SUB activity patterns at structurally analogous locations and the alignment of SUB population activity pattern shifts to the beginnings and endings of path segments evidences a role for this structure in encoding structurally-defined 'chunks' of the environment. Together, this evidence suggests that SUB may carry out a qualitatively different function during spatial navigation.

Structural Analogy and Trajectory-Defined Discrimination Versus Generalization

Perhaps the most striking result from this study is evidence for representation of analogous, but spatially separate locations through recurrence of SUB firing patterns. By 'analogous' we refer to two or more locations that are spatially separate from each other, yet logically linked by reference to location within topologically similar pathways and/or by head orientations taken during travel through their locations. For humans, city street grids bear regularity in the orientations and intersections among individual pathways and, therefore, subsets of environmental locations have 'analogy' with respect to how they align relative to environmental boundaries. Thus, streets and avenues in major cities may be organized by north-south and east-west affordances for travel and give rise to emergent concepts such as a 'northwest corner' or 'a block'. The observed spatial and directional firing patterns of SUB neurons and their organized recurrence across the maze used in our task is suggestive of the emergence of encoding that reflects recurrence in environmental sub-structure. Recurrence based on repetition within the structure of a single path has been reported for entorhinal cortex, retrosplenial cortex and posterior parietal cortex (Alexander & Nitz, 2015; Nitz, 2012; Derdikman et al., 2009) and the reported presence of 'axis-tuned' neurons in an earlier publication from our group (Olson et al., 2017) can be interpreted as reflecting recurrence of individual neuron firing patterns over all paths having the same orientation. We note that CA1 neurons under some circumstances exhibit tuning to related locations, but that, in the present case, CA1 neuron population patterns exhibited very little recurrence in firing patterns. Thus, the transition from CA1 to SUB would appear to reflect a substantive shift from encoding of location in the environment to the encoding of multiple locations according to structural and/or functional analogy.

Related to the phenomena described here are findings from earlier research showing that SUB generalizes in its spatial firing patterns across different environments sharing the same-shaped boundaries and/or a singular, prominent distal polarizing cue (Sharp & Green,

1994; Sharp, 1997; Brotons-Mas et al., 2010). In the present work, SUB neurons generalize in their spatial firing patterns across widely separated environmental locations that are analogous with respect to the shape and layout of paths taken through them. The analogous representations of SUB neurons sharply contrast with CA1 neurons. CA1 place cells are highly specific to experiences, segmenting experience by place, movement, and even trajectory dependence during navigation (O'Keefe & Dostrovsky, 1971; McNaughton, 1983; Markus & McNaughton, 1995; Wood et al., 2000; Frank et al., 2000; Ferbinteanu & Shapiro, 2003). Here, CA1 place cells, as previously reported, are highly specific to the animal's location and trajectory. CA1 firing patterns clearly distinguished not only all separate locations within the environment, but also the first section shared by the four outbound paths according to whether the animal would ultimately turn left (paths 1,2) or right (paths 3,4).

The factors that lead to the SUB representations of analogous locations is an open question, but orientation appears to be an important variable. On our maze, analogous locations sharing highly similar SUB firing patterns are often aligned in orientation, although they may also have opposing directions such as the second segment of paths 1,2 versus paths 3,4 and the end segments of the two perimeter paths. The axis-tuned neurons previously reported from a minor sub-population of the present dataset (Olson et al., 2017) may be an extreme form of this sort of analogous representation. This may reflect association of inputs from different sub-populations of anterior thalamic neurons having opposite tuning by head orientation ((Taube, 1995; Goodridge & Taube, 1997; Peyrache et al., 2017; Viejo & Peyrache, 2019; Frost et al., 2020). Future work on environments where functionally similar spaces are not parallel may help tease apart whether the structural or functional aspects of the locations drive such responses in SUB neurons.

Previous work by Frank and colleagues (Frank et al., 2000) showed a high prevalence of path equivalency in deep entorhinal cortex neurons. These neurons fired in spaces that were

analogous in their movement sequences, such as on left turns on similar sub-paths within a 'W-shaped' maze. The authors did not often see this pattern in CA1 neurons, however. Knowing both SUB and CA1 project to deep EC, the authors hypothesized SUB may play a role in this signal. Our data are consistent with this hypothesis and strengthen the possibility that analogous location representations are generated in SUB as a result of circuit interactions between EC, CA1, and anterior thalamic inputs that are tuned to head orientation.

An interesting quirk to our data exists in the trajectory dependence on the outbound routes. On the first segment leading into turn 1, CA1 population patterns almost completely differentiate trials in which the animal makes opposite choices at the upcoming turn. Even the SUB population demonstrates a much more moderate degree of trajectory dependence over the same locations. However, the segments leading to the final outbound turn (segment 3) show no trajectory dependence in either CA1 or SUB firing patterns. This difference could be due to the fact that the outbound path spaces subsequent to turn 1 are the same leading into both of the third turns (for paths 1,2 and 3,4). In contrast, different maze spaces lead into the first segment of all four outbound paths. We note here that the outbound turn 1 alternation pattern typically yields related alternation in the return paths leading into the first outbound segment.

Prior studies have either found strong evidence for trajectory-dependent modulation of place-specific activity (Frank et al., 2000; Wood et al., 2000; Ferbinteanu & Shapiro, 2003; Nitz, 2006) or the near absence of it (Bower et al., 2005). For multichoice mazes, prior work suggests that trajectory dependence was similar at the first and second choice points of a double-Y maze (Ainge et al., 2007; Grieves et al., 2016). Our data evidence the fact that trajectory dependence is dynamic in its expression even within a single environment and task structure. We hypothesize that the difference in its prevalence preceding different choice locations may be related to action stereotypy or to the navigational strategy employed by the animal. In our study, animals quickly traversed segment 1 and alternated at the first turn at a very high rate, 91%.

However, the animals often slightly slowed preceding turn 3 and executed less regular turn choice patterns across trials. That is, the alternation patterns at turn 1 were not strongly observed at turn 3 such that different orders of paths to goal locations 1-4 were observed (Figure 2DE). We therefore suggest that the trajectory dependence is strongly related to relative degree of separation of the behavioral patterns. In other words, the trajectory dependence appears to follow the navigational strategy and patterns of the animals' path selections in solving the task. Our data and associated hypotheses further predict that trajectory dependence should develop over learning and be more pronounced on tasks with more consistent transitions. We would predict that in our task, if path order was consistent, then trajectory dependence would appear for the locations leading into turn 3. If our theory is correct, it is also a potential indicator of the state of learning of the animal on a task.

Representational Scale, Alignment, & Partitioning of Maze Segments

SUB neurons are known to have larger firing field sizes than those of CA1 neurons in the open field and on a track (Sharp & Green, 1994; Kim et al., 2012). It has been theorized that this may be useful for information transfer, as individual neurons carry more information (Kim et al., 2012; Kintashi et al., 2020). Using a population correlation approach (Maurer et al., 2005) we have shown that the SUB population also encodes space at a larger scale than CA1 - a result that follows from the larger fields of individual neurons.

While larger scale was an expected finding, we also report that the scale of CA1 and SUB is dynamic, changing on different segments of the track. For larger track segments, the encoding expands such that similar patterns are observed across locations separated by larger distances. SUB carries a larger dynamic range than CA1 in this respect, but there appears to be parallel shifts in scale of representation between the two regions. This suggests a shared underlying cause. Differences in CA1 and CA3 scale are only known to exist across the longitudinal axis (septo-temporal axis) of the hippocampus (Maurer et al., 2005; Jung et al.,

1994; Kjelstrup et al., 2008; Royer et al., 2010). This has been theorized to be due to a differential relationship between place fields and running speed across the longitudinal axis (Maurer et al., 2005). We therefore hypothesize that the increased running speed on longer track sections may be responsible for the dynamic scaling reported here, but emphasize that this influence can apply to the same population of neurons as opposed to expression only across the longitudinal axis.

We also examined the locations at which spatial firing patterns in SUB and CA1 shift. From these analyses, we find that CA1 and SUB population firing patterns partition the environment in different ways with SUB population shifts biased to the locations of path intersections. The partitioning we observe is reminiscent of “chunking”, a proposed psychological phenomenon where information is grouped into intuitive components to facilitate memory (Miller, 1956). In our data, there are transition points of low representational scale separating areas of large scale. Before and after these transition points, SUB and CA1 patterns may persist across contiguous locations along the track. We note that, in such cases, the patterns preceding and following a transition point are different, and that this, in part, defines the transition point itself. Previous research has suggested SUB neurons’ activity results in a schematized chunking of space (Olson et al., 2017; Lee et al., 2018), and our results largely support this interpretation. Gupta et al. (2012) previously described similar dynamics in chunking for CA1 populations relative to inflection points in the animals behavior (e.g., turns and stopping points for reward). Our results complement their findings, showing that the activity patterns segmenting space in this fashion are prevalent even at a population level, occur with less frequency for SUB than for CA1, and, as stated, are biased to path intersections for SUB.

If chunking is indeed the outcome of spatially organized, non-continuous shifts in spatial representation, then an important question is how the information (space) is actually being grouped. This may lend insight into processing of the task and space. Instead of presupposing

the locations, we applied an unbiased analysis of all potential locations for transition between representational partitions (or “chunks”). Perhaps surprisingly, we found the transition locations were not the same for CA1 and SUB. SUB transition points near shifts between path segments (i.e., at corners) were consistent with the importance of orientation previously discussed. CA1 transitions, on the other hand, often occurred within individual path segments, for example, the halfway point along segment 1 of outbound paths. These results may hint at the different functions of the two hippocampal outputs. The SUB, with its more orientation-based partitioning of maze space, may be more specific to structural components of the full path network while for CA1 transition may more closely align to action sequences.

To report that SUB is both chunking space and representing analogous spaces may seem superficially contradictory, but in many ways, the two features are orthogonal. Analogous representations treat spatially separate locations similarly, while chunking groups locally contiguous space. Together, both encoding features function to assemble and associate particular kinds of places together. Indeed, progress along a learned route of a particular shape, irrespective of its location in an environment, is encoded independently of action in many posterior parietal and retrosplenial cortex neurons (Nitz, 2006; Alexander and Nitz, 2015). SUB activity patterns may thus contribute to such encoding through input patterns to retrosplenial cortex that recur for topologically similar, but spatially separated routes.

Encoding of Location

As with any set of neural analyses, the importance of the relative aspects of CA1 and SUB activity patterns depends on the transformation of information by downstream readers. Here, we have analyzed population activity using correlations of 0.5 as a baseline due to historical precedence (Maurer et al., 2005). While our results on representational scale, alignment, and chunking are robust and similar at a variety of thresholds between 0.3 and 0.7

(Supplemental Figure 2), what threshold is meaningful is ultimately determined by the neural networks receiving the signal.

The implications of what level of correlation is meaningful is especially important to SUB outputs, as differing levels will determine if SUB generalizes or separates locations sharing spatial variables. Odd/even trial population correlations at individual locations were extremely high in SUB and, if anything, higher than for CA1. The differences between these regions lies a step below the strong “place” correlations in population patterns for repeated visits to the same location while on the same trajectory. Thus, if downstream readers are extremely sensitive to small gradations in firing rates among a large population of neurons, many locations will therefore be differentiated and an encoding of location only may result. However, if output regions are less sensitive to variations in input patterns, the analogous locations will be read equivalently, and structural information will be the information transmitted. We cannot determine, as yet, whether structures such as retrosplenial cortex better resemble the location tuning of CA1 as opposed to the location and analogy encoding properties of SUB. However, we predict that population coding for different, but analogous locations in SUB often reaches values of 0.75 or higher, levels often considered adequate evidence for reliable encoding of individual locations (e.g., Maurer et al., 2005).

Anatomical Context

Considering the importance of downstream readers, it is worth considering outputs of SUB and their known activity patterns in light of these novel input signals from SUB. SUB projects to many regions considered important for spatial navigation. One particular region of interest may be retrosplenial cortex. Retrosplenial cortex primarily receives its “hippocampal” input from SUB (Jay & Witter, 1991) and, like SUB, has been shown to also represent conjunctions of spatial and movement variables (Alexander & Nitz, 2015). The conjunctions seen in RSC often combine allocentric with egocentric variables such as proximity to a border

and locations of turns (e.g. Alexander et al., 2019; Alexander and Nitz, 2015), an aspect not seen in SUB to date. This may further indicate that SUB acts in part as a step before retrosplenial cortex along the transformation of spatial information into action.

Finally, these results throw into doubt the idea that the activity patterns in SUB are best understood as a mere transformation of CA1. If this is not the case, it raises the question as to what the other key drivers of the activity in SUB are. Strong cortical inputs exist from entorhinal, perirhinal, and postrhinal cortices and subcortical inputs from nucleus reuniens, the medial septum, and anterior thalamus (Witter et al., 1990; Witter et al., 2000; O'Mara, 2006; Witter, 2006). Recent work has shown the importance of anterior thalamic inputs into SUB in guiding choice behavior at intersections. Temporary and permanent anterior thalamic lesions led to marked behavioral deficits and degraded spatial firing in SUB while CA1 place fields remain intact (Frost et al., 2020). Further research is needed into investigating the importance of these inputs for SUB function.

Limitations and Future Directions

While our task and data have brought to light many new interesting aspects of CA1 and SUB activity during spatial navigation, there were aspects of the experiment we would have liked to improve. We believe the Triple T maze is a strength of our design, allowing assessment of multiple spatial variables, their conjunctions, and the ability to assess generalization. That said, in order to more conclusively tease apart contributions of variables that are highly conjunctive, even more data and combinations are needed. Including all 8 reward locations and repeating the task in both directions would have moved us much closer to this goal, but this amount of data is largely infeasible in single recording sessions. Future work may need to record animals over multiple sessions in a day to collect the varied behaviors needed to better disentangle the forms of information represented in SUB.

Another limitation of our work is the anatomical distribution of our recordings. Most of our data was collected in proximal SUB. The proximal-distal gradient in SUB is well described and separates both activity patterns, sources of afferents, and projection targets (Naber & Witte, 1998; Kim et al., 2012; Aggleton & Christiansen, 2015; Cembrowski et al., 2018). It is of note that this data, while appearing extremely spatial, is in the region of SUB that largely projects to and from lateral entorhinal cortex, not medial. Conversely, the object vector cells recently discovered in SUB appear to have been located predominantly in distal SUB (Poulter et al., 2020). This leads us to speculate that the what/where division currently dominant in the field between lateral and medial entorhinal cortex may be a false dichotomy, and that in the context of more complex behaviors, lateral entorhinal cortex activity may be interpreted differently. Regardless, future work spanning the full proximal-distal extent of SUB on this or similar navigation tasks would be invaluable toward having a better understanding of the proximal-distal function of SUB for spatial navigation.

CONCLUSION

This work has studied the properties of neurons in dorsal CA1 and SUB while rats navigated a complex path network. We have found that both CA1 and SUB dynamically adapt their encoding properties over different phases of the task but chunk those spaces in different locations. SUB neurons showed a propensity for activity at analogous functional or structural locations on the track, and the SUB population often encoded these analogous locations similarly. The differences in specificity and generalization between these two hippocampal outputs are stark and suggest SUB and CA1 may play complementary roles. Differentiating small differences as seen in CA1 is crucial for memory of individual events, but grouping similar information as in SUB is vital for extrapolation and a holistic cognitive map. As such, we believe these results point to complementary roles for CA1 and SUB in episodic memory and navigation, and that these roles may expand more generally to episodic storage versus

consolidation of abstract information. We hope future work will further evaluate these two hippocampal outputs side by side to clarify their roles in navigation and memory.

METHODS

Subjects

Subjects were adult male Sprague-Dawley rats (N = 6). Rats were housed individually on a 12-h light/dark cycle. Prior to experimentation, animals were habituated to the colony room and handled for acclimation for 1-2 weeks. The rats were food restricted for motivational purposes at 85-95% of their free-fed weight. No water restriction occurred. All rats were required to reach a weight of 350g (5-10 months of age) before surgery and experimentation. All experimental protocols adhered to AALAC guidelines and were approved by UCSD IACUC and UCSD Animal Care Program.

Apparatus

All behavioral tasks were conducted on the “Triple-T” track maze. The Triple-T maze is a custom built environment made of black plastic with a running surface of a thin sheet of black rubber. This path-network is made of tracks that are 8cm in width. The overall dimensions of the environment are 1.6m x 1.25m and are elevated 20cm from the ground. Track edges are approximately 2cm in height, allowing the rat an unobstructed view to distal cues. Taller 10cm walls were included at internal sides of corners and to block potential shortcuts across small gaps between tracks near the reward locations.

Behavior

Rats were habituated to the Triple T maze with two 30 minute epochs of free exploration with rewards scattered throughout the maze. Animals were then trained to conduct outbound routes, traversing from the midpoint of one of the long edges of the maze (Figure 1A, location of

rat), through the center space, to one of the four reward locations (Figure 1A, colored dots). The outbound routes from the start to the reward locations consisted of straight paths interleaved with 3 left or right turns. The first and third turn directions were choice points but the second turn was forced. Total outbound route lengths were 140cm with turns at 51cm, 87cm, and 118cm. Rewards (1/4 - 1/2 piece of Cheerios cereal) were manually delivered at the reward sites. Animals were trained to return to the start location via the outside paths after completion of an outbound route. We refer to these behaviors throughout as return routes. Animals were trained over 1-2 weeks to make these route traversals and to do so without stopping mid-route. At this point in training, we implemented the visit-all working memory task to be used throughout experimentation. The animals were rewarded at any of the four locations, but needed to visit all four locations before rewards were again available at previously visited reward locations. Over at least 2 additional weeks, animals were trained by simple trial and error to a criterion of 80% for ballistic (uninterrupted) path traversals, regardless of reward performance. Only after this level of running performance were animals surgically implanted for experimentation.

Surgery

Rats were surgically implanted with 1-3 tetrode (twisted sets of four 12μm polyimide-insulated tungsten or nichrome wires) arrays integrated into custom microdrives. Each microdrive held four to twelve tetrodes. Under isoflurane anesthesia, animals were positioned in a stereotaxic device (Kopf Instruments). Following craniotomy and resection of dura mater, microdrives were implanted. Microdrives were implanted relative to bregma with targeting coordinates consistent across animals. SUB: A/P −5.6 to −6.6 mm, M/L ±1.6 to ±2.7 mm; dorsal CA1: A/P −3.8mm, M/L ±2.3mm.

Neural and Behavioral Recordings

Following a week for recovery from surgery, animals were retrained for at least 1 week before beginning recordings. This ensured adequate behavior and running ability with the new weight of the implant. Electrodes were moved ventrally in 40μm increments between most recordings to maximize the number of distinct units collected for each animal. Each microdrive had 1-3 electrical interface boards (EIB-16, Neuralynx) connected to a single amplifying headstage (20x, Triangle Biosystems). The headstages were tethered to a set of preamplifiers (50x) and a high pass filter (>150 Hz). These signals were input in the acquisition computer running Plexon SortClient software. Signals were recorded after digital filtering at 0.45–9 kHz, 1–15x amplification (to reach a total of 1,000–15,000x amplification of the signal), and 40 kHz digitization. Waveforms from single units were isolated in Plexon OfflineSorter software. Waveform parameters used were peak height, peak valley, energy, average voltage, 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 only partial spiking data of neurons. Waveform amplitudes were monitored to ensure systematic fluctuation did not result in confounds for isolating single units. Recordings typically lasted 30-60 minutes.

After single unit isolation was complete, cluster quality was quantified using a modified isolation distance score as described in Olson et al. (2017, Figure 2B). Briefly, isolation distance (Harris et al., 2000) measures the cluster separation using the Mahalanobis distance between the cluster center and the nth closest noncluster spike, where n is the number of spikes in the cluster. The units of this are equal to cluster variance. Equivalently, isolation distance is the radius of the cluster center to the circle containing double the number of spikes as actually classified in the cluster. This is normalized by the cluster variance. As such, this measure is undefined when the spikes recorded is not at least double the spikes included in the cluster. To avoid this issue, Olson et al (2017) adapted this measure to be the minimum of the isolation

distance as defined by Harris et al. and the distance to the noncluster spike 20% into the noncluster spike distance distribution. This modification defines the value for all neurons and reduces the isolation distance for clusters with many spikes. This modification makes the criteria stricter, but this conservative adjustment more accurately represents cluster quality for tetrodes with one high firing neuron. Isolation distance is not a criterion used for excluding units in this study. Instead, it is presented here to show the high quality of neurons identified.

Animals' positions were tracked during neural recordings using a camera located 2.6m above the recording room floor. Plexon CinePlex Studio software detected two LED lights on the animal's surgical implant separated by approximately 5cm. Location tracking was captured at 60Hz. At any given time point, position and orientation were determined using the average location of the two lights and the orientation of the vector between the lights. All animal movement data such as location, head direction, and derivatives are calculated from these values.

We recorded a total of 573 subiculum and 401 CA1 neurons from 6 rats. Relatively inactive neurons were excluded from analysis if their activity never averaged at least 3 spikes/sec at any location on the maze. Interneurons were attempted to be excluded from analysis by removing neurons that averaged more than 3 spikes/sec at all locations on the maze. After exclusions, our dataset consisted of 480 subiculum and 298 CA1 neurons.

Histology

Animals were perfused with 4% paraformaldehyde under deep anesthesia. Brains were extracted and sliced into 30μm - 50μm sections. Slices were Nissl stained to reveal the final depth of electrodes. Microdrive movement was used to reconstruct recording-depth profiles. These data were then compared to known anatomical boundaries of the regions of interest (Paxinos & Watson, 2006) to establish a final categorization of the source of each microdrive.

Filtering of Behavior for Fluid Route Traversals

To limit variation in behavior at each location, we only analyzed data for when the animal was performing a smooth, uninterrupted traversal of an entire route. We refer to these smooth traversals as uninterrupted trials. This permitted us to examine action potential firing rate data associated with stereotypical movements (forward running, turning) through all sections of all routes. The defined routes were the four internal outbound routes toward potential reward sites and the two return routes leading back to the internal entrance (Figure 1D).

A multistep process using custom MATLAB graphical user interfaces was used to identify uninterrupted traversals of each route. Users defined starting and ending gates for each route. The program then selects all trials crossing these locations with sustained running speeds of 3cm/s or greater throughout. Finally, a researcher verifies all identified trials did not contain either obvious interruptions in locomotion or significant deviation from the stereotyped path for that route (Figure 1D). This method separates stalled track traversals, reward periods, and behavior between trials from controlled action and spatial data. Individual recordings were excluded from analysis if the number of traversals on each internal route did not exceed 3.

2D Positional Firing Rate Maps

To assess activity as a function of 2D space, we calculated individual neurons' positional firing rates by dividing the total number of spikes at each location by the total occupancy time at that location. If only showing identified routes, only data identified as during an uninterrupted trial was used. Positional firing maps were smoothed using a 2D convolution with a Gaussian filter with s.d. of 1cm that also accounts for bins with no occupancy (Kraus, 2013).

Spatial Statistics

Many spatial statistics have been previously defined to summarize the spatially selective nature of neuron firing. First, we created 2D positional firing rate maps using 1cm square bins with no smoothing. We then calculated spatial information in bits per spike (Skaggs et al., 1993),

$$Spatial\ Information = \sum_{i}^{N} p_i \frac{\lambda_i}{\lambda} \log_2 \frac{\lambda_i}{\lambda}\ \text{bits/spike}$$

where i is each bin, pi is the occupancy of bin i, λi is the mean firing rate of bin i, and λ is the overall mean firing rate. Spatial selectivity (Skaggs et al., 1996) is large for neurons with firing fields that are a small portion of the environment and is simply the ratio of the max firing bin divided by the overall mean firing. Finally, spatial coherence (Kubie & Muller, 1989) assesses continuity of signals and is defined as the z-transform of the spatial autocorrelation between each bin and the average of its immediate neighbors. Here we used a Spearman correlation as the correlation and do not transform into Z coordinates.

Linearized Firing Rate Maps

We also aligned neural activity to progression through each of the identified routes. For each recording, custom MATLAB software is utilized to generate a spatial template matching the average trajectory of the animal along each route in the horizontal (2D) plane. This approach ensures the best possible matching of animal behavior and positions taken across recordings and trials. Position samples included in the identified ballistic route traversals were mapped to the nearest template bins. Then the linearized firing is found by dividing the total number of spikes at each location by the total occupancy time at that location. Linearized firing maps were smoothed using a 1D convolution with a Gaussian filter with s.d. of 1cm that also accounts for bins with no occupancy (Kraus, 2013). The linearized firing rate is then averaged over all traversals the animal made for that recording to calculate the mean linearized firing rate.

Individual Neuron Analogous Route Correlations

To assess individual neuron's similarity of representation across routes, a pairwise Pearson's correlation was calculated for each neuron between each outbound route and each other outbound route. The calculation was on the vector of mean linearized firing rates between the non-overlapping portions of the routes. The same analysis was then applied to the two return routes. The maximum correlation of all correlations was used for the population analysis, as the sparse nature of activity in HPC and SUB renders most values undefined or near-zero. Of interest is whether any high correlations exist among any of the comparisons.

Population Analogous Route Correlations and Correlation Matrices

Population-wide similarity of route representations were also taken. For each position along the linearized track space, individual neurons' mean firing rates were appended to create a population firing rate vector. Then, Pearson's correlations were calculated of the population firing rate vectors of two locations of interest. Locations chosen include locations with similar but spatially separated locations or functions with respect to the trained behavior (i.e. two return routes, or close to the reward locations). If more than two analogous locations existed, pairwise correlations were calculated and then averaged across all pairwise combinations.

As a control, the linearized firing rates for each neuron were split into odd and even traversals. For each neuron a new mean firing rate was calculated from the odd/even traversals and used to create two population firing rate vectors from non-overlapping trials. Correlations of the same space from these two odd and even vectors show the reliability of the signal and serve as a control for the limit of possible correlation given the consistency patterns of the population.

Correlation matrices were also constructed to visualize the relationship of each pairwise set of positions across the linearized track space. As specified above, the linearized firing rates for each neuron were split into odd and even traversals. For each neuron a new mean firing rate

was calculated from the odd/even traversals. For each pairwise combination of positions, the population vector of odd traversal mean firing rates was correlated with the population vector of even traversal mean firing rates. By splitting the trials into non-overlapping sets and correlating these values, the diagonal value of the correlation matrix reflects a form of reliability of population activity for each position.

Population Representational Scale, Alignment, and Transition Points

The correlation matrices exhibit where and how similar population neural activity patterns are across the maze. We developed new and adapted old metrics to quantify some of these patterns.

Representational scale was defined as the space surrounding a location that correlates above a given threshold with that location. This groups contiguous, similar space both in front of and behind the location. We used 0.5 as the correlation threshold, consistent with historical precedent (Maurer et al., 2005), for this analysis and its derivative analyses below, but a wide array of values resulted in similar results (Supplemental Figure 2). Representational alignment was operationalized as the difference in the amount of highly correlated space before and after the current location. More correlated space in front of the location is positive, while more space behind is negative. The derivative of the representational alignment shows how the alignment changes across the track space. The peaks of the derivative, termed the transition points, are points where the correlations move from backwards looking to forwards looking, indicating a separation in the representation of nearby locations.

Representational Analyses Shifted Population Bootstrap

To assess the magnitude and validity of the fluctuations of our newly defined representational scale and alignment metrics, we created a bootstrapped distribution for comparison. This distribution was created by appending all of the routes linearized firing rates,

as done in the correlation matrix, then shifting the mean firing rates of individual neurons independently to create a new population where local neural activity field characteristics remain, but population level neural field distributions are random and unassociated with track features. New correlation matrices were created from this shifted population and all representational analyses were conducted. This procedure was repeated 1000 times, and 1st and 99th percentiles of the results were used as statistical comparisons for results expected by similar neurons with similar field properties by chance.

Statistical Tests

Nonparametric tests were used throughout to avoid assumptions of normality in the data. The Mann Whitney U test was used to evaluate the statistical significance of behavior to chance and differences between SUB and CA1 across the different analyses, as well as comparisons of SUB and CA1 to odd trial or even trial control populations. The Kolmogorov-Smirnov test was used to assess if pairwise distributions of correlations from SUB and CA1 were significantly different. Representational scale, alignment, and transition points were compared to the 99th percentiles of a population created by bootstrapping the shifted linearized firing rates from the actual neural population. Circular median tests were used to compare CA1 and SUB transition points to the turn locations. No statistical methods were used to predetermine sample sizes. However, based on similar sample sizes reported in previous publications, we believe we have adequate power (0.8) or greater to detect significant effects.

Data and Code Availability

The data collected and analyzed in this study as well as the code used in all analyses are available from the corresponding author upon reasonable request.

Acknowledgements

Chapter 2, in full, is a reprint of the material in the preprint manuscript: Johnson, A. B., Olson, J. M., Chang, L., Tao, E. L., Wang, X., Nitz, D. A. Complementary Maps for Location and Environmental Structure in CA1 and Subiculum *BioRXiv* (2021). The dissertation author was the primary investigator and author of this paper.

Figures

(Continued on next page)

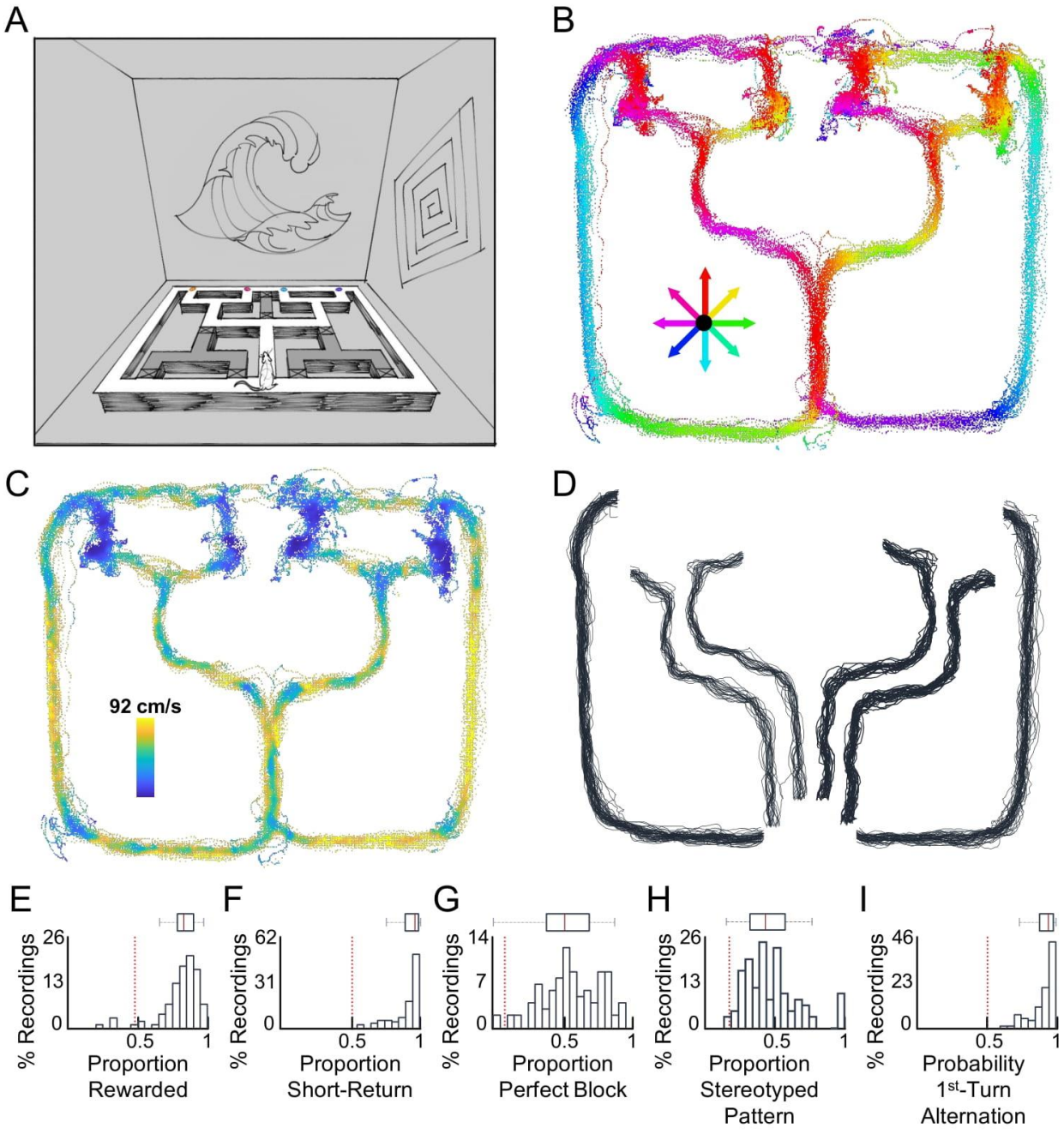


FIGURE 2.1: Robust Navigation of a Complex Environment

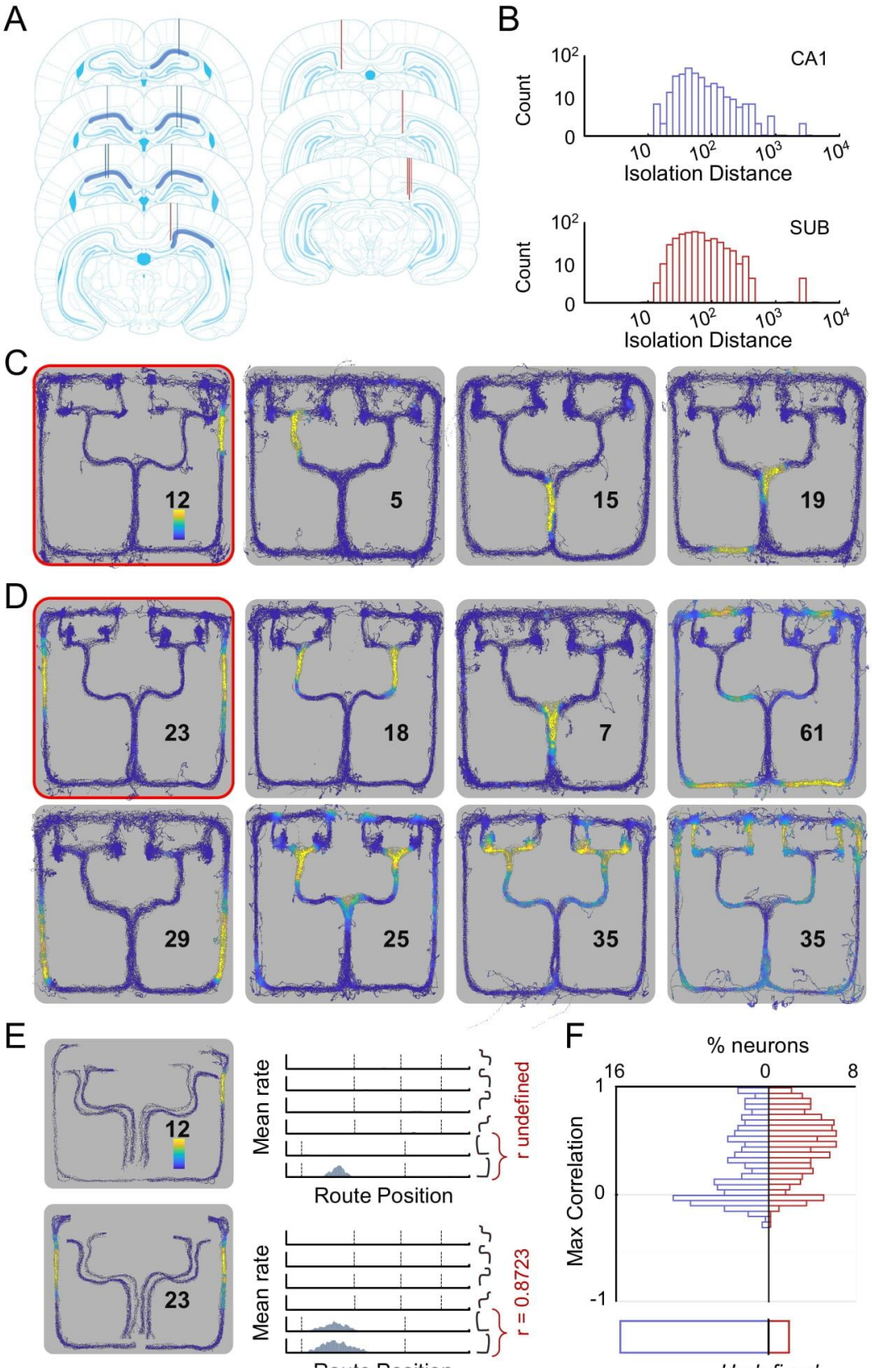


FIGURE 2.2: Individual Subiculum Neurons Are Active in Analogous Spaces

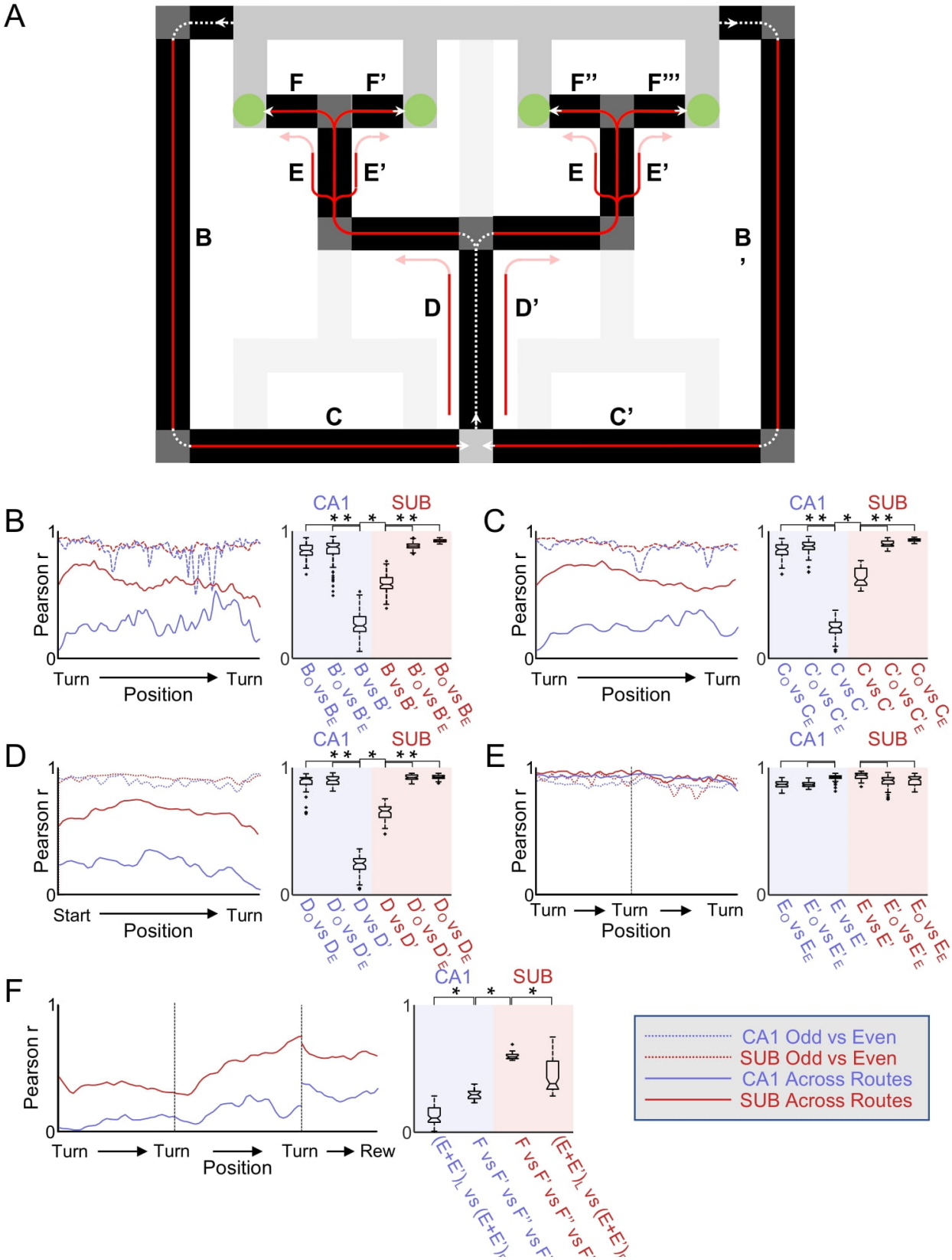


FIGURE 2.3: Analogous Responses in Subiculum Include

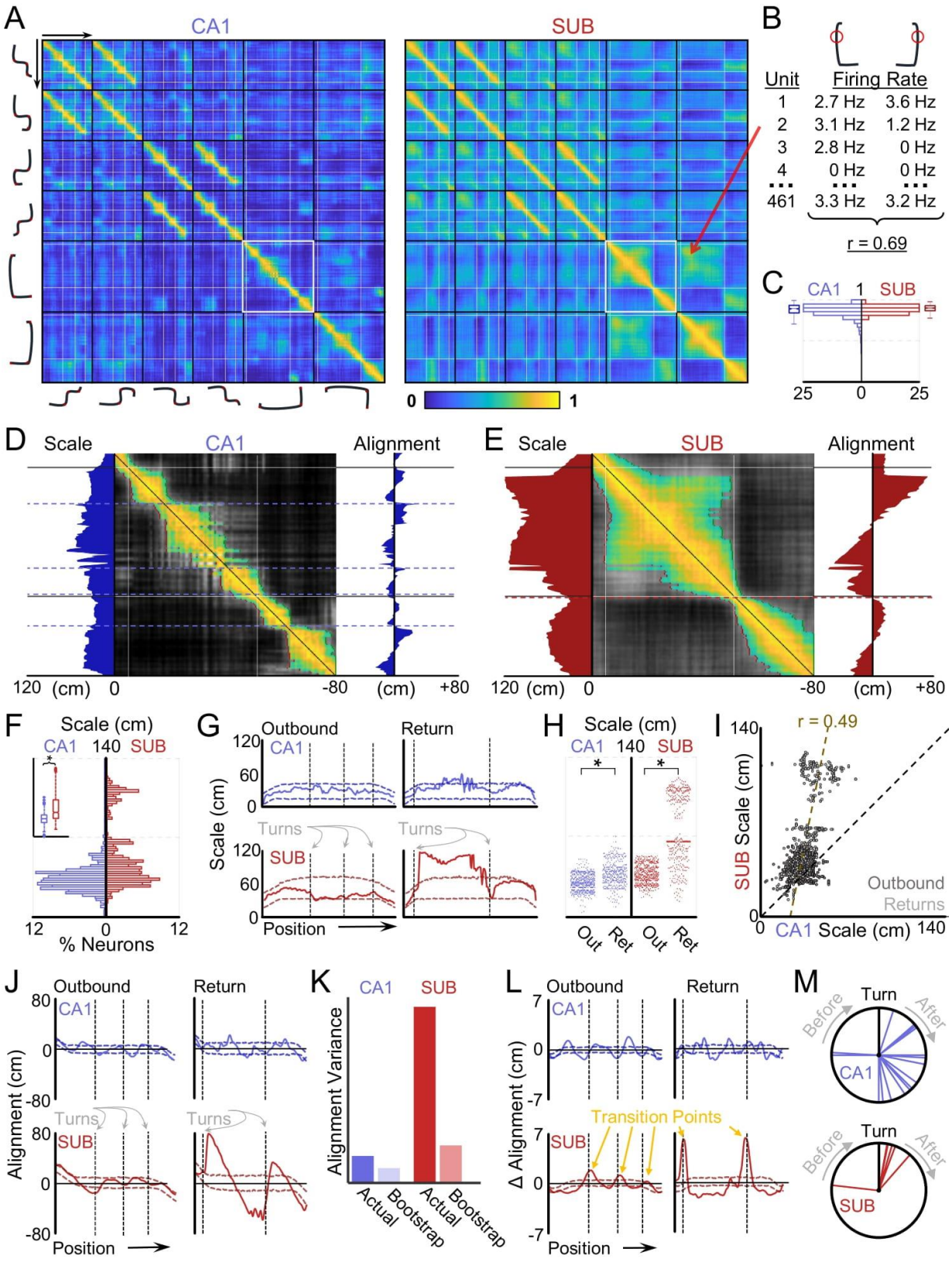
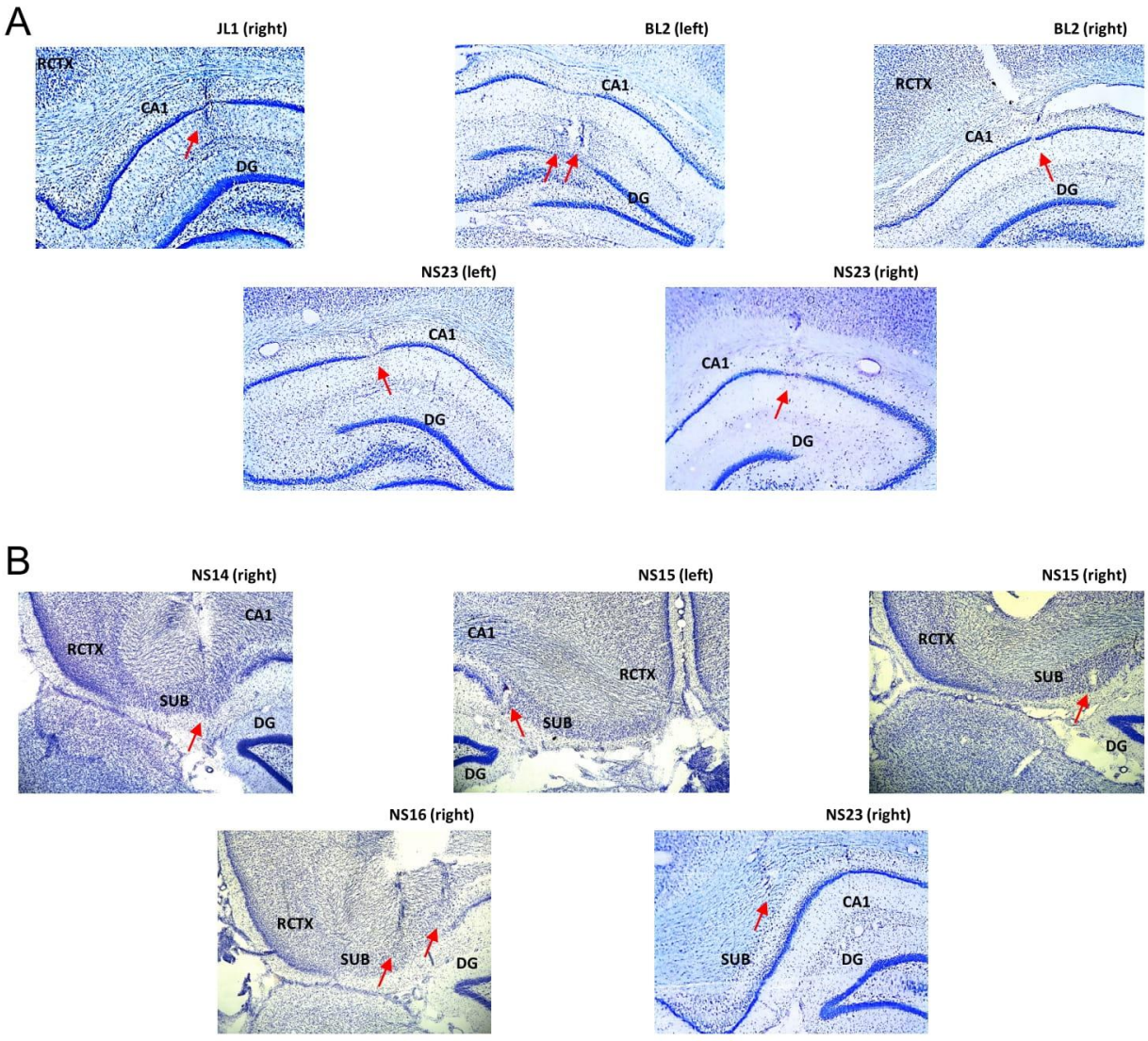
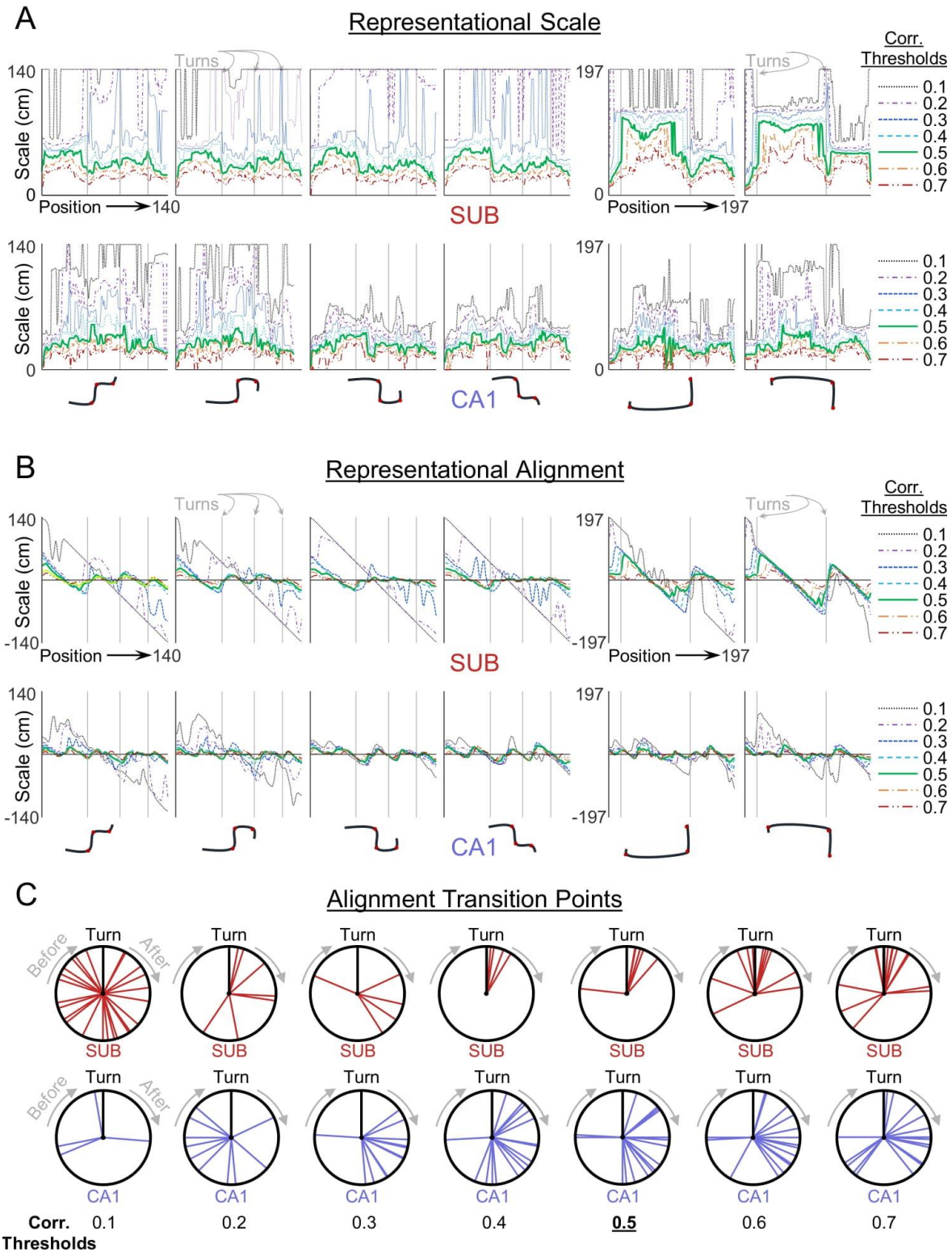


FIGURE 2.4: Subiculum and CA1 Populations Chunk Epochs at Different Locations



SUPPLEMENTAL FIGURE 2.1: Summary of Recording Site Histological Data



SUPPLEMENTAL FIGURE 2.2: Representational Scale, Alignment and Transition Points are Consistent across a Wide Range of Correlation Threshold Values

Figure Legends

Figure 1: Robust Navigation of a Complex Environment

A) Illustration of Triple-T navigational task recording environment. Animals begin at a starting point (center-bottom) and must navigate a three-turn sequence to arrive at one of four different reward locations. In the visit-all-4 reward schedule, rats must visit each of the four locations before revisiting any reward location. **B**) Behavioral tracking from a full sample recording on the Triple-T maze. Positional data is color-coded showing the animal's average head direction at each position. **C**) Behavioral tracking, as in B, but with color coding showing average velocity, from 0 in blue to the max, 92cm/s, in yellow. **D**) Behavioral tracking of identified routes. Shown are all data labeled as uninterrupted runs from the same recording session in the visit-all-4 reward schedule. In this setup, four outbound routes begin at the start point and end at four different reward locations. Two external routes are also defined encapsulating the paths from the rewards to the starting location. Routes are minimally translated and stretched for visualization purposes. **E-I**) Box plots of per behavioral session performance metrics (red bar: median; box limits: first and third quartiles; whiskers: range of non-outlier data points). In each, the dotted red line represents chance. **E**) Proportion of rewarded runs. **F**) Proportion of shorter return routes taken. **G**) Proportion of visit-all-4 reward blocks without a mistake. **H**) Proportion of the most commonly run block pattern from each recording. **I**) Proportion of left/right alternations at the first choice point.

Figure 2: Individual Subiculum Neurons Are Active in Analogous Spaces

A) Electrode placement in CA1 (blue, 3 rats) and SUB (red, 4 rats). Lines end at the terminal identified locations of tetrode bundle tracks. **B**) Isolation distance waveform discrimination quality metric for both CA1 (top) and SUB (bottom). **C**) Positional firing rate maps of example CA1 neurons, color mapped from 0 (blue) to the mean + 3 s.d. (yellow) for each neuron. The

max value is written in each map. **D**) Positional firing rate maps of example SUB neurons, colormapped as in C. **E**) Left: Two dimensional spatial maps of mean firing rates for individual neurons highlighted in C and D, mapped as a function of route and track position. Like Figure 1C, routes are minimally translated and stretched from the actual track location to separate each map for visualization purposes. Colormap scaling is identical to C. Right: Linearized mean firing rates as a function of routes for the corresponding neurons on the left. The routes for each graph are depicted on the right. Pearson correlations of the two return routes are shown. When one route has no activity, correlations are undefined (top). **G**) Population histogram of maximum pairwise correlations of routes for individual cells. If only one route had activity, the correlation was undefined.

Figure 3: Analogous Responses in Subiculum Include Decreased Trajectory Dependence

A) Schematic of Triple-T maze. Highlighted regions are analyzed in the following panels corresponding with the labels. White dotted lines show animal routes, with solid red portions indicating regions analyzed and shown in this figure. **B-F**) Left: Pearson correlations of population mean firing vectors, and Right: box plots of the Pearson correlation distributions. In all panels, CA1 data is blue and SUB is red. Solid lines are across route segment comparisons. Dotted lines are odd versus even correlations within one group. For all box plots, black bar: median; notch: comparison interval at 5% level; box limits: first and third quartiles; whiskers: range of non-outlier data points; +: outliers). Subscript O is for odd trial population data, while E for even trial population data. * denotes Mann Whitney U test with $P < 0.05$. B) CA1 and SUB population correlations between the long straight segments of the two return routes. **C**) CA1 and SUB population correlations between the final straight segments of the two return routes. **D**) CA1 and SUB population correlations of the center stem split according to upcoming turn direction. **E**) CA1 and SUB population correlations of the two straight segments preceding the

final outbound turn, split by upcoming turn direction. **F**) CA1 and SUB population correlations of non-overlapping outbound run segments.

Figure 4: Subiculum and CA1 Populations Chunk Epochs at Different Locations

A) Odd versus even trials population correlation matrices for CA1 (left) and SUB (right). The vector of mean firing rates for all recorded cells in the given region are correlated across each pairwise position and route combination. High correlations (yellow) indicate similar encoding of the two spaces. The routes that are linearized are depicted next to the corresponding rows/columns. Dotted white lines indicate turn apexes. **B**) Example population correlation calculation. The matrix value indicated by the red arrow on A is the correlation of the odd or even mean firing rates from all neurons at the corresponding route and position locations. Here, it is two corresponding locations from the two return routes. The color mapped value is the Pearson correlation of these two ensembles. **C**) Histograms of odd versus even trials population correlations for the same locations. This corresponds to the diagonal values in the population correlation matrices. **D-E**) Representational scale and alignment examples. **D**) Center: Subsection of population correlation matrix highlighted in A. Contiguous row-wise off-diagonal values above 0.5 are mapped to color on the same scale from A. The remainder of the submatrix is mapped black to white. The width of the above threshold region (representational scale) is shown on the left along the route space. The difference in forward/backward extent of the above threshold region (representational alignment) is shown on the right. Scales are in cm. Black lines show turn apexes, while dotted blue lines show segmentation transition points. **E**) Same as D, but for SUB. Chunking edge lines are in red not blue. **F**) Histogram of representational scale of CA1 (left, blue) and SUB (right, red). Inset: box plots of same data (bar: median; notch: comparison interval at 5% level; box limits: first and third quartiles; whiskers: range of non-outlier data points; +: outliers). * denotes Kolmogorov-Smirnov test with $P < 0.05$. **G**) Mean representational scale for the outbound (left) and return (right) runs for both

CA1 (top) and SUB (bottom). Dotted lines indicate bootstrapped 1st/99th percentile thresholds. **H**) Representational scale distributions for outbound and return routes. * denotes Kolmogorov-Smirnov test with $P < 0.05$. **I**) Scatterplot of CA1 (x axis) versus SUB (y axis) representational scale at identical locations for outbound (dark grey) and return (grey) routes. **J**) Mean representational alignment for the outbound (left) and return (right) runs for both CA1 (top) and SUB (bottom). Dotted lines indicate bootstrapped 1st/99th percentile thresholds. **K**) Variance in representational alignment for CA1 and SUB compared to bootstrapped 99th percentile. **L**) Mean derivative of the representational alignment for the outbound (left) and return (right) runs for both CA1 (top) and SUB (bottom). Dotted lines indicate bootstrapped 1st/99th percentile thresholds. Peaks (transition points) are locations of maximum change from reverse to forward representational alignment. **M**) Distributions of transition point distances relative to nearest turn apexes for CA1 (top) and SUB (bottom).

Supplemental Figure 1: Summary of recording site histological data.

A) Recordings of CA1 neurons (N = 401) were obtained from a total of six four-tetrode bundles in three animals. Numbers of total recorded neurons and numbers of neurons included above each figure. Red arrows depict tracks left by the bundles and their approximate endpoints. All of the recording sites were restricted to the dorsal CA1 while one (BL2-right) was additionally moved ventral to DG after main-experiment (DG data not included). B) Recordings of SUB neurons (N = 573) were obtained from a total of six four-tetrode bundles in four animals. Red arrows depict tracks left by the bundles and their approximate endpoints. Three of the recording sites were restricted to the SUB while three (NS15-left, the lateral bundle in NS16-right, and NS23-right) were in a transition zone bordering the CA1 sub-region. Abbreviations: RCTX (retrosplenial cortex), DG (dentate gyrus), SUB (subiculum)

Supplemental Figure 2: Representational Scale, Alignment and Transition Points are Consistent across a Wide Range of Correlation Threshold Values

A) Representational scale as a function of correlation thresholds for each of the four outbound and two return runs. Top Row: SUB population. Bottom Row: CA1 population. **B**) Representational alignment as a function of correlation thresholds for each of the four outbound and two return runs. Top Row: SUB population. Bottom Row: CA1 population. **C**) Representational alignment transition points as a function of correlation thresholds. Top Row: SUB population. Bottom Row: CA1 population.

Works Cited

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CHAPTER 3: Self-Motion Independent Mapping of Route Structure in Parietal Cortex

Abstract

Posterior parietal cortex (PPC) has been the target of neuroscientists for decades seeking to uncover its role in cognitive processes. Evidence from humans, non-human primates, and rats all have demonstrated PPC to be involved with the perception of space across many frames of reference defined by the animal's body (egocentric), and the environment itself (allocentric). This remains true across many species and rodent studies in particular afford neuroscience a flexibility in experimental design that is unattainable through other model subjects in that rat nervous systems can be recorded as the subject moved around relatively unrestrained. Rodent studies have demonstrated that the shapes of routes as defined by the specific self-motion sequence undertaken is a powerful modulator of PPC neuron activity in rats. It remains unknown to what extent space beyond self-motion is represented in the activity of PPC neurons. We studied the activity of PPC neurons on a structured path-network to investigate the role allocentric environmental structure has on individual neuron activity. We describe a population of parietal cortex neurons that exhibit activity patterns consistent with the encoding of structure of a route for instances when self-motion sequences are exactly opposite one another.

Main Text

INTRODUCTION

Moving across space, whether it is tapping a single finger onto a keyboard or locomoting the entire body across a field requires an appreciation of the space which one is moving at the appropriate level on which one is moving. Our awareness of these spaces comes from our nervous system's ability to associate relevant sensory stimuli together to mentally create relevant frames of reference on which our attention can be placed. Considering the space

between our hands and the keyboard as one frame of reference allows for decision making processes to rapidly calculate efficient pathways between the hands and each key which needs to be pressed, the shapes of these pathways having unique meaning within that frame of reference. Within the brain many regions of sensory cortex exhibit spatial responses with regard to the location of a stimulus relative position to the body (Holmes, 1918; Penfield & Boldrey, 1937; Montero & Torrealba, 1973). Association cortices, like posterior parietal cortex (PPC), integrate activity across many different sensory modalities (Krieg, 1946; Jones & Powell, 1970; Rushworth et al., 2005) and demonstrate neural responses which discriminate spatial location of sensation across many species (Gottlieb et al., 1998; Connolly et al., 2003; Merriam et al., 2003; Nitz, 2006; Bremmer et al., 2013). These responses are seen to manifest in a variety of ways across studies and species. The inherent multimodal nature of PPC, in one way suggests that it should be expected to find neurons that have their activity modulated by the specific experimental task being used.

PPC is densely interconnected with other regions of cortex which process visual information (Miller & Vogt, 1984; Montero, 1993), vestibular information (Guldin & Grüsser, 1992), somatosensory information (Burton, 1986), and other association cortices (Whitlock, 2008; Agster & Burwell, 2009). Of particular interest is the anatomical junction PPC is placed at within a circuit connecting efferent information flow of the hippocampal formation to motor cortices (Yamawaki, et al. 2016), along what could be described as a space-to-action pathway (Olson et al., 2019). This anatomical consideration would place PPC as a mediator for transforming spatial information from the hippocampus into a usable motor command by secondary motor (M2) and primary motor (M1) cortices. This framework with which to interpret rodent PPC data aligns with many previous studies investigating PPC function in other animals.

Clues to the general function of PPC in humans historically has come from lesion studies. Lesions to this region of the cortex create profound deficits in everyday life with regard

to working memory, spatial navigation, motor planning, attention, and perception (Holmes, 1918; Holmes & Horrax, 1919; Brain, 1941; Denny-Brown et al., 1952; Critchley, 1962, Levine & Mohr, 1978; Damasio & Benton, 1979; Bisiach et al, 1979). These deficits are rarely confined to a single sensory modality but rather seem to impact many sensory and motor systems. This mapping across reference frames can also been seen in neglect syndrome of human patients where the patients seem to lose their perception or attention to the side of an object across many frames of reference such as their own body (Bisiach et al., 1979) or an image they are tasked with paying attention to (Heilman & Valenstein, 1979).

Findings from human patients accompany studies performed using neurophysiological recordings in nonhuman primates which have shown direct involvement of PPC neurons in motor planning (Cui & Andersen, 2011), working memory (Chafee & Goldman-Rakic, 1998), and what has been described as a mapping of space for the intention to move some effector to (Andersen & Buneo, 2002). The specific cognitive processes being probed, as well as the tasks being performed, vary significantly across studies. Consistently PPC has been seen to involve itself with the perception of space and spatial relationships. These studies have helped imagine PPC as a region of the brain tasked with mapping one set of coordinates defined by one or several sensory modalities onto another set of coordinates which can be utilized by motor systems (Pouget & Sejnowski, 1997).

Based on anatomical homology with primates (Krieg, 1946) it has been theorized that PPC in the rodent brain should also have a critical function in spatial processing, particularly for attention and working memory processes (Corwin & Reep, 1998; Reep & Corwin, 2009). Lesion studies in rats have shown distinct motor deficits (Kolb & Walkey, 1987), perceptual memory deficits (Chiba et al., 2002), path integration deficits (Save et al., 2001), and route planning deficits (Kolb et al., 1994). One benefit to working with rodents as the previous studies do, is the relative flexibility in experimental design. Neurophysiology studies in rodents more so than in

primates are poised to incorporate interesting and meaningful spatial relationships into experiment design. Indeed studies directly investigating the spatial properties of rat PPC neuron activity have found encoding of route shape through comparing activity of neurons on equivalently shaped routes (Nitz, 2006). And various route-structures have been demonstrated as viable frames of reference to explain activity profiles (Nitz, 2009; Nitz, 2012).

Alongside studies outlining the theoretical understanding of PPC in animals as an area of spatial sensitivity are a number of studies that describe PPC neuron activity with regard to self-motion (Kawano et al., 1980; Andersen & Mountcastle, 1983; McNaughton et al., 1994; Wilber et al., 2014; Sasaki et al. 2020). These self-motion responses could be expected considering PPC is connected to cortical regions, RSC and M2, which also have large populations of neurons modulated by self-motion (Alexander & Nitz, 2015; Olson et al., 2019). These self-motion representations and how they are modulated in various contexts are central in many theories regarding the frames of reference along which PPC is uniquely sensitive to (Save & Poucet, 2000; Cohen & Andersen, 2002; Bicanski & Burgess, 2018).

From these theories on PPC integrating self-motion one could get the idea that the spatial frames of reference PPC neurons are sensitive to are restricted to being fundamentally egocentric. In many ways even studies into PPC responses to frames of reference such as route shape would suggest the shape being referenced needs to be equivalent in self-motion (Nitz, 2006). What has yet to be demonstrated is an explicit investigation into if spaces of similar structure but different, or opposite self-motion profiles elicit similar response profiles in PPC. If so this would suggest that PPC is capable of encoding space in a manner which is divorced from the commonly studied self-motion. It could be predicted, from previous studies, that self-motion in the form of linear and angular velocities dictate possible spatial coding in PPC. It could also be predicted that PPC neurons respond to structural similarity more generally as a frame of reference and thus will represent space in a self-motion independent fashion.

To better understand potential spatial firing properties we examined the neuron activity profiles of 236 rodent PPC neurons while rats (n=5) navigated on a path-network consisting of several interconnected pathways. The structure of the environment in addition to application of specific rules constituting a working memory task allow for an assessment of the animal's spatial knowledge based on how well they are able to utilize the appropriate routes. All the pathways shared a consistent structure of straight portions and 90° turns. The difference in shape for the routes comes from the combination of specific right-left turns that bring the animal to their destination. The first and third turn for each internal route consisted of a left/right decision-point which the animal could choose. This choice of environment allows investigation into how differently shaped pathways, which are similar in structural connectivity, influence PPC neuron activity. This is an important consideration to be made as structure is known to be encoded for in the spatial navigation system (Dabaghian et al., 2014; Johnson et al, 2021), and PPC is known to respond across several frames of reference with a bias for the particular shape of routes (Nitz, 2009; Nitz, 2012).

During periods of engaged task running we find a population of individual PPC neurons which remain significantly correlated in activity across periods of exact opposite self-motion sequences. At the population level we show significant variability across individual routes for the ability of self-motion to predict firing of neurons. Using the same method we demonstrate that PPC neurons highly correlated to structure of route are not tuned for self-motion, whereas PPC neurons exhibiting significantly negative correlations in activity patterns across routes of exact opposite self-motion profiles were significantly tuned for angular velocity. This research identifies a population of PPC neurons significantly tuned to a novel frame of reference; space beyond self-motion as defined through task-structure. This underlines the importance of task structure beyond what can be considered through self-motion similarity to be considered when

analyzing PPC datasets.

RESULTS

Animals Perform task very fluidly and engage with individual routes equivalently

We trained 5 male Sprague Dawley rats to navigate on a "Triple-T" structured path environment during the context of a working memory task (Johnson et al., 2021). The rats were trained to collect food reward at 4 specific locations (FIG 3.1A), and were only permitted a single direction of travel while navigating the internal pathways. Each pathway is defined by a unique action sequence of left or right turns and in that regard are very different from one another (Fig 3.1B), however all routes shared structural similarity in that the turn locations along each route located equivalently through each route (Fig 3.2C). The rats become very proficient in this task (FIG 3.1D) collecting a reward for about 84% of all traversals (s.d. = 6.88%) in a single recording. Animals frequently utilized a number of strategies including the use of the shorter return arm to return back to the main stem of the maze (Fig 3.1F mean = 0.9197 s.d. = 0.1075) alternation (FIG 3.1H mean = 0.8973 s.d. = 0.0916) at the first decision point, while not repeating a memorized pattern (Fig 3.1G mean = 0.4016 s.d. = 0.173).

Animals navigated at consistently high speeds (FIG 3.1I,J) and with consistent angular velocities (Fig 3.1K,L). The profiles of linear velocities were seen to be consistent across routes (Fig 3.1M top) whereas the profiles for angular velocities were seen to differ across route comparisons dramatically (Fig 3.1M bottom)

Individual PPC Neurons Variably Tuned to Self-Motion Across Routes

Frequently applied measures of activity and of calculating self-motion tuning were applied to each neuron. PPC neurons fired on average 5.6Hz during track running recordings (s.d. = 7.67Hz) (Fig 3.2B).

From the creation of spatial ratemaps many PPC neurons appeared to be strongly tuned to angular velocity on one or most of the triple-T's routes (Fig 3.2C). This was confirmed by GLM analyses demonstrating a significant decline in NMSE values for pGLMs lacking angular velocity (Fig 3.2D). Another population of PPC neurons had reliable activity profiles with equivalent strength of firing to their counterparts which were tuned to angular velocity. This second population of neurons however demonstrated no clear relationship between firing profiles and either linear nor angular velocities (Fig 3.2E). These neurons had characteristically elevated NMSE values and as expected were insensitive to pGLM analyses (Fig 3.2F).

The entire population of PPC neurons' GLM analyses exhibited a heterogeneity in accuracy across the different routes (Fig 3.2G). With minimum performance of cGLMs at mean NMSE = 0.9707 s.d.= 0.0337; correlation value = 0.1474 s.d. = 0.8696. Mean performance of cGLMs doing better at mean NMSE = 0.8408 s.d = 0.136; correlation value = 0.3214 s.d. = 0.1014. Maximum performance with mean NMSE = 0.6514 s.d. = 0.2496; correlation value = 0.4983 s.d. = 0.1424. This heterogeneity is reflected in pGLM accuracy across neurons showing performance for minimum impact pGLM LV NMSE = 0.7515 s.d. = 0.2663 correlation = 0.0659 s.d. = 0.0676; pGLM AV NMSE = 0.7107 s.d. = 0.2608 correlation = 0.0616 s.d. = 0.066 ; mean impact pGLM LV NMSE = 0.9004 s.d. = 0.1371 correlation = 0.2030 s.d. = 0.0959; pGLM AV NMSE = 0.8886 s.d. = 0.1366 correlation = 0.2216 s.d. = 0.0883 ; maximum impact LV pGLM NMSE = 0.9911 s.d. = 0.0184 correlation = 0.3740 s.d. = 0.1447; AV pGLM NMSE = 0.9919 s.d. = 0.0179 correlation = 0.4307 s.d. = 0.1461. Proportional change in NMSE and correlation are presented to give a better illustration of how scores vary across routes.

Identification of Environmental Structure Encoding in PPC

Using linearized rate vectors for each neurons we calculated the correlation value (Pearson's r) for each neuron across routes in a pairwise fashion. An example of a neuron with strong general route-route correlations is seen in Figure 3.3A (top). An example of a neuron with

generally negative correlation values with the exception of route comparisons hat share similar angular velocity profiles is seen in Fig 3.3A (bottom). We defined neurons as being significantly tuned to the structure of the environment on three specific examples: route 1 and route 4, route 2 and route 3, and the two return routes. Each of these comparisons highlight similarities in spatial structure while differing completely in angular velocity profiles (Fig 3.1M). For each comparison tuning was defined by neurons inter-route correlation being above or below the mean and 2 standard deviations for an equivalent distribution of correlation values coming from a collection of shuffled data. At the same time neurons needed to exhibit stable activity along the spatially defined route through the correlation of each route's data with itself split in an odd/even traversal fashion. Neurons that fell above or below two standard deviations for both criteria were selected out for further analyses (Fig 3.3B). In all three instances of oppositely shaped routes we found neurons that had significantly elevated correlation values (dubbed 'A' neurons in each instance). Not as surprising were the population of neurons, dubbed 'B' neurons, which responded with significantly negative correlation values for these path-comparison.

To test the relative strength of self-motion for the activity profiles within each selected A & B population a pGLM analysis was performed (Fig 3.3C). Neurons that were classified as 'A' for either their 1:4 correlation or their 4:1 correlation were included in the 'A' data on the left, a similar classification scheme was used for each 'A' and 'B' population throughout. The cumulative change in NMSE score was calculated for each population and tested against one another using a 2-tailed t-test. This revealed that 'A' and 'B' neurons consistently differed only with respect to their apparent angular velocity sensitivity with those in the 'B' classification regularly having elevated values compared to the 'A' population. A slight opposite trend could be seen for the population of 'A' and 'B' neurons for route comparison 1 & 4 when linear velocity is removed. This suggests that, consistent with previous reports (Nitz 2012), linear velocity may

still be important for modulating activity patterns across routes. Interestingly this trend does not occur for the comparison across the two return routes which share equivalently long pathways where high speeds are achieved by rats (Fig 3.1I).

DISCUSSION

PPC neurons, as recorded on the context of an environment with complex structure, are capable of encoding the general structure of a route beyond what can be described through self-motion. This sensitivity to structure presents itself on routes which share a general connectedness with the greater environment. This finding progresses our understanding of how allocentric frames of reference are construed within PPC neuron activity. PPC, while often studied with regard for self-motion defined space, is capable of spatial representations anchored to allocentric frames of reference relevant to route structure during spatial navigation. It could be expected that these spatial representations will be shown to be functionally related with spatial representations in connected cortical regions such as M2 (Olson et al., 2019) or RSC (Alexander & Nitz, 2015). In order to better examine how spatial representations in these regions differ tasks which afford sufficiently complex environments may be needed in order to provide a sufficient amount of frames of reference with which to consider the data. Even though neurons in M2, PPC, and RSC all are modulated by allocentric frames of reference it should be expected that the particular representations in each region will align to some frames of reference more than others depending on where it is recorded. These differences may be subtle and require multiple behavioral tasks to fully appreciate as well as seen previously that imposed structure on unrestrained running in the form of a laser to chase following a highly predictable pathway changes the temporal component of neuron activity (Alexander et al. 2020). With increased structure inherently comes with a restraint on the degrees of freedom with which an animal is allowed to move, complexity of the structure in the form of many paths interconnecting

is a relatively controlled mechanism to experimentally probe the relationship structure and complexity have on neural dynamics.

Previous studies have examined how activity profiles of PPC neurons reflect specific shapes of routes (Nitz, 2006; Nitz, 2009; Nitz, 2012). These ‘route-cells’ have been some of the purest forms of spatial representation described in PPC neurons. PPC neuron activity can reflect the specific shape of a route regardless of where, in space, that shape was ran. Finding that PPC neurons modulate responses along structural frames of reference such as route recurrence additionally gave tremendous insights into how structure can guide the frame of reference which PPC neuron activity is tuned to. From this an open question remained asking how the dynamics of PPC neurons would reflect routes that shared general features but were exact opposite in self-motion defined shape.

Structure of the environment, which is often discussed in this field as topology, is a known feature of the spatial navigation system which modulates the activity of neurons (Dabaghian et al., 2014; Rueckemann et al., 2021). However there has yet to be a comprehensive theory as to how the features of topology are encoded for in the first place. PPC neurons clearly demonstrate the ability to encode for positions along a structurally complex maze in a manner that corresponds to route position in a fashion which generalizes across routes of similar structure indifferent to the specific shape being dissimilar, a feature which may be construed as topology encoding by some.

Representations of structural similarity throughout the brain are beginning to be appreciated (Johnson et al., 2021). This posits shift in attention for neuroscience away from the classically defined ‘cell types’ which populate many regions of the spatial navigation system which respond to a very specific feature in the environment (O’Keefe, 1976; Taube et al., 1990; Lever et al., 2009). Many of these spatial representations, such as the head direction signal, occur in various forms throughout the spatial navigation system (Stackman & Taube, 1998;

Taube & Muller, 1998), work presented here suggests that at least for some of these regions redundant signaling may be attributed to the environment that is being utilized not being sufficiently complex to extract out all the frames of reference which are important to the spatial navigation system.

Finding that PPC neurons are capable of encoding routes of different shapes adds to a deeper understanding of the computational abilities of PPC neurons. These neurons as a population encode for self-motion as many other rodent studies have found (McNaughton et al., 1994), but do so in a dynamic fashion not well described by previous studies. PPC neurons are thought to integrate self-motion cues across time (Whitlock et al., 2012, Alexander et al., 2020), and this integration is proposed to be the base for PPC neurons ability to be tuned to various frames of reference (Save & Poucet, 2009). It will be an exciting advancement to see how timescales of integration are influenced by additional layer of structure during navigation and how that may serve as a foundation to the creation of the frames of reference observed in PPC.

What these routes do have in common most is the progression through each where deflections in self-motion occur. Seeing the space in this generalized frame of reference helps interpret the many examples of structurally-tuned PPC neurons which appeared to respond similarly to all internal routes in a fashion qualitatively different from the external routes (Fig 3.2 E). Many of these PPC neurons most likely had heterogeneous scores on the GLM decoding analysis for this reason; as a neuron tuned along this frame of reference might respond similarly for positions where self-motion is dissimilar, and may only respond on the internal or external routes in a reliable fashion.

The ability to utilize rodents as subjects in an experimentally flexible manner means that a variety of structural contexts ought to be able to be applied in researching PPC. It has recently been shown that PPC self-motion tuning can be significantly modulated by rats running a predicted path in space (Alexander et al., 2020) additionally those authors found that a neural-

network decoder's ability to predict individual activity profiles from self-motion alone had an increased window of elevated performance on those same structured runs. The ability of PPC to learn, associate, and represent spatial structures may underlie many of the studies performed on PPC neurons in other animals. For instance a primate being studied on a delay match-to-place experiment may have neurons recorded which generalize the structure of motor output about to be performed; and if an insufficient number of discernable motor patterns were being performed findings may be conflated with PPC neuron sensitivities to imposed spatial structure. Neural coding in this form in a structure interconnected with motor cortices may even underlie observations such as M1 neurons encoding for spatially defined pathlets (Hatsopoulos & Suminski, 2011). Additionally many more insights into the frames of reference PPC responds to across various tasks could be made by incorporating a diversity of effector patterns. Translating findings from rodent spatial navigation studies to primate working memory functions requires a lot of work, but can be guided by findings such as those presented here.

Future studies should look not only at replicating these findings in primate studies, but utilizing the experimental flexibility rodents afford. Previously PPC neurons have been described as being modulated by the predictability of a pathway being run (Alexander et al., 2020), the extent to which this extends to explicit environmental structure imposed on the animal by being placed on a maze like the triple-T has yet to be studied. PPC afferents include several neuromodulator structures such as basal forebrain (Lamour et al., 1982) which is known to encode task features such as epoch which could be used to define a general task-structure (Tingley et al., 2015). The extent to which this possible task structure and environmental structure interact should also be pursued in tasks designed with embedded task-environment structures.

CONCLUSION

We have presented data supporting the idea that spatial representations better explain a subset of activity profiles recorded in PPC neurons. These spatial representations are likely not all equivalent along which frames of reference they are tuned to. This heterogeneity in what spaces elicit responses across PPC neurons makes analyses that consider activity across all routes unable to determine self-motion determinates for PPC neurons with strong spatial correlations.

It has never been explicitly described in the rodent PPC, but environmental structure encoding of this form could be expected considering many known spatial representation qualities of cortical regions connected to rodent PPC. Rat PPC activity has been demonstrated to reflect where, in a route, the animal is located (Nitz, 2009); additionally certain spatial features such as segment number have been seen to modulate the activity of individual PPC neurons (Nitz, 2012). Furthermore PPC neurons have been seen to respond very strongly to imposed task structure. On experiments where rats were chasing a laser which would move about the environment randomly; when the laser was moved in a stereotyped pathway, and embedded path, PPC neurons' responses to self-motion changed. The integration window is known to widen as well when the task the animal is performing is given more structure, as running in a stereotyped or previously learned shape in an open arena (Whitlock et al., 2012; Alexander et al., 2020). This process could reflect the PPC's role in encoding long-term memory of spatial structures which the animal encounters (Poucet & Save, 2009). That is to say that by affording the animal additional layers of structure with which to interact with (e.g. interconnected paths on the triple-t) additional frames of reference emerge as relevant to the neurons of PPC. Presented here is work that furthers the understanding of what frames of reference PPC neurons are capable of having their activity tuned to.

It is not known if this process would be reflected in more abstract forms of task structure such as the organization of behavior. The triple-T task, along with other tasks used in neuroscience, allow the animal to self-organize a strategy which to accomplish the task. If PPC neurons are seen to trend toward more complicated forms of environmental structure, perhaps PPC neurons would also modulate their activity patterns surrounding the employment of certain structured behavioral strategies. Only recently have the analytical tools been developed to begin looking at simple decision processes and already reveal a rich changing of behavior strategies (Ashwood et al., 2020). Surely as advances in behavioral analyses progress the role of integrative brain regions such as the PPC with be further elucidated.

METHODS

Rats

Subjects were 5 male Sprague Dawley rats all under 6 months old prior to the initiation of training. Rats were initially started on an ad libetum feeding schedule. Following the initial habituation phase of training rats had their food intake lessened to reduce body weight down to approximately 90% baseline weight. This motivated state ensured rats learned the triple-T working memory task quickly. Weight was monitored throughout the experiment to avoid fluctuations.

Surgery

Following one month of pre-surgery training on the triple-T working memory task. Rats were surgically implanted with custom built microdrives each equipped with bundles of 12.5 um nickel chromium wires spun in groups of 4 into tetrodes. Rats were implanted unilaterally or bilaterally with microdrives positioned dorsal to PPC with wires initially positioned approximately 0.5mm deep into cortex. Rats were anesthetized with isoflurane and were held in a sterotactic device (Kopf Instruments). Coordinates for implants were determined through referencing

Paxinos & Watson Rat Brain Atlas (Paxinos & Watson 2014) and measuring distance from bregma. PPC coordinates were centered around (A/P -3.8 mm, M/L ±2.3 mm, D/V 0.5mm). Following a craniotomy and resection of dura mater microdrives were implanted. Multiple skull screws were placed on the skull including 2 screws used as references and attached to the microdrives with insulated copper wire. These implants were secured in place and secured with dental cement.

Triple-T Maze Environment

Experiments were conducted on a “triple-T” path-network maze. The track (Figure 3.1A ; 8-cm-wide pathways, overall perimeter 1.6 m × 1.25 m in length and width, painted black) stood 20cm high in the middle of the recording room. The track edges were only 2 cm in height, allowing an unobstructed view of the environment’s boundaries and associated distal visual cues. Access to certain areas of the maze were restricted by placing painted black cans at key junctions. The placement of these blockers configures the available space to a total of 4 internal pathways, defined by their terminus location, each measuring 140 cm in length with junctions located 51 cm, 87 cm, and 118 cm along each internal pathway (Figure 3.1A top). Two perimeter routes flank the internal portions of the maze and were defined, each 197 cm in length, based on which side of the maze they were on.

Spatial Working Memory Behavior Task

Rats were habituated to the “triple-T” maze for 2 periods of about 30 minutes prior to training. During the first habituation period the animal had access to the entire maze without any blockers present. The second habituation period took place the following day and only some of the possible internal pathways were made available. Following habituation rats were trained to traverse one of the four available internal pathways in for a food-reward. Following the collection of the food reward animals learned to utilize the perimeter routes of the maze to return to the

‘main stem’, the shared portion of each internal route’, and begin another traversal for another food reward. Rats were permitted to choose whichever route back to the ‘main stem’ they preferred and were also permitted to turn around only on the perimeter pathways. Rats often did not change their direction however often restricting their behavior to a single direction for each position of the maze, and maintaining consistent self-motion (Figure 3.1 I-L). Once animals regularly performed 80% or more non-interrupted traversals of all four internal pathways a reward schedule was implemented which required the rat to obtain each of the 4 potential rewards before the rewards were replaced. Rats quickly learned this find-all-4 rule and performed the task reliably quickly and with high accuracy.

Recording Sessions

Each microdrive implant had one electrical interface board (EIB-16 Neuralynx) connected to an amplifying headstage (20X, Triangle Biosystems). Raw signals were initially amplified and low-pass filtered (50X, 150Hz) and brought into a dedicated recording computer running Plexon SortClient software. Here, the signal was digitized at 40kHz, band-pass filtered (0.45 – 9kHz), and amplified between 1X and 15X to fit the shape of detected waveforms (for a total of 1,000X – 15,000X). Over time tetrode wires were moved in 40um steps ventrally through brain tissue to maximize number of unique neural units recorded across days from each animal. Single units were identified and isolated by hand using Plexon OfflineSorter software. Key waveform parameters for separation were peak height, peak-valley distance, energy, average voltage, and principals components.

Animal position data was collected at 60Hz using a ceiling-mounted camera, mounted 305cm above the recording room floor. Colored LED lights affixed to the implants of recorded animals were tracked using Plexon CinePlex Studio software to obtain X,Y coordinates. Lights were approximately 4.5cm apart and were positioned perpendicular to the heading of the animal.

Histology

Rats were perfused intracardially with a solution of 4% w/v paraformaldehyde in PBS during deep anesthesia. Following an injection of a lethal dose of pentobarbital brains were removed and sectioned into 30um slices. Brain slices were Nissle-stained to identify the location, trajectory, and depth of tetrode wires in PPC. Boundaries of PPC were defined based on previous electrophysiological studies and in accordance with Paxinos and Watson altas (Paxinos & Watson 2014). All tetrodes were determined to have been located in the PPC at the time of recording for the units to be included in this study.

Identification of Clean Traversals

To identify traversals made on the triple-T maze that demonstrated clean and uninterrupted running custom MATLAB graphical interfaces were utilized. First the user defines, in space the starting and ending 'gates' for each route defined for analyses. The MATLAB script automatically extracts traversals with sustained running speed at or above 3cm/s throughout the traversal. The user then verifies each individual run to ensure there are no obvious deviations from uninterrupted stereotyped running behavior. The selection of clean runs results in the data presented in Figure 3.1A (bottom). Through this method regions of the maze without stereotypical running behavior, such as the reward locations and spaces between defined routes, are not conflated in our analyses for the spatial firing characteristics of these neurons. Additionally individual traversals which introduce outlier behavior are not conflated in analyses which assume some degree of recurrence in behavior.

Route and Space Referenced Ratemaps

To analyze the action and spatial correlates for each neuron, individual neuron activity was mapped onto the position of each route through the use of custom MATLAB scripts. Previously identified stereotypically ran traversals were overlaid on one another for the

traversals belonging to each route. A spatial template was drawn for each route that equally binned space in steps of 10 pixels apart which corresponds approximately to 3.5cm. Firing rates were then calculated for each bin by dividing the total number of spikes by occupation time. Activity patterns were then smoothed with a Gaussian filter (σ = 6 cm AOC = 1).

Similar to the linearized route referenced ratemaps, two dimensional firing ratemaps were constructed for each neuron for the entire space of the maze for the entire experiment. For recording bins with a detected velocity at or above 3cm/s the X,Y coordinates are identified the number of spikes is divided by the occupancy time. This process is done for the entire experiment and averaged across each identified X,Y position. Raw two dimensional ratemaps were smoothed with a gaussian filter (σ = 6 cm^2 AOC = 1).

Generalized Linear Model

A series of GLMs were computed to assess the impact self-motion had on the activity profiles of individual neurons as rats performed the triple-T task. To begin only linear and angular velocity were chosen as those are the self-motion cues most associated with PPC activity. A complete GLM or cGLM was constructed for the max-normalized mean firing rate for each neuron which used both linear and angular velocities as predictors (glmfit function in MATLAB using the 'identity' link function). Coefficients were calculated for both linear and angular velocities (glmval function in MATLAB) to reconstruct the activity profile which was used to calculate the fit between the actual firing rate vector and the cGLM output assessed using the normalized mean squared error ('NMSE', as an output from the function 'goodnessOfFit' in MATLAB). To test the impact of each self-motion variable on the accuracy of the GLM a partial GLM, or pGLM was fit to each neuron in the same manner as above, but by dropping either linear or angular velocity from the model. Kruskall-Wallis tests with post hoc Boneferonni corrections were made comparing the distribution of values derived from the pGLMs relative to their respective cGLMs. NMSE scores derived from pGLMs were tested, using a 2-tailed t-test,

against cGLM NMSE scores for specific populations to determine which aspect of self-motion, if any, best explained cGLM performance.

Correlation Analyses of Topological Encoding

Individual neurons had their activity patterns compared across identical length routes, as in the 4 internal routes or the 2 external routes. through a pearson's r calculated for the mean firing rate profiles for each route across each positional bin. As a measure of reliability the correlations derived from same-path comparisons were made from the correlation between mean firing rate profiles for both the even traversals and the odd traversals. Correlations for non-identical path comparisons were calculated form the mean firing rate vectors without further splitting of the data.

Shuffled correlation values were created by randomly shifting each traversal of the linearized spike train for each route 100 times. These shuffled firing rate profiles had both inter and intra path correlations calculated as described above and the distribution of these correlation values was used in determining significant route-route correlations for each neuron. For each neuron topological encoding was determined if the route-route correlation was equal to or higher than the mean of the control distribution plus or minus 2 standard deviations.

Acknowledgements

Chapter 3, in full, is a reprint of the material as it appears in the following manuscript that is currently being prepared for submission for publication: Johnson, A.B., & Nitz, D.A. Self-Motion Independent Mapping of Route Structure in Parietal Cortex. The dissertation author was the primary investigator and author of this paper.

Figures

(Continued on next page)

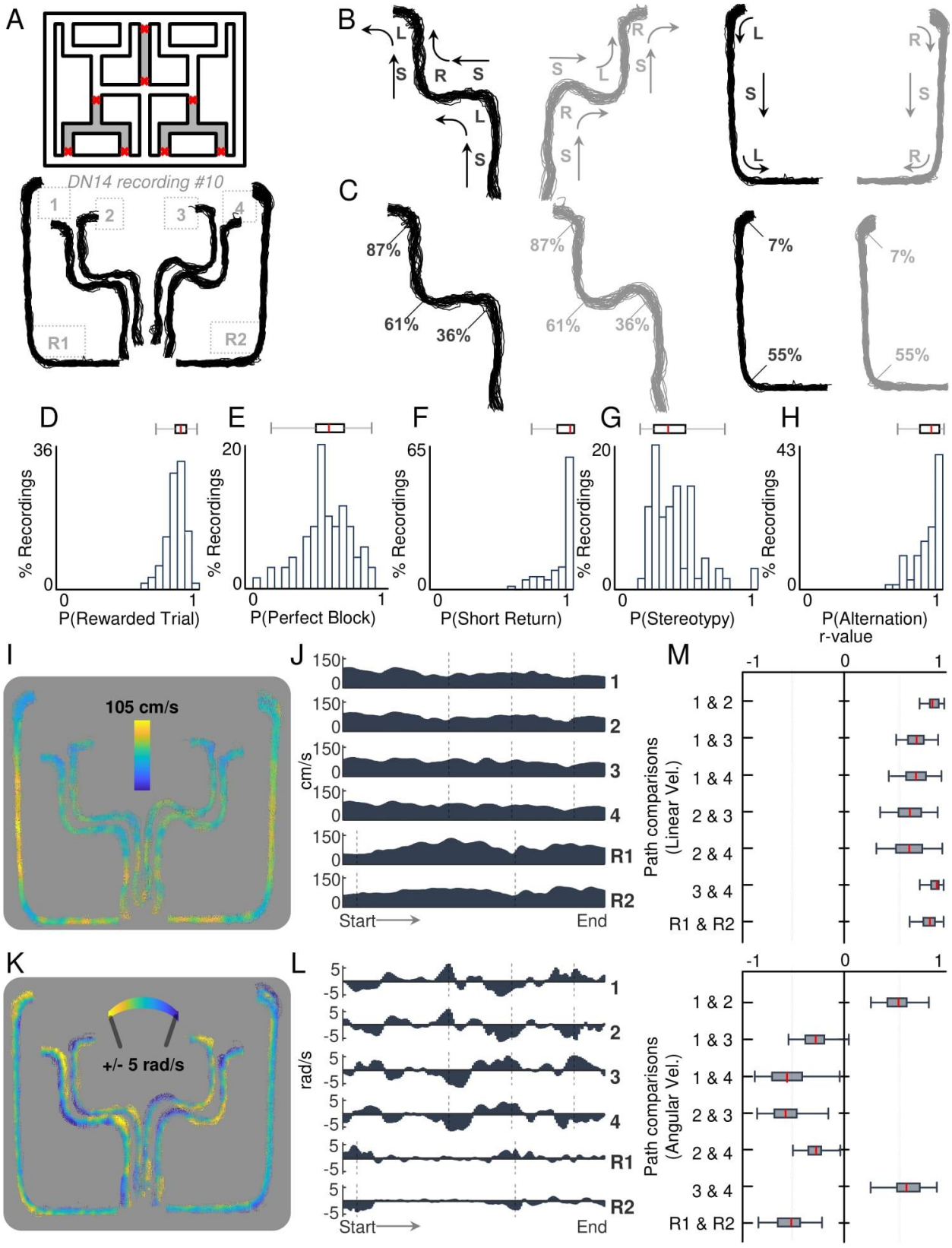


FIGURE 3.1: Components of Self-Motion During Spatial Working Memory Task

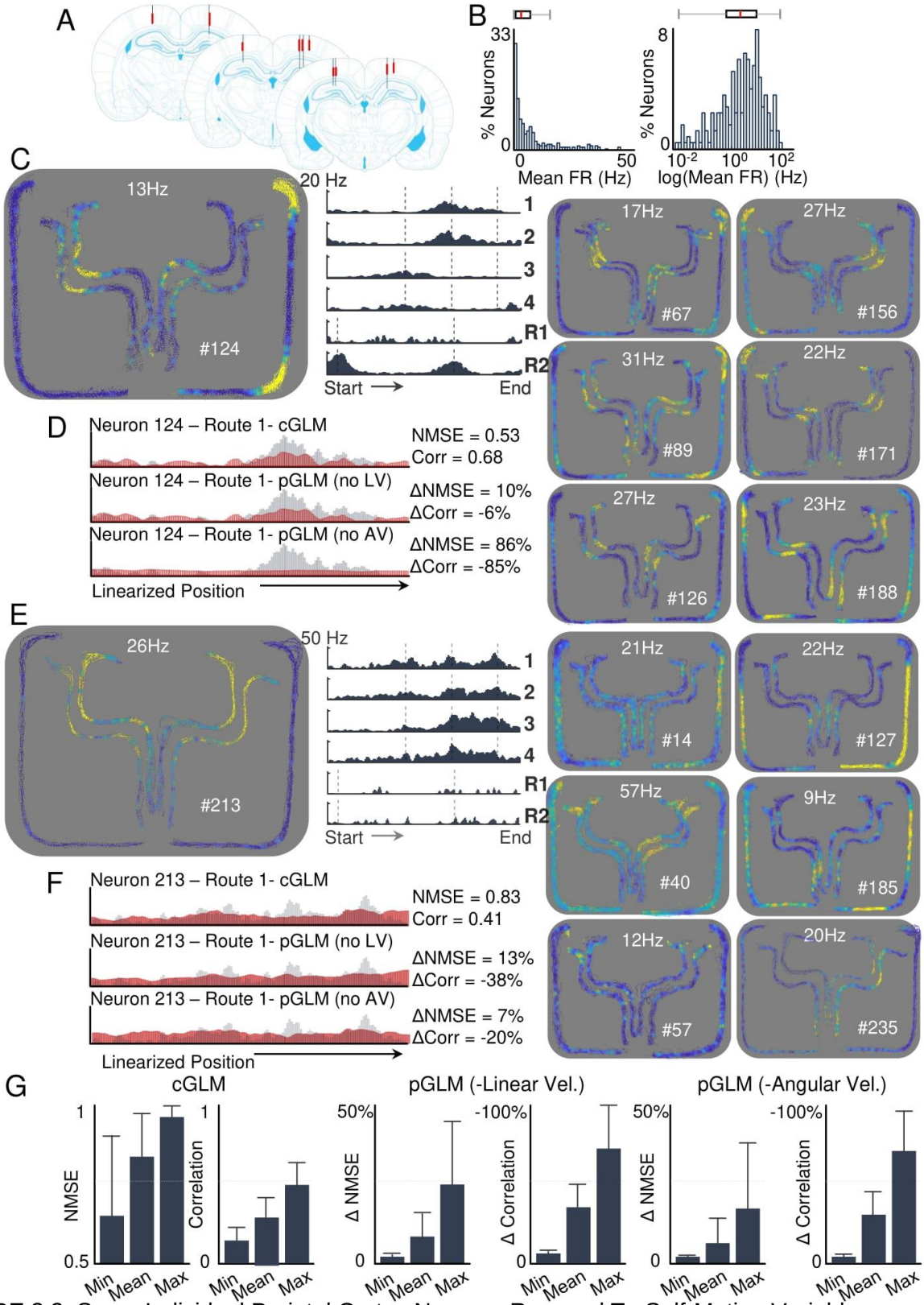


FIGURE 3.2: Some Individual Parietal Cortex Neurons Respond To Self-Motion Variables

Parietal Cortex Neurons Respond To Self-Motion Variables

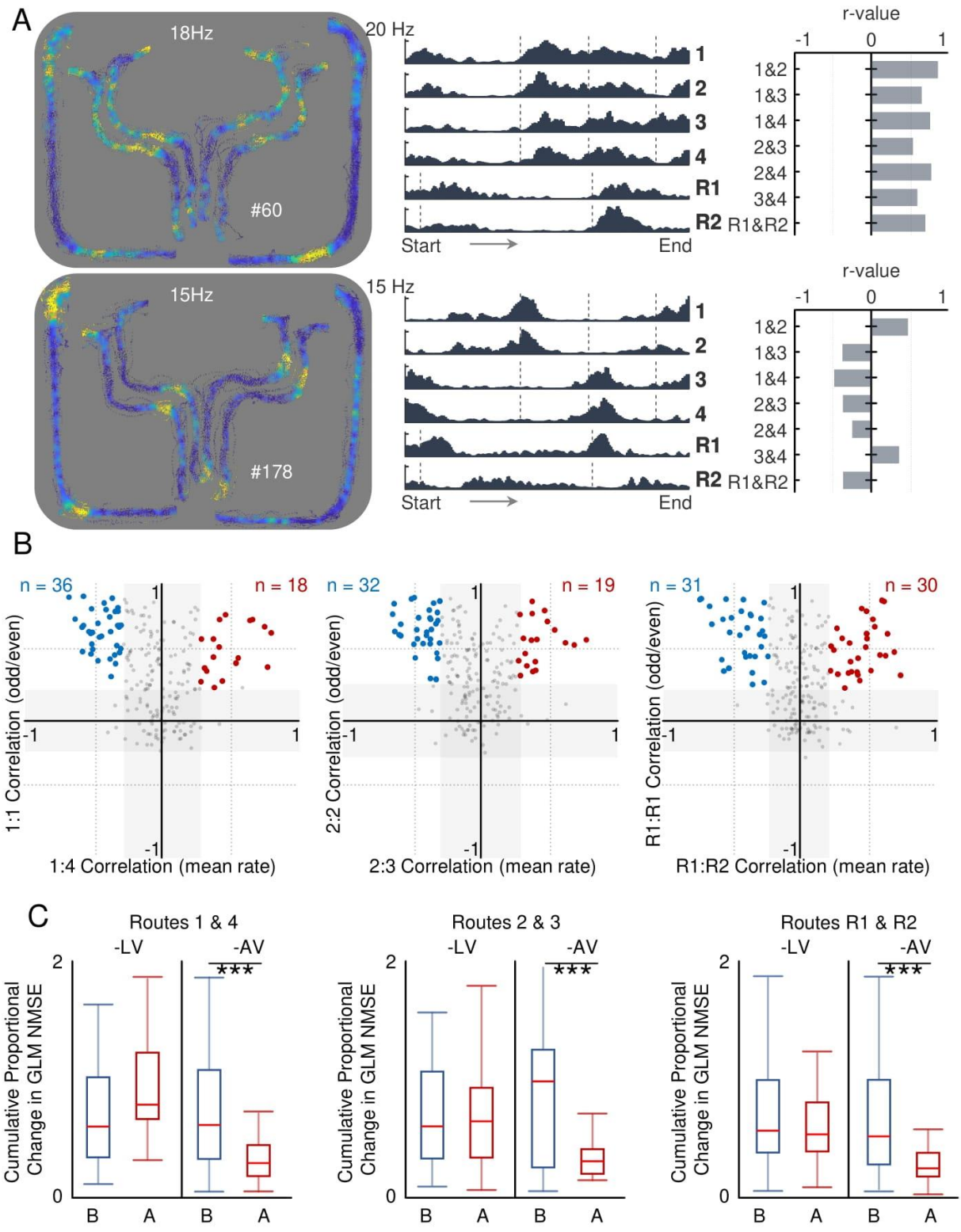
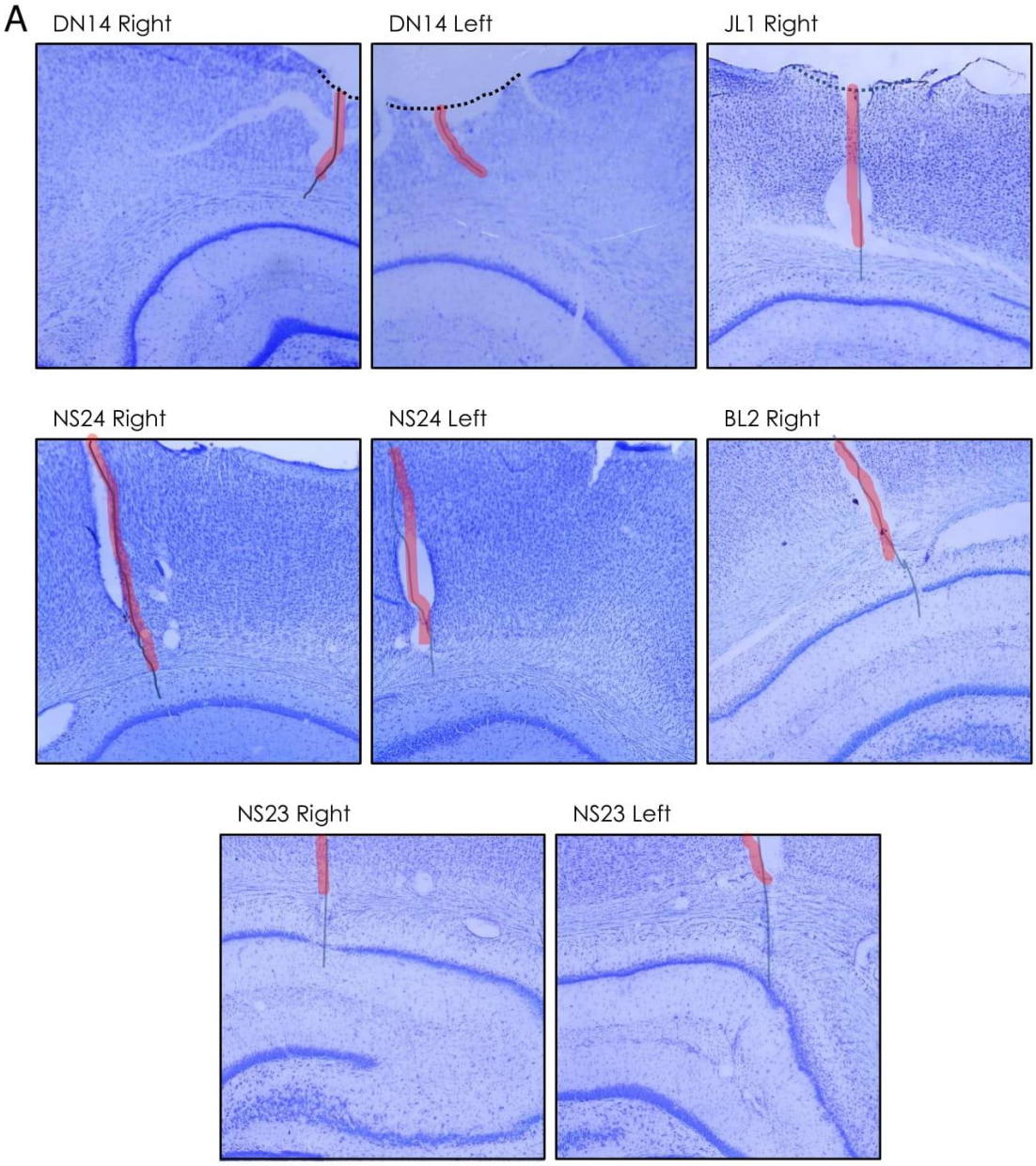
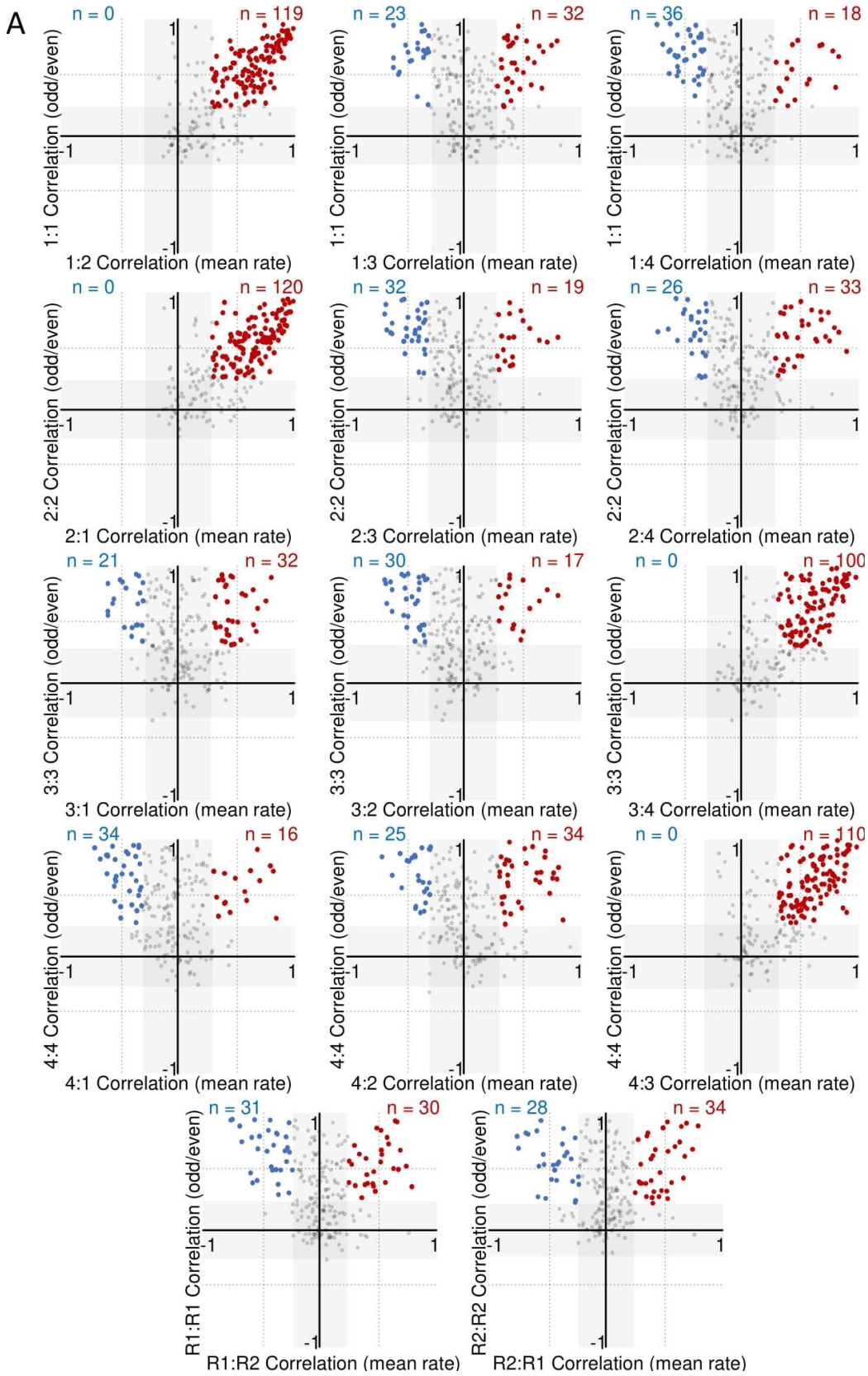


FIGURE 3.3: Path Structure and Path Shape Encoded by PPC Neurons



SUPPLEMENTAL FIGURE 3.1: Summary of Recording Site Histological Data



SUPPLEMENTAL FIGURE 3.2: Complete Correlation Projection Across all Routes

Figure Legends

Figure 3.1: Components of Self-Motion During Spatial Working Memory Task

A) Schematic of triple-T environment configured to the find-all-4 task. Blockers (red X's) were used to make certain portions of the environment inaccessible. Below are individual traversals of the maze separated in space to illustrate labelling individual paths based on their terminal location. **B**) Individual internal pathway pairs 1 and 4 and the external pathway pair of R1 and R2 can be considered exact opposite shaped routes when considering the specific direction of turning along each route. **C**) The same pathway pairs as in B, can also be seen as identical in general structure when considering where the turn locations are. **D-H**) Compilation of performance on the find-all-4 condition. Probability of getting a reward mean = 0.838 s.d = 0.0688 . Probability of completing a block without error mean = 0.566 s.d. = 0.1679. Probability of choosing the shorter of two return arms mean = 0.9197 s.d. = 0.1075. Probability of perfect blocks having the same sequence as the maximum performed sequence that recording mean = 0.4016 s.d. = 0.173. Probability of alternating at the first decision point mean = 0.8973 s.d. = 0.0916. **I**) Color-coded behavioral tracking illustrating average linear velocity, from 0 in blue to 105cm/s in yellow. **J**) Data from I presented with each route's space linearized along the x-axis from the start to the end for each route. **K**) Color-coded behavioral tracking illustrating average angular velocity, from -8 radians/s in blue to +8 radians/s in yellow. **L**) Data from K presented with each route's space linearized along the x-axis from the start to the end for each route. **M**) Route-route correlations for mean linear velocity (above) or mean angular velocity (below).

Figure 3.2: Some Individual Parietal Cortex Neurons Respond To Self-Motion Variables

A) Schematic of electrode placement with recordings determined to be in PPC highlighted in red. **B**) Mean firing rate for all neurons showing a logarithmic distribution consistent with previous reports of neurons in this cortical region. **C**) Color-coded ratemap of an example PPC

neuron tuned to angular velocity from no firing in blue to the mean firing plus one standard deviation, labelled at the top, in yellow. Additional example of PPC neurons seemingly tuned to angular velocities are tiled to the right. **D**) GLM analysis for neuron #124 route 1 activity. cGLM performance for route 1 had NMSE of 0.53 and a correlation of r=0.68. pGLMs were made without either linear or angular velocity and a proportional change in NMSE was recorded (10% increase for the pGLM without linear velocity, and 86% increase for the pGLM without angular velocity). **E**) Color-coded ratemap of an example PPC neuron which displayed an activity profile not readily explained by self-motion across all routes. Additional example of PPC neurons with activity patterns not explained by self-motion are tiled to the right. **F**) GLM analysis for neuron #213 route 1 activity. cGLM performance for route 1 had high NMSE of 0.83 and a low correlation of r=0.41. pGLMs were made without either linear or angular velocity and a proportional change in NMSE was recorded. Compared to the GLM analysis in D relatively small effects were noted (13% increase for the pGLM without linear velocity, and 7% increase for the pGLM without angular velocity). **G**) For each neuron the minimum, mean, and maximum cGLM NMSE and correlation scores across routes are presented. (Min NMSE mean = 0.6514 s.d.= 0.2496; Mean NMSE mean = 0.8408 s.d. = 0.136; Max NMSE mean = 0.9707 s.d. = 0.0337; Min r-value mean = 0.1474 s.d.= 0.0869 ; Mean r-value mean = 0.3214 s.d. = 0.1014; Max r-value mean = 0.4983 s.d. = 0.1424). Proportional changes to GLM scores were recorded for both pGLMs: subtracting linear velocity (Min NMSE mean = +0.7% s.d.= 1.69%; Mean NMSE mean = +8.45% s.d. =7.63%; Max NMSE mean = +24.47% s.d. = 20.76% ; Min r-value mean = -6.08% s.d.= 9.74% ; Mean r-value mean = -35.36% s.d. = 15.57%; Max r-value mean = -72.03% s.d. = 22.23%), and subtracting angular velocity (Min NMSE mean = +0.6% s.d.= 1.3% ; Mean NMSE mean = +6.48% s.d. = 6.65%; Max NMSE mean = +17.62% s.d. = 17.3%; Min r-value mean = -4.04% s.d.= 7.34%; Mean r-value mean = -31.34% s.d. =14.39%; Max r-value mean = -70.39% s.d. = 24.75%).

Figure 3.3: Path Structure and Path Shape Encoded by PPC Neurons

A) Two example neurons' color coded tracking data showing where the neuron exhibited no-firing in blue, and maximal firing in yellow. Linearized activity profiles were constructed and route-route correlations were made across mean firing rates. Top neuron demonstrated very high correlation values for all path comparisons in a manner not consistent with self-motion. Bottom neuron exhibited an activity profile consistent with angular velocity tuning and had route-route correlation values comparable to the angular velocity correlations across routes. **B**) Scatterplot of path-path activity comparisons. Shaded regions correspond to the mean plus two standard deviations for correlations derived from shuffled data. Neurons labelled in red are significantly correlated for represented path-pairs. Neurons in blue are significantly anti-correlated for represented path-pairs. The three path-pairs represented all are behaviorally opposite, but structurally identical. **C**) Cumulative change in NMSE was calculated across all routes for each neuron's GLM analysis. The population of neurons above, labelled A, and below, labelled B, were tested against each other in a 2-tailed t-test to compare relative contributions of each self-motion variable. Routes 1&4 -LV: p=0.9 ; -AV: p = 0.00024; Routes 2&3-LV: p=0.9 ; -AV: p = 0.00024; and the two return routes -LV: p=0.9 ; -AV: p = 0.00024.

Supplemental Figure 3.1 Summary of Recording Site Histological Data

A) Nissl stained brain sections of each animal's tetrode recording sites. Anatomical locations determined to be included in the dataset highlighted in red.

Supplemental Figure 3.2 Complete route-route Correlation Projections

Scatterplot of path-path activity comparisons. Shaded regions correspond to the mean plus two standard deviations for correlations derived from shuffled data. Neurons labelled in red are significantly correlated for represented path-pairs. Neurons in blue are significantly anti-

correlated for represented path-pairs. Neuron counts for each grouping (above or below) are presented above the route-route projections.

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CHAPTER 4: Considering Spatial Recurrence to Probe Self-Organized Behavior on a Latent Learning Task

Abstract

Animals employ many behavioral strategies when engaged with even simple tasks. These strategies have been shown to maximize the animal's exploratory capabilities, and can be as simple as alternating at a choice point. How these simple strategies, adapt to more sophisticated spaces however is understudied. We endeavored to explain rat choice behavior on a working memory task which employed two choice points in sequence. The same rats then had their task elaborated upon where there were 3 decision points in sequence. Presented here is evidence that rats employ a sophisticated understanding of physical and task structure on the triple-T working memory task. Additionally is evidence that rats anchor alternation behaviors based on spatial location. The latter is consistent with ideas that alternation strategies are useful for maximizing exploration.

Main Text

INTRODUCTION

From decisions about who to associate with, what to eat, when to go to sleep, or where to travel to in order to reach one's goals; decisions of all sorts permeate our everyday experience. Many of these decisions allow us to choose from an almost endless number of possibilities, when navigating across a field for instance there are a near infinite combination of locations - or routes, which all would deliver one from a shared starting location to a terminal location. Mentally running through each of these possibilities would result in very long mental processing times for even simple decision making tasks. Clues to how a potential knowledgebase is formed such that decision making can be more efficient currently is guided by studies showing that the structure of environments previously experienced strongly influences

navigational strategies (Barhortst-Cates et al., 2021; Coutrot et al., 2022). In particular having knowledge regarding spaces where path-junctions occur was highly related to navigational ability (Brunec et al., 2022). These studies highlight the importance of physical structures in guiding perceptions and utilizations of space. This inherently reduces the availability of certain routes through space thus demanding fewer considerations be made by decision making processes. While the previous example was explicitly one about spatial decision making it could be theorized that those sorts of decisions are actually insights into decision making more generally. It has been proposed that across domains decisions are often broken up into sequential binary decisions that the brain mentally navigates through (Sridhar et al., 2021). In this way the study of spaces and structures explicitly can inform theories into decision making mental-spaces and adaptions to bounds placed on problem-solving. Studies of this nature have historically been performed through the application of structured mazes.

The application of maze environments to study psychology and behavior is rich and begins for the purposes of this research in the late 19th century with the studies done by George Romanes. A friend of Charles Darwin's, he used maze structures to investigate the evolution of memory systems across species, and coined the phrase 'comparative psychology' (Romanes, 1882). The use of mazes in behavior experiments quickly became a foundational tool for studies probing memory systems, and designing appropriate maze structures began to be crucial in order to elicit 'naturalistic' behaviors such as navigation back to a home den through a labyrinth of tunnels (Kline, 1899; Small, 1901). One unexpected outcome of the implementation of these structured environments was the observation of animals organizing their behaviors around certain types of structures in unpredicted ways. First noted by Carr (Carr, 1917) animals had a robust tendency to alternate decisions at junctions which presented 2 choices. That is when animals approached a T-shaped junction on a maze the animals reliably chose opposite their previous choice. While Carr did not elaborate much on this behavior other researchers such as

Tolman took this behavior as crucial evidence of an adaptable navigation system (Tolman, 1925). This alternation behavior (AB) as it has since been known, became the central focus of research investigating memory systems across animals in accordance to theories put forward by Hull whereby the expression of AB was a result of a form of satiety with regard to the action just taken, and thus the choice to pursue a new action follows an avoidance for repetition (Hull, 1935; Hull, 1943). Since Hull, the nuances of why animals express AB have been debated in the literature for decades (Dember & Earl, 1957; Olton, 1979; Richman et al., 1986; Lalonde, 2002; Bak et al., 2017).

Throughout the last hundred year of AB research there remains a stubbornly consistent experimental design. In virtually all studies into AB the selected environment consists of a single decision point. Many manipulations can be made on the structure of this simple design for example: the start corridor and two possible choice corridors can be oriented in various angles, and can demonstrate effects of this on AB (Douglas et al., 1972). For most experiments the choice arms are oriented perpendicular from the start forming a T-shape (Deacon & Rawlins, 2006), although similar behavioral outcomes can be expected on a Y-shaped maze (Bak et al., 2017) with equal angles orienting each of the three arms. Rats and other animals (Schultz, 1964; Livesey, 1965; Hughes, 1967), will spontaneously exhibit AB on about 80% of traversals of such decision points. This phenomenon has been described as one of the most robust in all of psychology for the persistence of it across many experimental conditions (Dember & Earl, 1957).

Perhaps because AB is such a robust and predictable behavior it has been investigated by neuroscience for many decades probing the neural foundations of AB (Lalonde, 2002). While damage to some brain regions such as hippocampus elicit strong deficits in AB presentation lending to ideas AB is contingent on short-term memory (Ellen & Deloache, 1968; Stevens & Cowey 1973), lesioning cortical regions such as frontal cortex (Divac et al., 1975), entorhinal

cortex (Scheff & Cotman, 1977), retrosplenial cortex (Pothuizen et al., 2008), and sensory cortices (Stevens, 1973) occasionally impact AB presentation especially paired with experimental manipulations like rotations or extended inter-trial intervals, however in many studies these deficits are much weaker compared to hippocampus lesions, are transient, or vary dramatically across subjects.

The persistent display of and mysterious nature of AB at single decision-point environments have captivated the attention of scientists for a hundred years. Surprisingly while it was the implementation of structured environments which gave rise to this observation very few structural manipulations to the environment are implemented to probe AB. The structural manipulations which are used in studies involve changing many features of the maze around the decision point; the relative angles across choice arms (Douglas et al., 1972) which specifically was used to show the abolishment of AB when choice arms are parallel to one another, and the use of rotations or changing rooms (Sherrick & Dember, 1966; Dudchenko & Davidson, 2002, Cahill et al., 2015) to illustrate the aspects of AB which may be a result of memory.

One characteristic of AB is that animals will with above 80% probability do it, and in many experiments AB occurs with a ceiling effect (Dember & Earl, 1957; Douglas et al., 1974). This is perhaps suggestive that the experimental design is too simplistic and that ; this perspective would posit that finding a mechanism to reduce the certainty of AB without abolishing it completely would directly inform what dimensions of experimental complexity AB is sensitive to. One way to increase the 'load' or difficulty of the task is to implement additional decision points within the task; and evidence from rats navigating mazes consisting up to 14-decision points (Michel & Klein, 1978) shows clearly that rats have the capability for decision-making well beyond the single choice experiments commonly used, however this and other studies that employ multiple decision points maintain a single 'correct' pathway which anchors

the animal's behavior (Schmitzer-Torbert & Redish, 2004; Ainge et al., 2007). These studies increase the cognitive load of wayfinding, but do not always investigate AB or the effect of how AB is influenced by various experimental structures. If these studies were to structure the manner in which the decision points were connected it would allow for many additional dimensions of study from what is historically done with regard to decision-making behavior.

Already there is a push for data of this nature from the field of artificial intelligence (AI). In the field of AI automata are often designed to navigate spaces analogously to their animal counterparts (Barrera & Weitzenfeld, 2008). Recent years have seen AI research demand more complex environments and contexts with which to test their automata. A common experimental design would see that the AI learns a navigational strategy from one set of spaces and is expected to generalize navigational strategies to other spaces (Bechtold et al., 2018; Yaman et al., 2019; Zou et al., 2021). This poses a problem for those fields as behavioral phenomena such as AB have not yet been investigated on more complex environments. This gap in sophistication experiment design between AI and experiment design for behavioral science is surprising given the call for sophisticated experimental design over the years (Olten, 1979; Meketa, 2014), indeed it seems as though experimental design complexity should match the complexity we seek to appreciate from the systems studied – thus even sophisticated memory and navigational systems may appear overly simplistic from overly simplistic experimental design.

To investigate the behavior of animals on a sequential-choice task we examined the navigational choices of rats performing a working memory task on a triple-T maze (Olson et al. 2017; Johnson et al., 2021) under conditions of two decision points and three decision points. The structure of the maze complements a working memory 'find-all' task which was implemented and required animals to collect rewards at each of the routes' terminus repeatedly. The combination of environment and working memory task allow for the animal to organize their

decision sequences in potentially dozens of ways while still performing the task optimally, as measured by probability of collecting a reward. Beyond simple metrics of correct-or-not this experimental design allows for the investigation into how animals organize their choices, and to specifically see how AB presents itself on the task. All potential choice pathways shared a consistent structure of straight portions and three 90° turns in sequence. The difference in shape for the routes comes from the combination of right-left turns which bring the animal to their destination. Initially animals perform on a two-sequence decision making task 'find-all-4' where 4 possible destinations are possible from changing the first and third turns, the second being yoked to the first through a blocker. Following several weeks of recordings on the find-all-4 task animals were allowed access to four other pathways through the removal of previously established blockers. Animals were from then on required to incorporate the new decision point to navigate to the 8 possible destinations through combinations of all 3 turns. Elaborating the task in this fashion forces the animal to incorporate 4 new routes, all structurally analogous to the original 4.

During both the find-all-4 condition and the find-all-8 condition we report a strong tendency for rats to perform well above chance. We describe rats' performance in detail across the first week of learning the new context as well as many facets of the rats' behavioral strategies as they relate to performance. Of these findings the most robust observed were the rats' tendency for AB across every turn location. AB on subsequent turns after the first did not appear to have the same ceiling effect as seen on the first turn. This AB was seemingly only true if each turn was considered in a spatial sense and not true when only sequence was considered with regard to sequence of choice alone. The combination of sequential decisions which the animal alternates at intriguingly emerges as 2nd order alternation (AA BB as opposed to ABAB) for the intermediate turn of the find-all-8 condition. The presence of AB along with the

utilization of spatial features as navigational aids affords many insights into how rats use these stratagems to organize their behavior along task demands.

RESULTS

Structure of Performance Across Latent Learning Condition

Rats began data collection following at least 2 weeks of training, and did not demonstrate a systematic bias for any route on the find-all-4 task (Fig 4.1C), even though rats were slightly more likely to perform an error on route number 1 (Fig 4.2A). Rats performed the find-all-4 task with very high performance, averaging probability of 0.84 for obtaining a reward on any given trial. Additionally rats on the find-all-4 task completed a block of 4 trials without error on average 56% of the time. When the rat obtained a reward the animal averaged receiving 9.1 rewards before making an error. Upon making an error animals averaged making 1.6 errors in a row before re-obtaining a reward (Fig 4.1G). Suggesting that animals regularly were in bouts of high performance, but when errors occurred they were not usually isolated incidents.

On the find-all-8 condition rats had a slight systematic bias for the routes newly incorporated into the task scheme consistent with oversampling novelty (Fig 4.1F; Fig 4.2D,H). This bias reliably occurred after the first day of recording on the find-all-8 condition (Fig 4.2I). In spite of this, animals still performed with excellent performance averaging a probability of 0.76 for obtaining a reward on any given trial. Additionally rats on the find-all-8 task completed a block of 8 trials without error on average 16% of the time, considerably lower compared to the find-all-4 condition. When the rat obtained a reward the animal averaged receiving 6.1 rewards before making an error. Upon making an error animals averaged making 1.9 errors in a row

before re-obtaining a reward (Fig 4.1H). These metrics define how the doubling of difficulty in task design does not equate to a doubling in error performance, but a more subtle increase.

Temporally, when animals made an error there was a remarkable decline in probability of error presentation immediately preceding or following. However an increase in error incidence could be seen in a fashion which suggests for both the find-all-4 and find-all-8 pathways the block structure also structures when errors will occur (Fig 4.2B,E). This corresponds to a finding that the highest incidence of error occurred in the final position in the block across conditions (Fig 4.2J,K). The pattern of reward incidence seemed to follow a similar, though weaker pattern (Fig 4.2C,F).

Use of Environmental Spaces as a Heuristic

Two forms of environmental heuristics were measured. First was the heuristic of taking the short return path back following a trial run which signified a linking of decisions to upcoming return behavior. Second was the bias demonstrated by animals where the return route selected would predict the upcoming trial run independent of if that return was a short or long traversal.

Animals showed similar display of both behaviors on the find-all-4 condition with average probability of taking the short return at 0.77 and average probability of biasing their next decision at 0.81. Importantly, one rat AJ5, reliably chose the opposite pattern – that is taking the long return path back, and biasing subsequent decisions to be on the ipsilateral side. It should be noted that this combination of behaviors is functionally equivalent for granting the animal a spatial heuristic to orient their decision making process (Fig 4.3A; Fig 4.4A). All animals regressed their heuristics closer to chance levels (50%) following introduction to the find-all-8 condition averaging a probability of 0.53 for taking the short return path back and 0.66 for biasing decisions based on previous return route (Fig 4.3F; Fig4.4F). This suggests that overall biasing of upcoming choice based on return route is a stronger heuristic, and that taking the

shorter return route back could be epiphenomenal on the simpler task. The peri-event probabilities of these behaviors around themselves (Fig 4.3C,H; Fig 4.4C,H) and to rewards (Fig 4.3E,J; Fig 4.4E,J) showed no dramatic patterning, however in the find-all-8 condition only it does appear as though errors apply a negative pressure on using these heuristics (Fig 4.3I; Fig 4.4I). Across the latent learning paradigm these behaviors followed similar general trends (Fig 4.3K; Fig 4.4K) with all but one animal strongly performing the behaviors prior to the new pathways and one animal strongly not performing the behavior. Following the introduction to the new pathways all animals across days migrated their behavioral probabilities to be closer to chance, suggesting the find-all-8 condition itself may exert a negative pressure on using these heuristics.

Alternation Behavior

Animals had a strong tendency to alternate their sequential decisions in a spatial manner. That is, for sequential choices AB was reliably seen for each considered decision point. Strongest demonstration of AB was seen at the first decision point with a mean probability of 0.89 (Fig 4.5A), this persisted through the latent learning paradigm with a mean rate of 0.85 after learning the new pathways (Fig 4.5F). In both conditions AB at the first turn was correlated to overall success for the find-all-4 condition ($r = 0.6577$) and for the find-all-8 condition ($r=0.6428$) (Fig 4.5B,G). There was approximately a 10% decline in probability of AB at the first turn on the second day of the find-all-8 condition, but not for the first day (Fig 4.5K). When an alternation occurs there is no increase in preceding or subsequent trials to have alternations (Fig 4.5 C,H) A slight but consistent variability in AB was seen just preceding error trials, but no such relationship to rewards could reliably be seen.

Nearly equivalent strong demonstration of AB was seen at the spatially defined final decision point with a mean probability of 0.86 (Fig 4.6A), this persisted through the latent learning paradigm with a mean rate of 0.86 after learning the new pathways (Fig 4.6F,K). In

both conditions AB at the final space was correlated to overall success for the find-all-4 condition (r = 0.5568) and for the find-all-8 condition (r=0.6479) (Fig 4.6B,G). When an alternation at the final space occurs there is no increase in preceding or subsequent trials to have alternations (Fig 4.6 C,H). There is a consistent drop in AB at the final turn during error runs, this suggests that about 50% of errors during the find-all-4 condition coincided with a failure to perform AB (Fig 4.6D). A slight but consistent increase in AB at the final space was seen around rewarded trials. On the find-all-8 condition both of these effects were much less in intensity but similar trends persisted (Fig 4.6I,J). There was no discernable change across the time course of latent learning seen (Fig 4.6K).

If the final decision was considered independent as to the particular space AB appears to go away. That is to say animals definitely organize their AB behavior on the triple-T tasks in a spatial and *not* a task-based manner. In the find-all-4 condition rats alternated at the final choice with a mean probability of 0.56 and on the find-all-8 condition on average 0.51 (Fig 4.7A,F), much lower compared to other alternation schemes. Both the find-all-4 condition and the find-all-8 condition saw alternating based on choice and not space was weakly negatively correlated with performance at r= -0.4657, and r= -0.389 respectively (Fig 4.7 B,G). When looking at the peri-event plots for incidence of alternating based on choice, when it occurred for the find-all-4 and not for the find-all-8 there was a dramatic decrease in probability just preceding and proceeding that event, in both directions there was then a dramatic increase in incidence the next trial over (Fig 4.7C). This oscillation of probability suggests that there is a strong preference for animals to *not* alternate in a choice-based fashion and when it occurs there is a pressure to prevent it from occurring again. Neither condition saw a reliable display of alternation at the final decision point based on choice share a temporal relationship with either errors (Fig4.7 D,I) or rewards (Fig 4.7 E,J) and the incidence rate remained stable across the latent learning paradigm (Fig 4.7 K).

The final site of alternation was the second turn on the find-all-8 condition only, as on the find-all-4 condition this turn was yoked to the first. Like the final decision point, this turn can be considered with regard to the particular space being traversed (Fig 4.8A-E,K) which demonstrated equivalently strong tendencies for alternate as with the other spatially considered decision points mean probability of 0.74 and had a strong correlation to overall probability of obtaining a reward r = 0.6641; or with regard to the second choice point regardless of the spatial location (Fig 4.8F-J,L) which still had above chance levels of presentation, with a mean probability of 0.6 , but a slight negative correlation to reward probability r = -0.3178. Alternating by spatial location did not have a strong peri-event pattern, but did have an interesting oscillatory shape when looked at alternations around error trials. This may mean that there is a regular oscillation of propensity to alternate at this turn, and at particular moments within that cycle errors are more likely to coincide with disruptions in this alternation scheme (Fig 4.8D). A similar type of pattern was not seen structured around reward trials. Alternation based on choice position appeared to have a oscillatory pattern around the peri-event probability. This pattern resembled that of alternation by choice position for the final turn on the find-all-4 condition (Fig 4.7C). The spatial alternation tendency appears to develop after the first recording day of the latent learning paradigm (Fig 4.8K) whereas there is no change in the alternation based on choice position alone over time (Fig 4.8L).

Emergent Second Order Alternations

One unique aspect of the experimental design chosen is that patterns beyond simple AB can develop along certain perspectives of looking at the data. AB which is of the first order, for example left – right – left – right – left – right, and so on, has been very well described in literature. However, it is known animals can learn second order alternation patterns with training, for example left – left – right – right – left – left, and so on where each decision is repeated a single time before an alternation event. The provocative high-then-low-then-high

peri-event probabilities for alternations based on choice position made it a natural variable to investigate whether higher order alternations occurred at the second choice point for the find-all-8 condition. When considered from a spatial perspective animals decidedly do *not* perform second order alternations with a mean probability of 0.42. The peri-event probabilities show no dramatic trend is display of this type of behavior with the exception that error trials appear to coincide with a reduced probability of alternating in this fashion (Fig 4.9D). Animals furthermore do not change this behavior over more experience to the find-all-8 condition (Fig 4.9K).

More fascinating was when second order alternation was considered with regard to the choice position. Here what seems to be a clear bimodal distribution across trials was calculated (Fig 4.9F) with a mean probability of 0.53. Neither bootstrapped method produced a bimodal distribution. Although this distribution did not pass a Hartigan's dip test for significance it was close enough to be considered a trend (dip = 0.1). This may be due to the sample size having been too low (n = 44), as more experiments are run it would be interesting to see if this bimodality becomes more pronounced and more easily definable. Alternation of this type within a recording session did not seem overly correlated to performance with r = -0.0971. The peri-event probabilities revealed, however, that when the animal alternated in this fashion the immediate next trial had a strong pressure to break the alternation (Fig 4.9H), however no clear patterning around errors or rewards was noted (Fig 4.9I,J). Across days it appeared that every animal has highly variable for this measurement across days (Fig 4.9L), with animals rarely ever performing around chance level and either decidedly using this alternation scheme or decidedly not.

DISCUSSION

Reported here is evidence that rats learn a relatively complicated find-all task and perform very well with both 4 and 8 choices. Rats take only one day typically to reach terrific performance and to establish stereotypical behavioral schemas. Results from rat performance

should speak volumes to a general understanding of rats' abilities to perform challenging spatial navigation tasks, and have impacts that extend into the fields of neuroscience and beyond.

Novel Metrics for Behavioral Assessment in Multichoice Task

One barrier to expanding the repertoire of behavioral tasks employed is the relative difficulty in collecting baseline data and learning how to titrate performance expectations, often it is much easier to use tasks which have a hundred years of baseline data described (Carr, 1917) and redescribed (Bak et al., 2017) – in that way there is a pressure on research to *not* push the boundaries of behavioral design. The experimental design presented here was only made theoretically possible from prior studies utilizing the same environment, but not always with the exact same behavioral protocol (Olson et al., 2017; Olson et al., 2019; Johnson et al., 2021). In this way the stage had been set to collect data in a controlled fashion because of the gained intuition to design the proper training and recording protocols. This aspect to experiment design cannot be downplayed as a barrier for researchers seeking to employ novel behavioral designs and urges extensive pilot investigations into the optimal experimental design.

Looking at AB in similar triple-T sequential decision making tasks has only been performed in insects (Pasquier & Gruter, 2016; Okada & Kumano, 2022), but with varying task conditions, and in humans (Rothacher et al., 2020) however again the task structure did not allow for subjects to generate meaningful navigational strategies as they were only traversing the maze in a restricted manner. These previous studies have limited their investigation of specific behaviors, unlike the present work into naturalistic patterns of behavior. The current study Investigates AB at many levels and reveals some natural, and many potential experimental targets examples of AB changing around the time of missed trials. In the find-all-4 task almost half of error trials were also trials where the animal broke alternation at the final spatial decision. A much reduced effect was seen in the find-all-8 condition, suggesting that there is a heterogeneity in the types of errors being performed, especially across conditions.

The use of the external pathways also allows for exciting self-generated behavioral patterns to emerge. These pathways flank the internal ‘rewarded’ routes and do not need to be selected with any relation to the choice of route selected before or after. As reliable as alternation on the , one animal decidedly chose the long return route and had their choice-bias flipped on the find-all-4 condition. This is an interesting deviation from the rest of the animals and may point to a natural deviation in strategy planning. This animal did have this deviation removed over time with the introduction of the new pathways. This presents another novel finding. Animals’ biases for return routes to influence trial run choices was seen to diminish equally across both metrics of return route utilization. Why this pattern of behavior was seen is not clear, but it remains as a provocative metric which should be tracked during experimental manipulations.

Animals have been seen to be able to be trained on second order alternation tasks, though with some difficulty depending on the experimental design (Hunter & Hall, 1941). What has never been reported before was a naturalistic use of this higher order alternation ability in rats in a manner that rats would generate such a pattern of behavior themselves when not explicitly rewarded for it. With multiple nested decision points the ability to consider each decision with regard to the specific spatial location as well as the generalized choice position affords for more analytics to be applied when looking at choice data. Second order alternation as a scheme emerges from the second choice point in a fashion which is consistently not-chance, but inconsistently above or below chance levels. This surprising facet to alternation behavior on this experimental design gives a metric to analyze self-generated second order alternations on experimental designs with similarly arranged choice points.

These metrics further provide fields such as AI a look into naturalistic patterns of exploration. Triple-T environments have been employed for automata without knowledge of what would be expected of an animal and thus prevented naturalistic comparisons. With these

metrics, and a reliable task-structure, automata which are trained to explore triple-T environments (Yaman et al., 2019; Zou et al., 2021) can further have their navigation studied with a naturalistic lens.

Influence of Physical and Task Structures

Critically important to the interpretation of these behavioral data is a consideration of the task and environment structure. The problem posed to the animal and potential solutions to the problem of the find-all task presents, intuitively, an urge to organize subsequent choice in a way to optimize performance with as few rules as possible. Additionally it is known animals will structure behavior around the physical structures of the environment (Montgomery, 1951). Therefore by demanding a task which has a pressure to organize choices, and by providing physical structures which are navigated in a systematic way it can be expected to see the structure

The latent learning protocol further demonstrates a fascinating discovery in the ease of learning rats demonstrated incorporating double the number of choices on the find-all task. Additionally the fact that error distributions across time resembled the same as those seen on the find-all-4 with regard to block-structure (Fig 4.2B,E) suggests that the task structure itself was carried over and may have been a key factor in rats performing to proficiency within a single day of the new pathways being presented.

Use of return route structure was also seen to deviate across animals for the find-all-4 condition in a manner that all animals utilized the return routes, however, one animal did so in an opposite fashion. The meaningful incorporation of the return routes puts forward the importance of having the space of trial choices being made circuitous such that animals were uninterrupted across many trials. In doing so animals were afforded the ability to develop strong heuristics. Why these heuristics diminished so much during latent learning is unclear, however

further analyses may reveal a nuanced relationship between return route usage and trial run choice on the find-all-8-condition.

Increasingly being appreciated by neuroscience research, sometimes in experiments on the triple-T environment, are brain regions which have neurons that respond along complex frames of reference defined by the presence of structure in the environment (Olson et al., 2017; Johnson et al., 2021), or the degree of structure in an expected trajectory (Alexander et al., 2020). Previous studies have also identified many brain regions which are modulated by allocentric positions across frames of reference anchored to the maze environment itself (Alexander & Nitz 2015; Olson et al., 20219). Not fully explored are what specific features of space modulate activity across all of these brain regions. That is to suggest that certain structural features of space may regularly modulate these allocentric responses that have not yet been controlled for in a systematic way as see is possible on the triple-T (Olson et al., 2019). These findings present motivation to study more regions of the brain along the same experimental design while also providing motivation to see the influence of disrupting these brain regions on performance of triple-T tasks.

Future Directions

Investigations into behavior on the triple-T maze are still very early compared to the long history of single-decision tasks. These behavioral studies situate themselves uniquely in a position to adapt many of the same lesion, stimulation, and pharmacological experimental manipulations done over the past hundred years on the single-T maze. Alternation at turn 1 had a very high presentation (Fig 4.5A,F), alternation at the final decision point was equivalent to the first decision point for the find-all-8 condition, but was slightly diminished in the find-all-4 condition (Fig 4.6A,F), and in the find-all-8 condition the intermediate turn showed a slightly diminished rate of presentation (Fig 4.8A,F) with more dynamics around error trials than the previously described spatial alternations (Fig 4.8D). These are only a few of the trends

described here, and already present an interesting area of investigation not available on sing-decision tasks. Future studies which investigate manipulations previously performed on single-decisions might very well find more robust disturbances of AB, or even the presence of AB at some turns and not others. Thus the triple-T find-all tasks present a new chapter in behavioral studies.

Yet to be incorporated to behavioral assessments such as this are metrics of vicarious-trial-and-error (VTE). Due to the relative subjectivity in classifying this as a behavioral moment on the currently available tracking data. VTE events certainly do permeate many of these datasets to varying degrees with some being almost imperceptible, or easily confused with other behavioral stereotypy events which may be differentiable with the use of tools such as DeepLabCut (Mathis et al., 2018). VTE behavior is known to influence future decisions (Goldenberg et al., 2020), and is shown to dramatically impact the spatial navigation system (Johnson & Redish, 2007, Tang et al., 2021).

Previous researchers have had great success with advanced modelling techniques such as hidden Markov model generalized linear models (HMMGLMs) which are used to detect hidden decision making states which, even on tasks with only two options, are able to pick up dynamic moments of bias behaviors as well as other ‘strategies’ that were difficult to describe (Ashwood et al., 2022). These tools have been used to explain why animals lapse in 2-choice tasks, but it appears they may be better suited to datasets composed of many more choices, and thus more ‘types’ of errors which could be made. For each condition there are n! choices where n is the number of choices in the condition. The number of path combinations for perfect blocks in the find-all-4 task equals 24 possibilities, and the number of combinations in the find-all-8 condition explodes to 40320 possibilities. This presents a hurdle to behaviors which employ multiple combinations of possible ‘right’ answers, to what degree does regularity and patterning occur within experimental days. It is possible that at times the animal is engaged with one

strategy and then, for unexplored reasons, the animal decides to employ a different strategy such as alternating at various spatial locations along the maze, or the display of 2nd order alternation seen in the second choice point which had the most dynamic peri-event probability of any variable examined (Fig 4.9H) as well as the most variability across recording days. HMMGLM techniques would be uniquely situated to detect decision states as well as the behaviors which corresponded to them.

CONCLUSION

Rats naturally learn and employ strategies to perform on triple-T tasks with excellent performance. The utilization of alternations at each spatially defined location is the most apparent strategy employed along with, on the simpler task rat-specific heuristics involving return route to trial choice associations. These data highlight many key perspectives with which to analyze future behavioral data collected on triple-T environments.

The use of a T-maze in the contemporary neuroscience culture may not be the type of experimental design which generates much excitement on its own. However, we are currently at the cusp of a revolution in neuroscience which promises to deliver on a long called for increased sophistication with behavioral design (Olton, 1979). The triple-T maze affords physical structure upon which stereotypical behaviors are able to play out, and the find-all task affords the animal the motivation to navigate the space and use behaviors like AB to perform more optimally. Together this experimental design advances a field which has not progressed much in the last hundred years with regard to meaningful elaborations on task design.

Experimental questions will, it seems, continue to demand reliable behavioral metrics with which to probe systems of memory, attention, planning, and navigation. T-maze studies which have been previously explored in depth continue to guide investigations today despite many T-maze studies showing weak, unreliable, or transient results. Expanding the application

of behavioral tasks to ones which afford many more metrics additionally affords neuroscience an increased investigatory power.

Books have been written on AB as it has been known across its long use as an experimental method (Dember & Richmond, 1989), primarily on single decision points. It will be interesting to see how AB across multiple spaces as well as the relationship of AB across various distributed spaces is influenced by experimental design. Additionally the use of spatial features as a heuristic to guide future choices motivates future studies to more explicitly study the role spatial location of particular structures in decision-making behaviors.

METHODS

Rats

Subjects were 6 male Sprague Dawley rats all under 6 months old prior to the initiation of training. Rats were initially started on an ad libetum feeding schedule. Following the initial habituation phase of training rats had their food intake lessened to reduce body weight down to approximately 90% baseline weight. This motivated state ensured rats learned the triple-T working memory task quickly. Weight was monitored throughout the experiment to avoid fluctuations.

All rats were, at the time, undergoing simultaneous neurophysiological studies as well. As such these animals had all been surgically implanted with microwire electrodes (diameter = um). All rats had their brain tissue verified post-hoc for the absence of apparent lesions, abnormal gliosis, or other histological changes that would indicate a chronic issue throughout data collection. Due to this all animals' nervous systems were considered to be intact and not lesioned in any manner.

Triple-T Maze Environment

Experiments were conducted on a “triple-T” path-network maze. The track (Fig 4.1A-B (left) ; 8-cm-wide pathways, overall perimeter 1.6 m × 1.25 m in length and width, painted black) stood 20cm high in the middle of the recording room. The track edges were only 2 cm in height, allowing an unobstructed view of the environment’s boundaries and associated distal visual cues. Access to certain areas of the maze were restricted by placing painted black cans at key junctions. The placement of these blockers configures the available space to a total of 4 internal pathways, defined by their terminus location, each measuring 140 cm in length with junctions located 51 cm, 87 cm, and 118 cm along each internal pathway. Two perimeter routes flank the internal portions of the maze and were defined, each 197 cm in length, based on which side of the maze they were on.

Spatial Working Memory Task

Rats were habituated to the “triple-T” maze for 2 periods of about 30 minutes prior to training. During the first habituation period the animal had access to the entire maze without any blockers present. The second habituation period took place the following day and only some of the possible internal pathways were made available. Following habituation rats were trained to traverse one of the four available internal pathways in for a food-reward. Following the collection of the food reward animals learned to utilize the perimeter routes of the maze to return to the ‘main stem’, the shared portion of each internal route’, and begin another traversal for another food reward. Rats were permitted to choose whichever route back to the ‘main stem’ they preferred and were also permitted to turn around only on the perimeter pathways. Rats often did not change their direction however often restricting their behavior to a single direction for each position of the maze. Once animals regularly performed 80% or more non-interrupted traversals of all four internal pathways a reward schedule was implemented which required the rat to

obtain each of the 4 potential rewards before the rewards were replaced. Rats quickly learned this visit-all-4 rule and performed the task reliably quickly and with high accuracy.

Latent Learning of New Pathways

After several weeks of being recorded performing the find-all-4 task some rats were given one day where a set of blockers, preventing the rat from navigating to the four pathways on the same side as the 'main stem' (Fig 4.1B (right)), was removed. On this day the rats began a find-all-8 paradigm which shares the same rule structure as before, but now includes the newly available pathways. Initially rats were allowed to perform however they chose, all but one rat chose to collect the already learned pathways and seemed oblivious to the change in path-connectivity. Two of the rats perseverated on the already learned pathways and appeared, after as many as 20 traversals, to still not notice the change to their environment. For these rats small experimenter cues tapping at the location of the changed blocker were made a single time each. Once the change was noticed by the rats they did not require any further cueing. Rats were allowed to complete two entire blocks of the find-all-8 condition before being removed from the maze for the day. All subsequent days rats were tested on the find-all-8 condition

Recording Sessions & Performance Analyses

Animal position data was collected at 60Hz using a ceiling-mounted camera, mounted 305cm above the recording room floor. Colored LED lights affixed to the implants of recorded animals were tracked using Plexon CinePlex Studio software to obtain X,Y coordinates. Lights were approximately 4.5cm apart and were positioned perpendicular to the heading of the animal.

Coordinates extracted from Cineplex were fed into a custom built MATLAB GUI to define each traversal. First the user defines, in space, a gate which is the shared beginning point for each internal-pathway traversal as well as an ending gate for each internal route stemming from

the starting gate. The MATLAB script automatically extracts all traversals belonging to each route. The user then verifies that all routes were collected. Another custom MATLAB script uses the choice data from each recording to reconstruct the block structure imposed during the recording. Individual traversals are marked for their features including if it was rewarded, an alternation from the previous choice, and if the short return route was taken (when applicable).

Alternations for each decision point were made across two reference frames. The first considered each decision point unique to its location. This measure was calculated by considering the turn being made (left or right) differed from the previous traversal when the animal made a decision at that specific location. Another method of calculating alternation was through considering each decision point after the first agnostic to the spatial location. That is an alternation would be considered to be made if the rat chose to turn left at the second decision point and on the previous trial had turn right at whichever second decision point was encountered through that traversal. Second degree alternations were identified through identification of trials

Peri-event plots of behavior probability were calculated for trials within each recording from trial n to the end less n trials where n is equal to the number of blocks viewed around each event. For the find-all-4 recordings an n size of 8 was used, for the find-all-8 recordings an n size of 16 was used corresponding to the lengths of two full blocks before or after each event of interest. Binary vectors for each variable were used to identify when events such as error trials had occurred. n trials behind to n trials in front of the identified trial where the behavior of interest occurred were collected for each recording. Mean value across recordings with standard deviation across recordings is presented.

Tests for bimodality were performed using a Hartigan's dip test of unimodality. (Hartigan & Hartigan, 1985) bin width set to 0.05 for the range of 0 to 1. MATLAB code adapted from F. Mechler was used (Mechler, 2002) for the calculation of the dip statistic. p-values were

calculated by sampling a random distribution values equivalently sized to the test data generating a histogram of equal bin width and sample size, but without predefined structure. The dip statistic was calculated on and re-calculating the Hartigan's dip statistic. This was repeated 10,000 times to generate a null distribution. For this statistic p is equal to the sum of instances when the bootstrapped statistic was less than the actual data.

Bootstrapping

Two methods of bootstrapping were done, both required a shuffling of the choice data by the rats and re-running the previously mentioned analyses. The first took the vector of pathways chosen, both internal and external and created a collection of all run internal pathways choices and all external pathways. The position of either internal or external path was used for each randomly created path-vector where the choice at each position was randomly sampled from the pool of all traversals made. This method preserves the sampling probabilities without the presumption of learning the structure of the find-all tasks. The second method preserved the structure of blocks by creating the shuffled vector one block at a time. For each block beginning with the first internal route chosen (when P(reward) = 1) , a pool of routes was of all internal and external choices made up until the return route following the final reward being collected that block. This method of bootstrapping preserved the overall sampling probabilities of each route as well as overall reward rate without the assumption of any strategies being employed.

Acknowledgements

Chapter 4, in full, is a reprint of the material as it appears in the following manuscript that is currently being prepared for submission for publication: Johnson, A.B., & Nitz, D.A. Self-Considering Spatial Recurrence to Probe Self-Organized Behavior on a Latent Learning Task. The dissertation author was the primary investigator and author of this paper.

Figures

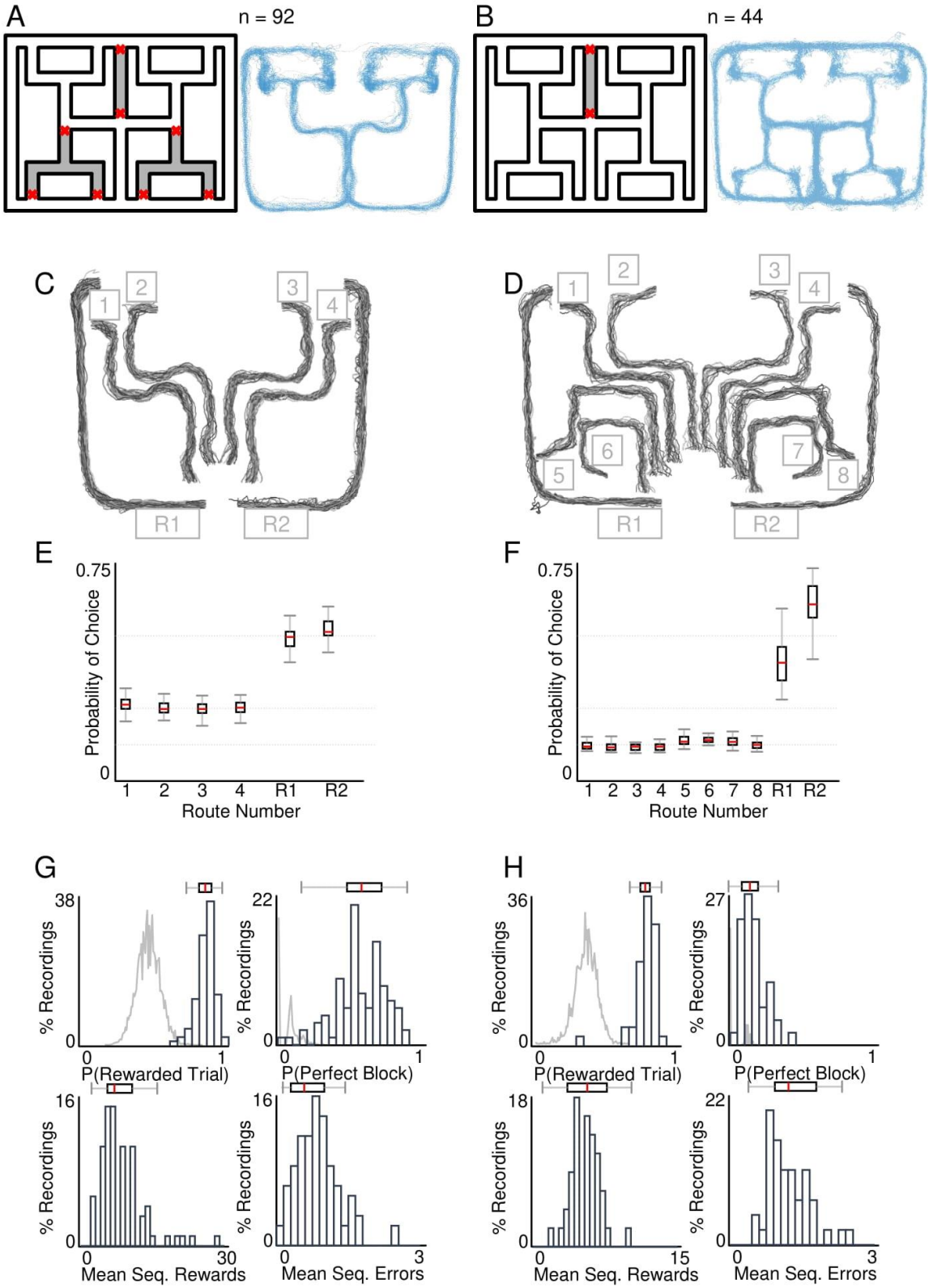

FIGURE 4.1: Triple-T Environment and Performance on Working Memory Task

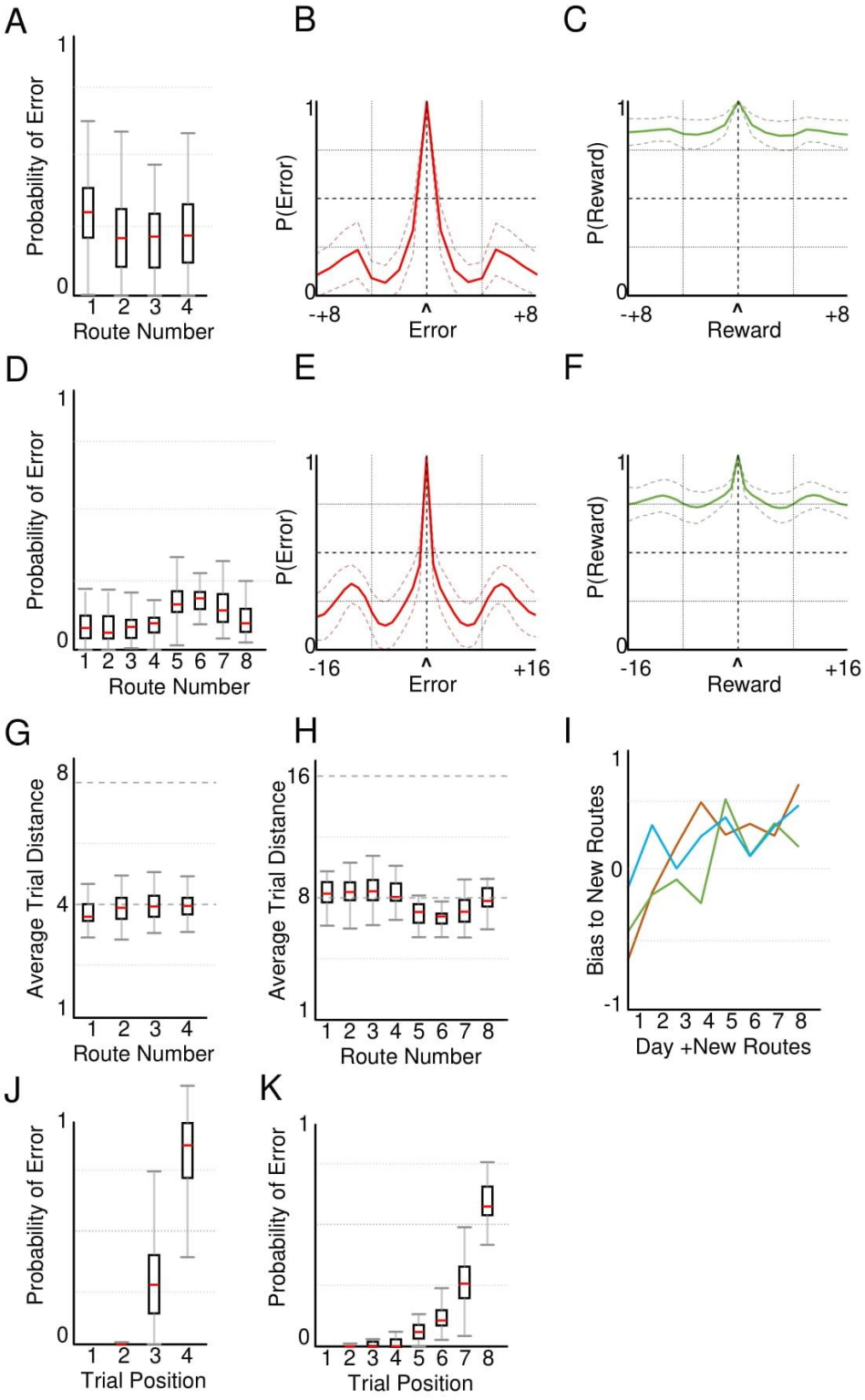

FIGURE 4.2: Assessment of Errors Across Routes and Trials

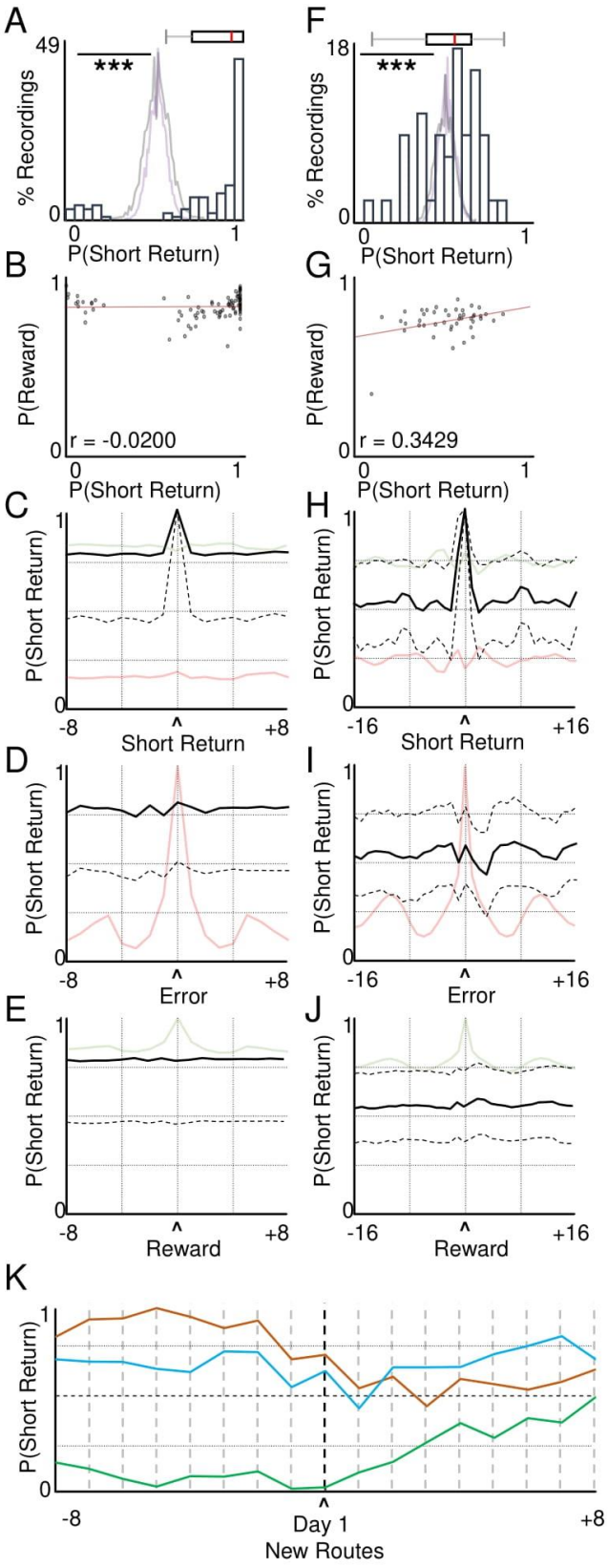

FIGURE 4.3: Detailed Examination of Choosing the Short Return Path Following a Traversal

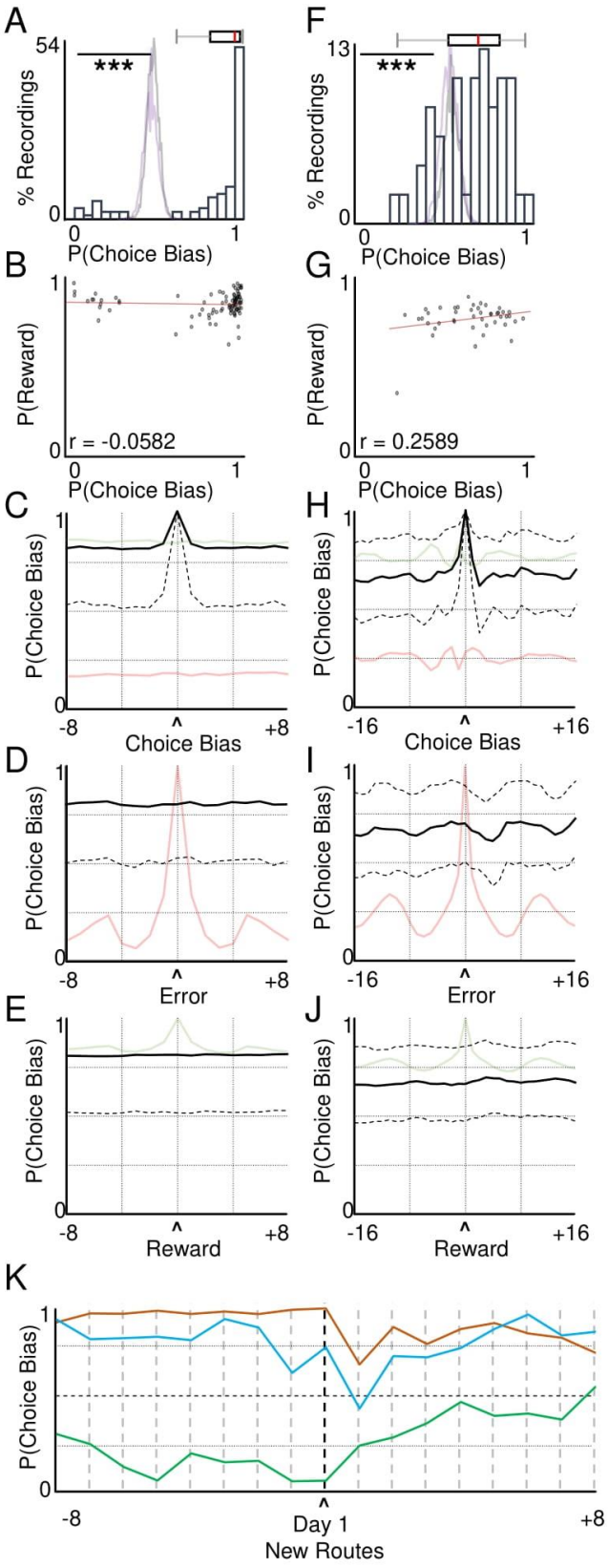

FIGURE 4.4: Detailed Examination of Choice Biased to Previous Return Route Chosen

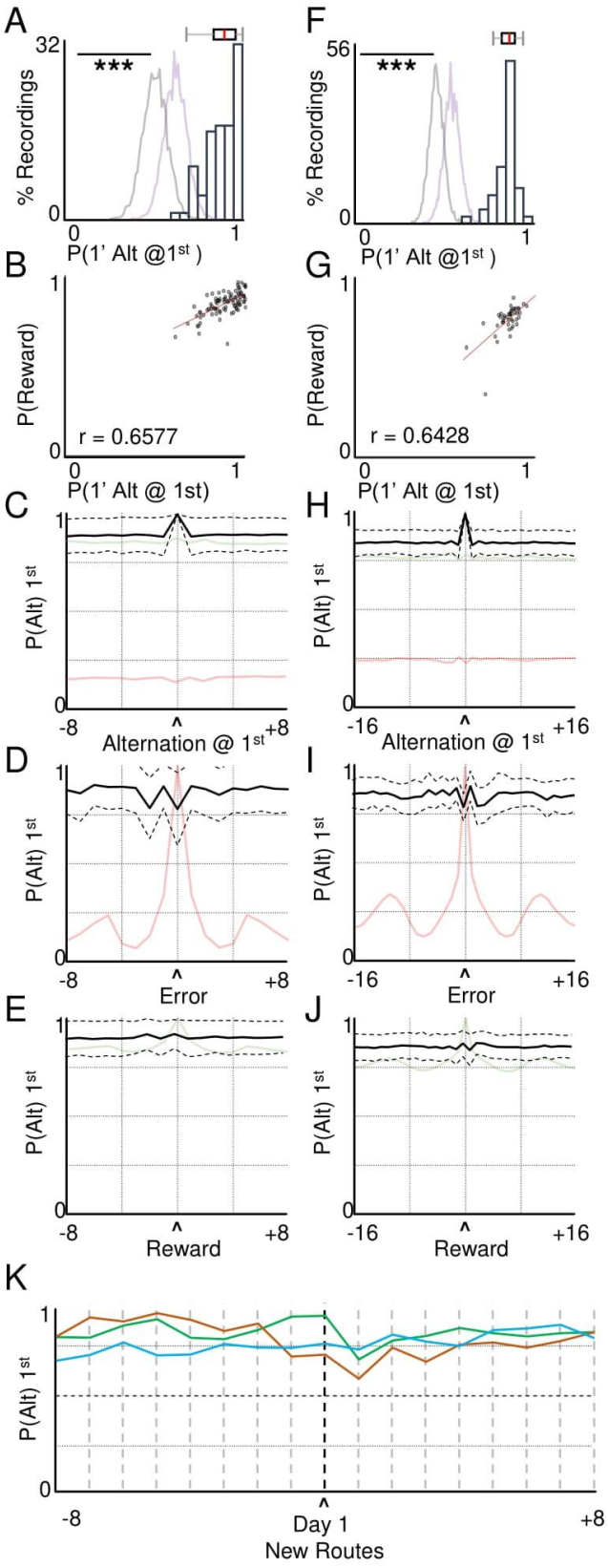

FIGURE 4.5: Detailed Examination of Alternation Behavior At the First Junction

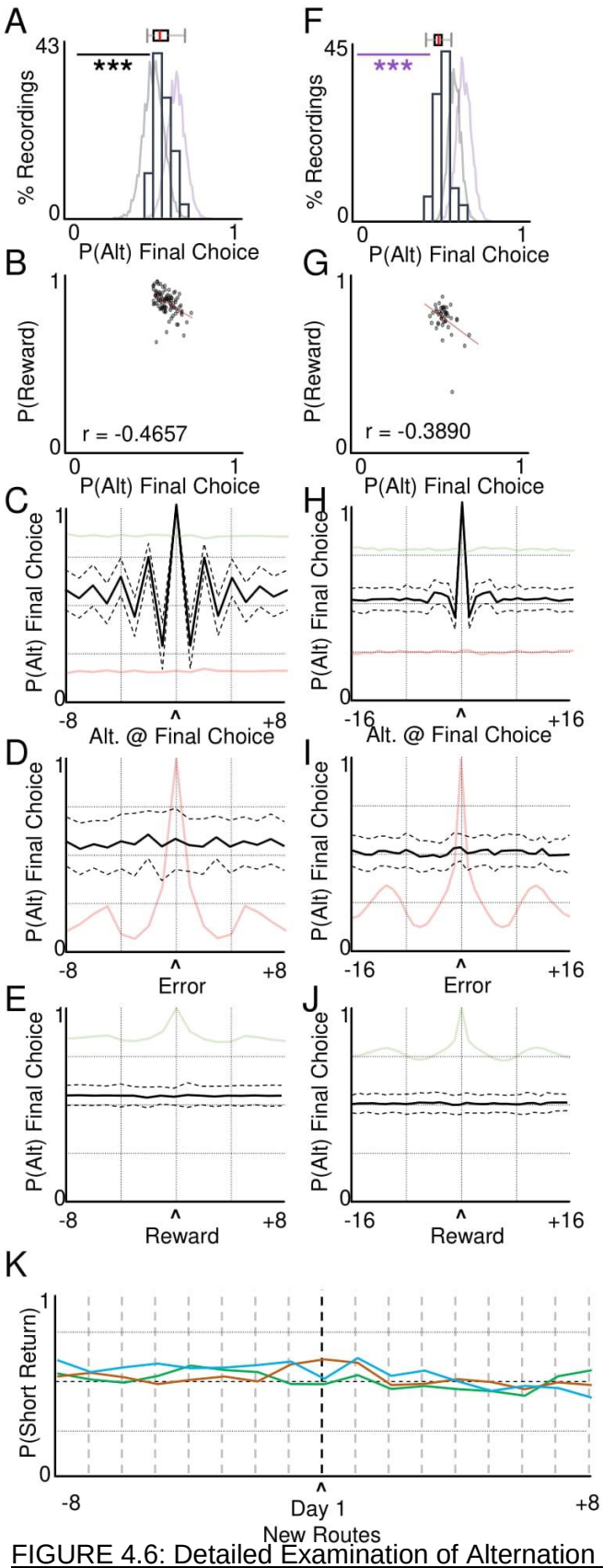

FIGURE 4.6: Detailed Examination of Alternation Behavior For Each Final Junction

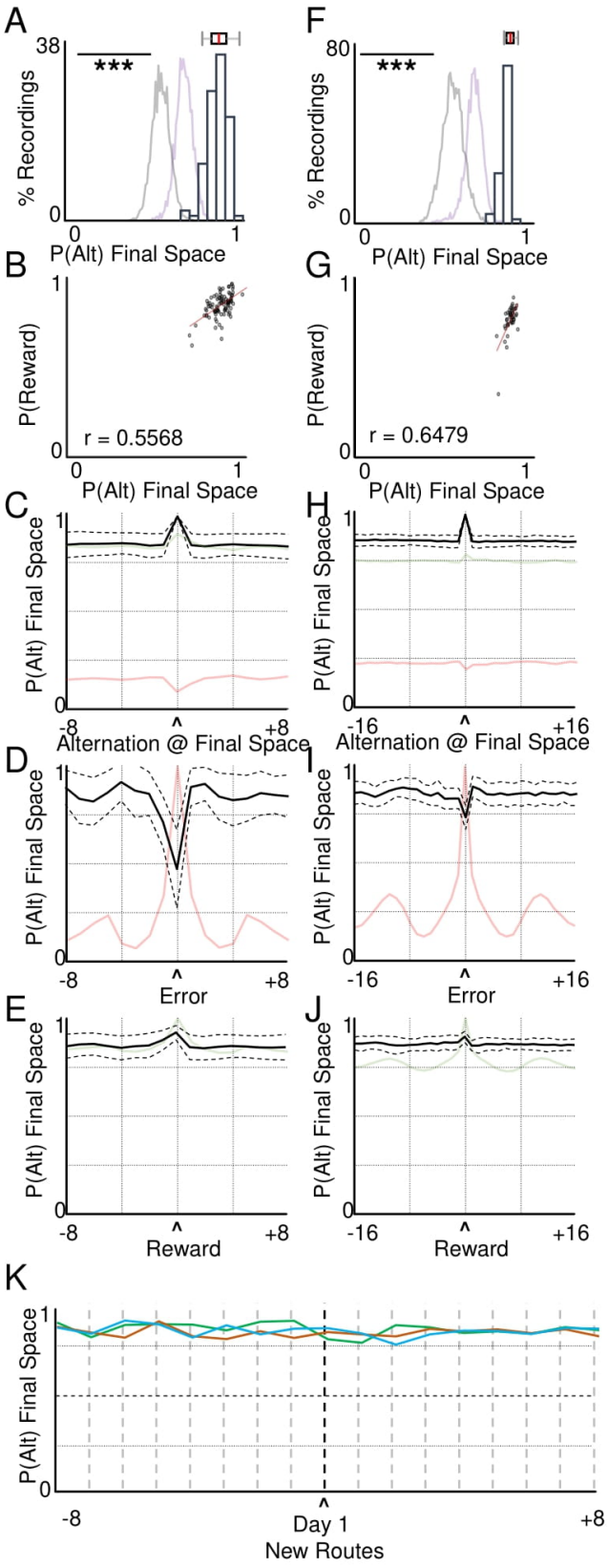

FIGURE 4.7: Detailed Examination of Alternation Behavior for the Final Decision

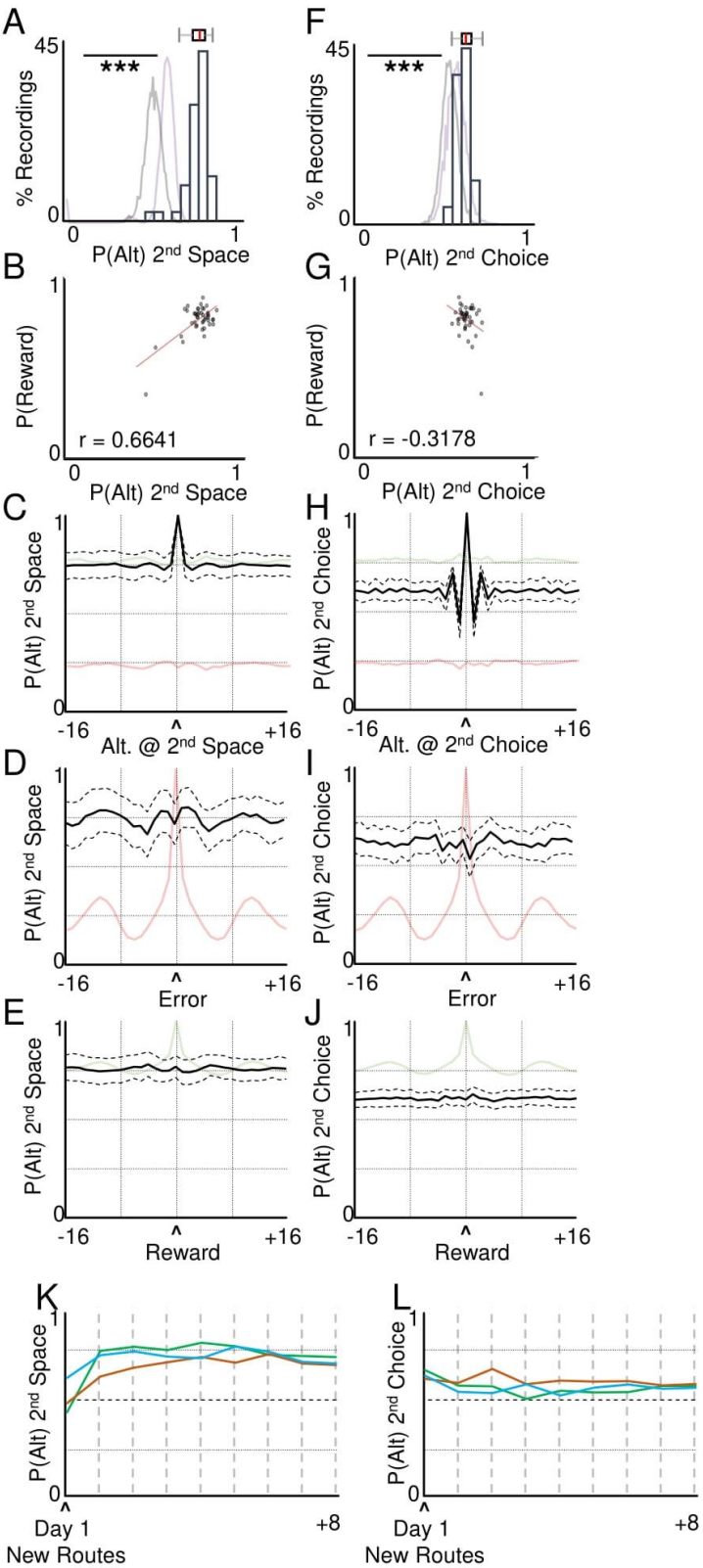

FIGURE 4.8: Detailed Examination of Alternation Behavior at the Second Decision in All-8 Condition

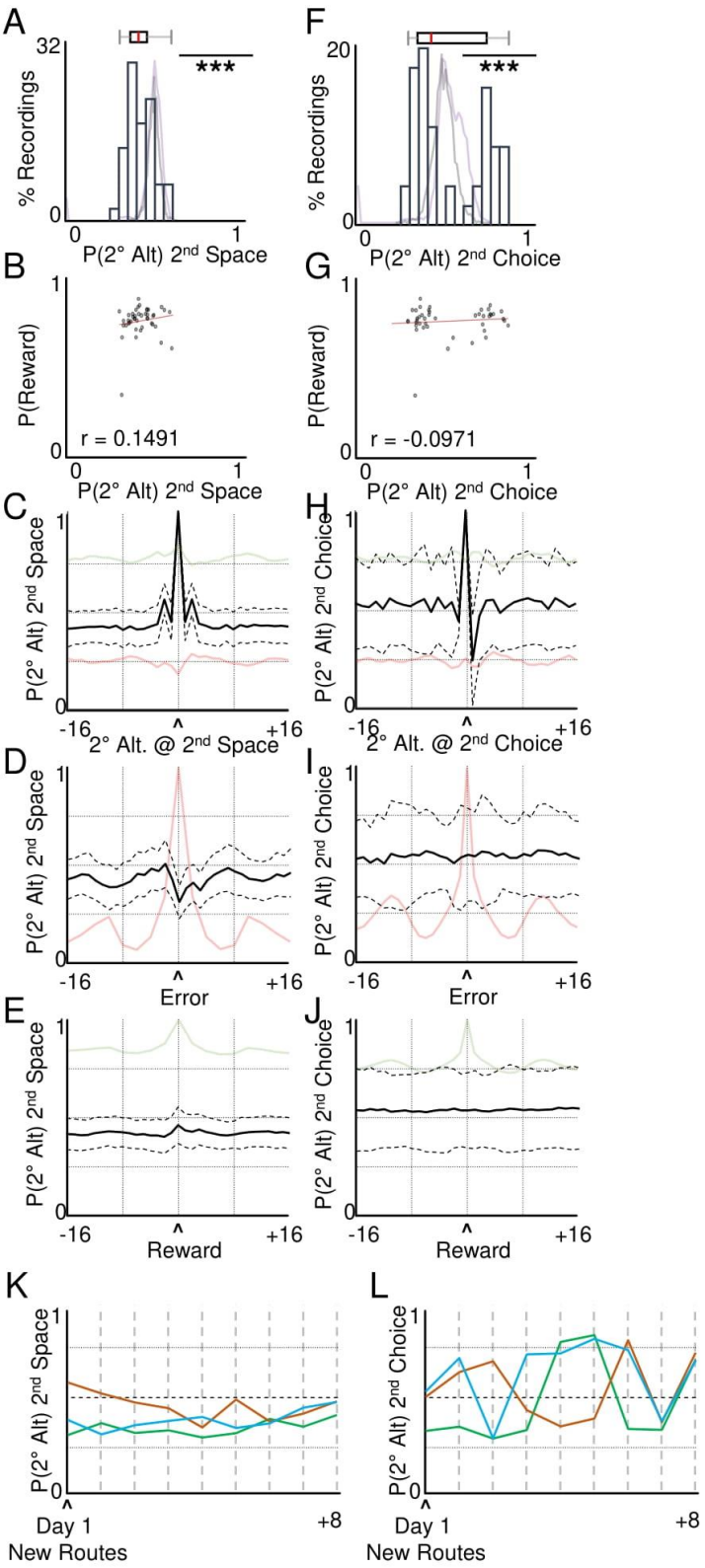

FIGURE 4.9: Detailed Examination of Second Order Alternation Behavior at the Second Decision

Figure Legends

Figure 4.1 Triple-T Environment and Performance on Working Memory Task

The find-all working memory task demanded of the animal that they navigate along the corridors of the triple-T in order to locate one of either 4 or 8 rewards. At the start all rewarded locations are baited, and one-by-one the animal collects them until they are all collected by the animal. At that point the rewarded locations are re-baited and a new block of trials begins being recorded. Animals are permitted to return to already-collected reward locations, however they are only rewarded for the first visit per place per block. Total number of recordings for the find-all-4 n = 92, total number of recordings for the find-all-8 n = 44.

A) Schematic drawing of triple-T environment set up for the find-all-4 condition. Blockers (red X's) were used to make certain portions of the maze inaccessible shown as shaded regions. Example tracking data collected from rat DN14 shown to the right of the schematic. **B**) Schematic drawing of triple-T environment set up for the find-all-8 condition. Only one corridor utilizing blockers (red X's) shown as shaded region. Example tracking data collected from rat AJ5 shown to the right of the schematic. **C)** Four possible routes were recorded as defined by their terminus location (Internal routes 1,2,3, and 4). Two external return routes (R1 & R2) flanked the internal routes and returned the animal back to the main stem of the maze shared by all four internal routes. **D**) Same as C but for the find-all-8 condition **E**) Probability of each route selection in the find-all-4 condition showing a relatively equal sampling of all routes. **F**) Same as E but for the find-all-8 condition. **G**) Basic performance metrics across recordings for probability of obtaining a reward (mean = 0.8429 s.d. = 0.0645, Bootstrapped(BS) KS.test $p < 0.001$), performing a block without any errors (mean = 0.5624 s.d.= 0.174 , BS KS.test $p< 0.001$). Metrics for how many rewards were obtained in sequence before an error (mean = 9.079 sequential trials s.d. = 4.387 trials), and how many errors were performed in sequence

before a reward was obtained (mean = 1.568 sequential errors s.d. = 0.3068 trials). **H**) Same as G but for the find-all-8. Probability of reward (mean = 0.762 s.d. = 0.0841, BS KS.test $p < 0.001$), probability of a perfect block (mean = 0.162 s.d. = 0.0854, BS KS.test $p < 0.001$), mean sequential errors (mean = 1.904 sequential errors s.d. = 0.7347 trials) , mean sequential rewards (mean = 6.077 sequential trials s.d. = 1.3549 trials).

Figure 4.2 Assessment of Errors Across Routes and Trials

A) Boxplot of probability of each route being chosen across recordings. **B**) Peri-event plot of probability of performing an error anchored to every instance of an error being performed. **C**) Same as B, but for rewards instead of errors. **D-F**) Same as A-C but for find-all-8 condition. **G**) Boxplot of trial-distance for each route calculated for each recording. **H**) Same as G, but for find-all-8 condition. **I**) Plot of bias score calculated for each of the 3 rats who underwent the latent learning protocol data across days from the first recording on the find-all-8 condition through 8 subsequent recording days. **J**) Boxplot of probability of an error occurring at each trial-position. **K**) Same as J, but for the find-all-8 condition.

Figure 4.3 Detailed Examination of Choosing the Short Return Path Following a Traversal

A) Histogram of the probability of choosing the short return (mean = 0.7738 s.d = 0.3177 dip =0.075 p = 0.4534). Gray line is a bootstrapped distribution made from a complete shuffle of the data (KS.test $p= 1*10^{-52}$). Purple line is a bootstrapped distribution made from shuffling data within each block (KS.test $p= 1*10^{-54}$) .**B**) Scatterplot correlating the probability of choosing the shorter return path for each recording with the probability of obtaining a reward for the recording. (Pearson's r = -0.02) **C**) Peri-event plot of probability of choosing the short return (black) anchored to each instance of having chosen the short return route. One standard deviation in either direction is a dashed black line. Red line denotes probability of performing an error. Green line denotes probability of obtaining a reward. **D**) Peri-event plot of probability of

choosing the short return (black) anchored to each instance of performing an error (red). **E**) Peri-event plot of probability of choosing the short return (black) anchored to each instance of obtaining a reward (green). **F-J**) Same as A-E, but for the find-all-8 condition. Probability of taking the short return (mean =0.5325 s.d. = 0.1686 , All Bootstrapped KS.test $p=1*10^{-7}$ Blocked Bootstrapped KS.test $p = 1*10^{-8}$). Correlation of probability of taking the short return to probability of getting a reward (Pearson's r = 0.34). **K**) Plot illustrating probability of taking the short return leading up to, during, and after the transition to the find-all-8 condition. Three rats shown were three used during the latent learning protocol (Green = AJ5; Blue = BL2; Orange = NS23).

Figure 4.4 Detailed Examination of Choice Biased to Previous Return Route Chosen

A) Histogram of the probability of biasing trial run based on previous return (mean = 0.8063 s.d = 0.2893 dip = 0.075 p = 0.4568). Gray line is a bootstrapped distribution made from a complete shuffle of the data (KS.test $p= 1*10^{-54}$). Purple line is a bootstrapped distribution made from shuffling data within each block (KS.test $p= 1*10^{-56}$) .**B**) Scatterplot correlating the probability of biasing trial run based on previous return for each recording with the probability of obtaining a reward for the recording. (Pearson's r = -0.0582) **C**) Peri-event plot of probability of choosing a trial run based on previous return (black) anchored to each instance of having biased their trial run. One standard deviation in either direction is a dashed black line. Red line denotes probability of performing an error. Green line denotes probability of obtaining a reward. **D**) Peri-event plot of probability of choosing a trial run based on previous return (black) anchored to each instance of performing an error (red). **E**) Peri-event plot of probability of choosing a trial run based on previous return (black) anchored to each instance of obtaining a reward (green). **F-J**) Same as A-E, but for the find-all-8 condition. Probability of choosing a trial run based on previous return (mean =0.6615 s.d. = 0.1826 , All Bootstrapped KS.test $p=1*10^{-16}$ Blocked Bootstrapped KS.test $p = 1*10^{-17}$). Correlation of probability of choosing a trial run

based on previous return to probability of getting a reward (Pearson's r = 0.2589). **K**) Plot illustrating probability of choosing a trial run based on previous return leading up to, during, and after the transition to the find-all-8 condition. Three rats shown were three used during the latent learning protocol (Green = AJ5; Blue = BL2; Orange = NS23).

Figure 4.5 Detailed Examination of Alternation Behavior At the First Junction

A) Histogram of the probability of alternating at the first decision point (mean = 0.8877 s.d = 0.0872). Gray line is a bootstrapped distribution made from a complete shuffle of the data (KS.test p= $1*10^{-80}$). Purple line is a bootstrapped distribution made from shuffling data within each block (KS.test p= $1*10^{-77}$) .**B**) Scatterplot correlating the probability of alternating at the first decision point for each recording with the probability of obtaining a reward for the recording. (Pearson's r = 0.6577) **C**) Peri-event plot of probability of alternating at the first decision point (black) anchored to each instance of having just alternated at the first decision point. One standard deviation in either direction is a dashed black line. Red line denotes probability of performing an error. Green line denotes probability of obtaining a reward. **D**) Peri-event plot of probability of alternating at the first decision point (black) anchored to each instance of performing an error (red). **E**) Peri-event plot of probability of alternating at the first decision point (black) anchored to each instance of obtaining a reward (green). **F-J**) Same as A-E, but for the find-all-8 condition. Probability of probability of alternating at the first decision point (mean =0.8483 s.d. = 0.0608 , All Bootstrapped KS.test p=$1*10^{-40}$ Blocked Bootstrapped KS.test p = $1*10^{-38}$). Correlation of probability of alternating at the first decision point to probability of getting a reward (Pearson's r = 0.6428). **K**) Plot illustrating probability of alternating at the first decision point leading up to, during, and after the transition to the find-all-8 condition. Three rats shown were three used during the latent learning protocol (Green = AJ5; Blue = BL2; Orange = NS23).

Figure 4.6 Detailed Examination of Alternation Behavior For Each Final Junction

A) Histogram of the probability of alternating at the final decision space (mean = 0.8552 s.d = 0.0531). Gray line is a bootstrapped distribution made from a complete shuffle of the data (KS.test p= $1*10^{-81}$). Purple line is a bootstrapped distribution made from shuffling data within each block (KS.test p= $1*10^{-68}$) .**B**) Scatterplot correlating the probability of alternating at the final decision space for each recording with the probability of obtaining a reward for the recording. (Pearson's r = 0.5568) **C**) Peri-event plot of probability of alternating at the final decision space (black) anchored to each instance of having just alternated at the final decision space. One standard deviation in either direction is a dashed black line. Red line denotes probability of performing an error. Green line denotes probability of obtaining a reward. **D**) Peri-event plot of probability of alternating at the final decision space (black) anchored to each instance of performing an error (red). **E**) Peri-event plot of probability of alternating at the final decision space (black) anchored to each instance of obtaining a reward (green). **F-J**) Same as A-E, but for the find-all-8 condition. Probability of alternating at the final decision space (mean =0.8648 s.d. = 0.0241 , All Bootstrapped KS.test p=$1*10^{-40}$ Blocked Bootstrapped KS.test p = $1*10^{-40}$). Correlation of probability of alternating at the final decision space to probability of getting a reward (Pearson's r = 0.6479). **K**) Plot illustrating probability of alternating at the final decision space leading up to, during, and after the transition to the find-all-8 condition. Three rats shown were three used during the latent learning protocol (Green = AJ5; Blue = BL2; Orange = NS23).

Figure 4.7 Detailed Examination of Alternation Behavior for the Final Decision

A) Histogram of the probability of alternating at the final decision choice (mean = 0.5565 s.d = 0.0485). Gray line is a bootstrapped distribution made from a complete shuffle of the data (KS.test p= $1*10^{-11}$). Purple line is a bootstrapped distribution made from shuffling data within each block (KS.test p= $1*10^{-10}$) .**B**) Scatterplot correlating the probability of alternating at the final decision choice for each recording with the probability of obtaining a reward for the

recording. (Pearson's r = -0.4657) **C**) Peri-event plot of probability of alternating at the final decision choice (black) anchored to each instance of having just alternated at the final decision choice. One standard deviation in either direction is a dashed black line. Red line denotes probability of performing an error. Green line denotes probability of obtaining a reward. **D**) Peri-event plot of probability of alternating at the final decision choice (black) anchored to each instance of performing an error (red). **E**) Peri-event plot of probability of alternating at the final decision choice (black) anchored to each instance of obtaining a reward (green). **F-J**) Same as A-E, but for the find-all-8 condition. Probability of alternating at the final decision choice (mean =0.5096 s.d. = 0.043 , All Bootstrapped KS.test p=0.7 Blocked Bootstrapped KS.test p = $1*10^{-8}$). Correlation of probability of alternating at the final decision choice to probability of getting a reward (Pearson's r = -0.389). **K**) Plot illustrating probability of alternating at the final decision choice leading up to, during, and after the transition to the find-all-8 condition. Three rats shown were three used during the latent learning protocol (Green = AJ5; Blue = BL2; Orange = NS23).

Figure 4.8 Detailed Examination of Alternation Behavior at the Second Decision in All-8 Condition

A) Histogram of the probability of alternating at the second decision space for find-all-8 recordings (mean = 0.7416 s.d = 0.0731). Gray line is a bootstrapped distribution made from a complete shuffle of the data (KS.test p= $1*10^{-36}$). Purple line is a bootstrapped distribution made from shuffling data within each block (KS.test p= $1*10^{-34}$) .**B**) Scatterplot correlating the probability of alternating at the second decision space for each recording with the probability of obtaining a reward for the recording. (Pearson's r = 0.6641) **C**) Peri-event plot of probability of alternating at the second decision space (black) anchored to each instance of having just alternated at the final decision choice. One standard deviation in either direction is a dashed black line. Red line denotes probability of performing an error. Green line denotes probability of obtaining a reward. **D**) Peri-event plot of probability of alternating at the second decision space

(black) anchored to each instance of performing an error (red). **E**) Peri-event plot of probability of alternating at the second decision space (black) anchored to each instance of obtaining a reward (green). **F-J**) Same as A-E, but considering probability of alternating at the second choice. Probability of probability of alternating at the second choice (mean =0.6073 s.d. = 0.0365 , All Bootstrapped KS.test $p=1*10^{-33}$ Blocked Bootstrapped KS.test $p = 1*10^{-19}$). Correlation of probability of alternating at the second choice to probability of getting a reward (Pearson's r = 0.3178). **K**) Plot illustrating probability of alternating at the second decision space leading up to, during, and after the transition to the find-all-8 condition. Three rats shown were three used during the latent learning protocol (Green = AJ5; Blue = BL2; Orange = NS23). **L**) Same as K, but considering probability of alternating at the second choice across recording days.

Figure 4.9 Detailed Examination of Second Order Alternation Behavior at the Second Decision

A) Histogram of the probability of alternating at the second order at the second decision space for find-all-8 recordings (mean = 0.4204 s.d = 0.0734). Gray line is a bootstrapped distribution made from a complete shuffle of the data (KS.test $p= 1*10^{-15}$). Purple line is a bootstrapped distribution made from shuffling data within each block (KS.test $p= 1*10^{-24}$) .**B**) Scatterplot correlating the probability of alternating at the second order at the second decision space for each recording with the probability of obtaining a reward for the recording. (Pearson's r = 0.1491) **C**) Peri-event plot of probability of alternating at the second order at the second decision space (black) anchored to each instance of having just alternated at the final decision choice. One standard deviation in either direction is a dashed black line. Red line denotes probability of performing an error. Green line denotes probability of obtaining a reward. **D**) Peri-event plot of probability of alternating at the second order at the second decision space (black) anchored to each instance of performing an error (red). **E**) Peri-event plot of probability of alternating at the second order at the second decision space (black) anchored to each instance

of obtaining a reward (green). **F-J**) Same as A-E, but considering probability of alternating at the second order at the second choice. Probability of probability of alternating at the second choice (mean =0.5254 s.d. = 0.1924 , All Bootstrapped KS.test $p=1*10^{-10}$ Blocked Bootstrapped KS.test $p = 1*10^{-10}$, dip = 0.1 p=0.0758). Correlation of probability of alternating at the second order at the second choice to probability of getting a reward (Pearson's r = -0.0971). **K**) Plot illustrating probability of alternating at the second order at the second decision space leading up to, during, and after the transition to the find-all-8 condition. Three rats shown were three used during the latent learning protocol (Green = AJ5; Blue = BL2; Orange = NS23). **L**) Same as K, but considering probability of alternating at the second order at the second choice across recording days.

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CHAPTER 5: The Utility of Structure: Spatial Similarities in Behavioral Neuroscience

This dissertation work has demonstrated in three ways the impact of a complex path-network environment on the representational qualities of nervous system activity, and on self-organized behavior. These impacts can be seen as the nervous system's ability to adapt representational qualities to environment's structure, and animals' ability to implement that structure in stereotypical ways to perform a working memory task. First, in chapter 2, with the comparison between CA1 neurons with subiculum (SUB) neurons with regard to spatially anchored activity patterns, data demonstrated that SUB is a brain region which has many neurons tuned across multiple spaces with similar physical structure. Second, chapter 3, with the investigation into posterior parietal cortex (PPC) neurons activity patterns across oppositely shaped trajectories, data showed that PPC extends known allocentric encoding beyond route cells to include routes of general structure which cannot be explained through self-motion. Finally, in chapter 4, with the behavioral analyses performed on animal behavior, data illustrated a novel display of alternation behavior (AB) distributed at similar T-shaped junctions. These studies each necessitated a particular structure exist in the external world to be perceived in order to influence the data in each way. That is to say, if similar experiments were performed on a single-T maze as opposed to the triple-T maze SUB neural responses may not be shown to represent structural analogy because the amount of structural analogy afforded to the system is relatively deprived. Similarly, PPC neurons appear to require complex routes to study its spatial representations appropriately. Without multiple routes involving different levels of similarity in self-motion it can become much more difficult to disentangle the effects of self-motion. The structurally complex environment was crucial for the behavior findings as well, the physical structure of embedded decision points, and the find-all task structure allowed for the exhibition of AB in a meaningful way.

Structure is ubiquitous in everything we experience through our day-to-day lives. Environments have structure in the form of physical boundaries, and tasks have structure in the form of a predictable events occurring in response to particular behaviors. What each use of the word ‘structure’ has in common is that within the domain which it is being applied, ‘structure’ refers to a meaningful arrangement of the respective features. The features of the environment are the physical boundaries around spaces which can define the structures around which one must navigate, like a T-junction. The features of the task are the reward contingencies, which give meaning to decision making and pressures the animal to organize their decision making processes to more optimally perform. To consider the environment with regard to structure the animal must both perceive features such as boundaries on their own, but also perceive the relationship to one another in some manner. Neural responses around particular features in the environment, like borders (Lever, et al. 2009) or landmarks (Deshmukh & Knierim, 2013) have been documented, but the application of a complex enough structure to fully appreciate the spatially tuned CA1, SUB, and PPC neural responses presented in the studies here had not been endeavored upon prior.

Environmental structure is the relationship across different features of space, and many of the possible structures perceived in an environment prompt the animal on how to behave. For example, the arrangement of borders in an environment define areas of accessibility and inaccessibility. The arrangement of the borders in this example is the environmental structure, and at different scales of perspective the structure of the environment can be described as a shape or multiple shapes. Some of these shapes, such as a T-shaped space, are meaningful in that they elicit predictable behaviors. Other shapes, such as the shape of a route taken to get to a reward, are meaningful especially in triple-T tasks where it can be generalized across all possible routes. Lastly, shapes such as the shape defined by the totality of navigable space are meaningful in that patterns of other shapes can be found to repeat within it, and lines of

symmetry naturally emerge which may assist in cognitive processes by breaking down more difficult navigational problems into simpler ones. Structural complexity defined from this perspective is an increased incidence of meaningful shapes that emerge from the total structure of that environment. A simple environment could consist of a linear path to traverse, the structure of which bounds the behavior of the animal to a single dimension and which can be described by the length of the corridor alone. Another simple environment could be an open arena, the structure of which affords the animal no bounds to their behavior and can be defined by the geometric shape of the boundaries alone. In these two examples, the minimal structure of the environment provides a single meaningful shape to the environment, but to dramatically different ends. A slightly more complex maze would be a maze consisting of a single T-shaped junction. Relative to the previous two examples, a single T-shaped junction lends itself to increased complexity as multiple scales of meaningful shapes emerge. Each linear portion of the maze is a shape unto itself, and the arrangement of the linear portions of the maze provides an additional T-shape. Even a small increase in complexity in the environment can allow for more meaningful tasks to be performed, which can shape behavior in dramatic ways.

Identifying what environmental structures guide behavior in stereotypical ways had been an active line of research (Carr, 1917; Mongomery, 1951; Douglas et al., 1974). However, in the last several decades, psychological research has gone from being dominated by behaviorist techniques to being biased toward contemporary neuroscience techniques which emphasize collecting unique forms of data. This current emphasis on specific neuroscience techniques include, for example, calcium transients (Harvey et al., 2012), recording from a genetically or anatomically defined subregion (Essig, et al., 2021), or utilizing sophisticated viral transfection techniques to influence neural activity with more specificity than a lesion through optogenetics and chemogenetics (Lammel et al., 2012; Roth, 2016). Relevant and high-impact data has changed from observing identifiable behaviors interpreted within their environmental contexts, to

observing biological correlates of particular events or behaviors. Sometimes these metrics and manipulations are variable, and require many instances of the behavior to be seen and validated. This has pressured the entire field to reduce structural-complexity of both tasks and environments used in experimentation. Less complexity has allowed for data to be collected and analyzed with greater speed and explained more thoroughly with ease. However, the trade off is that meaningful behaviors dependent on increased environmental complexity are not being captured. No domain of research suffered greater from this reductionist trend more than the study of spatial navigation.

A demand for using structurally complex environments could have been expected in 1976 with the discovery of place cells (O'Keefe, 1976), or expected in 1983 when place cells were seen to be directionally tuned (McNaughton et al., 1983), or again expected in the early 2000's with the discovery of trajectory dependence (Wood et al., 2000; Ferbinteanu & Shapiro, 2003), or at any point in the long study of the spatial navigation system of the brain. From the beginning of place cell study, and repeated throughout the decades has been evidence that particular structures and behaviors elicited dramatically different responses in place activity. Despite mounting evidence to the importance of structural context, the employment of such structures in recording studies has not become the standard; in fact very few papers specifically address the effect structures of different types have on the spatial navigation system, and fewer of them interpret their results as a direct result emergent from the structure of environment chosen (Leutgeb et al., 2005; Derdikman et al., 2009; Nitz, 2011; Dabaghian et al., 2014; Stensola et al., 2015). Interpretations of these data are rarely able to go beyond the fact that structure matters, and only in rare occasions (Nitz, 2011) do researchers include specific structural features in their interpretation of the data. Even more surprising is that despite several historical attempts to push the field in this direction (Olton, 1979) it is only recently that this

subject has come back in discussions (Fetsch, 2016). If this push for greater environmental structure is going to make a lasting impact or is merely a perennial trend is yet to be seen.

We know that spatial representations within the brain, such as with place cells of the hippocampus (HPC) (O'Keefe, 1976), consider more environmental and task features than border arrangement to manifest a representation of place. These features indeed involve the geometric shape the boundaries make (Leutgeb et al., 2005), but also the orientation in several frames of reference (Muller & Kubie, 1987; Deshmukh & Knierim, 2013), the specific connectivity places have to one another (Dabaghian et al., 2014), and even what experiences have previously occurred at that place (Kaufman et al., 2020, Poulter et al., 2021). In many ways the spatial navigation system of the brain considers nearly every imaginable feature of space in order to better represent it within the activity of neurons (Grieves & Jeffery, 2017). This growing constellation of observed spatial features encoded by various brain regions is a testament to the adaptive nature for which the spatial navigation system has evolved. What is critically underappreciated, however, is in what ways the nervous system is tuned to particular structures of these features. Studies on place cells as animals traverse maze environments with heavy recurrence (e.g. a spiral shape) clearly demonstrate that recurrence of structure can be a driving force for activity (Nitz, 2011). Findings such as this provide a deeper understanding of how recurrence of features in an environment organize spatial representations in the brain, and clearly demonstrate that environmental structure should be expected to play a major role in modulating brain activity.

In chapter 2 SUB neurons were found to have distinct 'break' points in their spatial representations around all corners. This novel consideration of the kind of space around which SUB neurons organize their activity was distinctly not seen in CA1 populations. This allows for interpretations of how space is being represented by each population differentially beyond the obvious display of analogous place fields in SUB neuron activity. It was found in chapter 3 that

about twenty percent (20%) of all PPC neurons had their activity best explained by spatial positioning. This adds to the interpretation of PPC neurons encoding for frames of reference at many levels beyond those defined by self-motion. Beyond route encoding, the fact that PPC neurons encode for general structure itself has many implications for future studies and data interpretation.

Findings from both chapters 2 and 3 give insights into the distributed spatial cognition system in the brain. It is important to consider these functional findings together with anatomical knowledge of a circuit that connects HPC to motor cortices via SUB, RSC, and PPC. This is what has been described as a space-to-action circuit (Vann & Aggleton, 2002; Yamawaki et al., 2016; Olson et al. 2019; Nitzan et al., 2020). Beyond the 'readout' of HPC place information there are strong recurrent connections from SUB directly back to CA1 necessary for some memory tasks (Xu et al. 2016, Sun et al. 2019). The multidirectional nature of these brain regions raises questions as to the relationship between the representations of place and potential representations of structure. From one perspective is a neural circuit which is poised to be impacted by the specific place representation of HPC efferent connections. Seeing HPC as a modulatory signal on efferent targets could perhaps explain the allocentric modulations of self-motion activity in RSC neurons (Alexander & Nitz, 2015) and M2 neurons (Olson et al., 2019). If the neurons presented in chapters 2 and 3 have their structural frames of reference defined by HPC place activity, then disruption of CA1 activity should reliably abolish SUB analogy encoding and PPC sensitivity to structure. This hypothesis would suggest that some integration of place activity is what gets construed as structure by the nervous system. From the other perspective this circuit is instead poised to directly inform and modulate place representations of HPC. This alternative view would help explain why lesions to PPC and SUB produce profound, but qualitatively different types of deficits during navigation as compared to HPC lesions (Morris et al., 1990; Save et al., 2005). If the neurons in chapters 2 and 3 are instead involved with the

creation of structured frames of reference that organize place cells, then a different set of expectations can be assumed. Disruption of CA1 place cells activity should instead not impact SUB representations of analogy and PPC sensitivity to structure, but disruptions of SUB or PPC should produce changes to CA1 place cells, perhaps only on a structurally complex maze such at the triple-T. Determining the 'flow' of information along this pathway will give a much better framework with which to interpret spatial representations of structure in the brain and their relationship to other cognitive processes.

Previous studies have attempted to describe SUB neuron activity as simply as CA1 neuron activity by studying SUB neurons along many of the same experimental paradigms that CA1 place cells are studied (Sharp. 1994; Kim & Frank, 2012; Lee et al., 2019. These paradigms commonly involve a relative lack in structural complexity. It should be expected from understudied brain regions that their neuron activity will respond to stimuli in unexpected ways. If, for instance, SUB is studied on unstructured environments or environments with simplistic structures it can be expected that SUB neurons will respond in an overly simplistic fashion resembling a low-quality place-cell in some contexts (Sharp & Green, 1994). There are many poorly studied brain regions that the field of neuroscience has yet to fully investigate. Chapter 2 highlights the importance of utilizing structured environments and tasks of sufficient complexity when investigating brain regions which are not well studied in order to potentially unveil surprising phenomena. Furthermore, both chapters 2 and 3 highlight the importance of re-exploring well studied brain regions such as CA1 and PPC on environments that are more complex compared to preceding investigations. Novel discoveries about CA1 such as a distinct lack of trajectory dependance at the subsequent decision after the first are surprising, and contradict some reports previously made (Ainge et al., 2007). What separates our study from previous studies is the combination of task design and environmental design that allows animals to self-generate their behavior during the find-all-4 task. This task difference for the triple-T

experiments may influence place representation and needs to be considered. It also could have been expected to see many place cells exhibit multiple place fields as the triple-T is a relatively large environment (Kjelstrup et al., 2008). However it also could have been expected to see those place fields distributed in a meaningful manner along the structure of the maze (Nitz, 2011; Grieves et el., 2017), which was not seen as compared to SUB neurons. The discoveries made in PPC neuron encoding additionally give much-needed context for studying PPC in rodents, and allow for a better interpretation for the many self-motion dominated rodent studies. These studies show that in addition encoding navigational behaviors (McNaughton et al., 1994) PPC neurons can encode postures (Mimica et al., 2018) , both of which are behavioral responses to particular structure in the environment.

Critical to all of these studies was a careful appreciation of the behavior being asked of our rats, which is specifically analyzed in chapter 4. The triple-T task afforded the experimental design an incredible possible number of explanatory variables and perspectives with which to look at the data. This is part of the hurdle scientists have had in designing coherent but complex structures for studies. As mentioned in chapter 4, it was only due to many years of pilot studies and experimenter observations (Olson et al., 2017; Olson et al., 2019) that made designing a meaningful task on the triple-T and identifying which behaviors were particularly relevant for their navigation possible. The triple-T environment allows for the find-all spatial working memory tasks consisting of serial left-right decisions. There were either two decisions in the find-all-4 condition or three decisions in the find-all-8 condition. The animals' ability to perform highly at the find-all task was utilized for neural recording experiments to ensure an even sampling of spaces. Surprisingly animals were able to learn and incorporate new pathways into the known find-all-4 schema quickly and efficiently after one day of a brief introduction. This finding again affords neurophysiological studies the ability to obtain a naturalistic even sampling of space,

and shows that rats are capable of much more difficult or complex tasks than currently get asked of them.

Beyond the behavior itself, how the rats *chose* to perform the find-all task, became fascinating in its regularity. Rats are known to exhibit AB regularly on T-maze tasks (Carr, 1917; Bak et al., 2017). This phenomenon, and how it modulates with environmental and task changes (Douglas, 1974), has been the source of study for almost 100 years. Interestingly, in our studies the rats chose to exhibit AB on the triple-T environment in a spatially organized manner which, when analyzed in some contexts reveals emergent spontaneous double-alternation behavior. These behavioral stereotypies become even more curious under the latent-learning transition from the find-all-4 to the find-all-8 condition. Rats learn and incorporate new pathways into a known task-schema immediately and better their performance as their behavior becomes more stereotyped. This incorporation of a new decision point quickly develops its own AB as well. This occurs without any diminishment to AB at either of the other two more experienced decision points. This is consistent with the rat's behavioral schema of choosing to alternate at every decision point when that space is encountered over subsequent trials.

One observation made from chapter 4 is that not all errors can be attributed to lapses in any one behavior in particular. This is especially true on the find-all-8 condition. Chapter 4 posits there exists some embedded patterning of behavior into larger strategy chunks. This evidence suggests that rats are strategizing about how to perform the task, and fluidly updating that strategy throughout the experiment. This hypothesis is backed by the occasional display of higher order alternation at the second decision point which could arise from nesting first order alternation at each location separately. The fact that this higher order alternation behavior, and others exhibit strong negative pressures for the behavior to be continued demand some temporal level of analysis beyond what is currently available. The data which chapter 4

describes agree with previous theories on rat alternation behavior that rats will bias their behavior to avoid more recently visited spaces in an effort to maximize exploratory behavior (Douglas, 1966; Phillmore & Klein, 2019). There is also a strong behavioral heuristic in the form of using the return arms on occasion. Exactly how spatial behaviors such as AB and the use of spaces as navigational heuristics interplay to form the rat's schema is a natural next step for this observation over time.

Chapter 4 also raised the question: do rats change their strategizing throughout the duration of the experiment? It has been seen that in simple two-choice perceptual response tasks mice perform in a manner that can be described by the shifting of several strategies across a single recording (Ashwood et al., 2022). Considering that the triple-T tasks performed involve several more decision points to consider it should be expected that the strategy of the rats ought to be more dynamic. To what extent these dynamics play out over time has yet to be seen. This is just one of the functions of how the triple-T, and related tasks, can be used to study spontaneous choice behaviors and strategy related activity in the brain.

In summary, work presented here gives a greater appreciation for the ability of the nervous system to perceive and represent structurally rich path-networks. This evidence is not surprising given the natural environments in which animals live and which the nervous system was evolved to perceive. However, the evidence is interesting because it supports the idea that some brain regions' unique ability to represent space necessitates that feature-rich environments be present in order to be observed. Experiments done which inadvertently deprive the nervous system of the richness in which it was evolved to interact through the application of substandard environments cannot reveal all of the responses that these brain regions, or behavior have to offer. The revelation of dynamics still unseen in neural activity and behavior and their relationship to structurally complex spaces may even reconcile research on the many

other cognitive functions associated with the spatial navigation system such as the episodic memory system, attentional systems, and planning systems.

Structural similarity can be seen in many ways across brain regions as demonstrated in chapters 2 and 3. Structural similarity can also been seen to organize behavior in regular ways as shown in chapter 4. These findings in summary can help guide neuroscience research in developing new, interesting, behavioral tasks which allow for the nervous system to both discriminate and generalize space. As seen with analogy encoding demonstrated by SUB neurons, by affording the animal a more structurally complex environment, allows for structurally relevant representations of space present themselves neuron activity. This was also seen in demonstration that PPC neurons respond in a way that is equivalent across structurally equivalent routes. Finally, by allowing our subjects to self-employ strategies to accomplish a structured working memory task within the triple-T path network it allowed for the emergence of never before described spontaneous behaviors. This is seen in how rats regularly structure their alternation behavior according to areas of topological similarity. Surely the application of environments which allow for many forms of structural similarity to be appreciated will allow neuroscience to continue to discover unique and surprising findings that shed light on the function of various brain regions, well studied and not. As more elaborate behaviors are employed and allow for the subject to self-organize their own behavioral strategy, two questions arise: (1) how are these strategies employed throughout the time course of an entire behavioral experiment, and (2) how do those strategies manifest in the activity of neural populations known to be associated with working memory and navigational processes? In order to answer these questions preliminary work that can reliably explain the naturalistic behavior of animals must be done. Already great strides are being made in the world of hybrid models using Hidden Markov Generalized Linear Models (HMMGLM) to describe the subtleties seen in choice behavior on a two-choice perceptual task (Ashwood et al., 2022). Application of these tools onto triple-T

datasets promises to identify epochs of time when the subject undertakes a consistent behavioral stereotypy. The ability to identify these times would also allow for the ability to compare neural activity across epochs to investigate behaviorally-structured neural activity.

As a result of the intersection across the spatial navigation system in the brain and other cognitive processes in the brain this research also poses many interesting questions for human pathology. That is to suggest that when inexplainable symptoms arise in cognitive systems, structure of the surroundings could give profound clues as to what an appropriate therapeutic approach to care may be. For example, freezing of gait in Parkinson's disease appears to be brought on by particular structures, such traversing a narrow doorframe (Stern et al., 1980). While the mechanism of this freezing is unclear, it could have foundations in the environmental structure being encountered itself. It may be that some structures elicit particular cognitive inflection-points, and in this way understanding the foundation of structure perception in the nervous system is critical to better understanding a source of this particular pathology. As another example, people living with memory impairment often have difficulty living in assisted communities as their structure is unfamiliar (Schiff, 1990). Many of the same systems utilized in the brain for memory are the same as used for spatial navigation. Further research into what types of structures anchor neural activity in brain regions affected by pathology will certainly inform what kinds of structures are most cognitively demanding and perhaps most difficult to navigate for the memory impaired.

Major Themes and Claims

Equivalent Structures Can Elicit Equivalent Behavior

Along what lines two stimuli are different and equivalent is a common source of inspiration for neuroscience research. A general reductionism in experimental design has been the result of an overabundance of attention placed on sophisticated methods of data collection.

These methods do not always afford for experiments with matched behavioral sophistication, and thus the scope of interpretation is bound to be simplistic. The real world is not simplistic, and various locations in the real world can be considered with regard to their similarity to one another. From our data, with sufficient structural complexity, we found that the nervous system is capable of appreciating equivalence across many frames of reference. The analogy encoding found in SUB neurons and PPC neurons generalizing in space against self-motion are two forms of evidence from distinct brain regions that structural equivalence is referenced in many brain structures. The spatially distributed nature of AB on the find-all tasks further demonstrates that spaces which have structural equivalence (e.g. T-junctions) can be seen as equivalent in many ways despite their spatially distributed nature. This dissertation demonstrates that places of equivalence will often elicit equivalent behaviors in the neural activity as well as decision-making patterns. While perhaps an unsurprising claim to make, it is more surprising that studies that allow for such a claim to be made are not more commonly performed. This claim also demands better experimental design from neuroscience. We cannot simply ignore evidence for the importance of structurally complex environments and continue to force the system being studied into an overly simplistic experimental design.

Structure to Place Interaction

The original motivation behind many of these studies was to observe how place representations would reflect, or not reflect, the structure of the triple-T maze. Unexpectedly, place cells did not systematically organize their activity around the structure of the triple-T with the exception of a lack of observed trajectory coding at the final turn for the find-all-4 condition. CA1 place cells did not organize their place fields around corners, which SUB neurons did. We observed that brain regions such as SUB and PPC organized their activity along frames of reference best described by the structure of the maze, while CA1 place cells did not. These findings are provocative and pose an interesting research topic as to the nature of place and

structure representations with regard to one another. From a space-to-action point of view, place representations would be generated by HPC and then broadcast to efferent targets, potentially to be decoded as representations of structure from the representation of place and sequences of places. From an action-in-space point of view, efferent copies of motor decisions would be associated with other information, such as vision and somatosensation. These associations could generate representations of the relevant structure within the cortex which could go on to influence neurons co-targeted by hippocampal efflux. This action-in-space system could provide a coherent representation of the context within which an action is being taken. SUB encodes structure in a form of activity that resembles place fields distributed in many meaningful locations, and yet the dynamics one synapse away with place cells are so different. It will be critical to understand the relationship between CA1 and SUB neurons in particular to elucidate a better understanding of how structure and place interact.

Structures as General Organizing Principals

Colloquially ‘structure’ is used to discuss the relative organization of features with one another. Structures have been shown to influence the activity patterns of neurons along intuitive lines. For example, the analogy tuning seen in SUB neurons is often reflected across an obvious line of symmetry with regard to the overall structure of the maze. Structure also influences PPC spatial representations in that PPC neurons respond similarly across similarly structured pathways irrespective of their specific shape. Additionally, structures which share the quality of being T-shaped junctions elicit AB in an equivalent fashion even though they are in different locations, occur at different points in the total decision making scheme, and are variably related to performing an error. Why do these very obvious and explicit features (symmetry, affordance, and shape) organize certain levels of the spatial navigation system and not others? One possibility is that the associations between perceived features as a whole may give rise to most, or all, possible structure representations. The brain could then have a process

to detect relevant structures at each level of neural computation and organize activity around them. This merely changes the question to identifying the process for which structural relevance is selected.

It seems useful to endeavor in constructing a framework in which to study structure as a concept itself. This would allow us to define common metrics which can be applied to both environmental and task design. These metrics could help put forward theories that link the many cognitive processes seen to involve the same brain regions, and help differentiate the many forms of redundant encoding seen in neural activity. It may be that functional specificity is not the correct perspective to get an appreciation for the uniqueness of each brain region – there is simply too much overlap in what each brain region is able to respond to in order to classify brain regions on what they 'do'. Experiments in this fashion often set out with an *a priori* hypothesis about a brain region's ability to encode a particular stimulus in order to receive a relatively binary answer for the stimulus and its feature set. Instead, it may be the difference in specific structural tuning that best defines functional brain regions. One brain region may respond to structures at a different level of complexity compared to another brain region, and that may be a more parsimonious way to describe them. Experiments that investigate this would need to employ thoroughly complex environmental and task structures to provoke many potential responses. Designing and considering sufficiently complex structures of task and environmental interactions through the experimental time course of neurophysiological studies may prove much more useful in describing meaningful differences across brain regions. Furthermore these studies have the potential to address specifically how relevant structures are identified from the set of all possible structures. Similar lines of thought have begun to take shape in the world of fMRI analyses where considering task similarity matrices are allowing for functional differentiation of many brain regions (Cohen et al., 2017).

As of now experimenters are restricted to their own intuition for how complex a task and environment can be reliably performed. Even then, it can take years of pilot studies to provide the foundation required to fully elaborate on meaningful patterns in behavior on such a task. If the concept of structural complexity could be formalized in a manner that was intuitive and quickly adaptable it would benefit the field greatly. Such a formalization may be structural complexity 'score', involving the structure of working memory task demands and the structure of spatially distributed choices. If such a value were to be computed, it would allow researchers the backing needed to endeavor into new tasks assured that they are not overly simplistic nor overly complex for the system they are studying.

The concept of structure can be applied to everything we can perceive, including objects and spaces, but as well as events and tasks. As more attention is applied to what is meant by 'structure' and proper tools are developed to investigate structure along behavioral and physical domains it is clear that representations of structure itself will emerge. These representations of structure will almost certainly be found to be powerful organizing forces for neuron activity.

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