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Effects Of Resting State On Perceptual Learning

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EFFECTS OF RESTING STATE ON PERCEPTUAL LEARNING

by

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EFFECTS OF RESTING STATE ON VISUAL CORTICAL NETWORKS DURING PERCEPTUAL LEARNING

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DISSERTATION

Presented to the Faculty of
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in Partial Fulfillment
of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

by

Sarah Eagleman
Houston, Texas

May, 2014

DEDICATION

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This work is dedicated to my brother Stephen Alwin (1986-2010)

who lives on in reactivations of networks in my brain.

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ABSTRACT

EFFECTS OF RESTING STATE ON VISUAL CORTICAL NETWORKS DURING PERCEPTUAL LEARNING

Sarah Eagleman B.A.*

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Psychophysical experiments in humans have demonstrated that improvements in perceptual learning tasks occur following daytime rests. The neural correlates of how rest influences subsequent sensory processing during these tasks remain unclear. One possible neural mechanism that may underlie this behavioral improvement is reactivation. Previously evoked network activity reoccurs – reactivates - in the absence of further stimulation. Reactivation was initially discovered in the hippocampus but has now been found in several brain areas including cortex. This phenomenon has been implicated as a general mechanism by which neural networks learn and store sensory information. However, whether reactivation occurs in areas relevant for perceptual learning is unknown.

To investigate how sleep affects perceptual learning at the level of single neurons and networks, an experimental paradigm was designed to simultaneously perform extracellular recordings in visual cortical area V4 along with sleep classification in monkeys. V4 is a midlevel visual area that responds to shapes, textures, and colors. Additionally, V4 is important for perceptual learning and shows significant attentional effects. In this experiment, two monkeys were trained to perform a delayed match-to-sample task before and after a 20 minute rest in a dark, quiet room. Whether monkeys

exhibit the same improvements in perceptual learning previously shown in humans is unknown. Here, monkeys did improve task performance following the 20 minute rest.

Additionally, whether neural networks in V4 could reactivate was explored in a passive fixation task. A reactivation of previously evoked sequential activity was observed in V4 networks following stimulus exposure in the absence of visual stimulation. This reactivation was time-locked to when the stimulus was expected to occur *after a cue*, which indicated to monkeys the trial was starting. Finally, whether the delayed match-to-sample task-evoked activity was spontaneously reactivated during the 20 minute rest period was tested. No evidence to suggest that reactivation occurs during this time was observed. Considering previous reactivation results, this suggests the cue is necessary to initiate the reactivation. In summary, this work represents an investigation of the neural correlates that underlie behavioral performance improvements following daytime rest. Results can provide a better understanding of how daytime naps improve perceptual learning.

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1. GENERAL INTRODUCTION

The brain "offline"

During the past 70 years, the visual system has proven to be a useful model to study the properties of how the external world is coded and processed in individual neurons and circuits. The majority of visual systems neuroscience has been dedicated to understanding what is happening in the brain when it is actively engaged with its sensory environment. However, the brain is not always engaged in actively processing the external environment and during periods in which it is not engaged, it is not silent. In fact, while the brain is resting it uses 20% of the body's resting metabolism when it only accounts for 2% of the body's weight (Attwell and Laughlin, 2001). Additionally, sleep can be found across all animal classes, including invertebrates, fish, amphibians, reptiles, birds and mammals (Campbell and Tobler, 1984). Sleep duration and complexity varies across these species based on their environmental situations. That is, some animals like dolphins sleep one hemisphere at a time so they can keep swimming and avoid predation, whereas, bears hibernate for months during periods when food is scarce (Lyamin et al., 2004; Shpak et al.; Siegel, 2009). It also appears that predators, like lions, sleep more deeply than their prey, like giraffes (Siegel, 2005, 2009). In these cases, sleep may serve to protect the animal from environmental conditions; however, studies in rodents, birds and humans show that sleep may also enhance our learning and memory (e.g. Born et al., 2006; Diekelmann and Born, 2010; Stickgold, 2005; Stickgold and Walker, 2007). What is the purpose of the activity during periods of time in which the brain is not engaged in the external environment and how does it impact subsequent sensory processing?

One hypothesis suggests that rest "resets" the brain, so that it is able to learn new information during its next period of sensory engagement (Tononi and Cirelli, 2003a, 2006).

This framework begins with the observation that synapses between cells are strengthened as neurons are activated together during sensory processing in the awake state (Cooke and Bliss, 2006; Hughes et al., 1956). This strengthening of synapses is suggested as a mechanism by which the brain learns and stores new information (Hebb, 1949). Because of a net strengthening of synapses, circuits may reach an asymptotic level of synaptic strength during wakefulness (Tononi and Cirelli, 2003b, 2006). During sleep, synapses are weakened, "reset" so the brain is primed to learn new information. Another way in which the brain may "reset" during sleep is by clearing out potential neurotoxins that can effect neural activity and cognition (Xie et al., 2013). Toxins, such as proteins linked to neurodegenerative diseases, decrease during sleep compared to the awake state (Bateman et al., 2006; Kang and Maunsell, 2012; Xie et al., 2013). Recent research has suggested that this decrease is due to the increase in interstitial space causing CSF to flush out these toxins (Xie et al., 2013). Psychophysical studies in humans demonstrate limits in behavioral performance with extended periods of wakefulness. For example, improvement in a visual discrimination task declines when stimuli are presented in the same visual location in multiple testing sessions over the course of a day (Mednick et al., 2002). Testing participants at a new visual location (which utilizes a new region of cortical tissue), or taking a nap recovers the performance (Mednick et al., 2002).

A second, non-exclusive hypothesis about the role of sleep proposes that sleep allows the consolidation of recent sensory experiences (Diekelmann and Born, 2010; Stickgold and Walker, 2007, 2013; Walker and Stickgold, 2004). This framework suggests that memories are redistributed and reorganized with sleep from the hippocampus to diffuse cortical areas (Born and Wilhelm, 2012; Buzsáki, 1998; Stickgold and Walker, 2007; Walker and

Stickgold, 2004). Functional magnetic resonance imaging studies in humans support this claim during associative encoding tasks: an increase in correlated activity between the hippocampus and lateral occipital (LO) complex (areas active during the task) was found during rest succeeding the task compared to rest preceding the task (Tambini et al., 2010). Further, the stronger the correlated activity between the hippocampus and LO during rest the better the subsequent memory performance (Tambini et al., 2010). Though both hypotheses about the purpose of activity during rest are under active investigation, details of the mechanisms at the individual neuron and network level remain unanswered.

Reactivation during resting states

In hippocampal networks, previously evoked activity reoccurs during sleep and quiescent awake periods (Diba and Buzsáki, 2007; Foster and Wilson, 2006; Gupta et al., 2010; Louie and Wilson, 2001; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994). For example, hippocampal cells that are active at the same time during a task exhibit stronger correlated activity after the task than before (Wilson and McNaughton, 1994). Further, a population of neurons activated in a sequence during a task show the same pattern of sequential firing during subsequent sleep (Skaggs and McNaughton, 1996). A reactivation composed of sequential firing of a previous experience is also observed during quiescent, awake periods (Diba and Buzsáki, 2007; Louie and Wilson, 2001), sometimes in the reverse direction from experience (Diba and Buzsáki, 2007; Foster and Wilson, 2006). Further investigations have revealed that this phenomenon happens in several other cortical networks (Hoffman and McNaughton, 2002a), including early visual cortex in anesthetized (Han et al., 2008; Xu et al., 2012; Yao et al., 2007), awake (Xu et al., 2012), and sleep states (Ji and Wilson, 2007). Studies analyzing brain activity in humans using functional magnetic

resonance imaging (fMRI) demonstrate that brain sensory cortical areas activated during visual stimulation are also active during mental imagery (Gandhi, 2001; O'Craven and Kanwisher, 2000; Slotnick et al., 2005; Wheeler et al., 2000). Thus, such reactivation of previously experienced activity appears to be a general property of neural networks and a candidate mechanism by which neural networks learn and store information.

Improvements in behavior and neural coding following rest

In addition to rehearsal of previously experienced stimuli, the brain is likely undergoing other processes during rest periods that optimize future neural coding. This is suspected because humans show improvement in learning and memory following daytime naps (Mednick et al., 2003, 2002; Tietzel and Lack, 2001, 2002; Tucker and Fishbein, 2008; Tucker et al., 2006). Even a nap as brief as 6-minutes can improve performance in list memory (Lahl et al., 2008). Improvements in visual discrimination tasks are observed following brief 60-90 minute afternoon naps (Mednick et al., 2003). In "nap" studies, multiple recordings including electroencephalograms (EEGs), electrooculograms (EOGs) and electromyograms (EMGs)—collectively called polysomnography—are employed to determine sleep stages. Because I did not employ polysomnography at all stages of this thesis work, I will not use the term "nap"; instead, I will use the more general term "rest" for periods in which there is a dark, quiet room, with no requirements of sensory engagement. During rest, behaviors indicative of sleep onset, such as extended eye closure and muscle atonia, are commonly observed. As discussed above, humans show learning improvements when they are allowed to rest (Tambini et al., 2010). Because humans improve behavioral performance after rest, neural correlates underlying those behavioral changes must exist.

What are the possible neural mechanisms that underlie this improvement? As stated earlier, the visual system is a useful model to study how sensory information is processed and utilized by the brain to affect behavior. Because humans show improvement in visual discrimination tasks after rest (Mednick et al., 2003, 2002), I can use the visual system to explore the neural correlates of this improvement. Potential changes that could occur include improved coding of sensory stimuli (in which cells are more sensitive and respond more discriminately to features of behaviorally relevant stimuli), improved response reliability, and improved synchronization with downstream targets. The ability to simultaneously record extracellular activity from multiple neurons allows several analyses of these properties in individual neurons and networks. Such analyses and their use to address the influence of rest on neural coding will be discussed in Chapter 3.

What has been missing to determine these neural correlates is an experimental paradigm that incorporates *in vivo* recordings and polysomnography in an animal model capable of exhibiting the complexity of behavior found in humans. Monkeys (*Macaca mulatta*, also known as rhesus monkeys*)* are capable of performing visual discrimination tasks equivalent to humans and enable us to perform the recordings necessary to answer questions about rest at the level of individual neurons and networks. In Chapter 3, I discuss the design and development of such an experimental paradigm using extracellular recordings in V4. The integrity of V4 is important for perceptual learning (Merigan and Pham, 1998; Schiller, 1994, 2013), and the activity of this area is modulated by higher cognitive processes such as attention (Connor et al., 1997; Desimone, 1998; Roe et al., 2012; Taylor et al., 2005; Williford and Maunsell, 2006). These properties, along with others discussed in the next section, make V4 a promising location to study the impact of rest on neural coding.

Visual cortical area V4

In the rhesus monkey, gross anatomical visual identification of V4 boarders are the lunate sulcus in the posterior portion and anteriorly by the superior temporal sulcus (Roe et al., 2012). However, specific functional, anatomical studies have shown that V4 is bordered posteriorly by V3 and anteriorly by V4A (Roe et al., 2012). Much debate surrounds whether V4 should be considered one area or a collection of smaller subareas (Roe et al., 2012; Stepniewska et al., 2005). A human homolog of V4 exists, but the exact boundaries are debated (Gallant et al., 2000; Hansen et al., 2007; Rizzo et al., 1992). An exploration of the connectivity patterns of V4 using tracer injections has revealed that V4 receives feedforward connections from early visual areas such as V1, V2 and V3, and projects information along the temporal and parietal lobes (Felleman and Van Essen, 1991; Ungerleider et al., 2008). The functional properties of V4 were first characterized in the 1970s, at which time it was proposed that V4 is an area responsible for color processing (Zeki, 1973). Further investigations showed that this area is much more complicated and heterogeneous—for example, V4 responds to color as well as complex contours, shapes, and patterns (Bouvier et al., 2008; Cadieu et al., 2007; Carlson et al., 2011; Desimone and Schein, 1987; Heywood and Cowey, 1987; Pasupathy and Connor, 2002; Roe et al., 2012; Schiller, 1994; Schiller and Lee, 1991). The connectivity of V4 and its anatomical location suggest it is involved in the construction of object and scene identification from the amalgamation of rudimentary features such as orientation, spatial frequency and contrast .

Another property of V4 is that it is the first area in the visual processing hierarchy to show strong attentional effects (Connor et al., 1997; Desimone, 1998; Fries et al., 2008; Moran and Desimone, 1985; Williford and Maunsell, 2006; Zhou and Desimone, 2011). The

input from lower visual areas as well as its sensitivity to attention suggest that V4 is positioned at a nexus between bottom-up and top-down influences. It is little surprise that lesions of V4 in monkeys and a homologous region in humans have revealed that this area is important for perceptual learning, specifically the detection and discrimination of visual features (Gallant et al., 2000; Rizzo et al., 1992; Schiller, 1994; Schiller and Lee, 1991). It has been recently suggested that the unifying purpose of this region is "context feature selection", meaning that given the complexity of the responses in V4, this area has the capacity to select relevant visual features of the environment for subsequent processing (Roe et al., 2012). In this thesis work, I begin with the hypothesis that V4 will exhibit hallmarks of rest-induced changes in neural coding, as it has important roles in attention and learning.

In this thesis, I describe the development of an experimental paradigm to study the behavior and neural coding before and after rest, during a task. Further, this paradigm can be used to study the neural properties of the rest period that affect subsequent coding and behavior. In Chapter 2, I test the hypothesis that previously evoked activity reoccurs during brief awake states when no stimuli are presented. Throughout this thesis I will refer to the reoccurrence of previously evoked activity as *reactivation*, which is synonymous with the term replay. I will use the term *rehearsal* to imply reactivation as a mechanism by which the brain learns and stores information. Chapter 3 contains a description of the resting state experimental paradigm that I have designed and developed to concurrently perform non-invasive polysomnography, recordings used for sleep staging, along with extracellular recordings in macaques. Using this paradigm, I tested the hypothesis that a 20-minute daytime rest improves behavioral performance. In Chapter 3, I also describe several analyses that may reveal how rest improves neural coding. Here, rest is defined as a period of time

when the brain is not actively engaged in sensory processing. Rest can be understood as a period of quiescent wakefulness. Though there is no polysomnography to determine sleep onset during rest, I observe extended eye closures and muscle atonia (specifically, a slack jaw). In Chapter 4, I discuss a subset of analyses using the data I collected in Chapter 3. I return to my investigation of reactivation in V4, testing the hypothesis that activity during a visual delayed match-to-sample task will reoccur during a 20 minute period of rest in a dark room.

"Daughter: Daddy, what is an instinct?
Father: An instinct, my dear, is an explanatory principle.
D: But what does it explain?
F: Anything—almost anything at all. Anything you want it to explain.
D: Don't be silly. It doesn't explain gravity.
F: No, but that is because nobody wants instinct to explain gravity. If they did, it would explain it. We could simply say that the moon has an instinct whose strength varies inversely as the square of the distance …
D: But that's nonsense, Daddy.
F: Yes, surely. But it was you who mentioned instinct, not I.
D: All right—but then what does explain gravity?
F: Nothing, my dear, because gravity is an explanatory principle. …
D: Daddy, is an explanatory principle the same thing as an hypothesis?
F: Nearly, but not quite. You see, a hypothesis tries to explain some particular something but an explanatory principle—like gravity or instinct—really explains nothing. It's a sort of conventional agreement between scientists to stop trying to explain things at a certain point."

– Gregory Bateson, Steps to an Ecology of Mind (1972, pp 38, 39).

2. IMAGE SEQUENCE REACTIVATION IN AWAKE V4 NETWORKS

This chapter is based upon Eagleman, S. L., & Dragoi, V. (2012). Image sequence reactivation in awake V4 networks. Proceedings of the National Academy of Sciences of the United States of America, 109(47), 19450–5.

Introduction

In natural environments the visual system is often exposed to successive, random image patches that are briefly inspected during periods of fixation. While the temporal coding of image sequences has been investigated during active vision by examining responses to sensory stimulation (Dragoi et al., 2000; Gutnisky and Dragoi, 2008; Hansen and Dragoi, 2011; Herikstad et al., 2011; Vinje and Gallant, 2000; Wang et al., 2011), as was discussed previously, whether and how cortical neurons and networks encode temporal image sequences in the absence of sensory stimulation is largely unknown. Here, I examined the possibility that during brief periods of quiescence stimulus-evoked responses could be 'rehearsed', or reactivated, by V4 cortical networks previously activated during stimulus presentation.

To reiterate, reactivation, also known as reverberation, of stimulus-induced neuronal activity is the phenomenon by which neurons in selected brain regions exhibit specific spiking patterns during periods of sleep and quiescent awake states resembling previously evoked responses. For instance, hippocampal cells firing together during a task period have been shown to exhibit increased correlations during subsequent sleep (Wilson and

McNaughton, 1994) compared to the period preceding the task. Subsequent studies have not only supported the fact that task-coactivated hippocampal neurons are reactivated together during post-task slow-wave (O'Neill et al., 2008) and REM (Louie and Wilson, 2001) sleep, but have also shown that the temporal firing pattern of responses reoccurs in the same order as during the task (Lee and Wilson, 2002; Skaggs and McNaughton, 1996). Although reactivation has been originally reported in the hippocampus as a mechanism of memory consolidation (Buzsáki, 1998; Marr, 1971; McClelland et al., 1995), it may constitute a fundamental property of neural ensembles in many brain areas. Indeed, in addition to hippocampus, reactivation has been reported in rat prefrontal cortex (Euston et al., 2007; Johnson et al., 2010; Peyrache et al., 2009), in motor and somatosensory cortex during quiescent awake states (Hoffman and McNaughton, 2002b), in rat primary visual cortex (V1) during slow-wave sleep (Ji and Wilson, 2007), and in rat and cat V1 immediately after stimulus presentation during anesthesia (Han et al., 2008; Xu et al., 2012; Yao et al., 2007).

An important issue is whether the reactivation of previously evoked neuronal activity can be demonstrated in the awake state, not only during sleep or anesthesia. Indeed, sleep or anesthetized states are characterized by high synchronous activity due to widespread oscillations in the same frequency band and a global decrease in brain activity (Destexhe, 2009). On the other hand, awake reactivation has been recently demonstrated during quiescent periods in hippocampal cells (Carr et al., 2011; Davidson et al., 2009; Foster and Wilson, 2006; Gupta et al., 2010; Karlsson and Frank, 2009), and has been shown to be influenced by the animal's current location (Davidson et al., 2009; Diba and Buzsáki, 2007; Foster and Wilson, 2006; Karlsson and Frank, 2009), to occur with elevated precision in novel environments (Diba and Buzsáki, 2007; Foster and Wilson, 2006), and to represent

pathways not previously experienced by the animal (Gupta et al., 2010). Furthermore, a more recent study (Xu et al., 2012) found reactivation in awake rat visual cortical cells in response to a moving dot stimulus swept across a linear path of adjacent receptive fields following a conditioning period. Nonetheless, the issue of whether neuronal populations can exhibit experience-dependent reactivation of evoked activity remains unclear. Specifically, reactivation of neuronal responses has been exclusively demonstrated when cells are activated sequentially in a temporal sequence. While sequential firing may be representative of neuronal firing in areas such as the hippocampus, where place cells fire in a specific temporal order as the animal explores the environment, sequential firing is less common in sensory cortex where neuronal responses represent incoming stimuli as a complex temporal spiking pattern. For instance, in visual cortex, neurons with non-overlapping receptive fields respond sparsely to successive fixation patches during natural viewing such that spikes from multiple neurons often occur coincidentally or at different times during the same viewing episode (Gutnisky and Dragoi, 2008; Vinje and Gallant, 2000). Whether neuronal networks can exhibit reactivation of complex, random patterns, such as those encountered in natural viewing conditions, is unknown.

Here, I examined the capacity of neuronal populations to exhibit reactivation in visual cortical area V4, where neurons respond to complex image features (Desimone and Schein, 1987; Gallant et al., 2000; Hegdé and Van Essen, 2005; Sheinberg and Logothetis, 2001) and play a key role in perceptual learning (Schiller, 1994; Schiller and Lee, 1991). Response reactivation was investigated by using a random presentation of image patches reminiscent of stimuli encountered during successive fixation episodes during natural viewing. I describe a novel form of rapid cortical reactivation at the network level induced in the awake state

precisely at the time when a stimulus is expected to occur. Specifically, I found that repeated, brief stimulation with random image sequences causes a significant 'memory trace' in a subsequent blank fixation trial and an increased similarity between the stimulus-evoked response and the network ongoing spiking pattern.

Methods

Behavioral Paradigm

All experiments were performed in accordance with NIH Guidelines for the Care and Use of Animals for Experimental Procedures and the Animal Welfare Committee at the University of Texas Health Science Center at Houston. Two male rhesus monkeys (Macaca mulatta) were trained to fixate on a centrally located fixation point (0.4 deg in size) within a 2 deg fixation window. To ensure fixation, eye position was continuously monitored using an eye tracker system operating at 1 kHz (EyeLink II, SR Research Ltd.).

Visual Stimuli

Stimulus trials consisted of 2 x 2 deg image patches randomly presented in a spatiotemporal sequence. The image patches were clipped from a larger image (10 x 10 deg) that covered the multiple receptive fields recorded within a session (I ensured that each receptive field was stimulated at least once during sequence presentation). A total of 25 image patches were presented for 120 ms each for a total of 3 s. The same sequence of image patches was presented throughout a given session. To determine whether response reactivation is stimulus specific in a subset of the sessions (13 out of 19) I added another

block of trials in which the same image patches were displayed in a new temporal order. Each block of trials contained pre-stimulus, stimulus, blank and post-stimulus conditions.

Electrophysiological Recordings

I used two recording techniques for multiple single-unit extracellular recordings in visual cortex area V4. First, in 8 of the sessions I used a custom Crist grid recording technique (Dragoi et al., 2002). Microelectrodes (tungsten, 1-2MΩ at 1kHz, FHC Inc.) were advanced transdurally through stainless steel guide tubes into V4. I recorded up to 10 units simultaneously with up to 12 electrodes in each session at depths between 200 and 400 μm. Recording sites were located between 1 mm and 2 mm of each other. Second, in 11 of the sessions I used laminar electrodes (U-probe, Plexon Inc) consisting of a linear array of 16 equally spaced contacts (100 μm inter-contact spacing). Each electrode contact was 25 μm in diameter and was coated with platinum iridium. The impedance at each contact is 0.3–0.5 MΩ. I recorded up to 19 units simultaneously in each session. Laminar electrodes were used either along with single contact electrodes or with multiple laminar electrodes. Real-time neuronal signals recorded from both electrode types (simultaneous 40 kHz A/D conversion on each channel) were analyzed using the Multichannel Acquisition Processor system (MAP system, Plexon Inc). Single-unit recordings were amplified, filtered, and viewed on an oscilloscope and heard through a speaker. Individual neurons were isolated through spike waveform sorting using Plexon's offline sorter program. Recording sites were selected on the basis of the quality of the signal (signal-to-noise ratio) and responsiveness to visual stimuli.

Receptive field mapping

Single units were identified at the beginning of each recording session and receptive fields mapped for all cells using reverse correlation stimuli. The range of receptive field sizes was 2–4 deg. Figure 2.1 shows an example of receptive field mappings from four channels recorded simultaneously in one session.

Neuronal Reactivation Analysis

All analyses used z-scored response-time matrices, using 10 ms time bins. I assessed reactivation both in neuronal populations and individual cells. I calculated the two-dimensional Pearson correlation coefficient between two matrices containing the averaged z-scored firing rates of all the cells in the recorded population as a function of time (in different stimulus conditions). The Pearson correlation coefficient between two response-time matrices is defined by:

$$CC = \frac{\sum_m \sum_n (A_{mn} - \bar{A})(B_{mn} - \bar{B})}{\sqrt{(\sum_m \sum_n (A_{mn} - \bar{A})^2 (\sum_m \sum_n (B_{mn} - \bar{B})^2}} \quad (1)$$

where *A* and *B* are matrices of z-scored firing rates across the population, $\bar{A}$ and $\bar{B}$ are the mean z-scored firing rates of each matrix, and *m* and *n* represent locations within the matrix (A_{mn}represents a value in matrix *A* in row *m* and column *n*). The correlation measures the degree of association between observed values. Correlation values range between -1 and +1. Negative values indicate that the firing rates are anti-correlated, i.e., high values in *A* are associated with low values in *B*. Positive correlation coefficients indicate that firing rates are positively correlated, i.e., high values in *A* are associated with high values in *B*. A correlation of 0 means there is no relationship between the firing rates in *A* and *B*. The correlation calculation can be saturated when neurons within the same population have largely different

firing rates, such as when one neuron has very low firing rates and another one has very high firing rates, hence responses were normalized using z-scores. However, I did not find a significant relationship between firing rate and correlations (Figure 2.6).

Mutual Information Analysis

I quantified how much information about image patches was conveyed in the population response. I binned neuronal responses from each cell individually in 120 ms bins (the duration of each image frame). I calculated mutual information using the information breakdown toolbox (Magri et al., 2009). I compared the mutual information values between populations showing statistically significant reactivation (using shuffled responses and bootstrapping) and those that did not show reactivation. Additionally, I confirmed that my information values were valid by shuffling the average firing rate responses and then performed the same analysis on the shuffled responses.

Results

Response Reactivation in V4 Populations

I performed extracellular recordings using multiple electrodes while monkeys performed a passive fixation task. The stimulus consisted of a 10 x 10 deg image encompassing multiple receptive field locations (Figure 2.1). The image was divided into 2 x 2 deg image patches, and each patch was presented serially in a random spatiotemporal sequence (Figure 2.2 and Figure 2.3A). Each receptive field was stimulated at least once during sequence presentation, and 25 image patches were presented throughout the 3-s movie (each image patch was flashed for 120 ms). Each 3-s *stimulus* trial was followed by a 3-s *blank* trial (Figure 2.3A)

triggered by the onset of a fixation point (the duration of the blank trial was equal to that of the stimulus trial). Each session was comprised of 150 stimulus and 150 blank trials. Baseline ongoing activity was assessed over 30 blank fixation trials prior to stimulus presentation (*pre-stimulus*), and this condition was repeated following the alternating stimulus-blank presentations at the end of the session (*post-stimulus*; these trials were identical to *blank* fixation trials). Sessions in which the monkeys did not achieve and maintain fixation for at least 70% of the trials were excluded. A total of 149 visually responsive cells were isolated across 19 sessions in two monkeys.

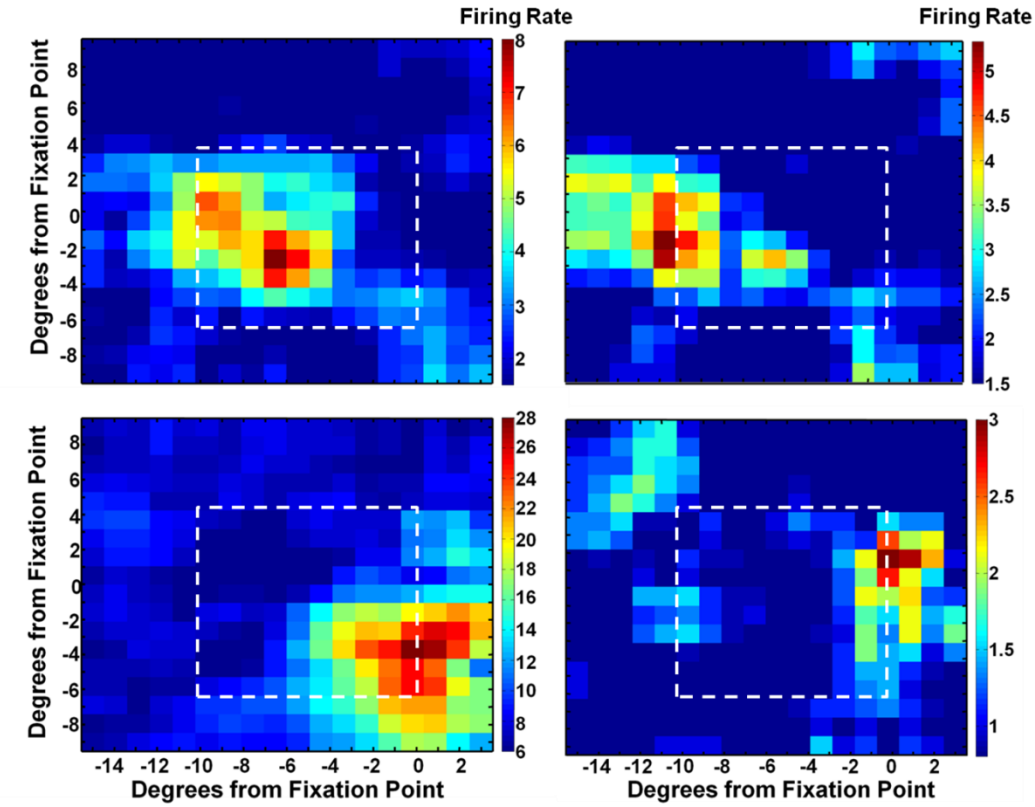

Figure 2.1. Examples of receptive field maps relative to the image position for four simultaneously recorded V4 cells.

Receptive fields were mapped by using reverse correlation stimuli consisting of four 0.5-deg–oriented gratings (0°, 45°, 90°, and 135°) briefly flashed across the receptive field locations. The dashed white lines represent the 10 × 10-deg image layout. The image was placed on the screen such as to stimulate all receptive field locations. Note that image patches were presented one at a time in a random spatiotemporal sequence; not all receptive fields would be active at the same time.

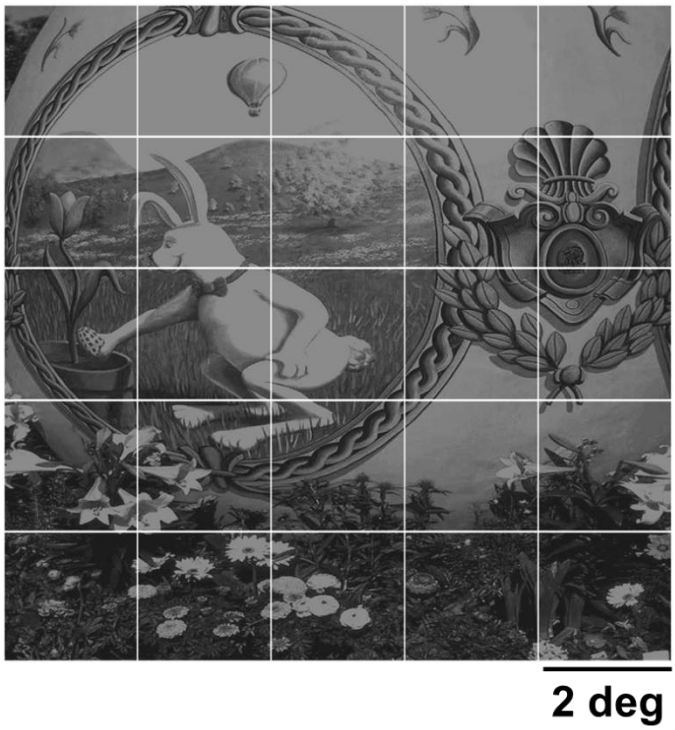

Figure 2.2. Example image used in the reactivation experiments.
Grayscale image was used to induce reactivation (the grid illustrates the size of each image patch). Each image patch was randomly presented at a different time at its original location in the image, thus creating a spatiotemporal image sequence.

Contrary to expectation, the firing rates of the neurons activated by the stimulus were increased not only when the movie was presented, but also during the alternating blank trials (Figure 2.3 B-C). Indeed, I analyzed the responses of the cells in my population throughout an extended time window starting with stimulus offset and ending with the subsequent blank presentation. By collapsing this time window analysis across trials for all the recorded cells (Figure 2.4), I found an increase in neuronal responses to the stimulus followed by a decrease in the inter-trial interval, and then a pronounced increase immediately after the onset of the fixation point in the subsequent blank condition ($P < 0.001$, Wilcoxon signed rank test; comparing the mean firing rates in the 3-s window before fixation onset in the blank condition and the 3-s window after the blank trial onset). The increase in firing rate in the

blank condition raised the possibility that neuronal responses may exhibit reactivation of the previously evoked spiking activity during the stimulus trial.

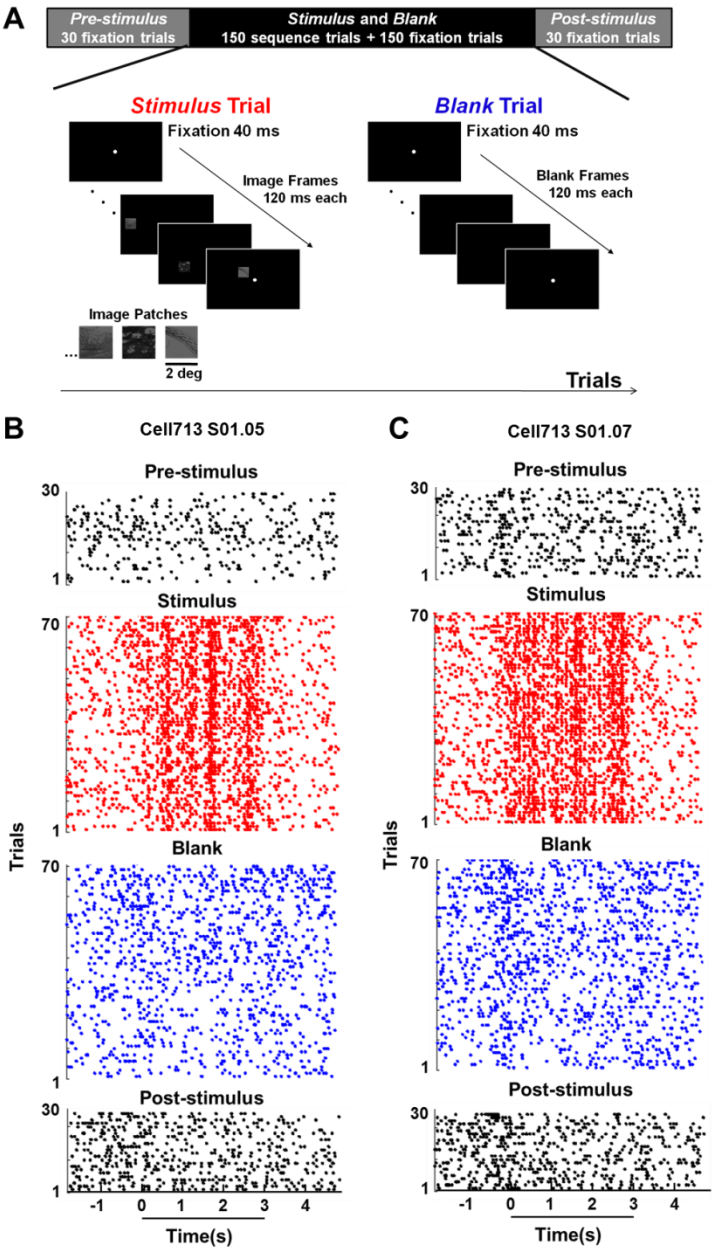

Figure 2.3. Experimental paradigm.
(A) Stimulus protocol – two monkeys performed a passive fixation task. The sequence presentation (*stimulus*) consisted of 2 x 2 deg patches of a natural scene presented serially in a random spatiotemporal sequence for 3 s. Each 3 s *stimulus* trial was followed by a *blank* fixation trial of similar duration, and was triggered by the onset of the fixation point. Baseline spontaneous activity was determined over 30 blank fixation trials prior to stimulus presentation (*pre-stimulus*). This condition was mirrored by 30 blank fixation trials following the alternating stimulus-blank presentations (*post-stimulus*; these trials were identical to the interleaved *blank* fixation trials). (B) Raster plots depicting the responses of one neuron in one session composed of successive blocks of pre-stimulus/stimulus/blank/post-stimulus trials. (C) Same as B for a different neuron.

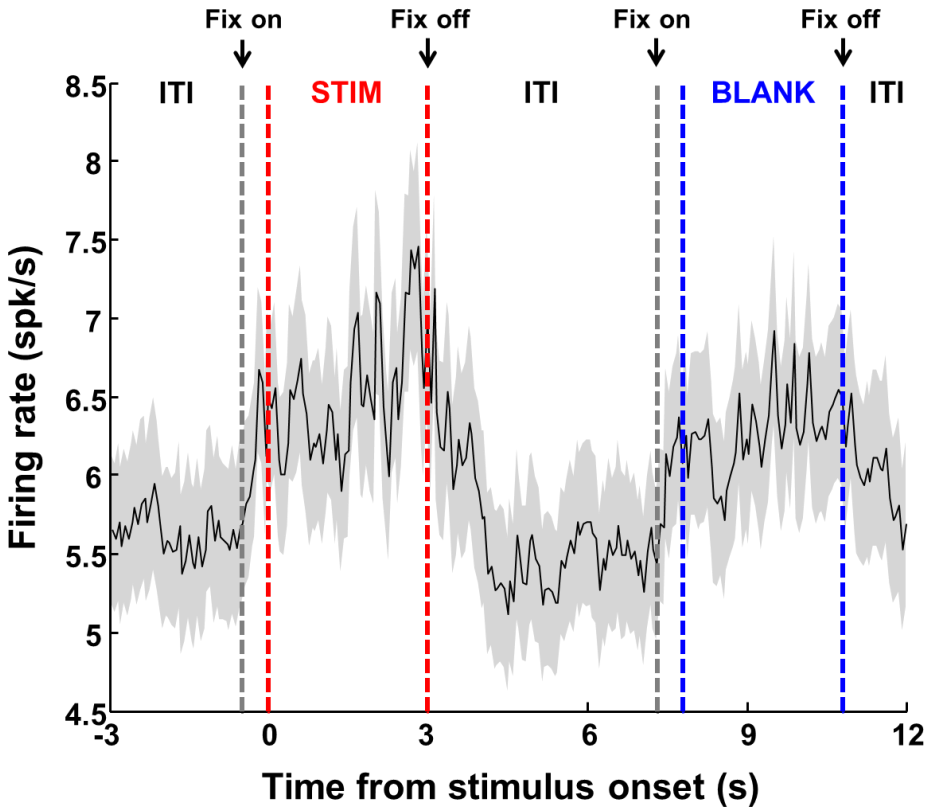

Figure 2.4. Neuronal response following stimulus presentation increases during subsequent blank

The peristimulus time histogram (PSTH) of the average firing rate across all trials and neurons in my population is shown relative to stimulus onset. Red lines indicate the onset and offset of the stimulus sequence; blue lines indicate the onset and offset of the following blank period. Gray lines indicate the onset of the fixation point in both stimulus and blank conditions (400 ms prior to stimulus or blank onset). ITI is the intertrial interval. Shaded envelopes represent S.E.M. of all visually responsive cells (n=149) in all sessions (19 sessions, 32 sequences).

Thus, I tested the hypothesis that repeated stimulus exposure causes a reactivation (at the same time scale) of the temporal pattern of stimulus-evoked neuronal responses across the population of cells in the absence of sensory stimulation. To quantify reactivation across the population of cells I measured the degree of similarity between the temporal pattern of neuronal firing in the stimulus and blank conditions. This was done by using the two-dimensional Pearson correlation coefficient (*CC*) after time-binning and z-scoring the neurons' average firing rates (Eq. 1; firing rates were computed for the entire 3 s of stimulus

presentation using 10 ms bins, and then averaged across trials for each condition; pre, stim, blank and post were all averaged and z-scored separately). Since cells with high average firing rates may impact my correlation measure more than those with low firing rates (de la Rocha et al., 2007), the responses of each cell were normalized across trials for each condition (using z-scores; Figure 2.5A; I found that the mean firing rates are uncorrelated with my Pearson correlation of z-scored response-time matrices, Figure 2.6). Since the increase in firing rates in the blank condition occurred at the same time as during stimulus presentation, I measured the correlation between the two response-time matrices using the same time scale. To determine the statistical significance of the correlation, I created a *pseudoblank* matrix by shuffling the blank z-scored neuronal responses across time bins and cells, which allowed us to compare the correlation between the stimulus and pseudoblank to the correlation between stimulus and blank (bootstrap method) – I found that 75% of sessions (24 out of 32) exhibited significant reactivation (Figure 2.7A, $p < 0.05$, this was calculated from a total of 32 sequence presentations [sequences]; 13 sessions contained blocks of two unique sequences).

I next assessed the magnitude of reactivation by comparing the correlation between the temporal responses across the network of cells during the stimulus and blank periods to that between stimulus and pre-stimulus. Clearly, my expectation was that the evoked response pattern would be more similar to the blank response than to the pre-stimulus response. Indeed, I found that the average response correlation between the stimulus and blank conditions was greater than that between stimulus and pre-stimulus (Figure 2.5B**;** $CC_{S\text{-}B} = 0.10$, $CC_{S\text{-}Pre} = 0.02$, $p < 0.01$, Wilcoxon rank sum test, results from 32 sequences). In addition, I compared the correlation between the stimulus and pre-stimulus conditions with

that between stimulus and post-stimulus, but failed to find a significant difference between the two ($CC_{S\text{-}Pre}$= 0.02, $CC_{S\text{-}Post}$ = 0.03, $p > 0.05$, Wilcoxon signed rank test; results from 32 sequences). I confirmed that these differences are not due to differences in eye movements between the different conditions (Table 2.1). Furthermore, I assessed whether reactivation was larger in the first half of stimulus presentation (i.e. the first 1.5 s) than in the second half of sequences that elicited significant reactivation (24 sequences, $CC_{S\text{-}B}$ first half = 0.15, $CC_{S\text{-}B}$ second half = 0.14, $p > 0.05$, Wilcoxon signed rank; both values were significantly different from the correlation between stimulus and pre-stimulus conditions $p < 0.001$ [first] , $p < 0.01$ [last], Wilcoxon rank sum).

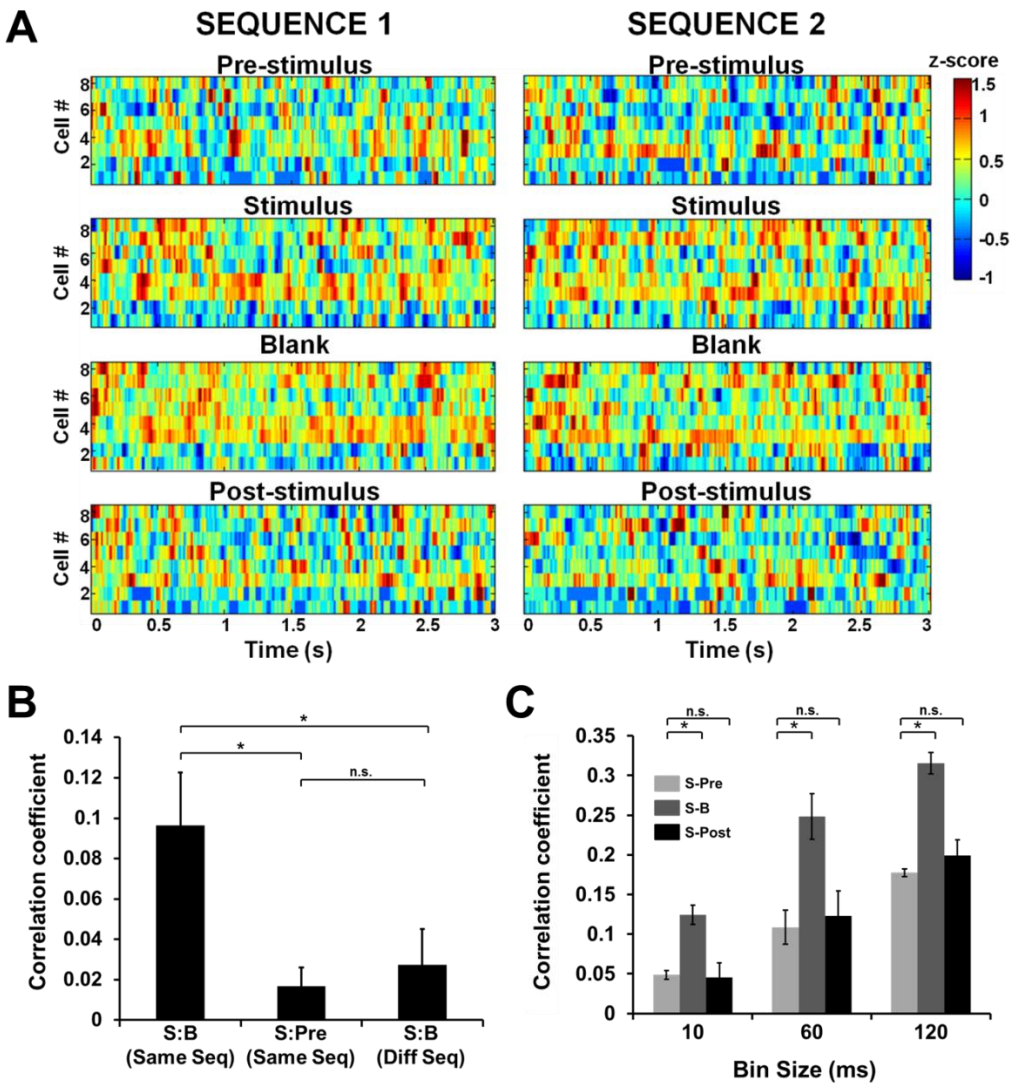

Figure 2.5. Response reactivation in visual cortical networks

(A) Averaged and z-scored response-time matrices for one population of cells in each condition for two different stimulus presentations (Sequence 1 and Sequence 2) are depicted. Neuronal firing rates were calculated for the entire 3 s of stimulus presentation using 10-ms bins, averaged across trials separately for each condition, and then normalized to obtain z-scores. (B) Stimulus specificity of response reactivation. I exposed the network of cells to two successive stimulus sequences and computed correlations between the stimulus-blank response-time matrices at the same time scale. I compared correlations between stimulus and blank periods within the same sequence and between sequences. (C) The effect of bin size on stimulus-blank correlations using 10, 60 and 120 ms bins. Correlations between stimulus and pre-stimulus (S-Pre), stimulus and blank (S-B), and stimulus and post-stimulus (S-Post) were compared for all bin sizes (* indicates $P < 0.05$, n.s. indicates $P > 0.05$; Error bars represent S.E.M.).

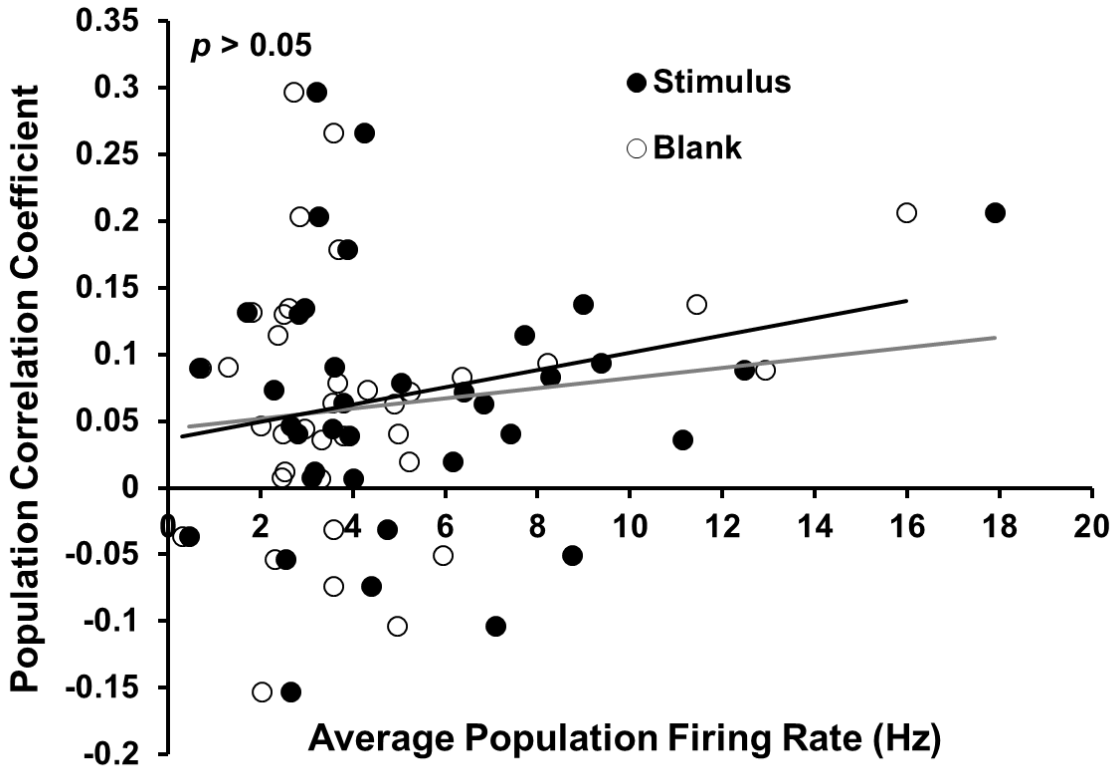

Figure 2.6. Stimulus–blank correlations are independent of firing rate
I examined the relationship between the average firing rate and average stimulus –blank correlation for each population of cells in each session. No significant trend between mean correlations and mean firing rates was observed for the stimulus (filled circles) and blank (open circles) conditions ($p > 0.05$ for both comparisons). Thus, correlation strength cannot be attributed to an overall change in firing rate.

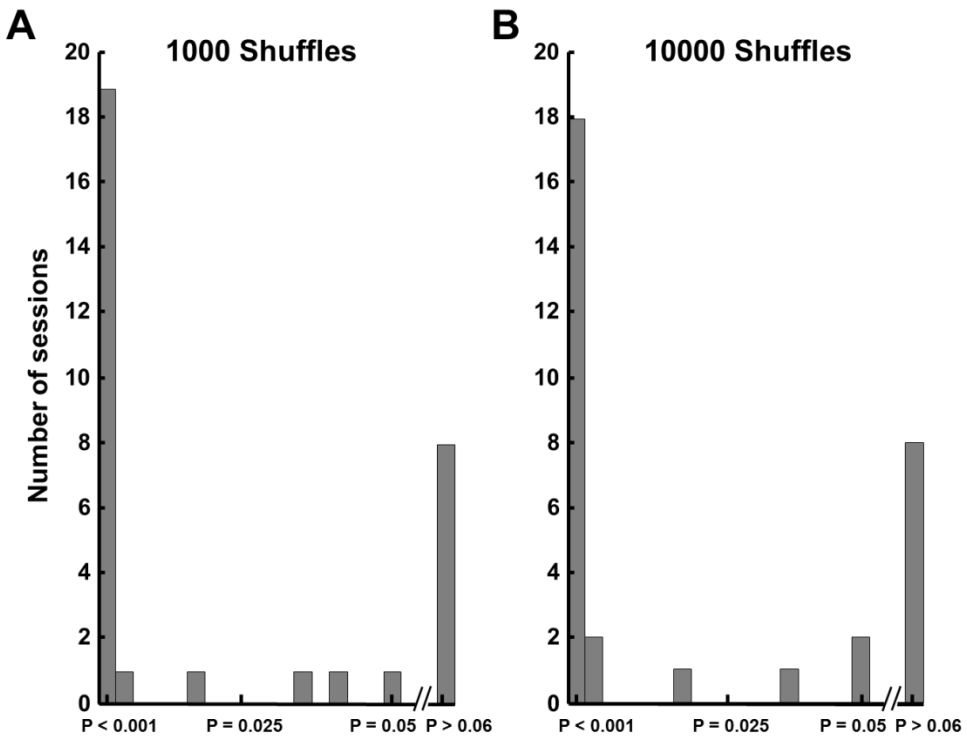

Figure 2.7. Distribution of significance values for shuffling and bootstrapping method
(A) To determine the significance of my reactivation, I compared the correlation between the sequence and blank period to the correlation between the sequence and a shuffled ('pseudoblank') period. I performed this comparison 1,000 times and interpreted values of <0.05 as significant correlation values. (B) I also performed this comparison 10,000 times.

	Eye Position Stim/Blank		Eye Position Stim/Pre		Eye Position Stim/Post	
Trace	**Correlation**	**P value**	**Correlation**	**P value**	**Correlation**	**P value**
Eye X axis	0.28 ± 0.10	0.40	0.29 ± 0.08	0.44	0.12 ± 0.10	0.61
Eye Y axis	0.38 ± 0.11	0.57	0.14 ± 0.08	0.53	0.19 ± 0.08	0.52

Table 2.1. Changes in eye position across conditions do not account for response reactivation
To determine whether eye movements had an effect on the neurons' capacity to show reactivation, I compared eye movements in the stimulus period to blank, prestimulus, and poststimulus conditions. I averaged the horizontal (x axis) and vertical (y axis) eye traces across trials in each condition (stimulus, blank, prestimulus, and poststimulus). I then computed the Pearson correlation coefficient between the averaged traces for stimulus and blank, stimulus and prestimulus, and stimulus and poststimulus conditions. The statistical significance of the correlation was assessed by using the shuffling and bootstrapping method. I found that none of the correlations were statistically significant, hence indicating that eye movements were not a confounding variable in my study. Errors are reported as SEM.

In principle, my results might have been influenced by the size of the time bin (10 ms) used to measure neuronal activity. I found that the increase in bin size causes a significant increase in stimulus-blank correlation (Figure 2.5C; 10 ms bins: $CC_{S\text{-}B}$ = 0.10; 60 ms bins: $CC_{S\text{-}B}$ = 0.22; 120 ms bins: $CC_{S\text{-}B}$ = 0.29; $F(2, 32) = 3.24$; $p < 0.05$, 1-way ANOVA). Specifically, the 60-ms and 120-ms binned stimulus-blank correlation coefficients were significantly greater than the 10-ms binned correlation coefficient, but not significantly different from each other. However, despite the fact that correlations increased with bin size, the difference between the stimulus/blank and stimulus/pre-stimulus correlations remained statistically significant for all bin sizes ($p < 0.0001$ [60 ms], $p < 0.001$ [120 ms], Wilcoxon rank sum; correlation values between the stimulus and post-stimulus responses were not significantly higher than those between stimulus and pre-stimulus, $p > 0.05$ [60 ms], $p > 0.05$ [120 ms], Wilcoxon rank sum). In addition, I found that 72% (23 out of 32) of the 60-ms binned and 69% (22 out of the 32) of the 120-ms binned sessions showed significant reactivation (using the pseudoblank and bootstrap method; results from 32 sequences).

To rule out the fact that reactivation in the blank condition could be due to a general increase in firing rates of neurons, possibly caused by a stimulus-independent increase in arousal or attention, I examined whether the effects described above exhibit stimulus specificity. I addressed this issue by exposing the network of cells to a second stimulus following initial stimulation. That is, after the initial completion of a pre-stim1/stim1/blank1/post-stim1 block of trials, I exposed the same network to a new block, pre-stim2/stim2/blank2/post-stim2, by presenting a new stimulus sequence (stim2) consisting of identical image patches, but presented in a new temporal order. Using the same correlation analysis as described above, I compared the correlation between stimulus and blank periods within the same sequence (stim1-blank1 and stim2-blank2) and between sequences (stim1-blank2 and stim2-blank1). I found a clear signature of stimulus specificity of reactivation – the stimulus-blank correlations within each sequence was significantly greater than those between sequences (Figure 2.5B**;** mean $CC_{Within} = 0.10$; mean $CC_{Between} = 0.03$, $p < 0.01$, Wilcoxon signed rank test; results from 13 sessions with 2 unique sequences). Importantly, this effect was not due to differences in recording stability as firing rates remained stable between sessions (Figure 2.8).

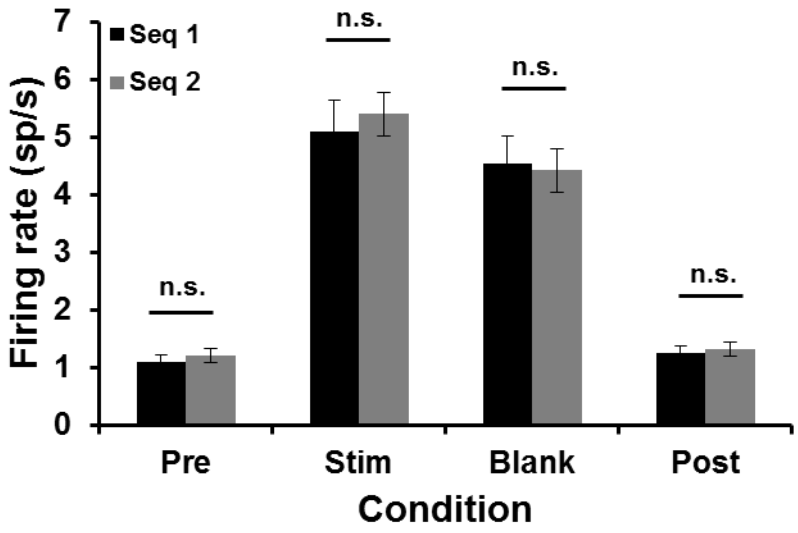

Figure 2.8. Firing rates do not change between successive sequence presentations
To determine whether the stimulus specificity of neuronal reactivation is related to possible differences in firing rates across different sequence presentations, I calculated the average firing rates during the 3-s stimulus period for all of the cells in my population (separately for each condition). Specifically, I calculated firing rates in 120-ms (for each image patch presentation) and then averaged them across bins and traisl for the presimulus (Pre), stimulus (Stim), blank (Blank), and postimulus (Post) conditions and for each block separately (the first sequence block firing rates are shown in black; the second sequence firing rates are shown in gray). I found no significant difference between firing rates associated with sequence 1 and 2, indicating that the difference in correlations (within and between sequences) is not due to differences in firing rates ($p < 0.05$, Wilcoxon signed rank performed for each condition pair separately, i.e., $Seq1_{Pre} - Seq2_{Pre}$, $Seq1_{Stim} - Seq2_{Stim}$, etc.). Notice that overall firing rates are relatively low due to the image patch presentations as cells responded sparsely when image patches were presented. Error bars represent SEM; n.s., $p > 0.05$.

Previous reports of response reactivation have shown that this phenomenon can occur in the forward or reverse direction (Louie and Wilson, 2001). To determine the direction of response reactivation in my study, I reversed the blank period along the time axis (Figure 2.9A) and performed the same correlation analysis by using only the sessions with statistically significant reactivation (24 sequences). I found that the average correlation between the stimulus and 'forward' blank responses was significantly higher than that

between the stimulus and 'reverse' blank responses (Figure 2.9B; $CC_{Forward}$ = 0.14, $CC_{Reverse}$ = -0.02, $p < 0.00001$, Wilcoxon signed rank test).

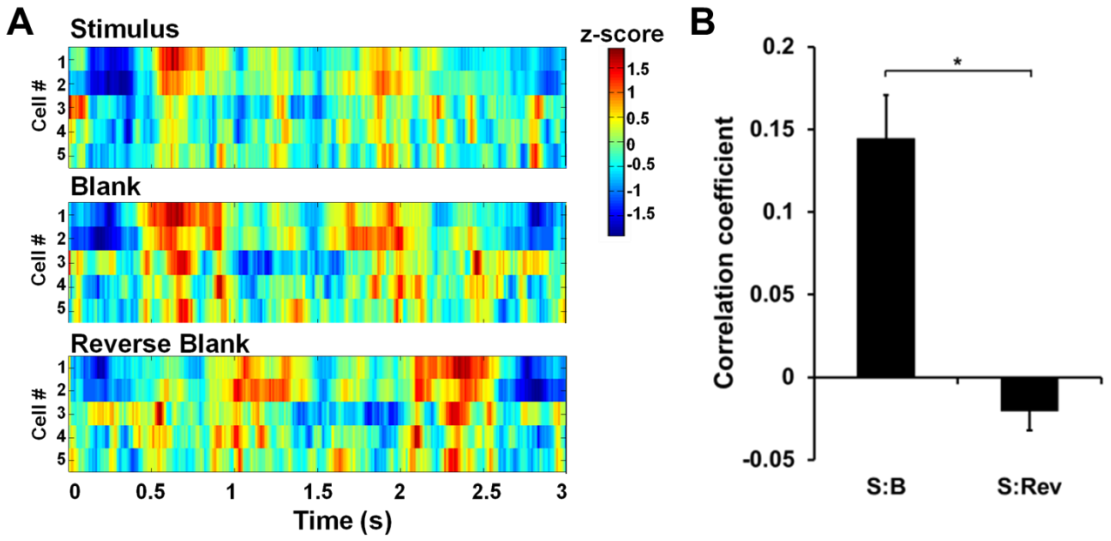

Figure 2.9. Reactivation only occurs in forward direction
(A) Averaged and z-scored response-time matrices of stimulus, blank, and reverse-blank conditions for one session. The 'reverse blank' condition is the blank response reversed along the time axis. (B) Correlation analysis comparing the forward and reverse reactivations. Only the sessions with a statistically significant effect were included in this analysis; * indicates $P < 0.05$; Error bars represent S.E.M..

My results so far depend critically on the temporal correlation between the population responses in the stimulus and blank conditions measured in a fixed 3-s window. Even though firing rates in the blank condition clearly increase immediately after the onset of the fixation spot (cf. Figure 2.4), it may be possible that correlations might have reached statistical significance even before the 3-s period following blank onset. To control for this possibility, I computed the correlation between the 3-s stimulus-evoked response and a 3-s moving window response sliding between stimulus offset and the end of the subsequent blank trial

only in those sessions showing significant reactivation (24 sequences). I used 60 ms time-binned, averaged, z-scored responses, and the time window was shifted in 60 ms increments until 4.2 s after blank onset (Figure 2.10). Interestingly, the correlation coefficient reached a maximum exactly at the starting point of the 3-s window corresponding to blank onset (Figure 2.10A; correlation values were normalized within each session). I further computed the statistical significance of the correlation as a function of time (using the pseudoblank and bootstrap method) and found that the only time window in which the correlation was significant (i.e. $p < 0.05$) was the 3-s blank window signaled by blank onset (Figure 2.10B). Altogether, these results further confirm the significance of the temporal correlation between the stimulus and blank trial responses.

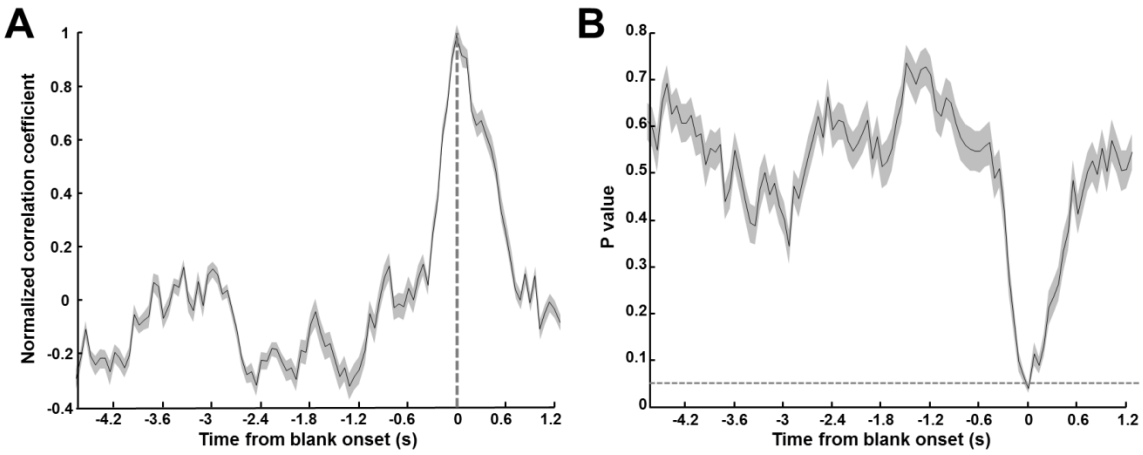

Figure 2.10.Correlation between the stimulus-evoked response and a 3-s moving window.
(A) Average normalized correlation between the 3-s stimulus-evoked response and a 3-s window (shifted every 60 ms) moving between stimulus offset and 1.2 s after blank onset (indicated by the gray dashed line). The peak correlation is observed at the expected onset of the stimulus during subsequent blank period. (B) Statistical significance of the correlation coefficient as a function of time. The only time window in which correlation is significant ($p \leq 0.05$) corresponds to the starting point of the 3-s blank window associated with blank onset. The figure represents the average p-value corresponding to each 3-s moving window (shifted every 60 ms). Shaded envelopes represent S.E.M.

Temporal Dynamics of Reactivation

I examined the temporal dynamics of stimulus-specific reactivation. To this end, correlations were calculated for blocks of two trials, and then normalized by the standard deviation of correlations for all conditions in each session. I performed this analysis on the 24 sequences (from 16 recording sessions) that showed significant reactivation. Figure 2.11A shows average normalized correlation values across sessions – stimulus-blank correlation increased with the number of stimulus exposures ($r = 0.42$, $p < 0.01$). In addition, I found a significant correlation between stimulus and blank trials (assessed using the bootstrap method, $p < 0.05$) even after few stimulus presentations – 73% of sessions were associated with a significant reactivation after 6 stimulus presentations; 94% of all sessions had a significant reactivation after 12 stimulus presentations. I also found that, on average, around 42% of sessions were associated with significant reactivation for each block of 2 trials (Figure 2.12). Furthermore, the probability of significant reactivation was increased as the neuronal population was exposed to more stimulus presentations ($r = 0.37$, $p < 0.01$).

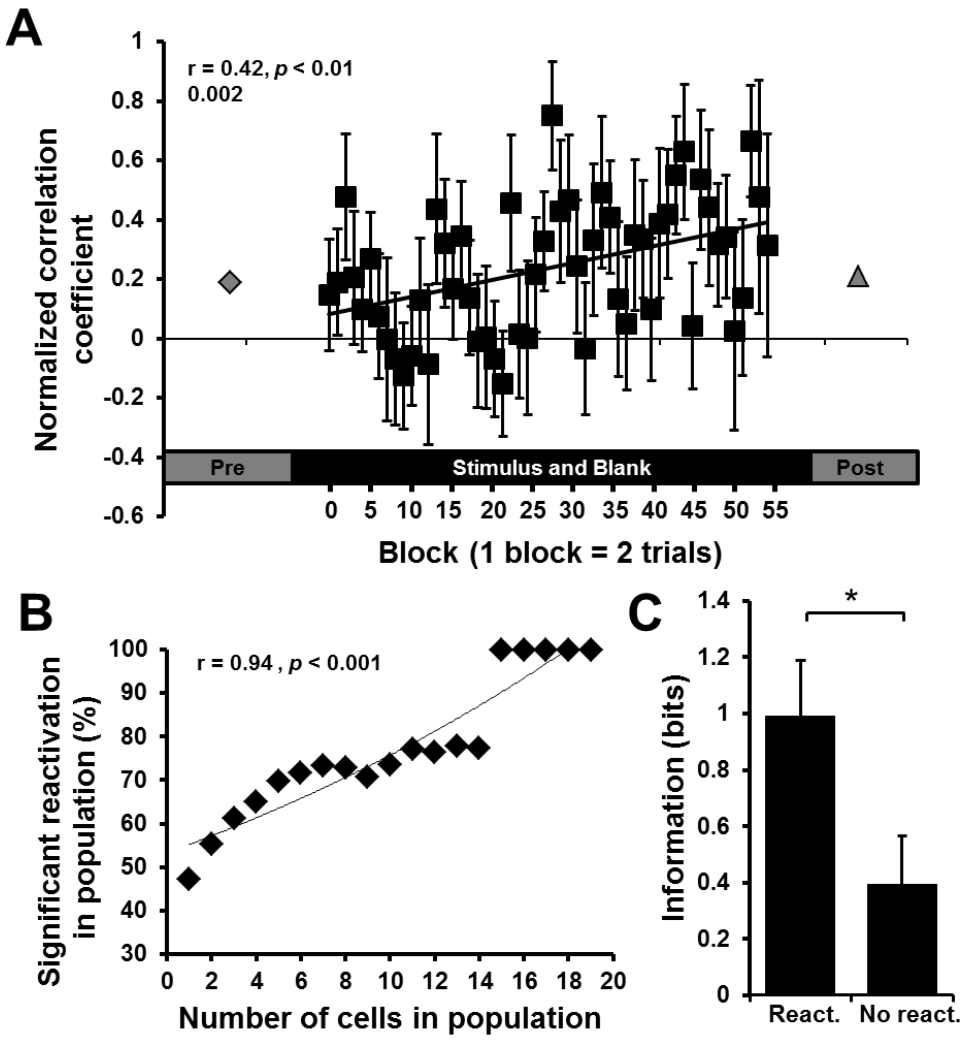

Figure 2.11. Temporal dynamics of response reactivation, effect of population size, and mutual information analysis

(A) Stimulus-blank correlation strength increases with the number of stimulus exposures. Each point represents the stimulus-blank correlation computed by averaging the z-scored network responses of two successive trials normalized by standard deviation of correlations within each session (normalized correlations were averaged across sessions). To eliminate variability in the total number of trials across sessions only the first 110 pairs of stimulus-blank trials were included in this analysis. The first and last points represent the mean correlations for stimulus-prestimulus and stimulus-poststimulus conditions computed for the 30 trials at the beginning and end of each session. Error bars represent S.E.M. (B) The probability of a significant reactivation event increases with the number of cells in the population. The percentage of combinations of cells showing significant reactivation was determined by comparing the $CC_{Stimulus,\ Blank}$ with $CC_{Stimulus,\ Pseudoblank}$ using the shuffling and bootstrap procedure. This analysis was exclusively performed on populations that showed significant reactivation. (C) Mutual information between population neuronal responses and image patches. Populations exhibiting significant reactivation carry more information about stimuli (* indicates $p < 0.05$, Error bars represent S.E.M.).

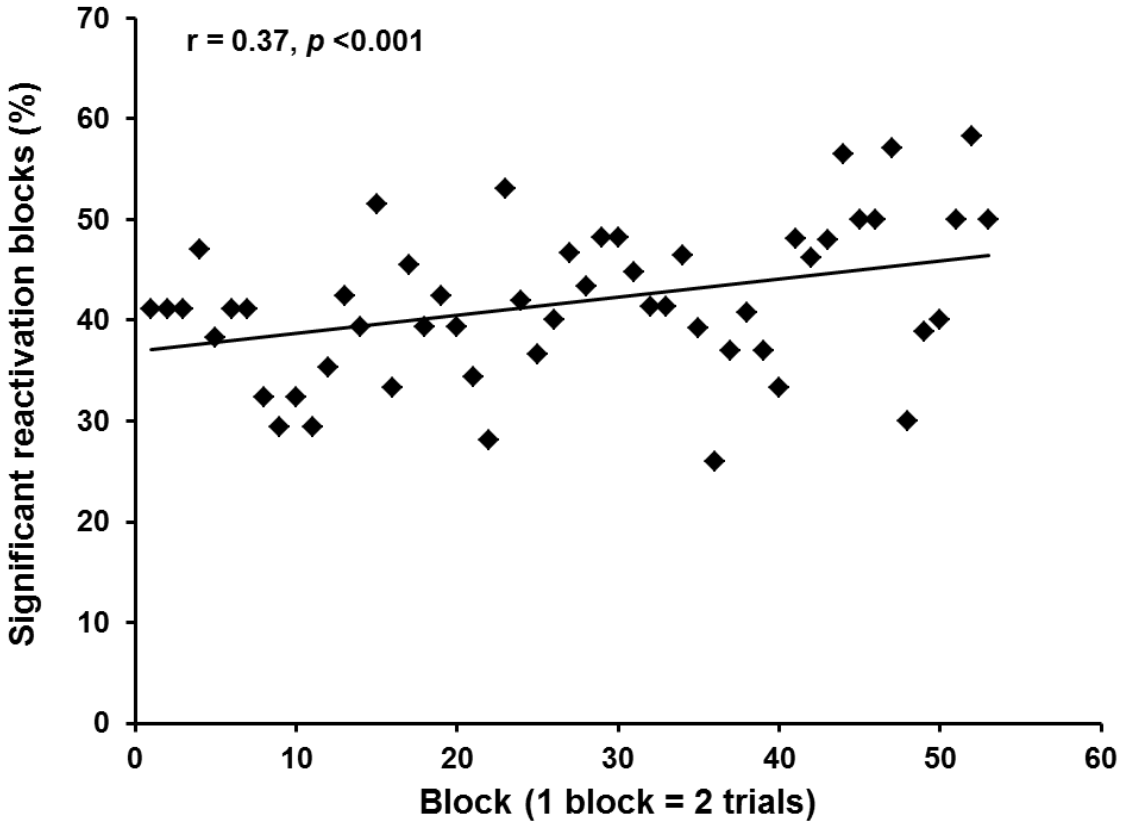

Figure 2.12. Increase in significant reactivation with the number of stimulus exposures
I determined the percentage of sessions that showed statistically significant correlation between stimulus and blank across trials. Specifically, I determined how many sessions showed significant reactivation for each block of two trials. I used stimulus and blank response-time matrices containing the average, z-scored responses calculated every two trials. To assess the statistical significance of the reactivation event, I used the shuffling and bootstrapping procedure. I found that, on average, 42% of session exhibited significant reactivation events on a block-by-block basis. That is, for every two stimulus presentations, there was a 42% chance that the neuronal population would exhibit reactivation. As the population is exposed to more stimulus presentations the probability of significant reactivation increases, as indicated by the trend line.

Reactivation Depends on Population Size

Does the strength of reactivation change when the number of cells in the network varies? To examine this issue, I employed a cell-dropping procedure to calculate the percentage of populations showing significant reactivation when the number of cells in the network is

gradually decreased (by using all possible combinations of simultaneously recorded cells). Specifically, after determining whether the stimulus-blank correlation was significant (for the entire network of *n* cells recorded within a session), I removed one cell from the population and recalculated the stimulus-blank correlation for the population of *n-1* cells, and then assessed the statistical significance of the correlation. This procedure was repeated until I was left with only one cell. The cell dropping procedure was repeated multiple times such that all possible combinations of cells were analyzed. I found that whereas 47% of single cells showed significant reactivation, the percentage of populations with statistically significant reactivation increased with the number of cells included in the population ($r = 0.94$, $p < 0.001$, Figure 2.11B; I only used neuronal populations that exhibited significant reactivation in the first sequence presentation, if multiple sequences were presented, when all neurons were considered). Furthermore, I extended my correlation analysis to local field potentials (LFPs). I observed significant reactivation as well as significant differences between the stimulus/pre-stimulus correlations and stimulus/blank correlations (Figure 2.13).

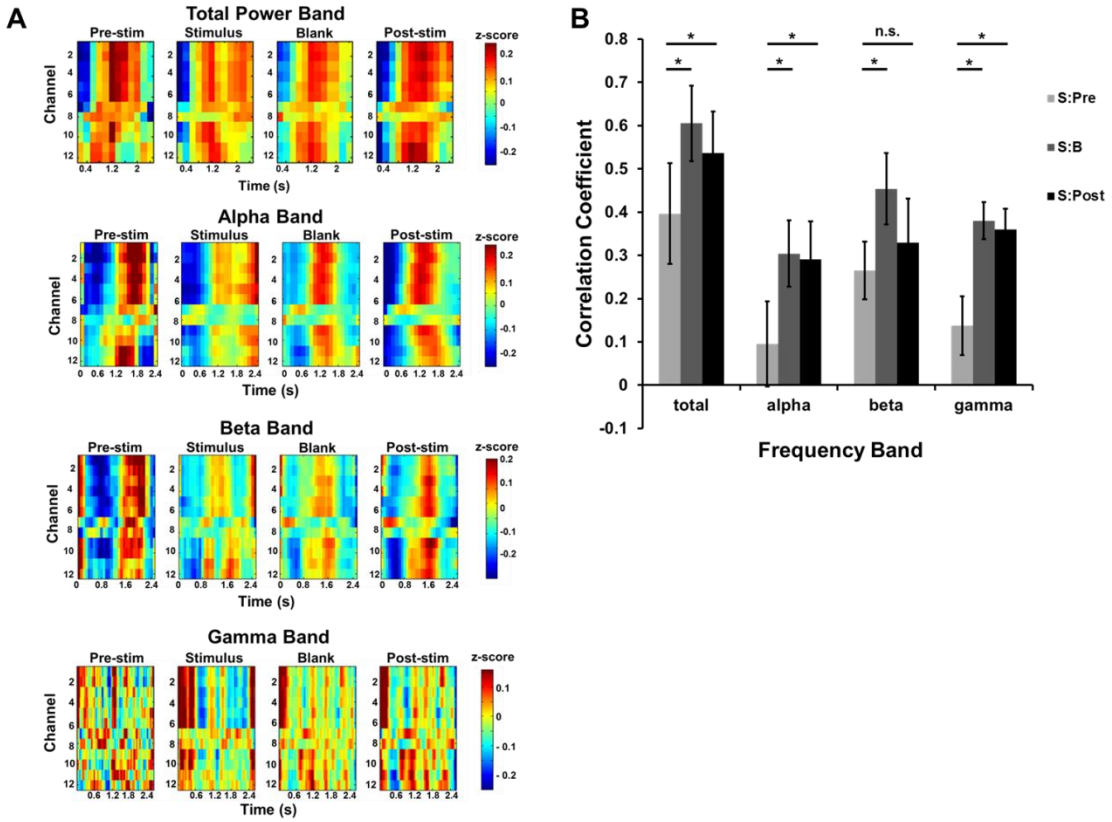

Figure 2.13. Reactivation effects in LFPs

I extended my analysis to LFPs, because LFPs reflect the summation of signals originating from ~250 μm around the electrode tip (Katzner et al., 2009). LFP traces were filtered using high-pass and low-pass filters or band-passed filtered for alpha (8-12 Hz), beta (15-35 Hz), or gamma bands (30-90 Hz, see LFP Pre-processing for filter descriptions); I then applied a 60 Hz notch filter if the frequency range included 60 Hz. I assessed power in time-binned intervals for filtered LFPs. The length of each bin varied for each band-pass filtered LFP to ensure accurate calculation of power for each frequency band (i.e., total: 200 ms; alpha: 120 ms; beta 80 ms; and gamma 30 ms). Similar to the spike data, the binned power plots were z-scored to eliminate channels with higher power biasing the calculation of the correlation. I also found that the correlation between the stimulus and post-stimulus was greater than that between the stimulus and pre-stimulus for the total, alpha, and gamma filtered LFPs, but not for the beta filtered LFPs (total power: $CC_{Stimulus,\ Prestimulus} = 0.40$, $CC_{Stimulus,\ Poststimulus,} = 0.54$, $p < 0.05$, alpha power: $CC_{Stimulus,\ Prestimulus} = 0.10$, $CC_{Stimulus,\ Poststimulus} = 0.29$, $p < 0.05$, beta power: $CC_{Stimulus,\ Prestimulus} = 0.27$, $CC_{Stimulus,\ Poststimulus} = 0.33$, $p > 0.05$, gamma power: $CC_{Stimulus,\ Prestimulus} = 0.14$, $CC_{Stimulus,\ Poststimulus} = 0.36$, $p < 0.05$, Wilcoxon signed rank). (**A)** Averaged and z-scored plots of total, alpha, beta, and gamma filtered LFPs (respectively, from top to bottom) during each session condition. (**B**) The correlation between stimulus and pre-stimulus is significantly lower than the correlation between stimulus and blank for all frequency bands. Additionally, the correlation between the stimulus and post-stimulus was significantly higher than the correlation between stimulus and pre-stimulus for alpha and gamma bands indicating that reactivation extended beyond the stimulus/blank fixation trials (* indicates $p <= 0.05$, n.s. indicates $p > 0.05$, Error bars represent S.E.M.).

Stronger Reactivation in Highly Informative Cells

I further examined whether the populations of cells exhibiting significant reactivation are those that are most informative about the stimulus. I tested this hypothesis by determining how much information about the stimulus is carried by the population response by computing mutual information between the population responses and image patches (Magri et al., 2009). I found that all information values were statistically significant regardless of whether the population responses exhibited reactivation or not. Interestingly, I found that populations exhibiting statistically significant reactivation carried more information about the stimulus (Figure 2.11C). That is, the populations of cells (24 sequences) showing statistically significant reactivation had a statistically higher average mutual information, 0.99 bits, while the populations of cells not showing reactivation (8 sequences) had an average mutual information of 0.39 bits ($p < 0.05$, Wilcoxon rank sum test, Figure 2.11C).

Discussion

I have demonstrated that populations of neurons in awake macaque visual cortex exhibit stimulus-specific, cue-triggered reactivation of previous evoked responses at the timescale of visual fixation. I found that the network reactivation of evoked activity is more robust in larger populations of cells and is observed in both multiple neuron responses and LFP activity. Additionally, I have demonstrated that the presence of reactivation is related to the capacity of neuronal populations to carry information about the stimulus.

One might argue that my results may be due to stimulus expectation, arousal, or attention, as neurons in visual cortex are known to increase their responses when a stimulus is expected or when attention is directed towards it (Kastner et al., 1999; de Oliveira et al., 1997). However, while I did observe a firing rate increase in blank trials, the fact that response

reactivation is stimulus specific (i.e., stimulus-blank within-sequence correlation is greater than that between sequences) and occurs exclusively in the forward direction argues against a general modulatory effect due to expectation, arousal, or attention. In addition, the fact that firing rates were normalized (using z-scores) prior to calculating correlation coefficients argues against a general modulatory effect on temporal correlations between stimulus and blank neuronal responses.

My study differs from previous stimulus entrainment reports involving repetitive stimulus exposure to induce firing at the same frequency as my stimulus presentation did not occur at a fixed receptive field location (Williams et al., 2004; Yakovlev et al., 1998). Indeed, my stimulus presentation is significantly different from that during entrainment – the presentation of image patches occurs at random locations within a 10 x 10 deg window, thus making it impossible for image patches to stimulate V4 receptive fields at a fixed frequency. Clearly, the temporal structure of my random stimulus presentation is captured by responses across the entire network, not the frequency-entrained responses of only one neuron. Furthermore, whereas entrainment studies describe how neuronal responses are modified immediately following stimulus exposure, my results demonstrate response reactivation exactly at the time when stimuli are expected to occur in the subsequent trial. One could also argue that the refresh rate of the monitor may be entraining neurons to exhibit reactivation. However, if this were the case, I would not find a significant difference between temporal correlations in responses occurring in the pre/stimulus-stimulus and blank/stimulus conditions, nor would I find stimulus specificity in my reactivation events (the same refresh rate is used throughout the experiments).

Previous studies using voltage-sensitive dye imaging (Han et al., 2008; Kenet et al., 2003) and single-cell electrophysiology in cat V1 (Yao et al., 2007) have shown that ongoing activity resembles orientation map responses to grating stimuli (Kenet et al., 2003) and natural movies (Yao et al., 2007), and can exhibit a 'memory trace' response immediately after the stimulus is extinguished (Yao et al., 2007). Furthermore, 'recall' responses were recently found in visual cortical networks following conditioning with a moving dot stimulus upon presentation of the first dot in the sequence (Xu et al., 2012). However, there are major differences between these findings and those in my study. In addition to the fact that most previous visual cortex reactivation studies have been performed in anesthetized V1 or during sleep, the spatial similarity between ongoing activity and stimulus-evoked response was either independent of stimulus history (Kenet et al., 2003) or was observed immediately after stimulus offset (Yao et al., 2007). In contrast, I found clear evidence for reactivation in the awake state in V4 networks at the expected time of stimulus onset while monkeys performed a fixation task. In agreement with the Xu et al study (2012), I was able to elicit response reactivation by using the fixation spot as a trigger stimulus. Thus, the onset of the fixation point at the beginning of a trial can be considered as a 'go' signal triggering response reactivation. This claim is supported by my finding that neuronal activity in the blank condition increases at the same time relative to the onset of the fixation spot as in the stimulus condition, and that the correlation between the evoked and ongoing activity reaches the maximum at exactly this point in time. The apparent necessity of the fixation point to elicit the reactivation suggests that the effect I have characterized is due to bottom-up mechanisms. Further investigation of this phenomenon during extended periods of

quiescence in the absence of a cue is required to determine whether the brain can elicit this activity through top-down mechanisms. I will explore this question in Chapter 4.

The relationship between the stimulus-induced and ongoing cortical activity revealed in my study has certain similarities with the 'replay' of neuronal activity in neural circuits mediating episodic (Carr et al., 2011; Louie and Wilson, 2001; Nádasdy et al., 1999) and sensorimotor (Dave and Margoliash, 2000) learning. In those studies, the temporal firing patterns of multiple neurons during learning are repeated either during sleep (Dave and Margoliash, 2000; Louie and Wilson, 2001; Nádasdy et al., 1999) or in the awake state (Foster and Wilson, 2006). However, there are major differences between classical replay and the effects shown here. For instance, in hippocampal circuits replay occurs at irregular intervals and the replayed sequences are often compressed (Foster and Wilson, 2006) or expanded (Louie and Wilson, 2001) in time. In contrast, my study reveals reactivation patterns occurring at the same rate as stimulus presentation that can be externally controlled by a trigger cue. Finally, an important departure from previous work is my demonstration that neuronal networks in sensory cortex exhibit reactivation after exposure to a complex, random temporal stimulation that is representative of stimuli encountered during natural visual experience.

Altogether, my results are consistent with Hebb's hypothesis (Hebb, 1949) that simultaneously activated neurons that share a common experience may form a 'cell-assembly' which exhibits cue-triggered recall. Since my stimulus sequence activates the receptive fields of neurons at different times, spike-timing-dependent plasticity (STDP) could alter the strength of intracortical synapses between successively activated neurons to increase their probability of spontaneous co-firing. Indeed, previous models and experimental work

have suggested that STDP could be a mechanism by which recurrent excitatory connections could be altered to allow the learning of temporal sequences (Bi and Poo, 1999; Hebb, 1949). Consistent with this hypothesis is the fact that the strength of network reactivation increases with the number of stimulus exposures. Finally, my results raise the possibility that the capacity of neuronal networks to reverberate may explain how the brain is able to learn and store events that occur in time following passive stimulus exposure during sensory experience (Chelaru and Dragoi, 2008; Dragoi et al., 2002; Gutnisky et al., 2009; Rao and Sejnowski, 2001; Sietz and Watanabe, 2003; Watanabe et al., 2001).

"…Maybe one day we'll wake up and this will all just be a dream"
–Mockingbird, Eminem 2005

3. DESIGN AND DEVELOPMENT OF SIMULTANEOUS SLEEP CLASSIFICATION AND EXTRACELLULAR RECORDINGS

Introduction

Behavioral improvements in learning and memory typically follow sleep (Gais et al., 2000; Hennevin et al., 1995; Stickgold, 2005; Stickgold et al., 2000, 2002; Wagner et al., 2004; Walker and Stickgold, 2004). Similar improvements can be observed after a brief afternoon nap (Backhaus and Junghanns, 2006; Lahl et al., 2008; Mednick et al., 2003; Nishida and Walker, 2007; Tambini et al., 2010). The brain is not quiet during these periods of time. Electrophysiological recordings from electrodes placed on the scalps of humans, called an electroencephalogram (EEG), reveal that the brain oscillates between measurably different stages of distinct neural activity during sleep. These distinct activity patterns revealed with EEG, coupled with recordings of eye movements, known as an electrooculogram (EOG), and muscle tone, known as electromyogram (EMG), are collectively called polysomnography. Using polysomnography, distinct activity patterns have been classified into rapid eye movement (REM) sleep, and non-rapid eye movement (NREM) sleep, which can be further classified into stages 1-4. Stages 3 and 4 of NREM sleep, known as slow-wave (SW) sleep, are characterized by a decreased behavioral response to the external environment and by an increase in electrophysiological delta oscillations (1-4 Hz) throughout the brain. Human psychophysical experiments have correlated the presence of stages of sleep during naps with improvements in behavioral performance during perceptual learning tasks (Aeschbach et al., 2008a; Karni et al., 1994; Mednick et al., 2003). However, that research has produced conflicting evidence. Improvements in visual discrimination tasks have been observed after naps containing SW activity (Aeschbach et al., 2008b), REM activity (Karni et al., 1994), and only when both are present (Mednick et al.,

2003). Thus, there is not a consistent sleep characteristic at the global level associated with improvements in perceptual learning.

More intriguing is that the definition of sleep has been expanded in recent years. Research has unveiled that sleep has rich local components at the level of individual neural networks as well as global, whole-brain characteristics (Huber et al., 2004; Tononi and Cirelli, 2003a). Further, this local sleep-like activity occurs during daytime, awake states (Vyazovskiy et al., 2011). The identification of local sleep-like activity merits a more detailed examination of neural networks. Despite the prevalence and impact of sleep on perceptual learning, little is known about the neural mechanisms underlying this improvement. This is primarily due to the lack of electrophysiological studies during experimental learning paradigms that include sleep.

This absence is primarily due to the difficulty in performing concurrent polysomnography and extracellular recordings in an animal model capable of performing complex psychophysical tasks similar to humans. Monkeys are an ideal model because they have a similar brain organization to humans and similar sleep patterns as humans – including daytime naps (Daley et al., 2006a; Hsieh et al., 2008). The difficulty of developing sleep paradigms incorporating polysomnography in monkeys includes complicated surgeries to implant recording electrodes and telemetry devices (Crowley et al., 1972; Daley et al., 2006a; Hsieh et al., 2008; Reite et al., 1965; Weitzman et al., 1965). Here, I have attempted to fill this gap by designing and developing a removable cap where I can simultaneously record EEGs, EOGs, and EMGs along with video, and I follow polysomnography rules outlined for human sleep characterization (e.g. electrode placement, filter settings, and sampling frequency) (Berry et al., 2013; Rechtschaffen and Kales, 1968). Given the demands of

recording time and constraints of controlling behavioral testing, I designed an experimental paradigm to study the neural correlates surrounding daytime naps instead of a night of sleep. To this end, I trained two monkeys to perform a natural image delayed-match-to-sample tasks before and after a daytime 20-minute nap.

I began by testing the hypothesis that monkeys improve with a 20-minute rest in the task. I refer to the period between the tasks as rest because I did not include polysomnography until later stages of the development of this paradigm. However, during the rest period I observed both monkeys to have their eyes closed and jaw slack for extended periods of time, indicating the presence of sleep onset.

The ultimate goal of this experimental design is to study the underlying neural correlates of behavioral improvement in visual cortex area V4. Thus, I chose a natural image delayed match-to-sample task. V4 neurons respond to shapes, textures and contours (Bouvier et al., 2008; David et al., 2006; Hayden and Gallant, 2013; Liebe et al., 2011; Roe et al., 2012) which are features present in natural scenes.

Potential neural correlates of improvement are expected in two well-defined neural response properties: improved discriminability of stimuli and increased response reliability. Using analytical techniques for electrophysiological data recorded from single and populations of neurons, I am able to quantify how well neurons respond selectively to stimulus features. Moreover, neurons do not always respond in the same way to the same stimulus; there is some inherent variability in responses (Holt et al., 1996; Shadlen and Newsome, 1998). Combining these two ideas, the more distinct and reliable a response is, the better downstream neurons receiving the response are able to determine what stimulus

was presented. These measures can be compared before and after rest to determine how rest influences neural coding.

The results discussed in this chapter all relate to development of an experimental paradigm to determine the neural correlates of behavioral improvement during tasks after a daytime nap. The second goal of developing these methods is to elucidate the characteristics of the individual and network activity during rest that correlate with neural and behavioral improvements. Using data from this experiment, I tested the hypothesis that reactivation of stimulus-evoked activity occurred during subsequent rest periods. This will be discussed in Chapter 4.

Methods

Behavioral Paradigm

All experiments were performed in accordance with NIH Guidelines for the Care and Use of Animals for Experimental Procedures and the Animal Welfare Committee at the University of Texas Health Science Center at Houston. To determine the neural correlates of how rest improves behavioral performance in a visual image orientation discrimination task, I exposed two trained male rhesus monkeys (*Macaca mulatta,* referred to as M1 and M2) to a task before and after a brief rest (M1: 23 sessions; M2: 6 sessions, Figure 3.1A,C). During rest periods monkeys remained in the experimental room with lights and monitors off and with a white noise background for 20 minutes. Eye closure was monitored using an eye tracker and/or video camera during this time. I coordinated the timing of my experiments such that monkeys were in the dark room around 2 pm, a time previously reported when monkeys were observed to nap (Daley et al., 2006b). Prior to experimentation, monkeys were trained to perform the natural image orientation discrimination task with all images used

during the experiment—this was meant to eliminate practice effects (Karni and Sagi, 1991, 1993). Acquisition of the task was assumed when the monkey could perform the discrimination task with an 80% correct performance for each image used for three days. To control for the effect of rest, I also implemented a *no rest* condition in which monkeys were not allowed to rest in between task sessions (Figure 3.1C). Animals were either presented videos for 20 minutes (M1 2 sessions, M2 6 sessions), or placed in a dark room with a random, auditory, buzzing noise (M1 2 sessions). As in the rest condition, both monkeys were monitored during this time either using the eye tracker for eye closure or video monitoring.

In my first design of the experiment with monkey 1, I preceded the first task by a 20-minute period of rest so I could utilize the data to explore both of my goals: improvement after rest in task performance and changes in the structure of activity during rest after task exposure. However, after comparing rest and no rest data from Task 1, I found that monkey 1 had better performance when Task 1 was preceded by a rest, than when it did not. Specifically, he exhibited significantly fewer false alarms (63% fewer false alarms with rest, $p < 0.01$, Wilcoxon rank sum test) and had a significantly higher percentage of correct responses (16% and 25% more correct responses at the two largest orientation differences, both $p<0.01$, Wilcoxon rank sum test). I still observed a significant difference between task performances; however, I reasoned that the actual difference in performance between tasks may be diminished by including this first rest period. Thus, in monkey 2 I incorporated a third experimental design where the monkey did not have a rest preceding Task 1 (Figure 3.1 C). To summarize, data from monkey 1 includes experiments with Rest1 – Task1 – Rest 2 –

Task 2 and Task 1 – No Rest – Task 2, and data from monkey 2 includes experiments with Rest1 – Task1 – Rest 2 –Task 2, Task 1 – No Rest – Task 2, and Task 1 – Rest – Task 2.

Visual Orientation Discrimination Task

Monkeys were trained to fixate a centrally located *fixation point* (0.4 deg in diameter). 400 ms after the monkeys achieved fixation, an 8-10 deg natural image (*target*) was presented for 300 ms over all of the receptive fields of the simultaneously recorded neurons (Figure 3.1 D). After a blank interstimulus interval of 1 s, the same image was presented (*test*) in the same position either at the same or at a rotated orientation. Monkey 2 had slightly different timing: 366 ms stimuli on the screen and 1.25 s interstimulus interval. The difference in timing was due to a difference in the refresh rates of the computer monitors used for stimulus presentation, 60 Hz was used for M1 and 75 Hz for M2. After the second image was presented the fixation point turned red indicating to the monkey to release or hold a response bar to indicate whether the images were presented at the same or different orientation, respectively. Correct responses were rewarded with juice. An equal number of rotated and not rotated trials were presented.

To accurately assess the monkeys' behavioral improvement and keep them motivated in the task, 5 orientations were used with the smallest orientations around each monkeys' discrimination threshold (3 – 20 deg M1, and 2 – 40 deg M2). Out of all of the rotated trials 25% were at the orientations threshold, 25% were within 3 degrees below, 25% were within 5 degrees above threshold, and 25% were at a larger orientation that the monkey could discriminate with almost 100% accuracy. I found that this combination of stimuli kept the monkey motivated throughout the experiment (hard orientation combinations would cause

the animal to not work) and still allows monkeys to improve after rest. Additionally, equal numbers of trials were used per stimulus because of the intended neural analyses. I ensured the monkeys were actively performing the task by calculating the false alarm rate which is the percent of same trials the monkeys correctly responded subtracted from 100. Sessions in which false alarms exceeded 60% during either Task 1 or Task 2 were discarded as I could not assess whether behavioral changes were due to changes in discrimination. I then assessed how well monkeys were performing at different levels of difficulty (varying degrees of rotation between *target* and *test*) by calculating the percent correctly identified.

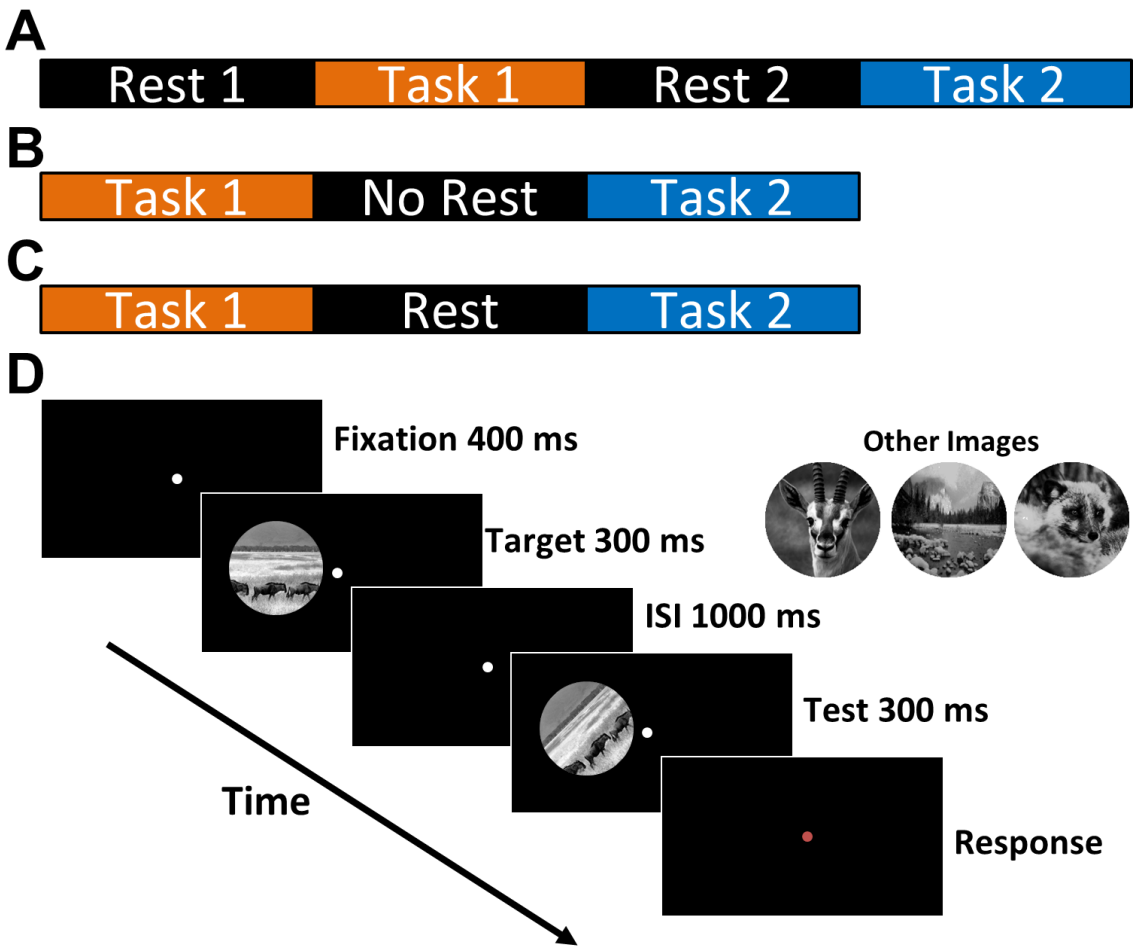

Figure 3.1. Experimental Design and image orientation discrimination task
(A) Experimental design to elucidate network changes that occur within task periods before and after rest. Task periods consisted of a natural image same-different task. During rest periods, monkeys were in a dark, quiet room for approximately 20 minutes. (B) Experimental design for control experiments where monkeys did not rest. During the period of no rest monkeys either watched a video for 20 minutes or were in a dark room for 20 minutes with intermittent auditory noise. (C) Experimental design used for monkey 2. Monkey was not able to rest until he completed Task 1. This session structure was implemented for monkey 2 to control for behavioral improvements observed in task 1 for monkey 1 when a rest preceded it. (D) Natural image same-different task consisting of two serially presented images. The second image was presented at the same or a rotated orientation. The monkey is expected to respond when the fixation point turned red by either holding or releasing a response bar if the images are at a different or same orientation, respectively. Timing is shown for M1; slightly different timing was used for M2 (see Methods).

Classification of rest and no rest sessions based on eye closure

I monitored eye closure throughout the duration of my experiments using an eye tracker and used it to quantify the rest the monkeys had between tasks. The eye tracker converts the position of the monkeys' eyes in to a voltage. Daily calibration aligned the eye position coordinates in the eye tracking software to those on the computer screen where I presented the visual stimuli by adjusting the output voltage of the eye tracking hardware. When the eye tracker is not able to detect the animals' eye position (which only occurs when it is closed), the eye tracker outputs a maximally negative voltage. In M1, this voltage was -1, and in M2 it was -4 V (Figure 3.2). The values differ between animals because I performed the experiments using two different eye trackers which had different voltage output (Eyelink II for M1 and Eyelink 1000 desktop mount for M2, SR Research). I recorded these voltage fluctuations and considered 80% of the minimum voltage value as times when the animal had his eyes closed. The threshold I use to determine whether the eyes are open or closed for each monkey is shown in Figure 3.2 by the dashed, horizontal red lines.

To elucidate the impact of rest on task performance I was interested in the amount of rest the animals had between tasks. Thus, I focused my analysis on Rest 2. I observed that during no rest sessions, both animals remained awake throughout the period. However, when examining eye traces during rest sessions, I observed that during some sessions the monkeys had their eyes open for most of the time. In fact, I observed two distinct types of eye closure behavior for all experimental sessions, one in which the monkeys closed their eyes for extended periods of time when they were left to rest, and one in which monkeys appeared to have their eyes open for most of the time. To classify sessions as *rest* and *no rest*, I calculated the percentage of time the monkey closed his eyes for 1 minute or longer

throughout the rest period. Sessions that had <5% closures were considered *no rest* sessions and those with >15% closures were considered *rest* sessions (Figure 3.3). Example traces of eye positions classified as *rest* and *no rest* are presented in Figure 3.2. Vertical lines with the 'LO' designation between them represent the periods of time when the lights were off in the experimental room during Rest 1 (only in M1 examples, Figure 3.2A) and Rest 2. Using this classification, M1 had 8 *rest* sessions and 9 *no rest* sessions (Figure 3.3A). M2 had 12 *rest* sessions and 6 *no rest* sessions (Figure 3.3B).

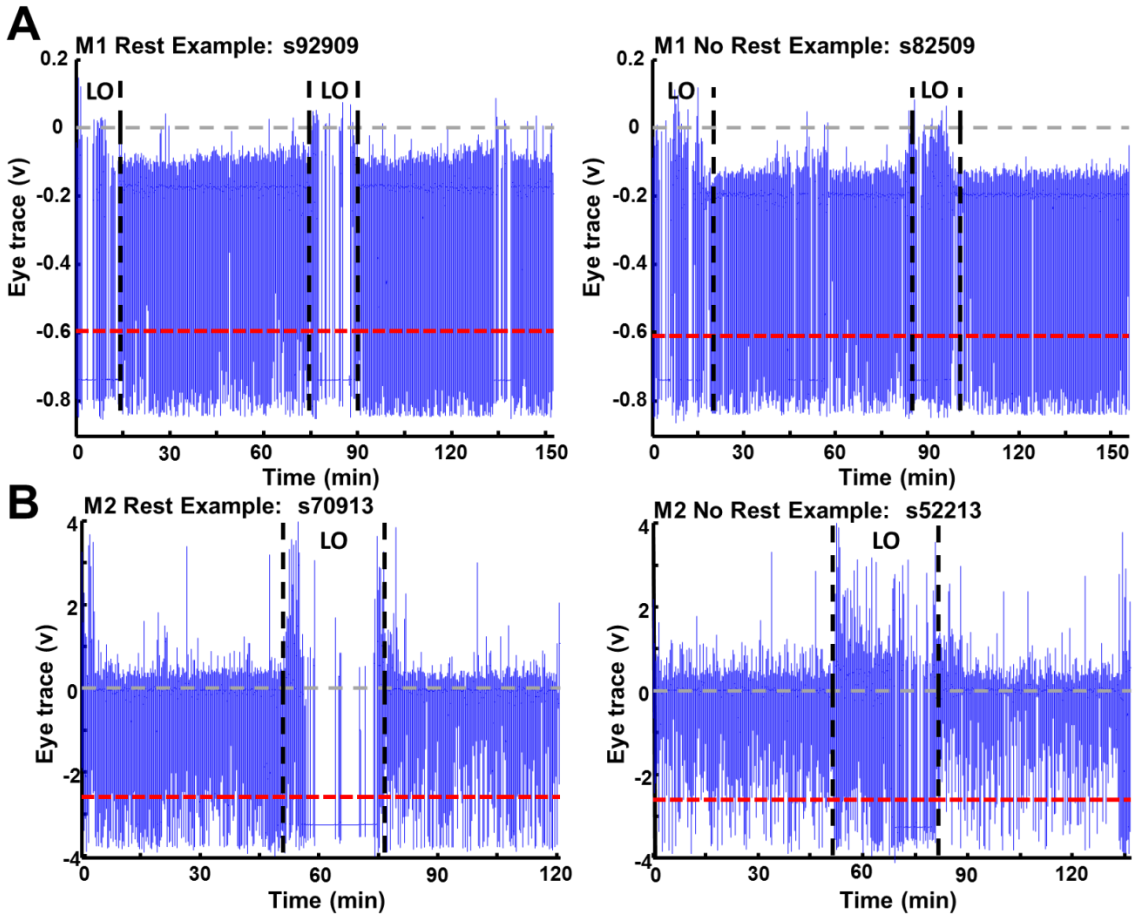

Figure 3.2. Examples of eye traces for rest and no rest sessions
During experimental recordings, I monitored eye position to ensure that the monkeys were fixating during presentation of stimuli, as well as to determine the percentage of time they closed their eyes during rest. The eye tracker converts the x and y coordinates of the monkeys' eye position into a voltage. When the eye tracker is unable to detect the pupil, which only occurs when the monkey's eyes are closed, the eye tracker outputs a negative voltage (-1 for M1 and -4 V for M2, as a result of using different eye trackers). I considered values below 80% of the minimum voltage (indicated here with the red dashed line) as periods when the eye was closed. Periods separated by vertical black dashed lines are when the monkeys were in the room with the lights off (LO). In the left column are example *rest* sessions (left) in which the monkey closed his eyes for extended periods of time, and in the right column are *no rest* sessions in which the monkey had his eyes open while in the dark room.

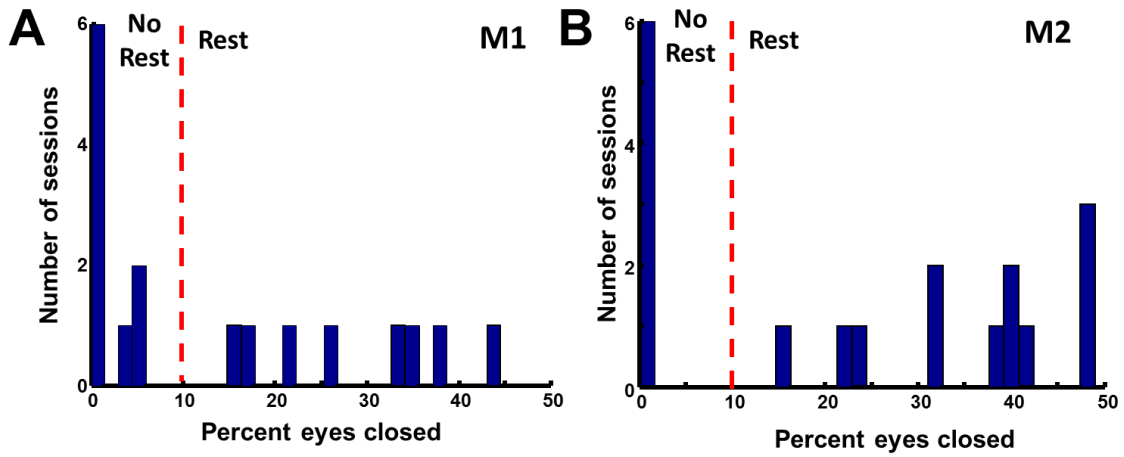

Figure 3.3. Distribution of eye closure across sessions and classification of *rest* and *no rest*

During *no rest* sessions both monkeys kept their eyes open for the duration of the task. However, during some *rest* sessions, both monkeys kept their eyes open for the duration of the time he was in the dark room (even though they was supposed to be napping). Therefore, to more accurately classify sessions into *rest* and *no rest* conditions, I calculated the percentage of time the monkey closed his eyes for 1 minute or longer during his 20-minute rest. I then classified *no rest* sessions as those with <5% of time with eyes closed and those with >15% of time with eyes closed as rest. The red dashed line indicates my boundary between *rest* and *no rest* classifications.

Polysomnography in monkeys

To accurately determine the neural correlates of behavioral improvement following rest and relate those correlates to research in the human nap literature, I needed to have the capacity to identify sleep stages in the monkeys. As previously discussed the combination of electroencelphalograms (EEGs), electrooculograms (EOGs), and electromyograms (EMGs)—collectively known as polysomnography—and how that can be used to determine sleep stages in humans and animals using the combined patterns of activity observed during a period of sleep. Previous research has successfully identified sleep stages in macaques identical to humans (Balzamo et al., 1998; Crowley et al., 1972; Daley et al., 2006a; Hsieh et al., 2008; Reite et al., 1965; Weitzman et al., 1965). However, all of these studies have used chronically implanted electrodes for their recordings, which require complicated surgical

procedures. Because I am interested in studying the effects of daytime napping on behavioral performance, I wanted a non-invasive and removable cap to conduct sleep staging along with extracellular recordings. A sleep cap combined with video monitoring similar to that used in humans allows for daily removal of polysomnography electrodes and implementation of daytime nap protocols that do not require complicated surgical techniques to obtain sleep data.

Incorporating polysomnography with extracellular recordings in monkeys is not well established. Thus, after consultation with a sleep physician at the University of Texas Medical School, Dr. Jeremy Slater, and a polysomnograph technician from Memorial Hermann Hospital, Carla Bodden, I designed and created a cap that incorporated EEGs, EOGs, and a chin EMG that could be used to determine sleep stages in the monkeys. Specifically, 6 mm cast silver, gold-plated, cup electrodes (Grass Technologies) were attached to an elastic cap (Figure 3.5) fitted to each monkey over the international standard 10-20 system of EEG sites corresponding to F3, C3 and O1 according to the AASM Manual for scoring human sleep (Berry et al., 2013). Electrodes were secured to separate straps that attached to the cap, positioning electrodes above the right eye and below the left eye to detect eye movements. An electrode located on the mentalis muscle was used to detect muscle tone. One ear clip electrode was placed on each ear lobe (RE, right ear and LE, left ear) and all EEG electrodes were referenced to RE and grounded to LE (Berry et al., 2013). EOGs were referenced to LE and grounded to RE. Electrodes were applied with Ten20 conductive paste. Recorded data was sampled at 500 Hz for all recording sites. EEGs and EOGs were low-pass filtered online at 150 Hz and EMGs were bandpassed filtered between 10 Hz and 250 Hz. Offline EEGs and EOGs were bandpassed filtered between 0.3 and 35 Hz.

Extracellular recordings

Extracellular recordings were recorded using identical procedures for M1 as discussed in Chapter 2. M2 data were recorded using the Cerebus system (Blackrock Microsystems). Further, all M2 data were recorded using single contact electrodes. As before, single units were identified and isolated manually offline using Offline Sorter (Plexon). Single units were identified using PCA analysis. Clusters that were significantly different from the background noise were further used in analyses (ANOVA for PC1 and PC2 between noise and single unit, $p < 0.05$). Cells included in the analysis were those that responded to the visual stimulus and had stable firing rates between the task periods. To identify visually responsive cells, I calculated the firing rate for all trials for all cells for the duration of the target stimulus (*evoked*, 300 ms) in Task 1. I then calculated the firing rate 300 ms before fixation onset (*baseline*) for all trials and cells. Visually responsive cells were those with evoked activity significantly greater than the baseline activity ($p<0.05$, Wilcoxon signed rank test). Additionally, I calculated the evoked activity in Task 2. Cells that had no significant change in firing rate between the task periods were considered stable ($p>0.05$, paired t-test).

Results

Behavioral performance improves after a 20 minute rest

Behavioral performance was assessed by calculating the percent of correct trials at varying orientation differences between *target* and *test* for Task 1 and Task 2. I used different orientations for each image and for each monkey based on the monkeys' performance as described previously. However, the same orientations were used for the same image for both *rest* and *no rest* conditions. To compare the monkeys' performances, I labeled the orientation differences based on the level of difficulty (LOD) for each monkey

(Figure 3.4). Small *target* and *test* orientation differences were harder for the monkeys to discriminate and thus given a LOD of 4, while large orientation differences were easier and thus were given an LOD of 1. Both monkeys improved their behavioral performance significantly after rest (Figure 3.4, left panels, M1: 11% more correct trials at LOD 2, $p < 0.001$; M2: 11% more correct trials at LOD 3, $p < 0.01$; Wilcoxon signed rank test with Bonferroni correction) but not when they did not rest (Figure 3.4 right panels, no significantly differences in behavior between Task 1 and Task 2 for either monkey were observed, all $p > 0.05$).

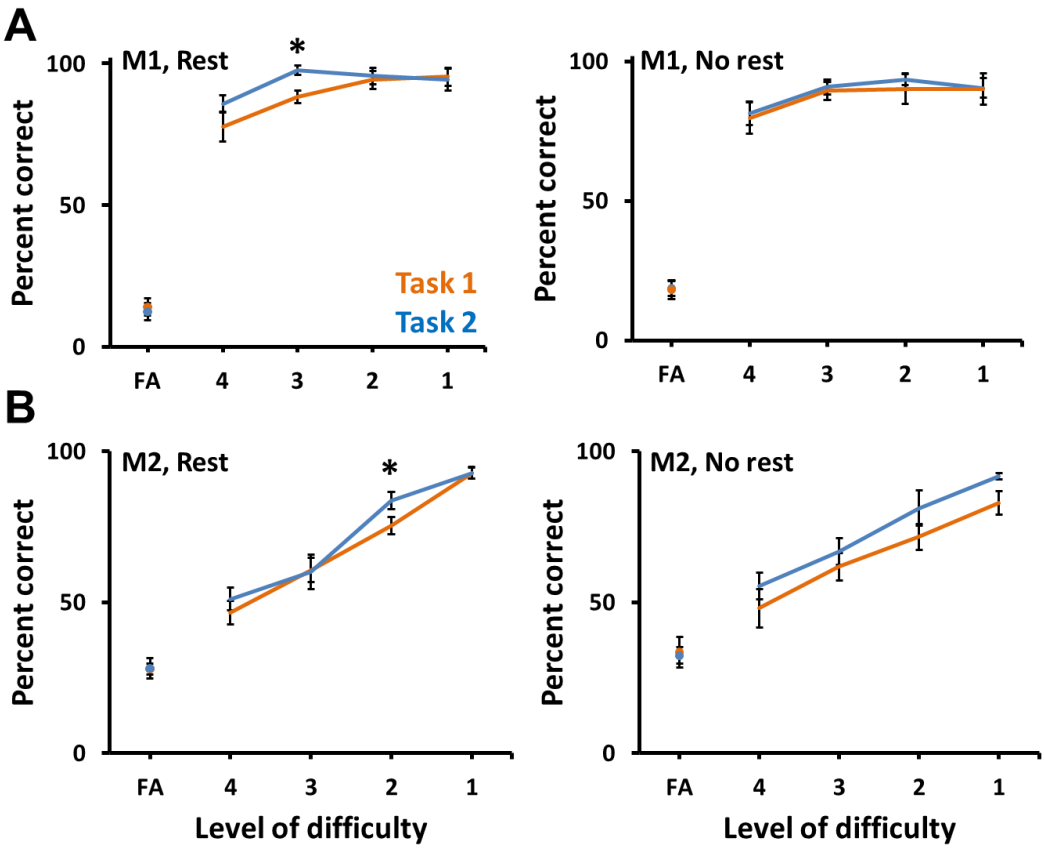

Figure 3.4 Behavioral performance improves with rest
Percent correct performance by orientation difference for monkey 1 (A) and monkey 2 (B) during Task 1 (orange) and Task 2 (blue) averaged over rest sessions (left) and no rest sessions (right). FA represents the false alarm rate – the percentage of same trials the monkey held the bar when he should have released it. Low false alarm rates indicate that the monkeys were actively performing the task. Both monkeys improved performance with rest but not without rest. Error bars represent S.E.M. and * represents $p < 0.01$.

Identification of sleep stages in macaque

To characterize the sleep stages of the monkeys during the 20 minute nap, I designed and created a custom polysomnograph cap that employs EEG, EOG and EMG recordings, all designed to be similar to those used in humans to stage sleep. After recording my resting state experiments in the M1, in a separate set of experiments (13 sessions) I allowed M1 to sleep for 45-60 minutes. Since this species of macaque has approximately a 56-minute sleep cycle, I reasoned that 45-60 minutes would allow me to observe all the stages of sleep.

Additionally, I included polysomnography in 16 sessions in M2 during the 20 minutes between tasks in both *rest* and *no rest* conditions. One sleep polysomnograph technician staged the sleep throughout the recordings in M1 using rules defined for staging humans sleep (Figure 3.5B) (Berry et al., 1968). Figure 3.5C shows an example of the activity associated with the transition of the monkey from an epoch scored as awake stage to an epoch scored as stage 2 sleep, as defined by the scorer. Note the decrease of high frequency activity in the EEG (F, C, and O) traces and decrease of muscle tone, indicated by decrease in activity in EMG recording on the chin. Analysis of sleep classification of the 12 sessions I recorded shows that the monkey was almost exclusively in *wake*, *stage 1*, or *stage 2* sleep during these 45 – 60 minute naps (Figure 3.6A). The sleep technician did observe REM sleep in 5 of the 12 sessions. Only 2 sessions had any slow-wave sleep (i.e., stages 3 or 4). Only one session had both REM and SWS. I assessed the time to the first scorable stage of sleep, known as sleep latency. I found a wide range of sleep latencies (Figure 3.6B, mean sleep latency = 7 ± 2.47 min). Previous reports of daytime naps in macaques found sleep latencies ranged from 8.6 to 20 minutes (Daley et al., 2006b). The average sleep latency in this experiment is close to the shorter time period previously reported. This may be because the monkey used in the current experiments was exposed to the experimental paradigm for 3 months prior to these experiments, and thus was well accustomed to falling asleep during this time.

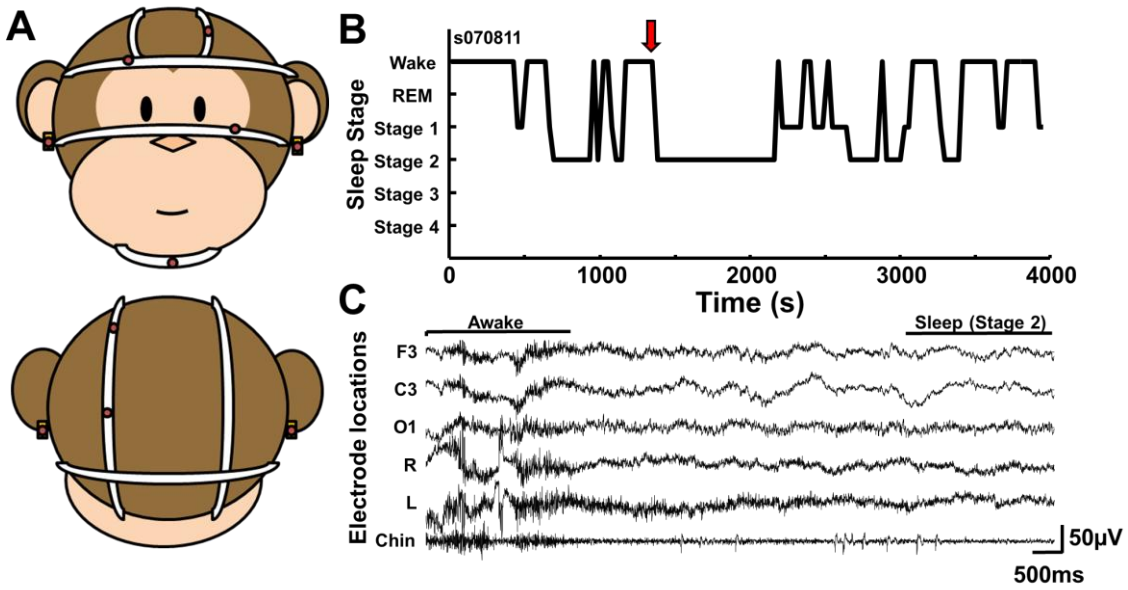

Figure 3.5. Polysomnography cap for macaques and sleep scoring
(A) Sleep cap designed for performing polysomnography recordings during daytime naps in monkeys. Recordings consisted of 3 electroencephalograms (EEGs) over F3, C3 and O1 locations, electrooculograms (EOGs) above right eye (R) and below left eye (L) and an electromyograms (EMG) over the monkeys mentalis muscle on the chin. Two ear-clip electrodes were used as ground and reference electrodes. (B) Example scoring of sleep by one polysomnograph technician. (C) Activity from electrodes at red arrow shown in B indicate a transition to sleep. Note the decrease in high frequency activity in EEGs and suppression of muscle activity (chin) as the monkey goes to sleep.

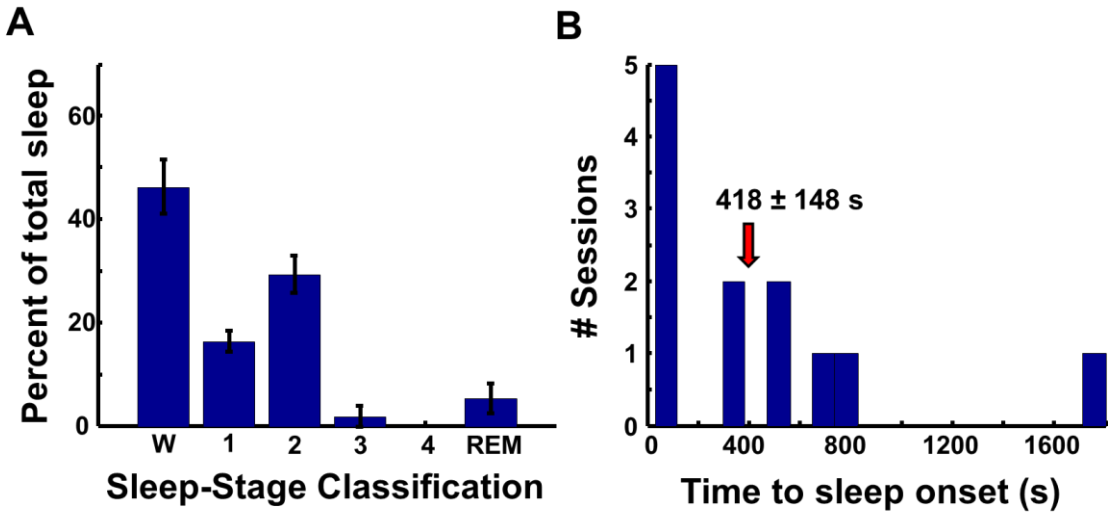

Figure 3.6. Sleep characterization from visual scoring of polysomnography
A total of 12 sessions from the polysomnography cap was scored by a polysomnograph technician using guidelines for human sleep scoring. (A) Average percentage of sleep stages over the 12 sessions. (B) Time to first epoch of scorable sleep, known as sleep latency, is shown. Red arrow shows mean sleep onset ± S.E.M.

Discussion

The goal of this work was to establish an experimental paradigm to elucidate neural correlates of perceptual learning improvement following daytime naps. To compare my work to human psychophysical literature, I needed to first test whether monkeys could show behavioral improvement in a perceptual learning task after rest. I trained two monkeys to perform a delayed match-to-sample natural image task before and after a 20 minute period where they were in a room with lights and monitors off. Quantification of the amount of time monkeys closed their eyes during the Lights Out period revealed that both monkeys had their eyes open with only periodic blinking during *no rest* sessions. Further, there were a few sessions in which the monkeys had their eyes open for the entirety of what was supposed to be a *rest* session. I therefore re-classified sessions into *rest* and *no rest* based on the percentage of time monkeys closed their eyes during the rest period. Using this classification, I observed a significant improvement in behavioral performance for both monkeys with *rest*.

This improvement was not seen in *no rest* sessions for either monkey. This is the first report demonstrating an improvement in a visual discrimination task after rest in monkeys. These behavioral results are similar to those observed in human nap studies and allow us to search for neural correlates underlying this effect.

To explore the neural correlates of this phenomenon, I implemented polysomnography to determine his sleep characteristics during daytime naps. Previous studies that performed polysomnography in nonhuman primates involve implantation of electrodes to determine sleep stages (Daley et al., 2006a; Hsieh et al., 2008; Reite et al., 1965; Weitzman et al., 1965). Since I was interested in studying the effects of daytime naps on perceptual learning and not a night of sleep, implantation of similar electrodes was not desired. Additionally, current polysomnography caps commercially available do not allow for simultaneous extracellular recordings. Thus, for these experiments, I designed a removable cap with EEG, EOG and EMG electrodes following rules standardized for humans (Berry et al, 2013). Scoring of the polysomnography was performed by one technician at Memorial Hermann Hospital trained to score human sleep. Analysis of the first scoring revealed that the daytime naps consist mostly of stages 1 and 2.

Given the improvement in behavioral performance, improved neural coding at the individual and network level in V4 after rest is expected. The ability to record both sleep stages as well as extracellular activity opens up the opportunity to explore whether similar improvements in V4 activity can be detected after rest. Many analytical techniques applied to electrophysiological data have been optimized to determine the coding capabilities of neurons whether by calculating their ability to discriminate stimuli or in the reliability of their response. Separate analyses have been established to investigate these measures in

individual neurons as well as populations; however, they have yet to be used to determine differences in coding properties of neurons with rest. At the level of individual neurons the discriminability of stimuli using concepts adapted from signal detection theory (Green and Swets, 1989) can be assessed. For example, I can calculate the difference in the mean firing rate of a single neuron in response to two different stimuli over the variance in the responses; this is known as d-prime (d'). The greater the difference in the mean responses and the smaller the variance, the higher the d' values and the more discriminability a neuron exhibits.

Another measure adapted from information theory (Shannon, 1948), mutual information, uses the same concept—how well neurons exhibit distinct firing rates—but infers this using probability. The basis of the information measurement is *entropy*, which relates to the variability in the response (specifically, the probability of observing each possible response). By considering the probability of observing a response for a particular stimulus and the variability within that response to each stimulus, I can assess how much information a neuron carries about various stimuli. A high probability of observing the same response for the same stimulus with low variability will give high information values, and vice versa.

Intrinsic to all of these discriminability measures is the known fact that neurons do not always exhibit the same response to the same stimulus. This variability in response can be measured directly. A specific example is the coefficient of variation (CV) (Yang and Maunsell, 2004). CV is calculated by dividing the standard deviation of the response by the mean response (Reinagel et al., 1999). Thus, the more reliably a neuron responds, the higher the CV. Given the nature of V4 as a feature selection extractor (Roe et al., 2012), demonstration of these improvements at the neural level would suggest that V4 is able to

modify its activity during rest such that individual neurons and networks are better able to encode behaviorally relevant features for the visual discrimination task I presented.

Because no neuron in the brain exists in isolation, examining *population* responses to stimuli is critical for determining how rest influences neural activity. This is especially true as rest might affect not only single neuron responses but also their responses in context of the rest of the population. To this end, measures have been established for studying the capability of networks to encode stimulus features, using principles similar to those for individual neurons. In populations, the same questions can be assessed: how efficiently are populations exhibiting distinct activity to different stimuli and how variable are the responses? Another way to think about the distinctness of the neural response is to ask how well one can determine which stimulus was presented, given the neural response. Determining the distinctness of neural responses to different stimuli can be achieved with a classifier. For example, to predict which test stimulus was presented, given a neural response, one can train a classifier with a distribution of stimuli and their measured neural responses from previously recorded trials. The performance of the classifier can be used as a measure to determine how unique the responses are to the different stimuli. The more distinct the responses, the more easily the classifier can separate out the responses by stimuli and the better performance it will have.

As with individual neurons, the variability in the population response can be computed. The degree of correlated variability is known as *noise correlations* (Abbott and Dayan, 1999; Averbeck and Lee, 2006; Averbeck et al., 2006). Noise correlations are calculated as the trial-by-trial correlated variability between two neurons. Restructuring correlated activity is one potential mechanism for how these features are extracted by which V4 could modify its

activity following rest. Since noise correlations can have opposing effects given the response properties of the neurons (Abbott and Dayan, 1999; Averbeck and Lee, 2006; Averbeck et al., 2006), it will be important to gently tease apart the relationships between neural codes and correlations in subsequent analyses. These analyses coupled with the sleep stages of monkeys during naps will provide an unprecedented view of how neural activity is modified following naps during perceptual learning.

Examination of the activity before and after rest is only one characterization that can be made from this activity. It is also possible to look at the extracellular activity during rest to search for neural correlates underlying the improvement in behavioral performance. In the next chapter, I will discuss a subset of analyses on the rest period activity. This work is a continuation of the research that I performed in Chapter 2, and the goal is to determine whether reactivation of task activity occurs during the 20 minute daytime rest period I described in this chapter.

"...Most animals have either keen eyes or sensitive eyes: cats have iridescent tapeta in their eyes for gathering the palest traces of light; but all that gathered scattery light in their eyes, then prevents cats from perceiving fine details. And hawks detect details, but since they do not have tapeta for collecting flickers, they must depend on the sun to boom down obvious light for them to see by. Your blessing is your curse and your curse is your blessing. Because you see details, you cannot see hints of light; because you see hints of light, you cannot see details. You would need diverse eyes if you wished to be equally penetrating and sensitive.

You would need to have eyes like the box jellyfish, with its sixteen light-sensitive eyes and eight acute cameralike eyes - all twenty-four eyes hanging down on stalks.

However, you would also need a brain.

But maybe that is not possible; maybe, in fact, the brainlessness of the box jellyfish is a direct consequence of its tremendous powers of sight. Perhaps neither the animal nor the prophet has been invented who could process so thorough a vision. It is disquieting enough to be hyperacute or hypersensitive; perhaps being both would very soon melt your brain and leave you quiescent, hanging transparently in the giant dancing green waters of the world."

-Please Don't Yell at the Sea Cucumber from "Things that Are" by Amy Leach 2012

4. REACTIVATION IN V4 DURING REST

Introduction

My discovery of reactivation in V4 cortical networks during awake states (Chapter 2) led me next to ask if reactivation was a general property of V4 networks. That is, does reactivation occur in other circumstances in V4? The time-locking of the reactivation event to the expected onset of the stimulus during blank trials suggested that reactivation in V4 may be dependent on an external cue.

To unpack this hypothesis, I first ask what events—external or internal—can trigger reactivation events in other regions of the brain. First, it is clear that external sensory input can cue reactivation in hippocampal place cells (Csicsvari et al., 2007; O'Neill et al., 2006). These cells respond selectively given the animal's physical position in space—for example, the animal's location along a linear track. Reactivation events during awake states are more likely to begin with place cells encoding the animals current location then other place cells involved in the sequential reactivation (Csicsvari et al., 2007; O'Neill et al., 2006). To the best of my reading, all of the examples of sequential reactivation in the hippocampal literature are either triggered by an external stimulus—*or* are associated with an internally generated event, such as sharp-wave ripples (SWRs) produced by the hippocampus (Maier et al., 2003). For example, reactivation events in the hippocampus during sleep are observed during or after SWRs (Lee and Wilson, 2002; Louie and Wilson, 2001). In other studies, during slow-wave sleep, SWRs appear to initiate and coordinate reactivation events in the hippocampus and primary visual cortex (Ji and Wilson, 2007). During the awake state, SWRs are associated with reactivation events in the hippocampus as well (Diba and Buzsáki, 2007; Foster and Wilson, 2006; O'Neill et al., 2008). The exception of this requirement of an initiation event is reactivation found in the hippocampus during REM sleep (Louie and

Wilson, 2001). Here, it is not immediately obvious what the trigger might be; however, the robust theta oscillations found during this time may be capable of initiating a sequence (Buzsaki, 2010).

In other words, examples of reactivation in the cortex suggest that *internal cues are capable of initiating reactivation*. For example, a spontaneous rehearsal of a sequence of squares causing a wave of electrical activity across visual cortex was observed in an anesthetized rat experiment using voltage sensitive dyes (Han et al., 2008). The wave of electrical activity was more likely to occur in the same pattern as the stimulus-evoked activity after stimulus presentation. When a human or animal is anesthetized, the brain exhibits slow oscillations between periods of high activity and low activity, commonly known as 'up' and 'down' states (Steriade et al., 1993). Such oscillatory activity itself, apart from internal events such as SWRs, has been proposed as a possible initiator and modulator of reactivation activity (Buzsaki, 2010). Thus, this innate oscillation of activity, especially the bursting 'up' states, in which cells are highly depolarized and exhibit bursting activity (Steriade et al., 1993) during anesthesia, could be the top-down influence that initiates this *spontaneous* reactivation.

A key question is whether internal initiation events can occur during resting states in cortex when SWRs and other obvious initiation events are notably absent. Reactivation in distributed cortical circuits involved in a sequential reaching task was demonstrated in monkeys during rest (Hoffman and McNaughton, 2002b). In this experiment, 12 x 12 arrays of electrodes were implanted in posterior parietal cortex (PP), motor cortex (M), somatosensory cortex (SS) and dorsal prefrontal cortex (PFC) in 1 monkey. The monkey had a 30-minute rest before and after the task. They found that cells in these distributed areas

showed more correlated activity after the task than before. Specifically, this was found within M, and SS and between PP and M (Hoffman and McNaughton, 2002b). This suggests that neurons in distributed brain continue to have coordinated activity after a task coactivates them, even in the absence of an obvious external cue. The presence of increased correlated activity within cortical areas after task exposure suggests that networks may be capable of reactivating sequences of task-evoked activity during resting states. However, this has yet to be tested experimentally.

To determine whether reactivation occurs during quiescent resting states in visual area V4, I examined two 20 minute rest periods occurring both before (Rest 1) and after (Rest 2) a delayed-match-to-sample task. Using a template-matching method, I tested whether stimulus-evoked activity was reactivated during a rest period after the task while the monkey was in a dark, quiet room. With this analysis, I am able to assess two properties of the neural activity that reflect reactivation: the similarity of the reactivation events to the task and the percentage of time a reactivation event is observed. Since reactivation has been shown to decay in cortex after stimulus offset (Han et al., 2008; Hoffman and McNaughton, 2002a; Xu et al., 2012), I reasoned that Rest 2, which occurs after exposure to the task, should show either a greater similarity of sequential activity to the task or a greater percentage of reactivation events than Rest 1.

Methods

Experimental Design

All experiments were performed in accordance with NIH Guidelines for the Care and Use of Animals for Experimental Procedures and the Animal Welfare Committee at the

University of Texas Health Science Center at Houston. Two male rhesus monkeys rested in an experimental room with the lights and monitors off and with white background noise for 20 minutes (*Rest 1*; Figure 4.1A). During this time, eye closure was monitored using an eye tracker (Eyelink II for M1 and Eyelink 1000 desktop mount for M2, SR Research) and infrared video monitoring. The monkeys then performed a delayed match-to-sample natural image task (Figure 4.1B). After the task, monkeys again remained in the room with lights and monitors off and white background noise for another 20 minutes (*Rest 2*). For task specifics, refer to the Visual Orientation Discrimination Task description in Chapter 3. Note that the timing of the task was different for each monkey. Both had a 400 ms fixation period before the target appeared. However, M1 had 300 ms stimulus presentation separated by a 1000 ms interstimulus interval (ISI) and M2 had 366 ms stimulus presentation separated by a 1250 ms ISI.

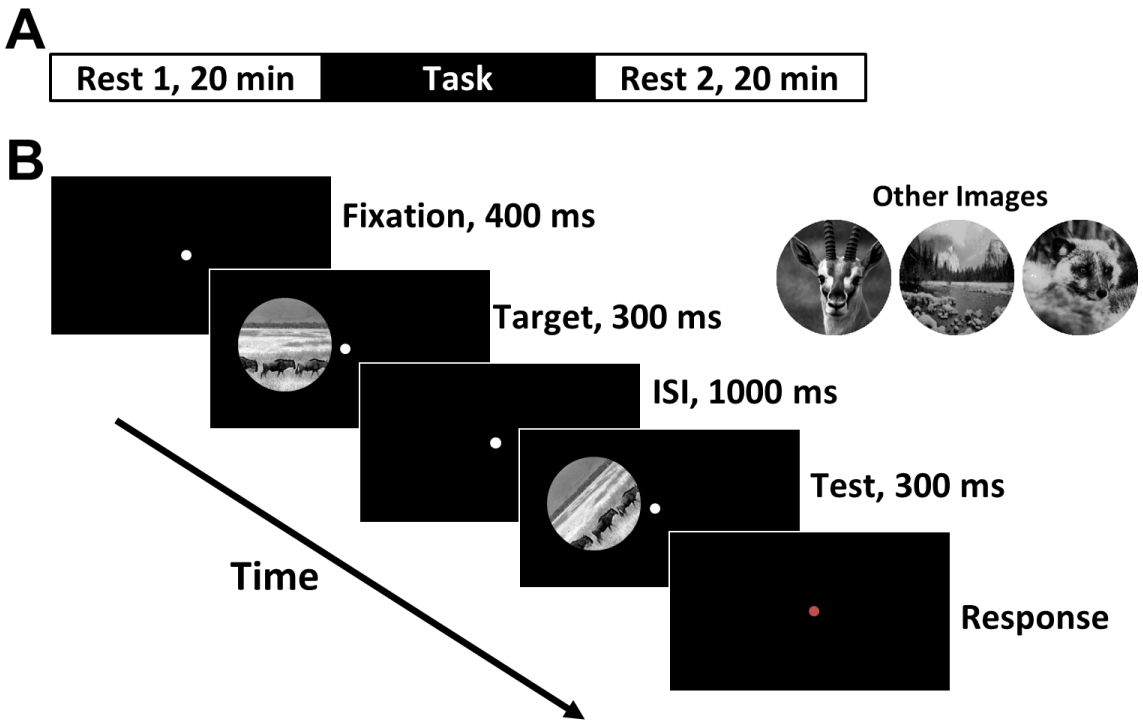

Figure 4.1 Experimental design
(A) Experimental design. To investigate whether reactivation occurs during periods of quiescence, monkeys were placed in a dark, quiet room before (Rest 1) and after (Rest 2) task exposure for a 20 minute rest. (B) Natural image delayed match-to-sample task. In this task, the same natural image was serially presented either at the same orientation or with the test image rotated. The monkeys released or held a response bar to indicate whether the images are the same or rotated, respectively. The degree of rotation between the images was adjusted so the task remained difficult for the monkeys. The timing for M1 is shown. Refer to Experimental Design for the timing of the task, as this was different for each monkey.

Electrophysiological recordings

For extracellular recording methods refer to the Electrophysiological Recordings section in Chapter 2. The same data described in Chapter 3 was used here. I recorded a total of 25 sessions with M1 with 100 cells responsive to visual stimuli and 6 sessions with M2 with 26 visually responsive cells (see Extracellular recordings in Chapter 3 for details on selection of visually responsive cells).

Reactivation analysis

To determine whether previously stimulus-evoked activity was reactivated during the 20 minute rest period, I used a template-matching method (Louie and Wilson, 2001; Ribeiro et al., 2004a; Tatsuno et al., 2006). This method can be used to assess the similarity between the task evoked activity and equivalent chunks of the rest period activity. To construct the template, I averaged the stimulus evoked activity per cell starting from 80 ms after *target* onset and ending 80 ms after *test* offset for both monkeys in 50 ms bins. This was the period of time in which I observed the start of the evoked response to the *target* and decrease in response to the *test*. The timing is consistent with previous reports of V4 response latencies (Schmolesky et al., 1998). The average firing rate for all simultaneously recorded cells made up the task template (Figure 4.2C). An equivalent time window during the rest period (chunk) for the same cells starting at the beginning of the time when the monkeys were in the dark quiet room was compared to this template (Figure 4.2A rest period activity, C and D are examples of rest period activity organized into the same dimensions as the template). The template (A) and equivalent rest chunk (B) were *M* x *N* matrices:

$$A = \begin{bmatrix} A_{11} & A_{12} & \dots & A_{1N} \\ A_{21} & A_{22} & \dots & A_{2N} \\ \vdots & \vdots & \dots & \vdots \\ A_{M1} & A_{M2} & \dots & A_{MN} \end{bmatrix}, B = \begin{bmatrix} B_{11} & B_{12} & \dots & B_{1N} \\ B_{21} & B_{22} & \dots & B_{2N} \\ \vdots & \vdots & \dots & \vdots \\ B_{M1} & B_{M2} & \dots & B_{MN} \end{bmatrix}$$

where *M* represents the number of simultaneously recorded cells and *N* represents the number of bins. The number of cells per template varied by the number of neurons recorded simultaneously per session. M1 had a 1.65 s template of 33 bins and M2 had a 1.95 s template of 39 bins. The difference in timing between the monkeys had to do with the different stimulus presentation times (M1: 300 ms image presentation, 1000 ms ISI; M2: 366 ms image presentation, 1250 ms ISI) given the differences in monitor refresh rates (M1: 60

Hz, M2: 75 Hz). Values in these matrices were then z-scored across bins for each cell separately using the following equations:

$$A_{MN} = \frac{A_{MN} - \bar{A}_M}{\sigma_{A,M}} \ , \ B_{MN} = \frac{B_{MN} - \bar{B}_M}{\sigma_{B,M}} \quad (2)$$

where $\bar{A}$ and $\bar{B}$ were the means calculated as:

$$\bar{A}_M = \frac{1}{N}\sum_{n=1}^{N} A_{MN} \ , \ \bar{B}_M = \frac{1}{N}\sum_{n=1}^{N} B_{MN} \quad (3)$$

and σ is the standard deviation calculated as:

$$\sigma_{A,M} = \sqrt{\frac{1}{N}\sum_{n=1}^{N}(A_{MN} - \bar{A}_M)^2} \ , \ \sigma_{B,M} = \sqrt{\frac{1}{N}\sum_{n=1}^{N}(B_{MN} - \bar{B}_M)^2} \quad (4)$$

The similarity between the two z-scored response matrices was computed using Pearson correlation (CC, see Equation 1 in Chapter 2). Positive correlations indicate similar activity, 0 means there is no related activity, and negative correlations indicate the rest activity exhibits an opposite pattern from the task activity. In addition, I calculated the significance of the correlation for each template comparison. This was performed by creating a *t-statistic* by transforming the correlation with *n*-2 degrees of freedom, where n is the number of cells in the template (Cohen et al., 2002). The confidence level (CL) is set by an asymptotic normal distribution:

$$CL = 0.5 * \log(\frac{1+CC}{1-CC}) \quad (5)$$

where CC is the Pearson correlation between the response matrices. The distribution has an approximate variance of:

$$\frac{1}{n-3} \quad (6)$$

where *n* is the number of matrix elements. This method of significance testing will be referred to as the *t-statistic* method.

The template was continuously compared to rest chunks by a sliding window of 50 ms (the bin width) for the entire duration of the rest period. Each time the template was compared to the chunk, a correlation and the significance of that correlation was calculated. This analysis was performed for the pre-task rest (*Rest 1*) and post-task rest (*Rest 2*) periods using the same task template.

For each rest period, the average total positive correlations (PC), the percentage of significant reactivations or percent matches (PM, $p<0.05$), and the average significant correlation values (SC, the correlation when $p<0.05$) were assessed. Because reactivation has been shown to decline with time after stimulus offset (Han et al., 2008; Hoffman and McNaughton, 2002a; Xu et al., 2012), the correlation values should be greater in *Rest 2* than *Rest 1*. Alternatively, more significant matches should occur in *Rest 2* compared with *Rest 1* if reactivation occurred during this time. Several variations on this calculation, described below, were performed to test whether reactivation occurs in V4 during extended, quiescent awake periods.

Results

Reactivation during rest

I used multi-unit extracellular recordings in macaque V4 to determine whether a reactivation of the stimulus-evoked response occurred during a 20 minute rest after task exposure. During the 20 minute rest, lights and monitors were turned off in the experimental room and white background noise was played. Monkeys were observed to close their eyes for various amounts of time throughout this period (Figure 3.2). An example of the neuronal activity during both rest sessions is depicted in Figure 4.2A. The task template (Figure 4.2B) captured the responses of all neurons to the stimulus, and differences between cells in the

interstimulus interval activity. Examples of the template-matching method 'matches' (Figure 4.2C) and 'non-matches' (Figure 4.2D) illustrates this method detects rest activity visually similar to the template.

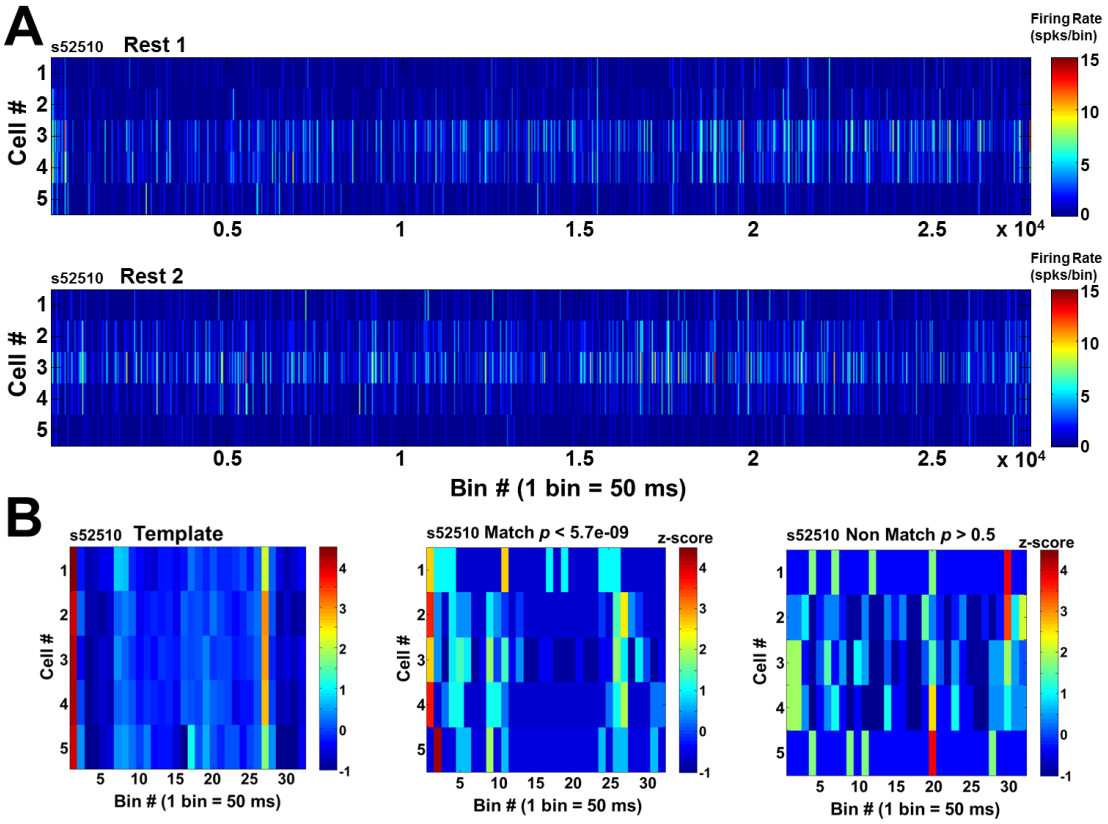

Figure 4.2 Examples of rest period activity and templates
A template matching procedure was used to determine whether reactivation occurred after task exposure during rest. (A) The responses of representative neurons across Rest 1 and Rest 2. (B) The template was created by combining averaged and z-scored activity from each cell during the task period stimulus presentation in to a matrix of responses. An equivalent window of time was compared during rest periods and a correlation was calculated. The template was moved across the rest period in a sliding window by one bin at a time. The significance of the correlation was calculated at each comparison A *p*-value of less than 0.05 was considered a match (C). A *p*-value greater than 0.05 was considered a non-match (D).

The important comparison to determine whether reactivation occurs during this time is that there is more similarity between rest activity and templates (higher positive correlations PC or significant correlations, SC) or a significantly greater percentage of reactivation events

(percent matches, PM) in *Rest 2* compared to *Rest 1*. Here, matches were considered significant using the *t-statistic method* to generate the *p*-value. The results from the *shuffling method* will be discussed next.

A significant difference in positive correlations (PC), significant correlations (SC), or percent matches (PM) was not observed between *Rest 1* and *Rest 2* in either monkey (Figure 4.3 A-C). No significant difference in total positive correlations (M1: PC_{R1} = 0.10, PC_{R2} = 0.10, n = 10, $p > 0.05$; M2: PC_{R1} = 0.09, PC_{R2} = 0.09, n = 6, $p > 0.05$, Wilcoxon signed rank), percent template matches (M1: PM_{R1} = 6.78%, PM_{R2} = 8.57%, $p > 0.05$; M2: PM_{R1} = 7.85%, PM_{R2} = 8.43%, $p > 0.05$), nor significant positive correlations (M1: SC_{R1} = 0.23, SC_{R2} = 0.24, $p > 0.05$; M2: SC_{R1} = 0.21, SC_{R2} = 0.21, $p > 0.05$). I also tested the hypothesis that reactivation may occur at the individual cell level, which would be demonstrated by increases in the same measures in Rest 2 compared to Rest 1 in individual cells. I did not find a significant increase in any of the measures in Rest 2 compared to Rest 1 (Figure 4.3 D-F). This was true for total positive correlations (M1: PC_{R1} = 0.17, PC_{R2} = 0.17, n = 91, $p > 0.05$; M2: PC_{R1} = 0.17, PC_{R2} = 0.17, n = 30, $p > 0.05$), percent matches (M1: PM_{R1} = 4.83%, PM_{R2} = 4.98%, $p > 0.05$; M2: PM_{R1} = 6.33%, PM_{R2} = 6.03%, $p > 0.05$), and significant positive correlations (M1: SC_{R1} = 0.45, SC_{R2} = 0.45, $p > 0.05$; M2: SC_{R1} = 0.42, SC_{R2} = 0.41, $p > 0.05$).

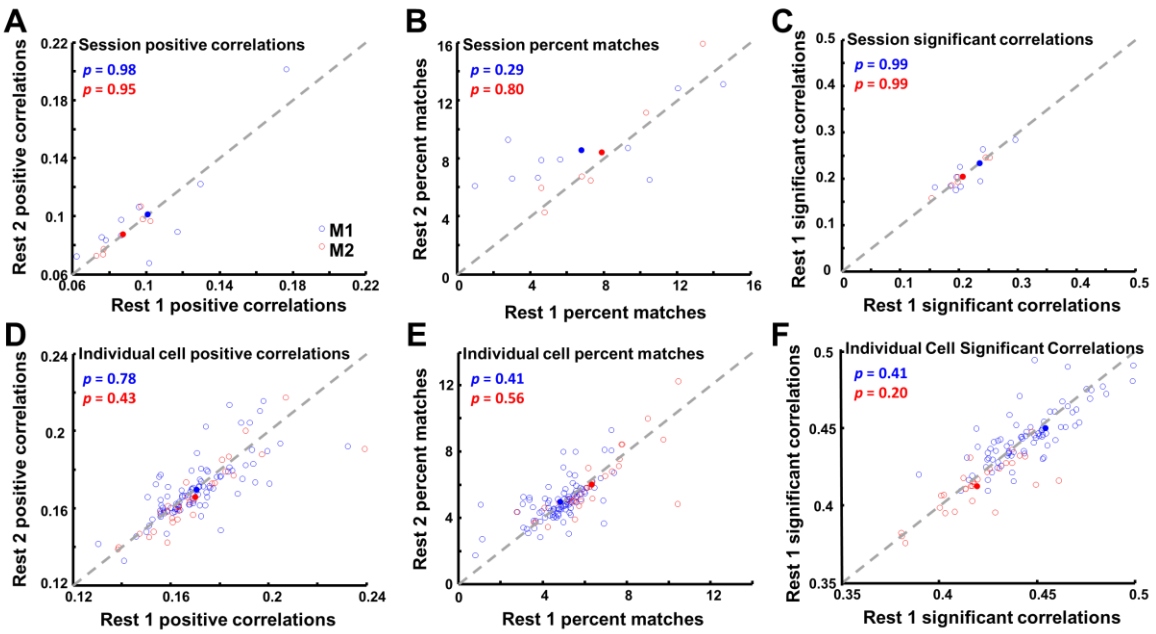

Figure 4.3. Reactivation template-matching analysis using z-score normalization yields no significant difference in reactivation between Rest 1 and Rest 2
A template matching procedure was used to determine if reactivation occurred in populations of simultaneously recorded cells or individual neurons during a rest period following the task. If reactivation occurred, then a higher average positive correlation, more significant reactivation events, matches, or higher significant positive correlations between the template and rest activity should occur following task exposure. I did not observe a higher overall positive correlation (A), greater percentage of matches (B), or higher significant correlations (C) in Rest 2 compared to Rest 1. The same analysis was performed on individual neurons testing the same hypothesis. Higher positive correlations (D), a greater number of matches (E), nor higher significant correlations were observed. Results for M1 are shown in blue and for M2 in red. All comparisons of Rest 1 to Rest 2 were not significant with all $p > 0.05$.

Normalizing firing rates to zero mean with unit variance using z-scoring does not maintain the differences in firing rates between cells. Thus, the correlation between z-scored neural responses only captures the firing rate fluctuations in response to the stimulus. Perhaps differences in mean firing rates between cells are important for accurately capturing reactivation. To that end, I investigated whether an alternative normalization method would cause differences in my reactivation estimates. Thus, I examined the correlation after

normalizing responses by dividing individual cells by their root mean squared (RMS) firing rates (Louie and Wilson, 2001; Ribeiro et al., 2004b) using the following equation:

$$A_{MN} = \frac{A_{MN}}{\sqrt{\frac{1}{N}\sum_{n=1}^{N} A_{MN}^2}} \quad , \quad B_{MN} = \frac{B_{MN}}{\sqrt{\frac{1}{N}\sum_{n=1}^{N} B_{MN}^2}} \tag{7}$$

As previously defined, *M* is the number of simultaneously recorded cells and *N* is the number of bins in response matrices for the task template, *A*, and equivalent chunk of time during rest, *B*. I tested whether there are significantly greater positive or significant correlations or greater percent template matches at the same timescale as the evoked activity in Rest 2 compared with Rest 1.

In this analysis, no significant difference between Rest 1 and Rest 2 was observed (Figure 4.4 A-C) in total positive correlations (M1: PC_{R1} = 0.10, PC_{R2} = 0.10, n = 10 $p > 0.05$; M2: , PC_{R1} = 0.09, PC_{R2} = 0.09, n = 6, $p > 0.05$, Wilcoxon signed rank test), percent template matches (M1: PM_{R1} = 7.02%, PM_{R2} = 9.08%, $p > 0.05$; M2: PM_{R1} = 7.40%, PM_{R2} = 8.53%, $p > 0.05$), nor significant positive correlations (M1: SC_{R1} = 0.24, SC_{R2} = 0.24, $p > 0.05$; M2: SC_{R1} = 0.21, SC_{R2} = 0.21, $p > 0.05$). I also tested whether this occurred in individual cells and again did not observed a significant difference between Rest 1 and Rest2 (Figure 4.4 D-F) in average positive correlations (M1: PC_{R1} = 0.17, PC_{R2} = 0.17, n = 91, $p > 0.05$; M2: PC_{R1} = 0.17, PC_{R2} = 0.17, n = 30, $p > 0.05$), percent template matches (M1: PM_{R1} = 4.85%, PM_{R2} = 4.97%, $p > 0.05$; M2: PM_{R1} = 6.33%, PM_{R2} = 6.03%, $p > 0.05$), and average significant positive correlations (M1: SC_{R1} = 0.45, SC_{R2} = 0.45, $p > 0.05$; M2: SC_{R1} = 0.42, SC_{R2} = 0.41, $p > 0.05$).

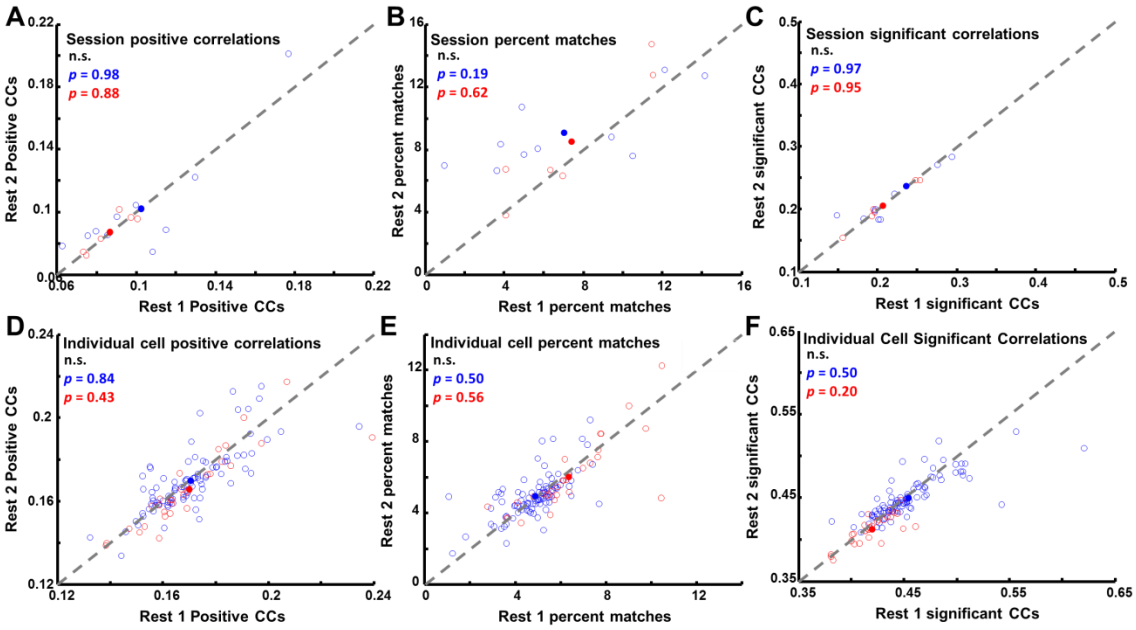

Figure 4.4. Template-matching analysis using root-mean-square normalization does not show evidence for increased reactivation during rest
Normalizing the data using z-scores does not preserve the firing rate differences between cells which may be important for reactivation analyses. Thus, values were normalized by dividing each cell by its root-mean-square (RMS) prior to the correlation computation. The total positive correlations, percent matches, and significant correlations were computed. No difference was observed in these measures between Rest 1 and Rest2. Specifically, no difference in populations in positive correlations (A), percent matches (B), or greater significant correlations (C) were observed. The RMS method was also used to test whether individual cells exhibited reactivation. No significant effects in any of these measures (D-F) were observed. All comparisons were not significant, all $p > 0.05$.

Because the reactivation analysis does not differ significantly between the z-score normalization method and RMS-method, I can conclude that maintaining the firing rate differences between cells does not influence correlations. The z-score method was used to test further possibilities of how reactivation may be occur in V4 to make results more consistent with those in Chapter 2.

Temporal dynamics of extended reactivation after stimulus offset

Previous reports have found that reactivation decays within an hour after exposure in the hippocampus (Kudrimoti et al., 1999; Pennartz et al., 2004) and visual areas (Han et al., 2008; Xu et al., 2012). A decay within as little as 10 minutes in cortical networks has also been reported (Hoffman and McNaughton, 2002a). Thus, by examining the entire rest period, it is possible that differences between *Rest 1* and *Rest 2* were not observed because time after the reactivation had already decayed was included in the analysis. To determine if this was true, the hypothesis that the correlation values decayed with time from task offset in Rest 2 was tested. Additionally, whether smaller percentage of matches were observed with increasing time from the task was tested. For comparison the same decay analysis was performed on Rest 1.

To test if there was a significant change in the correlations with time the total number of comparisons across 15 minutes of rest activity (18000 comparisons) were averaged in to 1 min bins (1200 comparisons per bin, 15 bins total). The average correlation was then calculated across all sessions. A regression between the average correlation vector and time (1 through 15 minutes) was performed for both Rest 1 and Rest 2 separately (Figure 4.5A). A significant negative correlation in Rest 2 would represent decay in reactivation following task offset. No significant correlation (CD) was observed for either monkey in either rest conditions (M1: $CD_{CCR1} = -0.07$, $p > 0.05$, $CD_{CCR2} = -0.23$, $p > 0.05$; M2: $CD_{CCR1} = -0.0442$, $p > 0.05$, $CD_{CCR2} = -0.24$, $p > 0.05$). Additionally, another way in which reactivation may decay is in the frequency of reactivation of events. To test this, the percentage of matches (positive correlations with p-values < 0.05) were computed across all comparisons starting from task offset to 15 minutes post task in 1 minute bins (1200 comparisons per bin, 15 bins

total; Figure 4.5B). A regression between the vector of percent matches was then correlated with time for each rest period individually to determine whether decay occurred. A significant negative correlation in Rest 2 would mean there was less frequent reactivation events with increasing time from stimulus offset. No significant correlation was observed between the percent matches and time for M1 (CD_{PMR1} = 0.0007, $p > 0.05$, CD_{PMR2} = 0.0003, $p > 0.05$). However, a significant increase in percent matches was observed for M2 for both rest periods (CD_{PMR1} = 0.38, $p < 0.05$, CD_{PMR2} = 0.37, $p < 0.05$). Both of these results demonstrate that the temporal proximity to stimulus offset does not decrease the likelihood of reactivation.

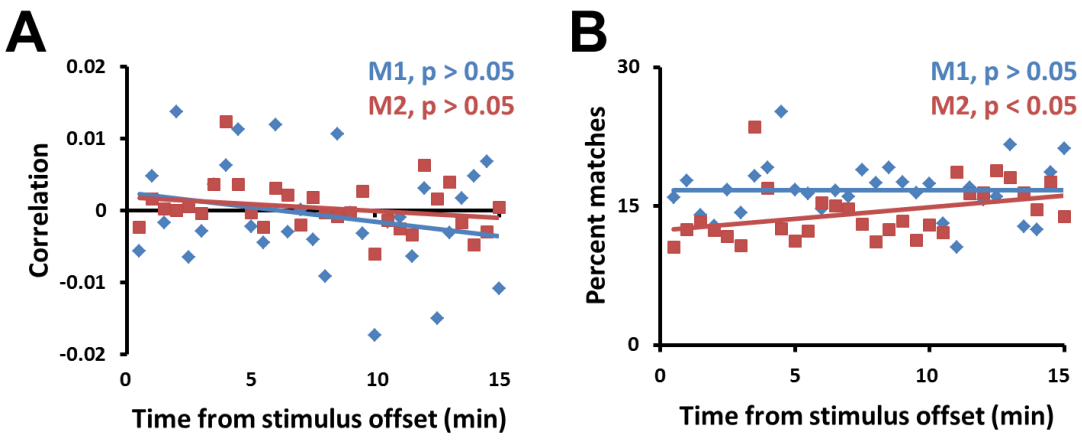

Figure 4.5. Temporal dynamics of reactivation after stimulus exposure
The possibility that reactivation was not found because it declined after stimulus offset was explored. (A) The Pearson correlation from the first comparison after from task offset to 15 minutes post task was averaged across all sessions for the Rest 2 period. (B) Additionally, the percentage of matches across all recorded sessions was calculated for the same comparisons in this time window. If either of these measures showed a significant negative trend with time, it means that reactivation declines after task offset. No decay in either measure was observed. A significant increase in the percentage of matches was observed in M2.

Since a significant increased frequency of percent matches was observed in M2, I further tested whether proximity to the task affected whether or not I observed reactivation. To this end, the first 10 minutes of Rest 1 was compared to the first 10 minutes of Rest 2. No difference between the first 10 minutes of each rest period in total positive correlations (M1: $PC_{R1} = 0.11$, $PC_{R2} = 0.10$, n = 9, $p > 0.05$; M2: $PC_{R1} = 0.09$, $PC_{R2} = 0.08$, n = 6, $p > 0.05$, Wilcoxon signed rank), number of significant reactivation events (M1: $PM_{R1} = 10.14\%$, $PM_{R2} = 9.16\%$, $p > 0.05$; M2: $PM_{R1} = 7.67\%$, $PM_{R2} = 8.49\%$, $p > 0.05$), or differences in the significant correlations (M1: $SC_{R1} = 0.24$, $SC_{R2} = 0.24$, $p > 0.05$; M2: $SC_{R1} = 0.20$, $SC_{R2} = 0.20$, $p > 0.05$) was observed. For comparison, the second 10 minutes of Rest 1 was compared to the first 10 minutes of Rest 2. There was also no significant difference observed between the rest periods. Specifically, no significant differences in total positive correlations (M1: $PC_{R1} = 0.11$, $PC_{R2} = 0.10$, n = 9, $p > 0.05$; M2: $PC_{R1} = 0.09$, $PC_{R2} = 0.08$, n = 6, $p > 0.05$), number of significant reactivation events (M1: $PM_{R1} = 9.69\%$, $PM_{R2} = 9.16\%$, $p > 0.05$; M2: $PM_{R1} = 9.88\%$, $PM_{R2} = 8.49\%$, $p > 0.05$), or differences in the significant correlations (M1: $SC_{R1} = 0.24$, $SC_{R2} = 0.24$, $p > 0.05$; M2: $SC_{R1} = 0.21$, $SC_{R2} = 0.20$, $p > 0.05$) were observed.

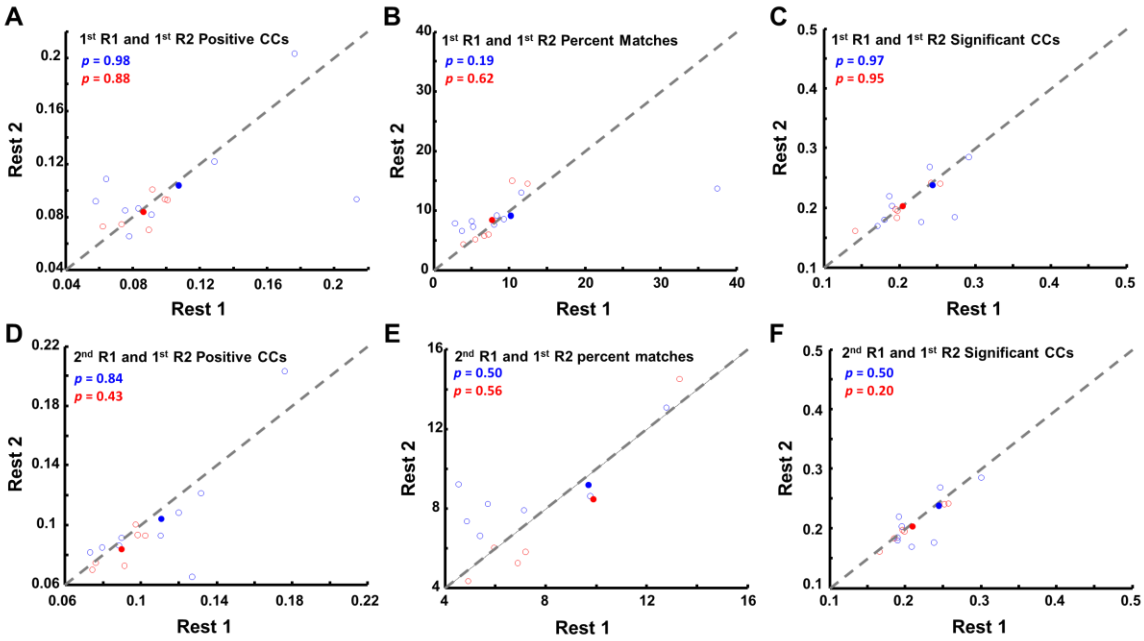

Figure 4.6 Reactivation during the first and second half of rest
To determine whether reactivation occurred within the first 10 minutes of Rest 2, the first 10 min of Rest 1 was compared using the template matching method measures (positive correlations, percent matches, and significant correlations) to the first 10 minutes of Rest 2 (A-C). For comparison, the second half of Rest 1 was compared to the first half of Rest 2 (D-F). Results are shown for M1 (blue) and M2 (red) separately. All comparisons were not significant, $p > 0.05$.

Reactivation and eye closure

Although previous work in the hippocampus has found reactivation during periods of awake, quiescent, immobility (Carr et al., 2011; Foster and Wilson, 2006; Karlsson and Frank, 2009; Louie and Wilson, 2001; Pavlides and Winson, 1989) and during running (Cheng and Frank, 2008; O'Neill et al., 2006), I reasoned that perhaps the ongoing demands of the visual system while the eyes are open (Barry et al., 2007) may inhibit reactivation from occurring. Previous work using fMRI in humans has shown that distinct areas are active during rest in a dark room when eyes are open or closed (Marx et al., 2003, 2004). Specifically, while the eyes were open, they found that areas involved in attention and eye movements were active such as the right precentral gyrus extending to the middle frontal

gyrus, in cerebellar structures, and bilaterally in the oribitofrontal cortex (Marx et al., 2003, 2004). When the eyes were closed, sensory cortices (visual, somatosensory, vestibular, and auditory) were active when eyes were closed (Marx et al., 2003, 2004). Specific activations were found in the inferior, middle and superior occipital gyri, the fusiform gyri, and the lingual gyri (Marx et al., 2003, 2004). They suggest that the activation of sensory cortices during eye closure represents an internally directed ("interoceptive") state compared to an externally directed ("exteroceptive") state when eyes are open (Marx et al., 2003, 2004). .

Thus, I tested whether significant correlations or percent matches changed when the monkeys had their eyes opened or closed. This analysis was only performed for M1 as the majority of sessions were recorded in this animal and I was unable to determine accurate eye closure for M2. No difference in the percentage of time the monkey had his eyes closed between Rest 1 and Rest 2 was observed (R1: 16 ± 4 %, R2: 17 ± 3%, $p > 0.05$, Table 4.1). Further, there was no difference in the average amount of time the monkey closed his eyes during these two rest periods (R1: 1.29 ± 0.27 s, R2: 1.47 ± 0.25 s, $p > 0.05$, Table 4.1).

Additionally, there was no significant difference between Rest 1 and Rest 2 (Figure 4.7), or a significant difference when the monkey had his eyes open or closed. Specifically, there was no difference between rest periods in total positive correlations (Closed: $PC_{R1} = 0.11$, $PC_{R2} = 0.11$, n = 8, $p > 0.05$; Open: $PC_{R1} = 0.12$, $PC_{R2} = 0.10$, $p > 0.05$), number of significant reactivation events (Closed: $PM_{R1} = 8.85\%$, $PM_{R2} = 9.97\%$, $p > 0.05$; Open: $PM_{R1} = 10.9\%$, $PM_{R2} = 9.07\%$, $p > 0.05$), or differences in the significant correlations (Closed: $SC_{R1} = 0.24$, $SC_{R2} = 0.25$, $p > 0.05$; Open: $SC_{R1} = 0.26$, $SC_{R2} = 0.25$, $p > 0.05$). Individual cells do not exhibit any significant differences between Rest 1 and Rest 2. There are no significant differences in total positive correlations (Closed: $PC_{R1} = 0.17$, $PC_{R2} = 0.17$, n =

81, $p > 0.05$; Open: $PC_{R1} = 0.17$, $PC_{R2} = 0.17$, $p > 0.05$), number of significant reactivation events (Closed: $PM_{R1} = 5.20\%$, $PM_{R2} = 5.15\%$, $p > 0.05$; Open: $PM_{R1} = 5.49\%$, $PM_{R2} = 4.91\%$, $p > 0.05$), or differences in the significant correlations (Closed: $SC_{R1} = 0.46$, $SC_{R2} = 0.45$, $p > 0.05$; Open: $SC_{R1} = 0.45$, $SC_{R2} = 0.45$, $p > 0.05$).

	Mean closed (sec)	% closed
Rest 1	1.29 ± 0.27	16 ± 4
Rest 2	1.47 ± 0.25	17 ± 3
P value	0.20	0.57

Table 4.1. Eye closure during rest
The amount of time the monkey had his eyes closed during each period of rest was evaluated by the average duration of time he closed his eyes during Rest 1 and Rest 2 and the percentage of the total time he closed his eyes while in the room with the lights off. These results are for M1 only.

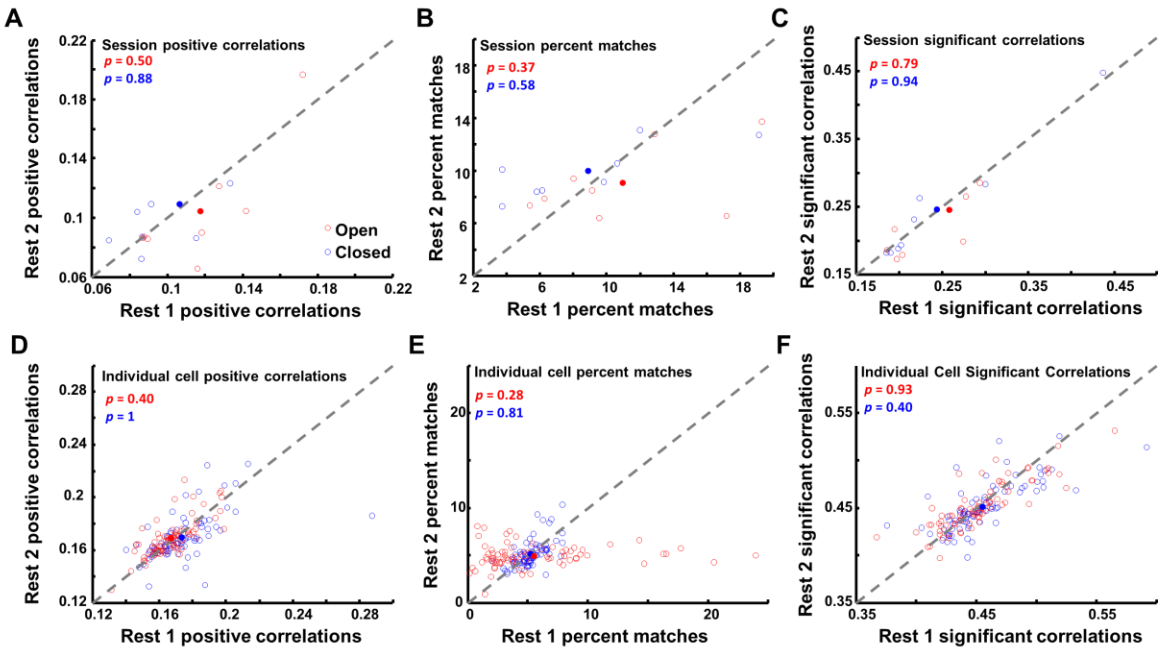

Figure 4.7 Reactivation does not depend on eye closure
I explored whether I did not find reactivation because it might only occur when the animal had his eyes closed, as this may represent a more rested state in visual cortex. I examined whether the overall positive correlations, percentage of matches, or significant correlations were greater in Rest 2 compared to Rest 1 when the monkey had his eyes open or closed. There is no significant increase in Rest 2 at the population level (A-C), nor a significant difference in these measures when the monkey had his eyes open or closed.

Examining compressed and expanded reactivation

Previous work in the hippocampus and early visual cortex (V1) has demonstrated that reactivation occurs at a compressed timescale, roughly twice the speed of the original experience, from the original experience during subsequent sleep (Ji and Wilson, 2007). I explored whether this was true in my data by altering the bin size of the rest period templates I was comparing to the task template. As an example, I explored whether reactivation occurred at twice the speed of the original experience by keeping the template bin size at 50 ms and comparing the template to 25 ms bins during the rest experience. Note that this does not alter the number of bins I am selecting for each comparison, only the size of the bin for the rest period. Using this analysis, I did not find any evidence of reactivation in the total

positive correlations (M1: PC_{R1} = 0.11, PC_{R2} = 0.11, n = 8, $p > 0.05$; M2: PC_{R1} = 0.11, PC_{R2} = 0.09, n = 5, $p > 0.05$), number of significant reactivation events (M1: PM_{R1} = 4.74%, PM_{R2} = 7.63%, $p > 0.05$; M2: PM_{R1} = 7.13%, PM_{R2} = 7.17%, $p > 0.05$), or differences in the significant correlations (M1: SC_{R1} = 0.26, SC_{R2} = 0.26, $p > 0.05$; M2: SC_{R1} = 0.26, SC_{R2} = 0.21 , $p > 0.05$). As in previous analyses I also compared individual cells using the same measure and again did not find any significant differences between Rest 1 and Rest 2 in total positive correlations (M1: PC_{R1} = 0.17, PC_{R2} = 0.17, n = 90 $p > 0.05$; M2: PC_{R1} = 0.17, PC_{R2} = 0.17, n = 30, $p > 0.05$), number of significant reactivations (M1: PM_{R1} = 4.41%, PM_{R2} = 4.53%, $p > 0.05$; M2: PM_{R1} = 6.28%, PM_{R2} = 5.88%, $p > 0.05$), nor differences in significant correlations (M1: SC_{R1} = 0.46, SC_{R2} = 0.45, $p > 0.05$; M2: SC_{R1} = 0.42, SC_{R2} = 0.41 , $p > 0.05$).

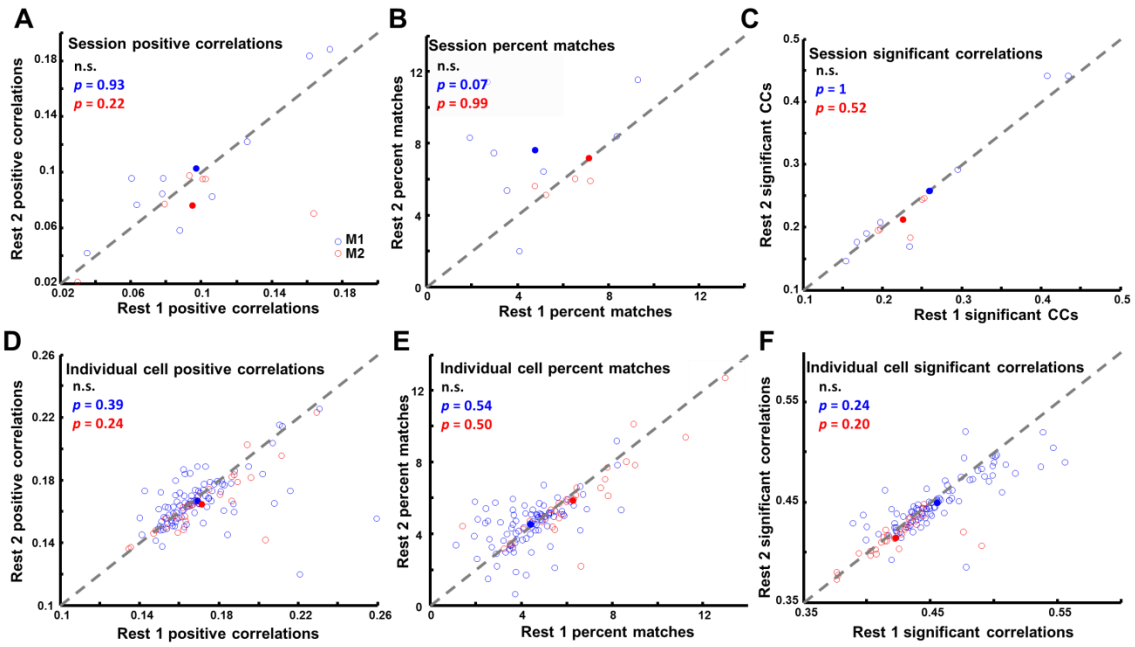

Figure 4.8. Reactivation does not occur at a compressed timescale from original sensory experience

Previous evidence examining reactivation in visual cortex found that it occurred at twice the speed of original sensory evoked activity. Using my template matching procedure I explored whether this was the case. I binned rest period activity using half the bin size compared to the task template. My assumptions were that I would find a greater number of matches or significant correlations in Rest 2 compared to Rest 1. I did not find reactivation at a faster rate than stimulus presentation in populations (A-C) nor individual cells (D – F).

Studies of reactivation during REM sleep has found it at an expanded timescale from the original experience – specifically at twice the experienced time (Louie and Wilson, 2001). I explored whether reactivation occurred at half the speed of the original experience by keeping the template bin size at 50 ms and comparing the template to 100 ms bins during the rest experience. Using this analysis, I did not find any evidence of reactivation in the total positive correlations (M1: PC_{R1} = 0.10, PC_{R2} = 0.10, n = 15, $p > 0.05$; M2: PC_{R1} = 0.08, PC_{R2} = 0.09, n = 6, $p > 0.05$), number of significant reactivation events (M1: PM_{R1} = 7.10%, PM_{R2} = 8.04%, $p > 0.05$; M2: PM_{R1} = 7.27%, PM_{R2} = 8.15%, $p > 0.05$), or differences in the significant correlations (M1: SC_{R1} = 0.23, SC_{R2} = 0.24, $p > 0.05$; M2: SC_{R1} =0.20, SC_{R2} =

0.20, $p > 0.05$). Individual cells do not exhibit any evidence in reactivation. There are no significant differences in total positive correlations (M1: $PC_{R1} = 0.17$, $PC_{R2} = 0.17$, n = 94, $p > 0.05$; M2: $PC_{R1} = 0.16$, $PC_{R2} = 0.16$, n = 30, $p > 0.05$), number of significant reactivation events (M1: $PM_{R1} = 4.75\%$, $PM_{R2} = 4.94\%$, $p > 0.05$; M2: $PM_{R1} = 5.76\%$, $PM_{R2} = 5.79\%$, $p > 0.05$), or differences in the significant correlations (M1: $SC_{R1} = 0.45$, $SC_{R2} = 0.45$, $p > 0.05$; M2: $SC_{R1} = 0.41$, $SC_{R2} = 0.41$, $p > 0.05$).

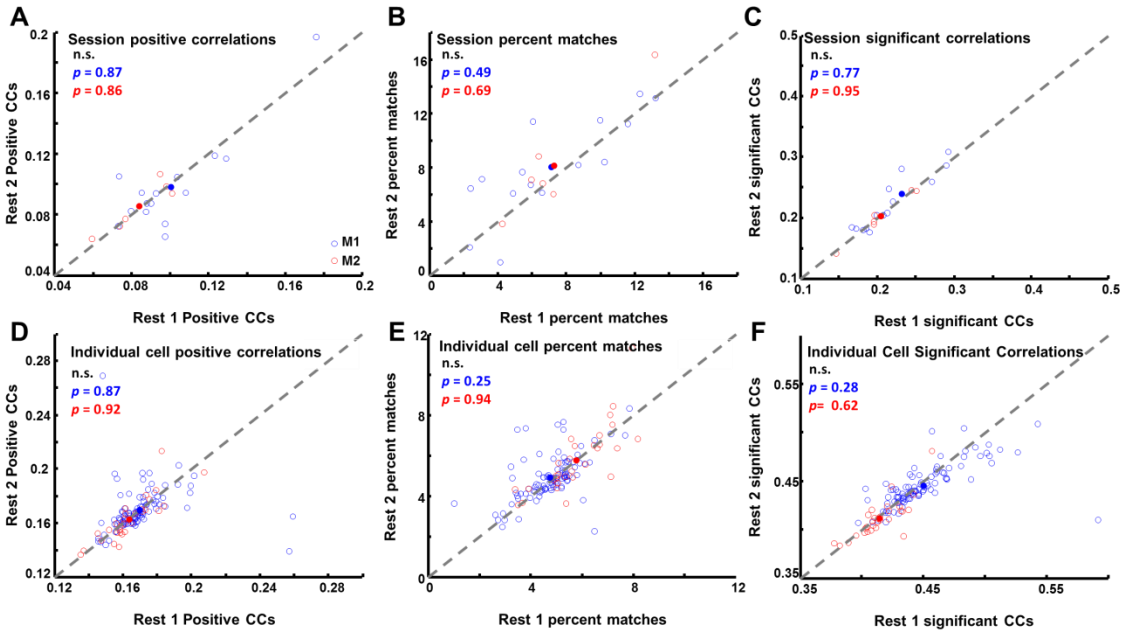

Figure 4.9. Reactivation does not occur at an expanded timescale from original sensory experience
I tested the alternate hypothesis that perhaps reactivation occurred at an expanded timescale than the sensory experience, specifically half the speed. To this end, I used my template matching method with rest bins twice the size of those used for the template. I did not find any evidence of reactivation at the population (A-C) or individual cell (D-F) level.

Note there is a slight difference in the number of sessions and number of cells that are used for each of these calculations. This is because increasing or decreasing the size of the bin either increases or decreases the probability of finding a significant number of spikes in the

rest window I am exploring. If the window size is cut in half, as in my compressed analysis, then I am less likely to pass the threshold of the minimum number of spikes I set per correlation comparison compared to the normal timescale comparison. This caused me to lose 3 sessions and 1 cell between the two monkeys for not having enough significant comparisons throughout the rest period to be considered in my overall analysis comparing the two rest periods. As a reminder, I only considered sessions that had 60% or greater comparisons out of the total number of comparisons. By this same reasoning, when I expanded the bin size during the rest period to twice that used in the same timescale I experienced the opposite issue where more comparisons passed my 6-spike minimum threshold and thus 6 more sessions were included and 6 more cells between the two monkeys. In a separate analysis, I selected only sessions that were present in all of these conditions and made the same comparisons (data not shown); however, no significant differences in total positive correlations, percent matches or significant correlations were observed between Rest 1 and Rest 2 were observed. Thus, I did not find evidence of reactivation in V4 during 20 minute rest periods when the monkeys were in a dark, quiet room.

Discussion

Whether reactivation of stimulus evoked activity during a delayed match-to-sample task was reactivated in V4 cortical networks during a rest period following task exposure was tested. Specifically, I wanted to test whether the sequential activity found in a rest period following the task more closely resembled the activity that occurred during the task than a rest period preceding it. A template-matching method was utilized to compare the task evoked activity to the rest period activity. Three different measures were used to test

whether reactivation occurred. These measures would reveal whether *Rest 2* activity was more similar to the task evoked activity (positive correlations [PC] or significant correlations [SC]) than *Rest 1*, or that a reactivation of task evoked activity occurred more frequently (percent matches[PM]) in *Rest 2*. Significantly greater values in Rest 2 compared to Rest 1 in any of these measures, would suggest that reactivation occurred in *Rest 2*.

Several variations comparing task template activity to rest did not reveal significantly greater similarity in any of these measures between Rest 1 and Rest 2. These include testing for reactivation at multiple timescales and testing whether reactivation depends on eye closure. Thus I conclude that reactivation of previously evoked sequential activity does not occur in V4 circuits during a 20 minute period of rest following a task.

It is possible that reactivation only occurs during particular brain states in visual cortex such as during slow-wave or rapid eye movement (REM) sleep when the brain is sufficient removed from processing sensory stimuli. Some preliminary evidence I recorded in a separate experiment indicated that the monkeys only entered Stage 1 and 2 during this 20 minute nap. To my knowledge, there has not been a demonstration of reactivation during early stages of sleep.

Interestingly, a decay in reactivation was not observed with time as was previously reported during rest in areas involved in a sequential motor task (Hoffman and McNaughton, 2002a). This study did not specifically test for reactivated sequences, only whether cells exhibited more correlated activity during rest following task exposure. Thus, it is possible that reactivation may exist in V4 in the sense that cells exhibit more correlated activity after stimulus presentation. This would mean that the network was primed for sensory

reactivation from task exposure, but lacked the capacity to exhibit sequential reactivation. A separate analysis replicating their analyses would have to be performed to determine this.

Reactivation can occur due to the activation of cells within a time window that enables spike-timing dependent plasticity (STDP). During this window synaptic changes occur that sequentially link neurons that participate in the same sensory event (Bi and Poo, 1999; Hebb, 1949). This process can modify the synaptic weights between neurons and enable the formation of a 'cellular ensemble' capable of reactivating a sequential response (Hebb, 1949). One difference between this experiment and that described in Chapter 2 is the stimulus presentation. In Chapter 2, patches of an image covering approximately a quarter of the receptive field were presented in a random spatiotemporal sequence. In the present experiment, one large image was presented over all the receptive fields twice. These different presentations may lead cells to fire in a sequential pattern in the first experiment and not in the second. Thus, the reactivation in the first experiment is due to the temporal sequence of neurons, where the second requires that a population of neurons exhibit the same activity with time. This second possibility may be more difficult or impossible for populations to exhibit.

Another key difference in the experimental design from the experiment I performed in chapter 2 is that I did not provide a cue to 'trigger' the reactivation sequence. Results found in chapter 2 demonstrated that the reactivation only occurred at the time that the stimulus was expected to occur after the onset of the fixation. If reactivation is a general property of awake V4 neural circuits, then sufficient internal events would be needed to initiate the reactivated sequential activity I reported in Chapter 2 when the cue is absent. Previous work that has observed reactivation in visual cortex during awake state (Xu et al., 2012) and

anesthetized state (Han et al., 2008; Yao et al., 2007) demonstrated that a cue was required to initiate the sequence. Evidence of reactivation during slow-wave sleep in visual cortex area V1 showed that the reactivation followed a sharp-wave ripple (SWR) event in the hippocampus (Ji and Wilson, 2007). Because it is possible that V4 may show reactivation during slow-wave sleep when SWRs are more likely to occur, this difference in reactivation suggests there may be two distinct types of reactivation.

I will categorize two distinct types of reactivation based on what initiates their sequence: bottom-up or top-down mechanisms. A top-down mechanism is one that is driven by internal events. Bottom-down influences are externally driven and arise from activity as it is processed from the external world up the layers of processing hierarchy. Both of these could have different functional roles and provide different mechanisms by which the brain utilizes reactivation for sensory processing. A bottom-up, externally driven mechanism of reactivation, similar to that observed during the awake state, could enable more reliable responses for behaviorally relevant events. A top-down, internally driven mechanism of reactivation, like those observed during slow-wave sleep, would allow coordination of multiple brain structures in order to synchronize and encode a sensory experience across networks. These two potential drivers of rehearsal and their relevance to how the brain receives and processes sensory information will be addressed in more detail in the next chapter.

5. GENERAL DISCUSSION

Development of simultaneous sleep classification and extracellular recordings in macaques

I designed and implemented an experimental paradigm to perform concurrent sleep classification and extracellular recordings. This involved a two-step process: one to determine if monkeys could show improvement in a perceptual learning task following a daytime rest, and two to integrate polysomnography into an extracellular recording setup. I initially wanted to determine whether monkeys showed improvement in a behavioral task following a 20 minute daytime nap. My resting state experiment included the first demonstration of improved behavioral performance in monkeys following a quiet rest in a dark room. Investigation of the underlying neural mechanisms that are important to the behavioral improvement observed following rest is underway and will be discussed in the Future section.

Examining reactivation in visual area V4

During the course of a day, our brains fluctuate between states of active processing of the sensory environment and quiescent, resting states. Given that the brain remains active during these quiescent times, raises the question: how might this activity impact subsequent neural coding? One possibility is that the brain rehearses previous experiences, as demonstrated, for example, by the sequential reactivation of cell ensembles that were activated during the initial sensory experience. Several examples of this have been found in the hippocampus during sleep (Lee and Wilson, 2002; Louie and Wilson, 2001; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994) and awake states (Carr et al., 2011; Davidson et al., 2009; Diba and Buzsáki, 2007; Foster and Wilson, 2006). Further, evidence of rehearsal in the early visual cortex (V1) has been found during anesthetized (Han et al., 2008; Xu et al.,

2012; Yao et al., 2007), sleep (Ji and Wilson, 2007), and awake states (Xu et al., 2012). However, several questions remain open about this rehearsal in cortical sites. Further, the capability of the brain to initiate reactivation versus requiring external signals to drive it affects the interpretation for the usefulness of this rehearsal. Finally, discovery and characterization of this phenomenon in an area responsible for perceptual learning, such as V4, has important implications for how the brain learns and stores information passively about the sensory environment.

My work is the first demonstration and characterization of reactivation in visual cortical area V4. Through this investigation, I have demonstrated several properties about rehearsal in V4. First reactivation in V4 observed during cued awake states is stimulus-specific, occurs in the forward direction, and is also observed in the local field potential activity. It does not occur spontaneously after stimulus exposure during quiescent awake states. Instead, it appears to require an external cue to *trigger* the sequence. The cue requirement suggests that during quiescent, resting states V4 does not receive sufficient internally generated signals to cause networks to reactivate. This does not mean that top-down mechanisms do not play a role. These areas involved in the sensory experience may require an external cue to initiate their effects on V4. This finding may in fact generalize to all cortical areas as this was the first attempt to find reactivation of sequential activity in an extended resting state without environmental cues, not during sleep, nor during a period of time without obvious initiation events such as SWRs (Carr et al., 2011; Davidson et al., 2009; Foster and Wilson, 2006). Thus, my results in Chapter 4 suggest that the reactivation I observed in Chapter 2 requires an external event to initiate the sequence and coordinate the reactivation. Further, V4 and possibly other cortical sites appear to require a deeper level of removal from the

external stimuli (for example, a stage like slow-wave sleep) to receive sufficient internally generated signals that have the capacity to initiate sequential rehearsal. A future investigation of reactivation in V4 during slow-wave sleep would need to be performed to verify these claims.

The divergence in network capabilities during the awake and sleep state to initiate reactivation opens up a much bigger picture about processes in the brain. Specifically, control of how reactivation occurs during these two states represents the primary goals of the brain during these times. Indeed, the two potential mechanisms by which reactivation is driven - external and internal - may serve two distinct purposes within neural circuits. For instance, these two mechanisms of driving reactivation may be the way in which the brain solves the stability-plasticity dilemma (Diekelmann and Born, 2010; Diekelmann et al., 2011). The stability-plasticity dilemma highlights the mystery that the brain can encode new sensory information without altering previously consolidated experiences. Integration of these two possible driving mechanisms (internal and external) with the two-stage model of memory consolidation (Diekelmann and Born, 2010) may help provide a mechanism of how the brain overcomes this issue. The two-stage model of memory consolidation proposes that there are two modes of storage within the brain, the first being temporary memory storage which can acquire knowledge quickly using temporary, local network changes, and the second being stable, long-term memory storage that involves coordination of diffuse brain networks spanning several areas (Diekelmann and Born, 2010). This means that local reactivation of experienced activity requires external cues to initiate the sequence. The cue requisite ensures that circuits are only reactivated for behaviorally relevant stimuli or salient stimuli. This is a possible mechanism by which the brain can filter information so as to avoid

overload by irrelevant sensory information. It can instead selectively rehearse items in the sensory environment grounded to relevant cues. Networks can then strengthen synapses locally between neurons encoding the sensory event. If this is the case, it follows that the brain does not rehearse everything it sees. Instead, it grounds importance to things in the environment that have meaning. The fixation point is something in my experimental paradigm that has meaning to the monkeys. It indicates to them that a stimulus is about to be presented on the computer monitor. Attention to this area is important for them as it signals upcoming rewards. The fixation point thus becomes a behaviorally relevant stimulus. Local, temporary stores of this information can be useful to create more accurate responses to external stimuli and strengthen networks involved in encoding relevant information.

Networks coactivated during sensory experience during awake states can be simultaneously stimulated with internal events during rest to cause more diffuse rehearsal during offline periods in distributed cortical sites. During deep sleep, when external distractors are removed, internal signals can be generated, sent, and received across several areas. Then, multiple areas that are remotely rehearsed during the day can be reactivated together at night. The spontaneous waves of activity that sweep across cortex during slow wave sleep can activate and deactivate ensembles, enabling the redistribution and re-organization of memories stored throughout the brain (Buzsaki, 2010, 2011; Diekelmann and Born, 2010). During SWS, ripples can coordinate reactivation in several areas as previously demonstrated in the hippocampus and visual cortex (Ji and Wilson, 2007). This intuitively makes sense that the awake state is not the right time for this to occur when the brain needs to either be actively engaged in its sensory environment or making only small modulations in preparation to engage. The 20 minutes I allowed the monkey to rest might not have been

enough time to allow the brain to generate these internal signals and reach a state where more diffuse areas could communicate with one another.

Future directions

Significant improvement in behavior with rest suggests that the experimental paradigm I developed has promise to reveal neural characteristics of this improvement. I here propose several analyses that can reveal the properties of the neural activity that are affected by rest and are important for identifying behaviorally relevant features of the stimuli. As I stated in the introduction, V4 has several properties that make this an interesting area to search for neural correlates of behavioral performance improvement following rest. The activity in V4 is strongly influenced by higher cognitive functions, such as attention (Desimone, 1998; Moran and Desimone, 1985; Zhou and Desimone, 2011). Additionally, the function of V4 is summarized as a 'context feature extractor' (Roe et al., 2012). These properties as well as the heterogeneity of responses within V4 identify it as an area capable of modulating the flow of visual information to select behaviorally salient features for subsequent processing.

I suggest two possible mechanisms by which neurons could exhibit greater selectivity for stimulus features. One method is by modulating firing rates, such that a neuron shows more preference for a particular stimulus orientation and less for others after rest. This would cause improvements in discriminability. Modulating firing rates for all neurons within the area would affect the capability of the network to encode stimuli. Another possible way V4 could accomplish this could be by the restructuring of correlated activity after rest. To determine whether this mechanism underlies the behavioral improvement, future analyses will examine noise correlations within V4 before and after rest along with the context of how

neurons are tuned to properties of the stimuli. Noise correlations can have different impact on coding based on the tuning properties of neurons (Abbott and Dayan, 1999; Averbeck and Lee, 2006; Averbeck et al., 2006). Here I expected to find that neurons similarly tuned to behaviorally relevant features would exhibit reduced noise correlations, increasing the information in the neural code, and vice versa, neurons oppositely or not tuned to behaviorally relevant features would exhibit increased or no change in noise correlations.

Examining neural changes in V4 enables me to look at the mechanisms that are involved in this improvement (i.e. whether this phenomenon results from top-down or bottom-up mechanisms). A possible top-down mechanism could be enhanced attention in the task following rest. Increases in gamma-band coherence within V4 (Taylor et al., 2005) and between V4 and prefrontal cortex (Gregoriou et al., 2009) found during attention directed tasks is another analysis I could use in conjunction with those I have described to explore before and after rest. Examination of coherent activity between V4 and its input areas, such as V1 or V2, could elucidate bottom-up influences. A more thorough examination of the concerted efforts of multiple areas is needed to tease apart the influences on V4 responses, but this preliminary analysis to identify features that change after rest is an important first step.

Research has revealed a rich local component of sleep, which expands our previous view about sleep as a global phenomenon. Thus, only focusing on the global aspects of sleep limits our ability to determine how sleep improves learning and memory. Indeed, local networks actively engaged in a task can exhibit different levels of sleep than surrounding brain regions (Huber et al., 2004) and this can even occur during awake states (Vyazovskiy et al., 2011). This suggests that there may be several unknown fundamental processes that

occur during resting states that may underlie our capacity to learn and store information about our sensory environment. The combination of identifying changes in neural activity during rest, and correlating these with behavioral improvements after rest, is imperative for teasing apart how and why rest influences perceptual learning.

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VITA

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