NEURAL DYNAMICS OF REACHING FOLLOWING INCORRECT, ABSENT, OR LAST-MOMENT PREPARATION

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Abstract

Moving is thought to require separate preparation and execution steps. While preparing, neural activity in primary motor and dorsal premotor cortices achieves a state specific to an upcoming action, but movements are not performed until the execution phase. In this work, we investigated the interactions between motor preparation and motor execution.

We first investigated whether the preparatory state (more precisely, prepare-and-hold state) is required for movement execution using two complementary experiments. We compared monkeys’ neural activity during delayed and non-delayed reaches, and in a delayed reaching task in which the target switched locations on a small percentage of trials. Neural population activity bypassed the prepare-and-hold state both in the absence of a delay and if the wrong reach was prepared. However, the initial neural response to the target was similar across behavioral conditions, regardless of whether there was a delay period. This means that there are consistent neural preparatory steps which are performed prior to movement even in the absence of a delay. This suggests that the prepare-and-hold state can be bypassed if needed, but there is a short-latency preparatory step which is performed prior to movement even without a delay. We suggest that this preparatory step may be best understood as a dynamical process rather than simply a particular, static neural state.

We next examined whether motor preparation and motor generation can be run in parallel. We instructed monkeys to reach to a particular target, and occasionally switched that target to a new location shortly before the monkey began initiating his reach. We found that the amount of time required to change a reach goal tends to remain constant regardless of whether that computation is being performed online
(during the execution of the initially-cued reach), or offline (prior to reach initiation). Examining neural activity during this task, we found that neural activity following a switch tends to explore dimensions which are not well-represented during the course of normal reaching. Furthermore, reaches can be initiated correctly even if their neural activity has not fully recovered from the switch, as long as neural activity in dimensions which are relevant to movement output has been corrected. This work reveals a potentially important way in which neural activity can simultaneously prepare one reach while executing another, again underscoring the view that motor preparation is itself a dynamical process which is independent of but complimentary to movement generation.
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Chapter 1

Introduction

1.1 Background

In order to successfully perform even a simple reaching movement such as picking up a coffee mug, the brain has a number of problems to solve (Wong et al., 2014). It must determine the goal of the movement. It must use sensory input such as vision and proprioception to identify the position of the arm and the position of the mug. It must take these positions into account in order to transfer that abstract goal into a proper set of muscle activations. It must monitor and correct the ongoing position of the arm. It must also be ready to respond to physical perturbations of the arm, or mug.

In order to accomplish these tasks, the motor system involves a large number of brain areas, comprising portions of the frontal and parietal lobes of cortex, as well as the cerebellum and numerous sub-cortical regions. In this work, we concentrate on neural activity recorded from dorsal premotor cortex (PMd) and primary motor cortex (M1) (Figure 1.1). We selected these areas for study for several reasons. First, both areas project to the spinal cord, suggesting that they may have a direct role in the control of movement. M1 and PMd collectively account for roughly 60% of corticospinal projections (Dum and Strick, 1991; He et al., 1995; Dum and Strick, 2002). Some studies have also used a technique called microstimulation to explore the link between neural activity and movements. During microstimulation, a small amount of
electrical current is released from the tip of an electrode placed into the brain area of interest. Short-duration microstimulation in M1 and PMd evokes muscle twitches (Strick and Preston, 1978; Weinrich and Wise, 1982), while long-duration microstimulation evokes complex arm movements and postures (Graziano et al., 2002). M1 and PMd are thought to be important for the preparation and execution of visually-guided reaches (Boussaoud and Wise, 1993; Weinrich and Wise, 1982; Weinrich et al., 1984; Tanji and Evarts, 1976).

In this study, we investigated the neural correlates of selecting a particular reaching goal and programming a movement. This process is often referred to as “motor preparation.” We wanted to investigate how different levels of motor preparation affected subsequent movement execution. Many studies have compared and contrasted the neural and behavioral correlates of motor preparation and execution, usually by separating these processes in time. In this work, I elected to probe the interaction of motor preparation and motor execution by designing behaviors which manipulated their relative timing.

In order to separate motor preparation from motor execution, studies have often
used delayed reaching tasks (Tanji and Evarts, 1976; Wise and Mauritz, 1985; Rosenbaum, 1980). In these tasks, subjects (typically monkeys or humans) initiate a trial and are then given information about what movement they will be asked to make in the future. This information can be either complete (touch a particular spot on a screen), or partial (e.g. information about reach direction but not extent (Riehle and Requin, 1989), touch one of several targets (Cisek and Kalaska, 2005; Bastian et al., 2003), information about which arm to use (Rosenbaum, 1980; Lecas et al., 1986)). The subjects must withhold from actually making the cued movement until a subsequent go cue.

This task reveals a behavioral signature of motor preparation. Namely, when subjects have advance knowledge of some parameters of their upcoming movement, the amount of time that they take to begin moving after the go cue (reaction time, RT) decreases (Figure 1.2) (Rosenbaum, 1980; Riehle and Requin, 1989; Churchland et al., 2006c). This suggests that reaching requires multiple neural steps. Some of these steps can be performed in advance of actually reaching, without triggering a movement. If subjects are given information about an upcoming reach, they can prepare for the reach by performing these steps ahead of time. Neural recordings in monkeys performing delayed reaches revealed that during the delay period, neural activity responds in a target-specific manner in several motor areas, including primary motor cortex (M1) (Tanji and Evarts, 1976), dorsal premotor cortex (PMd) (Weinrich and Wise, 1982; Weinrich et al., 1984), the supplementary motor area (SMA) (Tanji et al., 1980), and putamen (Alexander, 1987). This neural activity became a candidate for the neural substrate of the preparatory process.

PMd in particular has been functionally implicated in the behavioral RT decrease observed in the delayed reaching task (Churchland and Shenoy, 2007). In this study, they microstimulated with a small amount of current: enough to perturb neural activity without explicitly evoking movement. They found that they could largely erase the RT benefit of the delay period by microstimulating in PMd around the time of the go cue. Saccadic RTs were not affected nearly as much, indicating that this is principally an effector-specific manipulation, rather than a broad attentional modifier or a manipulation of the perception of the target or go cue. This suggests that the
delay period neural activity in PMd contributes to the behavioral preparatory effects observed in the delayed reaching task.

The exact nature of this preparatory process and its relationship to movement generation as a whole has been a topic of wide investigation in the motor field. Early studies, inspired by similar work in the saccadic system, claimed that preparatory activity was essentially sub-threshold movement activity (Georgopoulos et al., 1989; Riehle and Requin, 1993; Bastian et al., 2003). In this model, each neuron has a preferred reach direction. Some neurons may fire a great deal for upward reaches, but very little for downward reaches. Others may prefer leftward reaches, or downward reaches. A reach is triggered when a population of neurons which prefers a particular direction achieve a firing rate threshold. In this view, motor preparation excites the same neurons that would be involved in execution of the same movement, but in a sub-threshold manner. This preparatory activity does not have a high enough firing rate to trigger a movement, but reduces the amount that these cells need to increase their firing rate in order to trigger a movement after the go cue.

While this representational model is satisfying in its simplicity, a great deal of work in the past ten years has eroded its validity. First, a primary assumption of a representational model is that each neuron has a single preferred direction which does not vary in time or context. This should indicate that the relationship between firing patterns during preparation and during movement should be high: the same
target direction should be preferred in both. However, this appears not to be the case. The actual correlation between preparatory preferred direction and movement preferred direction falls off quickly after the go cue. Within 100 ms, there is only a 10% correlation in preferred direction of neurons (Churchland et al., 2010). In addition, many neurons in motor cortex have oscillatory firing rates, whose ostensible preferred direction can change drastically even over the course of movement (Churchland et al., 2010, 2012). Sometimes, individual neurons can display almost the same preparatory activity for two different movements, but vastly different activity during movement generation (Churchland et al., 2010). This strongly suggests that preparatory activity is not simply sub-threshold movement activity, but that the relationship between movement preparation and movement generation may be more nuanced.

A competing explanation of motor control takes a more mechanistic view. This explanation, originally dubbed the optimal subspace hypothesis, states that reaches are generated by some specific, time-varying pattern of firing rates across neurons. During preparation, neural activity achieves a particular state (optimal subspace) which will serve as a good jumping-off point from which to generate these patterns (Afshar et al., 2011; Churchland et al., 2006a). In this view, preparation and movement generation are causally linked, but the exact firing rate patterns observed during preparation and generation can be quite different.

There have been a number of recent studies in favor of this mechanistic view of motor control. First, if monkeys are trying to constrain their neural activity to be in a particular state during the preparatory period, the neural variability during this time period ought to be lower than neural variability prior to the target cue. Indeed, when researchers examined the fano factor (a measure of neural variability which accounts for the fact that variance tends to scale with mean firing rate), they discovered that neural variability decreases after the onset of a target cue (Churchland et al., 2006c). Furthermore, trials with a fast RT should have neural preparatory states which are close to or within the optimal subspace, whereas trials with a slow RT might be less well prepared. This would lead to a larger across-trial variability in long-RT trials, as these trials would be less neurally optimized, and tend to have firing rates which were further from the mean. This was also found to be the case: trials whose RT was faster
than the median had less variability in preparatory state than trials whose RT was slower than the median (Churchland et al., 2006c). Later work expanded this idea to show that trial-by-trial variation in preparatory state correlated with trial-by-trial variation in RT (Afshar et al., 2011). Trials whose neural preparatory state is slightly further along the path that neural activity will take to generate the movement tend to have a faster RT than trials whose neural state is slightly further back, just as racers who begin slightly closer to the start line will have an advantage over racers who start further back. Yet another study showed that preparatory activity on a single neuron basis correlated more strongly with what the neural population as a whole would be doing during movement than with any combination of kinematic variables (Churchland et al., 2010). This again makes the case that the purpose of preparatory activity is likely not to represent particular parameters of a movement but to set up future movement-generation neural activity.

Figure 1.3: Oscillatory dynamics observed during movement generation. The phase of the oscillation (moving up and right versus left and down) is set by the preparatory activity (red and green dots). The displayed plane is found using a technique called jPCA, which seeks to find 2-dimensional projections of neural firing rates which display the strongest oscillations. Adapted from Churchland et al. (2006c)

Investigations into the dynamics of movement generation have also implicated preparatory activity as important for setting the stage for subsequent movements.
Recent work has observed that there is a strongly oscillatory component to movement-generation neural activity (Churchland et al., 2012). The phase of this oscillation seems to be set by the preparatory activity (Figure 1.3). While there is always an oscillatory component to movement-related neural activity, the differences in phase may come down to a difference in the initial condition, where the initial condition is set by the preparatory state.

While this model of preparatory neural activity as the initial condition for a particular pattern of motor generation activity has had promising results, it leaves many questions which remain to be tested. One central question is whether motor preparation is necessary to generate a particular movement, or whether it is an optional optimization step. Another question is the degree to which motor preparation and motor generation are independent processes. Must they always be separated in time, or can they run in parallel, for example if a monkey changes his mind mid-reach?

\subsection{1.2 Summary of thesis work}

In this thesis, I first investigate the degree to which motor preparation is required for motor generation. I use two complimentary analyses to ask this question. In the first experiment I examine whether the preparatory process that we observe during a delay period is preserved in reaches without an explicit delay between the appearance of the reach target and the go cue. In the second experiment, I ask monkeys to perform a task in which they prepare for one reach but must perform a different reach. I ask whether they must re-prepare before being able to make a correct reach in this case.

This investigation reveals several new insights into the interaction between motor preparation and motor generation. First, I find that the neural “prepare and hold” state attained during the delay period is not attained in the absence of a delay. Even if a monkey has prepared the wrong reach, we do not observe “re-preparation” in the form of bringing neural activity to the correct prepare-and-hold state. This indicates that achieving the exact neural state which is attained during the delay period is not a requirement of making a correct reach, although it does lead to a faster reaction time if the prepare-and-hold state is attained during a delay period. This presents a potential
issue for strong interpretations of the Optimal Subspace Hypothesis, which suggest that what reach is performed is highly dependent upon the neural preparatory state. We do find, however, that some components of the putative preparatory process seem to be performed regardless of whether or not there is a delay period. This suggests that motor preparation may be better thought of as a time-varying dynamical system in its own right, rather than a single neural state or set of states from which it is possible to generate a particular movement.

I next examine whether motor preparation and motor generation can be run in parallel. I instruct monkeys to reach to a particular target, and occasionally “jump” that target to a new location shortly before the monkey begins initiating his reach. I find that the amount of time required to change a reach goal tends to remain constant regardless of whether that computation is being performed online (during the execution of the initially-cued reach), or offline (prior to reach initiation). Examining neural activity during this task, I find that neural activity following a switch tends to explore dimensions which are not well-represented during the course of normal reaching. Furthermore, reaches can be initiated correctly even if their neural activity has not fully recovered from the switch, as long as neural activity in dimensions which are relevant to movement output has been corrected. This work reveals a potentially important neural correlate for how to simultaneously prepare one reach while executing another, again underscoring the view that motor preparation is itself a dynamical process which is independent of but complimentary to movement generation.
Chapter 2

Investigating the Necessity of the Delay-Period Motor Preparatory State

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2.1 Summary

Moving is thought to require separate preparation and execution steps. While preparing, neural activity in primary motor and dorsal premotor cortices achieves a state specific to an upcoming action, but movements are not performed until the execution phase. We investigated whether this preparatory state (more precisely, prepare-and-hold state) is required for movement execution using two complementary experiments. We compared monkeys neural activity during delayed and non-delayed reaches, and in a delayed reaching task in which the target switched locations on a small percentage of trials. Neural population activity bypassed the prepare-and-hold state both in the absence of a delay and if the wrong reach was prepared. However, the initial neural response to the target was similar across behavioral conditions. This suggests that the prepare-and-hold state can be bypassed if needed, but there is a short-latency
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preparatory step which is performed prior to movement even without a delay.

2.2 Introduction

Movements are often prepared ahead of time. For example, when a soccer player makes a penalty kick, she takes time to set up the kick to improve her chances of scoring. At other times, movements may be performed without taking extra time to explicitly prepare. If an opposing defender appears just as the soccer player is about to kick, she may pass the ball to a teammate without taking time to carefully set up the pass. Because humans and other animals must contend with a variety of behavioral contexts, we are presumably capable of performing both prepared and relatively unprepared actions. However, how these behaviors relate to each other is not well understood. In this study, we examine this relationship to determine which aspects of motor control are consistent across different levels of preparation.

Motor control is often studied with a delayed-reach behavioral paradigm. In this paradigm, some or all aspects (e.g. direction, speed, extent) of an upcoming reach are revealed, but subjects must wait for a go cue before moving (Churchland et al., 2006a; Messier and Kalaska, 2000; Shen and Alexander, 1997; Riehle and Requin, 1989; Rosenbaum, 1980; Tanji and Evarts, 1976). This allows subjects to prepare their reaches ahead of time. The reaction time (RT) between the go cue and reach initiation decreases when the reach is pre-cued (Churchland et al., 2006c; Rosenbaum, 1980). Electrophysiological recordings have revealed that neurons in primary motor cortex (M1) and dorsal premotor cortex (PMd) change their firing rates (FR) in response to information about upcoming reaches (Tanji and Evarts, 1976; Weinrich et al., 1984; Cisek and Kalaska, 2002; Rickert et al., 2009). This activity is correlated with RT (Afshar et al., 2011; Churchland et al., 2006c) and other aspects of the reach, such as peak speed (Churchland et al., 2006a,b). Furthermore, electrically perturbing neural activity in PMd during the delay can largely erase the RT benefits of preparation (Churchland and Shenoy, 2007).

These observations have led to the idea that delay period neural activity reflects computations related to motor preparation (Crammond and Kalaska, 1994; Riehle
2.2. INTRODUCTION

Figure 2.1: (A) Optimal subspace hypothesis. For each reach, there is a corresponding neural preparatory state. After the go cue, the neural population activity takes a trajectory that begins in the preparatory state and generates the prepared reach. (B) Initial condition hypothesis cartoon. Gray trace, mean neural population trajectory; black traces, individual trial neural population trajectories. When a reach is pre-cued, neural population trajectories on individual trials move to the preparatory state. On each trial, the degree to which the neural state has advanced by the time of the go cue correlates with RT.

and Requin, 1989). In particular, the model asserts that this preparation causes the observed decrease in RT. This model can be explained using a state-space framework, which has helped to understand neural population activity across a variety of paradigms in recent years (Broome et al., 2006; Churchland et al., 2012, 2010; Harvey et al., 2012; Shenoy et al., 2011, 2013; Stokes et al., 2013). In this framework, neural population activity is projected into a neural state space, in which the FR of each neuron is a dimension in this space. The FRs across all neurons at a given time corresponds to a point in state space. The FRs over time form a trajectory through state space (Figure 2.1 A). The optimal subspace hypothesis (Churchland et al., 2006c) states that when a reach is pre-cued, neural activity moves to a preparatory region (set of neural states) that is beneficial for the upcoming reach. The model states that, while there is almost certainly some drift and variability in the neural preparatory state, neural activity should pass through or near this preparatory region to generate a correct reach. Furthermore, slight variations in neural preparatory state also correlate with RT (Figure 2.1 B) (Afshar et al., 2011). Trials in which the neural state happens to have progressed further in the direction that it will move after the go cue
have a slightly shorter RT than trajectories which are further behind.

While this framework has helped to elucidate some of the neural mechanisms of movement, it is limited by the use of just one task, the delayed reaching task. During the delay, subjects are not only preparing a reach, but are also holding the arm outstretched in front of them and withholding movement. The neural preparatory state observed during the delay is thus more precisely a prepare-and-hold state. It remains unclear to what degree neural activity in this prepare-and-hold paradigm can be generalized to reaches without an explicit delay. If reaching is dependent on the preparatory state (Churchland et al., 2012, 2010; Afshar et al., 2011), then initiating movement outside of the correct preparatory region ought to generate a different reach. If this were the case, we would expect either an incorrect reach or neural evidence of last-minute preparation in conditions where the monkey’s preparatory state is not correct.

We used two behavioral paradigms to investigate the neural correlates of reaching with different levels of neural preparation. First, we compared the neural activity of reaches performed with and without a delay, to examine what happens when monkeys are not given explicit time to prepare. Second, we compared the neural activity of delayed reaches and reaches where the target cue switched to a new location. We examined whether monkeys neural activity in the switch condition re-prepared by passing through or near the correct prepare-and-hold region.

In both cases, RT was lowest for conditions where the neural state is in the prepare-and-hold state at the time of the go cue and higher for conditions where the neural state was outside of the prepare-and-hold state at the time of the go cue. This indicates that the state is behaviorally beneficial. However, if the neural state was outside of the prepare-and-hold state at the time of the go cue, we determined that neural activity did not re-prepare by achieving the prepare-and-hold state for the performed movement. In fact, neural trajectories in these situations often passed quite far from the prepare-and-hold state. This suggests that achieving the prepare-and-hold state is not necessary for generating a reach. However, when examining the full time-course of neural trajectories, we found that the initial neural response to the target is similar between delayed and non-delayed reaches. These early responses may reflect some
early preparation which is performed even in the absence of a delay period.

2.3 Materials and Methods

2.3.1 Task Design

Two male rhesus macaques (Maccaca mulatta) (N and K) performed variants of a delayed reaching task. Animal protocols were approved by the Stanford Institutional Animal Care and Use Committee. Images were back-projected onto a vertical screen 30 cm in front of the monkey. Timing of task events was confirmed using a photo box. Hand position was tracked optically by detecting a reflective bead taped between the first and second knuckle of the monkeys middle two fingers (Polaris, Northern Digital Inc.). Eye position was monitored optically (Iscan).

In the delayed vs. non-delayed task variant, monkeys performed a reaching task composed of two block types. In the delayed reaching block, the monkeys either touched a central 9-mm radius square (K), or directed a cursor projected 10 cm above the monkeys hand into the square (N) to initiate the trial. After 700-1100 ms, one of 8 (N), 14 (K-single electrode), or 7 (K-array) peripheral targets appeared (target cue). After a randomized delay (0-900 ms), the central target extinguished (go cue), and the monkeys were permitted to move their hand (K) or the cursor (N) into the cued target. After holding the target for 500-600 ms, they received a juice reward. In the non-delayed reaching block, the monkeys initiated trials in the same manner, but the target cue and go cue were always simultaneous.

In the switch task, two trial types were randomly interleaved. In 80% of trials, monkeys performed delayed reaches as in the previous task. In 20% of trials, the monkeys eye-fixated and either touched (K) or directed a cursor inside (N) a central, 9-mm radius square. After 700-1100 ms, a peripheral target appeared. After a delay, this target disappeared and a second, 180-degree separated target appeared (target switch). This switch occurred either at a fixed interval of 400 ms (N), 450 ms (K-single electrode), or a random interval of 450-900 ms (K-array). After an additional delay of 0-900 ms, the central target disappeared and the monkeys were required to touch
and hold the second (new) target to receive a juice reward.

2.3.2 Behavioral Analysis

We generated mean reach trajectories for each reach direction and condition (delay, no-delay, switch) by averaging the x-y coordinates of each reach of a given type, aligned to movement onset. We defined the end of movement as the first time that reach velocity falls below 7% of maximum reach velocity. We used the hand position 30 ms after this as our end-point location, to ensure that the hand had fully stopped. We performed a one-way Multivariate Analysis of Variance (MANOVA) on the endpoint distributions. We examined the maximum reaching velocity for each reach direction and condition by performing a t-test on the distributions of maximum velocities for each condition pair. Velocity differences were normalized to the mean delayed-reach velocity.

2.3.3 Neural Recording

We recorded in Monkey K using single electrode recordings. Single electrode penetrations were guided by stereotactic coordinates, known response properties of PMd and M1, cortical microstimulation thresholds, and neural response to muscle palpation. Recordings were made anterior to the central sulcus, lateral to the spur of the arcuate sulcus, and posterior and medial to the precentral dimple, although some recordings were likely within the precentral dimple, based upon recording depth and stereotactic coordinates. Single electrode recordings were isolated online using the Plexon recording system. Only well-isolated single units were used. We recorded a total of 63 neurons over 42 days for the delayed / non-delayed task, and another 81 neurons over 67 days for the switch task.

We recorded using 96-electrode arrays in both Monkey N and Monkey K. Electrode arrays were implanted in PMd and surface M1 (Figure 2.2). Array recordings typically resulted in poorer isolation qualities than single electrodes, due to the static nature of the array, but allowed for a higher trial-count per neuron. We recorded waveforms on each channel which crossed a voltage threshold of -3.5 times the STD of the voltage,
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and spike sorted these waveforms offline using a custom spike sorter (Neurosort). In N, we performed a total of 13 days of recording for the pre-cue vs. non-pre-cue task and 18 days of recording for the switch task. In K, we performed a total of 7 days of recording for the pre-cue vs. non-pre-cue task and 3 days of recording for the switch task. Array data shown in this paper is from datasets recorded on November 5, 2010 and February 4, 2011 in N, and on July 18 and 19, 2012 in K. We selected datasets for analysis and publication based on maximizing recording quality and trial count.

2.3.4 Peri-Stimulus Time Histograms

For each unit, we calculated PSTHs to estimate mean FR over time. We aligned trials to several times: target onset, switch (if relevant), go cue, and movement onset. We binned spike times in 1-ms bins and averaged over trials of the same reach direction and condition. We convolved these average FRs with a 25-ms Gaussian to smooth the FR estimate. We interpolated between the different aligned events to yield a trace which estimates the FR over time over the course of a trial.

2.3.5 Dimensionality Reduction

Prior to reducing the dimensionality of our data, we performed a softmax normalization of each PSTH, dividing FR for each neuron by the maximum variance across conditions for that neuron. This helps to avoid being biased by high FR neurons, by
ensuring that each neuron has the same overall variability across conditions.

We elected to use Principal Component Analysis (PCA) to reduce the dimensions of our data. PCA imposes few assumptions on the underlying structure of the data, simply revealing dimensions which explain a large percentage of the variance. More complex methods, such as Factor Analysis (FA) or Gaussian Process Factor Analysis (GPFA) (Yu et al., 2009), often require additional assumptions on the data. For example, GPFA requires simultaneous recordings to accurately build a neural noise model, which we did not have in our single electrode datasets, and is optimized for predicting trial-by-trial neural activity, where here we concentrate on average neural activity. PCA has a strong precedent in the literature as a dimensionality reduction method for trial-averaged data (Churchland et al., 2012, 2010; Harvey et al., 2012; Rivera-Alvidrez et al., 2010). In addition, because we use enough dimensions to account for >90% of the neural variance in our distance quantification, the selection of dimensionality reduction method likely has little effect on the results.

For a given target and condition (e.g., switching, non-switching, delayed, non-delayed), we generated a matrix of PSTHs of dimension $n \times \sum_{c,t} c(t)$, where $n$ is the number of recorded neurons, and $c(t)$ denotes the selected conditions over time. We ran PCA on this space to reduce these dimensions to $k \times \sum_{c,t} c(t)$, where $k$ represents the dimensions across which the most neural variance was explained. We then either plotted trajectories in the first three dimensions of this space (delay/no delay task), or performed a second calculation to find an informative rotation of this space (switch task).

Because the switching conditions featured reaches to more than one target, the first principal components often represent neural activity which is the same across multiple reaches. Therefore, the first few principal components are often less informative about the differences between reach trajectories which we wish to observe. To find an informative projection, we reduced to a four dimensional space, using the first three principal component dimensions plus the dimension which best separates the prepare-and-hold states for the reaches we were comparing. Orthogonalizing this space gives us a rotation which allows us to visualize the neural difference between the different prepare-and-hold states. This allows us to observe re-preparation or the
lack thereof in the switching conditions. This rotation is purely for visualization and is not used for subsequent distance analyses.

2.3.6 Distance Analysis

To estimate the minimum neural distance between different conditions over time, we performed a modified Euclidean distance analysis. We selected points on one of the two trajectories we were comparing (delay/no delay task: delayed reach trajectory; switch followed by a delay: switch trajectory; switch without a second delay: non-switch trajectory) (reference trajectory), and calculated the Euclidean distance between that point and every point on the second trajectory, in the first 15 principal components (Figures 3-5). We elected to use 15 principal components for the main manuscript to err on the high side of estimated dimensionality in this system (Yu et al., 2009). These 15 dimensions account for >90% of the variance of the data in all datasets. We selected the minimum Euclidean distance across all points on the second trajectory as our estimate of neural distance between the two trajectories at that time. This ensured that we would never over-estimate the distance between the trajectories due to misalignment in time. A low distance indicates that the second trajectory achieves the selected state at some time, while a high distance indicates that the second trajectory never achieves the target state.

2.3.7 Bootstrap Procedure

To estimate the variability of the distance between traces, we performed a bootstrap analysis. For each reach direction and reach condition (delay, no delay, switch, non-switch), we resampled the trials recorded for that condition. We selected a new set of trials (randomly, with replacement) of the same size as our original set. We then calculated PSTHs from this resampled dataset, performed PCA on these resampled trajectories, and calculated the neural distance as described above. We collected 1000 resamples for each reach direction / condition set. This yields an estimate of the variability of the distance between neural trajectories.

It is possible that FR increases during the preparation and moving phases could
artificially lead to an increase in average neural distance. To control for this possibility, we performed a second bootstrap analysis, where we resampled a single condition twice (reference trajectories: delay/no delay task: delayed reach trajectory; switch followed by a delay: switch trajectory; switch without a second delay: non-switch trajectory). We generated one set of the same size as the delayed reach set, and a second set of the same size as the non-delayed reach or the switch trials. By comparing the minimum neural distance between these resampled trial sets, we can estimate how far apart we would expect neural population trajectories to be if they truly were generated from the same underlying distribution. To determine the likelihood that the observed distance between trajectories was higher than expected by chance, we calculated the percentage of resamples in which the resampled distance was greater than this control resampled distance.

2.3.8 Response Timing Analysis

To determine when motor cortical neurons began responding to each unit, we first characterized the trial-by-trial FR of each neuron. To avoid timing confounds of convolving with an acausal Gaussian filter, we defined a neuron’s FR at a given time as the number of spikes in a 40-ms bin preceding that time. We then calculated the mean FR for each unit across all trials for each target and condition (delayed or non-delayed), aligned to different times in the trial (target onset, go cue, and movement onset).

To determine the neural response time to target onset, we estimated the baseline position as the mean FR in the 50 ms before target onset. We then calculated the neural distance at each time as the Euclidean distance between the full-dimensional neural position at that time and the baseline state. We considered the motor cortex to have begun responding to a target when it crossed a threshold of 20 spikes/s more distant than the distance at target onset. To determine this threshold, we observed the range of observed distances between neural trajectories and the mean baseline activity prior to target onset (when distance is expected to be as low as possible). This ranged from 7-11 spikes/s across targets in Monkey N, and 5-9 spikes/s in Monkey K. We
selected a threshold of 20 spikes/s, which is approximately double that range. This minimizes the chances of a “false start” due to natural fluctuations in neural distance, while keeping the threshold relatively low.

We performed a similar analysis for the timing of response to the go cue, asking when neural activity became different from the mean position in the 50 ms before the go cue appeared (holding our 20 spikes/s threshold constant).

We next looked at the distance between the delayed and non-delayed neural trajectories at each time point after the target appeared. We set a threshold of 20 spikes/s greater than the distance at the time of target onset to serve as our divergence time. We performed all of these analysis for each target separately, using all units to generate our neural trajectories.

2.4 Results

2.4.1 The prepare-and-hold state is not achieved in the absence of a delay

We first investigated the necessity of the prepare-and-hold state by comparing reaches with and without a delay. We trained monkeys N and K to perform a reaching task with blocks of delayed and non-delayed trials (Figure 2.3 A). Mean reach trajectories were close (Figure 2.3 B-D), with overlapping end point distributions. While several end point distributions had significant differences (N: 4/8 reaches; K-array: 6/7 reaches; K-single electrode: 12/14 reaches; p<.05 One-Way MANOVA), the magnitude of these differences was small, representing less than 15% of the target diameter. The peak velocities of the reaches were also similar (Figure 2.3 E-G). For Monkey N-array, delayed and non-delayed reach velocities were not significantly different (p = 0.17, 2-sample unpaired t-test). For Monkey K, delayed and non-delayed reaches displayed a significant (but small magnitude) difference in reach velocity, with delayed reaches being an average of 3.1% faster than non-delayed reaches in the array dataset (p<.01, 2-sample unpaired t-test), and 0.75% faster in the single electrode dataset (p<.01, 2-sample unpaired t-test). Overall, reaches were quite similar across delayed
and non-delayed reach conditions.

We observed some behavioral differences in RT, consistent with previous studies (Figure 2.3 H-J). For Monkey K, long delay trials (>450 ms) had a significantly shorter RT than zero delay trials ($p < .001$, 2-sample t-test). The mean RT difference was 10.0 ms in K-array and 35.5 ms in K single electrode. Both monkeys showed an irregularity in their RT curves, with an RT increase for intermediate-length delays. This effect lasts longer in Monkey N, resulting in long-delay trials not having a significantly shorter RT than zero delay trials ($p > .05$). This may be due to the monkeys learning the statistics of our task, which contained more long delays (450-900 ms) than intermediate (50-450 ms) delays. For our neural analyses, we used only trials with a zero delay or a long (450-900 ms) delay, excluding reaches with intermediate delays from our analyses.

We recorded neural activity in M1 and PMd using tungsten microelectrodes in Monkey K, followed by two 96 electrode arrays (PMd, M1) in Monkeys N and K. We collected 125 units for N-array, 104 units for K-array, and 63 units for K-single electrode. We computed peri-stimulus time histograms (PSTHs) for each unit and target to estimate the mean neural activity for each reach direction (Figure 2.4 A-C). To compare delayed and non-delayed reaches in the neural population as a whole, we examined the neural population states in these two conditions. To gain intuition about the neural population state as it evolves through time (i.e., the neural-population trajectory), we normalized the PSTHs (to avoid bias toward high-FR neurons), and performed principal component analysis (PCA) on the PSTHs for a given reach direction. Plotting the first three principal components (PCs) yields a low-dimensional neural trajectory which can be visualized easily and still represents much of the variance of the original neural data ($>75\%$ of the total variance for every reach direction).

Tracing the neural trajectory for a delayed reach, we observe that the neural activity begins in a baseline state (Figure 2.4 D-F, gray trace). After the target cue, neural activity moves to a new part of the state space, representing the prepare-and-hold state for that reach. After the go cue, the trajectory follows a path into peri-movement activity, eventually slowing to a new hold state after reach completion.

We can compare this delayed reach neural-population trajectory to the non-delayed reach condition by plotting them in the same space (Figure 2.4 D-F, red
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Figure 2.3: (A) Task design. Monkeys performed trials broken into blocks of delayed and non-delayed reaches. In the delayed reach block, a delay of 0-900 ms separated target onset and go cue. In the non-delayed reach block, the target onset and go cue were simultaneous. (B-D) Mean reach trajectories for delayed reaches (black) and non-delayed reaches (red). Circles show 1 STD of end point positions. Starred reaches show significantly different endpoint distributions (p < 0.05) (E-G) Differences in maximum reach velocity for each reach direction. Positive values indicate delayed reaches were faster than non-delayed reaches. Gray bars show significantly different reach velocities (p < 0.05). (H-J) Mean +/- SEM RT vs. delay length in 100-ms sliding bins.
Figure 2.4: (A-C) Example individual neural PSTHs. Each color represents a different reach direction. Top: conditions with a delay. Bottom: conditions without a delay. (A) Unit where delay activity is quickly recapitulated in the non-delay condition. (B) Unit whose delay activity is skipped in the non-delay condition. (C) Unit whose delay and non-delay activity has similar tuning but different magnitude. (D-F) Example neural state-space diagrams. Gray trace: delayed reach. Red trace: non-delayed reach. Arrows show direction of time. (G-I) Median resampled distance between trajectories at different times, for the trajectories pictured in D-F. Error bars show 5th and 95th percentile of the distribution. Red ticks: median, 5th, and 95th percentile of the distance detected if neural trajectories were generated from the same underlying distribution. Stars show bootstrap significance.
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The neural-population trajectory in the non-delayed reach condition also begins at baseline. When the go cue (and target) turns on, the neural activity follows a path which resembles the peri-movement activity in the delayed reach condition. However, the two trajectories do not overlap for a large portion of their paths. In particular, the non-delayed reach neural trajectory does not appear to pass through the delayed reaches prepare-and-hold state. Instead, it bypasses that state, moving along a path that resembles the peri-movement trajectory. The two conditions take parallel but separate paths through state space, converging around the time of movement onset. In this projection, it thus appears that neural activity only achieves the prepare-and-hold state if there is a delay.

Though looking at a low-dimensional plot can be useful for gaining intuition about neural processes, neural activity occupies more than three dimensions (Yu et al., 2009). Therefore, it is critical to examine neural activity in a higher-dimensional state. This helps to ensure that the intuition gained in the reduced-dimensional view is not an artifact of the projection and allows a more precise quantification of the trajectories differences and similarities.

We developed a distance quantification that could be computed in arbitrary (N) dimensions. We cannot simply compare the distance between trajectories at each time for two reasons. First, the delayed reach condition contains more time points than the non-delayed reach condition. Second, a misalignment in time could result in an overestimation of the distance between trajectories. Instead, we selected relevant times on the delayed reach neural trajectory (target onset, go cue, movement onset, and after the movement has finished), and then found the closest point on the non-delayed reach neural trajectory (across all times), to err statistically conservatively (on the side of estimating the trajectories to be as close as possible). The Euclidean distance between these two points represents the minimum possible distance between the trajectories at this time. A zero distance indicates that the non-delayed reach neural trajectory passes through the selected point on the delayed reach trajectory, while a large distance indicates that the non-delayed reach neural trajectory never comes near the selected point on the delayed reach neural trajectory. Because neural activity is variable across trials, our trajectories will never be exactly the same, so
we will never see a true zero distance. To determine whether the distance is significantly higher than expected, we compared our estimated distance between delayed and non-delayed conditions to the distance between resamples pulled only from the delayed condition. This distance tells us how far apart we would expect the neural trajectories to be by chance if the data were truly pulled from the same underlying distribution. To determine the confidence interval of the distances at different times, we performed a bootstrap analysis by resampling our underlying trials for each condition, re-generating our neural trajectories from this resampled data set, and calculating neural distance as above. The distance distributions for the example trajectories plotted in Figure 2.4 D-F are shown in Figure 2.4 G-I.

Even at the time of target onset, many reach directions display significantly higher (p<.05) distances than expected if the trajectories were pulled from the same distribution (N-array: 8/8 targets; K-array: 4/7 targets; K-single electrode: 6/14 targets). This may reflect the block structure of the task, which allows the monkeys to predict whether the upcoming trial will contain a delay. This could lead to anticipatory differences in the baseline state. However, the magnitude of the baseline distance is still low. The mean +/- STD distance between trajectories, across targets, normalized by expected distance is: 2.1 +/- 0.4 for N-array, 1.6 +/- 0.2 for K-array, and 1.6 +/- 0.3 for K-single electrode. This reflects that neural activity is still similar at this time. At the time of the go cue, the distance between the trajectories is significantly larger than expected. The normalized mean +/- STD distance between trajectories across targets is: 6.4 +/- 1.1 for N-array, 6.7 +/- 2.4 for K-array, and 2.2 +/- 0.3 for K-single electrode (all targets, all datasets p<.01). This indicates that the neural population trajectory does not achieve the prepare-and-hold state during non-delayed reaches. At the time of movement onset, the distance between the neural trajectories is lower, reflecting that they have begun to re-converge. However, most reaches still have significantly higher than expected distances at this point. The normalized mean +/- STD distance between trajectories across targets is: 3.9 +/- 0.8 for N-array, 4.2 +/- 0.4 for K-array, and 1.8 +/- 0.3 for K-single electrode. By the end of the reach, the trajectories have largely re-converged. The normalized mean +/- STD distance
between trajectories, across targets is: 2.0 +/- 0.4 for N-array, 2.1 +/- 0.7 for K-array, and 1.5 +/- 0.1 for K-single electrode. This distance analysis indicates that the prepare-and-hold state is not achieved in the absence of a delay.

2.4.2 Neural activity can move from one prepare-and-hold state to another during a delay

We next investigated whether monkeys must re-prepare if they have prepared the wrong reach. We trained monkeys N and K to perform a delayed-reaching task variant (Figure 2.5 A). 80% of trials (non-switch trials), were delayed reach trials with the same parameters as the previous task. 20% of randomly interleaved trials were switch trials. Following a delay of 400 ms (N-array), 450 ms (K-single electrode) or 450-900 ms (K-array) after the initial target onset, the initial target turned off and a second target appeared 180 degrees separated from the initial target. There was then either a second delay (0-900 ms), or the monkey was allowed to reach immediately.

We compared the kinematics of the reaches between switch trials and non-switch trials. The mean reach trajectories were similar, with overlapping end point distributions (Figure 2.5 B-D). The end point distributions were often significantly different (N-array: 1/2 targets; K-array: 3/4 targets; K-single electrode: 4/4 targets; p < 0.05 One-Way MANOVA), though the magnitudes of the differences were small (N-array: 8 +/- 8%; K-array: 7 +/- 4%; K-single electrode: 4 +/- 2%; mean +/- STD Euclidean distance between mean reach endpoints, as percentage of target diameter) (Figure 2.5B-D). The peak reach speed was also similar between switch and non-switch trials (Figure 2.5 E-G), with no target having a greater than 5% difference in velocity.

The target switch had a significant effect on RT when the switch and go cue were simultaneous. In this case, the monkeys had a longer RT for the switch condition. Moreover, the monkeys seemed to suffer an RT penalty for preparing to the wrong reach; the switch RT is even longer than the RT for zero-delay trials (Figure 2.5 H-I) (19 ms, N-array, 14 ms, K-array, p < 0.01 2-sample unpaired t-test). When given sufficient time to re-prepare, however, this RT deficit is largely erased. In the Monkey K-single electrode data, however, the RT for zero-delay switch trials (red trace, Figure
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Figure 2.5: (A) Task design. 80% of trials were delayed reaches. In 20% of trials, the initial target switched locations after 400 ms (N), 450 ms (K-single electrode) or 450-900 ms (K-array). The go cue either arrived immediately, or there was a second delay of 0-900 ms. (B-D) Mean reach trajectories for non-switch reaches (black) and switch reaches (red). Circles represent 1 STD of end point positions. Starred reaches show significantly different endpoint distributions (p<.05) (E-G) Difference in reach velocity between different reach directions. Positive indicates non-switch reaches were faster. Gray bars show significantly different reach velocities (p<.05). (H-J) Mean +/- SEM RT curves for non-switch (black) and switch (red) trials. In switch trials, delay length represents time from the target switch, rather than time from the initial target onset.
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2.5. It is lower than the RT for trials without a delay and without a switch (mean of 9.6 ms faster, p < .001 2-sample unpaired t-test). We believe that this is because, while collecting the single-electrode switching data, we held the switch time constant at 450 ms, which could have resulted in the monkey learning to anticipate that a switch was more likely at a particular time. To correct for this possibility, when later collecting the array data we instead switched the targets at a variable time between 450-900 ms. When we made this behavioral modification, the RT for zero-delay switch trials increased to longer than the RT for trials with no delay. While Monkey N also performed the task with a fixed 400-ms switch time, he did not appear to learn to anticipate the switch.

To determine the dynamics of re-preparation, we first examined conditions in which the target switch was followed by a second delay. We calculated PSTHs for the switching and non-switching conditions for each neuron and target (N-array: 119 units, 2 target; K-array: 114 units, 4 targets; K-single electrode: 83 units, 4 targets) (Figure 2.6 A-B). In both example PSTHs, neural activity first moves to the prepare-and-hold state of the cued target. After the switch, neural activity moves to the new targets prepare-and-hold state. Movement-generation activity looks similar between switch and non-switch conditions.

To observe the population neural state after the target switch, we again performed PCA on the normalized PSTHs to find an informative, low-dimensional projection of neural state (see methods for full details). We plotted the neural trajectories in this space, which still accounts for >45% of the variance of the original data (Figure 2.6 C-D). When the initial target turns on, the switch condition (red trace) and non-switch condition (gray trace) move to different prepare-and-hold states, because different targets are cued. After the switch but before the go cue, the switch condition neural population trajectory moves from its initial prepare-and-hold state to the new prepare-and-hold state. After the go cue, movement generation activity appears quite similar between the switch and non-switch conditions.

Calculating the distance between the neural trajectories in the full-dimensional neural state space confirms the divergence and convergence of the neural trajectories observed in the low-dimensional projection (Figure 2.6 E-F). The neural distance
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Figure 2.6: (A-B) Example neural PSTHs. Traces are color coded by final reach direction. Top: conditions without a switch. Bottom: conditions with a switch. (C-D) Example state-space diagrams. Gray trace: non-switch condition. Red trace: switch condition. After the target switch, neural activity moves from prep state 2 to prep state 1, and then remains close to non-switch trajectory through the movement. (E-F) Median resampled distance between trajectories at different times, for the trajectories pictured in C-D. Error bars show 5th and 95th percentile of the distribution. Red ticks: median, 5th, and 95th percentile of the distance measured if neural trajectories were generated from the same underlying distribution. Stars show bootstrap significance.
between conditions is initially low, rarely differing significantly from the expected distance distribution. The normalized mean +/- STD distance between switch and non-switch trajectories across targets is 1.4 +/- 0.09 for N-array, 1.4 +/- 0.2 for K-array, and 1.4 +/- 0.2 for K-single electrode (p > .01, all targets). The distance then increases after the appearance of the first target cue (when the two trajectories are preparing different reaches) and is significantly greater than expected if the trajectories were pulled from the same distribution. The normalized mean +/- STD distance between trajectories across targets is: 4.2 +/- 0.8 for N-array, 8.6 +/- 2.9 for K-array, and 3.0 +/- 0.5 for K-single electrode (p < .01, all targets). After the target switch, the distance between the two conditions returns to a level more comparable to the distance at baseline. The normalized mean +/- STD distance between trajectories is: 1.6 +/- 0.1 for N-array, 2.0 +/- 0.5 for K-array, and 1.7 +/- 0.1 for K-single electrode. The distance remains low through the remainder of the trial. The normalized mean +/- STD distance between trajectories at time of movement onset is: 1.6 +/- 0.10 for N-array, 1.5 +/- 0.08 for K-array, and 1.4 +/- 0.3 for K-single electrode.

2.4.3 If a target switch and go cue are simultaneous, neural activity does not pass through the prepare-and-hold state for the new target

We next examined whether reaches are also re-prepared in conditions with a simultaneous target switch and go cue. Observing the population neural state using PCA (Figure 2.7 C-D), the neural trajectories initially look qualitatively similar to the previous switch-with-re-preparation case. When the first target turns on, the switch condition and non-switch condition move to opposing prepare-and-hold states. In this condition, the go cue and target switch are given at the same time. When this happens, the switching neural population trajectory does not divert to the new, correct prepare-and-hold state. Instead, it takes a path that appears to parallel the non-switching trajectory. The two trajectories gradually converge over the remainder of the trial.

This low-dimensional impression is borne out in the high-dimensional distance
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Figure 2.7: (A-B) Example neural PSTHs. Traces are colored by final reach direction. Top: non-switch conditions. Bottom: switch conditions. (A) Delay period activity is recapitulated after the go cue in the switch condition. (B) The preparatory state is not achieved after the go cue in the switch condition. (C-D) Example state-space diagrams. Gray trace: non-switch condition; red trace, switch condition. After the target switch, neural activity does not divert through the correct prepare-and-hold state, instead converging gradually with the non-switch movement trajectory. (E-F) Median distance between trajectories at different times, for the trajectories pictured in C-D. Error bars show 5th and 95th percentile of the distribution. Red ticks: median, 5th, and 95th percentile of the distance measured if neural trajectories were generated from the same underlying distribution. Stars show bootstrap significance.
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analysis (Figure 2.7 E-F). The distance between the trajectories starts out low. The mean +/- STD normalized distance between different trajectories is: 1.3 +/- 0.001 for N-array, 1.4 +/- 0.2 for K-array, and 1.2 +/- 0.08 for K-single electrode (p >.01, all targets). Once the target turns on, the trajectories diverge, and the distance at the time of the go cue is high. The normalized mean +/- STD distance between trajectories across targets is: 4.6 +/- 1.1 for N-array, 6.2 +/- 1.9 for K-array, and 2.9 +/- 0.4 for K-single electrode (p<.01, all targets). This implies that the state achieved by the correctly prepared trajectory during the delay is never achieved by the switch trajectory. The distance between the trajectories remains relatively high at the time of movement onset. The mean +/- STD normalized distance between trajectories, across targets is: 2.5 +/- 0.2 for N-array, 3.8 +/- 0.3 for K-array, and 2.4 +/- 0.07 for K-single electrode. This is despite the fact that the reaches themselves are kinematically similar (Figure 2.5). By the time the monkeys have finished reaching, the neural trajectories have largely re-converged. The mean +/- STD normalized distance between trajectories, across targets is: 1.7 +/- 0.06 for N-array, 1.6 +/- 0.08 for K-array, and 1.3 +/- 0.05 for K-single electrode. This analysis indicates that even if monkeys have prepared an incorrect reach at the time of the go cue, their neural activity still does not need to re-direct through the correct prepare-and-hold state to make a correct reach.

2.4.4 The initial target response is similar between delayed and non-delayed reaches

Given that the prepare-and-hold state is not achieved in the absence of a delay, we wanted to investigate whether any neural response properties were similar between delayed and non-delayed reaches. To address this question, we compared the time course of neural responses under different behavioral conditions.

We first examined the timing of neural responses to command cues. For delayed reaches, we asked how long it takes for neural trajectories to respond to the target versus the go cue. We used a distance analysis to determine neural response time to different targets. We estimated neural FRs on each trial as the number of spikes
in a 40-ms bin preceding each time point. For delayed reaches to a given target, we estimated the distance over time between the average baseline activity and the average neural trajectory for that target (Figure 2.8 A-B, green trace). Looking forward from the time of target onset, we defined the neural response time as the first time that the neural distance from baseline crosses a threshold of 20 spikes/s further than the neural distance at the time of target onset. We selected this threshold to be approximately double the expected fluctuation in the distance metric, as measured during the baseline period. We repeated this analysis across all target directions to yield an across-target estimate of neural response time (Figure 2.8 A-B, vertical green line). We repeated this analysis to generate a go cue response time, examining when the neural distance from the prepare-and-hold state starts increasing after the go cue (Figure 2.8 A-B, black trace and vertical black line).

![Figure 2.8](image_url)

In both N-array and K-array, the neural response to the target cue was faster than
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to the go cue (mean +/- STD difference in response times across reach directions: N: 49 +/- 13 ms, p<0.01; K: 47 +/- 13 ms, p<0.01; paired t-test) (Figure 2.8 A-B, black vs. green trace). This suggests that target identity may reach the motor cortex faster than the putative go command. If this is true, then neural responses during a non-delayed reach should first resemble the initial target-cue response of a delayed reach, as target identity arrives first. Only after the go command reaches the motor cortices should delayed and non-delayed reaches diverge.

We therefore compared the neural responses of delayed and non-delayed reaches. Delayed and non-delayed reaches had a similar (though still significantly different in K) neural response latency to the target cue (mean +/- STD difference in response times across reach directions: N: 0 +/- 2 ms, p>0.05; K: 2 +/- 2 ms, p<0.05; paired t-test) (Figure 2.8 A-B, blue vs. green trace), suggesting that the presence or absence of a delay does not strongly influence the arrival time of target information. Given the timing similarity, is the initial response itself also similar? To determine the similarity of responses, we calculated the neural divergence time between delayed and non-delayed trajectories. This was defined, for a given target, to be the first time the distance between delayed and non-delayed neural trajectories was >20 spikes/s greater than the distance at baseline. We found that this divergence time is later than the initial target response times (N: 59 +/- 17 ms later; K: 65 +/- 21 ms later, p<0.01, paired t-test, both monkeys) (Figure 2.8 A-B, red trace). This means that the initial neural response to the target cue are highly similar across delayed and non-delayed reaches.

Delayed and non-delayed reach neural activity could remain similar until the go cue signal arrives. If this is true, then the divergence time should be close to the go cue response time of delayed reaches. And indeed, we see that for both monkeys, the divergence time is closer to the delayed reaches go cue response time, though still significantly different in K (mean +/- STD difference: N: divergence 10 +/- 17 ms longer, p >0.05; K: divergence 17 +/- 9 ms longer, p <0.01; paired t-test) (Figure 2.8 A-B, red trace vs. black trace).

Even though the prepare-and-hold state is not achieved during non-delayed trials, the initial response is highly similar between delayed and non-delayed reaches. This
suggests that the early neural target responses could be sufficient to distinguish where to reach and generate a correct movement.

2.5 Discussion

We investigated whether neural activity always engages preparatory activity before generating movement. We first asked whether neural activity always achieves a specific prepare-and-hold state. First, we compared delayed and non-delayed reaches. These reaches were kinematically similar. However, non-delayed reaches did not achieve the same neural prepare-and-hold state as delayed reaching movements. Second, we asked whether reaches are re-prepared if the target switches locations. When the target switch was followed by a delay, the monkeys neural activity moved from the initial prepare-and-hold state to the new one. However, when the target switch and go cue were simultaneous, we did not observe the same effect. Instead, the switch condition neural population trajectories took a parallel but separate path through neural state space. The switch and non-switch neural population trajectories did not fully re-converge until the end of the trial. These results show that achieving a single, specific neural prepare-and-hold state is not necessary for generating a correct reach.

We then asked whether any neural preparatory activity was similar between delayed and non-delayed reaching conditions. Specifically, we examined the time course of the neural responses to different behavioral cues. We found that the neural target response has a similar latency in delayed and non-delayed reaches. These responses are similar to each other until about 50 ms after they begin. The time that the responses diverge is similar to the go cue response time in the delayed reach case. These findings are in line with previous findings that cross-condition neural tuning is similar following target onset in both delayed and non-delayed reach conditions (Crammond and Kalaska, 2000). Our findings suggest that there may be two separate inputs to motor cortex in this task. Target information arrives relatively quickly, while go cue information takes slightly longer, even if these cues are given simultaneously from an external perspective. If the go cue fails to arrive soon after the target cue, then
neural activity moves to the prepare-and-hold state. When the go cue arrives, movement generation activity is engaged, regardless of whether the prepare-and-hold state was reached. This preserved neural target response, combined with the fact that reach kinematics are similar across delayed and non-delayed reaches, suggests that the early neural target response is sufficient to generate correct reaching dynamics.

2.5.1 Prepare and Hold

It is worth noting that preparatory activity identified in this and previous studies (Cisek and Kalaska, 2002; Riehle and Requin, 1989; Tanji and Evarts, 1976; Wise and Mauritz, 1985) is observed when monkeys are simultaneously preparing a movement and holding a static posture. Our study indicates that fully attaining this state is potentially unique to this prepare-and-hold task, rather than a necessary step in all forms of voluntary motor generation. While there are certainly times when preparing for but withholding from movement may be warranted (particularly behaviors which require a short RT, such as swatting a fly, or dodging a dodge ball), it is likely that neural activity in this case is not universally representative of all movement generation activity. Previous studies have demonstrated that achieving this state decreases RT (Afshar et al., 2011; Churchland et al., 2006c). However, if achieving this state is not necessary, it remains unclear why neural activity attains this particular state (instead of, for example, pausing at the state at which the delayed and non-delayed neural target responses diverge). It could be that this state allows motor preparation and avoids motor initiation, while other preparatory states would trigger a movement. It is also possible that this state is better optimized to generate a specific reach, having been given more time to prepare. Further work will be required to determine the significance of this particular state during prepare-and-hold paradigms.

2.5.2 Visual Effects

To make even simple movements, the brain must perform many computations (Wong et al., 2014). First, subjects must use sensory processing to identify potential targets and barriers. Subjects must then decide where and when to move, based on this
sensory input and task goals. Finally the movement must be prepared and executed. Studies in the Frontal Eye Field (FEF), an area involved in the selection and execution of saccadic eye movements (Schall, 2002), have suggested that visual target selection has a fixed duration which does not co-vary with RT (Schall and Thompson, 1999; Thompson et al., 1996). In contrast, saccadic motor preparation takes a variable length of time and likely is the primary cause of RT variability in saccadic eye movements in a visual-search task. This observation of a two-step visuo-motor transform in the FEF agrees on a basic level with our findings that target-related neural responses are observed earlier than go-cue related neural responses. Indeed, target selection must certainly precede motor generation regardless of motor effector.

However, there are also many differences between FEF and PMd/M1. First, motor-related responses in FEF tend to display a rise to threshold form of motor generation saccades are generated when neural activity has reached a specific threshold (Hanes and Schall, 1996). In contrast, neurons in PMd and M1 often display both increasing and decreasing FRs during movement preparation, which is not well predicted by a rise-to-threshold model. Furthermore, directional tuning during preparation is only loosely correlated with directional tuning during movement (Churchland et al., 2010). Second, FEF contains separable populations of cells which primarily display visual-related activity or motor-related activity (Bruce and Goldberg, 1985; Sato and Schall, 2003), while these processes tend not to be separated on a neuron-by-neuron basis in the motor cortex (Churchland et al., 2010). Finally, the task used in the FEF studies described above was a visual search task in which when to move was not explicitly instructed. In our task, monkeys were instructed when to begin moving. It is thus presumably necessary that some form of trigger signal (or hold signal) be transmitted to the motor cortex, to allow the reach to be delayed until the go cue (Kaufman et al., 2014b).

2.5.3 An updated model of motor cortical dynamics

The results in this paper offer an enhancement and expansion of previous models of motor cortical dynamics. The optimal subspace hypothesis (Churchland and Shenoy,
2.5. DISCUSSION

2007) suggested that there is an optimal preparatory region from which to generate a given movement. The initial condition hypothesis (Afshar et al., 2011; Churchland et al., 2010, 2012) further refined this model to suggest that the neural preparatory state serves as the initial condition for a dynamical system that generates the reach.

These previous models both concentrated on movement generation dynamics. These models acknowledge preparatory state as the initial condition for the movement-generation dynamical system but do not concentrate on the mechanism of how this state is achieved. It is clear, however, that neural activity behaves differently during different epochs (e.g., preparatory period vs. movement period) (Kaufman et al., 2014a). For example, neural activity approaches the prepare-and-hold state during the delay, but moves away from this state after the go cue. Therefore, motor cortical dynamics likely change as a function of task context and inputs, such as target information and the go cue (Shenoy et al., 2013).

We now describe a conceptual model of motor cortical activity that explicitly takes into account the distinct dynamics we observe during different task epochs. We propose that two principal dynamical systems are engaged during reaching: a preparatory system driven by target information, and a movement generation system driven by the go cue.

During a delayed reach, target information is transmitted to motor cortex first (Figure 2.9 A, blue trace). This input sets the dynamics of the network, illustrated with a gray vector flow field. This preparatory dynamical system gives rise to the delay-period neural activity observed in this and previous studies. The preparatory dynamical system contains a putative attractor corresponding to the observed prepare-and-hold state. Given enough time, the neural state will converge to the prepare-and-hold attractor. When the go cue arrives, this changes the dynamics from preparatory dynamics to movement-generation dynamics. The neural state at the time of the change serves as the initial condition for this new dynamical system (Figure 2.9 B, blue trace), consistent with the Initial Condition Hypothesis. Different initial conditions will yield slightly different motor-generation trajectories, as dictated by the dynamics. Given that RTs tend to be lower for fully prepared reaches, it seems
Figure 2.9: (A) When preparatory dynamics are engaged, neural activity approaches an attractor. Given a full delay (blue trace), neural activity reaches the attractor. Otherwise (red trace), neural activity approaches the attractor but may not converge. (B) The arrival of the go cue engages movement-generation dynamics. The neural state at the time of this transition (transparent red and blue traces) serves as the initial condition for the movement-generation neural trajectory (solid red and blue traces). (C) In target switch trials, neural activity moves to an attractor for the initially cued reach. (D) When the target switches, the attractor moves to a location corresponding to the preparatory state for the new target. If there is time, neural activity converges with this attractor (blue trace), otherwise approaches the attractor but may not converge (red trace). (E) The arrival of the go cue engages movement-generation dynamics. The neural state at the time of this transition serves as the initial condition for this new dynamical system.
reasonable to assume that the prepare-and-hold state represents a low RT initial condition. Being in this state could allow the subsequent movement-generation trajectory to initiate movement more quickly, consistent with the Optimal Subspace Hypothesis.

In a non-delayed reach condition, one might initially think that either neural activity must go all the way to the prepare-and-hold state, or skip preparatory dynamics entirely and go directly into the movement-generation dynamical system. Our results indicate, however, that neither of these cases is true. Neural activity does not achieve the prepare-and-hold state during non-delayed reaching conditions. At the same time, the first portion of the neural response in the non-delayed reach condition looks much like the delayed-reach target response. We suggest that during non-delayed reaches, the preparatory dynamical system is engaged first, for a short period of time (Figure 2.9 A, red trace). This could be caused by the target information reaching motor cortex more quickly than the go cue information. Preparatory dynamics are not engaged long enough to fully achieve the prepare-and-hold state. They are engaged long enough, however, to ensure that when the go cue arrives the subsequent trajectory can generate the correct reach (though usually with an RT penalty) (Figure 2.9 B, red trace).

In behavioral conditions that involve a target switch, the progression from preparatory dynamics to movement dynamics is likely similar. When the first target turns on, this sets a preparatory dynamical system with a putative attractor corresponding to the prepare-and-hold state for this target (Figure 2.9 C). When the target location changes, this moves the attractor to the prepare-and-hold state for the new target. Given sufficient time, neural activity will move to this new preparatory state (Figure 2.9 D, blue trace). When the go cue comes, this engages the movement-generation dynamical system, and a movement is generated with neural activity that is similar to a standard delayed-reach (Figure 2.9 E, blue trace).

If the go cue and target switch are given simultaneously from an external perspective, the target information is transmitted more quickly to the motor cortex. This allows a short period of time when the new targets preparatory dynamics are engaged, similar to the non-delayed reach case. The neural state moves part of the way
toward the new preparatory state (Figure 2.9 D, red trace). When the go cue information arrives, the system changes into movement-generation dynamics. The neural population state at that time serves as the new initial condition, and the population state begins moving along a movement-generation trajectory (Figure 2.9 E, red trace).

Several details of this two dynamical system model remain to be investigated in future work. For example, we cannot currently predict how neural dynamics will respond to perturbations while a reach is being executed. If the target goal changes mid-reach, or if the arm is perturbed unexpectedly, this will likely require on-line coordination of processes often ascribed separately to either motor preparation (e.g. determining and remembering task goal, setting up a reach), and motor generation (e.g. executing a reach to the correct target). We also cannot currently tell whether the transmission of the go cue is automatic. It could be that the go cue is always transmitted to motor cortex at a particular latency. Alternately, whatever system is sending the go cue could be monitoring the preparatory state of motor cortex, and only transmit the go cue when it detects that the neural state is sufficient to generate the correct reach, as hypothesized in early studies of the Optimal Subspace Hypothesis (Churchland and Shenoy, 2007).

Our results indicate that movement-generation neural trajectories can drive highly similar movements despite being initiated from different preparatory states. Many factors could relate to this effect. First, the preparatory region required for a given movement could be relatively broad, such that multiple states can result in the same movement. Neural trajectories are unlikely to achieve the same preparatory state from trial to trial, due to the variability of FRs. Therefore, some flexibility in required initial state could be beneficial, to allow a correct reach to be generated despite preparatory variability. Alternatively, target-specific information could be retained during the movement period, resulting in slightly different movement-generation dynamical systems for each reach. This could allow slight errors in initial state to be corrected by the movement-generation dynamical system itself. Further studies will be required to determine whether one or both of these possibilities is at work.

Previous work has uncovered neural features of movement generation which are preserved across reach directions (Churchland et al., 2012). This work has informed
our investigation and interpretation, but also presents several methodological differences with our study. First, the prior work concentrated on across-target comparisons of delayed reaches. The present study, in contrast, compares delayed, non-delayed, and switch conditions for the same target. The previous work also uses a new method called jPCA, which accounts for a considerable percentage of the variance in the data (50-70% in the top 2-3 jPCA planes), but there still remains some neural variability to be accounted for. The previous study gives evidence for a single movement-generation dynamical system which depends only upon neural preparatory state (as opposed to unique dynamics for each reach). It is still possible, however, that there remain areas of variability which cannot be explained by this model. The evidence we present here cannot decisively distinguish whether or not movement dynamics are fully independent of target identity; additional investigation will be required to address this question.

This study represents one of the first substantial forays into examining not just motor preparation and execution, but the interaction between the two processes. We determined that fully achieving a specific prepare-and-hold state is not necessary, but that there are also aspects of delay-period activity that are preserved even without a delay. We propose that motor preparation may be more accurately defined as the engagement of a specific set of preparatory dynamics, rather than the achievement of a particular neural state. The set of states which are produced by these dynamics serve as initial conditions which are sufficient to generate a correct reach. This has helped advance our understanding of the nature of the dynamics of motor cortex, and how task constraints affect these dynamics.
Chapter 3

Probing the interaction of motor preparation and generation with a last-moment target switching task

3.1 Summary

In this study, we investigate the behavioral and neural effects of overlapping motor preparation and execution. We train monkeys to perform a delayed reaching task where the reach target occasionally switches locations after the go cue but before movement onset. We find that the resulting behavior depends on the length of time between the target switch and movement onset. If the target switches shortly before movement begins, the reaches begin toward the initial target and are corrected online. If there is more time between the switch and movement, reaches can begin toward the new target immediately. We find that we can explain a fair amount of the behavioral variability in the reach simply based on how far neural activity had progressed toward initiating the original reach at the time of the target switch. We also wanted to investigate the neural mechanisms of correcting neural activity following a target switch. We found that reaches could be initiated correctly even if their neural activity remained significantly different from non-switch reaches to the same target. However, this neural correction seemed to occur more quickly in dimensions
which were putatively important for driving muscle activity, and neural distance in putative "muscle potent" dimensions performed better than total neural distance at predicting the reaching behavior.

3.2 Introduction

In section 2.5.3, we hypothesized two potentially distinct functional modes of the motor cortex: motor preparatory dynamics, mediated by input specifying a reach target, and motor execution dynamics, mediated by a go cue input. We further hypothesized that, when motor preparation precedes motor execution, the neural state at the time of the go cue serves as the "initial condition" for the motor execution dynamics. This initial condition may set important parameters for the execution of the reach (Churchland et al., 2010, 2012; Afshar et al., 2011). We wanted to further test the nature of these two processes, by manipulating their temporal relationship. In order to accomplish this goal, we took inspiration from two different classes of experiments: countermanding tasks and metronome tasks.

Countermanding tasks probe the timing and neural correlates of reach generation (Lappin and Eriksen, 1966; Logan and Cowan, 1984). In these tasks, a go cue is occasionally followed by a stop cue, indicating that subjects should refrain from reaching. Depending on the relative timing of the go and stop signals, subjects will either correctly suppress their reach or will make a false start, being unable to suppress their initial reach. This process is typically modeled as a race between a relatively slow movement initiation process and a faster stop process (Verbruggen and Logan, 2009; Mirabella et al., 2006; Verbruggen and Logan, 2008). If the go process wins, neural activity crosses a ‘point-of-no-return’ which causes the monkey to initiate a reach. If the stop process wins, neural activity will not cross the point-of-no-return and the monkey will correctly refrain from reaching. The timing of the go process is measured using non-countermanded reaches. The time it takes to complete the stop process must be inferred based on false-start trials, as successful stop trials will not involve any overt movement (Verbruggen and Logan, 2008). Neural responses to the putative stop signal on successfully countermanded trials can be found in PMd (Mirabella
et al., 2011).

Metronome tasks study the progression of the motor preparatory process. In these tasks, human subjects are instructed to listen to a metronome, and begin a reaching movement on the fourth beat. Typically, the subjects only have access to partial information about the upcoming reach. For example, at the start of the trial, they may know that they will be reaching to one of two possible targets. The final reach target is disambiguated at variable times before the final metronome beat. If the reach is disambiguated a long time before movement onset, subjects will make normal reaches to the cued target. But if the disambiguation occurs close to the go cue, subjects will display different reaching behavior depending upon the nature of their ambiguous information. If the two possible targets are far apart, they will guess which target to reach to. If the two possible targets are close together, then they will make an intermediate reach between the two potential options (Ghez et al., 1997).

Countermanding tasks are primarily useful to examine the process of reach generation. We wanted to use an analogous approach to instead examine reach selection: where to reach, as opposed to when to reach. To accomplish this, we trained monkeys to perform a reaching task. In a small subset of trials, the cued target location changes at a random time between the go cue and movement onset. As in countermanding tasks, we can classify trials as correct or incorrect, based on whether the initial portion of the reach is toward the original or the new target. However, this task gives us additional information which countermanding tasks are blind to. For incorrect trials, we can tell that the monkeys started reaching toward the wrong target, similar to a false start in the countermanding task. However, because our task allows for online correction of reaches, we can also tell at what time the monkeys begin correcting this initial error. For correct trials, our task also has more precise behavioral timing information than the countermanding task. On a correctly countermanded trial, the hand never moves, so the countermanding process is entirely covert. In our correct trials, we can tell not only that the monkeys started reaching toward the correct target, but also at what time they begin making the reach. This gives us a good view of the time required to reprogram a reach, as well as a way to behaviorally align correct and incorrect trials.
Our task can also be considered to be analogous to a metronome task. Instead of tightly controlling the time of movement initiation, we instead can sort trials post-hoc based on the time between the target switch and movement onset. This enables us to more easily adapt this task into a nonhuman primate model system, where we can simultaneously measure the activity of individual neurons. Similar to the metronome task, we investigated the time required to select a new reach, with the added benefit of simultaneously recording activity from multiple neurons while the task is being performed.

In analyzing the neural activity in our task, we took inspiration from a few recent studies. These studies seek to make sense of neural activity in complex tasks by finding projections of neural firing rates (neural dimensions) which correlate only with particular task parameters (time, strength of decision-related evidence, etc.) (Machens et al., 2010; Brendel et al., 2011; Kaufman et al., 2014a). In particular, one recent paper has suggested that there are some patterns of neural activity which are output to the muscles (muscle potent dimensions), while others are not (muscle null dimensions). For example, if a muscle responds based on the sum of the firing rates of two neurons, then decreases in one neuron’s FR that are offset by increases in the other neuron’s FR will have no effect on muscle output. Kaufman et al. (2014a) demonstrated that during the delay period, neural activity was restricted to putative “muscle null” dimensions, whereas during movement, neural activity explored both muscle potent and muscle null dimensions. They suggest that this sort of neural organization may enable a given brain area to perform internal computations in output null dimensions without interfering with the current output of that area.

Our data yield new insights into the nature of reach selection. Unlike a truly winner-take-all process, incorrect reaches can be corrected online – initiating a given reach does not reflect complete commitment to that reach. Rather, it seems that the process of target selection can be performed in parallel to a simultaneously-executed reach, only affecting reaching behavior once the new target has been selected. Neural data recorded during this task supports the hypothesis that target selection is performed in dimensions which are orthogonal to motor output, making this the first study that we are aware of to directly indicate the use of output null dimensions for
computation during active reaching.

3.3 Materials and Methods

3.3.1 Behavior

Figure 3.1: To initiate a trial, monkeys touched an illuminated center hold target projected on a vertical screen. After 500-700 ms, a second target appeared, indicating where the monkey would be asked to reach next. After a delay period of 0-500 ms (S) or 0-900 ms (K), the center target disappeared, serving as a go cue. On 80% of trials, the monkeys would then reach to the cued target. On 20% of trials (switch trials), the initially cued target changed locations at a random time after the go cue but before the monkey began reaching. The monkey needed to touch the new target to receive a juice reward.

All research protocols were approved by the Stanford Institutional Animal Care and Use Committee. We trained two monkeys (K,S) to perform a variant of a delayed reaching task (Figure 3.1). The monkeys sat in a custom primate chair (Christ Instruments) and touched targets projected onto a vertical screen approximately 30 cm in front of them. The position of the monkey’s hand was monitored optically using a reflective bead taped between the first and second knuckles of the middle two fingers. The monkeys performed two categories of trial: switch trials and non-switch trials. During non-switch trials, the monkeys touched and held a center target to initiate a
3.3. MATERIALS AND METHODS

trial. After 500-700 ms, a second target would appear 10 cm away from the center target. The monkeys were required to withhold from reaching for a delay period of 0-500 ms (S) or 0-900 ms (K). After the delay, the center target would disappear, providing a go cue. The monkeys could then reach to the peripheral target to receive a juice reward. Switch trials proceeded in an identical manner to non-switch trials through the time of the go cue. At a random time following the go cue but before the minimum RT of the monkey, the initial peripheral target would turn off and a second peripheral target would turn on, at a separation of 180, 135, 90 or 45 degrees from the original target. The monkey would need to reach to the new target in order to receive their reward.

Throughout this work, we will use the term “switch pairs” to refer to any given pair of targets, taking into account order of appearance. For example, if one trial type involves first a right target, then a switch to a left target, that would be one switch pair. If another trial type involved a switch from the left target to the right target, that would be a separate switch pair, as the order of the targets’ appearance is different. In order to ensure a sufficient trial count for each switch pair, we typically allowed only a subset of possible switches on any given day. For example, one day might include four targets arranged in a square, but restrict to only 180-degree switches, leading to a total of four switch conditions.

We classified switch trials as correct or incorrect based on the initial angle of the reach. We first calculated the distribution of initial angles for non-switch reaches to the correct target. If the angle of a switch reach was greater than two standard deviations different from the mean of this expected distribution in the direction of the incorrect target, it was labeled an incorrect reach. Otherwise, it was labeled a correct reach.

To determine when on-line switches occurred, we used a velocity test (Figure 3.2). For a given switch pair, we used non-switch reaches toward the first (incorrect) target to generate an expected reach velocity distribution. We subtracted the velocity in the direction of the second (correct) target from the velocity in the direction of the first (incorrect) target. This enabled us to tell more precisely whether reaches were in the direction of the first or second target in the case where the angle between
the targets were small. We then performed this same velocity calculation for each switch trial, and asked at what time the velocity ceased to be within three standard deviations of the mean expected non-switch velocity (with the added constraint that the following 10 ms must also fall outside of the non-switch velocity distribution, to exclude occasional noisy deviations). We classified this as the switch time. The incorrect reaching distance was classified as the distance between the hand position at the time of the switch and the original center hold position. A zero value would indicate that the hand started reaching toward the new target immediately. A large value indicates that the reach progressed a long way toward the original target before correcting.

![Figure 3.2](image.png)

**Figure 3.2:** Example trace showing how we find the time of behavioral correction. Gray traces show mean $+/−$ 3 standard deviations of the reach velocity for non-switch trials to the first target. Black trace shows the hand velocity for a switch trial. The time of behavioral correction is measured as the first time that the velocity on the switch trial is more than three standard deviations outside of the non-switch velocity distribution (red dashed line). Inset: blue traces are non-switch reaches to the first target, black trace shows the hand trajectory for the example switch trial. Red dot indicates the position at the time of detected behavioral correction.

### 3.3.2 Neural Recordings

We used two techniques to simultaneously record from multiple neurons in motor cortical areas. Monkey K was chronically implanted with two 96-electrode arrays, one
in M1 and one in PMd. Monkey S was implanted with a cylinder above PMd. 3-mm diameter burr holes were drilled in the skull enclosed by this cylinder, leaving the dura intact. We recorded neural activity with a linear array of 16 electrodes (Plexon U-probe) which was lowered acutely each day through one of these burr-holes.

Many of our analyses required us to calculate the firing rate of recorded neurons on individual trials. Because we often wanted to use a causal filter (not taking into account spikes that haven’t happened yet) in order to do behavioral predictions, we opted to use a simple calculation for our estimated firing rate at a given time: We counted the firing rate of a neuron at a given time as the number of spikes that neuron fired in a 60-ms bin leading up to that time point.

### 3.3.3 Predicting Magnitude of Reaching Error

We performed a leave-one-out analysis to predict the distance switch trials would reach to the wrong target based on neural activity at the time of the switch. We created a projection vector for neural activity using non-switch reaches to the original target (Figure 3.5 A). We connected the average neural state (FR of each neuron) at the time of the go cue to the average neural state near the time of movement onset. The exact latency relative to movement onset was selected for each monkey to maximize the Rsquared of the regression across all datasets for that monkey (Monkey K - 50 ms before movement onset, Monkey S - at the time of movement onset). For each switch trial, we then found the neural state at the time of the switch and projected that neural state onto our projection vector. This yielded a readout of how far that trial’s neural state had advanced towards the onset of movement at the time that the target switched.

We then used that “neural commitment” value to predict the distance that the monkey reached to the wrong target before correcting. Because the neural trajectory could progress somewhat towards triggering a movement before actually committing to the first part of the movement, we fit a function that would enable a linear fit to the “incorrectly” initiated reaches, while allowing all “correctly” initiated reaches to be estimated to be zero (Figure 3.5 B). We fit the function $\max(0, m \times x + b)$ using
an iterative least squares algorithm. We calculated accuracy using a leave-one-out process, training our fit using all but one trial and then testing accuracy on the left out trial, for each trial for a given switch pair. This showed us how well switch-trial reaching behavior could be predicted by degree of commitment to the original reach.

3.3.4 Dimensionality Analysis

We wanted to determine whether there were dimensions occupied by the neural switch response that were not occupied by the movement response. For each dataset, we gathered the mean firing rate for each neuron during (non-switch trial) reaches to each target, from 100 ms before the onset of movement to 500 ms after the onset of movement, and target-onset locked activity from 0 to 250 ms after the onset of the target (during the delay period). We also gathered the mean firing rate during target switch conditions, from the time of the switch to 200 ms after the switch. We then performed PCA on all of the PSTHs to de-noise the data slightly, restricting ourselves to the number of dimensions which explained 95% of the total neural data.

We then performed PCA on only the movement-related neural activity, to divide dimensions into movement-related dimensions and “other” dimensions. We took the switch aligned neural activity and projected it into this space, calculating what percent of the switch response variance was in each dimension. We divided the dimensions in half, assigning the first n/2 dimensions to be putative “movement related” dimensions, and referring to the remaining n/2 dimensions as ”switch dimensions.” We found that <10% of the movement variance was in the “switch” dimensions, but 40-50% of the switch response occupied these dimensions, suggesting that there are significant components of the switch-related neural activity which are not usually explored during reaching.

3.3.5 Neural Convergence

To calculate how long it took for neural activity to fully account for the target jump, we selected only the correctly-initiated trials. Because these trials display similar behavior to non-switch trials, they also ought to also have similar neural activity to
that of non-switch trials. We first calculated the average non-switch neural trajectory by taking the average FR for each recorded neuron across all reaches to that target. We then asked how long it took for neural activity in target switch trials to converge with this mean non-switch trajectory, using two different metrics.

**Full-Dimensional Neural Distance**

First, we asked how long it took for the Euclidean distance between individual switch trial neural trajectories and the mean non-switch neural trajectory to drop to zero (Figure 3.8 A). For each switch trial, we calculated the firing rate of each neuron as a function of time from the switch. We then calculated the distance from the mean non-switch neural trajectory for the new target at each time point. To determine which time points to compare on each trajectory, we aligned the switch trajectory and the non-switch trajectory at the time of movement onset. So, if the target switch occurred 100 ms prior to movement onset, we calculated the neural distance between the switch trajectory at the time of the switch and the average non-switch trajectory 100 ms before movement onset, and then stepped forward in time to get distance estimates for up to 400 ms following the switch. Because true euclidean distance will never truly reach a “zero” value, we subtracted the distance between single trial non-switch trajectories and the mean non-switch trajectory. A zero distance therefore indicates that the distance between the switch trajectory and the mean non-switch trajectory is the same as expected if there had not been a target switch. To calculate the convergence time, we performed a t-test on the distances measured across switch trials and asked when the distance distribution first fails to be statistically different from zero (following the time of maximum distance, to eliminate some early false positives).

**Separating Axis Projection**

We next asked how long it took for individual switch trial neural trajectories to converge to the mean non-switch neural trajectory, in the dimension separating the mean trajectories to the first and second target (separating projection) (Figure 3.8 E).
To calculate this projection, at a given time point, we drew a line connecting the mean neural activity for reaches to the originally-cued and new target. We then projected switch trials on to this line, again aligned to the time of movement onset. A value of 1 indicates that neural activity lies near the original target’s neural trajectory, while a value of 0 indicates that neural activity lies near the new target’s neural trajectory. To calculate the convergence time, we performed a t-test on the distances measured across switch trials and asked when the distance distribution first fails to be statistically different from zero (following the time of maximum distance, to eliminate some early false positives).

Comparing Classifier Accuracies

As one method of directly comparing the relative importance of the full-dimensional neural distance vs. the separating axis projection, we asked how well each of these values classifies whether switch trials are initiated in a correct or incorrect direction. We calculated the neural distance and the separating projection value at the time of movement onset for all switch trials of a given type. We then ran a leave-one-out binary classifier to ask how well these metrics distinguish between correct and incorrect reaches.

3.4 Results

We trained two monkeys (K,S) to perform a variant of a delayed reaching task (Figure 3.1). The monkeys touched a central target projected on a screen to initiate a trial. After 500-700 ms, a second target appeared, indicating where they would need to reach next. Following a delay of 0-900 ms (K) or 0-500 ms (S), the central target disappeared, providing a go cue. On 80% of trials, the monkeys then reached to the cued target to receive a juice reward. The remaining 20% of trials were switch trials. In switch trials, at a random time after the go cue but before movement onset, the original target disappeared and a new target appeared. The monkeys were required to touch the new target to receive their juice reward.

The monkeys display a variety of behaviors in this target switching task. Some
Figure 3.3: (A-B) Example switch-trial reaches for a day with 180-degree switches (A) and a day with 90-degree switches (B). Blue traces indicate the reach was initiated toward the correct target, red traces indicate that the reach was initiated toward the incorrect target. Both datasets are taken from Monkey S. (C) % correct reaches versus time between switch and movement onset for all switch trials recorded across all datasets and both monkeys.
reaches are initialized directly toward the new target (Figure 3.3 A-B, blue traces), while others are initialized toward the original target (Figure 3.3 A-B, red traces). We can examine the time required to switch which target the monkey begins reaching toward by plotting the percentage of correctly-initiated reaches as a function of the time from the switch to movement onset (Figure 3.3 C). If the target switches less than 100 ms before the start of movement, almost no reaches are initiated toward the correct target. If the target switches more than 200 ms before the start of movement, almost all reaches are initiated toward the correct target. The percentage of correct reaches crosses 50% at slightly before 150 ms (Monkey K - 139 ms, Monkey S - 143 ms).

However, switching a reach target is not a purely binary process. Among incorrectly-initiated traces, some go quite far toward the original target before correcting, while others are corrected almost immediately (Figure 3.3 A-B, red traces). This behavioral variability seems to be tightly linked to how much time has passed between the target switch and the beginning of movement. The less time there is between the switch and movement onset, the further the monkeys reach in the wrong direction before correcting (Figure 3.4 A-B). Furthermore, for a given switch pair there appears to be a relatively consistent amount of time between the target switch and online reach correction (Figure 3.4 C-D). In fact, the amount of time that the correction takes does not appear to be strongly related to the time between the switch and the movement. Whether the neural switching process is performed almost entirely before the movement or almost entirely during the movement, it seems to take a consistent amount of time.

This indicates that there is no single “point-of-no return,” at which monkeys fully commit to a given reach. Rather, monkeys can switch their reach goal regardless of the underlying motor state of the arm. If the target switches early in the reach generation process, then the process of changing the reach goal can be completed before movement starts. If the target switches later in the reach generation process, then more of the original reach will be performed before correcting online.

We wanted to examine this idea neurally. We wanted to see how well we could use neural activity to predict the subsequent reaching behavior. We first asked whether
3.4. RESULTS

Figure 3.4: (A-B) Distance reached to the wrong target as a function of the amount of time between the target switch and the beginning of movement, for the same days as displayed in (Figure 3.3 A-B). Colors indicate different switch pairs. (C-D) Time from the target switch to the first observed behavioral correction, for reaches that initiated toward the wrong target.
Figure 3.5: (A) Cartoon of the method for classifying neural commitment to a reach. We used non-switch trials for a particular target to generate a projection vector connecting the average neural activity at the time of the go cue and the average neural activity around the time of the reach (red line). We then projected the neural state of switch trials at the time of the switch onto this vector (gray dots). Trials that were further along this vector tended to be closer to initiating movement than trials which were further back at the time of the switch. (B) Example function fit for one switch pair from Monkey K. (C-D) Histogram of R-squared regressing commitment to move versus distance reached to the incorrect target for all switch pairs recorded for Monkey S (C) and K (D). Mean Rsquared values indicated by red arrows.
3.4. RESULTS

we could neurally identify trials that were closer to triggering a reach. We further asked whether those trials’ reaches would tend to go further in the wrong direction. First we constructed a method to neurally quantify how close a particular switch trial was to initiating a reach at the time of the target switch. For a given switch pair, we created a projection vector using non-switch trials to the original target (Figure 3.5 A). This vector connected the average neural state at the time of the go cue to the average neural state near the time of movement onset. This vector represents the progression of neural activity as it starts initiating a movement. We then projected the full-dimensional neural state of individual switch trials at the time of the switch onto this vector. This gives us a trial-by-trial quantification of how close to movement onset the neural state was at the time of the switch. We then performed a leave-one-out function fit, using this “neural commitment” quantification to predict how far the hand would reach in the wrong direction before correcting. We fit a linear function with the added constraint that all below-zero values would be set to zero, to account for the nonlinearity between correctly-initiated reaches and incorrectly-initiated reaches (Figure 3.5 B shows an example fit for one switch pair).

We were able to explain a fair amount of the behavioral variance simply from this one-dimensional neural readout. For monkey S, where we recorded an average of 12 simultaneous units, we explained an average of 26% of the variance in distance reached to the wrong target (Figure 3.5 C). For Monkey K, where we recorded an average of 90 simultaneous units, we explained an average of 46% of the variance in distance reached to the wrong target (Figure 3.5 D). This confirms that a strong determinant of how much of the original reach is performed before correction is related to how close the monkey is to making the original reach before the switch command arrives. If monkeys are close to initiating the original reach when the target switches, then they will perform more of the originally-cued (incorrect) reach before correcting. If neural activity has not progressed as far, then less of the original (incorrect) reach will be performed. This suggests that the reach goal can be changed at seemingly arbitrary times with regards to the reach initiation process. Reach selection can be performed on top of reach generation.

We wanted to further explore the mechanism by which selecting a new reach can be
performed on top of an ongoing reach generation process. If the same motor areas are engaged in both reach selection and reach generation, how can these processes overlap without interfering with each other? One possibility is that different populations of neurons are involved in the process of motor preparation and motor generation. While previous studies have indicated that many neurons in PMd and M1 display both preparatory activity and movement generation activity (Tanji and Evarts, 1976; Mauritz and Wise, 1986; Lecas et al., 1986), we wanted to confirm that this was the case in our task. Using non-switching reach trials to each target, we calculated the trial-by-trial firing rate of each neuron during the delay period and during the movement epoch. We used a 1-way ANOVA to determine whether the firing rate distributions for reaches to different targets were significantly different (p<.01). If they were, then we said that that cell was tuned during that epoch. We found that >50% of cells we recorded were tuned during both the delay period and during the movement epoch (Figure 3.6). This indicates that the movement and preparatory processes are not primarily separated on a neuron-by-neuron basis.

Figure 3.6: Percentage of neurons tuned in only the delay period epoch, only the movement epoch, both epochs, or neither epoch, for all neurons recorded from Monkeys S and K. Total # of neurons in each category displayed above the bars.

If the same neurons perform both reach selection and reach generation, how can these processes overlap without interfering with each other? A recent paper may provide insight into this issue (Kaufman et al., 2014a). This paper suggested that
3.4. RESULTS

the neural dimensions (i.e., linear combinations of neural firing rates) involved in preparing a reach may be distinct from those dimensions which generate a motor output. This might suggest that re-programming a movement could be performed in parallel to executing a movement, as long as the neural dimensions governing these processes are orthogonal.

We therefore examined the neural dimensions that are explored following a target switch. We asked whether these dimensions tend to be similar to or different from the dimensions explored during movement. We used non-switch trials to find the average firing rate for each neuron during the movement epoch. We used switch trials to calculate the mean firing rate following the time of the target switch. We then ran Principal Components Analysis on only the movement-related data, to order neural dimensions by the % of movement variance explained. We projected the switch-related neural trajectories into this space and calculated what percent of variance was explained by each dimension for switch-related neural activity. (Figure 3.7 A).

We divided these movement-PCA dimensions in half. The first half of the dimensions, which explain a large percent of movement-related neural activity, we termed our "movement" dimensions. The second half we termed our "switch" dimensions. We found that a fair amount of switch-related neural activity occurred in dimensions which contain only a small percentage of movement epoch neural variance. (Figure 3.7 B-C). This suggests that a reasonable percentage of switch-related neural activity may occupy dimensions with relatively little impact on motor output.

While this gives us a global view of the neural dimensions explored in this task, we wanted to look closer at the time course of the neural switching process. We took advantage of the correctly-initiated switch trials for this analysis. For correct switch trials, we have a good idea of what the average neural trajectory ought to look like for both the initially-cued reach and for the reach the monkeys actually make. In each case, the neural trajectories ought to resemble the non-switch neural trajectories to that target. For incorrect switch trials, we don’t have as clear a view of what the neural activity is trying to do after a switch, as the movement itself is different from the non-switch reaches.

We first asked how long it takes for correct switch trials’ neural activity following a
Figure 3.7: (A) Dimensionality from an example dataset. Dimensions are selected using PCA on average movement-related neural activity across all reach targets. Blue line - cumulative % variance explained for movement-related activity. Red line - cumulative % variance explained for switch-related activity. (B) The % total variance in the “switching” dimensions, defined as the second half of movement-PCA-ordered dimensions. Dimensions which typically explain 40-50% of switch variance often explain very little movement variance, pointing out that the switch conditions are exploring dimensions not well-explored during movement. Red arrows show distribution means.
3.4. RESULTS

Firing Rate, Neuron 1
Firing Rate, Neuron 2
Firing Rate, Neuron 3

Figure 3.8: (A) Cartoon of full-dimensional distance metric. At each time point, we calculated the distance between an individual switch trial and the average neural trajectory for non-switch trials to the new (correct) target. (B-C) Euclidean distance from new trajectory as a function of time from the switch, for correctly-initiated trials (black trace - mean distance; gray bars - standard error) for two example switch pairs. Red dots show the distance at the time of movement for individual trials. The x value of the red dots indicates the time between the target switch and movement initiation. (D) Histogram of the time from the switch to full-dimensional neural convergence, for each switch pair. Red arrows indicate the mean convergence time across switch pairs for each monkey. (E) Cartoon of projection-vector distance metric. At each time point, we projected the neural state of an individual switch trial onto a vector connecting the trajectories to the new and old targets at that time. (F-G) Projection values as a function of time from the switch, for correctly-initiated trials. Switch pairs are the same as in (B,C). Red dots show the projection at the time of movement for individual trials. (H) Histogram of the time from target switch to neural convergence in the separating dimension. Red arrows indicate the mean convergence time across switch pairs for each monkey.
target switch to converge with the average non-switch trajectory for the new, correct movement. For each switch trial, we calculated the euclidean distance between that trial’s neural trajectory and the average non-switch trajectory to the new target (Figure 3.8 A). We normalized this distance to the distance expected if there were no switch, by subtracting off the average distance between individual non-switch trajectories and the mean non-switch trajectory.

We observed that after the target switch, the neural distance between switch and non-switch trajectories begins increasing (Figure 3.8 B-C). This suggests that the switch process is not simply a linear progression from one pattern of neural activity to another, and is likely related to the switch-related activity which occurs in orthogonal dimensions to movement-related activity. Eventually, the distance begins decreasing, converging to zero at an average of 233 ms (S) / 192 ms (K) after the target switch (Figure 3.8 D).

Counterintuitively, if we look at the distance between the trajectories at the time of movement onset, we see that for some of the correctly-initiated switch trials, the neural distance has not yet converged to zero (Figure 3.8 B-C, red dots). Even though the behavioral output is similar, the neural activity is actually quite different (more different, in fact, than the overall distance between non-switch trajectories to different targets, though I should quantify this). So how can such different neural activity yield such similar behaviors?

We reasoned that some neural dimensions may have a greater impact on behavioral output than others. Perhaps neural activity could be reasonably different in “muscle null” dimensions without a strong negative effect, whereas differences in muscle potent dimensions would need to be low in order to produce similar behaviors. Do the switch condition neural trajectories converge more quickly in dimensions that are relevant to the output of the arm?

We asked whether switch trial neural activity converges faster in dimensions that are relevant for distinguishing between different reaches. We reasoned that the most important dimension for determining motor output at any given time ought to be the dimension which separates the new and old non-switch trajectories. We therefore examined the progression of switch trials in this separating dimension (Figure 3.8
E). At each time point, aligned to movement onset, we generated a projection vector connecting the non-switch neural trajectories for reaches toward the old and new targets. We then projected switch trials onto this vector. A projection magnitude of zero indicates that the switch trial’s projection resembles the new trajectory. A larger projection value indicates that the switch trial is closer to the original trajectory.

After the switch, the projection magnitude begins decreasing, as opposed to the increase in distance that we observed in the full space (Figure 3.8 F-G). This decrease continues until the average magnitude has converged to zero, which takes an average of 136 ms (S) / 165 ms (K) (Figure 3.8 H). Interestingly, this convergence time is similar to the time in which we tend to see a majority of correctly-initiated trials behaviorally. We also see that most correctly-initiated trials are initiated after the projection magnitude has converged, whereas some correct trials are initiated when the total neural distance is still quite high.

If this projection dimension is important for determining the motor output of a reach, then it should also be the case that projection magnitude at movement onset should accurately predict whether the switch trials will be correct or incorrect. Conversely, because full-dimensional distance will include both distance in output null and output potent dimensions, we might expect that this metric would not perform as well. We built two classifiers to predict whether switch trials are correct or incorrect. In the first, we looked at the euclidean distance between the neural state of switch trials and the average neural state for non-switch reaches to the correct target at the time of movement onset. In the second, we looked at the magnitude of the projection of switch trials onto the vector separating non-switch neural trajectories for reaches to the correct and incorrect targets at the time of movement onset.

As we predicted, the projection vector performed better than the full neural distance at classifying correct vs incorrect switching trials (Figure 3.9 A-D). In 80% of switch pairs (S and K), the projection performed better than the full-dimensional distance, with an average difference in performance of 14% (S) and 8% (K) (Figure 3.9 C-D). We also performed an analysis to see whether the particular projection vector we chose performed better than chance. We selected 1000 random vectors, and asked how well they classified switch trials. In 62% (S) and 60% (K) of pairs, the chosen vector performed better than chance.
CHAPTER 3. LAST-MOMENT TARGET SWITCHING

Figure 3.9: (A-B) Classifier accuracy for each recorded switch pair using full-dimensional euclidean neural distance between the switch trials and the correct trajectory at the time of the reach, compared with classifier accuracy using just the magnitude of the projection onto the separating vector between the new and old trajectories, for monkey S(A) and K(B). Histograms show the range of performance for each metric separately. (C-D) Relative performance between the projection and the distance metric for Monkey S(C) and K(D). Projection performance was significantly better than distance performance for both monkeys. (E) Projection performance vs randomly-selected vectors for all switch pairs. Projection performed better than 95% of randomly-selected vectors in 62% (S) and 60% (K) of switch pairs.
3.5 Discussion

In this investigation, we probed the time course of motor preparation, and asked whether new movements can be prepared during the performance of a different movement. We trained monkeys to perform a task in which the cued target location occasionally switched locations at a random time after the go cue but before movement onset. By examining how close to the go cue correct movements to the new target were initiated, we determined that movements can be prepared relatively quickly, taking a minimum of 100 ms and a maximum of 200 ms. Interestingly, we also found that preparing a new movement did not seem to be dramatically different between cases where the arm is moving and cases where the arm is still. Rather, it seemed that there is a preparatory process which takes a certain length of time, and the movement of the arm is updated after this preparatory step is complete. If a new reach is fully prepared before the arm starts moving, then the monkey will start its reach in the correct direction. On the other hand, if the monkey starts reaching before the new reach has been fully prepared, then the reach will start out toward the original target. Once the preparatory process is complete, the monkey will begin correcting its reach online, changing direction to reach toward the new target.

This raises an obvious question: if the same neurons are involved in both reach planning and reach generation, how can reaches be re-planned without immediately affecting motor output? To answer this question, we took inspiration from the results of a recent study (Kaufman et al., 2014a). This study suggests that certain patterns of neural activity may signal to downstream areas. Patterns of neural activity which are orthogonal to these output dimensions might be reserved for internal computations which are not directly output to downstream brain areas (or muscles). In line with this perspective, we found that a significant portion of the neural response to the switch occurred in dimensions which were not well-explored during the course of normal reaching. Furthermore, we found that distance in putatively output-potent
dimensions did a better job of predicting whether reaches would be initiated toward the correct or incorrect target than total neural distance.

3.5.1 The evolving definition of motor preparation

While the term motor preparation has been used extensively in motor literature, our understanding of the nature of this seemingly simple process has developed considerably since the first studies described the phenomenon. Motor preparation can perhaps best be operationally defined as neural activity in motor areas of the brain which does not itself generate movement, but which somehow sets up or optimizes these brain areas’ activity in anticipation of future movements. One recent hypothesis, the initial condition hypothesis, has posited that preparatory activity serves as the initial condition for a dynamical system which generates a reach; in the strongest formulation of this hypothesis, the movement that is generated is a function of the preparatory activity - if the preparatory activity changes, the reach itself will also be affected.

While our earlier work has shown that achieving the exact preparatory state that we usually observe during the delay period is not a necessary condition to generate a reach, there remain very early responses to visual cues in motor cortex which might be sufficient to serve as an initial condition even for non-delayed reaching conditions.

While this view of preparation and movement as a two-step process does a good job of explaining simple reaching behavior, it does not necessarily generalize well to the behavior that we observe here. If the reach is determined by the preparatory state, then what happens if you need to change your reach goal online? In order to enable this sort of online correction behavior, it seems to be necessary to allow motor preparation to happen not just in anticipation of a future movement, but also simultaneously with an existing movement. When we consider that reach preparation and reach execution are not well-separated at the neuron level in PMd and M1, accomplishing simultaneous preparation and execution becomes a complicated problem. The solution that we suggest here, where the neural dimensions which are used to prepare for a reach are orthogonal to dimensions of neural activity which execute a reach different, may serve to illustrate a more general principle of computation in the brain as a
whole.

### 3.5.2 Target switching: generalized or specific?

Many studies of target-jump paradigms have either explicitly or implicitly attempted to relate neural switch responses to neural responses to the appearance of a target without a switch. Some have tried to show that a similar sub-population of cells respond to the appearance of a particular target regardless of reach condition. While this study concentrates on identifying particular multi-neuron firing rate patterns rather than specific sub-populations, we nonetheless also make the claim that the neural response to a target switch shares something in common with neural responses to target onset more generally. However, we see some evidence in this study that, while this similarity may be true to a first approximation, the full story may well be more complicated. The target switch response explores some dimensions which are not strongly occupied either during the delay period or during movement. In other words, there seem to be significant ways in which the neural response to a target switch is unique. This offers some potentially interesting insights into motor cortical activity, namely, that its full capabilities are not fully described by a simple delayed reaching task. Continuing to probe the system under new and varied behavioral paradigms will be critical to test the full computational range of this system.

### 3.5.3 Decision making

Decision making, whether it is perceptual decisions about the nature of observed stimuli or value-based decisions about internal preferences or desires, is intimately tied with motor action. After all, the final result of a decision is often to take action: electing to reach for an apple or a candy bar, deciding to throw a dodgeball or wait for a better opportunity, using your right or left hand to pick up a mug of coffee. Many brain areas which have been implicated in decision making have also been shown to play a role in generating movements. These areas include the Frontal Eye Field, Superior Colliculus, and even PMd. Our results could suggest a mechanism for how motor areas such as PMd could enable changes of mind when evidence for one
CHAPTER 3. LAST-MOMENT TARGET SWITCHING

choice (in our case, a particular reach target) gets outweighed by new evidence for a different choice (such as a new target). The evidence that we explore is as non-ambiguous as we could make it, as only one target is illuminated at a time. However, future investigation could involve providing partial evidence for several choices and observing the degree to which PMd activity during a reach reflects evidence for a possible (but uncertain) change of mind. During a delay period, preparatory activity in PMd reflects the presence of multiple targets in a way that seems to correlate with the probability of selecting a given reach. It therefore seems possible that this would continue to be the case even during the execution of a reach. In this manner, the motor system could remain maximally prepared to update based on a change of mind.

3.5.4 Other brain areas

While this study has focused on the neural activity in M1 and PMd, the process of motor generation is a distributed over a many brain areas (Dum and Strick, 2002, 1991). In particular, the task of identifying and selecting a particular reaching goal is unlikely to be entirely restricted to PMd and M1. One area which has been implicated in the process of visuomotor transformation is the posterior parietal cortex (PPC). In humans performing a target switching task, temporary disruption of parietal lobe activity via Transcranial Magnetic Stimulation (TMS) interfered with subjects' online corrections of their initial reach (Desmurget et al., 1999). Portions of the parietal cortex have also been implicated in visual decision-making tasks, usually in the context of selecting a saccade target but also occasionally during reaching.
Chapter 4

Generalization of work and future directions

4.1 Somatosensory vs. visual perturbations

In this work, we concentrated principally on visual perturbations of the target location. There remain many other forms of potential perturbations which must be taken into account before we can have a truly complete view of motor function. One primary example of this is somatosensory and proprioceptive perturbations. These often take the form of a force deflecting the arm off its path, and are highly relevant stimuli that must be accounted for in a robust reaching system.

There are multiple time courses of the motor response to a sudden force applied to the arm. The fastest is a short-latency stretch reflex (20-40 ms) which is thought to be mediated by a monosynaptic reflex pathway in the spinal cord. Later, we observe a long-latency stretch reflex (45-75 ms), which is thought to be cortically mediated (Palmer and Ashby, 1992) and is modified by task constraints (Lewis et al., 2006; Shemmell et al., 2009). For example, if a subject is preparing to reach to a rightward target, they will exert more force resisting a leftward perturbation (away from the target) than a rightward perturbation (toward the target).

Subjects can respond to somatosensory perturbations not only by updating their reach path to the original target, but also by selecting a new target as a result of the
perturbation (Pruszynski et al., 2014). For example, if subjects have a choice of two possible targets, one straight ahead and one off to the side, they will usually select the center target as their initial reach goal. However, if their arm is pushed to the side while reaching, such that the side target is now closer to their arm, they will often change their goal mid-reach and reach instead to the (now easier) side target (Figure 4.1) (Pruszynski et al., 2014). Recent studies of this process have indicated that changing the reach goal actually takes slightly longer than simply updating the reach path to the original goal (Nashed et al., 2014). This may indicate that these processes have different neural correlates. More work on the intersection of somatosensory perturbations and target selection is needed to fully pull apart the manner in which these two processes interact.

![Figure 4.1](image)

**Figure 4.1: Cartoon of possible responses to a force applied to the arm during reaching.**

Black line, unperturbed reach to the center target. Green line: a reach that responds to a deflecting force by updating the reaching path to the original target. Red line: a reach that responds to a deflecting force by selecting a new, more accessible reach target.

### 4.2 The trigger signal

In this work, we hypothesized that one primary input signal for setting motor cortical dynamics is a trigger signal. This signal gives the final command of when to begin
executing a reach. One open question about the nature of this signal is that of monitoring. Does the brain area which sends the trigger signal actively monitor the state of motor cortical activity? We know that reaction times are higher for unprepared or incorrectly prepared reaches (Chapter 2). One possible explanation for this is that these conditions’ neural state is somehow less efficient for initiating a movement: the trigger signal is always sent at approximately the same time, and the RT differences are primarily due to the differing states of the motor cortex itself. Another explanation could be that there is some brain area monitoring the state of motor cortex, and this area delays the trigger signal until the preparatory state has been sufficiently corrected.

Our behavioral results indicate that reaction times in reaching tasks are often slower than they strictly need to be. When the target switches during the reaction time epoch, we observe correct reaching behavior in some reaches in as little as 100 ms after the switch. For reaches initiated more than 200 ms after the switch, the reach is almost always initiated correctly (Figure 3.3 C). Yet, if the switch comes at the same time as the go cue, reaction times tend to be much higher than 200 ms. During trials with a simultaneous switch and go cue, RTs averaged over 250 ms in both monkeys we recorded from (Figure 2.5 H-J). This suggests that monkeys are actually delaying the onset of their reach beyond what is strictly necessary to correct their neural activity. This delay could be a result of the triggering area monitoring motor preparatory activity and waiting until this activity has been sufficiently corrected before sending the command to move. The delay could also be a result of the triggering area receiving information that a switch has happened, and simply waiting an amount of time which it knows to be sufficient before sending the command to move.

Further work also remains to characterize the extent and nature of the trigger signal. While we concentrated on ballistic reaches in this study, there are many movements which have a more fluid and ongoing nature (often oscillatory), such as walking or riding a bike. During walking, must each leg movement be triggered, or is there a single trigger signal to begin walking? Must the end of this movement also be triggered? An additional behavior that could have multiple potential relationships to a
triggering signal is coordinated arm movements. If one is performing a task that involves bilateral coordination, such as trying to catch a ball with both hands, is there a single trigger signal which is sent to both hemispheres simultaneously, or is each motor effector controlled separately? Expanding our repertoire of behaviors in which we study movement will likely yield a great deal of insight as to the exact nature of command mechanisms of the motor system.

4.3 Single Trial Analyses

In Chapter 3, we performed several analyses which attempted to use simultaneously recorded neural data to allow for single-trial analyses. As the field continues to push forward on techniques to record activity from many neurons simultaneously, more work needs to be done to figure out how best to leverage this data. One well-documented issue is that of noise (Yu et al., 2009). Neural spiking can be approximated by a poisson process, where the spiking variability increases as the firing rate increases. This effect can throw off some forms of dimensionality reduction, such as Principal Components Analysis, which will tend to be biased toward over-representing more variable (and therefore higher firing rate) neurons. This can be mitigated by performing dimensionality reduction on trial averaged data, as we did here, but this limits our ability to characterize single-trial data with these techniques. A better strategy for dimensionality reduction on single-trial data is to use a method that allows neurons to have both shared and private noise, such as Factor Analysis.

Another challenge for single-trial data analysis is that of temporal smoothing. Techniques that have been developed in order to analyze single trial data, such as Gaussian Process Factor Analysis (Yu et al., 2009), often have a large temporal smoothing component. This smoothing, especially when performed by an acausal filter (which incorporates both past and future action potentials into its estimation of firing rate), can interfere with the ability to precisely determine the time course of a neural activity. Depending upon the question being asked, this may be more or less important. For example, in our task we were very careful to use causal filters (which only take into account past spikes to estimate FR) when we estimated at
what time neural activity betan responding to the target cue and the go cue. Some
brain areas may also care more about precise spike timing than others. While firing
rates have often been considered sufficient to explain neural activity in cortical areas,
many other areas have displayed spiking behavior which follows more precise timing
patterns. For example, the phenomenon of phase procession in the hippocampus de-
scribes neurons that tend to fire at particular phases relative to the theta rhythm
of the local field potential (LFP) (O'Keefe and Recce, 1993; Skaggs et al., 1996). A
simple firing rate analysis in areas where spike timing is important may miss criti-
cal information. Thus, the technique used for analyzing single trial data will likely
need to be strategically chosen based on the brain area, the behavioral task, and the
particular research questions being investigated.

4.4 The Importance of Timing

When studying neural responses to external signals, it is important to consider the
timing of the neural response, even when considering neural firing rates (as opposed to
precise spike timing). We found that examining the relative timing of motor cortical
responses to the target cue versus the go cue provided a critical clue for how to
reconcile our results with broader results in the motor field. Especially for areas which
are not primarily sensory areas, the timing of neural responses may vary both with
the type of sensory input and also with the information content of the signal itself. For
example, we found that motor cortical responses to visual cues had a shorter latency
when those cues were target-related, and a longer latency when the cue indicated it
was time to initiate a movement. Previous work has suggested that motor cortical
responses to somatosensory cues occur at a lower latency than visual cues. Measuring
the timing of neural responses to external cues is likely to be important for gaining a
thorough understanding of how different brain areas make sense of information from
the outside world and how they transform that information into action.
Appendix A

Publications

A.1 Journal Articles


   This work appears as Chapter 2. I collected the data, designed and performed all analyses with KVS, and wrote the manuscript with KVS.

A.2 Refereed conference articles and abstracts


   This work became Ames et al. (2014).


   This work became Ames et al. (2014).

This work became Ames et al. (2014).


Part of this work is contained in Chapter 3


Part of this work is contained in Chapter 3
References


REFERENCES


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