Spike-Time Neural Codes and their Implication for Memory

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Abstract

The possibility of temporal coding in neural data through patterns of precise spike times has long been of interest in neuroscience. Recent and rapid advancements in experimental neuroscience make it not only possible, but also routine, to record the spikes of hundreds to thousands of cells simultaneously. These increasingly common large-scale data sets provide new opportunities to discover temporally precise and behaviourally relevant patterns of spiking activity across large populations of cells. At the same time, the exponential growth in size and complexity of new data sets presents its own methodological challenges. Specifically, it remains unclear how best to (1) discover precise spike-time coordination in data sets that challenge existing analysis techniques, and (2) determine whether detected coordination is relevant to behaviour. Here, we introduce a new approach for analyzing the structure of spike-time coordination, in which patterns of spikes are represented as complex-valued vectors. This approach discovers clusters of similar spike patterns, makes effective links between spike timing and behaviour, and provides insight into the structure of putative spike-time codes.

Keywords: Dimensionality reduction, spike-time codes, working memory, navigation
Summary for Lay Audience

Neurons in the brain represent and transmit information by firing “spikes” at specific times and at measurable rates. While firing rates are known to play a role in behaviour and cognition, it is still debated whether the precise times of spikes are meaningful. Due to recent and rapid advancements in experimental neuroscience, it is now possible to record the spikes of hundreds to thousands of cells simultaneously. These increasingly common large-scale data sets provide new opportunities to discover patterns of spikes with specific relationships to behaviour. However, they also introduce methodological challenges related to analyzing such large amounts of data. First, it is unclear how best to discover precisely repeating patterns of spikes in data sets that challenge existing analysis techniques. Second, once such patterns are found, it is not clear how to determine whether they are relevant for behaviour. Here, we introduce a new approach for analyzing the structure of spike-time patterns. This approach discovers instances of similar spike-time patterns in neural data, provides insights into the structure of these patterns, and makes meaningful links between spike timing and behaviour.

In Chapter 1, this approach is applied to spiking data from macaque monkeys performing a sophisticated working memory task that takes place in a virtual environment. Neural activity was recorded from the lateral prefrontal cortex, a brain region known to be important for working memory, while the subjects performed a memory guided navigation task. This approach discovered sequences of spiking activity that represent task-relevant information held in working memory.

In Chapter 2, this approach is used to study the phenomenon of “phase precession” in the rodent hippocampus. In this phenomenon, specific sequences of spiking activity are thought to function like a “GPS” that represents the rodent’s location. Here, we develop a mathematical description of these sequences that allows us to study how they transform over time and across cells. The mathematical properties of these transformations show that the sequences represent more than just current position. Instead, they represent trajectories between past positions and possible future positions, linking the roles of phase precession in navigation and memory formation.
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Co-Authorship Statement

I share co-first authorship on both papers presented in this work. I am deeply grateful for the hard work of my collaborators, Dr. Megan Roussy, who is the experimentalist behind the data presented in Chapter 1, and Dr. Federico Pasini, who developed the H Operator presented in Chapter 2.

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List of Abbreviations, Symbols, and Nomenclature

1. CA1: Cornu Ammonis 1: A subregion of the hippocampus, which is a brain structure in the temporal lobe implicated in learning and memory.

2. DFT: Discrete Fourier Transform: A discrete transform used in Fourier analysis, which transforms a sequence of data samples into a complex-valued function of frequency.

3. Dorsoventral axis: The dorsal pole refers to the top of the brain, while the ventral pole refers to the bottom. The dorsoventral axis is the vertical axis running between these two poles.

4. LFP: Local Field Potential: Electric potential recorded from many types of electrodes implanted in brain tissue (as opposed to EEG, recorded from electrodes placed on the skin, or ECoG, which are recorded from subdural surface electrodes). LFP signals are recorded from the extracellular space in the brain at the depth of the electrode and reflect the activity of the local population.

5. LPFC: Lateral Prefrontal Cortex: A subregion of the prefrontal cortex, which is a brain region in the frontal lobe involved in cognitive control (including memory, planning, attentional selection, etc). The lateral PFC is situated towards the outside of the brain (as opposed to medial, which is situated towards the middle of the brain).

6. NHP: Non-Human Primate

7. ODR: Oculomotor Delayed Response Task: Subjects must remember a cued location on a computer monitor during a delay period, after which they respond by making a saccade to the remembered location.

8. PCA: Principal Component Analysis: A technique for reducing the dimensionality of data. A data matrix is projected onto a subset of eigenvectors of the covariance matrix describing the data. Emphasis is placed on discovering dimensions that explain the most variance.

9. Saccade: A quick movement of both eyes that shifts the gaze between two points of fixation.

10. WM: Working Memory: The ability to remember and manipulate information in the mind for short periods of time, usually to complete a task or achieve a current goal. WM is updatable, a key feature distinguishing it from short-term memory.
Introduction

Single neurons represent and transmit information through action potentials, or “spikes” – discrete events that occur at specific times and at measurable rates. These spikes are coordinated over time, and across large populations of cells, to form the intricate patterns of activity that support cognition. Despite many decades of study, the underlying structure and principles that govern this coordination remain elusive. An important question, which remains up for debate, is to what extent the precise timing of individual spikes plays a role in neural computation.

Firing rate data has been studied extensively and correlated with behaviour. In computational approaches, these firing rates are commonly generated by simulated spike trains of individual neurons that are modelled as Poisson processes [1]. As such, the timing of individual spikes is often considered to be random. This assumption drives the idea that it is these rates, generated by random spiking, that form the basis for the neural code [2, 3]. On the other hand, spike timing is known to play an important role in synaptic plasticity, the learning mechanism that modifies neural circuit function [4, 5]. Further, increasing evidence suggests that the precise timing of individual spikes contributes to neural coding in multiple systems [6–10], and has a dramatic effect on behaviour [11–14].

Much of this recent evidence for temporal coding has emerged as a consequence of rapid advances in neural recording technologies. The size of simultaneously recorded populations has been increasing exponentially [15]. In the early 2000s, recording from “many” cells at a time meant “more than 20” [16] – now, it means hundreds or thousands. For example, this year [17] recorded from an average of 1,700 well-isolated single units from multiple visual areas at once. It is now possible to study large neuronal populations on the timescale of behaviour in awake animals performing complex tasks. However, in doing so, a new challenge arises: that of interpreting such vast amounts of noisy data.

Specifically, the best way to analyze patterns of spikes from hundreds to thousands of single units remains unclear. This challenge has two underlying causes: (1) meaningful spike patterns across many neurons may be very high-dimensional, and (2) it is unclear how best to apply traditional dimensionality reduction techniques for continuously sampled variables, like PCA, to detect precise coordination in large-scale spiking data. To address this challenge, we developed a novel technique for analysis of spike train patterns using complex variables. This technique provides a straightforward approach for dimensionality reduction on spikes, while also providing a natural, algebraic language for spike patterns that can range from very simple, such as reliably repeating sequences, to very intricate.

In Chapter 1, this approach is applied to spiking data from rhesus macaque monkeys performing a sophisticated visuospatial working memory (WM) task which takes place in a virtual environment. The approach relates temporally precise sequences of spiking activity in the lateral prefrontal cortex (LPFC) of two macaque monkeys to WM content. The sequences were predictive of task performance and could be used to decode the trial conditions. Further, sub-anesthetic doses of ketamine were used to establish a causal link between the sequences and WM. Taken together, these results reveal a striking link between spiking activity in primate LPFC and WM behavior. Further, these results reveal the utility of a mathematical approach to spike times that can create rigorous links to behaviour.

In Chapter 2, one of the clearest biological examples of a spike-time code is explored: the phenomenon of phase precession in the rodent hippocampus. A complex-valued operator
which describes this phenomenon is developed and studied as a putative spike-time code. The algebraic symmetries of this operator suggest a clear link between the roles of phase precession in navigation and memory formation. Further, a novel spike-based decoder derived from this operator predicts the trajectories of simulated animals in a two-dimensional environment with high accuracy, even in the presence of realistic biological noise.

An important question concerns the choice to develop a method based on complex-valued variables when the data in question may more intuitively be thought of as real-valued pairs: (neuron number, time of spike). There are several considerations guiding this choice that will be examined in depth in the discussion. In brief, complex numbers offer a natural way to reference spike times to internal brain rhythms when spike patterns occur in the presence of population oscillations, as is the case in Chapter 2. Further, it allows for a representation of duration and relative timing that does not depend on “clock time”. For example, time windows relevant for analysis can be determined by fluctuations in population excitation, or by an experimental trial structure that may take variable amounts of time. In this way, the approach allows for patterns to be stretched or compressed in time. Finally, the symmetries available in the complex plane that do not exist on the real line may provide further insight into the structure of relevant patterns, and allow for more natural extensions of the method to patterns with higher-dimensional structure.
Chapter 1

Neural sequences in primate prefrontal cortex encode working memory content

Working memory (WM) is the ability to maintain and manipulate information in the mind for short periods of time, generally seconds [18]. We use WM frequently in our day-to-day lives; for example, remembering the password to a Zoom meeting just long enough to type it in, or remembering your friends’ orders when making a coffee run. Importantly, WM is updatable: you can update your mental representation of your friend’s order from a muffin to a donut when they change their mind. This ability to update and manipulate remembered information is a key feature that distinguishes WM from short-term memory.

Previous lesion and electrophysiology studies have demonstrated that the primate lateral prefrontal cortex (LPFC) is important for WM function for both non-human primates [19, 20] and humans [21, 22]. Impaired LPFC function results in specific WM deficits, but does not affect the ability to perform similar tasks that do not require WM [23, 24]. However, despite decades of study, the specific neural mechanisms that underlie WM remain up for debate.

Traditionally, these WM circuits are probed through delayed response tasks, in which the subject must maintain a mental representation during a delay period which then guides a choice during the subsequent response period [25]. A commonly used version of this task is the oculomotor delayed response task (ODR). In this task, the subject fixates on a dot at the center of the screen, a cue location appears briefly, then disappears for a delay period – after which the subject responds by making a saccade to the remembered location. The ODR task was crucial for revealing the contribution of single neurons to WM coding. In this type of task, single neurons display elevated and persistent firing throughout the delay period, and are selective for a particular stimulus [19, 26, 27]. These cells form the basis of the “persistent firing” code, which is the mechanism traditionally understood to support working memory.

However, when the task is complicated, or the delay period lengthened, neural responses become more varied [28–30]. For example, Bateuv et al. showed in 1979 that only a small subset of cells fire persistently throughout a 10 second delay, while many cells fired consistently during select parts of the delay period only. Returning to this previous literature, we hypothesized that the observed precise temporal scale of firing was meaningful.

To test this hypothesis, our collaborators, Dr. Megan Roussy and Dr. Julio Martinez-Trujillo, developed a sophisticated WM task which takes place in a virtual environment. In this task, 1 of 9 target locations is presented in a virtual circular arena. After a 2 second delay
period, the subjects navigate to the remembered location using a joystick. Unlike traditional ODR tasks, this task allows for free visual exploration and has a clear spatiotemporal structure. We hypothesized that the complexity of this task may necessitate more intricate patterns of neural activity. We recorded neuronal activity from around 200 cells at a time in order to study these patterns across large populations of neurons. Specifically, we recorded from two 96-channel microelectrode Utah Arrays (Blackrock Neurotech, UT, USA) implanted in the left LPFC of both rhesus macaque monkeys trained for the task.

We observed clear bands of spiking activity in single trial data that played out on a behavioural timescale and spanned both arrays. These sequences of spiking activity emerge clearly from single-trial recordings when the neurons are sorted by peak firing time. These single-trial population sequences are made up of temporally precise single-cell contributions. The contribution of a single cell is comprised of a brief (100-200ms) elevation in firing, far above its background rate.

Neural sequences have been observed before in other animals, and increasing evidence suggests that precise spike times do play a role in behaviour, from the initiation of motor acts to the neural coding of time and place in the hippocampus [7, 8, 10, 31–36]. Based on this literature, we hypothesized that neural sequences could also support WM in a complex, naturalistic environment. We then developed a new computational approach to probe the relationship between these spike sequences and WM content. The following manuscript is the result of this work.
Neural sequences in primate prefrontal cortex encode working memory in naturalistic environments

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Working memory is the ability to briefly remember and manipulate information after it becomes unavailable to the senses. The mechanisms supporting working memory coding in the primate brain remain controversial. Here we demonstrate that microcircuits in layers 2/3 of the primate lateral prefrontal cortex dynamically represent memory content in a naturalistic task through sequential activation of single neurons. We simultaneously recorded the activity of hundreds of neurons in the lateral prefrontal cortex of macaque monkeys during a naturalistic visuospatial working memory task set in a virtual environment. We found that the sequential activation of single neurons encoded trajectories to target locations held in working memory. Neural sequences were not a mere successive activation of cells with memory fields at specific spatial locations, but an abstract representation of the subject's trajectory to the target. Neural sequences were less correlated to target trajectories during perception and were not found during working memory tasks lacking the spatiotemporal structure of the naturalistic task. Finally, ketamine administration distorted neural sequences, selectively decreasing working memory performance. Our results indicate that neurons in the lateral prefrontal cortex causally encode working memory in naturalistic conditions via complex and temporally precise activation patterns.

Introduction

Working memory is the ability to briefly maintain and manipulate information ‘in mind’ to achieve a current goal (Baddeley, 1986; Baddeley, 2003). Brain circuits supporting working memory differ from those for sensory processing in that they must represent precise information in naturalistic contexts in the absence of sensory inputs (Goldman-Rakic, 1990; Arnsten, Wang, & Paspalaras, 2012; Wang, 2021; Roussy et al., 2021a). They also differ from long-term memory circuits in that the information is only maintained for short time intervals - just long enough to complete a specific task. Despite five decades of study, the neural mechanisms underlying working memory remain controversial.

The primate lateral prefrontal cortex (LPFC) has been widely implicated in working memory function as evidenced by previous lesion and electrophysiological studies in macaque monkeys (Curtis & D’esposito, 2004; Constantinidis, et al., 2018; Leavitt et al., 2017a; Pasternak et al., 2015). A long-supported mechanism for coding of working memory representations in LPFC of primates during delayed response tasks is persistent firing in single neurons selective for the memorized information (Fuster & Alexander, 1971; Constantinidis, et al., 2018). During such tasks, subjects must remember the location or features of a sample item for a few seconds after its disappearance, and then produce a behavioral response, e.g., a saccade to a remembered location. However, most delayed response tasks used to explore the neural mechanisms of working memory lack the spatiotemporal structure of naturalistic behavior (i.e., they use simple stationary displays and constrain eye movements during memory maintenance). During many natural behaviors involving working memory eye gaze is unconstrained and the visual scenery is rich and dynamic (Roussy, et al., 2021a).

Studies using delayed response tasks with increased spatiotemporal complexity report few single neurons with persistent firing during the entire delay period. Instead, many neurons fire transiently, during brief time intervals (Batuev et al., 1979; Lundqvist et al., 2016; Roussy et al., 2022). Thus, researchers have proposed alternative mechanisms to persistent firing, such as short-term synaptic storage (Stokes, 2015; Pals et al., 2020), or dynamic coding (Lundqvist et al., 2016; Parthasarathy et al., 2019). However, evidence in favor of such mechanisms is highly debated (Wang, 2021). Here, we hypothesize that a mechanism for working memory coding in naturalistic conditions must preserve the spatiotemporal structure of natural behavior while being robust to interference by concomitant sensory and motor signals. We specifically hypothesize that working memory coding during naturalistic tasks, in the presence of eye movements and rich visual scenery, relies on sequential activation of neurons in primate LPFC.

Neuronal sequences, consisting of temporally precise patterns of neural activity, have been reported to encode the varying spatiotemporal structure of motor signals in the high vocal center (HVC) of songbirds (Chi & Margoliash, 2001; Tang et al., 2014; Srivastava et al., 2017; Okubo et al., 2015; Daliparthi et al., 2019), and of spatial trajectories to remembered locations during navigation in the parietal cortex (Har...
vey et al., 2012) and the hippocampus of rodents (Itskov, et al., 2011; Eichenbaum, 2014; Zhou et al., 2020). Early investigations in macaque monkeys suggested that the spiking activity of a few single neurons in LPFC could have a precise and informative spatiotemporal structure (Abeles et al., 1993). However, sequences of single unit spiking activity have not been directly observed or causally linked to working memory during naturalistic behavior in primates (Wang, 2021).

We use microelectrode arrays to record neuronal activity in LPFC layers 2/3 of macaque monkeys during a naturalistic working memory task set in a 3D virtual environment. We find that temporally precise sequential patterns of neural activity in LPFC, extending over behaviorally relevant timescales of several seconds, represent important task variables for the successful maintenance of and navigation to remembered target locations in the 3D environment. These neural sequences robustly and flexibly represent trajectories to remembered locations during shifts in eye positions toward various elements of the environment. Sequences were not found when we examined an oculomotor delayed response task (ODR) which has been commonly used to explore working memory in previous studies. Further, pharmacological blockade of NMDA receptors with anesthetics as doses of ketamine demonstrates a causal link between sequences and working memory.

**Results**

We trained two rhesus macaque monkeys on a visuospatial working memory task that took place in a virtual circular arena containing naturalistic elements (see Fig. 1a, b). We recorded neuronal activity using two 96-channel microelectrode Utah Arrays (Blackrock Neurotech, UT, USA) implanted in the left LPFC of both animals (Brodmann area 8a, 9/46 (Petrides, 2005)) (see Fig. 1c). The task began with a three second presentation of a target in one of nine possible locations in the arena (cue epoch). The target then disappeared, and after a two second delay period, the animal was required to navigate towards the cued target location using a joystick (see Fig. 1d). Virtual navigation within the environment was exclusively available during the navigation epoch. Animals were able to successfully perform this naturalistic working memory task (average correct trial rates across sessions were: NHP B: mean = 87%, NHP T: mean = 57%; chance = 11%) (Fig. 1e; Extended Data Fig. 1a-d). Eye movement was recorded throughout the task using a video eye tracker. Animals made frequent saccades to explore different scene elements throughout all trial epochs. Firing rates across the recorded neuronal population were poorly tuned for the direction and amplitude of saccades (Roussy et al., 2021b; Roussy et al., 2022) (see Extended Data Fig. 1e-k for eye behavior analyses).

**Neural sequences in LPFC neurons.** Precise patterns of neural activity have been identified as a mechanism for representing complex processes in mammalian brains (Buzsáki, 2010); however, such patterns have not been identified during visuospatial WM tasks in primates. We hypothesize that WM representations during our naturalistic task are maintained by temporally precise neural sequences (Fig. 1f). Neural sequences are typically described by temporally precise activation of neurons above their background rates of activity. We observed that LPFC neurons exhibited brief (duration of 80% of max firing value = 220 ms) elevations of spike rate above their background levels of firing (Extended Data Fig. 2) at specific points during the task. To identify potentially relevant population-level patterns in these elevations of spike rate, we sorted neurons by their normalized peak firing time. Sequential patterns emerge in single trials, visualized here using spike density functions (Fig. 1g) and population rasters (Fig. 1h).

A code that relies on neural sequences implies temporally precise activation of single neurons (see Fig. 2a for schematic) (Buzsáki, 2010; van der Meij & Voytek, 2018). We examined the firing properties of 3543 neurons in 17 recording sessions (mean of 208, median of 229 simultaneously recorded neurons per session). Many neurons transiently fired during the same time in single trials of the same target condition (Fig. 2b, c, d, more examples in Extended Data Fig. 3). To quantify this regularity, we calculated the standard deviation (time consistency) of peak firing time between correct trials of the same condition for each neuron (Extended Data Fig. 4a). 20% of neurons (699 neurons) demonstrated a standard deviation below 1000 ms and 65% (2297 neurons) demonstrated a standard deviation below 1500 ms.

We additionally shuffled the peak firing times for each neuron within each trial to generate random firing time estimates across trials. The distributions of standard deviations for correct trials were shifted to lower values relative to the corresponding shuffled distributions (example session in Fig. 2e, all neurons in Extended Data Fig. 4a). The area of non-overlap between the lower tails of the real and shuffled distributions represents the neurons with peak firing times occurring more
regularly than expected by chance within trials of the same condition. On the other hand, the real and shuffled distributions overlapped considerably for incorrect trials (example session in Fig. 2e; all neurons in Extended Data Fig. 4a), suggesting that neurons’ peak firing occurred at less consistent times during single trials of the same condition when animals made mistakes. Indeed, the difference between means of the real and shuffled distributions (Fig. 2f) was lower for incorrect trials than correct trials (correct: median = 270.9 ms, incorrect: median = 71.4 ms. Wilcoxon Signed-Rank Test, $p = 0.001$) (Fig. 2g; Extended Data Fig. 4b, c).

Using 11 sessions in which there are sufficient correct and incorrect trials in all nine conditions, we show that the standard deviation of neurons’ peak firing time (n = 2051) during correct trials (mean = 1358 ms) is significantly lower than incorrect trials (mean = 1828 ms; 1-way ANOVA, post-hoc, $p = 3.8E-09$; Extended Data Fig. 4a). This suggests that increased temporal precision of firing in single neurons is needed for correct task performance.

**Neural sequences are predictive of trajectory to remembered targets.** We have demonstrated a pattern of sequential activity that spans the trial duration and is driven by the temporal consistency of the firing neurons. Next, we examined whether these identified sequences are related to working memory; more precisely, whether sequences can encode the contents of working memory during the delay epoch of the task, when the cue has disappeared, and navigation was not permitted. We developed a computational method to analyze spike sequences in single trials, allowing for efficient unsupervised discovery of neural sequences that are consistent within the same target condition. We represented individual sequences of peak firing during the delay epoch in each trial across the population of recorded neurons as complex-valued vectors. We performed dimensionality reduction on the resulting correlation matrix (Fig. 3a; Extended Data Fig. 5a-c). The resulting component values are projected into a 3-dimensional space where each colored circle represents a cluster centroid for a different target condition (Fig. 3b).

Using a supervised distance-based classifier, we could correctly predict target condition within a single trial based on the clustering of condition centroids during the delay epoch (9 locations: median = 15% above chance, $p = 9.7E-05$; Extended Data Fig. 5d, see Methods - Projection Classification Analysis). We then observed that the condition centroids reliably formed three distinct groups based on the three primary trajectory directions to targets (i.e., left, center, right). Based on this observation, we hypothesized that this grouping may relate to task behavior since movement trajectories typically fall within these three directions. A supervised classifier based on this hypothesis can correctly predict target condition column (left, center, right) based on delay-epoch spiking activity within a single trial (median = 40% above chance, $p = 1.7E-05$; Extended Data Fig. 5e). Further, an unsupervised classifier developed from our analysis could predict the target column within a single trial based on the emergent clustering of projected data into column-based clusters - without any training required (median = 33% above chance, $p = 1.7E-05$; Extended Data Fig. 5f).
Data Fig. 5f). Taken together, these results demonstrate that these patterns of spiking activity contain a unique temporal structure for different target conditions that may be related to remembered target locations.

To explore the direct relationship between sequences and task relevant behavior during working memory, we compared the distances between centroids during the memory delay epoch to distances between movement trajectories (i.e., the trajectories the animals used to reach the remembered target location). We calculated the Spearman correlation between matrices containing the Euclidean distance between condition centroids, and the Frechet distance between average traveled trajectories to targets in the virtual arena (see Fig. 3c-f; see Extended Data Fig. 6 for alternative methods). The Frechet distance between two trajectories is a measure of similarity between them that takes into account the location and ordering of the points along the trajectories (Alt & Godau, 1995). The distance matrices were more positively correlated compared to those obtained when shuffling the target locations, suggesting that the separation between neural sequences in multidimensional space parallels the discriminability between trajectories to targets held in working memory (observed: median = 0.50, shuffle: median = 0.34, Wilcoxon Signed-Rank Test: p = 0.02). Moreover, the relationship between sequences and target trajectories predicts whether information is successfully maintained during the working memory delay period, with higher correlations for correct than incorrect trials (correct: mean = 0.45, incorrect: mean = 0.30. T-test, p = 5.7E-04) (Fig. 3g).

We compared the correlation results during the working memory delay period with those from a temporally equivalent period of a perceptual task, where the target did not disappear during the entire trial and therefore the animals did not need to represent the trajectory in working memory. The correlation was higher during the working memory delay epoch than during the perceptual task control delay epoch (Fig 3h; Spearman Correlation; working memory: mean = 0.51 perception: mean = 0.33, T-Test, p = 1.4e-05), indicating sequences were more correlated to behavior during working memory.

We further asked whether behaviorally relevant sequences were composed of neurons considered tuned for the remembered target location using conventional criteria (i.e., differences in integrated firing rates amongst locations). Sequences composed of tuned neurons were equally correlated with behavior than sequences composed of untuned neurons (1-way Anova, p = 0.94; Extended Data Fig. 6d). The latter indicates that classically considered untuned neurons can take part in the sequences.

Single neurons show trial-to-trial variability in their responses. One may ask whether sequences could be robust to this phenomenon, (i.e., not all the neurons active during trial n sequences for one remembered target location will be active in trial n+1, n+2, etc). Remarkably, the correlation between neural sequences and target trajectories during correct trials remains stable even after removing 70% of neurons from the population. 80 - 90% of neurons must be removed for this correlation to significantly change, at which point the correct trial correlation becomes equal to the incorrect trial correlation (Extended Data Fig. 6d, e). The latter indicates sequences are robust to ‘single trial absentee neurons’ and therefore to trial-to-trial response variability.

Furthermore, neural sequences during working memory were different from those that occur during the cue and navigation epochs (Fig 4a-c) and they were most predictive of target condition (Spearman Correlation; cue: mean = 0.46, delay: mean = 0.60, navigation: mean = 0.51, all: mean = 0.49; 1-way Anova, p = 0.03) (Fig 4d). Sequences were also more correlated to target trajectories when we limit a neuron’s contribution to a sequence to one epoch (i.e., one neuron is only allowed to participate in a single epoch sequence – considering a single max firing time) compared to contributing to multiple epoch sequences (i.e., allows for multiple peak firing times) (Spearman Correlation; single: mean = 0.48, multiple: mean = 0.37; T-Test, p = 0.006) (Extended Data Fig. 6c). This latter result indicates sequences are most informative when different neurons contribute to different epochs, suggesting unique contributions of neurons in different trial periods.

Neural sequences are specific to naturalistic working memory.

Neural sequences may operate as a mechanism for working memory coding in the naturalistic conditions of our task. To test this prediction, we conducted the same set of analyses exploring macaque LPFC single neuron temporal precision and population sequences in a classic oculomotor delayed response task (ODR) (Extended Data Fig. 7a, b). The task we used included 16 possible target locations. Here animals must fixate on a dot on a blank screen, then a peripheral target is flashed for a short time period. After target offset, the animals keep fixating on the dot for a few seconds while remembering the spatial location of the target. Upon the fixation dot offset, the animals make a saccade towards the remembered location to obtain a reward (Leavitt, 2017b, Leavitt, 2018). Saccades are ballistic movements that practically ‘teleport’ the fovea from the starting to the end point, without perceptual of the travelled path during eye movement (Bremmer et al. 2009).

As opposed to the VR navigation task, when neurons during the ODR task were ordered by peak firing time, the patterns of activation were often disrupted or incomplete (Extended Data Fig. 7c), suggesting that the organization of spiking activity may be different from the VR task. This may be related to neurons during the ODR delay epoch exhibiting less temporally consistent peak firing times from trial to trial. For many instances, real and shuffled distributions of standard deviations were overlapping (Extended Data Fig. 7d). Indeed, the difference in means between real and shuffled distributions was significantly smaller in the ODR task compared to our naturalistic VR task (ODR1: median = 93.2. ODR2: median = 31.6, VR: median = 270.9; Kruskal Wallis, p = 1.2e-06) (Extended Data Fig. 7e).

To further explore this issue, we applied the complex-valued dimensionality reduction analysis described above to the ODR task data. Condition centroids were clustered in quadrants based on position of target location as reported previously using spike rate-based analysis (Leavitt, 2018) (Fig. 4c). We calculated the correlation between the matrices of centroid distances and target locations Euclidean distances. The correlation was significantly smaller in the ODR than in the naturalistic VR task (ODR: median = 0.22, VR: median = 0.43. Wilcoxon Rank Sum, p = 0.004) (Fig. 4f).

These results indicate that sequences are more correlated to behavioral performance during the naturalistic VR tasks than during the classic ODR task used by previous studies.
The naturalistic VR task is different in several ways. First, it measures visuospatial working memory in a dynamic and more spatiotemporally complex environment. Second, it allows for free visual exploration via saccades. Third, it requires 3D navigation to a target location. Neural sequences may be best utilized in the episodic and dynamic spatiotemporal context of our VR working memory task.

Neuronal sequences represent abstract trajectory. The previous analyses demonstrate that working memory sequences contain information about the trajectories to remembered locations suggesting that sequences map into behavioral paths. Indeed, condition centroids were more highly correlated to the Frechet distance between the traveled trajectories to target locations (median = 0.50) than to the Euclidean distance between target locations (median = 0.43; Wilcoxon Sign Rank Test, $p = 0.01$); thus, sequences better represent trajectories to targets than target location alone. Real trajectories are also more correlated to condition centroids than ideal trajectories to targets (calculated by Euclidean distance from start to target location) (Extended Data Fig. 8a) (median = 0.43; Wilcoxon Sign Rank Test, $p = 0.01$). Here one must consider that traveled trajectories are imperfect and can be distinct from ideal trajectories. Real trajectories reflect idiosyncrasies of remembered trajectories and the virtual environment, and may reflect perceived curvature of the arena and obstacles in space (i.e., arena walls) (see Fig. 3d for example trajectories).

One may argue that the observed sequences represent activation of neurons with mnemonic ‘place fields’ similar to sequential activity of place cells in the hippocampus (Itskov et al., 2011; Eichenbaum, 2014; Zhou et al., 2020). Inconsistent with this idea, the sequences are differentiable between memory delay and navigation evidenced through classification analysis (mean decoding = 76%, median decoding = 87%, compared to chance (33%): T-Test, $p = 9.2e-08$) (Fig. 4b, c).

If sequences were primarily reflecting motor planning during the delay period or neural replay of planned trajectories during the response/navigation period, one may anticipate neural sequences during the delay and response epochs from the same trial to be highly correlated, and that this correlation would be higher than sequences from different trials. This was not the case. Delay and navigation epoch sequences were equally correlated between different trials as they were within the same trial (Extended Data Fig. 8b, c). These results indicate that neural sequences in macaque LPFC represent remembered trajectories to target locations, and that such representation is specific to the working memory delay period of the task. The latter makes the representation distinct from classical replay found in structures such as the hippocampus (Skaggs et al. 1996). LPFC neurons appear to represent more abstract qualities of target trajectory.

Ketamine disrupts neuronal sequences and impairs working memory performance. In order to demonstrate a causal link between neuronal sequences and working memory we used ketamine, a N-methyl-D-aspartate (NMDA) receptor non-competitive antagonist that induces selective working memory deficits in humans and animals (Frohlich & Van Horn, 2014; Roussy et al., 2021b, Wang et al., 2013). We injected subanesthetic doses of ketamine (0.25 mg/kg - 0.8 mg/kg) intramuscularly while animals performed the task (see experimental timeline in Fig. 5a, Roussy et al., 2021b). Ketamine drastically reduced performance of our virtual working memory task without affecting performance on a perception control task. Working memory performance recovered 30 minutes to 1 hour post-injection in the late post-injection period (Pre-Injection:...
Discussion

We recorded the responses of hundreds of single neurons in the macaque LPFC during a complex visuospatial working memory task set in a naturalistic virtual environment. We report three major findings: (1) sequences of population activity represented trajectories to remembered locations in the environment (2) neural sequences of single neuron spiking activity were predictive of behavioral performance (3) NMDA receptor dysfunction caused by ketamine and disruption of neuronal sequences leading to deficits in working memory.

*Fig. 4. Working memory sequences are unique to naturalistic behavior. a. Representation of maximum firing times per neuron during the three task epochs being converted to complex phase values and used in our complex vector dimensionality reduction method. b. Epoch specific sequences projected in 3D space. Colored dots correspond to epochs and represent a single trial. c. Decoding accuracy using unsupervised classifiers for predicting the target epoch. Dots represent data from different sessions. d. Decoding accuracy using unsupervised classifiers for predicting the target column (left, middle, right) for sequences during task epochs. e. Condition cluster centroids for an ODR task projected in 3D space. Centroid colors correspond with their position in the virtual environment (see inlet). f. Correlation values for centroid distance and the distance between target locations for the virtual reality task and the ODR task. Dots represent data per session. *p<0.05, **p<0.01, ***p<0.001.

After ketamine injection, the differences in standard deviation distribution means for peak firing times between the real and shuffled data decreased suggesting that neurons fired with less time consistency after ketamine (Pre-Injection: median = 171.6, Early Post-Injection: median = 40.2, Late Post-Injection: median = 100.4; Kruskal Wallis, \( p = 8.5e-05 \)) (Fig. 5a; Extended Data Fig. 9a, b).

*Fig. 5. Ketamine manipulation distorts neural sequences and working memory. a. Experimental timeline for ketamine injection. Pre-injection period is depicted in green, early-post injection in blue, and late-post injection (recovery) in pink. b. Task performance as percent of correct trials for each injection period. Color dots represent median values per injection period for working memory data and gray dots represent median values per injection period for perception control data. Asterisks indicate significance between working memory injection periods. c. Difference in real and shuffled distribution means between ketamine injection periods. d. Condition centroids projected in 3D space. Centroid colors correspond with their position in the virtual environment (see inlet). Ellipsoids are illustrated guides to indicate behaviorally relevant groupings of targets in the pre-injection and late post-injection periods. These results indicate a causal link between NMDA receptor dysfunction caused by ketamine and disruption of neuronal sequences leading to deficits in working memory.
tor antagonism by ketamine disrupted neuronal sequences, selectively impairing working memory performance.

**Neural sequences and working memory coding.** Prefrontal neural activity during tasks that require holding a single item in working memory during a delay response period have demonstrated persistent activity that represents the memoranda (Leavitt et al., 2017a). One major shortcoming of the persistent firing hypothesis is that it may not be able to support working memory representations with rich spatiotemporal structure (Steveninck et al., 1997; Lestienne & Strehler, 1987; Lundqvist et al., 2016). Indeed, in tasks during which sequences of multiple items need to be held in working memory, persistent firing is rare (Lundqvist et al., 2016). A recent study reported that during a multi-item spatial working memory task in which monkeys had to remember a series of spatial locations in sequential order, temporally organized neuronal populations represented the order in which items were remembered (Xie et al., 2022). These studies demonstrate that additional mechanisms may be needed to support coding of working memory representations when the memoranda have spatiotemporal structure.

Our paradigm differs from those used in previous studies. We did not use multiple memoranda; instead, our subjects remembered a single target location and the trajectory to the location in a 3D virtual naturalistic environment. Importantly, our study did not restrain eye position, allowing for naturalistic exploration of the scene while information is being held in working memory. The rationale behind studies restraining eye position is to avoid the interference caused by eye position signals and changes in the retinal image and consequently in visual inputs, on the working memory representation (Suzuki & Gottlieb, 2013). However, in naturalistic conditions working memory coding must be robust to such changes. To our knowledge, working memory coding has not been tested under naturalistic conditions.

Previous studies in macaques have tried to approach the idea of transiently active neurons maintaining working memory through shared temporal relationships by exploring spike train patterns of several neurons. However, due to methodological constraints, these studies were unable to record large numbers of simultaneously active neurons and thus unable to demonstrate sequence coding (Prut et al., 1998). Our study has overcome this limitation by recording from hundreds of simultaneously active neurons, revealing precise sequences of single unit spiking activity that encode specific working memory content.

Studies in mice that simultaneously record from many neurons have reported neuronal activation sequences during short-term memory tasks in the posterior parietal cortex and dorsomedial striatum (Harvey et al., 2012; Akhlaghpour et al., 2016). In the rodent hippocampus, sequences of place cell activation signal trajectories to remembered locations that are stored in long-term memory (Skaggs & McNaughton, 1996). Thus, sequential activation of neurons to encode spatiotemporal episodes appears to be a general coding mechanism across species.

However, the neural sequences we report in this study differ in several ways from those described in the rodent. First, they occur in the LPFC, a brain area that appears during brain evolution in anthropoid primates (Passingham & Wise, 2012). More specifically, the sequences reported here occur within the supragranular layers 2 and 3, where working memory representations have been reported (Bastos et al., 2018; Finn et al., 2019). The expansion of layers 2/3 is found in anthropoid primates and is accompanied by changes in the morphology; size (Gilman et al., 2017) and proportion of different interneuron types (Torres-Gomez et al., 2019) relative to other species and brain areas. Thus, LPFC layers 2/3 may have evolved a microcircuitry for holding internal working memory representations that can be supported by persistent firing, when spatiotemporal structure is poor and concomitant visual and motor signals are not present; or by neuronal sequences, when spatiotemporal structure is rich and distracting signals are present, as in our naturalistic task.

We propose LPFC layers 2/3 neuronal sequences may allow primates to represent short-term spatiotemporal episodes ‘in the mind’. Such episodes can be dissociated from sensory and motor signals and may be key to an enriched virtual world that enables enhanced cognitive control, planning and creativity observed in anthropoid primates (Passingham & Wise, 2012). Importantly, this form of episodic working memory may correspond to the episodic working memory buffer proposed by theoretical and behavioral studies of working memory in humans (Baddeley, 2000).

**Causality and potential mechanisms.** Through pharmaceutical manipulation, we identify that sequence generation relies on NMDA receptor function. The interactions between inhibitory interneurons and excitatory pyramidal cells play an important role in LPFC prefrontal circuits during working memory tasks (Wang et al., 2004). Therefore, the precise activation of pyramidal cells may be dependent on a temporally coordinated ‘release of inhibition’ by interneurons (Cannon, et al., 2015; Kosche et al., 2015). We have demonstrated in past research that NMDA receptor antagonism using the same doses of ketamine as in this study selectively decreases the firing of narrow spiking neurons (Roussy et al., 2021b). A parsimonious explanation for our findings is that ketamine induced loss of firing in narrow spiking interneurons (e.g., PV basket or chandelier cells) which in turn impaired their ability to coordinate sequences in pyramidal cells ultimately causing deficits in working memory. The fact that the effect of ketamine was selective for the working memory task further support our view that the sequential activation mechanisms reported here is particularly important for mental representations that ‘live’ within the LPFC microcircuits.

There may be various benefits of a sequence-based code. It would be more energy efficient than one that relies on continuous activation of neurons in a population. A temporal code may also be robust to interference by other concomitantly occurring signals, as is the case during naturalistic tasks. Temporal specificity may also add complexity to prefrontal networks, allowing for higher dimensional representations and flexible cognition.

Finally, neuronal sequence codes in the LPFC could be the substrate of working memory episodes that can be played in the mind and the neural correlates of the episodic buffer component of working memory systems in the human brain (Baddeley, 2000). The episodic working memory buffer was proposed as an upgrade of the classic working memory model that contained a visuomotor sketchpad, a phonological loop and an executive or attentional controller (Baddeley, 1986; Baddeley, 2000). The episodic buffer can bind experiences into
working memory episodes. Such episodes can exist temporarily ‘in the mind’ and be ‘erased’ without undergoing long term storage. The latter make them distinct from long term episodic memories (Tulving, 2002) which engage hippocampal circuits (Burgess, Maguire, & O’keefe, 2002).

Conclusion. We demonstrate robust and behaviorally relevant temporal organization of spiking activity in layers 2/3 of LPFC during a naturalistic working memory task. Neuronal sequences during periods of working memory maintenance represent the spatiotemporal structure of the information held in working memory. Sequences were disrupted by low doses of ketamine which caused impaired behavioral performance. We conclude that layers 2/3 LPFC circuitry in primates contains the neural substrates for temporarily representing working memory episodes ‘in the mind’ without necessarily engaging sensory, motor and even long term memory systems. Such representations provide primates with a powerful tool for planning the future and adapting to the uncertainty of changing environments.
Extended Data Figures
Fig. 6. Task behavior. 

a, Response time for correct trials. Black lines indicate the median values for each animal. Dots represent data for each session. 
b, Optimal trajectory for correct trials. Calculated by real trajectory length/Euclidean distance between the start location and target location. The dashed gray line indicates the optimal value of 1. Black lines represent median values and dots represent data for each session.
c, Example animal trajectories for two target locations. Red circles indicate the target, green lines represent correct trials and gray lines represent incorrect trials. Black lines represent median values and dots represent data for each session.
d, Distance from target for incorrect trials. Calculated as the Euclidean distance from the animal’s end location to the correct target location. Since ‘Unreal’ units are arbitrary, distance values are normalized by the distance (in ‘Unreal’ units) between two targets. A normalized distance of 1 indicated by the dashed gray line is the distance between two target centers. Dashed purple and red lines represent mean values.
e, Percent of eye data points falling on screen for different task epochs. Black lines represent mean values for each group and dots represent data from each session.
f, Heat maps of eye fixation position on screen during delay for two target examples. Eye fixation is concentrated in task relevant areas on screen but is not primarily concentrated on the target location. 
g, Percentage of total fixations during the delay epoch that land within the target location for correct and incorrect trials. The black lines represent median values and the dots represent data for each session.
h, Decoding accuracy for predicting target location from eye fixation location during the cue and delay epochs. The black lines represent median values and the dots represent data for each session. The gray line indicates chance performance for 9 classes.
i, Decoding accuracy for decoding target location from eye fixation position during the delay period for different ketamine injection periods. Decoding is shown for 3 classes (33.33% chance). The gray circles indicate median values.
j, Main sequence values during the delay period for saccades that fall on and off target location. 
k, Proportion of neurons tuned for saccade landing position in retinocentric and spatiocentric reference frames as well as the proportion of neurons tuned for target location during the delay period. Overlapping sections indicate neurons that are selective for both remembered target location and saccade position. Panels ‘i’ and ‘k’ have been adapted from Roussy et al., 2021. Panels ‘a’, ‘b’, ‘c’, ‘f’, ‘g’, ‘h’, and ‘j’ have been adapted from Roussy et al., 2022 (bioArx). *p < 0.05, **p < 0.01, ***p < 0.001.
**Fig. 7. Neural sequences.** Alignment of peak firing times for all neurons (blue) compared to shuffled peak firing times (gray).

**Fig. 8. Example time consistent neurons.** a-f. Example single neurons. Left column represents the activity of a neuron over trial time over all trials of a certain condition. Pink lines separate task epochs. Right column shows a histogram of max firing times per trial.
Fig. 9. Time consistent neurons. a, Trial-trial standard deviation in max firing time for each neuron across sessions during correct and incorrect trials and for shuffled correct and incorrect data. The Red crosses indicate group means. b, Difference in real and shuffled distribution of the deviation in neuron action potential timing between trials. Presented for correct and incorrect trials for NHP T. The red lines represent median values. Dots represent data for individual sessions. c, Difference in real and shuffled distribution of the deviation in neuron action potential timing between trials. Presented for correct and incorrect trials for NHP B. The red lines represent median values. Dots represent data for individual sessions. *p < 0.05, **p < 0.01, ***p < 0.001.

Fig. 10. Classification of sequential coding. a, Representation of max firing times per neuron during delay being converted to complex phase values and used in our complex vector dimensionality reduction method. b, Large dots represent projected target centroids in 3D space. Smaller dots represent individual trials. c, Large dots represent projected column centroids in 3D space. Columns contain pooled trials between right, left, and center targets. Smaller dots represent individual trials. d, Supervised classification of target location using centroid distances. Each line represents decoding accuracy over number of dimensions considered for one session. e, Supervised classification of target column (left, right, center) using centroid distances projected into three dimensions. f, Unsupervised classification of target column (left, right center) using centroid distances. Each line represents decoding accuracy over number of dimensions considered for one session.
**Fig. 11. Deviations of correlation method.**

**a.** Method summary outlining different ways to calculate sequences. Crossed-out epochs indicate epoch data that was not used as part of the complex vectors for a given method. Asterisk indicates the method used in the main figures and text. 

**b.** Correlation based on each method outlined in ‘a’. Dots represent data per session. Colored dots represent mean and correspond to the table in ‘a’. 

**c.** Correlation when neurons are only considered in one epoch sequence or when all neurons participate in all sequences. This reflects the possibility of one instance of peak activity versus multiple occurrences of increased firing. Dots represent data per session. 

**d.** Spearman correlation using either tuned and matched untuned units. 

**e.** Spearman correlations for correct trials between delay neural sequences and target trajectories after removing 10 - 90% of neurons from the sequence. The dashed gray line represents 0 difference. *p < 0.05, **p < 0.01, ***p < 0.001.

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**Fig. 12. Temporal organization of neural activity during ODR task.**

**a.** Depiction of ODR task with 16 targets. 

**b.** Surgical images showing location of Utah arrays implanted in left LPFC of NHP JL and NHP F. 

**c.** Two trial examples of simultaneously recorded population activity. Normalized firing rate for each neuron is arranged by max firing time. 

**d.** Real and shuffled distributions of max firing time trial-to-trial deviation for an example session. 

**e.** Difference in the means between real and shuffled distributions for the virtual reality task and the ODR task. Gray lines indicate median values and dots represent data per session. *p < 0.05, **p < 0.01, ***p < 0.001.
Fig. 13. Trajectory analysis. a. Correlation between condition centroids and target location, ideal trajectories (i.e., Euclidean distance from start location to targets), or actual trajectories to targets locations. Dots represent data per session. b. The correlation between delay and navigation epoch neural sequences between trials for an example session. The diagonal represents sequence correlations within the same trial. If neural sequences repeated between delay and response epochs in the same trial, a clear diagonal of increased correlation values would be present. c. Correlation between delay and navigation neural sequences within the same trial and between different trials for all sessions. *p < 0.05, **p < 0.01, ***p < 0.001.

Fig. 14. Ketamine and saline control analysis. a. Percent of correct trials for ketamine and saline sessions over injection periods for NHP T. Dots represent data for individual sessions and error bars are SEM. b. Percent of correct trials for ketamine and saline sessions over injection periods for NHP B. Dots represent data for individual sessions and error bars are SEM. c. Difference in the means between real and shuffled distributions of standard deviation values for neuron max firing time between trials. Presented for each ketamine injection period for NHP T. Dots represent data for each session. d. Difference in means between real and shuffled distributions for each ketamine injection period for NHP B. Dots represent data for each session. e. Correlation between the distance between condition cluster centroids and distance between target trajectories. Correlation values are presented for ketamine injection periods for NHP T. Dots represent data per session. f. Correlation values for ketamine injection periods for NHP B. Dots represent data per session. g. Difference in mean values between real and shuffled distributions for saline injection periods. Dots represent data for each session (NHP T and NHP B combined). h. Correlation between the distance between condition cluster centroids and distance between target trajectories. Correlation values are presented for saline injection periods for both animals combined. *p < 0.05, **p < 0.01, ***p < 0.001.
Fig. 15. Neural recording setup. 

a, Graphic of presurgical planning procedure showing 3D reconstructed skull and brain based on CT and MRI scans. Electrode array positioning is illustrated in blue with a red outline. The craniotomy is outlined by the larger red box. 

b, 3D modeled brain with electrode array placement in pink. 

b, Surgical images of array implantation in NHP B and NHP T. 

c, Example of spike sorting for one electrode channel. Upper panel represents PCA space and the lower panel represents individual threshold crossing event waveforms. The blue and red clusters represent what we would classify as a single unit. The green cluster would be classified as a multiunit. 

e, Example of spike sorting for one electrode channel. The blue cluster would represent a single unit. The green cluster would represent multiunit activity. This figure is modified from Roussy et al., 2021.)
Methods

We used the same two adult male rhesus macaques (Macaca mulatta) in the main experiment as well as the ketamine and saline experiments (age: 10, 9; weight: 12, 10 kg). The oculomotor delayed response task was recorded from two different male macaques using one multielectrode Utah array implanted in each animal (Leavitt, 2017b, Leavitt, 2018).

Ethics statement. Animal care and handling (i.e., basic care, animal training, surgical procedures, and experimental injections) were pre-approved by the University of Western Ontario Animal Care Committee. This approval ensures that federal (Canadian Council on Animal Care), provincial (Ontario Animals in Research Act), regulatory bodies (e.g., CIHR/NSERC), and other national standards (CALAM) for the ethical use of animals are followed. The oculomotor delayed response task experiment complied with Canadian policies and regulations and was preapproved by the McGill University Animal Care Committee (Leavitt, 2017b, Leavitt, 2018). Regular assessments for physical and psychological well-being of the animals were conducted by researchers, registered veterinary technicians, and veterinarians.

Experimental setup. Animals performed the task in an isolated room with no illumination other than the monitor. The room contained no AC power lines and was radiofrequency (RF) shielded. The task was presented on a computer LDC monitor positioned 80 cm from the subjects' eyes (27" ASUS, VG278H monitor, 1024 × 768 pixel resolution, 75 Hz refresh rate, screen height equals 33.5 cm, screen width equals 45 cm). Eye positions were monitored using a video-oculography system with sampling at 500 Hz (EyeLink 1000, SR Research). Stimulus presentation was controlled through a custom computer program (through Unreal Engine 3). Subjects were seated in a standard enclosed primate chair (Neuronitek) during the experiment and were delivered juice through an electronic reward integration system (Crist Instruments). Prior to the experiments, subjects were implanted with custom fit, PEEK cranial implants which housed the head posts and recording equipment (Neuronitek). See Blonde et al., 2018 for more information. The head posts were attached to the primate chair for head fixation. The experimental setup for the oculomotor delayed response task is outlined in both Leavitt et al. 2017b and Leavitt, 2018.

Task. The virtual task environment was developed using Unreal Engine 3 development kit, utilizing Kismet sequencing and UnrealScript (UDK, May 2012 release; Epic Games). Details about this platform and the recording setup can be found in Doucet et al., 2016. Movement speed through the environment was fixed. Target locations within the virtual arena were arranged in a 3 × 3 grid and spaced 290 unreal units apart (time between adjacent targets is approximately 0.5 seconds). The optimal trajectory was calculated for correct trials as the navigation start time to the time in which the animal reaches the correct target location.

The optimal trajectory analysis was calculated for correct trials. It is calculated as the real length of the animal's trajectory to correct target location divided by the optimal trajectory (i.e., the Euclidean distance from the start position to the target location).

For incorrect trials, we calculated the distance from the animal's final position to the correct target location. Distance values were modified from arbitrary 'Unreal' units (the unit system in Unreal Engine Development Kit, Unreal Engine 3, Epic Games) to 'Unreal' units divided by the distance between two targets to increase interpretability. A new value of 1 would represent 290 unreal units (the distance between two adjacent targets).
**Eye behavior.** Percent of eyes on screen measures the number of eye data points falling on the screen divided by the total number of eye data points. Off screen data points occur when the animal looks off screen or closes their eyes (as occurs during blinking).

Eye data was classified into fixations and saccades based on a method outlined in Corrigan et al., 2017 that was developed for use in a similar virtual environment. The percent of fixations on target was calculated by the number of fixation events falling within a trial’s target location divided by total number of fixation events. We used a linear classifier (SVM) (Libsvm 3.14, Fan et al., 2008) with 5-fold cross validation to predict target location from eye fixation position data.

The main sequence was calculated by separating saccades into bins of 3° of amplitude, starting at 2° and computing the medians for each bin. The proportion of single units tuned for eye position in both retinocentric and spatiocentric reference frames was calculated using a quadrant binning pattern for a 40°×30° field. A bin had to have at least ten saccades to be acceptable and sessions had at least three acceptable bins.

**Spike processing.** Neuronal data was recorded using a Cerebus neuronal Signal Processor (Blackrock Microsystems) via a Cereport adapter. The neuronal signal was digitized (16 bit) at a sample rate of 30 kHz. Spike waveforms were detected online by thresholding at 3.4 standard deviations of the signal. The extracted spikes were semi-automatically resorted with techniques utilizing Plexon Offline Sorter (Plexon Inc.). Sorting results were then manually supervised. Multiunits consisted of threshold-crossing events from multiple neurons with action potential-like morphology that were not isolated well enough to be classified as a well-defined single unit (for spike sorting example see Extended Data Fig. 10d, e). We collected behavioral data across 20 working memory sessions (eight in animal T, twelve in animal B) and neural data across 17 sessions. This yielded a total of 3950 units recorded: 2578 single neurons (346 in animal T, 2232 in animal B) and 1372 multiunits (512 in animal T, 860 in animal B). We collected behavioral data across 18 ketamine-working memory sessions (nine in animal T, nine in animal B) and neuronal data from 17 ketamine-working memory sessions with one session from animal T removed due to incomplete synchronization of neuronal data during the recording. This yielded a total of 2906 units recorded during ketamine-working memory sessions: 1814 single neurons (259 in animal T, 1555 in animal B) and 1092 multiunits (533 in animal T, 559 in animal B).

**Spike density function.** Spike density functions (SDFs) were generated by convolving the spike train with a Gaussian kernel (standard deviation=100 ms).

**Time consistent neurons.** To qualify time consistent neurons, we created SDFs combined between electrodes arrays over the entire trial time using neurons with firing rates above 0.5 Hz. SDFs were created for each condition that contained at least five trials. We calculated the peak firing time for each neuron in the population, calculated the standard deviation of the peak firing time for each neuron over all trials in a condition and created a probability distribution from the standard deviation values. We shuffled the peak firing times for each neuron from trial to trial so that the peak firing time no longer aligned for any one neuron. We created a shuffled probability distribution. We calculated the difference in mean values between the real and shuffled distributions to get the mean difference value. To calculate the standard deviation values plotted in Extended Data 4a, we calculated trial-trial standard deviation of peak spike time for the target condition in which each neuron fired the most consistently during correct trials (i.e., lowest deviation). The same conditions were used for shuffled data and for incorrect trials.

ODR1: This data was collected from the same animals and electrodes as our naturalistic VR task. This task contained 16 targets (Extended Data Fig. 7a) and was a variation of a traditional ODR task in which the fixation point changes location across trials, resulting in many different task conditions with varying combinations of fixation location and target location. For this reason, we grouped trials with target locations within the same quadrant (same direction saccade). To match the task structure of the VR task, we did not use data from the fixation period.

ODR2: This data was collected from NHPs JL and F (Extended Data Fig. 7b) using one Utah array implanted in the left LPFC (same region as NHP B and T). This task contained 16 targets with a consistent central fixation point (Extended Data Fig. 7a). To match the task structure of the VR task, we did not use data from the fixation period. Since the ODR2 task had jittered delay epoch timing, we used trials with delay periods > 1000 ms and included the first 1000 ms of the epoch.

**Sequence representation.** Each trial was represented as a complex-valued vector by mapping the time of maximum spike density of each neuron to a phase value between - and . For the main analysis, only cells with peak firing time during the delay (working memory) period were included in the sequences. To compare across trial epochs, cue and navigation sequences were also considered, including only the cells with peak firing time in the cue and navigation periods respectively.

**Dimensionality reduction.** For each recording session, a correlation matrix was created by computing the correlation coefficient between each pair of phase vectors representing single trial sequences. The eigenspectrum of this matrix was computed, and the eigenvalues sorted in descending order of modulus. The correlation matrix was then projected onto the eigenvectors corresponding to the first three sorted eigenvalues to generate a low-dimensional summary of the data in 3D-space. The points in this projection each correspond to one trial, and their positions are determined by the relative similarity of the corresponding sequences. The centroids of the clusters corresponding to each trial condition were then determined, and the matrix of Euclidean distances between the centroids was computed.

The points corresponding to a specific target location l defined a cluster, and the centroids were computed, resulting in one coordinate triple, c_l = (x_l, y_l, z_l)∈R^3, corresponding to each target location. The matrix, D, of Euclidean distances between each pair of centroids was then computed, with 

\[ D_{ij} = \sqrt{((x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2)}. \]

In this way, the spiking data from each recording session was reduced to a 9x9 distance matrix.

**Correlation analysis.** For each recording session, mean trajectories followed by the subject to each target location were
obtained by averaging the group of correct trajectories to that target (excluding outlier trajectories with z-score > 1 of mean Frechet distance to other trajectories in the group) (Alt & Godau, 1995). A 9x9 distance matrix was then created from the Frechet distances between each of the mean trajectories. In this way, each recording session was described by two 9x9 normalized distance matrices, one representing the relationships between target centroids in 3-space, and the other representing distances between behavioral trajectories to the target locations in the virtual environment. The correlation between the two distance matrices was then computed, which measures the similarity between the neuronal representations of the targets and the physical trajectories to them.

**Target columns.** Since centroids of targets in the same column tended to cluster together, some subsequent analyses group sequences by target column rather than trial condition. In these analyses, the clusters corresponding to targets in the same column were combined to produce 3 centroids instead of 9. Furthermore, due to this structure observed in the data, a null model for comparison was created by shuffling the target columns while preserving the target rows, thereby destroying the correlation while preserving more of the structure of the data.

**Dimensionality reduction for ODR.** When repeating the analysis for the oculomotor delayed response task, 16 targets were included so the data was reduced to a 16x16 distance matrix describing the relationships between the neuronal representations of the target locations. Furthermore, since the ODR task does not contain a navigation component, the distance matrix of the projection centroids was compared to the matrix of Euclidean distances between targets (rather than a Frechet distance matrix).

**Projection classification analysis.** We used a simple classifier based on our computational approach with 5-fold cross-validation to classify sequences on a single trial basis. The classifier assigns labels to points in the projected 3-space, assigning each trial in the test set (20% of trials) to the centroid of trials in the training set (80% of trials) which has the minimum Euclidean distance to the trial in question. In the supervised version, the training set centroids are determined using the trial condition labels. The unsupervised version uses K-means clustering to determine the training set centroids.

The same method is used for all classifiers. To classify single trials by trial condition (9 targets), the supervised classifier was necessary. To classify single trials by target column (3 columns), the unsupervised version was used. To classify single sequences by trial epoch (3 epochs), the cue, delay, and response sequences (see ‘Sequence Representation’) were all used to create the correlation matrix (i.e., the correlation between each pair of sequences from all 3 epochs was used to generate the projection), and the centroids are defined by sequence epoch clusters rather than trial condition clusters.

**Trajectory analysis.** We repeated the centroids analysis described above, but replaced the distance matrix describing mean trajectories with two other task-relevant measures. First, we constructed geometrically ‘ideal’ trajectories straight from the start location to each target, and repeated the analysis using Frechet distance between trajectories. Second, we used the matrix of Euclidean distances between target locations. Unlike the mean trajectory analysis, these measures only described the task set up and did not include behavioral data.

To determine whether the sequences represented motor planning during the delay that was replayed during navigation, we compared the sequences specific to the delay epoch with those from the navigation epoch. For each recording session, we computed the correlation between each delay-navigation sequence pair. We then considered the pairs in which the delay sequence and navigation sequence corresponded to the same trial separately from the pairs where the two sequences came from different trials. To consider this measure across recording sessions, we computed the distributions of correlation values for each group (same trial versus different trial).

**Single versus multiple contribution.** In the main analysis, we assume each cell contributes only once to the sequence representation, at its time of peak firing. As such, each cell participates in only one of the cue, delay, or navigation epoch sequence. To test the validity of this assumption, we repeated the analyses for sequences in which each cell participated in all three epochs, by considering the time of max firing within each epoch.

**Removing cells.** For percentages increasing from 10% to 90% in 10% increments, we removed a percentage of cells contributing to the delay sequence in each trial. The correlation analysis was then performed for the sequences with removed cells. This process was repeated for 10 iterations of both correct trials and incorrect trials, and correlation values were averaged across iterations to produce the plot. The difference between results for correct and incorrect trials is also plotted.

**References**


Chapter 2

An algebraic approach to spike-time codes in the hippocampus

The previous chapter demonstrated the utility of a new mathematical approach to spike-time codes. This approach linked temporally precise sequences of spiking activity in the LPFC of macaque monkeys to specific WM content. Here, we turn to a different set of neural sequences: place field activity in the rodent hippocampus. We derive an analytical expression to describe this phenomenon, and use the mathematical properties of this description to provide insight into what these precisely coordinated spike sequences may encode. The operator describing this phenomenon bears notable similarities to a DFT – being carried out by the brain. Its symmetry properties reveal a behaviourally relevant space-time symmetry that contributes to neural coding in this phenomenon.

During navigation and learning on a physical track, specific cells in the rodent hippocampus develop highly structured responses. These cells fire preferentially when the rodent traverses specific locations along the track. As such, they are termed “place cells”, and thought to function as a hippocampal “GPS system” [37–39].

The firing responses of these cells are more complicated than just a location-specific increase in firing rate. In fact, the timing of individual spikes (or spike bursts) during these responses are highly structured relative to the population activity in this brain region. During navigation, the rodent hippocampus exhibits a regular population rhythm [40–43]. This 8 Hz oscillation is termed the “theta rhythm”, and functions as the metronome which ties together the place cell representations across cells and over time.

Here, we study the structure of these place cell firing responses, known as the “phase precession” phenomenon. It is thus named because individual cells begin firing at a specific phase of the theta rhythm, then “precess” – fire at earlier and earlier phases of theta in subsequent cycles [44, 45]. The physical location on the track in which a cell fires is known as its “place field”. Within a single theta cycle, the population of active place cells forms a sequence, with cells nearing the end of their place field firing first, and cells at the beginning of their place field firing near the end of the cycle. These sequences have been shown to compress events that play out on a behavioural timescale (for example, the traversal of a segment of a track) down to a timescale that is relevant for synaptic plasticity (one theta cycle, 125ms), and have thus been implicated in learning and memory formation [46–50]. A third dimension structuring this phenomenon takes place along the dorsoventral axis of the CA1 region of the hippocampus.
Along this axis, place field size increases linearly [51, 52].

The three dimensions of phase precession (single neurons across cycles, local populations within a single cycle, and global populations spanning the dorsoventral axis) have been well documented experimentally [37,38,44,45]. Significant work has been done to relate this phenomenon to synaptic plasticity and memory formation [45, 47]; however, the fact that this phenomenon represents one of the clearest experimental examples of a precise spike-time neural code has been largely overlooked. Here, we investigate the nature of this code by deriving an operator expression mapping points along a trajectory to complex-valued spikes. The mathematical symmetries of this operator reveal a space-time symmetry in the phase precession code that unites its role in spatial navigation with its role in memory formation. This operator demonstrates the utility of an algebraic approach to spike-time codes that goes beyond dimensionality reduction, as introduced in Chapter 1, to investigate the structure of putative codes. The mathematical description of this structure enabled the construction of a novel decoder, which decodes the trajectories of a simulated rodent from only an initial position and a pattern of spike-times, and operates on much less data than is typically used by decoders based on average firing rate. The following manuscript is the result of this work.
An algebraic approach to spike-time neural codes in the hippocampus

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Although temporal coding through spike-patterns has long been of interest in neuroscience, the specific structures that could be useful for spike-time codes remain highly unclear. Here, we introduce a new analytical approach, using techniques from discrete mathematics, to study spike-time codes. As an initial example, we focus on the phenomenon of “phase precession” in the rodent hippocampus. During navigation and learning on a physical track, specific cells in a rodent’s brain form a highly structured pattern relative to the oscillation of population activity in this region. Studies of phase precession largely focus on its role in precisely ordering spike times for synaptic plasticity, as the role of phase precession in memory formation is well established. Comparatively less attention has been paid to the fact that phase precession represents one of the best candidates for a spike-time neural code. The precise nature of this code remains an open question. Here, we derive an analytical expression for an operator mapping points in physical space to complex-valued spikes by representing individual spike times as complex numbers. The properties of this operator make explicit a specific relationship between past and future in spike patterns of the hippocampus. Importantly, this mathematical approach generalizes beyond the specific phenomenon studied here, providing a new technique to study the neural codes within precise spike-time sequences found during sensory coding and motor behavior. We then introduce a novel spike-based decoding algorithm, based on this operator, that successfully decodes a simulated animal’s trajectory using only the animal’s initial position and a pattern of spike times. This decoder is robust to noise in spike times and works on a timescale almost an order of magnitude shorter than typically used with decoders that work on average firing rate. These results illustrate the utility of a discrete approach, based on the structure and symmetries in spike patterns across finite sets of cells, to provide insight into the structure and function of neural systems.

I. INTRODUCTION

The brain encodes sensory information, makes decisions, and generates motor outputs through patterns of activity across large populations of neurons. It remains unknown, however, whether these patterns are made up of precisely coordinated and meaningful spike times [1], or whether the timing of the spikes is random and only their average rate is meaningful [2, 3]. Recent experimental results in the songbird singing [4–7] and the motor system [8] indicate that precise spike timing can dramatically influence behavior [9–12]. In this work, we introduce an approach to study specific, individual spike patterns, using methods from discrete mathematics. As a first demonstration, we consider one of the clearest experimental examples of a spike-time pattern observed in the brain – phase precession in the rodent hippocampus.

A. Phase precession

In the rodent hippocampus during navigation on a linear track, the timing of single neuron spikes exhibits a precise relationship with the large-scale population rhythm in this brain area [13, 14]. Neurons in the CA1 region of the hippocampus fire spikes when the animal is in a specific location of an environment, creating a neural representation of that location [15–17]. The spiking regions of individual cells, called their “place field”, tile the environment (Fig. 1, top). During navigation, population activity in the hippocampus oscillates at a rhythm of 8 Hz, which is termed the θ-rhythm [18–21]. Place field sizes are ordered across the dorsal-ventral axis of the CA1 region, with cells closer to the ventral pole having progressively larger place fields [22, 23]. As a result, the brain assembles a map of physical space along an axis of cortical space, using the theta oscillation as a metronome to tie these two representations together. The computational consequences of both this mapping and the precise spike timing relative to the θ-rhythm, however, remain an open question in neuroscience.

In studying the relationship of action potential timing to the θ-rhythm, researchers discovered that a single neuron begins spiking at the peak of the population θ-rhythm, with the timing of spikes occurring earlier and earlier in following cycles [13]. This phenomenon is called “phase precession” because spikes start at a specific phase of θ and then progress to earlier and earlier phases while the animal traverses the place field (Fig. 1, middle). This process is critical to formation of mem-
ory traces [24–26], which are then thought to be transferred to neocortex for consolidation and long-term storage [27, 28]. The spike-time pattern formed by a population of cells exhibiting phase precession (Fig. 1, middle) is central to this process of memory trace formation, as the pattern is thought to compress behavioral sequences that last for several seconds onto the timescale relevant for synaptic plasticity [14, 24].

![Diagram of Place fields, Phase procession, and Complex-valued spike times](image)

**FIG. 1.** Phase precession in the rodent hippocampus. (top) Depicted are five neurons (N1-N5) with active place fields during navigation from left to right on a linear track. (middle) While the rodent runs through the place field of cell N3 (blue), the phase of its spikes systematically advances with respect to the population \( \theta \)-rhythm (n.b. opposite in sign to the local field potential, relative to which phase is defined), starting close to \( \pi \) at the beginning of the place field, advancing to 0 at the place field center, and ending near \(-\pi\) at the end of the place field. (bottom) A complex-valued representation of the pattern formed by the phase precession of multiple neurons N1-N5 with place field centers spaced across the track creates a compressed sequence of spike times in each individual \( \theta \)-cycle.

Phase precession and the compression of temporal sequences of neural activity have been well described in the dorsal pole of CA1 [14], which represents the finest spatial scales [22, 23]. Absent, however, is a description of what this sequence structure means for the global pattern of hippocampal activity across multiple dorsoventral levels. In other words, what can this experimental example tell us about spike-time codes across a whole brain region? The population \( \theta \)-rhythm itself, which was long thought to be synchronous throughout hippocampal CA1, has also recently been found to be systematically organized as a wave traveling from the dorsal to the ventral pole of CA1 [29, 30]. The combination of phase precession at multiple levels of the dorsoventral axis, along which the size of place fields increases linearly [31], with the wave-like organization of the \( \theta \)-rhythm along this same axis raises the possibility that a sophisticated structure is apparent in the global pattern of spike times across the hippocampus. Understanding such a global structure in spike times could provide insight into how the hippocampal neural code is organized during the process of memory trace formation.

In the following sections, we derive equations describing spike times in the population of neurons across the hippocampus during this phenomenon. By representing spike phases relative to the \( \theta \)-rhythm in terms of complex numbers (Fig. 1, bottom), we arrive at an operator expression relating physical space to spike times in the hippocampus. We show that this operator leads to a symmetry between past and future spike patterns in hippocampal populations, and that this symmetry reflects a specific trajectory in space and time. Further, this symmetry provides a specific meaning to the recent observation that the \( \theta \)-rhythm is a wave traveling across the dorsoventral axis [29]. Based on our operator expression, we then introduce a spike-based decoder that can correctly predict the animal’s location. This decoder requires only the animal’s starting position and spike times, and it operates on a timescale almost an order of magnitude shorter than current decoders that work on average firing rate [32, 33]. Importantly, this decoder, which is derived from the operator expression, allows us to relax key simplifying assumptions made in developing the mathematical approach. Taken together, these results provide fundamental new insight into mathematical approaches to spike-time codes, in addition to the specific temporal code exhibited during phase precession in the hippocampus.

**II. RESULTS**

**A. Analytical approach**

We start by introducing our notation for the hippocampal spike pattern. Experimental observations show that place field length varies along the dorsoventral axis and is constant on cross-sections of the axis [22, 23]. In order to parametrize these quantities, we introduce the variable \( \ell \in [0,1] \) to represent position along the dorsoventral axis, with \( \ell = 0 \) being the dorsal pole. To good approximation, place field length increases linearly along the dorsoventral axis [31]:

\[
L_\ell = L_0 + (L_1 - L_0)\ell, \tag{1}
\]

where place field lengths range from less than one meter at the dorsal pole \( L_0 \) to approximately 10 meters at the ventral pole \( L_1 \) [31].

The total phase precession of a place cell during a single traversal of its place field spans approximately a full \( \theta \) cycle [14]. More precisely, the spikes of a cell systematically advance their phase with respect to the \( \theta \) oscillation, with a total phase gain approaching \( 2\pi \). It is well documented that the phase of a spike (or the mean phase of a spike burst) of an active place cell within a theta
cycle reflects the fraction of the cell’s place field the rodent has traversed at the moment of the spike [13, 14]. Note that the spike could represent a single action potential or the centroid for a burst of spikes, as typically considered in studies of phase precession [14, 24]. Here we construct a model which makes the relationship between the animal’s position within place fields and spike phases precise. Taking \( \phi \in [-\pi, \pi] \), the spike happens at a phase \( \phi \) such that the expression
\[
\frac{-\phi + \pi}{2\pi}
\]
equals the fraction of the place field covered at the time of the spike. Setting the space coordinate of the place field centre to be \( c \), the spike phase can then be retrieved as
\[
\phi = -2\pi \frac{x - c}{L_\ell}.
\]
(3)

Importantly, while the phase offset of \( \theta \) will change linearly with \( \ell \) as the \( \theta \) wave travels over the dorsoventral axis, reaching \( \pi \) at the ventral pole [30], here we represent each spike’s phase with respect to the local \( \theta \)-rhythm, instead of referencing all spikes to the phase of the \( \theta \) traveling wave at the dorsal pole.

A spike happening at the physical space-time coordinates \((x_s, t_s)\) can thus be mapped to a complex valued spike phasor using (1) and (3). The mapping from physical space and time to complex-valued spikes can be seen as an operator \( H : \mathbb{R}^2 \to \mathbb{C} \):
\[
H : (x_s, t_s) \to \exp \left( -2\pi i \frac{x_s - c}{L_0 + (L_1 - L_0) \ell} \right).
\]
(4)

As expected from experimental evidence [24], only spatial position appears in the \( H \) operator. This closed-form analytical expression now allows us to understand the functional significance of phase precession in terms of symmetries in this discrete pattern of interleaved spikes in the hippocampus.

**B. Space-time symmetries in the \( H \)-operator**

We now (1) describe the time evolution of the spike pattern in terms of the \( H \)-operator, (2) define operations equivalent to shifting the spike pattern across \( \theta \)-cycles in time or across place cells in space, and then (3) use these operations to study symmetries in this space-time representation in the hippocampus. While we focus in this section on the well-studied case of rodents navigating on a linear track, the analytical form for the operator \( H \) allows us to generalize quite naturally to spike patterns in two dimensions, as we will show later.

First, we describe the time evolution of the spike pattern in terms of the \( H \)-operator. If a cell’s total phase precession spans a full \( 2\pi \), then it oscillates with precisely one more cycle than the \( \theta \)-rhythm during place field traversal. Assuming that in the \( j \)-th \( \theta \)-cycle the animal’s velocity \( v_j \) is constant (without requiring constant velocity on a longer timescale), the spiking frequency \( f_\ell \) of a place cell at dorsoventral location \( \ell \) is:
\[
f_\ell = f_\theta + \frac{v_j}{L_\ell} = f_\theta + \frac{v_j}{L_0 + (L_1 - L_0) \ell}.
\]
(5)

Note that (5) defines the local slope of this relationship without requiring global information about the full phase precession.

Second, we define an operation equivalent to shifting the spike pattern across \( \theta \)-cycles in time. Let \( c_k \) be the position of the place field center of cell \( k \), and let \( x_{(j,k)} \) denote the position at which cell \( k \) fires during \( \theta \)-cycle \( j \). The phase corresponding to this spike (or burst) is given by \((S_j)_k = H(x_{(j,k)}) = \exp(-2\pi i x_{(j,k)} - c_k)\). If we let \( T_\ell = 1/f_\ell \) denote the time between consecutive spikes of the same cell, then the spike phase of the cell \((S\ell)_k\) in the subsequent theta cycle can be described in terms of \((S\ell+1)_k\):
\[
(S_{\ell+1})_k = \exp \left( -2\pi i \frac{x_{(j+1,k)} - c_k}{L_\ell} \right)
= \exp \left( -2\pi i \frac{x_{(j,k)} + v_j T_\ell - c_k}{L_\ell} \right)
= \exp \left( -2\pi i \frac{x_{(j,k)} - c_k}{L_\ell} \right) \exp \left( -2\pi i \frac{v_j T_\ell}{L_\ell} \right)
= H(x_{(j,k)}) \exp \left( -2\pi i \frac{v_j}{L_\ell f_\ell} \right)
= (S_j)_k \exp \left( -2\pi i \frac{v_j}{L_\ell f_\ell} \right).
\]
(6)

This equation indicates that, to advance \( S_j \) to the next \( \theta \)-cycle, we rotate clockwise by an angle of \( 2\pi \frac{v_j}{L_\ell f_\ell} \).

Third, we now specify and study the symmetry in the phase precession spike pattern. We fix the dorsoventral location \( \ell \) so that all place fields under consideration have length \( L_\ell \). In order to illustrate this idea, we now consider a simplified scenario in which \( v \) is constant during a single run and a population of neurons in which a new cell starts firing at phase \( \pi \) on each \( \theta \)-cycle. In this case (Fig. 2c-g), the phase difference \( \Delta \phi = -2\pi \frac{v_j}{L_\ell f_\ell} \) between spikes of the same cell in two consecutive \( \theta \)-cycles equals the difference in phase between the spikes in the same \( \theta \)-cycle of two cells with consecutive place field centers:
\[
\Delta \phi_n = \frac{2\pi}{T_\theta} (T_\theta - T_\ell) = 2\pi \left( 1 - \frac{f_\theta}{f_\ell} \right) = 2\pi \left( 1 - \frac{L_\ell f_\theta}{v} \right).
\]
(7)

Therefore, the following rotation transforms the spike of cell \( k - 1 \) in \( \theta \)-cycle \( j \) into the spike of cell \( k \) in \( \theta \)-cycle \( j \) (Fig. 2b):
FIG. 2. Phase precession schematic with computation of $\Delta \phi_x$ and $\Delta \phi_y$. (a) Schematic depicting the relationship between phase, time, and space in the hippocampus. In theta cycle $j$, cell $k$ spikes near $-\pi$ phase, indicating place field $k$ began in the recent past. The time between the spikes of cell $k$ in $\theta$ cycles $j$ and $j+1$ is given by $T_\theta$, which is slightly shorter than the length of the $\theta$ cycle, $T_\theta$. In that time, the rat travels a distance of $vT_\theta$ along the track. The place field center is $c_k$ and the rat's physical positions at the time of the spikes are denoted. (b) This diagram demonstrates the relationships between the vector operations for the population of neurons under consideration, where $H$ maps a physical position along the track to the spikes of the full population using the shift between cells, and $H'$ propagates the spike pattern of the population into the next $\theta$ cycle using the shift in time. (c) Depicts the difference between $\Delta \phi_x$ and $\Delta \phi_y$. (d) When these two quantities are equal, the spike pattern of the population is invariant. Advancing forwards one $\theta$ cycle simply rotates the labels of the spikes clockwise by one spike. (e) The $x$ and $y$ axes represent time and spike phase, respectively. The vertical black bars demarcate theta cycles. The diagonal black lines represent the phase of the $\theta$-cycle. The blue and red circles represent the spike phases of two cells at the same location along the dorsoventral axis. These phases are determined by the intersection points of the $\theta$-cycle phase (diagonal black lines) with the coloured lines, the slope of which is determined by the spiking frequency of the place cell. (f) Computation of $\Delta \phi_y$. A close-up view shows the invariant case when the phase difference between the spikes of two cells with consecutive place fields, $\Delta \phi_y$, is exactly opposite to the phase difference between spikes of the same cell in consecutive theta cycles $\Delta \phi_x$. The diagonal black lines have slope $2\pi/T_\theta$ by construction, since they represent the phase of the $\theta$ oscillation across by one cycle. This means $\frac{\Delta \phi_y}{T_\theta} = \frac{\Delta \phi_x}{T_\theta}$. (g) Here, the same values are represented on the unit circle in the complex plane. Rotating clockwise by $\Delta \phi_x$ propagates the spike of a cell into the subsequent theta cycle. A rotation of $\Delta \phi_y$ counterclockwise is a shift forwards in space: it transforms the spike of cell $k$ into the spike of cell $k+1$, which has the next place field.

Equations 6 and 8 allow us to derive an explicit expression for the symmetry in the hippocampal spike code. Take $\tau = (f_\text{t} - f_\text{d})$ to be the temporal frequency of the phase precession phenomenon itself (with units rad/s) and $\chi = 1/L_\text{t}$ to be the spatial frequency determined by the length of a place field (with units rad/m). When $v = \tau/\chi$, the discrete spike pattern is invariant, i.e. $(S_j)_{k} = (S_j)_{k+1}$, $\forall k \in [1, N_A]$, where $N_A$ is the number of actively spiking cells (see Methods - Complex-Valued Spikes). Here, the quantity $\tau/\chi$ has an important physical meaning: this invariant, with units of m/s, represents a fixed trajectory linking past to future locations on a specific space-time scale. Further, the temporal frequency determining this space-time scale is $\tau = (f_\text{t} - f_\text{d})$, representing two key phenomena internal to the hippocampus, the $\theta$-rhythm itself and the spiking frequency of a place cell during phase precession. Finally, while we focused on the case of constant speed, the spike patterns resulting from an arbitrary time-varying movement profile (reflecting changes in speed) involve a straightforward extension of this calculation.

This mathematical approach becomes even more revealing when considering this invariant at multiple dorsoventral levels in the hippocampus. The quantity $\tau/\chi$ is independent of $\ell$. This quantity defines a space-time scale projecting a trajectory into the future. This specific space-time scale remains invariant whether the local populations represent the smallest spatial scales ("dorsal", Fig. 3a right) or the largest spatial scales ("ventral", Fig. 3a right). As the $\theta$-wave sweeps across CA1 during each oscillation cycle (color scale, Fig. 3a), the local populations across the dorsoventral axis repre-
FIG. 3. Relationship between dorsoventral location and phase precession. (a) A mouse brain depiction is coloured by the phase offset of the $\theta$-rhythm along the dorso-ventral axis, along which the $\theta$-rhythm travels as a wave with total phase offset $\pi$ [30]. (b) Phase precession is depicted for three cells at different locations along the dorso-ventral axis using the same colour scheme.

This decoding algorithm allows us to study how the spike train structure described by the $H$-operator may encode the position of the animal under realistic conditions, including biological noise in spike phase, varying speed of the animal, and phase precession in two dimensions. We first applied the decoding algorithm to linear trajectories within a 2D environment (Fig. 5a, solid line and dots), which were well handled by the algorithm. Further, the general form of this operator provides a straightforward generalization to decoding two-dimensional trajectories with arbitrary curvature (Fig. 5b, black solid line and black dots). The trajectories decoded by the algorithm are robust to noise in the spike phase (Fig. 5b, blue dots, and Fig. 5d). In contrast, random spike phases resulted in decoded trajectories extending far from the correct path and in different directions on each iteration (Fig. 5b, red dots). These results show that a decoding algorithm inspired by the $H$-operator introduced in this work can faithfully decode spatial locations from the temporal pattern of spikes alone, and on the timescale of a single $\theta$-cycle.
this operator leads to a clear space-time symmetry between past and future spike patterns in the hippocampus. This theoretical approach unites the role of the hippocampus in spatial navigation with its role in memory formation. Finally, we introduced a spike-based decoder that can successfully predict an animal’s spatial location under realistic noisy conditions, and on the timescale of a single $\theta$-cycle ($\sim$125 ms), a timescale almost an order of magnitude shorter than typically used with rate-based decoders [32, 33].

A. Difference from previous work

Theoretical work on phase precession has largely focused on its role in memory formation, since these spike-time structures compress behavioural timescales onto the scale of a single $\theta$-cycle [14]. This sequence compression has been causally implicated in the process of memory formation by pharmacological manipulation of NMDA receptors [24], can allow temporal-order learning [36], and has been studied in both spiking network models [37] and analytically [38]. Further, [38] studied how phase precession (where cells spike at a frequency just higher than the population) can be consistent with the oscillation in the population spiking activity. Finally, a position-theta-phase model is proposed in [39] to investigate the modulation of firing rate by running speed. These studies demonstrate interest in developing theoretical approaches to phase precession. This previous work, however, placed less emphasis on studying phase precession as a spike-time code. Due to the lack of theoretical work on this topic, many critical aspects of phase precession remain unexplored, including what behaviourally relevant features could be encoded by phase precessing populations and how phase precession generalizes beyond one-dimensional linear tracks (as considered in 14, 24). In this work, we have utilized approaches from discrete mathematics to understand the spike-time structure involved in phase precession. Our operator expression, which relates points on a trajectory to complex-valued spikes in a hippocampal population, provides not only a formula for the spike times in the simplified one-dimensional scenario considered in the analytical approach, but also a straightforward generalization to two (or more) dimensions. Further, this operator expression reveals a symmetry in the hippocampal representation, which encodes not just the animal’s position but also trajectories linking past and future positions. Finally, we show that the dorsal-ventral sweep of theta plays a specific role in this code: the travelling theta wave unites local representations of trajectory across CA1 in the context of an expanding spatial map.

III. DISCUSSION

In this work, we have introduced a discrete mathematical approach to spike patterns that are a key component of memory formation [13, 24]. A central idea in neuroscience is that memories are stored across large groups of neurons [34]. Neural activity is composed of discrete spiking events, but nearly all models of memory focus on the continuous rate of spikes, rather than their discrete timing. This approximation has been convenient because precise spike times are generally considered to be stochastic; however, more and more recent experimental work suggests that precise timing of spikes across groups of cells may be critical to memory formation [24, 26, 35].

By starting with a specific experimental observation in the hippocampus, where the clearest demonstration of a precise spike-time pattern has been found, we have introduced a novel, discrete approach to the problem of spike times in memory. We first introduced a model for this precise pattern of spike times, and we then derived an analytical expression for the operator mapping points in physical space to complex-valued spike times. We find

FIG. 4. Encoding and decoding spike phases: a) A 2D space tiled with place fields. Each neuron is represented by a 2D Gaussian defining the firing rate of the cell at each position in the place field. The values of the Gaussian for a particular cell along the trajectory define the black 1D curve in b). The intersections of the LFP (red) with this curve define the phase of a spike (or spike burst). These intersections occur on the rising phase before the peak firing rate (which occurs at the center of the place field) and on the falling phase after the peak. c) Depicts the decoding step to update the position estimate $\hat{x}_T$ at time $T$ for a simple case with only two place fields.
B. θ traveling wave in the hippocampus

These results provide insight into the global organization of hippocampal activity during memory formation and the recent observation that θ is a wave that travels from dorsal to ventral portions of the hippocampus [29, 30]. The hippocampus is thought to be where a “cognitive map” [16, 40] emerges, such that information about an animal’s location during navigation serves as the brain’s “GPS system” [41]. If the central function of the hippocampus were only to precisely encode spatial location, we might expect the temporal organization of activity during a θ cycle to sweep from the ventral, coarsest spatial scale of representation to the dorsal, finest spatial scale, analogous to a radar sweep that would initially scan across coarse scales and progressively narrow its focus to identify an object’s location. If, on the other hand, the fundamental function of the hippocampus is linking past and future, then a sweep from the dorsal to the ventral pole would represent a trajectory linking past and future positions at a fixed space-time scale within a larger and larger spatial map. Interestingly, recent experiments have reported that neural activity can encode possible future positions and that this neural activity can be precisely coordinated both within and between theta cycles [42–44]. Though these reports are not directly aligned with the phenomenon studied here, these experiments represent potential avenues for connecting this mathematical approach with analysis of neural recordings in future work. Importantly, our mathematical results crystallize the role of the θ traveling wave in the hippocampus, which is to establish memories as a link between past and future [27]. This role, which is in agreement with the direction of propagation observed in experimental data [29, 30], also provides one of the first clear computational roles for traveling waves in the brain [45].

C. A discrete approach to spike-time codes

Spike times are often considered to be random and modelled as stochastic point processes. As such, it is not the events themselves, but rather the rates at which they occur, that are most often related to behaviour. This may be due, in part, to the lack of mathematical tools capable of accounting for the precise timing of spikes. Approaches from discrete mathematics may provide a new avenue for exploring how spike timing may contribute meaningfully to neural computation. By studying specific spike time patterns, and leveraging their algebraic properties (such as symmetries), it may be possible to uncover general theoretical principles for the role of spike timing in neural computation. In this work, we introduce an operator approach to phase precession, one of the best known examples of a specific spike-time pattern. Representing neural spikes as complex numbers allows for the exploration of symmetries, like the rotations described above, not easily observable in a linear variable. Many possibilities exist for future work to develop general methods for arbitrary spike-time codes in populations of the hippocampus and neocortex.

FIG. 5. Spike-train decoding based on the $H$-operator. (a) 1D trajectory in 2D environment. A linear trajectory (solid line) is plotted for a simulated rodent running from left to right (color changing from blue to yellow in time), along with dots for decoded positions in each theta cycle (same color code). Simulated place cells have 1 m fields, leading to 169 active neurons on this trajectory. (b) An example 2D trajectory (solid line) is plotted along with the results from several decoding models: the $H$-model (black dots), the $H$-model with $\pi/16$ phase noise, and a null model in which each spike is assigned a random phase value. Place cells have 1 m fields, and 388 cells are active along the 3.36m trajectory. (c) For the example trajectory in b), mean error is plotted as a function of the number of place fields active along the trajectory. Different numbers of place fields were randomly generated to tile the 2D space surrounding the trajectory, resulting in different numbers of cells with place fields overlapping the trajectory. (d) Decoding error for the same trajectory is plotted as a function of added phase noise. For each value of phase noise, 10 iterations were used to produce the plot. Solid lines represent mean decoding error averaged over realizations, and shaded regions represent standard error. (e) Mean error is plotted as a function of trajectory length.
Appendix A: Methods

1. Complex-Valued Spikes

We begin by recalling the $H$-operator and notation from the main text. For cells at a fixed location $\ell \in [0, 1]$ along the dorsoventral axis of CA1, all place fields have length $L_\ell = L_0 + (L_1 - L_0)\ell$. Suppose that cell $k$ has place field center $c_k$ and spikes at the physical position $x_{(j,k)}$ in theta cycle $j$. Then the spike phase of cell $k$ in theta cycle $j$ is given by:

$$\vec{S}_j^k = H(x_{(j,k)}) = \exp \left( -2\pi i \frac{x_{(j,k)} - c_k}{L_\ell} \right)$$  \hspace{1cm} (A1)

If the total phase precession of a cell during place field traversal spans a full $\theta$ cycle, the cell is active for precisely one more cycle than the $\theta$ rhythm during the traversal. Assume that in each $\theta$ cycle, $j$, the rat’s velocity is constant and given by $v_j$; however, this velocity need not be constant along the whole trajectory. Then, the spiking frequency in $\theta$ cycle $j$ of a place cell at dorsal-ventral location $\ell$ can be written as:

$$f_\ell = f_0 + \frac{v_j}{L_\ell}$$  \hspace{1cm} (A2)

Note that this equation defines a local slope (Fig. 2e,f, red and blue lines), and does not require global information about the full phase precession (i.e., it still holds when the cell does not precess a full $2\pi$). Let $T_\ell = \frac{1}{f_\ell}$ denote the time between spikes of the same cell in two consecutive theta cycles. Then, the physical distance travelled between those two spikes is $v_j T_\ell$, as in Fig. 2a. This quantity can be used to define the shift required to propagate the spike phase of cell $k$ from theta cycle $j$ into cycle $j + 1$. In this way, it is unnecessary to compute $(\vec{S}_{j+1})_k$ from the $H$-operator directly: it can also be estimated from $x_{(j,k)}$, $T_\ell$, and $v_j$ as follows.

$$\begin{align*}
(\vec{S}_{j+1})_k &= H(x_{(j+1,k)}) \\
&= \exp \left( -2\pi i \frac{x_{(j+1,k)} - c_k}{L_\ell} \right) \hspace{1cm} (A3) \\
&= \exp \left( -2\pi i \frac{x_{(j,k)} - v_j T_\ell - c_k}{L_\ell} \right) \hspace{1cm} (A4) \\
&= \exp \left( -2\pi i \frac{x_{(j,k)} - c_k}{L_\ell} \exp \left( -2\pi i \frac{v_j T_\ell}{L_\ell} \right) \right) \\
&= \exp \left( -2\pi i \frac{x_{(j,k)} - c_k}{L_\ell} \right) \\
&= (\vec{S}_j)_k \exp \left( -2\pi i \frac{v_j}{L_\ell f_\ell} \right)
\end{align*}$$

Therefore, rotating clockwise by an angle of $2\pi \frac{v_j}{L_\ell f_\ell}$ propagates the spike of cell $k$ forward to the next theta cycle.

Now, let $v_j = v$ be constant throughout the trajectory, and suppose a new neuron begins spiking every theta cycle. In this case, the phase difference $\Delta \phi_\ell = -2\pi \frac{v}{\pi f_\ell}$ between the spikes of the same cell in two consecutive theta cycles equals the difference in phase between the spikes in the same theta cycle of two cells with consecutive place field centers. This quantity is given by $\Delta \phi_\ell$ and can be computed from Fig. 2f as: $\Delta \phi_\ell = \frac{2\pi}{\tau_\ell} (T_\ell - T_0) = 2\pi (1 - \frac{L_0}{L_1}) = 2\pi (1 - 1 - \frac{L_0}{L_1})$. Here, the positive slope represents moving from the spike of the red cell to that of the blue cell. Since the red cell has precessed more than the blue cell, we know the place field of the red cell began before that of the blue cell; i.e., if blue denotes cell $k$, then red denotes cell $k - 1$.

We can therefore define the following rotation to transform the spike of cell $k - 1$ in theta cycle $j$ into the spike of cell $k$ in theta cycle $j$:

$$\begin{align*}
(\vec{S}_j)_k &= (\vec{S}_j)_{k-1} \exp \left( 2\pi i \left( 1 - \frac{f_0}{f_\ell} \right) \right) \hspace{1cm} (A6)
\end{align*}$$

Equivalently, $(\vec{S}_j)_{k-1} = (\vec{S}_j)_k \exp \left( -2\pi i \left( 1 - \frac{f_0}{f_\ell} \right) \right)$, shifts backwards in space, moving from one cell to another with a place field that began earlier in the trajectory.

As in Fig. 2g, we define the shift from one cell to the next by the rotation of $2\pi (1 - \frac{L_0}{L_1})$ counter clockwise. Importantly, this phase difference, $\Delta \phi_\ell$, is exactly opposite $\Delta \phi_\ell$. We can therefore shift forwards in time (i.e. propagate the spike of a cell forwards one theta cycle) by rotating clockwise, and shift forwards in space (i.e. shift to the cell with the subsequent place field) by rotating counter clockwise. Furthermore, when considering a population of $N_A$ active cells at dorsal-ventral location $\ell$, in which a new cell begins spiking every theta cycle, the phase pattern of the population remains invariant across the trajectory. In this case, the spike phases of the neurons are equally spaced around the unit circle in the complex plane, and advancing forwards in time simply rotates the neuron indices from spike to spike. These rotations can be used to define the following vector operations, depicted in Fig. 2b, which consider the aforementioned population of cells as a whole.

First, map the animal’s position along the track to the population spike pattern in one $\theta$ cycle using the operation: $\mathcal{H} : \mathbb{R} \to \mathbb{C}^{N_A}$ maps $x_{(j,1)} \to \vec{S}_j$, with

$$\begin{align*}
(\vec{S}_j)_k &= (\vec{S}_j)_1 \exp \left( 2\pi i k \left( 1 - \frac{f_0}{f_\ell} \right) \right) \hspace{1cm} (A5)
\end{align*}$$

Here, cell $1$ is the active cell with the earliest place field center; i.e. in theta cycle $j$, cell $1$ reaches the end of its place field and spikes close to phase $\pi$. This operation maps the physical location of a single spike to the spike pattern of the full population.

Second, map the spike pattern in one $\theta$ cycle to the spike pattern in the next $\theta$ cycle using the shift between $\theta$ cycles: $\mathcal{H}_\ell' : \mathbb{C}^{N_A} \to \mathbb{C}^{N_A}$ maps \vec{S}_j \to \vec{S}_{j+1}$ with

$$\begin{align*}
\vec{S}_{j+1} &= \vec{S}_j \exp \left( -2\pi i \frac{v}{L_\ell f_\ell} \right) \hspace{1cm} (A6)
\end{align*}$$

between the spikes of the same cell in two consecutive theta cycles.
These vector operations can be composed, as illustrated in Fig. 2b. As such, in the simplified case of constant speed, it is unnecessary to recompute the population spike pattern of the \( N_A \) active cells individually in each \( \theta \)-cycle using the \( \mathcal{H} \) operator. Instead, the \( \mathcal{H} \) operator can be used to define the population spike pattern from one physical position, and then the \( \mathcal{H}' \) operator can be used to propagate the spike pattern forwards in time. In this case, the spike pattern is invariant in time; i.e., \( (S_j)_{k+1} = (S_j)_k \) for all cells \( k \) and \( \theta \)-cycles \( j \), as is made clear by re-expressing \( (S_j)_{k+1} \) as two specific rotations of \( (S_j)_k \):

\[
(S_j)_{k+1} = (S_j)_k \exp \left( 2\pi i \left( 1 - \frac{f_\theta}{f_\ell} \right) \right) \\
\quad \text{(shift forward one cell)} \\
= (S_j)_k \exp \left( -2\pi i \frac{v}{L_\ell f_\ell} \exp \left( 2\pi i \left( 1 - \frac{f_\theta}{f_\ell} \right) \right) \right) \\
\quad \text{(shift forward one \( \theta \)-cycle)} \\
= (S_j)_k \text{ precisely when} \\
-2\pi i \frac{v}{L_\ell f_\ell} + 2\pi i \left( 1 - \frac{f_\theta}{f_\ell} \right) = 0 \quad (A7)
\]

In this case, we have that:

\[
v = 1 - \frac{f_\theta}{f_\ell} \implies \frac{v}{L_\ell} = f_\ell - f_\theta \implies v = (f_\ell - f_\theta)L_\ell,
\]

which yields the relationship \( v = \tau/\chi \) where \( \tau = (f_\ell - f_\theta) \) is the difference between the cellular frequency and the \( \theta \) frequency, and \( \chi = 1/L_\ell \) is the spatial frequency determined by the length of the place fields.

2. Spike-Based Decoding Algorithm

To implement the decoder, we first generate a trajectory in a two-dimensional space tiled with \( N_C \) place fields. Place field centers \( \{c_k\}_{k=1}^{N_C} \) are randomly generated and uniformly distributed across the space. The place fields all have the same diameter \( L \) reflecting cells in a specific dorsal-ventral location in the hippocampus. The trajectory is given by the positions \( \{x_j\} \) corresponding to \( \theta \)-cycles \( j \in [1, N_T] \). Note that the time-resolution of the generated trajectory is much finer than the length of a \( \theta \)-cycle. Within each cycle, the simulated animal travels over a short line segment, which we represent by a set of points, \( x_j \). The decoded position, \( \hat{x}_j \), is a point representing the decoder’s estimate for the position of set \( x_j \).

Since the sequence of line segments \( \{x_j\} \) captures both position and time, there is no need to assume the animal moves at a constant speed. As such, we implement trajectories with varying speeds, accelerations, lengths, and curvatures. We used a simple model to generate phase precession along the trajectory using a Gaussian place field and sinusoidal theta rhythm. The combination of the firing rate profile and the theta oscillation determines at which phase the spike occurs in each \( \theta \)-cycle. Following previous work [24], the moment that the sinusoidal oscillation crosses the level of excitatory input determined by the place field at each spatial location determines the spike. This moment is on the rising phase for the first half of the place field and on the falling phase for the second. Note that the spike could represent a single action potential or the centroid for a burst of spikes, as typically considered in studies of phase precession [14, 24].

This method is used to generate a matrix of spikes, \( S \), where entry \( S_{j,k} \in [-\pi, \pi] \) gives the spike phase of cell \( k \) during \( \theta \)-cycle \( j \). Note that, while \( N_C \) represents the total number of place fields tiling the space, only a small fraction of them are active during any given \( \theta \)-cycle. The number of cells which are active in a specific theta cycle, \( N_{A,j} \), is given by the number of place fields which overlap the line segment \( x_j \). If cell \( k \) does not spike during \( \theta \)-cycle \( j \), \( S_{j,k} = \text{NaN} \).

The decoder takes as input the initial position, \( x_1 \), and the matrix of spikes \( S \). Decoding proceeds as follows:

For \( j = 1 \), \( \hat{x}_j = x_1 \), the first point in the line segment \( x_1 \). For \( j \in [2, N_T] \), where \( N_T \) is the total number of \( \theta \)-cycles in the trajectory,

\[
\hat{x}_j = \hat{x}_{j-1} + \frac{\pi}{N_{A,j}} \sum_{k=1}^{N_C} F_{j,k} \frac{(-1)^\alpha (\hat{x}_{j-1} - c_k)}{\|(-1)^\alpha (\hat{x}_{j-1} - c_k)\|} \quad (A8)
\]

where

\[
\alpha = \begin{cases} 
0 & S_{(j,k)} > 0 \\
1 & S_{(j,k)} \leq 0 
\end{cases}
\]

and \( F_{j,k} \) represents the fraction of place field \( k \) traversed in theta cycle \( j \). This fraction is determined by:

\[
F_{j,k} = \frac{L}{2\pi} (S_{(j,k)} - S_{(j-1,k)} + \pi).
\]

For each \( \theta \)-cycle, the vectors \( (\hat{x}_{j-1} - c_k) \) are computed for each active cell \( k \). Each of these vectors forms a line segment from the animal’s current estimated position, \( \hat{x}_{j-1} \), to the center of a place field corresponding to an active cell. The directions of these vectors are determined by the exponent \( \alpha \); i.e., the animal moves towards the center of a place field with positive spike phase, and away from the center if the spike phase is negative. Note that here spike phases are defined with respect to the local field potential, which is opposite to the population oscillation discussed in the main text. The vectors are then normalized to unit length and multiplied by \( F_{j,k} \), so the magnitude of the vector is determined by the fraction of the place field traversed during that \( \theta \)-cycle. The sum of these vectors estimates the animal’s movement during that \( \theta \)-cycle. However, it overestimates the amount of distance covered by the animal due to overlap between place fields. The normalizing factor \( \frac{\pi}{N_{A,j}} \) was chosen experimentally to reduce the effects of this overlap. Finally,
To compute the accuracy of the decoded trajectory, we consider the minimum Euclidean distance from each estimate $\hat{x}_j$ to the line segment $x_j$. Define the line segment $x_j := \{x_{j,i}\}_{i=1}^{N_j}$, where $N_j$ denotes the number of points in the line segment, determined by the speed of the animal’s movement during $\theta$-cycle $j$. Then, the accuracy of the estimate for a single theta cycle is given by:

$$d_j := \min_i \|x_{j,i} - \hat{x}_j\|$$

For the entire trajectory, we then define the mean error $e_m := \frac{1}{N_T} \sum_{j=1}^{N_T} d_j$ and cumulative error $e_c := \sum_{j=1}^{N_T} d_j$.

In Fig. 5d, the mean error is plotted for one example trajectory with varying amounts of active place fields and added phase noise. In Fig. 5e, the mean error is plotted for 500 random trajectories of varying lengths. To produce this plot, the number of place fields tiling the space was held constant, and 500 randomly generated trajectories were decoded in separate realizations of the decoding algorithm. Note that trajectories near the boundary of the space result in increased error due to edge effects, which are straightforward to address but not considered here. The example in 5b was chosen to avoid the boundaries, but the trajectories in 5d were completely random.

ACKNOWLEDGMENTS

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Discussion

In this work, we investigate both experimentally and mathematically the role of spike-time sequences in neural computation. In Chapter 1, sequences of neural activity in primate LPFC were shown to encode working memory content. In Chapter 2, sequences of place cell activity in rodent hippocampus were analyzed as a spike-time code. These two sets of sequences are qualitatively and functionally different. The WM sequences play out over a behavioural timescale of seconds, while the place-cell sequences compress behavioural information into the timescale of a single theta cycle. The place-cell sequences follow a complex and highly structured organization along multiple dimensions: single cells across time, local populations within a single theta cycle, and global populations across the dorsoventral axis. The organization of the WM sequences is much simpler: they span a population of cells over the timescale of an experimental trial. In both cases, each cell contributes only once to a population sequence. Further, both types of sequences have an important link to memory. The WM sequences presented in Chapter 1 encode task-relevant WM items as a parallel code alongside cells exhibiting canonical persistent firing delay activity. The hippocampal sequences presented in Chapter 2 encode not just current location but also trajectories that link past and projected future positions. These sequences thus contain a memory trace of the rodent’s recent past alongside estimates of the near future, which extend in both directions as the theta wave sweeps from the dorsal to ventral pole.

Sequences are a biologically plausible code

One important question is whether a neural code based on sequences is biologically plausible. The possibility for downstream neurons to decode sequential information has been well studied, and forms the basis for a subfield of computational neuroscience. The response properties of cortical neurons in vivo enable them to track temporal variations in their synaptic inputs with high precision, despite the fact the inputs are stochastic in nature [53, 54]. Further, through spike-time dependent plasticity, neurons can learn to detect specific and repeating patterns of spikes embedded within random and ongoing spiking inputs [55]. Neurons can also learn to spike at exact times relative to randomly spiking but oscillating inputs [56], and recurrent neural networks can learn mappings between inputs and output spike sequences using a biologically plausible learning rule [57]. This previous computational work supports the possibility that sequences of spiking activity form a plausible neural code, which can be decoded by downstream neurons and used for computation.

Neural sequences have also been found in previous experimental work. Temporally precise sequences of neural activity were discovered in songbird premotor cortex, and related to the production of specific syllables during singing [7, 31]. Further, the sequential firing of place cells in rodent hippocampus discussed in Chapter 2 has a long history of study [37, 45, 58–61], and work on this topic contributed to a Nobel Prize in 2014. However, neural sequences have never before been found in primates [62]. As such, we believe that neural sequences are not only a plausible mechanism for computation, but that the data presented in Chapter 1 is the first experimental discovery of a code that has been the subject of significant interest and theoretical modelling for the past 20 years.
Implications of new large-scale data sets

In the past, the ability to detect precisely coordinated patterns of spikes was limited by the size of recorded populations. Recent advances in neural recording techniques have solved this problem – it is now common to record from hundreds of neurons at a time, and in the next 5 years, data sets with thousands of simultaneously recorded cells will likely become routine [15, 63]. These new data sets will enable researchers to ask increasingly complex questions related to temporal coding; however, they also present new methodological challenges. The exponential growth in size of recorded populations challenge existing analysis paradigms and theoretical frameworks [64]. Further, mounting evidence points to neural responses that are more distributed and sparse than previously thought, and that multiplex to encode multiple stimuli at once [65]. Understanding these distributed and sparse responses is an important avenue for future work, but it is limited by researchers’ ability to detect precise coordination in large-scale data. These patterns of spiking activity may be very high dimensional, and thus difficult to detect. Further, it is unclear how to apply dimensionality reduction techniques designed for continuously sampled variables to discrete spiking data of this kind. As such, once coordination is discovered, it can still be a challenge to find meaningful relationships to behaviour.

Previous work has focused on detecting structured or sequential activity in spiking data [66–72]. Many such techniques rely on parameter tuning, are computationally expensive, or were designed for single neurons and are thus difficult to extend to large data sets. Here, we instead focus on the challenge of relating sequences to behaviour. In both Chapter 1 and Chapter 2, the sequences considered are clearly defined. As such, the novelty of the methods presented is not sequence detection (which is relatively simple in these cases), but rather the ability to find behaviourally relevant clusters of similar sequences, and analyze sequence structure from the perspective of temporal coding.

The benefit of complex numbers

The choice to develop a method based on complex numbers may seem counter-intuitive, especially in the case of spike patterns as simple as the sequences presented in Chapter 1. In this case, the complex representation of a single trial sequence was created by linearly mapping the time at which each cell reached its maximum spike density to a corresponding phase value. One could argue that since spike times were linearly mapped to phase values, the computations performed thereafter could equivalently be done with real-valued spike times. This is true. However, it may not always be so, as demonstrated in Chapter 2, when rotations in the complex plane play an important role for understanding the structure of spike patterns and how they are transformed in time.

One benefit to the complex-valued approach is related to the problem of defining time windows relevant for analysis. When analysing long recordings, that can be up to several hours, it is necessary to subdivide the data in some way. The structure of experimental trials provides one good way to do this. However, as experimental tasks become more sophisticated, it is increasingly common to have trials of variable lengths. For example, some working memory tasks involve a variable length delay period, or responses that may take different amounts of time. In these cases, the complex-valued approach offers an advantage, because it provides a
representation of relative timing that does not depend on “clock time”. One possible extension of the analysis in Chapter 1 exemplifies this point. Since the navigation period took different amounts of time depending on the trial and the target presented, the complex representation offers a simple way to consider the full navigation sequences without requiring complicated time-warping procedures [72]. In this way, the assumption that relevant spike patterns may be stretched or compressed in time is inherent to the method design.

Importantly, this assumption is not just useful for comparing across trials, but is relevant for analyses within single trials as well. When studying how spike patterns could evolve in time, or transform with behaviour, it is necessary to define the base scale of such spike patterns. For example, does a relevant spike pattern have a temporal resolution of 1 second? 5 ms? It is neither clear how best to define these relevant time windows, nor that the brain operates on “clock time” at all. The complex approach offers a possible solution, related to the population oscillations found in the brain. In the case of such an oscillation, as considered in Chapter 2, complex numbers provide a natural way to reference the timing of spikes to the internal population rhythm. This rhythm reflects the oscillating states of excitation in a brain region, and as such, serves to define windows of time in which cells are more or less likely to spike. In this way, the oscillation can help to define time window boundaries that are biologically relevant, rather than externally imposed.

Further, these oscillations need not be as well-defined and constant as the theta rhythm considered in Chapter 2. The method is equally applicable to spike patterns in the presence of oscillations with variable frequency. In this case, patterns of real-valued spike times can appear unrelated, while the corresponding phase patterns are identical.

A complex-valued approach also offers the potential to bridge the analyses of spike train data with other types of neural recordings. For example, the local field potential at an electrode could be used to define the oscillation to which spike patterns of cells at the same electrode are referenced. In fact, the method presented here was initially developed to detect spatiotemporal patterns in continuous measures of population activity, like LFP or optical imaging recordings. As such, further development (planned for future work) could allow for the possibility of a cohesive framework through which to study multiple types of neural data that can be recorded in parallel during an experiment.

Conclusions

In Chapter 1, we present a dimensionality reduction technique for sequences of spikes represented as complex-valued vectors. In contrast to traditional methods like PCA which emphasize variance, this technique creates a “similarity space” in which patterns with similar structure cluster together. These clusters can be leveraged to make links with behaviour, and form the basis for a simple but effective distance-based decoder. Further, this technique suggests a new approach to large-scale spiking recordings, which exclusively considers the timing of spiking responses (rather than changing firing rates across/between trials). This approach also suggests a first step towards the study of complicated multiplexing; for instance, allowing for the possibility that multiple sequences coexist or interact to form more complicated patterns of spikes.

A key next step for future work is generalizing this approach to more intricate patterns of
activity by relaxing key assumptions, especially the assumption that each cell only contributes once to the pattern. Here, this assumption makes sense in both Chapter 1 and Chapter 2. In the WM sequences presented in Chapter 1, firing during the sequence is significantly higher than the background rate (see Extended Data Fig. 7), and allowing cells to contribute multiple times to the sequence impairs the relationship to behaviour (see Extended Data Fig. 11e). In the study of phase precession, it is common to assume as we did in Chapter 2 that each cell bursts only once in each theta cycle [47]. However, this assumption may not hold in other systems, a possibility which partly motivates the choice to base the approach on complex numbers. In Chapter 1, for example, the mapping between spike times and spike phases is linear; as such, the results presented are equivalent to computing with vectors of spike times. However, the symmetries available in the complex plane, as demonstrated in Chapter 2, offer more possibilities for generalizing to higher dimensional patterns than considering spike times on the real line.

In Chapter 2, we introduce a novel approach to the study of spike-time codes by presenting a specific application to phase precession. Mathematical descriptions of the phenomenon have been suggested before [73]. The operator approach introduced here is intended, not just to describe the phenomenon, but to reveal key features of the putative spike-time code through the study of its mathematical properties (like symmetries). For example, we noticed the operator functioned similarly to a DFT performed by the hippocampus. Due to this structure, there are space-time symmetries inherent in the code which represent behaviourally relevant information that links the well-studied roles of phase precession in memory and navigation. These symmetries also suggest a computational role for the travelling wave of theta in this brain region. The dorsal to ventral sweep of theta corresponds to the expansion of the spatial map in which trajectories are represented at a fixed space-time scale. The operator approach also enabled the construction of a novel spike-based decoder that, by leveraging the structure of the spike-time code, can operate on far less data than is traditionally used in rate-based decoding. Important next steps include testing this decoder on neural recordings and expanding this approach to other systems.

In summary, this work introduces first steps towards a cohesive approach for the analysis of spike train patterns. This approach includes a dimensionality reduction technique designed to link spike patterns to behaviour, unsupervised spike-based and distance-based decoding algorithms, and an operator approach that enables the in-depth study of spike pattern structure. This work also suggests several avenues for future study, including testing these approaches in data from other systems, and relaxing assumptions from these initial examples which may not be applicable in the general case. Hopefully, the approach introduced in this work can provide initial steps towards the goal of developing a mathematical language for spike-time patterns, to aid in both the analysis of large-scale data, and the study of computation with spike-time neural codes.
Bibliography


Alexandra Busch

EDUCATION
Masters of Science (in progress) 2021/01 - Current
Western University, Applied Mathematics
Advisors: Dr. Lyle Muller and Dr. Ján Mináč

Bachelor of Arts 2016/09 - 2020/10
Western University
Major in Mathematics, Major in Arts and Humanities
Minor in English Language and Literature

RESEARCH CONTRIBUTIONS

My main contribution was developing a novel spike-based decoding algorithm to predict two-dimensional trajectories from a model of spiking activity of hippocampal place cells. I also contributed to writing, making figures, and defining vector equations.


My contribution involved developing a dimensionality reduction technique for complex-valued representations of the spike sequences observed in the data. This low-dimensional summary of the data allowed us to relate sequences of precise spike times to behaviour and task performance.


FUNDING HISTORY
BrainsCAN Graduate Studentship 2021/09-2023/08
Western University - Master's Studentship valued at $25000 each year for 2 years.

Western Graduate Research Scholarship 2021/01-current
Western University - Applied Mathematics. (Amount varies by term.)

Western University Summer Research Internship 2020/05-2020/09
Western University - Applied Mathematics ($5700)
During this research internship, I worked with a team to study the spread of Covid-19 by applying tools from Network Theory (a branch of mathematics that studies the structure and properties of networks) to contact-tracing and mobility data. My role included data analysis and giving presentations on our progress, potential new data sources, and basic network theory concepts.

**SCHOLARSHIPS AND AWARDS**

**Essay Finalist ($100)**  
Ayn Rand Institute - 15th place in an international essay contest with around 700 applicants.

**Kay Maclver Memorial Award ($500)**  
Western University - Awarded by the department for the best English essay by an undergraduate on the topic of Canadian Literature.

**Bounce Scholarship ($1700)**  
Western University - Awarded to an undergraduate in the Faculty of Arts and Humanities with the highest academic achievement in a course in Restoration and Eighteenth-Century British Literature

**UWO In-Course Scholarship Year 2 ($700)**  
Western University - Awarded based on academic merit.

**The Baldwin Family Scholarship for English Language and Literature ($900)**  
Western University - Awarded to students entering second year of an Honours Bachelor degree program containing English Language and Literature who achieved above 80% average in their first year.

**Western University Scholarship of Excellence ($2000)**  
Western University - Top high school admission average.

**Faculty of Arts and Humanities Scholarship of Excellence ($2000)**  
Western University

**Dean's Honor List**  
2016-2020

**EMPLOYMENT**

**Teaching Assistant, Western University**  
Duties include teaching tutorials (for Calculus 1414 and Math 1600), working in the drop in math help center (for first- and second-year math courses), marking, and proctoring exams.

**Marker for Calculus 2032, Western University**  
Duties included grading assignments and exams.

**Gymnastics coach, Gymworld**  
I coached both recreational and competitive gymnastics for children aged three to sixteen. Duties involved lesson planning, coaching, attending competitions, and helping my athletes develop and work towards their short- and long-term goals in the sport.
ACTIVITIES

Editor, Association for Women in Mathematics (Western Chapter) 2022
As editor for the new AWM chapter at Western, my role was to write and edit content for the blog and other communications, as well as to help with events and facilitate outreach workshops.

Public Outreach Facilitator, Anova 2018-2019
I completed a volunteer internship at Anova, London's women's shelter and sexual assault center, as part of an experiential learning credit at Western. I facilitated a number of public outreach workshops on sexual violence, healthy relationships, workplace inclusivity etc, to diverse audiences throughout the community. I also helped with the research, planning and writing for a proposal to make the shelter pet friendly.

Panelist, Curious Careers, Let's Talk Science Dec, 2022
This year I volunteered as a panelist in a Curious Careers webinar hosted by Let’s Talk Science. The webinar was aimed at youth in grades 6-8. We discussed “digital careers” and answered questions from students about planning for future jobs.