Dynamic Reorganization of Neuronal Activity Patterns in Parietal Cortex

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Dynamic Reorganization of Neuronal Activity Patterns in Parietal Cortex

Abstract

Neuronal representations change as associations are learned between sensory stimuli and behavioral actions. However, it is poorly understood whether representations of learned associations stabilize or continue to change following learning. We tracked the activity of posterior parietal cortex neurons for a month as mice stably performed a virtual-navigation task. The relationship between the activity of neurons and task features was reliable within single days but often changed over weeks. The pool of neurons which were informative about task features (trial type and maze locations) differed across days, with change accumulating over time. Despite changes in individual cells, the population activity had statistically similar properties on each day and stable information could be decoded for over a week. As mice learned additional associations, new activity patterns emerged in the neurons used for existing representations without affecting the rate of change of these representations. We propose that dynamic neuronal activity patterns could balance plasticity required for learning with stability for memory.

posterior parietal cortex (PPC)

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Acknowledgments

shout out to the homieszzz

Chapter 1

General introduction and background

A major function of neurons in the cortex is to form associations between stimuli in the sensory environment and behavioral actions. In some cases these associations can be formed between arbitrary stimuli and responses, such as, for example, when learning to relate landmarks in an environment with navigation actions or when learning behaviorally-relevant categories of sensory cues(Harvey et al., 2012) (Freedman and Assad, 2016; Harvey et al., 2012). These associations are mediated by patterns of neuronal activity that develop through the course of learning. (**?**) Past work has studied these patterns to understand how sensorimotor associations are represented in cortical neurons. Nearly all this work has focused on measurements of neuronal activity at single snapshots in time. For example, in typical experiments, activity from one neuron or set of neurons is recorded on one day and activity from other neurons is recorded on subsequent days. Information about sensorimotor associations is thus built up based on snapshots from different sets of neurons measured at separate time points. A fundamental question therefore remains unanswered: does neuronal activity converge to a stable pattern following learning or does neuronal activity change even after expert performance has

been achieved on a learned task? The answer to this question is essential to understand how sensorimotor associations are represented in cortex.

Here we tested if cortical representations of learned associations between arbitrary stimulus-action pairings stabilized after these associations had been learned to expert levels. We also tracked the same neurons during learning of novel stimulus action pairs and compared change during learning to change during stable behavioral performance. We developed methods to track the activity of populations of neurons and behavioral patterns across weeks as mice performed a navigation-based decision task in virtual reality. We focused on activity in the posterior parietal cortex (PPC), which is essential for performing this task and in rodents is considered to function in learned sensorimotor associations, including during navigation (Harvey et al., 2012; McNaughton et al., 1994; Nitz, 2006; Whitlock et al., 2012). We found that activity patterns in individual neurons changed greatly over the course of days and weeks, such that the population of neurons that provided the most task-relevant information drifted over time. Despite changes in single cells over time, the PPC population maintained a steady state with the same statistics of population activity properties across weeks, such as, for example, a consistent distribution of the fraction of neurons active at each point in the trial. Information about the task could be decoded above chance for weeks using a fixed decoder, indicating that a stable readout of population activity could exist, despite changes in individual neurons. Also, representations of newly learned cue-response relationships were incorporated without perturbing existing representations. We propose that drift in neuronal activity patterns could be important for mediating a tradeoff between

stable encoding of information and flexibility for incorporating new information.

1.1 The Memory Engram and Fixed Representations in Cortex

A common framework for memory proposes that there is a direct and fixed mapping of neuronal activity patterns with sensory stimuli and behavioral actions. In this framework, learned associations are thought to develop through the linking of these fixed representations (Messinger et al., 2001; Sakai and Miyashita, 1991; Veit et al., 2015). During learning, synaptic and other biophysical changes are thus hypothesized to minimize errors in the link between sensory stimuli and behavioral outputs, eventually converging to a stable solution upon reaching expert performance on a task (Ganguly and Carmena, 2009; Peters et al., 2014a). This view proposes that a memory engram is a collection of the same neurons that are activated every time the learned association is recalled (Tonegawa et al., 2015). This framework is appealing due to its conceptual simplicity and because artificial networks that converge to a fixed mapping of inputs to outputs through learning have proven highly successful in solving complex tasks (Abbott and Sussillo, 2009; Buonomano, 2005; LeCun et al., 2015).

1.2 Synaptic Plasticity and Dynamic Representations in Cortex

Alternatively, experimental data have revealed that synaptic connections between neurons in various brain regions continually change over time, with only a small fraction of synaptic connections, and in some cases none, persisting over weeks and months (Attardo

et al., 2015; Stettler et al., 2006; Trachtenberg et al., 2002). These results have motivated theoretical models that propose continuous change in neuronal circuits as a mechanism to optimize a tradeoff between stability and flexibility by sampling from multiple solutions of activity patterns and connectivity that similarly convey relevant information (Ajemian et al., 2013; Kappel et al., 2015; Rokni et al., 2007). These models present computational benefits such as limiting the likelihood of overtraining and convergence to local optima.

1.3 Convergence to a Fixed Representation During Learning

1.4 Variable Rate of Change to Balance Learning and Memory

1.5 Tracking the Activity of Cells Over Days

Recently developed methods to track the activity of the same neurons over time have led to a growing body of literature examining how representations change with learning (Huber et al., 2012; Komiyama et al., 2010; Poort et al., 2015). However, relatively little work has examined the stability of these representations after learning. Pioneering studies have investigated the stability of representations of sensory stimuli or the patterns of activity that accompany motor actions, in most cases over the course of several days. Studies in sensory cortex have revealed that representations of basic stimulus features, such as visual stimulus orientation, are generally stable over the examined periods (Andermann et al., 2010; Mank et al., 2008; Margolis et al., 2012; Peron et al., 2015b; Poort et al., 2015; Rose et al., 2016; Tolias et al., 2007). In motor cortex, the stability of activity patterns

for generating motor actions is a controversial topic. Many studies have noted stability in motor cortex activity but some have identified subtle shifts in tuning over days (Chestek et al., 2007; Ganguly and Carmena, 2009; Huber et al., 2012; Padoa-Schioppa et al., 2004; Peters et al., 2014a; Rokni et al., 2007; Stevenson et al., 2011). Also, a recent study has reported a mix of stable and changing features in the activity in songbird HVC during the generation of learned birdsong (Liberti et al., 2016). The largest changes in neuronal activity patterns over time have been noted in the hippocampus, in which upon repeated exposure to the same environment, place cell activity was gained and lost in individual cells without apparent shifts in place field locations (Kentros et al., 2004; Ziv et al., 2013). This work was performed in the absence of a task that required hippocampal activity and results have in part been attributed to learning and forgetting of environments (Attardo et al., 2015; Ziv et al., 2013). Therefore, the emerging literature on the stability of neuronal activity patterns over time provides hints that a degree of instability may exist. However, no studies have shown a major reorganization of activity patterns that are required for a learned behavior, leaving open the possibility that any instabilities noted might not be relevant for key features of behavioral output. In addition, much work has been limited by the available methods to confidently track neurons across days because it is difficult with electrophysiology or with supra-cellular resolution epifluorescence imaging to provide reliable metrics of cell identities on each day (Peron et al., 2015a).

1.6 Virtual Reality for Precise Behavioral Control

Chapter 2

Development of Methods for Tracking Neurons and Behavior Over Days

2.1 Introduction

2.2 A virtual reality system for mouse behavior

2.2.1 Detailed description

2.3 Fixed association decision task

2.3.1 Task description

2.3.2 Training procedure

2.3.3 Behavioral characterization

2.4 Pre-processing of imaging data

2.4.1 Within-session processing

Custom-written MATLAB software (available upon request) was designed for streamlined motion correction, definition of putative cell bodies, and extraction of fluorescence traces (ΔF/F). Following motion correction based on the Lucas-Kanade method (Greenberg and Kerr, 2009), putative cell bodies were first identified by eye and then binary masks were calculated based on the correlation of fluorescence timeseries between pairs of pixels located within 60 μm of one another in the neighborhood of the identified cell. A continuous-valued, eigenvector-based approximation of the normalized cuts objective (Shi and Malik, 2000) was applied to the pixel correlation matrix, followed by discrete

segmentation using k-means clustering. A separate neuropil mask was identified to accompany each cell body, defined using the same normalized cuts and segmentation method. Neuropil contamination was removed from the cell fluorescence time series by subtracting the background time series scaled by a contamination factor. The contamination factor was calculated by regressing the cell fluorescence against the background time series (Supplementary Figure S2E). ROI selection and neuropil subtraction were manually verified and manipulated when necessary using an interface tool that allowed examination of anatomical information and fluorescence traces corresponding to each cluster. The event rate was estimated using a previously described deconvolution algorithm (Vogelstein et al., 2010) to minimize the impact of indicator kinetics. This metric describes the relative firing rate of each neuron over time but cannot be used to confidentially identify single spikes. We therefore refer to deconvolved traces as an estimated event rate.

2.4.2 Across-session processing

Binary masks for all fluorescence sources were identified on each day separately and then aligned across days using a semi-automated custom tool. The algorithm ranked cells across imaging days with their most likely matches based on proximity after alignment and anatomical image correlation (a 60 μm box around the centroid of the cell). Matches were then verified by eye. This method has advantages over other commonly used approaches. Other approaches often use a single map of ROI masks for all days, such that this map is transformed on each day to best fit that day's imaging alignment. Slight

deviations in the axial plane of the image or other sources of in-plane distortion could lead to slight offsets in masks from day-to-day relative to the ideal alignment. Such slight offsets could result in contamination from activity in other cells, dendrites, and axons. Our approach identifies signal sources on each day and thus avoids any potential contamination from other signal sources. We then align the signal sources identified on each day to those from other days. The only error that could result is in incorrectly calling two signal sources as the same across days. However, to prevent such errors we visually compared the anatomical images to make sure the signal sources appeared to correspond to the same cell. We also checked that the signal source masks had approximately the same size and shape across days. If a cell could not be confidently identified on a given day, the data were excluded on that day. As a result, our approach resulted in an incomplete map of all cells across all days. We note that cells had to have some activity (calcium transients) in order to be identified on a given day. This activity requirement for the identification of each cell could potentially result in an underestimation in the extent to which cells gain and lose task related activity. Cells were more likely to have a defined mask on days that were nearby in time due to variable activity and viral expression of the indicator GCaMP6m (Supplementary Figure S2).

Chapter 3

Dynamic reorganization of neuronal activity patterns in parietal cortex blah

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This and the following chapters are a modified version of a submitted manuscript.

3.1 Introduction

Here we tested if, in the mouse posterior parietal cortex (PPC), representations of learned associations between arbitrary stimulus-action pairings stabilized after these associations had been learned to expert levels. We developed methods to track the activity of populations of neurons and behavioral patterns across weeks as mice performed a navigation-based decision task in virtual reality at near-perfect levels. We focused on activity in PPC because it is essential for performing this task and in rodents is considered to function in learned sensorimotor associations, including during navigation (Harvey et al., 2012; McNaughton et al., 1994; Nitz, 2006; Whitlock et al., 2012). We found that

activity patterns in individual neurons changed greatly over the course of days and weeks, such that the population of neurons that provided the most task-relevant information drifted over time. Despite changes in single cells over time, the PPC population maintained a steady state with the same statistics of population activity properties across weeks, such as, for example, a consistent distribution of the fraction of neurons active at each point in the trial. Information about the task could be decoded above chance for weeks using a fixed decoder, indicating that a stable readout of population activity could exist, despite changes in individual neurons. Also, representations of newly learned cue-response relationships were incorporated without perturbing existing representations. We propose that drift in neuronal activity patterns could be important in PPC for mediating a tradeoff between stable encoding of information and flexibility for incorporating new information.

3.2 Results

We trained mice to perform a two-alternative forced-choice task based on navigation through a T-maze in visual virtual reality (Harvey et al., 2012) (Figure 1A). At the beginning of the T-stem, mice saw one of two possible visual cues (white walls or black walls). Mice then ran through a short delay period portion of the T-stem in which the walls were identical between trial types. Upon reaching the T-intersection, mice had to report a choice about the cue?s identity by making a left or right turn to receive a reward. Mice learned this task over 4-6 weeks of training and reached expert behavioral performance

that was mostly stable over weeks (Figure 1B).

3.2.1 Tracking behavior and neuronal activity over weeks

During the task, we imaged the activity of hundreds of layer 2/3 PPC neurons simultaneously using volumetric two-photon calcium imaging (Peron et al., 2015b). Imaging locations were identified based on stereotaxic coordinates, and separate experiments revealed that these coordinates corresponded to a location anterior to cortical regions identified using retinotopic mapping. Here we call this region PPC and note that recent work from the Allen Brain Institute calls this regions VisA and that this regions is medial to what previous work has called secondary visual area A (Methods). Imaging sessions were performed typically every day with occasional one-day gaps between sessions (Supplementary Figure S1). On each day we identified the same field-of-view so that we could track activity patterns of neurons across time (Figure 1C-D). We developed a conservative approach to ensure as best as possible that we identified the same neurons across imaging days. First, we identified fluorescence signal sources (putative cells) on each day independently. Signal sources were selected on the basis of temporally correlated fluctuations between pixels, rather than using manual, anatomical selection methods that can fail to separate nearby cells, dendrites, and axons (Hamel et al., 2015; Peron et al., 2015a). Second, to match putative cells across all imaging days, we used a custom algorithm based on distance between regions-of-interest (ROIs) and similarities in the fluorescence images surrounding each ROI. Finally, we visually compared each identified cell across all days to ensure that each cell appeared consistent in the anatomical

images and had highly similar ROIs assigned to it (Figure 1D). We only considered cells on days in which they were identified; other days, in which we could not with high confidence identify the cell, were excluded from our analysis, such that not every cell was identified on every day. This approach was aimed to minimize mislabeling of neurons across days and was intended to be conservative compared to previously developed methods (Huber et al., 2012; Liberti et al., 2016; Peron et al., 2015b; Peters et al., 2014; Poort et al., 2015; Ziv et al., 2013) (see Supplementary Figure S2 and Methods for a full discussion).

3.2.2 Necessity of PPC activity for post-learning performance of the task

The activity patterns of PPC neurons on a single imaging day were consistent with those reported previously (Harvey et al., 2012; Morcos and Harvey, 2016). On each day, individual neurons were transiently active, with different neurons active at different time points, such that PPC activity tiled the entire duration of a trial (Figure 1E). Many of these responses were reliable and selective for a particular trial type. For example, some cells were more active on black cue-right turn trials than on white cue-left turn trials or vice versa.

The activity in the PPC appeared necessary for the mouse to perform the behavioral task. We virally expressed channelrhodopsin-2 in parvalbumin-expressing inhibitory interneurons at a location centered at the PPC and activated these neurons to inhibit excitatory activity on a subset of trials. Inactivation of PPC decreased the mouse?s behavioral performance from 85

% correct to just above chance levels (Figure 1F). These results were obtained days or weeks after the mouse achieved plateau behavioral performance, suggesting that PPC activity was necessary for performing the task even in the post-learning phase. These results were in agreement with our earlier work that used pharmacological methods to inactivate the PPC and other studies showing a role for the rodent PPC in visual decision tasks (Goard et al., 2016; Harvey et al., 2012; Licata et al., 2016; Raposo et al., 2014). We note, however, that although inactivation was centered on PPC, such activity manipulations may not be isolated solely to PPC, as has been shown in other systems.

3.2.3 Reorganization of sequential activity across maze locations

To compare the activity patterns of neurons across days, we first focused on sequential activity throughout a trial. On each day, a sequence of neuronal activity was present (Figure 2A, top left, center, and bottom right panels). To determine whether this sequence of activity was the same from day to day, we sorted neurons based on where in the maze they had a reliable peak of activity. We then used the same sorting to look for the same sequence of activity on earlier or later days. Strikingly, the sequence of activity that was present on one day was largely different on other days (Figure 2A). Cells that had a significant peak of activity in the maze on a given day were unlikely to have a significant peak of activity at the same or nearby position after long intervals (Figure 2B). Over time this likelihood of a consistent peak position approached levels expected from a random reorganization of neuronal identities (Figure 2B). These changes resulted from cells with a peak of activity on one day either losing that peak of activity or having a shift in the peak?s location on

subsequent days, both of which increased in likelihood as a function of time from when a peak was identified (Figure 2D). In some cases, peaks shifted by distances larger than one meter. The loss of peaks of activity was offset by an approximately constant rate at which cells initially lacking a peak of activity gained an activity peak (Figure 2D), resulting in a consistent fraction of active cells with significant peaks over the imaging period of weeks (22.0 ± 0.5

3.2.4 Different populations of neurons with trial type-specific activity patterns across days

We also investigated changes in activity patterns that could be related to information about the trial type. Specifically, we asked if the neurons that had different activity patterns on trials with different cues and choices, and thus provided information potentially useful for solving the task, were the same across time. For each neuron on each day, we used a decoder to quantify how well that neuron?s activity across the entire duration of a single trial could predict the trial type (white cue-left turn vs. black cue-right turn, correct trials only). On a given day, a significant fraction of active neurons had a decoding accuracy above chance (29.1 ± 1.1

We also examined if the neurons with selective activity for one trial type (e.g. black cue-right turn trials) switched to having a preference for the other trial type (e.g. white cue-left turn trials) (Figure 3F-H). The most highly selective cells for each trial type over time often lost their selectivity or gained additional selectivity, at other points in the maze, for the

other trial type (Figure 3F-G). We tracked the neurons that had the strongest preferences for each trial type on a given day (Figure 3H). Over days, the trial type preferences of these neurons approached that of the entire population. Only a small fraction of neurons switched from having statistically significantly higher activity on one trial type to having statistically significantly higher activity on the opposite trial type (4.7 ± 1.7

3.2.5 Using a generalized linear model to compare relationships between neuronal activity and behavioral features across days

These findings together provide evidence that major changes and reorganization of neuronal activity patterns occurred during stable performance of a behavioral task. However, these analyses only considered two aspects of the task (position in the maze and trial type) and did not include other task features that could potentially be represented in the neuronal activity. For example, PPC has been considered to be important for movement planning and could thus have activity related to the running patterns of the mouse (Andersen and Cui, 2009; Nitz, 2006; Whitlock et al., 2012). In addition, PPC receives inputs from visual areas and might have activity related to the movement of visual stimuli projected on the screen, such as during turning compared to forward motion (Harvey et al., 2012; Oh et al., 2014). We wanted to understand whether behavioral variability across days could explain the changes in neuronal activity or alternatively if these changes were due primarily to single neurons having different relationships between their activity and the behavior of the mouse across time. We therefore developed an approach to describe an individual neuron?s activity on single days based on a large

number of variables that described the task and mouse?s behavior. We developed a generalized linear model (GLM) in which we modeled the activity of an individual neuron based on the running patterns of the mouse on the spherical treadmill, the virtual maze position (visual scene), the trial type, reward events, and whether the mouse was in the inter-trial interval period (Friedman et al., 2010; Park et al., 2014) (Supplementary Figure S3 and S4). We fit the relationship between a cell?s activity and these behavior and task features to develop a model of that cell?s activity-behavior relationship. We tested the quality of this model by predicting the cell?s activity based on the behavioral and task features in a subset of trials not used for fitting. If the predicted activity closely matched the real activity, we concluded that our model could describe the activity-behavior relationship for the cell on that day. Across cells, models were able to explain a large fraction of neuronal activity (57.9 ± 2.6

We used these models to compare the relationship between a cell?s activity and behavioral features across days. Using the model of a cell?s activity-behavior relationship fit on a single day, we predicted the cell?s activity based on behavior features in other days (Figure 4A and B). If the model developed on one day was able to predict activity on a subsequent day, then we concluded that a consistent activity-behavior relationship existed. In contrast, if a model developed on one day failed to predict the activity on subsequent days, then we concluded that a consistent activity-behavior relationship was absent. This approach has the potential to track stable relationships between neuronal activity and behavior features across days that traditional approaches might miss. For example, if a

neuron had activity related to the running patterns of a mouse and if these running patterns changed relative to position in the maze across days, the GLM could potentially reveal a stable activity-behavior relationship over time that would be missed if only maze position were analyzed. Importantly, behavioral features, such as running speed and trial duration, were variable across trials, but maintained a similar distribution and range of values on each day, suggesting that models should be transferable across days (Supplementary Figure S6). We limited effects due to fitting procedures, such as regularization, and due to correlated task variables by fitting and testing bidirectionally for each pair of days (see Methods for a full discussion).

3.2.6 Changing activity-behavior relationships in single neurons over days

Models of activity-behavior relationships developed on a given day were, on average, able to predict activity patterns well on neighboring days, but did a poor job of predicting activity patterns as the time between the compared days increased (Figure 4C). Over long intervals, model predictions eventually reached that of a null model (for intervals greater than 17 days), indicating that a cell?s activity-behavior relationship was generally inconsistent over weeks (Figure 4C). We also quantified similarity of models for a given cell across days using Kendall rank correlations of model parameters and found a comparable decay over time (Supplementary Figure S5F). The changes in these activity-behavior relationships were made up of cells that lost well-modeled relationships, gained well-modeled relationships, and switched relationships across days. To quantify the prevalence of these events, we used a statistical threshold, based on shuffled data, to binarize model performance into

models that predicted activity patterns above chance levels (significant predictions) and models that provided poor predictions of activity (Methods). We then compared pairs of models fit on separate days for a given cell. If the models developed on one day provided good predictions of the activity patterns on the other day, then the cell was considered to have a consistent activity-behavior relationship (Figure 4D). Instead, if one model with a significant prediction of activity could be developed for one day?s activity but not for the other day?s activity, then the cell was considered to have lost or gained an activity-behavior relationship (Figure 4D). If models with significant predictions could be developed on both days but these models provided poor predictions of activity on the other day, then the cell was considered to have switched activity-behavior relationships (Figure 4D). The likelihood that a cell lacking an activity-behavior relationship gained such a relationship remained constant throughout the imaging period of weeks (26.8 ± 0.01

The rates at which neurons had changes in their activity-behavior relationships varied greatly (Figure 4F). For each neuron, we calculated the likelihood that a model developed on one day provided a significant prediction of another day?s activity (Figure 4G, left) and fit an exponential to this likelihood over time to define a metric of consistency for each cell (Figure 4G, right). Some neurons had slow decays and thus had relatively consistent activity-behavior relationships, whereas others had fast decays indicative of rapid changes. Over a 20 day interval, the large majority of neurons had a low likelihood of consistent models (Figure 4H). Only 7.3

The changes in activity-behavior relationships did not reflect independent relationships

across days. Rather, consistent relationships in the recent past were predictive of consistent relationships in the short-term future. Neurons with a consistent activity-behavior relationship for two or more consecutive days prior to a particular imaging day were more likely to maintain that relationship than neurons which had a consistent relationship for only one of the immediately preceding imaging days (Figure 4J). These results suggest that neurons potentially operated with modes of activity that tended to persist for neighboring days such that a neuron?s activity on each day was not independent of the recent history of its activity. However, the predictive power of recent consistency fell off after ten days, suggesting there was not a separate population of permanently consistent cells (Figure 4J).

The GLM analyses therefore suggest that the changes in activity we observed were likely due to unstable activity-behavior relationships, rather than changes in behavioral patterns across days. We further supported this finding by comparing the similarity of population activity patterns on trials with the most similar or least similar behavioral patterns across all days. The population activity was more similar on those trials with more similar behavioral features, measured as correlations between population activity vectors (Supplementary Figure S7A). However, the difference in activity similarity between the most and least similar behavioral trials was small compared to the population activity changes across time (Supplementary Figure S7A). In addition, we did not observe any evidence that the mouse forgot and re-learned the task on each day because mice performed at near perfect levels, even on the first few trials of each day (Figure 1B,

Supplementary Figure S7B).

3.2.7 Consistent statistical features of population activity on each day

Despite these changes in the activity of individual neurons, we noticed that consistent patterns were present in the population activity on each day. As shown above, neuronal activity that tiled the full trial duration was present on each day and was made up of different neurons across days (Figure 2A). The distribution of population activity across the trial was not uniform, but this distribution was highly similar across all days in a given population of neurons (Figure 5A-B). In addition, on each day, a decoder for trial type based on population activity achieved similar levels of performance and had similar distributions of performance across time points in the trial (Figure 5C-D). Interestingly, in each population of neurons from different mice, differences between mice were maintained across days. For example, the population of neurons in one mouse (red) had higher decoding accuracy of trial type than did the population of neurons in another mouse (green) across all days (Figure 5D). These differences were maintained with a similar shape across the duration of the trial (Figure 5C). Many other properties of population activity had similar distributions on each day, including for neuron-neuron activity correlations (Figure 5E-F), trial-trial population activity correlations (Figure 5G-H), estimated population firing rates (Figure 5I-J), and decoding accuracy of trial type for individual neurons (Figure 5K-L). These results indicate that even though properties of activity in individual neurons changed across time, the statistical features of population activity were largely consistent over weeks. Therefore, the population appeared to have a

?set point? of similar activity each day, using different neurons, and neurons in different ways, to achieve this steady state.

3.2.8 Decoding of information from dynamic neuronal representations

The changes in neuronal activity-behavior relationships over time raise questions about how information could be read out from such a dynamic neuronal population over days and weeks. One possibility is that the cells with the most consistent activity-behavior relationships are those that preferentially carry information for the readout. Alternatively, it could be possible that the activity in the cells with less consistent activity-behavior relationships also allows decoding of information over time. We investigated this issue by testing various decoding strategies for reading out the trial type on the basis of population activity.

We first trained and tested a linear decoder on each day separately using all neurons (as in Figure 5C-D). We found trial type information could be decoded throughout the duration of the trial, with higher decoding accuracies at the end of the trial, when the mouse executed a turn at the T-intersection (Figure 6A). We then compared the decoding performance using the cells with the most consistent activity-behavior relationships and the least consistent relationships, defined by exponential fits of model performance decay over time (from Figure 4G, see Methods). The cells with the least consistent activity-behavior relationships had better decoding accuracy throughout the majority of the trial than did the cells with the most consistent relationships. In the final segment of

the trial, when the mouse executed a turn, decoding accuracies were similar between groups (Figure 6B).

To analyze the stability of information in activity patterns, we tested decoding performance across days. We trained a decoder on a given day and tested it on subsequent days (Figure 6C). When considering a random subset of cells, decoding performance decreased as the interval between compared days increased. This result was present throughout the duration of the trial (Figure 6C). In the cells with the most consistent activity-behavior relationships, decoding performance was low and consistent across time for the majority of the trial (Figure 6C, left and middle). For the final segment of the trial, the decoding performance in these cells was high and consistent over days (Figure 6C, right). As expected, the performance of a decoder trained on one day and tested on other days decreased as a function of time for the cells with the least consistent activity-behavior relationships (Figure 6C). Interestingly, however, over intervals within one week, these cells performed better in the majority of the trial than the cells with the most consistent relationships (Figure 6C, left and middle). Together, these results indicate that the information in the population was not stable over time, but some information remained in the population for days and weeks, even in neurons with the least consistent activity patterns.

If the relevant information for the task needs to be read out near the time the mouse executes a turn, then it might be beneficial to weight strongly the activity of the cells with the most consistent activity-behavior relationships. In contrast, if information in the

T-stem is more relevant for behavior, then weighting the activity of the cells with the less consistent relationships might be beneficial. In light of this reasoning, we returned to our optogenetic inactivation experiments and now inhibited PPC activity either during the first half of the trial or the second half of the trial. Interestingly, we found that when PPC activity was inhibited in the first half of the trial, the mouse?s performance was greatly impaired (Supplementary Figure S8). In contrast, inhibiting PPC activity during the second half the trial had no significant effect on the mouse?s performance (Supplementary Figure S8). This result is consistent with what has been reported previously in other tasks (Goard et al., 2016; Licata et al., 2016; Raposo et al., 2014). We note that it is difficult to interpret what this finding means in terms of PPC?s role in the task: PPC activity could be involved in the transformation of the sensory information into a behavioral action plan or in some aspect of visual processing or potentially other computations. Regardless, this finding suggests that it is possible that the task-relevant information in the cells with the least consistent activity-behavior relationships could be of importance for PPC?s role in this task.

These results suggest that to maintain high levels of information across time, an ideal readout would need to change in a coordinated manner with the encoding network. However, we wanted to test if it might be possible to identify a stable readout in the form of a single decision plane that could be used across all days to separate trials of different types on every day. We combined all imaging days together and trained a decoder on a subset of trials and tested this single decision boundary on other trials that were distributed

across all days. We compared the performance of this combined-day decoder using only the neurons with the least consistent activity-behavior relationships, the most consistent relationships, or a random sample of all neurons (Figure 6D). In these cases, the cells with the least consistent relationships provided information at a similar level as the other groups and allowed decoding of trial type above chance levels. As expected, when the identities of the cells were shuffled independently on each day, the ability to find a stable readout decreased significantly, indicating that the decoding performance required the structure of the population activity over days (Figure 6D). This analysis therefore reveals that, for the binary classification of trial type over the time intervals examined, it is possible to find a stable readout of population activity that performs above chance levels, even from the most dynamic population of neurons. However, to achieve higher performance, a readout that changes dynamically with the encoding network would likely be necessary.

3.2.9 Incorporation of representations for newly learned associations into existing population activity patterns

3.3 Discussion

Our results reveal that during stable performance of a learned navigation task, the activity patterns in PPC were variable over the timescales of days and weeks. These findings indicate that through the course of learning, neuronal activity patterns in PPC did not converge to a single representation. Rather, there appeared to be a set of activity patterns

in the PPC population that were similarly sufficient for the task. Importantly, many statistical features of the population activity were consistent across days, such that the population appeared to reach a set point. However, the PPC neurons that were used in this representation, and how these PPC neurons were used, changed across days.

3.4 Acknowledgements

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3.5 Author contributions

L.N.D and C.D.H. conceived of the project. N.L.P. designed and performed inactivation experiments and analyzed associated data. S.N.C. and M.M. designed cell selection algorithm and gui for extracting single session ROI maps. L.N.D. designed across session alignment algorithm and gui for aligning ROI maps across days. L.N.D and C.D.H designed all other experiments and analyses, and L.N.D. performed these experiments and analyses. L.N.D. and C.D.H. wrote the manuscript.

Chapter 4

Monitoring neuronal activity over days during learning blah

4.1 Introduction

Given that changing neuronal activity patterns either cause some instability in information coding or require compensation by a dynamic decoding network, it is important to consider what advantages might arise from these changes. Computational models suggest that an advantage of dynamic neuronal representations could be the flexibility of incorporating new information into the population (Ajemian et al., 2013; Rokni et al., 2007).

4.2 Results

4.2.1 Task Design for Learning a Stimulus-Action Pair

We therefore tested how existing representations were affected by the learning of a new association. We trained the same mice that had already stably performed the task described above to learn a new association. We introduced a third possible cue (crosshatch pattern)

that required a specific turn at the intersection for the mouse to receive a reward (the turn direction was randomly selected for each mouse) (Figure 7A). After a mouse had learned the novel third trial type, we introduced a fourth cue (triangle pattern) that required the mouse to turn the opposite direction of that required for the third cue (Figure 7A). Mice learned the novel cue-response associations while maintaining high performance for the original two cue-response associations (Figure 7B).

4.2.2 Decoding Trial Type Information Over the Course of Learning

We first asked whether the neuronal activity patterns were different between trials in which the mouse saw the novel cues and those in which it saw the familiar cues. Using a decoder based on population activity, the novel trial types were separable from the familiar trial types (Figure 7C). These differences in activity between trial types could be visualized on a single day based on population activity in a dimensionality-reduced space (Figure 7D). We tested if the addition of new learned associations altered the rate at which neuronal activity patterns changed during performance of previously learned trial types. We might expect learning to increase the rate of change as new information is incorporated into network activity. Surprisingly, we found that the rate of change was comparable between the days with stable performance of the two familiar trial types and during learning of the novel trial types (measured based on the performance of models of cells'□□ activity-behavior relationships across days, as in Figure 4C-E) (Figure 7E-F).

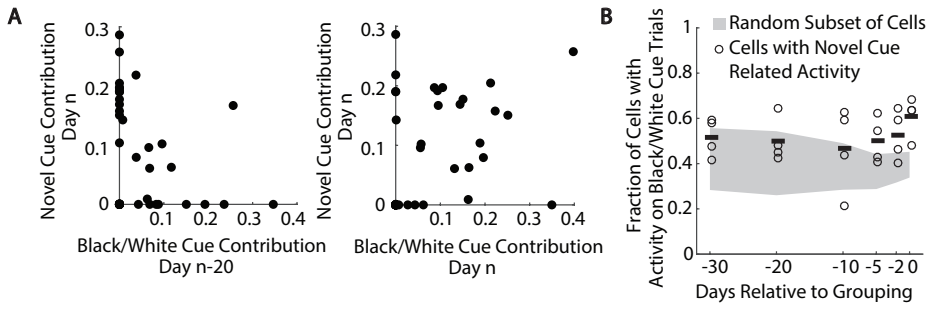

Figure 4.1 | Behavioral training procedure for the fixed association evidence accumulation task. a, Sequence of mazes used for behavioral training. Asterisks indicate reward location. Only some example mazes are shown (for example, right choice and not left choice maze in maze 1). **b,** Distribution of net evidence corresponding to different difficulties used in training the final task (maze 8; see **d**). **c,** Screen captures of the virtual environment at cue 1, cue 6, and the turn in maze 8. **d,** Behavioral performance across sessions for three example mice. Colors correspond to the maze colors indicated in **a**. Shapes correspond to the net evidence probabilities in **b**.

4.2.3 Rate of Change During Learning Compared to Stable Behavior

This similar rate of change could have occurred because the cells with activity related to the novel trial types were different from the cells with activity related to familiar trial types. We therefore analyzed if the cells with activity related to novel cues had activity related to familiar cues on previous days. Surprisingly, cells with activity related to novel cues were more likely to come from the group of cells which recently (within the past ten days) had activity related to the familiar cues than from a random sample of neurons (Figure 7G-H). The evolving pool of cells involved in representing task features was thus more likely to incorporate new task relevant information than the group of cells presently without task relevant information. This finding suggests that new information can be incorporated into the pool of task relevant activity as this pool continuously shifts over time, without disrupting baseline functionality. We speculate that the ability to incorporate new

information using 'multitasking' neurons could allow for flexibility during learning, such that the network's ongoing changes provide a framework for the addition of new associations.

4.3 Methods

Training for novel trial type associations was performed during imaging. Mazes were identical to maze 5. On each day, mice were presented with novel trial types after 40 trials of the original trial types (black cue-right turn and white cue-left turn). After novel trial types were introduced, familiar trial types and novel trial types were interleaved such that there were equal fractions of left and right turn trials. Mice were first presented with a 3rd cue (crosshatch) and after mice performed all three trial types at above 80 % for three consecutive days, we introduced a 4th cue (triangles). For mouse 1, the 3rd cue instructed left turns and the 4th cue instructed right turns. For mouse 2, the cue-turn relationship for novel trial types was reversed. White and black cues maintained consistent cue-turn relationships for both mice. Mice learned novel trial type cue-turn relationships by trial and error while maintaining previously learned relationships for black and white cues.

4.4 Discussion

We speculate that the changes in neuronal activity reported here reveal key features of how associations are formed and represented in PPC. In particular, our work reveals a potential strategy for a population of neurons to achieve stability in the maintenance of

learned associations while also allowing flexibility to incorporate new information.

Chapter 5

Discussion and future experiments

5.1 Dynamic reorganization of activity in cortex

Our work provides, to our knowledge, the strongest evidence to date that neuronal representations of behaviorally-relevant information undergo major changes over long time scales outside of the context of learning. In contrast to other studies in sensory and motor regions that reported no changes or subtle shifts in activity over a few days, our results revealed a major reorganization of neuronal activity patterns during a learned task over the course of weeks (Margolis et al., 2012; Peron et al., 2015b; Peters et al., 2014b). For example, in HVC during birdsong, activity patterns had a 0.8 correlation coefficient over a five-day period (Liberti et al., 2016). In contrast, the changes we identified over long intervals approached those that could be expected by random reorganization. The magnitude of changes we observed appear consistent with those in hippocampal place cells upon repeated exposure to the same environment (Ziv et al., 2013). Our work demonstrates that the changing representations were likely used to perform the task, based on our inactivation results, which was not tested in chronic studies of hippocampal

representations. It is important to note that we found a wide range in the stability of encoding properties in single neurons. Although our decoding experiments revealed that information could be read out from the cells with the least consistent activity-behavior relationships, it will be important to test experimentally if downstream networks preferentially weight the inputs from the neurons with the most consistent relationships.

5.2 A strategy For maintaining and updating relevant activity patterns

We speculate that the changes in neuronal activity reported here reveal key features of how associations are formed and represented in PPC. In particular, our work reveals a potential strategy for a population of neurons to achieve stability in the maintenance of learned associations while also allowing flexibility to incorporate new information. We illustrate this idea by focusing on activity patterns that separate one relevant task parameter, trial type. Over weeks, individual neurons did not maintain different activity between trial types, such that many neurons gained or lost selectivity and a small fraction of neurons switched trial type preferences. Despite these seemingly random changes over weeks, information about trial type could be read out at above chance levels from the population using a single decoder. The decoder's success was because the majority of neurons did not change in a way that their activity had opposite implications for a decoder on different days. That is, most neurons that had higher activity on black cue-right turn trials eventually lost selectivity and less frequently switched to having higher activity on

white cue-left turn trials at the same location in the maze. Many changes therefore occurred in a null dimension relative to the dimension important for trial type (Ajemian et al., 2013; Rokni et al., 2007). Importantly, these changes need not be coordinated in an 'intelligent' or 'structured' way to occur specifically in a null dimension. Rather, this effect is characteristic of a high dimensional activity space because in high dimensional spaces most dimensions are orthogonal to each other. Most random change in neuronal representations would thus occur in an orthogonal dimension to the one relevant for reading out trial type (Buonomano and Maass, 2009). We note, however, that more information would be accessible to the readout if it functioned as a dynamically adaptive decoder. It is possible that over long timescales an ideal decoder could also slowly drift in response to the dynamically encoded information, but could change at a slower pace. In such a case, the readout network could have a lag in its changes and/or a slower rate of change, afforded by the properties of a high dimensional space where drift in any direction will often occur in an orthogonal direction to the optimal decision boundary. Over long timescales, learned associations that are not practiced could be lost if the readout is not updated for an extended interval. On the other hand, learned associations that are often practiced could maintain a tight link between drifting activity patterns and the relevant readout. Importantly, a slow drift, such as could be produced by background synaptic plasticity, has been shown to provide computational advantages such as avoiding local minima and providing exploratory strategies described in the field of reinforcement learning (Kappel et al., 2015). In this framework, neuronal activity is continuously

remodeled in order to maintain the most up-to-date, relevant information for behavior while discarding irrelevant, previously learned memories over long timescales.

5.3 Balancing stability and flexibility according to role

The importance of representations that mediate a tradeoff between stability and flexibility likely varies depending on the function of the population of neurons. In areas closely connected to sensory coding or the generation of motor actions, there may be a greater need for stability and less need for plasticity, which could explain why studies of sensory and motor cortex have described less drastic changes than those we report. However, it will be important to test the stability of sensory and motor representations over time windows similar to those we used here. We focused on the PPC, which receives multisensory input, has recurrent connections with frontal regions, and has outputs to motor-related structures, suggesting that it is an association area (Harvey et al., 2012; Oh et al., 2014). Our previous work found little evidence of purely visual responses in PPC during passive viewing, suggesting that its activity is not driven solely by sensory input (Harvey et al., 2012). Also, PPC is multiple synapses from movement-generating regions, such that it is unlikely important for directly driving actions. In contrast to sensory and motor regions, association regions, like the PPC, probably require flexibility for learned behaviors as one of their key properties, in which case malleable activity patterns in neuronal populations would be highly advantageous. Consistent with this idea, studies of the turnover rate of dendritic spines have revealed that spines in another association

region, hippocampus, turn over at a faster rate than those in primary somatosensory cortex and have a much lower percentage of stable spines (Attardo et al., 2015). Analogous to learning new information in association areas on top of stable sensory and motor representations, work in artificial networks has demonstrated the utility of training additional layers on top of previously learned deep nets to perform complex tasks (Kümmerer et al., 2014). It will be of interest to directly compare the rates of activity changes in populations of neurons across different brain regions, including in cortical and subcortical areas, to test if abstract learned representations drift at faster rates than activity patterns that represent sensory stimuli or generate motor actions.

5.4 Stability in the emergent properties of the network

Our work provides evidence that individual PPC neurons do not have specified roles in network activity. Instead, not only did we observe that neurons lost or gained activity-behavior relationships over time, but we also found some neurons that switched their activity-behavior relationships, suggesting that they provided different information to the network at different time points in their lifetime. We found that neuronal activity was best described by combinations of behaviorally relevant features similar to recent work suggesting information in cortical neurons is multiplexed, such that neurons participate in many information channels (Cromer et al., 2010; Rigotti et al., 2013). In addition, the changes in activity we observed suggest that a neuron's activity might not be confined to a specific class of activity pattern, thus supporting recent evidence for category-free

neuronal populations in the rodent PPC (Raposo et al., 2014). Together our work and others suggest that the role of individual neurons could be less important than the overall population activity pattern, such that individual neurons participate in many information streams and can change their participation over time (Yuste, 2015). Consistent with this idea, we identified that although the activity-behavior relationships of individual neurons changed over time, the PPC population activity had similar statistics of activity on each day, using different neurons or the same neurons in different ways. This result suggests that the population activity reached a set point of activity that was necessary for the PPC's role in the task. This idea is conceptually similar to the homeostatic ideas that have been revealed in the stomatogastric ganglion (Marder, 2011; O'Leary et al., 2014; Prinz et al., 2004). In that system, innovative work has revealed that the same firing patterns of neurons can be achieved through different combinations of ion channels and ion channel expression levels. We speculate that a similar idea could be present in neuronal populations given the underlying changing activity of individual neurons.

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