1-1-2013

The Role of REM Sleep in Ocular Dominance Plasticity Consolidation

Michelle Claire Dumoulin
University of Pennsylvania, michelle.dumoulin@gmail.com

The Role of REM Sleep in Ocular Dominance Plasticity Consolidation

Abstract
Despite decades of research, the function of sleep remains controversial. One theory is that sleep plays a role in consolidating plasticity induced during prior waking. Ocular dominance plasticity (ODP) in the cat visual cortex is induced during waking monocular deprivation (MD) and consolidated during subsequent sleep. Extracellular signal-regulated kinase (ERK) signaling is required for ODP and is elevated during post-MD sleep, but its requirement during sleep is unknown. In **Chapter 2**, we investigated whether ERK activity is required during sleep for ODP consolidation by inhibiting the upstream activator of ERK (MEK) with intracortical infusions of U0126 into V1 during post-MD sleep. ERK inhibition abolished ODP consolidation, as measured by extracellular single unit recording. Furthermore, ERK inhibition reduced phosphorylation of eukaryotic initiation factor 4E (eIF4E) and post-synaptic density protein 95 (PSD-95) levels. MAP kinase-interacting kinase 1 (Mnk1) is activated by ERK and directly phosphorylates eIF4E; inhibition of Mnk1 mimicked the effects of ERK inhibition. These results show that activation of the ERK-Mnk1 pathway during post-MD sleep is required for ODP consolidation, and that this pathway promotes the synthesis of plasticity-related proteins such as PSD-95. However, sleep can be broadly subdivided into rapid eye movement (REM) and non-REM (NREM) sleep, but the relative contributions of these states to ODP and the ERK pathway are unknown. In **Chapter 3**, we examined whether REM sleep is required for ODP consolidation and ERK activation by depriving animals of REM sleep following six hours of waking MD. REM sleep deprivation (RSD) abolished ODP consolidation, as measured by optical imaging of intrinsic cortical signals, and reduced ERK phosphorylation in V1. These effects were not seen in a group that received NREM-fragmented sleep (as a control for the nonspecific effects of RSD). Furthermore, ODP and ERK phosphorylation correlated with the degree of beta-gamma activity in V1 during REM sleep, suggesting that neuronal activity patterns during REM promote ERK activation and ODP consolidation. Together, the findings in the following chapters suggest that, following the induction of cortical plasticity during waking, the ERK-Mnk1 pathway is activated during REM sleep, promoting the synthesis of plasticity-related proteins to consolidate cortical plasticity.

Degree Type
Dissertation

Degree Name
Doctor of Philosophy (PhD)

Graduate Group
Neuroscience

First Advisor
Marcos G. Frank

Keywords
consolidation, extracellular signal-regulated kinase, MAP kinase-interacting kinase 1, ocular dominance plasticity, protein synthesis, REM sleep

THE ROLE OF REM SLEEP IN OCULAR DOMINANCE PLASTICITY CONSOLIDATION

Michelle C. Dumoulin

A DISSERTATION

in

Neuroscience

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2013

SUPERVISOR OF DISSERTATION

_____

Marcos G. Frank, Ph.D

Associate Professor of Neuroscience

GRADUATE GROUP CHAIRPERSON

_____

Joshua I. Gold, Ph.D.

Associate Professor of Neuroscience

DISSERTATION COMMITTEE

Marc F. Schmidt, Ph.D., Associate Professor of Biology

Larry A. Palmer, Ph.D., Professor of Neuroscience

Allan I. Pack, M.B.Ch.B, Ph.D., John Miclot Professor of Neuroscience, Genomics and Computational Biology

David M. Raizen, M.D., Ph.D, Assistant Professor of Neurology

ACKNOWLEDGEMENT

I would like to thank my mentor, Marcos Frank, for his guidance and support throughout my time in the lab. The work presented in this document would have been impossible to achieve without the dedicated teamwork of past and present lab members, including Julie Seibt, Sara Aton, Adam Watson, Tammi Coleman, and Leslie Renouard. I would also like to thank Marc Schmidt, Larry Palmer, Allan Pack, and David Raizen for serving as my thesis committee, and Mike Nusbaum, Rita Balice-Gordon, and Josh Gold for their guidance as NGG chairs at different stages of my years in the program; each of these individuals has provided guidance not only for my thesis but for my career. In addition, many thanks to Jane Hoshi and her unfailing organizational skills keep the NGG running smoothly. I would also like to thank the University of Pennsylvania Center for Sleep and Circadian Neurobiology for collaborating on the biochemical analysis presented in this work, and the ULAR husbandry and veterinary staff, whose support and care for our animals has made these studies possible. Finally, I would like to thank my classmates, for providing intellectual stimulation and friendship throughout our time in the NGG, and Morgan Bridi, for supporting me whenever times got tough.

ABSTRACT

THE ROLE OF REM SLEEP IN OCULAR DOMINANCE PLASTICITY CONSOLIDATION

Michelle C. Dumoulin

Dr. Marcos G. Frank

Despite decades of research, the function of sleep remains controversial. One theory is that sleep plays a role in consolidating plasticity induced during prior waking. Ocular dominance plasticity (ODP) in the cat visual cortex is induced during waking monocular deprivation (MD) and consolidated during subsequent sleep. Extracellular signal-regulated kinase (ERK) signaling is required for ODP and is elevated during post-MD sleep, but its requirement during sleep is unknown. In **Chapter 2,** we investigated whether ERK activity is required during sleep for ODP consolidation by inhibiting the upstream activator of ERK (MEK) with intracortical infusions of U0126 into V1 during post-MD sleep. ERK inhibition abolished ODP consolidation, as measured by extracellular single unit recording. Furthermore, ERK inhibition reduced phosphorylation of eukaryotic initiation factor 4E (eIF4E) and post-synaptic density protein 95 (PSD-95) levels. MAP kinase-interacting kinase 1 (Mnk1) is activated by ERK and directly phosphorylates eIF4E; inhibition of Mnk1 mimicked the effects of ERK inhibition. These results show that activation of the ERK-Mnk1 pathway during post-MD sleep is required for ODP consolidation, and that this pathway promotes the synthesis of plasticity-related

proteins such as PSD-95. However, sleep can be broadly subdivided into rapid eye movement (REM) and non-REM (NREM) sleep, but the relative contributions of these states to ODP and the ERK pathway are unknown. In **Chapter 3**, we examined whether REM sleep is required for ODP consolidation and ERK activation by depriving animals of REM sleep following six hours of waking MD. REM sleep deprivation (RSD) abolished ODP consolidation, as measured by optical imaging of intrinsic cortical signals, and reduced ERK phosphorylation in V1. These effects were not seen in a group that received NREM-fragmented sleep (as a control for the nonspecific effects of RSD). Furthermore, ODP and ERK phosphorylation correlated with the degree of beta-gamma activity in V1 during REM sleep, suggesting that neuronal activity patterns during REM promote ERK activation and ODP consolidation. Together, the findings in the following chapters suggest that, following the induction of cortical plasticity during waking, the ERK-Mnk1 pathway is activated during REM sleep, promoting the synthesis of plasticity-related proteins to consolidate cortical plasticity.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2:

Chapter 3:

LIST OF ILLUSTRATIONS

Chapter 1:

Chapter 2:

Chapter 3:

Chapter 4:

CHAPTER 1: INTRODUCTION

The function of sleep has long been a topic of debate. This work will explore the theory that plastic changes in brain circuitry are induced by waking experience and consolidated during subsequent sleep. In this chapter, the concepts of learning, memory, and brain plasticity will be introduced, and ways in which sleep may influence these processes will be discussed.

Learning and Memory. The ability of organisms to encode, store, and retrieve information about their changing environments is critical to survival. In humans, two primary categories of memory exist: declarative (explicit) and non-declarative (implicit). Declarative memories include conscious memories for facts and events. Non-declarative memories consist of procedural memories for skills, perceptual priming (in which exposure to a stimulus influences responses to a later stimulus), associative memories (such as classical conditioning), and non-associative reflexive learning (reviewed in Squire and Zola, 1996).

The formation of these different forms of memory involves distinct brain regions. Studies of patient H.M, who underwent bilateral hippocampal resection, provided some of the first evidence that the hippocampus is required for the formation of new declarative memories (Scoville and Milner, 1957). Interestingly, the hippocampus is only required for the retrieval of recently acquired memories, whereas remote memory is intact following hippocampal lesions in both human and rodent studies (Scoville and Milner, 1957; Anagnostaras et al., 1999). Evidence suggests that the time-limited role of

the hippocampus in memory is due to the redistribution of stored information from the hippocampus to other brain regions, such as the cortex, a process referred to as system consolidation (Bontempi et al., 1999; Frankland et al., 2004; Frankland and Bontempi, 2005). Further studies of hippocampal lesions in an animal model revealed that in addition to declarative memory, the hippocampus is also necessary for the formation of spatial memory (Morris et al., 1982).

In contrast with declarative memory, the acquisition of non-declarative memories is not impaired by the loss of hippocampal function. The encoding and storage of these memories involves plasticity in extra-hippocampal brain regions such as the striatum, cerebellum, amygdala, and/or neocortex, depending on the learning task (reviewed in Squire and Zola, 1996).

Hippocampal Plasticity. The discovery that the hippocampus plays an important role in memory led to the development of models of hippocampal plasticity. The first of these models was repetitive electrical stimulation of the hippocampal perforant path, which resulted in the potentiation of responses at hippocampal CA3 synapses in both anesthetized and awake rabbits, a phenomenon now referred to as long-term potentiation (LTP) (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973). Additional work in hippocampal slices *ex vivo* has since demonstrated that high frequency stimulation induces LTP, whereas low frequency stimulation produces the opposite effect, synapse weakening, termed long-term depression (LTD) (Dunwiddie and Lynch, 1978). Many forms of hippocampal LTP and LTD, as well as their underlying mechanisms, have now been described.

Classical forms of both LTP and LTD require N-methyl-D-aspartate (NMDA) receptor activation (Collingridge et al., 1983; Dudek and Bear, 1992) and subsequent calcium influx (Lynch et al., 1983; Mulkey and Malenka, 1992). High levels of calcium influx during LTP induction activate calcium/calmodulin dependent kinase II (CaMKII) and calcium-stimulated adenylyl cyclases 1 and 8, which are required for LTP (Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992; Giese et al., 1998; Wong et al., 1999). Cyclic adenosine monophosphate (cAMP) production by adenylyl cyclase activates protein kinase A (PKA), which is also necessary for LTP (Matthies and Reymann, 1993). PKA and CaMKII-mediated phosphorylation of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) receptors during LTP induction increases their membrane insertion and trafficking to the synapse, respectively; CaMKII may also increase AMPA receptor conductance and open probability (Oh et al., 2006; Correia et al., 2008; Lu et al., 2010; Kristensen et al., 2011), increasing the amplitude of excitatory postsynaptic currents in response to presynaptic neurotransmitter release. These kinases also promote activation of the extracellular signal-regulated kinase (ERK) cascade, which is required for LTP (English and Sweatt, 1997). The long-term stabilization of LTP depends upon both transcription and translation (Stanton and Sarvey, 1984); both of these events can occur downstream of ERK activation (via the transcription factors CREB and Elk-1, and the Mnk1 translation pathway) (Impey et al., 1998; Davis and Vanhoutte, 2000; Kelleher et al., 2004; Panja et al., 2009). In contrast, during LTD, low levels of calcium influx activate the protein phosphatases calcineurin and protein phosphatase 1 (Mulkey et al., 1993, 1994; Morishita et al., 2001), which can dephosphorylate AMPA receptors and promote their endocytosis (Kameyama et al., 1998; Lee et al., 1998, 2000; Beattie et al., 2000).

Given the importance of the neocortex for both procedural learning and long-term memory storage, studies of the hippocampus cannot fully explain how learning occurs in the intact brain. During mammalian development, primary sensory areas of the cortex undergo periods of heightened plasticity, referred to as 'critical periods.' Studying cortical plasticity during critical periods allows natural sensory stimuli, rather than artificial electrical stimulation, to be easily be applied, and has revealed many principles underlying plasticity in the brain.

Visual cortical plasticity. Plasticity in the primary visual cortex (V1) has been extensively studied, and can be induced by several manipulations of visual experience. Ocular dominance plasticity (ODP) is a model of visual cortical plasticity that was first discovered in kittens (Wiesel and Hubel, 1963a). In kittens with normal vision, the majority of cells in V1 respond to input from either eye. However, if vision is occluded in one eye by suturing the eyelid shut (monocular deprivation, MD) for several days or weeks during a critical period of development that begins around postnatal day 28, V1 neurons become almost exclusively responsive to visual stimuli presented to the non-deprived eye (Hubel and Wiesel, 1970). Short (<24 hour) MD causes protein synthesis-dependent plasticity within layer II/III of V1, whereas longer periods of MD are required for plasticity in layer IV and the thalamocortical cells projecting from the lateral geniculate nucleus (LGN) in the cat; eventually over the course of MD, cell bodies and arborizations of deprived-eye responsive LGN cells shrink (Wiesel and Hubel, 1963b; Trachtenberg et al., 2000; Taha and Stryker, 2002) (Figure 1.1). In contrast, thalamocortical plasticity occurs on the same time course as intracortical plasticity in the mouse (Liu et al., 2008; Coleman et al., 2010; Khibnik et al., 2010), underscoring the

importance of considering the species-specific visual system organization when studying ODP. Nonetheless, many of the cellular and molecular mechanisms underlying ODP have been determined and are shared across species.

Mechanisms of deprived eye response depression. ODP occurs in distinct stages: first, responses to visual stimulation of the deprived eye become depressed, and then responses to stimulation of the nondeprived eye are enhanced (Frenkel and Bear, 2004). The depression of deprived eye responses in V1 results from decorrelation of inputs from the two eyes, rather than loss of retinal illumination or retinal ganglion cell firing (Rittenhouse et al., 2006; Linden et al., 2009). The initial depression of deprived eye responses in rodents shares mechanisms of hippocampal LTD, including NMDA receptor activation, subsequent dephosphorylation of AMPA receptors, and AMPA receptor internalization (Bear et al., 1990; Heynen et al., 2003; Yoon et al., 2009). Expression of the immediate early gene encoding the activity regulated cytoskeletal-associated protein (Arc), which interacts with endocytic machinery to accelerate AMPA receptor internalization (Chowdhury et al., 2006), is also required for deprived eye response depression during ODP in the mouse (McCurry et al., 2010). Metabotropic glutamate receptor activation is important for LTD in visual cortical slices (Kato, 1993; Haruta et al., 1994; Hensch and Stryker, 1996); however, the role of mGluRs in ODP is less clear, as blockade of mGluRs does not prevent synapse weakening V1 of the kitten *in vivo* (Hensch and Stryker, 1996). Further complicating our understanding of plasticity in V1 is the possibility that mechanisms for depression may differ between cortical layers. In mouse V1 slices *ex vivo*, layer II/III LTD requires endocannabinoid CB1 receptor activation, whereas layer IV LTD requires PKA and AMPA receptor endocytosis; both require NMDA receptor activation (Crozier et al., 2007).

Endocannabinoid antagonism during ODP *in vivo* also abolishes depression in layer II/III but not layer IV of the mouse (Liu et al., 2008), but it is unclear whether these layer-specific mechanisms also exist in the cat visual cortex.

Mechanisms of metaplasticity. How can a single sensory stimulus (decorrelated visual input) produce both depression and potentiation in visual cortical circuits? The theoretical idea that the threshold for synaptic modification slides according to the history of synaptic activity has been proposed as an answer to this question (Bienenstock et al., 1982; Bear et al., 1987). In support of this hypothesis, decreased activity in V1 neurons (by dark rearing) causes a shift in the threshold for plasticity induction, such that the optimal stimulation frequency for induction of both LTP and LTD is reduced (Philpot et al., 2003, 2007). One potential mechanism underlying metaplasticity is a shift in the NR2A:NR2B subunit ratio of NMDA receptors. NR2B-containing NMDA receptors have slower kinetics compared to NR2A-containing receptors, such that a relative increase in NR2B should lower the strength of the stimulus required to induce potentiation (Bear, 2003). In support of this idea, decreased activity in visual cortical neurons *in vitro* and *in vivo* increases levels of *NR2B* gene expression (Lee et al., 2008). Furthermore, genetic knockout of NR2A lowers the optimal stimulus frequency for both LTP and LTD in slices, and ODP induction in NR2A knockout mice *in vivo* produces immediate potentiation rather than depression (Philpot et al., 2007; Cho and Khibnik, 2009). Together, these studies suggest that MD decreases activity in V1 neurons, causing initial LTD. Decreased neuronal activity also decreases the NR2A:NR2B ratio, which then lowers the threshold for LTP and allows subsequent potentiation to occur.

Mechanisms of nondeprived eye response potentiation. Mechanisms of potentiation in V1 have been elucidated using both ODP *in vivo* and LTP in V1 slices *ex*

vivo. In addition, stimulus-specific response potentiation (SRP) is a plasticity model induced *in vivo* by repeated presentations of the same visual stimulus, which induces only LTP in V1 (Cooke and Bear, 2010). Together, these models have provided insight into the mechanisms of non-deprived eye response potentiation during ODP.

Several well-established molecular mechanisms of LTP are required for ODP. For example, work in the cat has shown that protein kinase A (PKA) activation downstream of NMDA receptor activation is required for ODP (Beaver et al., 2001). In addition, CaMKIIα activation (by autophosphorylation after Ca^{2+}/calmodulin binding) is necessary for ODP in the mouse (Taha et al., 2002), and ERK activation is required in the rat for ODP *in vivo* and LTP in V1 slices *ex vivo* (DiCristo et al., 2001). Multiple pathways can activate ERK during plasticity, but ERK phosphorylation in V1 due to light exposure following a period of dark rearing is dependent upon PKA activity (Cancedda et al., 2003), suggesting that PKA may activate ERK during ODP as well.

Downstream effectors of ERK during neuronal plasticity include gene transcription, mRNA translation, ion channel phosphorylation, and cytoskeletal alterations (reviewed in Adams and Sweatt, 2002). A number of lines of evidence suggest that ERK promotes gene transcription during ODP. The transcription factor cAMP response element binding protein (CREB) is activated by MD in rodents (Pham et al., 1999) and is required for ODP in ferrets (Mower et al., 2002). CRE-mediated gene expression in V1 following light exposure depends upon ERK and PKA activity (Cancedda et al., 2003), and ERK is translocated to the nucleus of V1 cells after MD (Takamura et al., 2007). Furthermore, light exposure promotes ERK-dependent mitogen and stress activated protein kinase (MSK) and histone H3 phosphorylation, as well as and histone H3 and H4 acetylation, events involved in the epigenetic regulation of gene

transcription (Putignano et al., 2007). Together, these results suggest that ERK plays a role in gene transcription during plasticity in V1. One gene that may be transcribed as a result is tissue plasminogen activator (tPA), a secreted protease whose mRNA and proteolytic activity is elevated during ODP in rodents and whose inhibition impairs ODP in kittens and mice (Mataga et al., 1996, 2002). Proteolysis of extracellular matrix components, such as chondroitin sulfate proteoglycans (CSPGs), by tPA may be permissive for ODP (Berardi et al., 2003). However, in cats that were re-exposed to binocular vision after a long period of MD, the digestion of CSPGs did not promote the recovery of vision in the deprived eye, suggesting that tPA may act on other targets (Vorobyov et al., 2013).

Although ERK appears to play a role in gene transcription during ODP, it may also have additional functions. Light exposure following dark rearing elevates ERK phosphorylation in both cell nuclei and dendritic spines in V1, suggesting that ERK may act on substrates local to the synapse (Boggio et al., 2007), such as proteins involved in translation. Early ODP in V1 requires translation (Taha and Stryker, 2002), and the time course of ERK requirement during LTP in V1 is consistent for a role in protein synthesis (DiCristo et al., 2001). Furthermore, *Arc* is required for SRP in the mouse, suggesting that it plays a role in nondeprived eye response potentiation (McCurry et al., 2010), and Arc synthesis requires activation of the ERK-mediated translation pathway for LTP in the dentate gyrus *in vivo* (Panja et al., 2009). These studies suggest that ERK may promote the synthesis of plasticity-related proteins required for ODP.

Interestingly, there are several mechanisms of LTP in the hippocampus that are not shared with ODP in V1. For instance, activation of the Ca^{2+} stimulated adenylyl cyclases 1 and 8, which are required for hippocampal LTP and memory (Wong et al.,

1999), are not required for ODP (Fischer et al., 2004). Furthermore, protein kinase C (PKC) and protein kinase G (PKG), which are required for hippocampal LTP (Lovinger et al., 1987; Malinow et al., 1989; Zhuo et al., 1994), are not required for ODP (Beaver et al., 2001; Daw and Beaver, 2001).

Other factors influencing ODP. The neuronal firing patterns driving long-term plastic changes during ODP can be modulated by several factors. For example, activity in local inhibitory circuits can shape neuronal activity. The critical period for plasticity in V1 coincides with maturation of parvalbumin-positive inhibitory cells, and mutations in mice that accelerate the maturation of inhibitory networks cause early closure of the critical period (DelRio et al., 1994; Huang et al., 1999). The consequences of the maturation of inhibitory circuitry, however are unclear; conflicting reports suggest that the early OD shift in GABAergic neurons may be distinct from those of excitatory neurons (but see Kameyama et al., 2010). Using calcium imaging of genetically identified GABAergic neruons in mice, GABAergic neurons have been found to shift in the same direction as glutamatergic neurons (toward the non-deprived eye), but more slowly, during ODP (Gandhi et al., 2008). In contrast, neuronal recordings of inhibitory fast-spiking interneurons demonstrate an initial counter-intuitive shift *towards* the deprived eye, followed by a return to binocularity in cats (Aton et al., 2013) or a complete reversal to favor the non-deprived eye in mice (Yazaki-Sugiyama et al., 2009). Differences in methodology between these studies may result in sampling of different subsets of GABAergic neurons, which may explain these discrepancies, but ultimately the function of inhibition during ODP is still unclear.

Neuromodulatory signaling may also influence ODP. Long-term local blockade of cholinergic M_1 (but not nicotinic) acetylcholine receptors, noradrenergic β receptors, or

serotonin-1 and -2C receptors in V1 during MD prevents a shift in ocular dominance (Shirokawa and Kasamatsu, 1986; Gu and Singer, 1989, 1993, 1995; Wang et al., 1997). These effects may be due neuromodulatory inputs activating downstream intracellular signaling cascades that intersect with signaling pathways required for ODP. Alternatively, neuromodulators may influence activity-dependent signaling pathways by altering neuronal activity patterns (reviewed in Gu, 2002). Similarly, T-type calcium channel activation in the cortex alters neuronal firing patterns, and is also required for ODP (Uebele et al., 2009).

Many mechanisms underlying neuronal plasticity have been elucidated. However, the brain undergoes dramatic changes in activity across the sleep-wake cycle that may influence plasticity. Therefore, an understanding of how the intact brain encodes, stores, and retrieves memories is incomplete without investigating the contributions of each arousal state to plasticity.

Sleep. Sleep is a reversible state of behavioral quiescence with an increased threshold for arousal in response to sensory stimuli. Prior to rigorous scientific investigation, sleep was long assumed to be a state of suspended brain activity. However, this view changed when Hans Berger (1929) developed the human electroencephalogram (EEG), which measures the summed electrical activity of many cortical neurons. Berger demonstrated that during waking, the brain exhibits high-frequency, low-amplitude activity. EEG recordings in sleeping human subjects subsequently showed that brain activity was not absent during sleep, but that the patterns of activity changed to slow, high-amplitude oscillations termed delta waves (0.1-4 Hz) and spindles (7-14 Hz) during sleep (Loomis et al., 1935a, 1935b; Davis et al., 1937, 1938) (Figure 1.2). It was later

found that delta rhythms could be subdivided into two distinct oscillations, now referred to as slow (<1 Hz) and delta (1-4 Hz) rhythms (Steriade et al., 1993). Intracellular recordings during this state show that, like the EEG signal, individual cortical neurons oscillate between periods of hyperpolarization with no action potential firing and periods of depolarization and rapid action potential firing (Steriade et al., 2001).

After the discovery of slow brain oscillations during sleep, Aserinsky and Kleitman (1953) noticed that human subjects cycle between periods of slow eye movements and periods of rapid, jerky eye movements during sleep. They discovered that this 'rapid eye movement' (REM) sleep (also referred to as paradoxical sleep, PS) was characterized by EEG activity resembling waking (Figure 1.2). Intracellular recordings of cortical neurons in the cat have since shown that individual cortical neurons fire tonically, similar to waking, during REM sleep (Steriade et al., 2001). Another electrophysiological hallmark of REM sleep is hippocampal theta (5-7 Hz) rhythm resembling alert waking (Green and Arduini, 1954; Jouvet et al., 1959). In addition, ponto-geniculo-occipital (PGO) waves are phasic potentials that occur sequentially in the pons, LGN, and occipital cortex during REM sleep in cats (Brooks and Bizzi, 1963; Mouret et al., 1963), and evidence suggests that similar event also occur in humans (McCarley et al., 1983; Peigneux et al., 2001). Motor tone during REM sleep, measured by the electromyogram (EMG), is completely absent with the exception of occasional brief muscle twitches (Dement, 1958; Jouvet et al., 1959; Gassel et al., 1964). Over the course of a night's sleep, subjects first enter sleep characterized by slow oscillations (now referred to as non-REM (NREM) sleep), followed by REM sleep, and cycle between these stages during the night (Dement and Kleitman, 1957).

Why sleep? As a state in which animals are not foraging for food or seeking out mates, and are vulnerable to predation, sleep appears disadvantageous. However, sleep-like states have been described in invertebrates, fish, reptiles, amphibians, birds, and mammals. Furthermore, birds and terrestrial mammals exhibit both NREM and REM sleep (Siegel, 2008). The phylogenetic conservation of sleep suggests that it serves a vital function, although the identity of this function remains a subject of debate.

Sleep function. The function of sleep was eloquently described by William Shakespeare as "Sleep that knits up the raveled sleave of care/ The death of each day's life, sore labor's bath, / Balm of hurt minds, great nature's second course, / Chief nourisher in life's feast…." (Macbeth 2. 2. 36-39). This description exemplifies the long-held assumption that sleep restores and repairs the brain and/or body. Although these ideas are intuitive, strong scientific evidence to support them is lacking (reviewed in Frank, 2006). Two theories of sleep function have received more support: the idea that sleep promotes brain development, and that sleep consolidates brain plasticity that is induced during waking experience.

The role of sleep in brain development. Roffwarg and colleagues (1966) observed that Infants spend about 16 hours asleep per day, with a large proportion of REM sleep (~50% of total sleep time), and sleep time declines gradually throughout life to less than six hours per day in 70-85 year-olds (13.8% REM sleep). This led to the hypothesis that sleep, especially REM sleep, promotes normal brain development, an idea that has since gained support from animal models.

REM sleep has been implicated in the developmental regulation of LTP in both the visual cortex and hippocampus. In V1 slices *ex vivo*, theta burst stimulation of the white matter induces LTP in layer II/III, but only within a critical period of development (Kirkwood et al., 1995). REM sleep deprivation (RSD) in rats extends the ages over which this form of LTP can be induced, although it cannot rescue this form of LTP in the adult rat (Shaffery et al., 2002, 2006a; Shaffery and Roffwarg, 2003). Development of parvalbumin immunoreactivity in the LGN is delayed by RSD (Hogan et al., 2001) and low-frequency white matter stimulation that normally produces LTD instead produces LTP in RSD animals (Shaffery et al., 2006b), suggesting that REM sleep promotes the maturation of inhibition in the visual system. Brain-derived neurotrophic factor (BDNF) signaling during REM sleep may be responsible for the maturation of inhibition, as BDNF infusion during RSD prevents the extension of the critical period for this form of plasticity (Shaffery et al., 2012). REM sleep may also promote the maturation of hippocampal circuits required for LTP. RSD prior to LTP induction in hippocampal slices from young rats destabilizes LTP maintenance, possibly due to decreased expression of mature postsynaptic components of glutamatergic synapses (Lopez et al., 2008).

The role of REM sleep in brain development has also been demonstrated using ODP as a model. RSD during long-term MD in kittens enhances the difference between deprived-eye and non-deprived eye cell size in the LGN, suggesting that REM sleep promotes the normal development of brain circuitry and counteracts the effects of exogenously induced plastic changes (Oksenberg et al., 1996; Shaffery et al., 1998). This finding was mimicked by lesion of the PGO wave generator prior to MD (Shaffery et al., 1999). Since lesion of the PGO-wave generator decreases LGN volume and cell size in the absence of MD (Davenne and Adrien, 1984; Davenne et al., 1989), these

studies suggest that PGO waves are the REM sleep component that are responsible for maturation of the visual system.

The role of sleep in consolidating memory and plasticity. Sleep has also been proposed to stabilize and enhance plasticity induced during prior waking, referred to as consolidation. This theory has gained much support from both the human and animal literature, using models of both hippocampus-dependent and hippocampus-independent learning and plasticity.

Sleep and consolidation: Humans. Various hippocampus-dependent memory tasks benefit from sleep. Recall of a declarative memory task, learned word pair associations, improves over a night of sleep (Ekstrand, 1967; Ellenbogen et al., 2006, 2009), and improvement is greater when sleep occurs soon after learning (Payne et al., 2012). Similarly, sleep following vocabulary learning enhances and sleep deprivation impairs subsequent recall in high school students (Gais et al., 2006). Recall of other declarative memory tasks, in which a face is paired with an inanimate object or a spatial location, is better if learning is followed by a period of sleep (Talamini et al., 2008; Tucker et al., 2011b). Hippocampus-dependent route learning and virtual maze learning also benefit from sleep over the course of a night or a nap (Ferrara et al., 2006; Wamsley et al., 2010; Nguyen et al., 2013).

Hippocampus-independent procedural memory tasks also benefit from post-training sleep. For example, after training on a procedural visuo-motor saccade task or sequential finger-tapping task, subjects do not improve over the course of daytime waking, but have substantial performance improvements after a night of sleep (Walker et

al., 2002, 2003; Kuriyama et al., 2004; Gais et al., 2008a; Tucker et al., 2011a). Furthermore, learning a visual discrimination task requires sleep within 30 hours of acquisition (Stickgold et al., 2000a, 2000b). Naps prevent the deterioration of performance on this task (Mednick et al., 2002), and consolidation is blocked by glutamatergic receptor antagonists given specifically during sleep, suggesting that activity of excitatory neurons during sleep drives consolidation (Gais et al., 2008b).

Sleep and consolidation: Rodents. Many hippocampus-dependent learning and plasticity models in rodents have demonstrated a role for sleep in consolidation. For example, sleep benefits and sleep deprivation impairs performance on the spatial object recognition test, in which animals must recognize an object that has been moved to a new location, in both rats and Djungarian hamsters (Palchykova et al., 2006a; Binder et al., 2012; Inostroza et al., 2013). Sleep also enhances performance on a temporal object recognition task, in which rats must recognize which of two objects they saw first (Inostroza et al., 2013). Furthermore, performance on the passive avoidance task in mice is impaired by post-learning sleep deprivation (Patti et al., 2010). In mice, hippocampus–dependent contextual fear conditioning increases subsequent NREM sleep; moreover, sleep immediately following training enhances and sleep deprivation inhibits recall (Graves et al., 2003; Hellman and Abel, 2007; Cai et al., 2009; Hagewoud et al., 2010). Increasing cAMP levels (by phosphodiesterase 4 inhibition) during sleep deprivation prevents memory impairment on this task, suggesting that LTP occurs during sleep via cAMP signaling (Vecsey et al., 2009). Consistent with this finding, sleep after training on the Morris water maze occludes a PKA-dependent form of LTP in the rat hippocampus (Habib and Dringenberg, 2010; Habib et al., 2013).

An attractive mechanism for sleep-dependent consolidation of hippocampal plasticity is the re-activation of neuronal ensembles during sleep that were active during prior waking, termed replay. Hippocampal place cells that fire during waking experience also tend to fire in the same temporal order during subsequent NREM sleep (Wilson and McNaughton, 1994; Nádasdy et al., 1999; Lee and Wilson, 2002; Ji and Wilson, 2007). The repetition of newly acquired neuronal activity patterns during sleep could potentially drive activity-dependent plasticity mechanisms, leading to consolidation. However, replay is observed after extensive training, and also occurs during waking (reviewed in Carr et al., 2011). Therefore, it is unclear whether replay contributes to the acquisition of new memories, and whether these contributions occur specifically during sleep.

Findings in rodents support a role for sleep in hippocampus-dependent learning, but evidence that sleep contributes to hippocampus-independent learning is inconsistent. For example, cued fear conditioning is not enhanced by sleep or impaired by sleep deprivation in mice (Graves et al., 2003; Cai et al., 2009) but is impaired by sleep deprivation in rats (Kumar and Jha, 2012). In addition, sleep fragmentation in mice, and sleep deprivation in mice and Djungarian hamsters, impairs performance on the hippocampus-independent novel object recognition task (Mumby et al., 2002; Palchykova et al., 2006a, 2006b; Rolls et al., 2011). In contrast, sleep does not improve performance on this task in rats (Inostroza et al., 2013). These discrepancies may arise from inter-species or methodological differences, leaving the role of sleep in hippocampal-independent learning an open question.

Sleep and consolidation: ODP in the cat. Frank et al. (2001) used ODP as a model to demonstrate the importance of sleep to the consolidation of cortical plasticity. In this study, critical period kittens underwent MD and were kept awake in lighted

conditions for six hours, ensuring that all animals received the same amount of decorrelated visual input to induce cortical remodeling. ODP measurements were then made either immediately, or following six hours of sleep or sleep deprivation in complete darkness (to prevent further visual stimulation). Six hours of MD alone was sufficient to induce a slight shift in OD; this shift was enhanced by subsequent sleep and destabilized by sleep deprivation, indicating that sleep both stabilizes and enhances ODP.

Further work on this model has elucidated some of the mechanisms underlying sleep-dependent ODP consolidation (Figure 1.3). Analysis of firing properties in response to stimulation of either eye revealed that depression of deprived-eye responses occurs during waking MD and is maintained during subsequent sleep, whereas potentiation of non-deprived eye responses occurs during sleep (Aton et al., 2009a). Several mechanisms known to promote potentiation are required during post-MD sleep for consolidation to occur. For example, blockade of NMDA receptors and PKA inhibition during post-MD sleep both abolish ODP consolidation; downstream of NMDA receptor activation, ERK, CaMKII, and the AMPA receptor subunit GluA1 (on the CaMKII site, S831) are phosphorylated during the first two hours of post-MD sleep (Aton et al., 2009a). Seibt and colleagues (2012) demonstrated that translation via the mammalian target of rapamycin (mTOR) pathway during sleep is also required for ODP consolidation. Sleep upregulates phosphorylation of proteins involved in translational control (eukaryotic initiation factor 4E binding protein, 4E-BP1; eukaryotic elongation factor 2, eEF2) and the synthesis of the plasticity-related proteins Arc and BDNF. In contrast, levels of *Arc* and *BDNF* mRNA decrease during sleep, indicating that sleep promotes the translation, but not transcription, of proteins involved in plasticity.

Although many mechanisms of sleep-dependent ODP have been revealed in recent years, a number of questions remain to be addressed. For instance, ERK phosphorylation occurs during sleep in the remodeling cortex, but is it required for consolidation? If so, which intracellular signaling pathways does it activate, and are these events involved in potentiation, depression, or both? In **Chapter 2**, we address these questions by inhibiting ERK phosphorylation during post-MD sleep and examining the resulting electrophysiological and biochemical changes.

Differential roles for REM and NREM sleep in consolidation. As discussed above, NREM and REM sleep have strikingly different electrophysiological characteristics, suggesting that they may play different roles in consolidation. Evidence from human studies suggests that NREM sleep promotes hippocampus-dependent learning and REM sleep enhances hippocampus-independent learning, although this distinction is less clear in animal models.

The role of REM sleep in consolidation: Humans. NREM sleep, but not REM sleep, after training benefits recall on a variety of hippocampus-dependent tasks in humans. For example, the most improvement on the declarative face-object pairing, word recognition, and mental spatial rotation tasks occurs over the course of early (NREM-rich, REM-poor) sleep (Plihal and Born, 1997, 1999; Drosopoulos et al., 2005). Furthermore, improvement on hippocampus-dependent virtual maze navigation correlates with EEG power during NREM sleep (Wamsley et al., 2010; Nguyen et al., 2013). In contrast, studies have largely failed to find a connection between REM sleep and hippocampus-dependent memory. The effects of long periods of intensive

declarative learning (such as during a college examination period) on REM sleep are minor or absent (Holdstock and Verschoor, 1974; Meienberg, 1977). Pharmacological suppression of REM sleep after learning word pair associations and consonant trigrams does not impact recall the next morning (Castaldo et al., 1974). Manual REM sleep deprivation also does not impair recall of previously learned words, word pairs, or a nonverbal declarative memory task (Ekstrand et al., 1971; Chernik, 1972; Lewin and Glaubman, 1975; Smith, 1995). The few studies that have reported a connection between declarative memory and REM sleep used quite complex recall tasks, such as learning stories, prose passages, and meaningless sentences (Empson and Clarke, 1970; Tilley and Empson, 1978).

In contrast, human studies generally support a role for REM sleep in procedural learning. Following learning of new motor skills such as trampolining, REM sleep amount increases (Buchegger and Meier-Koll, 1988; Buchegger et al., 1991). Training on motor learning tasks such as the mirror tracing and tower of Hanoi tasks increase subsequent REM sleep intensity (Smith et al., 2004). Improvements on the mirror tracing task are greatest following late (REM rich) sleep (Plihal and Born, 1997), and daytime naps improve performance on this task only when the nap contains REM sleep (Backhaus and Junghanns, 2006; Tucker et al., 2006). Performance on a visual discrimination task improves following REM-containing, but not REM-lacking, naps (Mednick et al., 2003) and nighttime sleep (Karni et al., 1994). Furthermore, performance on this task correlates with amount of REM sleep during the last quarter of the night (Stickgold et al., 2000b) and EEG power (in the 8-12Hz band) during REM sleep (Suzuki et al., 2012). Performance on another procedural motor task, learning a finger-tapping sequence, is correlated with REM sleep amount and is impaired by

cholinergic blockade during late (REM-rich) sleep after learning (Fischer et al., 2002; Rasch et al., 2009). Learning non-declarative verbal priming tasks is impaired by REM sleep deprivation following acquisition and performance improvements are greatest following late (REM rich) sleep (Smith, 1995; Plihal and Born, 1999).

The role of REM sleep in consolidation: Rodents. Despite evidence in the human literature that REM sleep consolidates hippocampus-independent memories, much of the work in rodent models has focused on the effects of RSD on hippocampal learning and plasticity. For example, RSD after training impairs learning of a complex maze (Rideout, 1979), the Morris water maze (Youngblood et al., 1997, 1999; Yang et al., 2008), shuttle avoidance (Pearlman, 1969), and multiple versions of the radial arm maze (Legault et al., 2004; Aleisa et al., 2011), although RSD after training on the inhibitory avoidance task has variable effects on retention (Fishbein, 1971; Miller et al., 1971; Shiromani et al., 1979; Patti et al., 2010). Furthermore, RSD for just four hours immediately following learning impairs learning of the Morris water maze (Smith and Rose, 1996) and the win-shift radial arm maze (Legault et al., 2010), but is not effective until 9-20 hours after learning the shuttle avoidance task (Smith and Butler, 1982; Martí-Nicolovius et al., 1988) (but see Leconte et al., 1974) indicating that there is a time window during which REM sleep is required for consolidation that depends on the task.

Some of the mechanisms by which RSD may impair hippocampal learning have been identified. RSD prior to plasticity induction impairs LTP induction and maintenance in hippocampal CA1 slices *ex vivo* (Davis et al., 2003; Ravassard et al., 2009), as well as in CA1 and the dentate gyrus *in vivo* (Aleisa et al., 2011). This may result from decreased membrane excitability in CA1 pyramidal neurons, decreased hippocampal

synaptic transmission, and/or decreased ERK phosphorylation, all of which are caused by RSD (Yang et al., 2008; Ravassard et al., 2009).

All of the studies described above employed variants of the "flowerpot" method to achieve RSD. Using this experimental design, an animal is placed on a platform situated over a water bath. The platform is large enough for the animal to enter NREM sleep, but muscle atonia at the onset of REM sleep causes the animal to fall from the platform and wake up (Jouvet et al., 1964). Control animals in these experiments are placed on a large platform over water on which the animal can enter REM sleep, providing a similar novel environment but no RSD. This technique is highly effective at eliminating REM sleep, and can be continued over long periods of time. However, despite its wide use, this method cannot control for the nonspecific effects of RSD. Repeated attempts to enter REM sleep increase time spent awake and fragment NREM sleep, which are not controlled for in the large-platform condition (Vimont-Vicary et al., 1966; Morden et al., 1967). Sleep fragmentation alone, accomplished by housing rats on an intermittently activate treadmill, impairs hippocampal LTP and performance on the Morris water maze (Tartar et al., 2006), as well as decreasing hippocampal CA1 pyramidal cell excitability and input resistance (Tartar et al., 2010), mimicking some of the effects of RSD. Together, these studies suggest that the impairments in hippocampal learning and plasticity observed after RSD may be attributable to the effects of sleep fragmentation rather than the removal of REM sleep *per se.* In support of this interpretation, manual RSD with a yoked animal to control for sleep fragmentation did not produce the same deficit on shuttle avoidance learning that was observed using the flowerpot technique (Van Hulzen and Coenen, 1979), whereas studies using manual

RSD without controlling for sleep fragmentation have found that RSD impairs maintenance of hippocampal LTP *in vivo* (Romcy-Pereira and Pavlides, 2004).

Nonetheless, some studies support a role for REM sleep in hippocampus-dependent learning without the confounding effects of sleep fragmentation. For example, pharmacologically decreasing REM sleep impairs learning on 2-way active avoidance task without major changes to NREM sleep and wake (although NREM sleep fragmentation was not examined) (Fogel et al., 2010). Moreover, many events have been observed during spontaneous REM sleep that may promote plasticity. For example, replay has occurs in hippocampal neurons during REM sleep (Louie and Wilson, 2001). In addition, plasticity-related intracellular signaling events including ERK phosphorylation, cAMP production, PKA activity, and CREB phosphorylation are all elevated during spontaneous REM sleep (Luo et al., 2013). The reactivation of neuronal circuitry and plasticity-related intracellular signaling pathways during REM sleep may therefore promote consolidation.

The hippocampus is involved in acquisition of many tasks, but newly acquired memories are thought to be transferred to the cortex for long-term storage, as previously discussed. This process may occur during REM sleep. Inducing hippocampal LTP *in vivo* or exposing animals to an enriched environment causes waves of immediate early gene expression that migrate away from the hippocampus during REM sleep, suggesting that plasticity is transferred from hippocampal to cortical circuitry during REM sleep (Ribeiro et al., 1999, 2002, 2007). However, LTP induced at CA1-medial prefrontal cortex synapses is *enhanced,* rather than inhibited, by subsequent RSD (Romcy-Pereira and Pavlides, 2004). These studies raise intriguing possibilities for the

role of REM sleep in system consolidation, but ultimately more detailed investigations are required to determine how REM sleep affects plasticity outside of the hippocampus.

The role of REM sleep in consolidation: ODP. Whether REM sleep consolidates hippocampus-independent plasticity has not been addressed in the animal literature. Sleep-dependent ODP in the cat is a model uniquely suited to answer this question, since many of its underlying mechanisms have been described, and REM sleep time is much greater in the cat than in rodent models. The requirement of REM sleep in this model has not been directly tested, and several studies have provided conflicting evidence. Frank et al. (2001) found that the extent of the OD shift correlates with the amount of NREM, but not REM, sleep that the animal received following MD. Consistent with this finding, six hours of post-MD RSD did not impair ODP, although the strong drive to enter REM sleep in young kittens causes the accumulation of substantial REM sleep amounts over the six-hour period that may explain this negative result (Frank et al., 2001; Aton et al., 2009a). In contrast, changes in neuronal firing rates during post-MD sleep occur during both NREM and REM sleep, suggesting that both of these states may contribute to ODP consolidation (Aton et al., 2009a, 2013).

The respective contributions of NREM and REM sleep to ODP will be thoroughly explored in **Chapter 3**. We will describe the effects of brief RSD on physiological measures of ODP, as well as its underlying biochemical mechanisms. Together, the studies described in the following chapters shed light on the mechanisms underlying sleep-dependent ODP consolidation, and the respective roles of REM and NREM sleep in these processes.

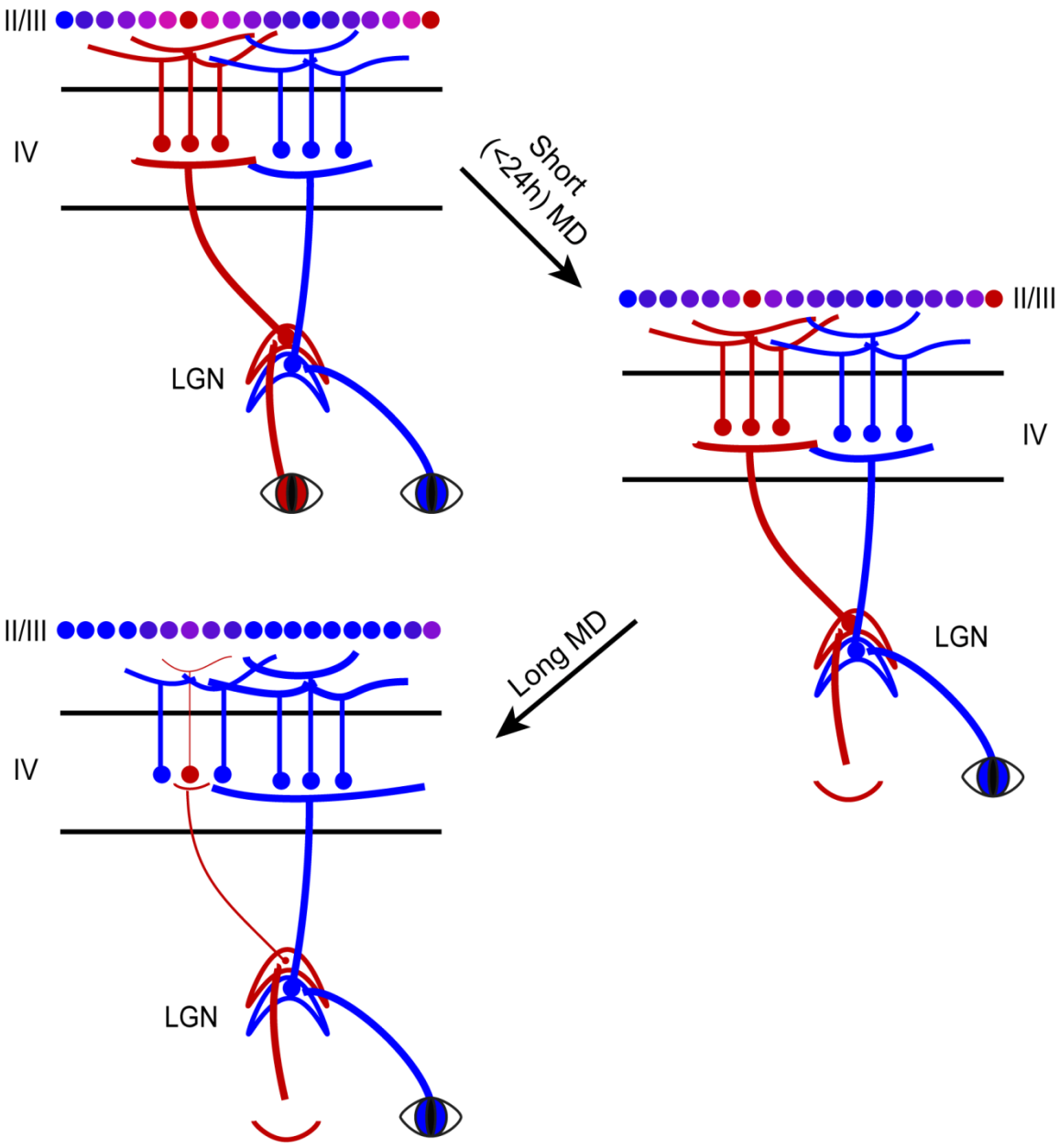

Figure 1.1. Ocular dominance plasticity in the cat. During short (<24h) MD, loss of patterned neuronal activity from the deprived eye causes decorrelated synaptic input. Depression of deprived eye responses and potentiation of nondeprived eye responses occur within layer II/III without changes in layer IV or the LGN. After longer periods of MD, the OD shift occurs in layer IV and thalamocortical cells of the LGN.

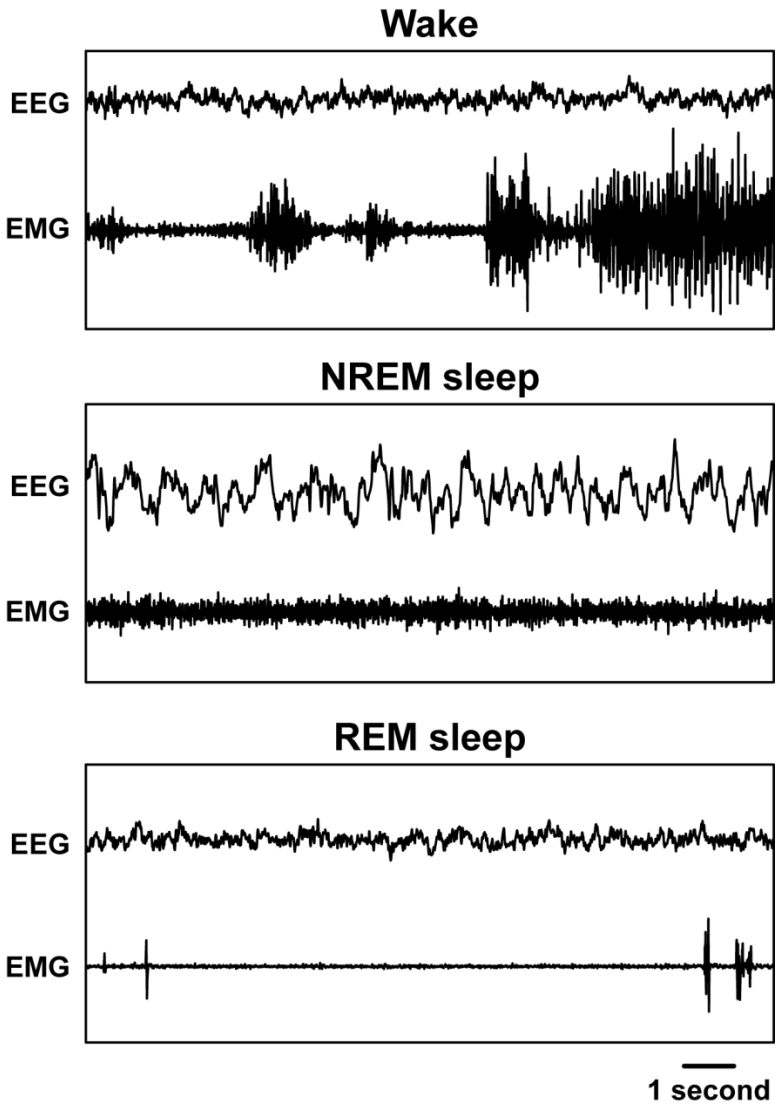

Figure 1.2. Polysomnography recordings in the cat. EEG activity during waking is low amplitude and high frequency, while the EMG signal is high. In contrast, high amplitude EEG signals predominated by low frequency oscillations (slow wave activity, 0-1 Hz; delta activity 1-4 Hz) occur during NREM sleep. The EMG signal during NREM sleep is lower than waking, but some motor tone persists. During REM sleep, EEG activity is low amplitude and high frequency, resembling waking. However, the EMG signal during REM sleep is absent with the exception of occasional brief muscle twitches.

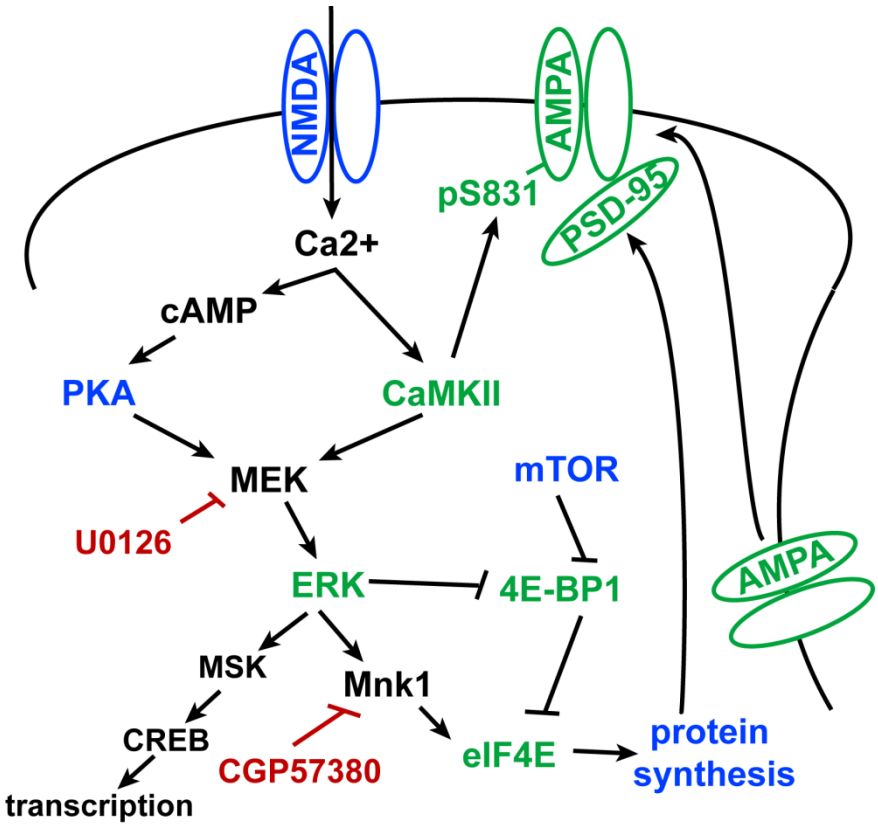

Figure 1.3. Intracellular pathways implicated in sleep-dependent ODP consolidation. Blue: steps shown to be *required* during sleep for ODP consolidation (i.e. infusion of specific inhibitors during post-MD sleep abolishes ODP). Green: events that occur during post-MD sleep, but whose requirement for sleep-dependent ODP consolidation has not been demonstrated. Black: events implicated in ODP but whose contributions during sleep have not been specifically investigated. Although ERK phosphorylation increases during post-MD sleep, the requirements of ERK and its downstream kinase Mnk1 have not been determined. In **Chapter 2**, we will investigate the requirement of the ERK-Mnk1 pathway in sleep-dependent ODP consolidation by intracortical infusion of two inhibitors (red): U0126 and CGP57380. In **Chapter 3**, we will investigate which of these molecular pathways are activated during NREM and REM sleep.

CHAPTER 2: EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) ACTIVITY DURING SLEEP CONSOLIDATES CORTICAL PLASTICITY *IN VIVO*

Michelle C. Dumoulin[1], Sara J. Aton[1], Adam J. Watson[1], Leslie Renouard[1], Tammi Coleman[1], and Marcos G. Frank[1]

[1]Dept of Neuroscience, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Abstract.

Ocular dominance plasticity (ODP) in the cat primary visual cortex (V1) is induced during waking by monocular deprivation (MD) and consolidated during subsequent sleep. The mechanisms underlying this process are incompletely understood. Extracellular signal-regulated kinase (ERK) is activated in V1 during sleep after MD, but it is unknown whether ERK activation during sleep is necessary for ODP consolidation. We investigated the role of ERK in sleep-dependent ODP consolidation by inhibiting the ERK-activating enzyme MEK in V1 (via U0126) during post-MD sleep. ODP consolidation was then measured with extracellular microelectrode recordings. Western blot analysis was used to confirm the efficacy of U0126 and to examine proteins downstream of ERK. U0126 abolished ODP consolidation and reduced both phosphorylation of eukaryotic initiation factor 4E (eIF4E) and levels of the synaptic marker PSD-95. Furthermore, interfering with ERK-mediated translation by inhibiting MAP kinase-interacting kinase 1 (Mnk1) with CGP57380 mimicked the effects of U0126. These results demonstrate that ODP consolidation requires sleep-dependent activation of the ERK-Mnk1 pathway.

Introduction.

Ocular dominance plasticity (ODP) is a canonical model of synaptic remodeling *in vivo* that has revealed many general principles underlying experience-dependent plasticity (reviewed in Berardi et al., 2003). In kittens, occluding vision in one eye (monocular deprivation, MD) during a critical period of development causes cells in the primary visual cortex (V1) to shift from being predominantly binocular to being primarily driven by nondeprived eye stimulation, as measured by single neuron recordings (Wiesel and Hubel, 1963a; Hubel and Wiesel, 1970). ODP can be induced with as little as six hours of MD during waking, and is consolidated during subsequent sleep (Frank et al., 2001). Although the mechanisms underlying sleep-dependent consolidation are largely unidentified, they include NMDA receptor and protein kinase A (PKA) activation (Aton et al., 2009a), as well as translation via the mammalian target of rapamycin (mTOR) pathway (Seibt et al., 2012). The functional role of other kinases activated during post-MD sleep is unknown. For example, extracellular signal-regulated kinase (ERK) is phosphorylated downstream of NMDA receptor activation during post-MD sleep (Aton et al., 2009a), but it is unclear whether ERK activity during sleep is required for ODP consolidation.

ERK has been implicated in many forms of neuronal plasticity, and is therefore an attractive candidate mechanism for regulating sleep-dependent plasticity. For example, ERK is necessary for visual cortical long-term potentiation (LTP) and long-term depression (LTD) *in vitro* (DiCristo et al., 2001; McCoy and McMahon, 2007; McCoy et al., 2008). Furthermore, ERK and mTOR co-regulate translation (via eukaryotic initiation factor 4E (eIF4E) and eIF4E binding protein (4E-BP1) phosphorylation) in multiple forms of hippocampal plasticity (Kelleher et al., 2004; Banko et al., 2006; Gelinas et al., 2007;

Tsokas et al., 2007). ERK promotes eIF4E phosphorylation by activating an intermediate kinase, MAP kinase-interacting kinase 1 (Mnk1) (Waskiewicz et al., 1997; Ueda et al., 2004). Mnk1 phosphorylation is elevated during hippocampal LTP (Gelinas et al., 2007) and required for hippocampal LTP consolidation (Panja et al., 2009). However, the precise role of the ERK-Mnk1 pathway in sleep-dependent plasticity has not been previously explored. Continuous infusion of ERK inhibitors (during both sleep and wake) for 1 week blocks ODP *in vivo* (DiCristo et al., 2001), but this approach does not reveal the specific role of ERK activity in sleep, and the molecular mechanisms downstream of ERK were not investigated. There are also no previous studies of Mnk1 in the induction or consolidation of ODP.

We hypothesized that activation of the ERK-Mnk1 transduction pathway during sleep is required for ODP consolidation. To test this hypothesis, we inhibited ERK (with U0126) or Mnk1 (with CGP57380) activity in the sleeping, remodeling visual cortex. We found that neither compound altered sleep architecture, but both profoundly reduced the enhancement of plasticity normally observed after sleep. In addition, ERK and Mnk1 inhibition during sleep both reduced eIF4E phosphorylation and PSD-95 levels. These results demonstrate that the ERK-Mnk1 pathway plays an important role in sleep-dependent synaptic plasticity *in vivo*, and suggest it may do so by regulating synthesis of plasticity-related proteins. A version of the work presented in this chapter has been previously published (Dumoulin et al., 2013).

***Materials and Methods*.**

All animal experiments were approved by the University of Pennsylvania IACUC and were performed as required by USDA regulations. Cats were bred in-house and housed with their queens on a 12:12 light:dark cycle prior to experimentation.

Surgical procedures for polysomnography and intracortical infusion. Seven female and 12 male cats were anesthetized and prepared for surgery as described previously (Frank et al., 2001; Jha et al., 2005). A craniotomy was performed bilaterally over V1 and the dura was partially reflected. Cannula guides with two attached EEG electrodes (Plastics One; Roanoke, VA) were positioned over V1 (Figure 2.1). An internal "dummy" cannula (solid stainless steel) was inserted into the cannula guide and remained in place until the start of the infusion to protect the cortex from infection. Five bilateral frontoparietal EEG and three nuchal EMG electrodes attached to an electrical socket were also implanted. Cannula assemblies and electrodes were affixed to the skull with bone screws and dental acrylic. Following a minimum of four days post-operative recovery and treatments, male and female animals were randomly assigned to the experiments described below.

Monocular deprivation and infusion procedures. At the peak of the critical period for visual cortical plasticity (Postnatal day 28-35), cats were singly housed in a recording chamber. As previously described (Jha et al., 2005), EEG and EMG signals were routed from the animal to an amplifier system (Grass Technologies; Warwick, RI) via an electrical cable tether/commutator attached to the implanted electrical sockets. The bipolar electrode pair attached to each cannula guide recorded EEG activity local to the infusion site. Polygraphic signals were high-pass filtered at 0.1 Hz (EEG) or 10Hz

(EMG), and low-pass filtered at 100Hz. Signals were digitized at 200Hz and recorded on a personal computer running commercial sleep-recording software (VitalRecorder; Kissei Comtec America, Inc.; Irvine, CA). After at least six hours of baseline recording, animals were briefly anesthetized with isoflurane and the right eyelid was sutured shut. Animals were then kept awake for 6 hours in lighted conditions, ensuring that all cats received 6 hours of monocular vision to induce cortical remodeling (Frank et al., 2001). At the end of this period, the dummy cannula was removed from the cannula guide and replaced with a sterile stainless steel cannula (28G internal diameter with the tip extending 1mm below the pial surface) attached to sterile tubing (see Figure 2.1). Tubing and cannulas were pre-filled with drug or vehicle solution. Animals were then allowed sleep *ad libitum* in complete darkness for 1-6 hours, during which time U0126 (5.26mM; Promega; Madison, WI) (Schafe et al., 2000), CGP57380 (2mM; Tocris Bioscience; Ellisville, MO) (Panja et al., 2009), or vehicle was infused bilaterally into V1 at a rate of 0.3 μL/min (Harvard Apparatus; Holliston, MA). U0126 and CGP57380 were first dissolved in DMSO (Sigma-Aldrich; St. Louis, MO), then diluted to their final concentrations 1:1 in ACSF (Harvard Apparatus) prior to infusion. An additional group of cats ('MD-only') received infusions of vehicle in one hemisphere and U0126 in the other during the MD period; the drug-infused hemisphere was alternated between animals. These animals did not receive post-MD sleep.

We chose U0126 because this compound potently inhibits the kinase that activates ERK (mitogen-activated protein kinase kinase, MEK) without nonspecific effects on other kinases (including the plasticity-related kinases PKC, CaMKII, and PKA) (Favata et al., 1998; Roberson et al., 1999). Similarly, CGP57380 inhibits Mnk1 without nonspecific effects on other kinases, including ERK and PKC (Knauf et al., 2001).

Tissue collection and western blotting. Western blotting was used to determine the efficacy of kinase inhibitors on target proteins and to examine the downstream effects of kinase inhibition. Eight cats were used in these experiments (Vehicle: 3 cats; U0126: 3 cats; CGP57380: 2 cats). Immediately following one hour of post-MD sleep with intracortical infusion, animals were anesthetized with isoflurane and sacrificed with an intracardiac injection of pentobarbital/phenytoin. V1 tissue near to (within 1mm) and far from the cannula was rapidly collected and frozen on dry ice. Whole-tissue extracts were prepared and analyzed as previously described (Aton et al., 2009a). Briefly, tissue was homogenized in lysis buffer containing 100 mM NaCl, 10 mM $Na_4P_2O_7 \cdot 10H_2O$, 10 mM $NaPO_4$, 50 mM NaF, 1 mM Na_3VO_4, 5 mM EDTA, 5 mM EGTA, 1% SDS, and phosphatase and protease inhibitor cocktails (1:100; Sigma). Lysates were then centrifuged to remove cellular debris and boiled in gel loading buffer (2.5%SDS) for 10 minutes. Protein concentration was quantified using a micro-BCA assay (Thermo Scientific; Rockford, IL). Proteins (40 μg/well) were separated on pre-cast polyacrylamide gels (Bio-Rad; Hercules, CA), and transferred onto nitrocellulose membranes (0.45 μM pore size; Invitrogen; Carlsbad, CA). Membranes were blocked at room temperature in Odyssey blocking buffer (Li-Cor; Lincoln, NE) then incubated overnight with primary antibodies diluted in blocking buffer and 0.1% Tween-20 (Sigma-Aldrich) at 4°C. Primary antibodies were raised in rabbit and obtained from Cell Signaling Technology (Danvers, MA), unless otherwise noted. The following primary antibodies were used: anti-ERK1/2 (1:1,000), anti-phospho-ERK1/2 (Thr202/Tyr204 and Thr185/Tyr187, respectively) (1:500), anti-CaMKIIα (1:1500) (Abcam; Cambridge, MA), anti-phospho-CaMKII (Thr286) (1:1000) (Abcam), anti-eIF4E (1:1,000), anti-phospho-

eIF4E (Ser209) (1:500) (Millipore; Billerica, MA), anti-CREB (1:500), anti-phospho-CREB (Ser133) (1:500) (Millipore), anti-eEF2 (1:1,000), anti-phospho-eEF2 (Thr56) (1:500), and anti-PSD-95 (1:500) (Thermo Scientific). We attempted to measure phosphorylation of additional ERK substrates that regulate transcription and translation (Elk-1 and Mnk1, respectively), but these antibodies did not work in feline tissue. Mouse anti-β-actin (1:10,000) (Sigma-Aldrich) was included with each primary antibody as a loading control. Membranes were incubated with fluorescently conjugated goat anti-rabbit (800 nm) and anti-mouse (700nm) antibodies simultaneously (Li-Cor) (1:20,000 in blocking buffer/0.1% Tween) for one hour at room temperature in the dark. Each membrane was scanned at both 700 nm (β-actin loading control) and 800 nm (protein of interest) and quantified with the Odyssey infrared scanner and image quantification software (Li-Cor). Variation across gels was controlled for by normalizing to a common sample run on multiple gels. Quantified proteins from U0126- and CGP57380-infused groups were compared with the same vehicle control group.

Single unit recording. Separate groups of cats (15 cats total) were used to determine the role of ERK and Mnk1 in sleep-dependent ODP consolidation. Immediately following six hours of intracortical infusion, cats were prepared for single unit recording (Jha et al., 2005). Animals were anesthetized with isoflurane and Nembutal and the skull above V1 was removed. Animals were paralyzed with a continuous intravenous infusion of Flaxedil and ventilated. The dura above V1 was removed and a 16-electrode array (FHC, Inc.; Bowdoin, ME) was lowered into V1 at sites near to (within 1mm) and far from the cannula. Contact lenses were used to focus the eyes on a monitor positioned 40 cm away. Extracellular single unit recordings were obtained while presenting one eye at a time with a blank screen or full-field reversing, drifting square gratings at eight stimulus

orientations. Each presentation was repeated four times per recording block. Between recording blocks, the electrode array was advanced deeper into the brain in 100μm steps or moved to a new location. Two to three penetrations were made at each recording site.

Units were sorted off-line using principal component analysis (Offline Sorter; Plexon, Inc.; Dallas, TX) and analyzed as previously described (Jha et al., 2005; Aton et al., 2009a). For comparison, unit data from four cats with binocular vision and unmanipulated sleep (referred to as 'normal' animals) were reproduced with permission from earlier studies (Aton et al., 2009a; Seibt et al., 2012). Briefly, each unit was assigned an ocular dominance (OD) category of 1-7 according to its left eye/right eye firing ratio. Visually unresponsive neurons (those that responded more strongly to blank screen than to gratings) were excluded from the analysis. OD scores were then used to calculate scalar measures of ODP (Issa et al., 1999). The contralateral eye bias index (CBI) is a weighted average of OD scores from the population of neurons sampled, where higher values indicate a stronger bias towards the contralateral eye. This measure was calculated separately for near and far recording sites in each hemisphere and converted to the non-deprived eye bias index (NBI) (where higher values indicate a stronger bias for the non-deprived eye) for clarity (Aton et al., 2009a). CBI values were also used to calculate the shift index (SI), a measure of overall changes in OD across both hemispheres of an animal (Equation 2.1). The monocularity index (MI) was also calculated, where a value of 0 indicates that all recorded neurons respond equally to both eyes and 1 indicates a complete loss of binocular responses. Scalar ODP measures from U0126- and CGP57380-infused groups were compared with values from the same vehicle control group.

$$SI = CBI_{\text{ipsilateral to deprived eye}} - CBI_{\text{contralateral to deprived eye}}$$

EQUATION 2.1

In addition to analyzing unit recordings on a population level, firing properties of individual units were examined. These properties include normalized peak firing rate, normalized spontaneous firing rate, evoked response index (ERI), and orientation selectivity index (OSI). For each unit, the peak and spontaneous firing rates were normalized to the average firing rate of all neurons that were recorded at the same location, as described previously (Aton et al., 2009a). The OSI, a measure of orientation tuning, was calculated as the unit's response strength at its preferred orientation versus the orthogonal (90°) orientation. The ERI, a measure of how selectively a neuron fires in response to visual stimulation versus spontaneous activity, was calculated using Equation 2.2. Neuronal properties were averaged within each recording site (Aton et al., 2009a; Seibt et al., 2012).

$$ERI = 1 - \frac{\textit{Spontaneous firing rate}}{\textit{Peak firing rate}}$$

EQUATION 2.2

Sleep/wake analysis. Vigilance states were manually scored as rapid eye movement (REM) sleep, non-REM (NREM) sleep, or wake in 8-second epochs by a trained experimenter (M.C.D). States were scored based on frontoparietal EEG and EMG signals (SleepSign for Animal; Kissei Comtec America, Inc.; Irvine, CA) according to previously described criteria (Frank et al., 2001). Percentage of total recording time and average bout duration for each vigilance state was calculated for baseline, MD, and

post-MD periods. The number of sleep-wake transitions and the total number of state transitions were also calculated for the post-MD period. Fast Fourier transforms were performed on post-MD frontoparietal and V1 EEG recordings and averaged in 2-hour bins to quantify changes in EEG activity during the infusion. For each hemisphere, power was averaged within the delta (0.5-4 Hz), sigma (10-15 Hz), theta (5-8 Hz), and high frequency (15-40 Hz) bands and normalized to mean baseline power (Jha et al., 2005). Two vehicle-infused hemispheres were excluded from the power analysis because the EEG signals at the cannula sites were unusable.

Statistical Analysis. Statistical tests were performed using SigmaPlot 11.0 (Systat Software, Inc.; San Jose, CA). Groups were compared using 1-tailed Student's t-tests, one-way ANOVAs, or 2-way ANOVAs, as indicated. The use of planned, 1-tailed t-tests (α=0.05, 95% confidence level) was specified prior to analysis, since we were interested in whether the drugs specifically impaired ODP and its underlying biochemical changes (Zar, 1999). Nonparametric tests (Mann-Whitney U tests, ANOVAs on ranks) were used in cases where data were not normally distributed.

Results.

U0126 inhibits ERK phosphorylation. To confirm the efficacy of U0126, we measured ERK phosphorylation in V1 infused with U0126 or vehicle for one hour of post-MD sleep, at a time when ERK phosphorylation is normally high (Aton et al., 2009a) (Figure 2.2 A). Western blot analysis revealed that near the cannula, U0126 significantly decreased ERK1 and ERK2 phosphorylation to 8.13±2.53% and 5.71±2.18% of vehicle levels, respectively (Figure 2.2 B, C). Furthermore, within U0126-infused hemispheres, ERK1/2

phosphorylation was lower near to the cannula than far from the cannula ($p=0.009$, Mann-Whitney U test; data not shown). There was no difference between near and far sites in vehicle-infused hemispheres ($p>0.05$, Mann-Whitney U test; data not shown).

Similar to ERK, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylation is elevated in the sleeping visual cortex following MD (Aton et al., 2009a). To verify that the effects of U0126 could not be explained by nonspecific inhibition of CaMKII, western blots for total and phosphorylated CaMKIIα in vehicle- and U0126-infused V1 tissue were performed. U0126 did not alter CaMKIIα phosphorylation (Figure 2.2 B, C), consistent with the previous observation that U0126 does not affect CaMKII phosphorylation in the hippocampus (Roberson et al., 1999).

ERK inhibition during sleep impairs ODP consolidation. Inhibition of ERK in the sleeping, remodeling visual cortex abolished the normal effects of sleep on ODP. Ocular dominance (OD) histograms revealed that the distribution of recorded cells was biased towards the non-deprived eye in vehicle-treated hemispheres and far from the cannula in U0126-infused hemispheres. In contrast, U0126 blocked this shift near the site of the infusion (Figure 2.3 B).

The extent of the OD shift was quantified using the non-deprived eye bias index (NBI) and the shift index (SI) (see Methods) (Figure 2.3 C). The NBI ranges from 0 (only responsive to the deprived eye) to 1 (only responsive to the non-deprived eye), while the SI ranges from -1 (only responsive to the deprived eye) to 1 (only responsive to the non-deprived eye). Both the NBI and SI were significantly lower in U0126- than vehicle-infused V1 when measured near the cannula. Furthermore, within U0126-infused hemispheres, the NBI was significantly lower near the cannula than far from the cannula.

These results confirm that ERK activity in V1 during sleep is required for ODP consolidation.

We have previously shown that mammalian target of rapamycin (mTOR) activity is required for the sleep-dependent consolidation of ODP, but not its induction during wakefulness (Seibt et al., 2012). We therefore examined whether ERK was necessary for ODP induction during waking MD. We infused vehicle or U0126 into V1 during 6 hour MD in awake animals, then obtained single unit recordings near to the cannula and compared the resulting monocularity index (MI) with normal animals (Figure 2.4). We examined the MI in this case because it is a more sensitive measure of this early stage of ODP than the NBI (Aton et al., 2009a). Consistent with previous results (Frank et al., 2001; Aton et al., 2009a), 6 hours of MD with vehicle infusion produced a small but significant increase in the MI relative to cats with normal binocular vision. MD-only hemispheres infused with U0126 also had a significantly higher MI than normal controls, indicating that ERK activity specifically during wake is not necessary for ODP induction.

U0126 infusion during sleep impairs eIF4E phosphorylation. ERK can promote plasticity by phosphorylating many substrates, including those involved in transcriptional and translational control (reviewed in Adams and Sweatt, 2002). However, it is unknown which of these pathways are activated by ERK during sleep-dependent consolidation. Therefore, we used western blot analysis to measure proteins phosphorylated downstream of ERK activation (eIF4E; eukaryotic elongation factor 2, eEF2; cAMP response element binding protein, CREB) in U0126- and vehicle-infused V1. These proteins regulate mRNA translation initiation (eIF4E), translation elongation (eEF2), and transcription (CREB). We also measured levels of post-synaptic density protein 95 (PSD-95), a post-synaptic scaffolding protein whose expression increases AMPA

receptor currents during experience-dependent plasticity *in vivo* (Ehrlich and Malinow, 2004). Sleep promotes the synthesis of PSD-95 in V1 (Seibt et al., 2012), but it is unknown whether this requires ERK activity.

U0126 significantly decreased eIF4E phosphorylation and levels of PSD-95 (Figure 2.5). In contrast, we did not find a significant effect of U0126 on eEF2 or CREB phosphorylation compared to vehicle. Together, these results suggest that ERK activity in the sleeping, remodeling cortex promotes the synthesis of proteins such as PSD-95, which may contribute to consolidation.

Mnk1 inhibition during sleep mimics ERK inhibition. Mnk1 is a direct target of ERK that phosphorylates eIF4E (Waskiewicz et al., 1997). Since U0126 infusion attenuated eIF4E phosphorylation, we investigated whether directly inhibiting Mnk1 activity could mimic the effects of U0126. To do this, we infused the Mnk1 inhibitor CGP57380 into V1 during post-MD sleep. We first collected V1 tissue after 1 hour of post-MD sleep to confirm the efficacy and specificity of the drug. CGP57380 infusion significantly decreased eIF4E phosphorylation and PSD-95 levels near to the cannula site without affecting ERK phosphorylation (Figure 2.6A, B). CGP57380 did not affect phosphorylation of any other proteins examined (p-CaMKIIα, p-CREB, p-eEF2: $p>0.1$, CGP57380 n=4 hemispheres, vehicle n=6; t-test; data not shown).

We then measured the effects of infusing CGP57380 during six hours of post-MD sleep on ODP. Single-unit recordings revealed that CGP57380 attenuated sleep-dependent plasticity, mimicking the effects of U0126. As shown in Figure 2.6 C, CGP57380 prevented the normal sleep-dependent shift in the OD histogram toward the non-deprived eye near the cannula site. CGP57380 also significantly reduced scalar measures of ODP (the NBI and SI) compared to vehicle and compared with sites far

from the cannula (Figure 2.6 D). These results confirm that Mnk1 activation is required during sleep for ODP consolidation.

Single unit firing properties following drug infusion. We have previously shown that post-MD sleep enhances V1 neuronal responses to non-deprived eye stimulation (Aton et al., 2009a; Seibt et al., 2012). Since ERK activity is required for LTP in rodent V1 (DiCristo et al., 2001), we hypothesized that the ERK-Mnk1 pathway is also required for sleep-dependent enhancement of non-deprived eye responses. To test this, we compared the normalized peak firing rate in response to stimulation of each eye between normal control, vehicle-infused, U0126-infused, and CGP57380-infused hemispheres. As expected, relative to normal controls, responses to the non-deprived eye were significantly enhanced in vehicle-infused V1; however, U0126 and CGP57380 infusion prevented this enhancement (Figure 2.7). No differences across groups were observed in response to deprived eye stimulation. These results confirm that ERK and Mnk1 promote sleep-dependent ODP consolidation by enhancing non-deprived eye responses, rather than attenuating deprived eye responses.

The effects of U0126 and CGP57380 on plasticity could not be ascribed to non-specific effects of these drugs on visual cortical neurons. As shown in Table 2.1, there were no significant differences between groups on any measure of single unit firing characteristics (percent of visually responsive units, evoked response index (ERI), orientation selectivity index (OSI), and normalized spontaneous firing rate).

Sleep architecture is unaffected by drug infusion. U0126 and CGP57380 also had no significant effects on global sleep architecture. 2-Way ANOVAs revealed no significant main effect of drug ($p \geq 0.320$) or drug×time interaction ($p \geq 0.423$) for the state amount,

state duration, or number of state transitions during post-MD sleep (main effects: drug treatment, post-MD time; Vehicle N=4 animals, U0126 N=4, CGP57380 N=3) (Figure 2.8). We observed significant main effects of time over the course of the post-MD period: NREM sleep amount ($p<0.001$) and bout duration ($p<0.001$) decreased, REM sleep amount ($p=0.02$) increased, wake amount ($p<0.001$) and bout duration ($p=0.013$) increased, sleep to wake transition number increased ($p=0.002$), and total number of transitions between arousal states increased ($p=0.004$). 2-Way ANOVAs also showed no main effect of drug ($p\geq0.205$) or drug×time interaction ($p\geq0.292$) for frontoparietal EEG power in delta, theta, sigma, and high frequency (HF) bands in these animals (Figure 2.9). We observed significant main effects of post-MD time on frontoparietal EEG power: NREM sleep power in the theta ($p<0.001$), sigma ($p=0.024$), and HF bands ($p=0.019$) decreased, REM sleep theta power decreased ($p=0.012$), and REM sleep HF power increased ($p=0.011$). However, these changes to sleep architecture and EEG power occurred in all treatment groups and reflect changes similar to those previously observed during post-MD sleep (Frank et al., 2001; Jha et al., 2005; Seibt et al., 2008). There were also no significant differences between groups in EEG activity at the infusion sites. 2-Way ANOVAs (Vehicle n=5; U0126 n=7; CGP57380 n=6 hemispheres) revealed no effect of drug ($p\geq0.098$), time ($p\geq0.630$), or drug×time interaction ($p\geq0.334$) on delta, theta, sigma, or HF power within V1 (Figure 2.9 A, B).

***Discussion*.**

We show that activation of the ERK-Mnk1 pathway during sleep is necessary for ODP consolidation. More specifically, inhibition of ERK or Mnk1 during sleep in the visual cortex abolishes the normal potentiation in non-deprived visual pathways

observed after sleep. The effects of ERK inhibition during sleep cannot be explained by indirect effects of drug infusion, since sleep architecture and neuronal firing properties in V1 were unaffected. This loss of plasticity is accompanied by reductions in phosphorylated eIF4E and levels of PSD-95. Phosphorylation of eIF4E correlates with increased 5' cap-dependent mRNA translation rates. Although the underlying mechanism is unclear, phosphorylation of eIF4E promotes its dissociation from the 5' cap, which may speed translation by facilitating polysome formation or increasing the pool of available cap-binding factors (Scheper and Proud, 2002). In addition to regulating translation via eIF4E phosphorylation, ERK-Mnk signaling is required for translation initiation complex formation during plasticity *in vivo* (Panja et al., 2009). PSD-95 is a synaptic protein whose expression controls the localization of AMPA receptors to the post-synaptic membrane during plasticity *in vivo* (Ehrlich and Malinow, 2004). Therefore, the current study suggests that the ERK-Mnk1 pathway is activated in the sleeping, remodeling cortex and promotes consolidation by upregulating the synthesis of plasticity-related proteins such as PSD-95.

Mechanisms of ERK-dependent consolidation. ERK regulates multiple pathways that can ultimately lead to the stabilization of plasticity, including transcription and translation (reviewed in Adams and Sweatt, 2002). In hippocampal LTP, translation is controlled by concomitant activation of the ERK and mTOR pathways via eIF4E and 4E-BP1 phosphorylation (Kelleher et al., 2004; Gelinas et al., 2007; Tsokas et al., 2007; Connor et al., 2011). Seibt et al. (2012) showed that protein synthesis via mTOR is required during sleep for ODP consolidation; however, the role of ERK in sleep-dependent translation was not explored. Although we did not directly measure initiation complex formation or translation, our finding that U0126 and CGP57380 inhibit eIF4E phosphorylation and decrease PSD-95 levels is consistent with a dual role for ERK and

mTOR in the synthesis of plasticity-related proteins during sleep-dependent consolidation.

Translation initiation, combined with decreased elongation via eEF2 phosphorylation, can promote the synthesis of a specific pool of proteins including CaMKIIα and Arc (Scheetz et al., 2000; Belelovsky et al., 2005; Park et al., 2008). Phosphorylation of eEF2 is elevated in V1 during post-MD sleep but is not blocked by mTOR inhibition (Seibt et al., 2012). Here, we found that eEF2 phosphorylation is also unaffected by U0126, suggesting that ERK and mTOR promote a general increase in translation during sleep, while an additional factor restricts the pool of translated mRNAs by increasing eEF2 phosphorylation. One candidate for this factor is PKA, which is required for sleep-dependent ODP consolidation (Aton et al., 2009a) and can promote eEF2 phosphorylation (Redpath and Proud, 1993; Diggle et al., 2001).

In addition to regulating translation, ERK can also translocate to the nucleus and activate downstream transcription factors such as CREB (reviewed in Adams and Sweatt, 2002). Phospho-ERK nuclear translocation occurs in rat V1 after 24 hours of MD, implicating ERK as a regulator of transcription during ODP (Takamura et al., 2007). However, we found that CREB phosphorylation is not blocked by U0126 during the first hour of post-MD sleep, suggesting that ERK does not exert transcriptional control during sleep. This finding agrees with previous observations that the time course of the effects of U0126 on LTP in V1 is consistent with a role for ERK in translation rather than transcription (DiCristo et al., 2001). This result is also consistent with our previous findings that transcription of the genes *c-fos, arc,* and *bdnf* in V1 is reduced during sleep (Seibt et al., 2012). Therefore, while it is possible that ERK may lead to CREB phosphorylation on a longer timescale (between 1 and 6 hours of sleep), or that ERK

may activate other transcription factors (such as Elk-1), our results suggest that ERK does not promote transcription during sleep.

ERK and synaptic potentiation. ODP occurs through decreased V1 responsiveness to deprived eye inputs, which is initiated during waking and maintained during sleep, as well as enhanced responsiveness to non-deprived eye inputs, which occurs during post-MD sleep (Aton et al., 2009a; Seibt et al., 2012). ERK activation is required for LTP in V1 (DiCristo et al., 2001), but is also necessary for various forms of LTD, including NMDA receptor- and metabotropic glutamate receptor-dependent hippocampal LTD (Thiels et al., 2002; Gallagher et al., 2004), cerebellar LTD (Kawasaki et al., 1999; Endo and Launey, 2003), and muscarinic acetylcholine receptor-dependent visual cortical LTD (McCoy and McMahon, 2007; McCoy et al., 2008). Therefore, we considered the possibility that the ERK-Mnk1 pathway may promote ODP either by enhancing non-deprived eye responses or depressing deprived eye responses during sleep. Analysis of neuronal firing properties revealed that U0126 and CGP57380 selectively inhibited the strengthening of non-deprived eye responses in V1. Therefore, we conclude that ERK and Mnk1 activation during sleep promote ODP by potentiating synaptic responses in favor of the non-deprived eye.

Potential mechanisms of ERK activation. Although we have demonstrated a requirement for ERK in sleep-dependent plasticity, the cellular events that initiate ERK phosphorylation during sleep remain unclear. ERK can be activated by numerous mechanisms, including neuronal activity and activation of neuromodulatory inputs (Reviewed in Adams and Sweatt, 2002). Aton et al. (2009) demonstrated that ERK activation during post-MD sleep requires NMDA receptor activity. Therefore, our results support a model in which cortical activity patterns unique to sleep activate NMDA receptors, thereby promoting ERK activation. However, cortical activity patterns and

neuromodulatory tone vary greatly across sleep states, raising the question of whether ERK activation may occur preferentially during NREM or REM sleep. One possibility is that the combination of neuronal activity and high cholinergic tone during REM sleep could activate ERK via NMDA and muscarinic acetylcholine receptors. In support of this hypothesis, REM sleep deprivation has been shown to decrease ERK phosphorylation in the rat dorsal hippocampus (Ravassard et al., 2009), and spontaneous REM sleep elevates hippocampal ERK phosphorylation (Luo et al., 2013). Furthermore, Lopez and colleagues (2008) observed a trend for decreased hippocampal PSD-95 in young rats that had been deprived of REM sleep. However, it remains to be seen whether these effects of REM sleep deprivation hold true in the cortex, and whether REM sleep deprivation impairs ODP consolidation. Ultimately, more detailed investigations of kinase function during wake, REM sleep, and NREM sleep in the remodeling cortex are required to fully elucidate the mechanisms underlying cortical consolidation.

Conclusions. Although a number of mechanisms underlying neuronal plasticity *in vitro* are known, it is unclear how many of these mechanisms function *in vivo*. The present study furthers our understanding of how the ERK-Mnk1 pathway functions in the intact brain, and reveals a novel mechanism underlying sleep-dependent cortical consolidation.

Table 2.1: Firing properties of V1 neurons were unaffected by U0126 and CGP57380. Average (±SEM) firing properties of units recorded near to the site of drug infusion are shown. No significant differences were observed across drug treatments for any property. ERI, OSI, spontaneous firing rate: One-way ANOVA p>0.25; % visually responsive neurons: ANOVA on ranks p>0.6; Vehicle n=7 hemispheres, U0126 n=7, CGP57380 n=6.

	% Visually responsive neurons	Evoked Response Index		Orientation Selectivity Index		Normalized Spontaneous Firing Rate	
		Left Eye	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye
Vehicle	99.05	0.73	0.62	0.75	0.69	0.65	0.59
	±0.70	±0.02	±0.07	±0.04	±0.04	±0.06	±0.11
U0126	99.80	0.71	0.67	0.72	0.67	0.57	0.61
	±0.20	±0.05	±0.02	±0.02	±0.02	±0.07	±0.06
CGP57380	99.26	0.78	0.71	0.72	0.75	0.51	0.52
	±0.48	±0.04	±0.06	±0.03	±0.02	±0.10	±0.11

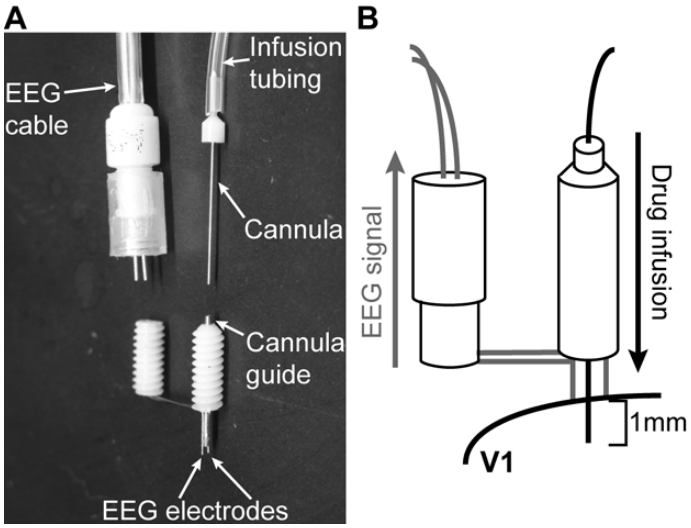

Figure 2.1. Cannula assembly. (A) The cannula guide and attached EEG electrodes were implanted into V1 during survival surgery. An internal 'dummy' cannula (solid stainless steel; not shown) was screwed into the cannula guide to protect the cortex from foreign material until experimentation. At the beginning of the baseline recording period, an EEG cable was plugged into the electrical socket connected to the two EEG leads flanking the cannula. At the beginning of the infusion, the dummy cannula was removed and replaced with a sterile stainless steel cannula (28G internal diameter) attached to sterile infusion tubing. The tubing and cannula were filled with drug or vehicle solution prior to cannula placement. (B) Schematic showing the placement of the cannula 1mm below the pial surface in V1.

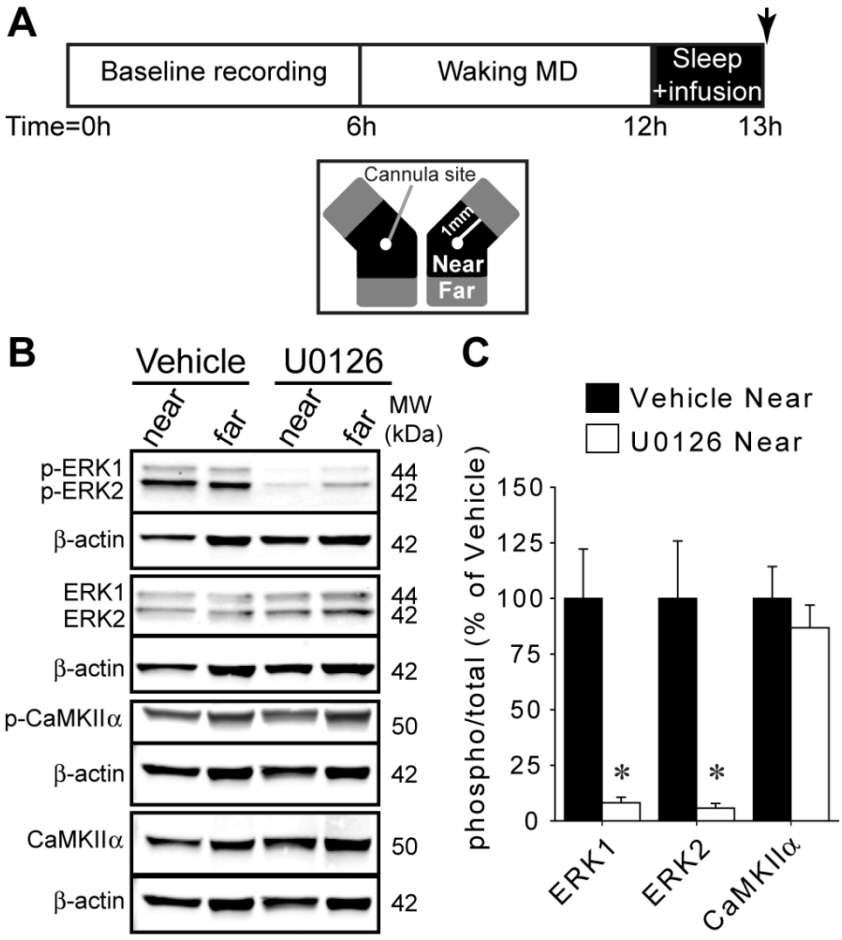

Figure 2.2. U0126 infusion blocks ERK, but not CaMKIIα, phosphorylation. (A) Top: Experimental design. Arrow indicates time at which V1 tissue was collected. Bottom: Schematic of bilaterally infused V1 tissue. Tissue was collected near (within 1mm) and far from the cannula site. (B) Representative western blots showing total and phosphorylated ERK and CaMKIIα in V1 after one hour of sleep with vehicle or U0126 infusion. Each protein of interest is shown above β-actin bands from the same blot. (C) Average (±SEM) ERK and CaMKIIα phosphorylation near the infusion sites. Phospho-ERK levels were decreased by U0126, while CaMKIIα phosphorylation was unaffected. *p=0.002, Mann-Whitney U test; n=6 hemispheres/group.

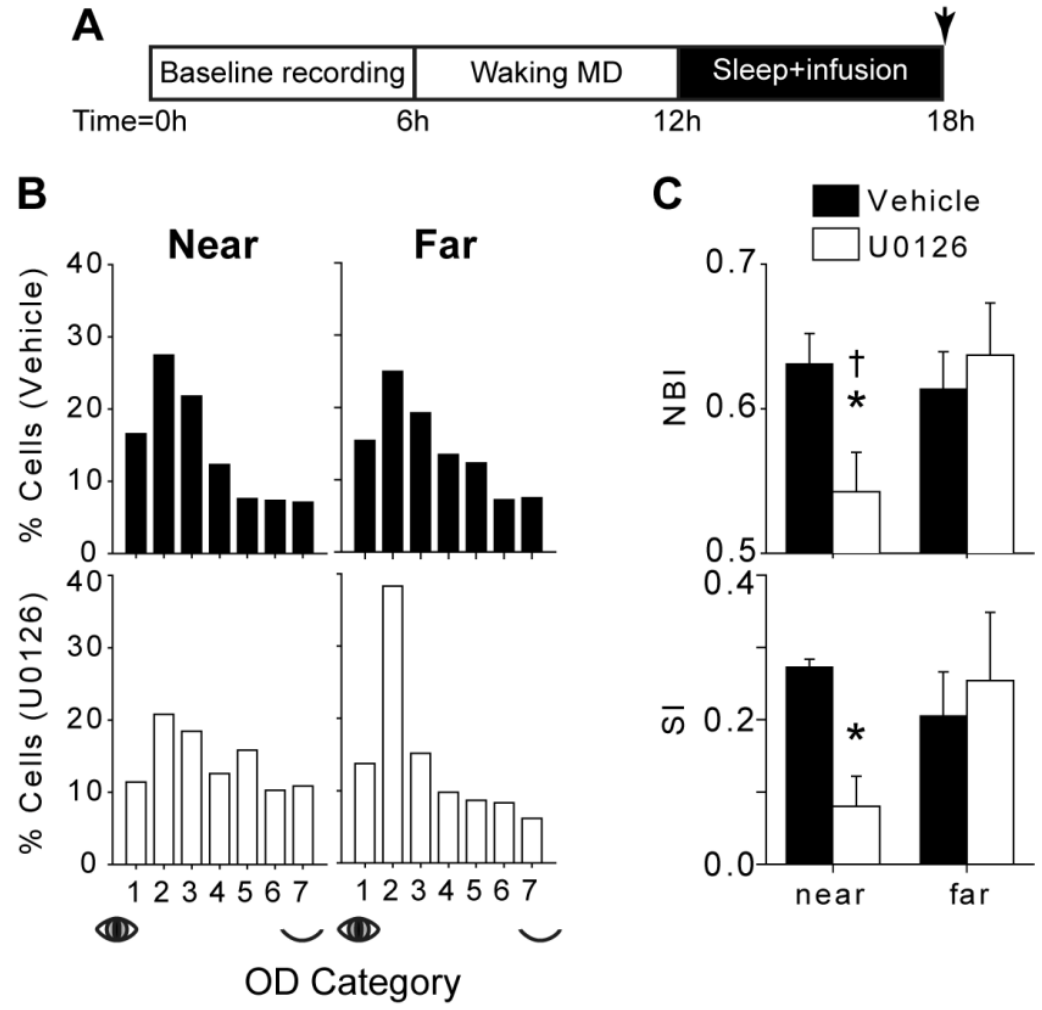

Figure 2.3. ERK inhibition abolishes sleep-dependent plasticity. (A) Experimental design. Arrow indicates the time point at which the animal was prepared for single unit recording. (B) OD histograms showing the distribution of single unit eye preference near and far from vehicle and U0126 infusion sites. Vehicle near n=423 cells; U0126 near n=342; vehicle far n=416; U0126 far n=277. (C) Average (±SEM) NBI and SI (n=7 hemispheres/group). U0126 infusion significantly attenuated the NBI and SI near the cannula compared to vehicle. The NBI was also significantly lower near vs. far from the cannula. *p<0.02, U0126 near vs. vehicle near; †p<0.03, U0126 near vs. U0126 far; t-test.

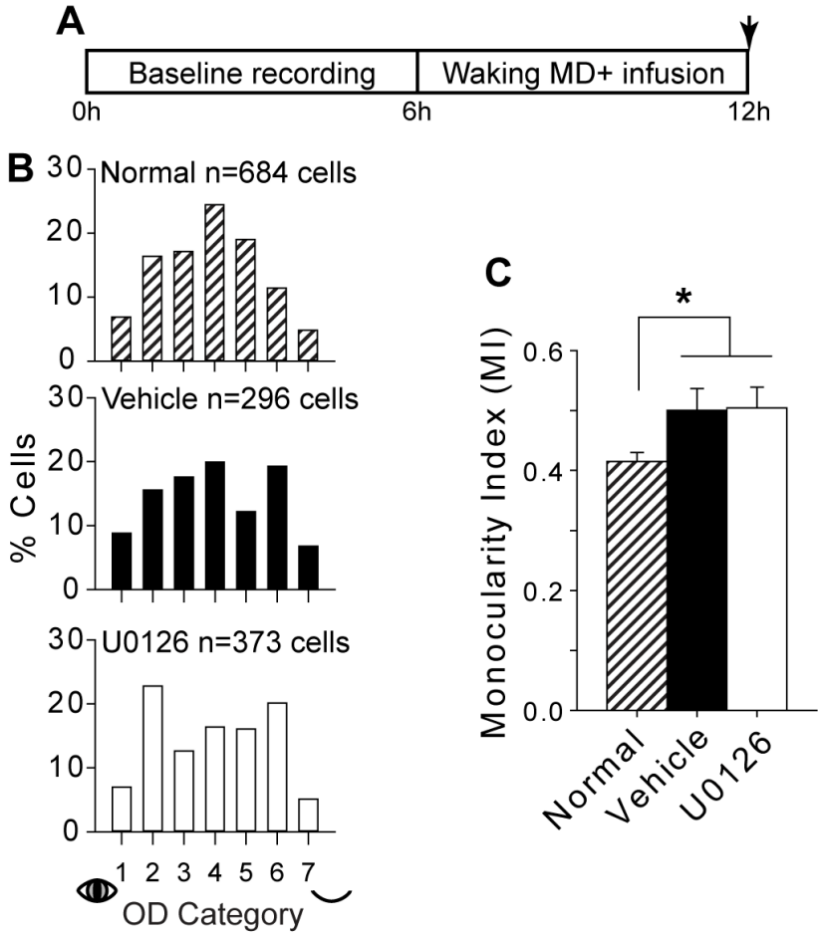

Figure 2.4. ERK inhibition during waking MD does not impair ODP. (A) Experimental design. Arrow indicates the time at which MD-only animals were prepared for unit recording. Normal animals did not undergo visual or sleep manipulation. (B) OD histograms from normal (top), vehicle-infused MD-only (middle), and U0126-infused MD-only (bottom) hemispheres. Recordings from infused hemispheres were obtained near to the cannula. (C) Mean (±SEM) MI values for each group. Binocularity decreased (indicated by increased MI) in both vehicle- and U0126-infused hemispheres compared to normal, indicating that ERK inhibition during waking does not impair ODP. *$p<0.05$, One-way ANOVA followed by Holm-Sidak post-hoc test vs. normal; Normal n=8 hemispheres, Vehicle n=4, U0126 n=4.

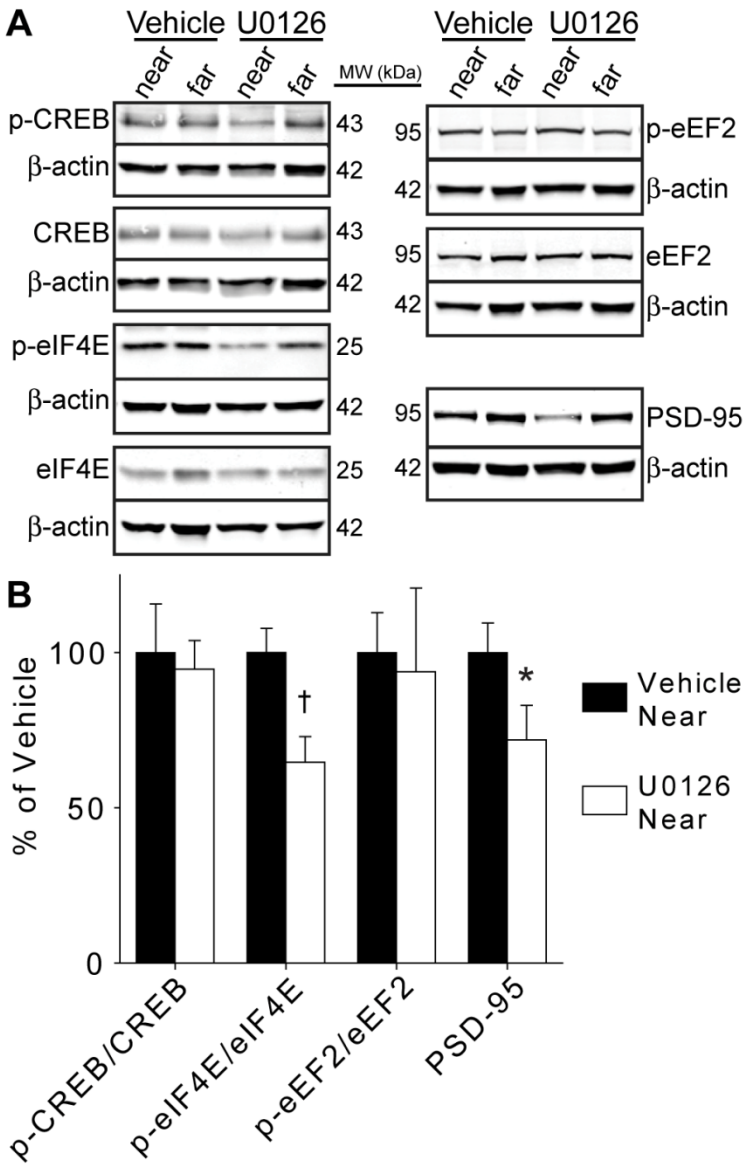

Figure 2.5. U0126 blocks downstream molecular effectors of ERK. (A) Representative western blots from vehicle- and U0126-infused V1. Bands for each protein of interest are shown above β-actin bands from the same blot. (B) Average (±SEM) levels of p-CREB, p-eIF4E, p-eEF2, and PSD-95. U0126 significantly decreased eIF4E phosphorylation and PSD-95 levels vs. vehicle. *p=0.041, †p=0.0054, n=6 hemispheres/group, t-test.

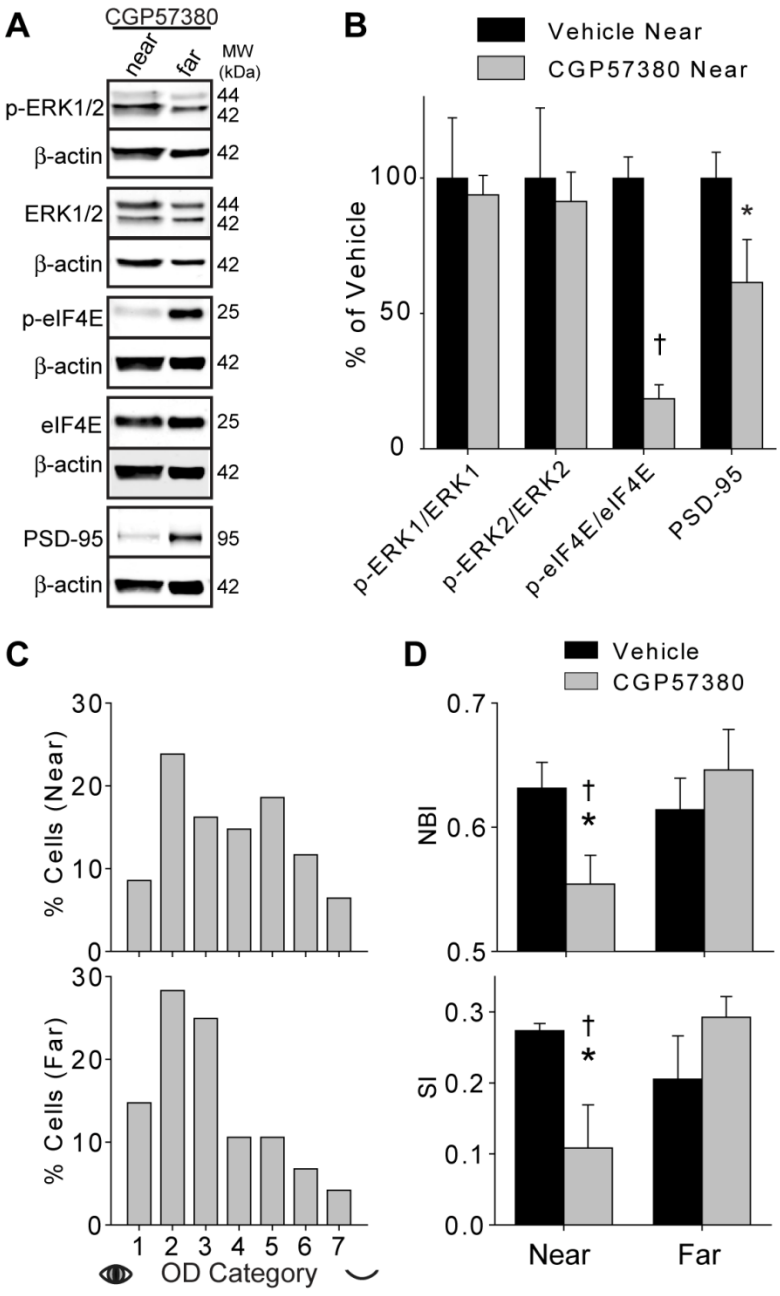

Figure 2.6. Mnk1 inhibition abolishes sleep-dependent plasticity. (A) Representative western blots. Each protein of interest is paired with β-actin bands from the same blot. (B) CGP57380 significantly decreased mean (±SEM) eIF4E phosphorylation and PSD-95 levels without affecting ERK phosphorylation. Vehicle values are reproduced from

Figures 2.2 and 2.5. *p=0.0275; †p≤0.001; CGP57380 n=4 hemispheres; t-test. (C) OD histograms revealed an attenuated shift near to, but not far from, the site of CGP57380 infusion. Near n=420 cells; far n=502. (D) Average (±SEM) NBI and SI (CGP57380 n=6 hemispheres). CGP57380 significantly impaired the OD shift near to, but not far from, the infusion site. Vehicle data are reproduced from Figure 2.3. *p<0.03, CGP57380 near vs. vehicle near; †p<0.03, CGP57380 near vs. CGP57380 far; t-test.

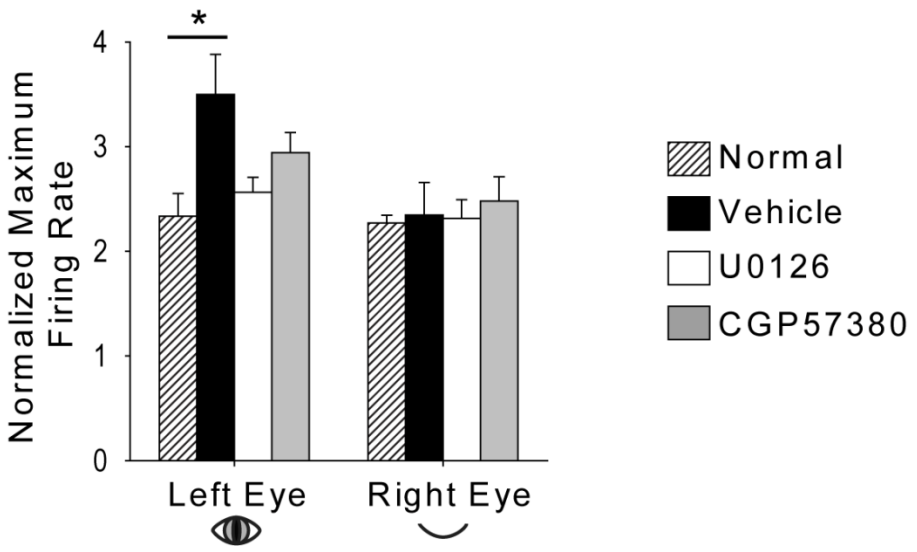

Figure 2.7. ERK and Mnk1 inhibition prevent enhancement of neuronal responses to non-deprived eye stimulation. Average (±SEM) normalized peak firing rates in response to left- and right-eye visual stimulation are shown. Left (non-deprived) eye responses were enhanced in vehicle-treated V1 compared to normal animals that did not undergo manipulations of vision or sleep. This increase was blocked near to the site of U0126 and CGP57380 infusion. *$p<0.05$, ANOVA on ranks followed by Dunn's post-hoc test. Normal n=8 hemispheres; vehicle n=7; U0126 n=7; CGP57380 n=6.

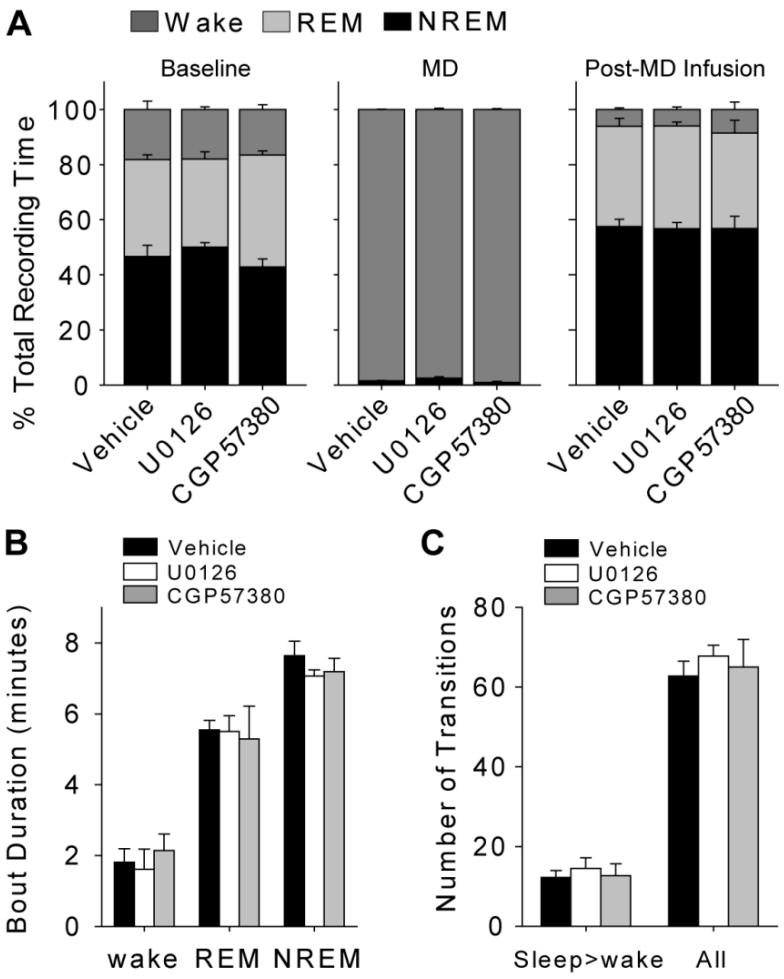

Figure 2.8. Sleep architecture is unaffected by drug infusion. Post-MD sleep characteristics are displayed as 6-hour averages for simplicity, but were divided into 2-hour bins for statistical analysis (see Results; no significant effects of drug or drug×time interaction were observed). Sleep architecture was determined using frontoparietal EEG and EMG recordings. Vehicle N=4 cats; U0126 N=4; CGP57380 N=3. (A) Mean (±SEM) amounts of wake, REM sleep, and NREM sleep during baseline, MD, and post-MD recording periods. (B) Mean (±SEM) duration of wake, REM sleep, and NREM sleep bouts during the post-MD infusion period. (C) Average (±SEM) number of transitions from sleep to wake and total number of transitions between all arousal states during the post-MD period.

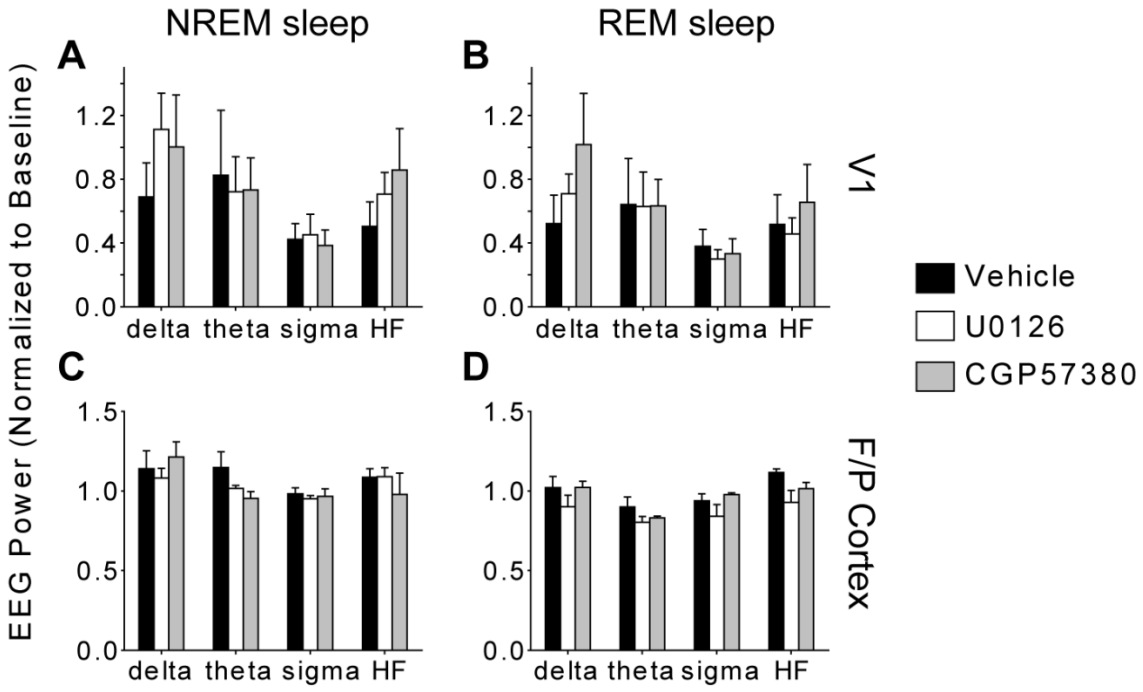

Figure 2.9. Drug infusion does not alter sleep EEG power. Data represent Fourier transformed EEG data, normalized to baseline values and averaged into standard frequency bands (delta, theta, sigma, and high frequency (HF)). Each frequency band is displayed as mean±SEM and averaged across the entire 6h recording period for simplicity, but was divided into 2-hour bins for statistical analysis (see Results; no significant drug or drug×time interaction effects were observed for any frequency band). (A-B) EEG power in V1, recorded from bipolar leads at the cannula site, did not differ between drug- and vehicle-infused cortex. (C-D) Drug infusion did not alter EEG activity in the frontoparietal (F/P) cortex.

CHAPTER 3: RAPID EYE MOVEMENT (REM) SLEEP CONSOLIDATES CORTICAL PLASTICITY *IN VIVO*

Michelle C. Dumoulin[1], Julie Seibt[1], Sara J. Aton[1], Tammi Coleman[1], Leslie Renouard[1], and Marcos G. Frank[1]

[1]Dept of Neuroscience, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Abstract.

REM sleep is hypothesized to promote brain plasticity, but the underlying mechanisms remain unclear. Ocular dominance plasticity (ODP) in the primary visual cortex (V1) is a canonical form of cortical plasticity *in vivo* induced by monocular deprivation (MD) during wakefulness and consolidated during subsequent sleep. The role of REM sleep in ODP consolidation has not been directly examined. We investigated whether REM sleep is required for ODP consolidation by implanting cats with EEG and EMG electrodes and inducing ODP with six hours of waking MD. Animals were then allowed sleep *ad libitum*, REM-deprived sleep, or NREM fragmented sleep (as a control for nonspecific effects of REM deprivation). REM sleep deprivation (RSD) was achieved by manually awakening cats upon REM sleep onset. Cats were then prepared for optical imaging of intrinsic cortical signals to measure ODP, or cortical tissue was collected to examine biochemical changes resulting from RSD. We found that RSD attenuated ODP consolidation and decreased levels of extracellular signal-regulated kinase (ERK) phosphorylation. These changes did not occur in the NREM fragmentation control group. Furthermore, ODP consolidation was rescued with recovery sleep following one hour of RSD, and one hour of undisturbed sleep was sufficient to protect

against the effects of subsequent RSD. ODP consolidation and ERK phosphorylation correlated with beta-gamma EEG activity during REM sleep, but not with REM sleep amount, suggesting that cortical activity patterns during REM sleep consolidate cortical plasticity.

Introduction.

The function of sleep remains one of the core mysteries of neuroscience. Investigating this mystery is complicated by the fact that, in terrestrial mammals and birds, sleep is subdivided into rapid eye movement (REM) sleep (also known as paradoxical sleep) and non-REM (NREM) sleep. The existence of REM sleep is a particularly puzzling phenomenon. During REM sleep, cortical single-neuron and EEG activity resemble waking, but in contrast to waking REM sleep is characterized by skeletal muscle atonia and phasic activations of sub-cortical and cortical brain areas (Aserinsky and Kleitman, 1953; Dement, 1958; Jouvet et al., 1959; Brooks and Bizzi, 1963; Mouret et al., 1963; Steriade et al., 2001).

Although the function of REM sleep is unknown, it has historically been thought to promote learning and memory. In humans, many studies support a role for REM sleep in the consolidation of hippocampus-independent procedural learning acquired during prior waking. REM sleep amount and intensity increase following learning of new motor skills and procedural memory tasks, and these measures correlate with subsequent performance (Buchegger and Meier-Koll, 1988; Buchegger et al., 1991; Plihal and Born, 1997, 1999; Stickgold et al., 2000b; Fischer et al., 2002; Smith et al., 2004; Rasch et al., 2009; Suzuki et al., 2012). Moreover, a number of procedural

memory tasks require REM sleep after training for subsequent performance improvement (Karni et al., 1994; Smith, 1995; Mednick et al., 2003; Backhaus and Junghanns, 2006; Tucker et al., 2006). However, animal models of brain plasticity have not been used to investigate the role of REM sleep in hippocampus-independent consolidation.

In animal models, REM sleep deprivation (RSD) alters the expression of developmentally-regulated cortical plasticity by impairing the maturation of cortical and sub-cortical visual circuits (Oksenberg et al., 1996; Shaffery et al., 1998, 2002, 2006a). Much less is known, however, about the role of REM sleep in consolidating experience-dependent cortical plasticity *in vivo*. Ocular dominance plasticity (ODP) in the cat primary visual cortex (V1) is a canonical form of cortical plasticity that has revealed many underlying principles of cortical plasticity (Reviewed in Berardi et al., 2003). In this model, occlusion of vision from one eye (monocular deprivation, MD) during a critical period of development induces V1 to shift from being predominantly binocular to being preferentially responsive to the open (non-deprived) eye (Wiesel and Hubel, 1963a; Hubel and Wiesel, 1970). ODP is induced with as little as six hours of MD during waking and is subsequently consolidated by sleep (Frank et al., 2001). Activation of extracellular signal-regulated kinase (ERK) (see **Chapter 2**), protein kinase A (PKA) (Aton et al., 2009a) and protein synthesis (Seibt et al., 2012) are all required during sleep for ODP consolidation. However, the relative contributions of REM and NREM sleep to these events, and to ODP consolidation, have not been explored.

In the current study, we directly investigate whether REM sleep is required for ODP consolidation and its underlying mechanisms. We achieved this by inducing plasticity in V1 with waking MD, then allowing animals either normal or REM-deprived

sleep and measuring ODP. We also included a NREM fragmentation (NF) group to control for the nonspecific effects of RSD. We found that RSD impaired ODP and ERK1/2 phosphorylation, indicating that REM sleep consolidates cortical plasticity by activating the ERK signaling cascade. The extent of the ODP shift and ERK phosphorylation correlated with beta-gamma EEG activity during REM sleep, suggesting that cortical activity patterns during REM sleep consolidate ODP.

Materials and Methods.

All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and performed in accordance with USDA regulations. Kittens were obtained from our on-site breeding colony and housed with their queens on a 12:12 light:dark cycle prior to experimentation. Whenever possible, animals from the same litter were distributed across groups to control for inter-litter variability.

Surgical procedures for polysomnography. Cats were anesthetized and prepared for surgery as described previously (Frank et al., 2001; Jha et al., 2005). Briefly, the animal was anesthetized with midazolam and propofol (IV) before being intubated and transferred to isoflurane anesthesia. Six EEG and three nuchal EMG electrodes attached to an electrical socket were implanted and affixed to the skull using bone screws and dental acrylic. Two EEG leads were placed over V1 in each hemisphere of animals used for tissue harvesting. Animals used for imaging had a single EEG lead placed over V1 in each hemisphere to minimize the risk of dimpling the cortical surface with EEG screws. The remaining EEG screws were placed in frontoparietal regions.

Following at least four days post-operative recovery and treatments, male and female animals were randomly assigned to the experiments described below.

Polysomnography and monocular deprivation. At the peak of the critical period for visual cortical plasticity (Postnatal day 28-35), cats were singly housed in a recording chamber. The floor of the chamber consisted of a motorized platform that could be activated to wake the animal up. The implanted electrical socket was attached to an electrical cable tether/commutator, which routed EEG and EMG signals to an amplifier system (Grass Technologies; Warwick, RI). Signals were high-pass filtered at 0.1 Hz (EEG) or 10Hz (EMG), low-pass filtered at 100Hz, digitized at 200 Hz, and recorded using commercial sleep-recording software (VitalRecorder; Kissei Comtec America, Inc.; Irvine, CA). At the end of a baseline recording period lasting at least six hours, animals were anesthetized with isoflurane and the right eyelid was sutured shut. From induction to recovery from anesthesia, this process took no longer than 15 minutes. After recovery from anesthesia, animals were kept awake for 6 hours in lighted conditions, ensuring that all cats received 6 hours of monocular vision to induce cortical remodeling (Frank et al., 2001).

Sleep manipulations. Following six hours of MD, animals were allowed to sleep in complete darkness for 1-2 hours. During the sleep period, animals underwent one or more of the following manipulations: undisturbed sleep, REM sleep deprivation (RSD), or NREM sleep fragmentation (NF). During RSD, the EEG and EMG signals were monitored by an experimenter. Each time an animal attempted to enter REM sleep, indicated by decreased EEG and EMG amplitude, the animal was awoken by activation of the motorized floor of the chamber. During NF, animals were awoken the same

number of times as the RSD animals (see Results); however, these animals were only awoken during NREM sleep, and were allowed undisturbed bouts of REM sleep.

Optical imaging of intrinsic cortical signals. ODP was measured by optical imaging of intrinsic cortical signals in 16 male and 15 female cats. Immediately following the sleep period, animals were prepared for optical imaging as previously described (Jha et al., 2005). Briefly, animals were anesthetized with isoflurane (±N_2O) and the skull above V1 was removed. Animals were paralyzed with a continuous intravenous infusion of Flaxedil and ventilated. The dura above V1 was removed and the brain was covered with 3% agarose and a glass coverslip. Eyedrops were used to dilate the pupils (1% atropine) and retract the nictitating membranes (10% phenylephrine). Contact lenses focused the eyes on a monitor positioned 40 cm away. A Dalstar 1M30 CCD camera (Dalsa; Waterloo, ON, Canada) was focused 600 μm below the pial surface with a 135/50 mm lens combination and the cortex was illuminated with red light. Reflected light was filtered (610 nm) and images were captured (30 frames/second) while presenting one eye at a time with a full-field rotating, drifting square grating (0.2 cycles/°; 2 cycles/second; 1 full rotation/minute). Both clockwise and counter-clockwise visual stimuli were presented. Vitals were monitored throughout imaging to ensure that heart rate and expired CO_2 levels were comparable during left and right eye stimulation.

Images were saved after temporal (four frames) and spatial (2×2 pixels) binning. Optical maps were generated as previously described (Jha et al., 2005) and cropped to remove vascular artifacts. The OD ratio at each pixel was calculated according to Equation 3.1, where *I* is the ipsilateral response and *C* is the contralateral response. OD ratios were grouped in seven bins, where bin 1 contains pixels most responsive to the

eye contralateral to the imaged hemisphere, 4 corresponds to pixels responding approximately equally to either eye, and 7 corresponds to pixels mostly responsive to the ipsilateral eye. The number of pixels in each bin was used to calculate scalar measures of ODP: the contralateral eye bias index (CBI), monocularity index (MI), and shift index (SI). The CBI is a weighted average of OD scores from the population of pixels sampled, where N_x is the number of pixels in category x and N_T= total number of pixels sampled (Equation 3.2). The CBI ranges from 0 to 1, where higher values indicate a stronger bias for the contralateral eye. This measure was converted to the non-deprived eye bias index (NBI) (where higher values indicate a stronger bias for the non-deprived eye) for clarity (Aton et al., 2009a). The CBI was used to calculate the shift index (SI), a measure of overall changes in OD across both hemispheres of an animal (Equation 3.3).

$$OD\ Ratio = \frac{I}{I + C}$$

EQUATION 3.1.

$$CBI = \frac{(N_1 - N_7) + {}^2/_3(N_2 - N_6) + {}^1/_3(N_3 - N_5) + N_T}{2 \times N_T}$$

EQUATION 3.2.

$$SI = CBI_{\text{ipsilateral to deprived eye}} - CBI_{\text{contralateral to deprived eye}}$$

EQUATION 3.3.

The monocularity index (MI) was also calculated, where a value of 0 indicates that all imaged pixels respond equally to both eyes and 1 indicates a complete loss of binocular responses (Equation 3.4).

$$MI = \frac{(N_1 + N_7) + {}^{2}/_{3}(N_2 + N_6) + {}^{1}/_{3}(N_3 + N_5)}{N_T}$$

EQUATION 3.4.

These values were calculated for all imaged cats. Values from six age-matched cats that underwent MD with no subsequent sleep (MD-only), three of which were reproduced with permission from a previous study (Aton et al., 2009a), were used for comparison. Maps from one hemisphere in the MD+1h sleep and one hemisphere in the MD+2h sleep groups were excluded from the analysis due to poor signal quality.

Tissue collection and western blotting. Brain tissue was collected from 14 male and 12 female cats for western blot analysis. Immediately following one hour of post-MD sleep, RSD, or NF, animals were anesthetized with isoflurane and killed with an intracardiac injection of pentobarbital/phenytoin. Primary visual, auditory, and motor cortex (V1, A1, and M1, respectively) and lateral cerebellar tissue was rapidly collected from each hemisphere and frozen on dry ice. In one sleep and one RSD animal, only V1 tissue was collected. Whole-tissue extracts were prepared and analyzed as previously described (Aton et al., 2009a). Briefly, tissue was homogenized in lysis buffer (100 mM NaCl, 10 mM $Na_4P_2O_7 \cdot 10H_2O$, 10 mM $NaPO_4$, 50 mM NaF, 1 mM Na_3VO_4, 5 mM EDTA, 5 mM EGTA, 1% SDS) containing phosphatase and protease inhibitor cocktails (1:100;

Sigma-Aldrich; St. Louis, MO). Cellular debris was removed by centrifugation and lysates were then boiled for 10 minutes in gel loading buffer (2.5%SDS). Protein concentration in each sample was determined with a micro-BCA assay (Thermo Scientific; Rockford, IL). 40 μg protein/well was separated on pre-cast polyacrylamide gels (Bio-Rad; Hercules, CA), and transferred to nitrocellulose membranes (0.45 μM pore size; Invitrogen; Carlsbad, CA). Membranes were blocked in Odyssey blocking buffer at room temperature (Li-Cor; Lincoln, NE), then incubated overnight at 4°C with primary antibodies diluted in blocking buffer and 0.1% Tween-20 (Sigma-Aldrich). Primary antibodies were raised in rabbit and obtained from Abcam (Cambridge, MA) unless otherwise noted; mouse anti-β-actin (1:10,000) (Sigma-Aldrich) was also included with each primary antibody as a loading control. The following primary antibodies were used: anti-ERK1/2 (1:1,000) (Cell Signaling Technology; Danvers, MA), anti-phospho-ERK1/2 (T202/Y204 and T185/Y187, respectively) (1:500) (Cell Signaling), anti-GluA1 (1:900); anti-phospho-GluA1 (S831) (1:200) (Millipore; Billerica, MA); anti-CaMKIIα (1:1500), anti-CaMKII (1:200) (Enzo Life Sciences, Inc.; Farmingdale, NY), anti-phospho-CaMKIIα/β (T286 and T287, respectively) (1:1000), anti-cofilin (1:400), anti-4E-BP1 (1:400) (Cell Signaling), and anti-phospho-4E-BP1 (S65) (1:500) (Cell Signaling). Membranes were incubated simultaneously with both fluorescently conjugated goat anti-rabbit and anti-mouse antibodies (Li-Cor) (1:20,000 in blocking buffer/0.1% Tween) at room temperature in the dark for one hour, then scanned and quantified with the Odyssey infrared scanner and quantification software (Li-Cor). Blots were scanned at 700 nm to detect the β-actin loading control and 800 nm to detect each protein of interest. Variation across gels was controlled for by normalizing to a common sample

run on multiple gels. Cofilin blots were not run for one animal in the 1h sleep and one animal in the 1h RSD groups, as not enough protein was obtained.

Sleep/wake analysis. Vigilance states were manually scored by a trained experimenter as rapid eye movement (REM) sleep, non-REM (NREM) sleep, or wake in 8-second epochs based on EEG and EMG signals (SleepSign for Animal; Kissei Comtec America, Inc.; Irvine, CA) according to previously described criteria (Frank et al., 2001). Percentage of total recording time and average bout duration for each vigilance state was calculated for baseline, MD, and post-MD periods. Fast Fourier transforms were performed on V1 EEG recordings and power across the spectrum was normalized to baseline power. For the correlation analysis, power during REM sleep was averaged within the theta (5-10 Hz) and beta-gamma (20-40 Hz) bands and normalized to mean baseline power. One animal in the 1h RSD group was excluded from the spectral power analysis because the EEG signal was not usable.

Cortisol measurements. Whole blood was collected from the jugular vein of awake cats at the indicated time points, and Normosol-R was administered subcutaneously to replace fluid volume. Blood was allowed to clot at 4°C, then spun at 2500 rcf for 7 minutes. Serum was collected and stored at -20°C until analysis. Cortisol was quantified by a solid-phase competitive enzyme-amplified chemiluminescent immunoassay on the Immulite 2000 (Siemens; Erlangen, Germany) at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania. Cortisol levels below the detection limit of the assay (1µg/dL) were assigned a value of 1.

Statistical analysis. Statistical tests were performed using SigmaPlot 11.0 (Systat Software, Inc.; San Jose, CA). Groups were compared using Student's t-tests or one-way ANOVAs, as indicated. Fisher's least significant difference test was used for post-hoc analyses in which only three groups were compared (Hsu, 1996); the Holm-Sidak post-hoc test was used in cases where more than three groups were compared. Correlation analysis was performed using the Pearson product moment correlation. Nonparametric tests (Mann-Whitney U tests, ANOVAs on ranks with Dunn's post-hoc tests, and Spearman rank order correlation) were used in cases where data were not normally distributed.

Results.

Post-MD sleep architecture. To determine whether REM sleep is necessary for ODP consolidation, we induced cortical plasticity with six hours of waking MD (Frank et al., 2001), then either manually deprived animals of REM sleep or allowed them undisturbed sleep. However, any method that experimentally removes REM sleep has nonspecific effects on other arousal states, including increased wake time and disrupted NREM sleep (Vimont-Vicary et al., 1966). Therefore, we also included a control group, referred to as NREM fragmentation (NF). These animals were awoken the same number of times as the REM sleep deprivation (RSD) group (RSD: 21.25±1.2 awakenings, N=12; NF: 20.2±0.47, N=10; p=0.428; t-test). However, NF animals were only awoken during NREM sleep and were allowed undisturbed bouts of REM sleep.

To determine the efficacy of RSD and NF, we examined post-MD sleep architecture (Table 3.1). As expected, both the RSD and NF animals spent more time awake than sleeping animals. In contrast to RSD animals, however, NF animals had normal amounts of REM sleep. NREM sleep continuity, measured by the length of NREM bouts, was also decreased by both RSD and NF (Figure 3.1B). Finally, we examined the NREM power spectra in V1 of animals in each group to determine whether RSD impaired the normal rebound in slow wave and delta power that occurs after a period of sleep deprivation. As shown in Figure 3.1 C, all groups displayed a rebound over baseline in these frequency bands (indicated by values above 1), but this rebound was blunted by both RSD and NF. Therefore, RSD dramatically reduced REM sleep amounts, while the nonspecific effects of RSD (increased wake, decreased NREM bout duration and low frequency power) were controlled for by the NF group.

RSD impairs ODP. To measure ODP after sleep, RSD, and NF, we performed optical imaging of intrinsic cortical signals in V1 while visually stimulating one eye at a time (Figure 3.2). Three scalar measures of ODP were calculated based on the responsiveness of each pixel to the non-deprived and deprived eyes (see Methods): the non-deprived eye bias index (NBI) and monocularity index (MI), calculated for each hemisphere individually, and the shift index (SI), which is a measure of plasticity across both hemispheres in the same animal. The NBI ranges from 0-1 (0.5 indicates no shift), the MI ranges from 0-1 (0 indicates complete binocularity), and the SI ranges from -1 to 1 (0 indicates no shift). RSD decreased all three of these measures compared to sleeping animals. Furthermore, the ocular dominance shift after RSD was comparable to the shift observed in animals that underwent MD without subsequent sleep (Figure

3.2C-E, reference line). The ocular dominance shift was not impaired in NF animals, indicating that the effects of RSD on ODP cannot be attributed increased wake time or disruptions to NREM sleep.

RSD attenuates ERK phosphorylation. Aton et al., (2009) found that ERK1/2, CaMKIIα/β, and GluA1 (S831) phosphorylation was elevated in remodeling V1 after 1-2 hours of post-MD sleep. To determine whether these biochemical changes require REM sleep, we collected tissue from V1 after post-MD sleep, RSD, and NF for western blot analysis (Figure 3.3). Compared with the sleep group, RSD impaired ERK1/2 phosphorylation. In contrast, ERK1/2 phosphorylation was normal in the NF group, indicating that this effect cannot be attributed to nonspecific effects of RSD. We also measured levels of cofilin, which is concentrated at excitatory postsynaptic terminals (Racz and Weinberg, 2006). Cofilin levels in V1 were decreased by RSD, but not NF, compared to normally sleeping animals. RSD did not significantly affect CaMKIIα/β phosphorylation or GluA1 phosphorylation on the CaMKII site (S831) (Barria et al., 1997).

Previous work from our lab has shown that protein synthesis in V1 during sleep is required for ODP consolidation (Seibt et al., 2012). 4E-BP1 phosphorylation, which increases after 1h of post-MD sleep, may underlie this sleep-dependent translation. We therefore also measured 4E-BP1 phosphorylation in the same tissue samples, but found no differences across groups (Sleep: 100±27.8%; RSD: 76.11±16.7%; NF: 64.64±19.3%; p=0.692; ANOVA on ranks).

REM sleep is a global brain state, raising the question of whether REM sleep activates ERK specifically in remodeling cortical areas (i.e. V1), or whether REM sleep increases ERK phosphorylation across the brain. To address this question, we performed western blot analysis on primary auditory cortex (A1), primary motor cortex (M1), and lateral cerebellum obtained from the same animals used in the analysis of V1 tissue (Figure 3.4). Interestingly, we found that ERK phosphorylation in A1 and M1 followed the same trend as V1, but these trends did not reach significance. When the values from A1 and M1 were pooled, RSD significantly impaired ERK2 phosphorylation. Therefore, the effect of REM sleep on ERK phosphorylation is greatest in cortical regions undergoing plasticity, but may not be entirely limited to these regions. No effects of RSD on cerebellar ERK phosphorylation were observed.

Recovery sleep after RSD rescues ODP. We have demonstrated that REM sleep following ODP induction is required for consolidation, but this raises many additional questions. For instance, can ODP impairments by RSD be reversed by subsequent REM sleep? Furthermore, does REM sleep immediately following ODP induction protect the cortex from the effects of later RSD? Therefore, we formed additional groups in which animals received 2h RSD, 1h RSD followed by 1h undisturbed recovery sleep (RSD+recovery group), or 1h undisturbed sleep followed by 1h RSD (delayed RSD group) (Figure 3.5 A). During their respective RSD periods, the RSD+recovery and delayed RSD groups had the same changes to REM sleep and wake time as the 2h RSD group (Table 3.2). The RSD+recovery animals showed a rebound in REM sleep during the recovery period, during which they received significantly more REM sleep

than animals in the 1h sleep group (RSD+recovery: 42.58±3.5% total recording time, N=8; 1h Sleep: 31.21±2.2%, N=12; p=0.01; t-test).

Two hours of RSD did not produce any additional ODP impairment compared with the 1h RSD group, as measured by the NBI (1h RSD=0.625±0.02; 2h RSD=0.605±0.008; p=0.346), the MI (1h RSD: 0.309±0.02; 2h RSD: 0.270±0.02; p=0.196), and the SI (1h RSD: 0.249±0.02; 2h RSD: 0.210±0.01; p=0.160) (1h RSD n=10 hemispheres; 2h RSD n=8; t-test). Similarly, a second hour of undisturbed sleep did not further enhance the OD shift compared to the MD+1h sleep group (1h sleep: 0.696±0.02; 2h sleep NBI= 0.667±0.02; p=0.321), MI (1h sleep: 0.439±0.03; 2h sleep: 0.353±0.03; p=0.088), or SI (1h sleep: 0.409±0.03; 2h sleep: 0.346±0.03; p=0.225) (1h sleep n=9 hemispheres; 2h sleep n=7; t-test). However, the NBI and MI were higher in the RSD+recovery group than the 2h RSD group, indicating that one hour of recovery sleep after RSD is sufficient to rescue ODP (Figure 3.5). Furthermore, the NBI was also higher in the delayed RSD group than in the 2h RSD group, indicating that once consolidated, the OD shift is not destabilized by RSD.

Since ODP consolidation was normal following RSD+recovery and delayed RSD, we expected that ERK phosphorylation would also be normal in these groups. Western blot analysis of V1 tissue revealed that ERK phosphorylation was not attenuated in the RSD+recovery and delayed RSD groups (Figure 3.6).

REM sleep beta-gamma EEG activity correlates with ODP and ERK phosphorylation. REM sleep is required for ODP consolidation, but the characteristics of REM sleep that are responsible are unclear. The SI is a measure of ocular dominance shift across the

whole animal (see Methods). Therefore, we examined the correlation between the SI and REM amount, REM theta power in V1, and REM beta-gamma power in V1 for each animal that received 1-2 hours of undisturbed sleep. Interestingly, the SI did not correlate with REM sleep time ($p=0.272$; $r=-0.484$; Pearson correlation; N=7 animals) or REM sleep theta power ($p=0.907$; $r=-0.0548$; Pearson correlation; N=7 animals). However, the SI did correlate with REM sleep beta-gamma power (Figure 3.7 A). In addition, we obtained usable EEG recordings within V1 in 14 hemispheres from sleeping animals that were used for tissue collection. We found that in these hemispheres, ERK1 phosphorylation correlated with REM sleep beta-gamma activity, although this did not reach significance for ERK2 phosphorylation (Figure 3.7 B).

RSD does not elevate serum cortisol. RSD can induce stress in rats, as measured by blood glucocorticoid concentration (McDermott et al., 2003). Lopez et al. (2008) observed a nonsignificant increase in glucocorticoid levels after RSD that also occurred in a control group similar to the NF manipulation used here, suggesting that NF controls for any stress effects of RSD. Nevertheless, to address the possibility that stress in the RSD group might account for the observed ODP impairment, we measured serum cortisol from a subset of animals at various time points during the experiment (Figure 3.8). Cortisol was elevated at the end of the MD period compared to baseline. Even so, peak cortisol levels after MD were more than six-fold less than levels reported to have only minor effects on ocular dominance plasticity when sustained for several days (Daw et al., 1991). Furthermore, cortisol returned to baseline levels following both post-MD sleep and RSD, indicating that RSD does not cause stress compared to normal sleep. Although we were only able to obtain cortisol measurements from two NF animals,

cortisol levels in these animals matched closely with measurements obtained from RSD animals (1.74 and 1.37 μg/dL), further demonstrating that any nonspecific effects of RSD are controlled for by NF.

We also investigated whether cortisol has overt circadian rhythms by collecting blood from normal cats (with unmanipulated sleep and visual experience) in four time-bins across the day: 6-10AM, 10AM-2PM, 2-6PM, and 6-10PM (Figure 3.8 B). No differences in serum cortisol were observed across the day.

Discussion.

The role of REM sleep in ODP consolidation. The current study demonstrates that REM sleep is required for the consolidation of cortical plasticity. Our results cannot be explained by the nonspecific effects of RSD on wake and NREM sleep, since plasticity in animals undergoing NREM fragmentation was unaffected. However, previous work using our model suggested that NREM, but not REM, sleep is responsible for ODP consolidation. For instance, ODP consolidation correlates linearly with NREM, but not REM, sleep amount (Frank et al., 2001). This apparent discrepancy is resolved if a threshold amount of REM sleep is required for consolidation, after which REM sleep does not provide any additional benefit. In addition, six hours of RSD does not impair ODP (Aton et al., 2009a), but the long period of RSD in this study combined with the strong REM sleep drive in kittens may have allowed REM sleep to accumulate to a level sufficient for ODP consolidation. This interpretation is supported by our finding that ODP did not correlate with REM sleep time, but did correlate with beta-gamma EEG activity in

V1. Therefore, neuronal activity during REM sleep, rather than the amount of REM sleep, determines the extent of the OD shift.

The current study also conflicts with the findings of Oksenberg and colleagues (1996), who showed that long-term MD combined with RSD in kittens *enhanced* an anatomical measure of ODP in the LGN (cell-size disparity). The methodological differences between these studies (age, RSD and MD duration, RSD method, and measure of ODP) make them difficult to compare. However, this discrepancy can be explained by the difference in MD duration. Short-term (<24h) MD induces an ocular dominance shift in extragranular layers of V1, but not in thalamocortical connections, in the kitten (Trachtenberg et al., 2000). Long-term MD produces an OD shift in V1 *and* increases cell-size disparity in the LGN by decreasing the size of deprived-eye LGN cells (Wiesel and Hubel, 1963b; Hubel and Wiesel, 1970; Tieman, 1984). Elimination of ponto-geniculo-occipital (PGO) waves (which normally occur during REM sleep (Brooks and Bizzi, 1963; Mouret et al., 1963)) during MD is sufficient to enhance the LGN cell-size disparity, mimicking the effects of RSD (Shaffery et al., 1999). Therefore, the findings of Oksenberg and colleagues indicate that the loss of PGO waves as a result of RSD enhances the effects of long-term MD in the LGN. In contrast, the length of MD used in the current study is too short to induce thalamocortical plasticity, and the effects of RSD reflect a requirement for REM sleep specifically for plasticity *within* V1. These studies are not contradictory, but rather suggest that REM sleep serves two roles: promotion of normal maturation of thalamocortical circuitry via PGO waves, and consolidation of experience-dependent plasticity in local cortical circuits.

Despite evidence in the human literature that REM sleep consolidates hippocampus-independent memories, much of the work in animal models has focused

on the effects of REM sleep on hippocampal learning and plasticity. REM sleep after training is necessary for performance improvements on a variety of hippocampal-dependent tasks in rodents (Pearlman, 1969; Leconte et al., 1974; Rideout, 1979; Smith and Butler, 1982; Martí-Nicolovius et al., 1988; Smith and Rose, 1996; Youngblood et al., 1997, 1999; Legault et al., 2004, 2010; Yang et al., 2008; Aleisa et al., 2011). These learning impairments may be due to deficits in the induction and maintenance of long-term potentiation (LTP), decreased synaptic transmission, decreased membrane excitability, and/or impairments in intracellular signaling cascades in the hippocampus caused by REM sleep deprivation (Davis et al., 2003; Romcy-Pereira and Pavlides, 2004; Yang et al., 2008; Ravassard et al., 2009; Aleisa et al., 2011). However, these studies do not control for the nonspecific effects of increased wake time and fragmented NREM sleep during RSD. Sleep fragmentation alone impairs hippocampal LTP and performance on the Morris water maze (Tartar et al., 2006), as well as decreasing hippocampal CA1 pyramidal cell excitability and input resistance (Tartar et al., 2010), suggesting that the impairments of hippocampal learning and plasticity observed after RSD may be due to sleep fragmentation rather than the removal of REM sleep *per se.* The current study controls for the effects of sleep fragmentation, and our novel results demonstrate how REM sleep can promote the hippocampus-independent learning that has been observed in humans.

ERK phosphorylation as a mechanism for REM sleep-dependent consolidation. ERK activation is required during sleep for ODP consolidation (see **Chapter 2**). ERK phosphorylation was greatly reduced by RSD (but not NF), suggesting that REM sleep consolidates ODP by promoting ERK phosphorylation. Our observation that ERK

phosphorylation in cortical areas other than V1 is slightly reduced after RSD raises the intriguing possibility that REM sleep may promote an overall trend toward ERK activation, and that this is amplified in areas induced to remodel during prior waking. This interpretation is consistent with the findings that RSD reduces ERK phosphorylation in the rat dorsal hippocampus (Ravassard et al., 2009) and ERK phosphorylation is increased during spontaneous REM sleep in mouse hippocampal CA1 and dentate gyrus (Luo et al., 2013) in the absence of plasticity-inducing stimuli.

Possible roles for ERK. There are many ways in which ERK can enact plastic changes, including activation of the transcription and translation pathways (reviewed in Adams and Sweatt, 2002). Recent work from our lab has shown that activation of the ERK-Mnk translation pathway is required for sleep-dependent ODP consolidation (**Chapter 2**). This pathway is required for translation initiation complex formation during plasticity *in vivo* (Panja et al., 2009) and the phosphorylation of eukaryotic initiation factor 4E (eIF4E) during sleep-dependent ODP (**Chapter 2**), which is associated with increased rates of mRNA translation (Scheper and Proud, 2002). Therefore, our results suggest that REM sleep promotes the synthesis of plasticity-related proteins (such as cofilin) via the ERK-Mnk pathway. ERK can play a dual role with the mammalian target of rapamycin (mTOR) in promoting translation during plasticity (Kelleher et al., 2004). Since mTOR activity during sleep is also required for ODP consolidation (Seibt et al., 2012), ERK and mTOR may work together during REM sleep to promote protein synthesis. However, we are unable to directly test the activation of mTOR following post-MD sleep or REM sleep deprivation, as commercially available antibodies against mTOR do not work in feline tissue (Seibt et al., 2012).

Recovery from REM sleep deprivation. The ODP impairment by 1h RSD can be reversed with subsequent REM sleep. We anticipate that the effects of 2h RSD are also reversible, since systemic administration of the benzodiazepine triazolam severely reduces REM sleep for the first two hours of a 6h post-MD sleep period without impairing plasticity (Seibt et al., 2008). Furthermore, Aton et al. (2009) found that after MD, intracortical infusion of the NMDA receptor blocker APV for the first 3h of a 6h sleep period was sufficient to impair plasticity. If the NMDA receptor activation required for ODP consolidation occurs during REM sleep, this suggests that ODP consolidation cannot be rescued after 3h RSD. However, this hypothesis is difficult to directly test, since the strong pressure to enter REM sleep in kittens results in decreasing efficacy of RSD as the RSD period is lengthened (Frank et al., 2001).

Concluding remarks. The current study identifies REM sleep as an essential player in ODP consolidation. However, these findings do not rule out a role for NREM sleep. In fact, our finding that CaMKII and GluA1 phosphorylation (two well-studied plasticity-related molecules whose phosphorylation is elevated during post-MD sleep (Aton et al., 2009a)) are unaffected by RSD indicates that these phosphorylation events occur specifically during NREM sleep. Together, these results suggest a model in which NREM sleep initiates short-term plastic changes involving post-translational protein modifications and AMPA receptor trafficking, whereas REM sleep promotes longer-term stabilization of these plastic changes via protein synthesis. This is consistent with our

observation that REM sleep during the first post-MD hour is sufficient to stabilize ODP and protect against the effects of subsequent REM sleep deprivation.

Although we have shown evidence that ERK signaling is one of the underlying mechanisms for REM-dependent consolidation, the characteristics of REM sleep responsible for ERK activation remain to be determined. One candidate mechanism is the replay of neuronal activity, which has been observed during REM sleep in the hippocampus (Louie and Wilson, 2001). Consistent with this idea, neuronal firing rates in V1 increase during both NREM and REM sleep following MD, and the extent of the OD shift is positively correlated with principal excitatory neuron firing rates in V1 during both states (Aton et al., 2009a, 2013). Our finding that REM sleep beta-gamma activity correlates with the ODP shift and ERK phosphorylation may reflect re-activation of cortical circuits during REM sleep that were previously active during waking. In addition, the high levels of acetylcholine during REM sleep may promote consolidation, as acetylcholine signaling is required for ODP (Gu and Singer, 1989, 1993). Future studies investigating these mechanisms will promote our understanding of the function of REM sleep, and of sleep in general, in cortical consolidation.

Table 3.1. Sleep architecture: One hour sleep groups. Average (±SEM) percent of total recording time spent in waking, NREM sleep, and REM sleep during baseline, MD, and post-MD periods is shown. There were no significant differences across groups during the baseline and MD periods. In the RSD group, post-MD REM sleep time was significantly reduced, and wake and NREM sleep time were significantly increased. The increase in wake time was also observed in the NF group. *p<0.05, ANOVA on ranks followed by Dunn's post-hoc test vs. sleep group.

	Group	% Wake	%REM sleep	%NREM sleep
Baseline	MD+1h Sleep	18.21 ± 1.5	36.67 ± 2.0	45.12 ± 1.1
	MD+1h RSD	20.17 ± 3.2	37.98 ± 2.0	41.85 ± 1.9
	MD+1h NF	23.07 ± 0.9	36.50 ± 1.7	40.43 ± 1.9
MD	MD+1h Sleep	99.43 ± 0.1	0.00 ± 0.0	0.57 ± 0.1
	MD+1h RSD	99.07 ± 0.2	0.00 ± 0.0	0.94 ± 0.2
	MD+1h NF	99.06 ± 0.1	0.00 ± 0.0	0.94 ± 0.2
Post-MD Sleep	MD+1h Sleep	6.37 ± 2.1	31.21 ± 2.2	62.43 ± 3.8
	MD+1h RSD	**19.59 ± 3.5***	**3.76 ± 0.5***	**76.66 ± 3.6***
	MD+1h NF	**17.97 ± 1.3***	26.26 ± 1.6	55.77 ± 4.1

Table 3.2. Sleep architecture: Two hour sleep groups. Average (±SEM) percent of the first and second post-MD hour spent in each arousal state is shown. During their respective RSD periods, the RSD+recovery and delayed RSD groups had significantly reduced REM sleep and increased wake amounts. *p<0.05, One-way ANOVA followed by Holm-Sidak post-hoc test vs. MD+2h sleep group.

	Group	% Wake	%REM sleep	%NREM sleep
Hour 1	MD+2h Sleep	1.85 ± 0.7	27.10 ± 6.1	71.05 ± 6.0
	MD+2h RSD	**23.25 ± 3.8***	**1.95 ± 1.1***	74.78 ± 3.3
	MD+RSD+recovery	**15.46 ± 1.9***	**4.15 ± 1.4***	80.43 ± 2.8
	MD+delayed RSD	4.66 ± 1.4	22.00 ± 4.4	73.38 ± 4.0
Hour 2	MD+2h Sleep	1.80 ± 0.5	36.83 ± 2.7	61.43 ± 3.0
	MD+2h RSD	**30.20 ± 5.9***	**3.35 ± 1.3***	66.45 ± 7.1
	MD+RSD+recovery	2.88 ± 0.4	42.58 ± 3.5	54.55 ± 3.6
	MD+delayed RSD	**21.11 ± 2.3***	**2.04 ± 0.4***	**76.86 ± 2.4***
Total	MD+2h Sleep	1.83 ± 0.3	31.96 ± 2.8	66.24 ± 2.5
	MD+2h RSD	**26.73 ± 3.6***	**2.65 ± 1.1***	70.61 ± 4.1
	MD+RSD+recovery	**9.17 ± 1.0***	**23.36 ± 2.3***	67.49 ± 3.0
	MD+delayed RSD	**12.89 ± 1.3***	**12.02 ± 2.3***	75.12 ± 2.2

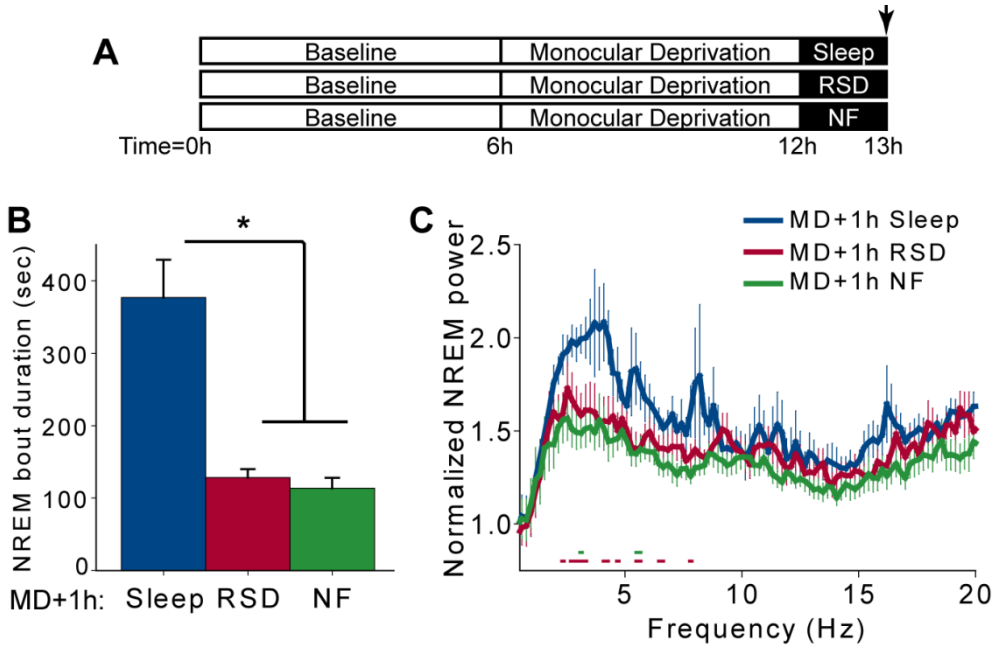

Figure 3.1. NREM fragmentation controls for the nonspecific effects of RSD. Sleep N=11; RSD N=12; NF N=10. Error bars represent SEM. (A) Experimental design. Animals underwent a baseline recording period, then MD during waking in lighted conditions. This was followed by one hour of sleep, RSD, or NF in complete darkness (see Methods). Arrow represents the time at which animals were prepared for optical imaging, or when tissue was collected for western blot analysis. (B) NREM bout duration during post-MD sleep was decreased in the RSD and NF groups. *$p<0.05$, ANOVA on ranks followed by Dunn's post-hoc test. (C) EEGs from V1 leads were Fourier transformed, and the power spectrum during post-MD NREM sleep was normalized to baseline. RSD and NF both reduced power at low frequencies compared to the sleep group, but did not differ from each other. Significance was determined by ANOVAs on ranks followed by Dunn's post-hoc test, and is indicated by red lines ($p<0.05$, RSD vs. Sleep) and green lines ($p<0.05$, NF vs. Sleep) at the bottom of the graph.

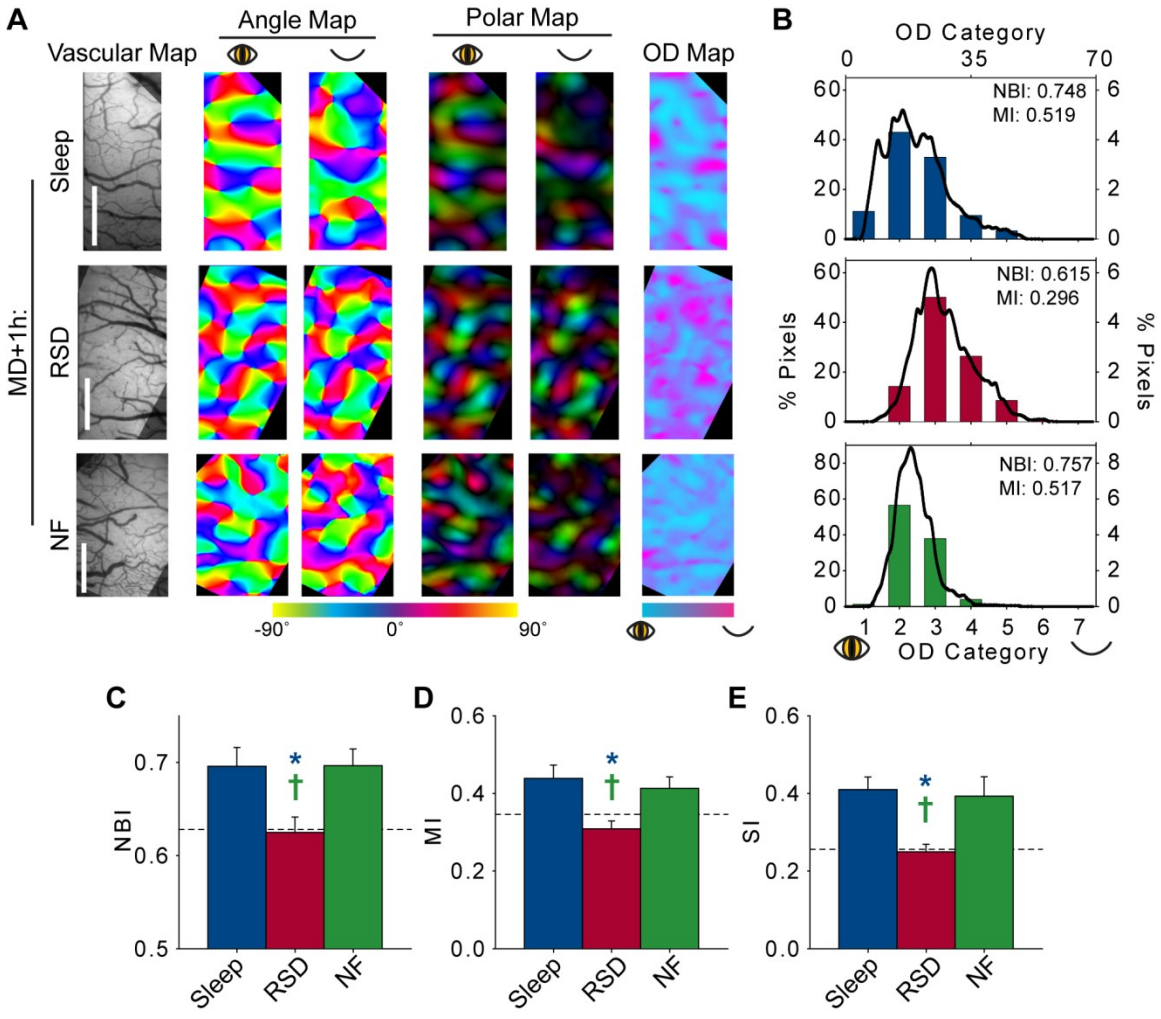

Figure 3.2. REM sleep deprivation impairs ocular dominance plasticity consolidation. (A) Representative optical maps obtained from animals that received sleep, RSD, or NF following MD. Vascular maps show the pial surface of V1, cropped to remove out-of-focus areas and large vessels; white scale bar indicates 1mm. Angle and polar maps are color-coded (see color bar at bottom) such that the color of each pixel indicates the visual stimulus orientation that maximally drives that pixel. In the polar maps, pixel brightness indicates the magnitude of the response. Ocular dominance (OD) maps show pixels predominantly driven by the non-deprived eye as cyan and those predominantly driven by the deprived eye as magenta. (B) Pixels from each map were

binned into 70 OD categories, and the percent of pixels in each category was plotted (top & right axes); these categories were collapsed into 7 ocular dominance categories (bottom & left axes) to produce the classical 7-point OD score of Hubel & Wiesel (Wiesel and Hubel, 1963a). (C-E) Average (±SEM) scalar measures of the OD shift were compared across groups. Hemispheres from RSD animals were significantly less shifted than those from sleeping and NF animals, as measured by the NBI (*p=0.011, Sleep vs. RSD; †p=0.009, NF vs. RSD), MI (*p=0.003, Sleep vs. RSD; †p=0.013, NF vs. RSD), and SI (*p<0.001, Sleep vs. RSD; †p=0.034, NF vs. RSD). One-way ANOVA followed by Fisher's LSD test; MD+1h Sleep: n=9 hemispheres; MD+1h RSD: n=10; MD+1h NF: n=10.

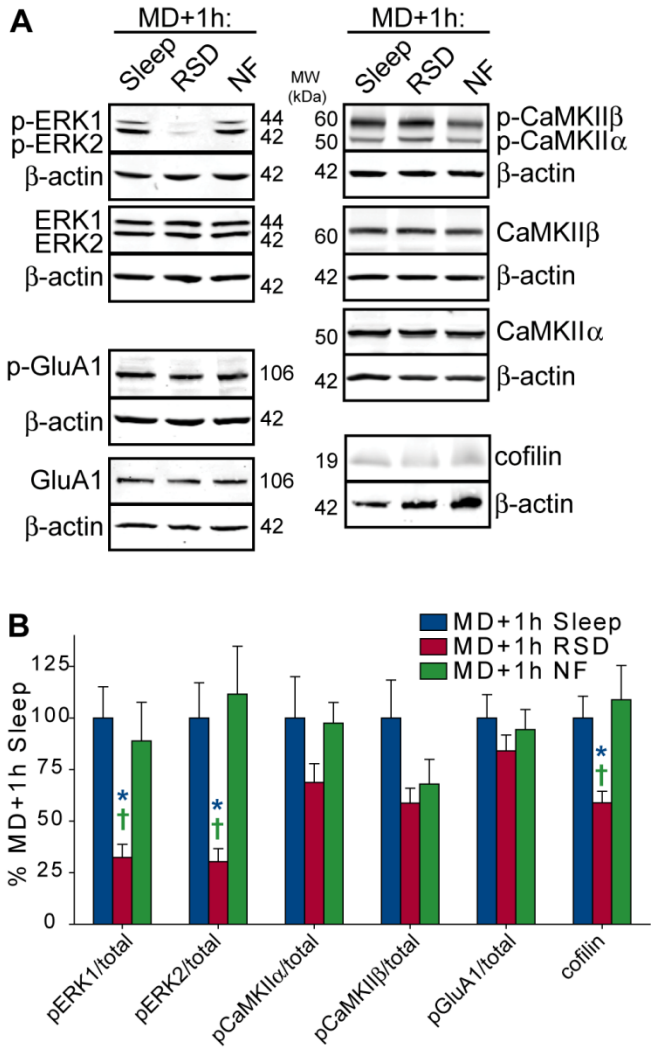

Figure 3.3. REM sleep deprivation decreases ERK phosphorylation and cofilin levels in V1. (A) Representative western blots for total and phospho-ERK1/2, CaMKIIα/β, GluA1, and total cofilin. (B) Average (±SEM) protein levels (displayed as a percentage of MD+1h Sleep levels). Compared with MD+1h Sleep and NF hemispheres, ERK1/2 phosphorylation and cofilin levels were significantly reduced by RSD. *p<0.05, sleep vs. RSD; †p<0.05, NF vs RSD; ANOVA on ranks followed by Dunn's post-hoc test. Sleep n=12 hemispheres; RSD n=14; NF n=10.

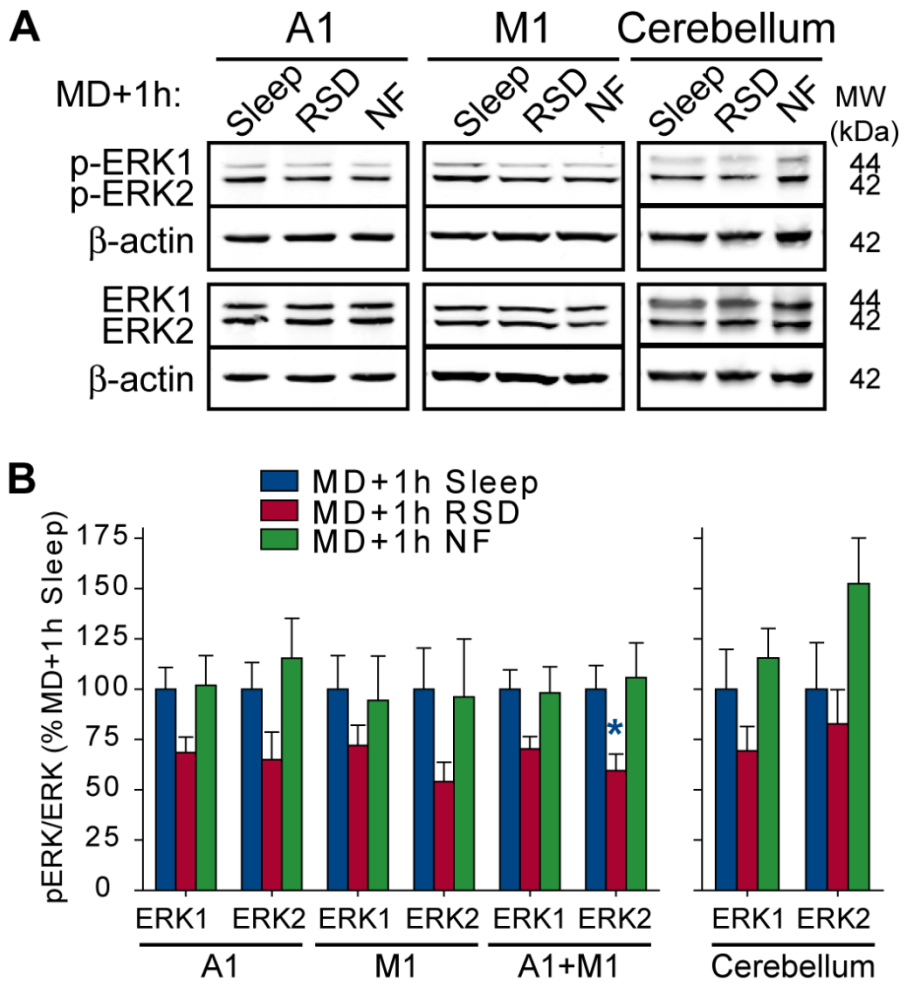

Figure 3.4. RSD has minimal effects on ERK phosphorylation in non-remodeling brain regions. (A) Representative western blots for phospho- and total ERK1/2 in primary auditory cortex (A1), primary motor cortex (M1), and cerebellum. (B) Average (±SEM) ERK phosphorylation is slightly but not significantly decreased in A1, M1, and cerebellum following RSD. ERK2 phosphorylation was significantly lower in the RSD group than in the MD+1h Sleep group when A1 and M1 data were pooled (*$p<0.05$, ANOVA on ranks followed by Dunn's post-hoc test). MD+1h Sleep n=10 hemispheres, MD+1h RSD n=12, MD+1h NF n=10.

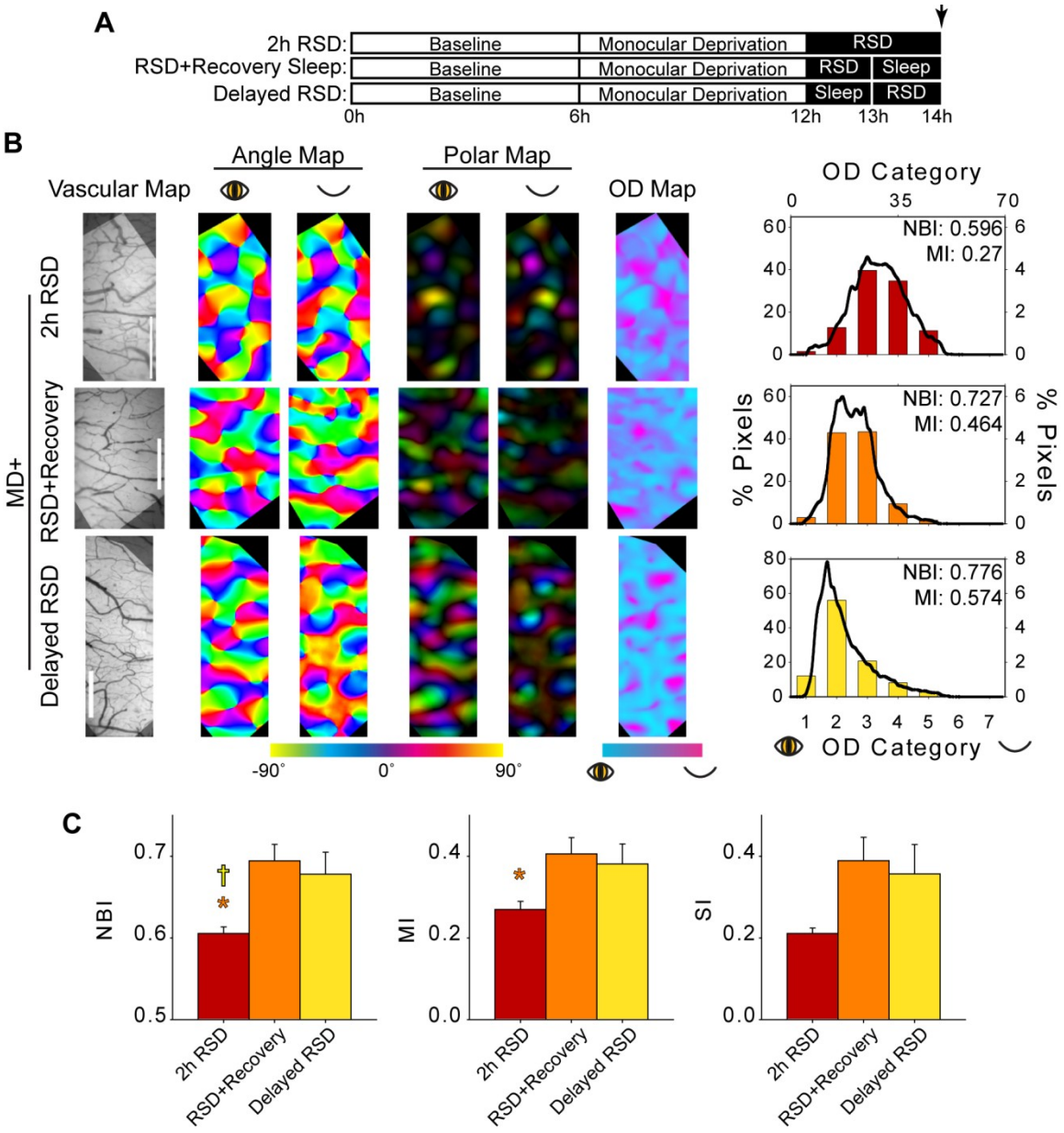

Figure 3.5. ODP is not impaired by RSD followed by recovery sleep or delayed RSD. (A) Experimental design. Groups received either two hours of post-MD RSD, one hour of RSD followed by one hour recovery sleep, or one hour of normal sleep followed by one hour of delayed RSD. Arrow indicates time at which animals were prepared for

optical imaging. (B) Vascular maps, angle maps, polar maps, and OD maps were obtained and pixel distributions were calculated as described for Figure 3.2. (C) Average (±SEM) scalar measures of ODP. Compared with the 2h RSD group, the NBI was higher in the RSD+recovery (*p=0.005) and delayed RSD (†p=0.017) groups. The MI was higher in the RSD+recovery than the 2h RSD group (*p=0.02) as well. One-way ANOVA followed by Fisher's LSD test; 2h RSD n=8 hemispheres; RSD+recovery n=8; delayed RSD n=8.

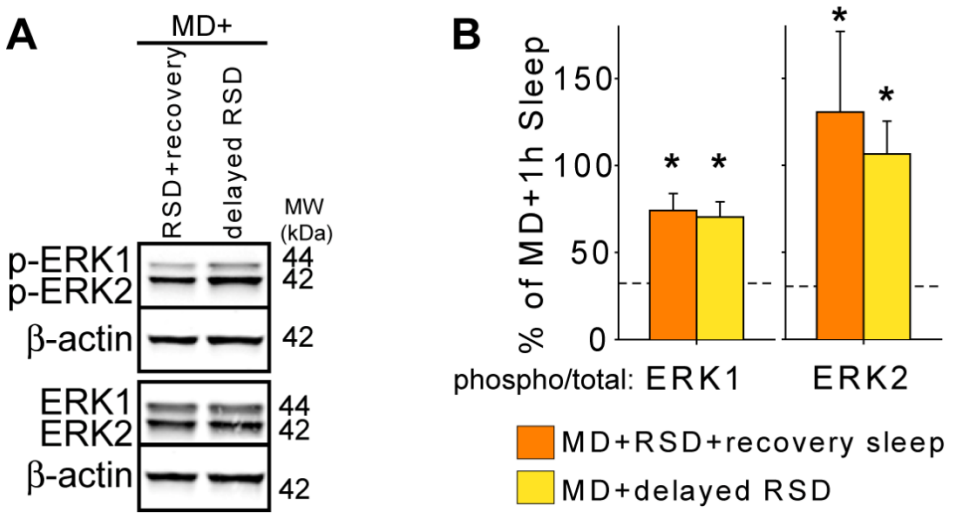

Figure 3.6. ERK phosphorylation is normal in the RSD+recovery and delayed RSD groups. (A) Representative western blots for total and phosphorylated ERK1/2. (B) Average (±SEM) ERK phosphorylation levels, displayed as a percentage of MD+1h sleep values, were compared with ERK phosphorylation levels after 1h RSD (dashed reference line). Both RSD+recovery and delayed RSD groups had significantly higher ERK phosphorylation than the 1h RSD group (*$p<0.05$) but did not differ from each other. ANOVA on ranks followed by Dunn's post-hoc test; MD+1h RSD n=14 hemispheres; MD+RSD+recovery n=8; MD+delayed RSD n=8.

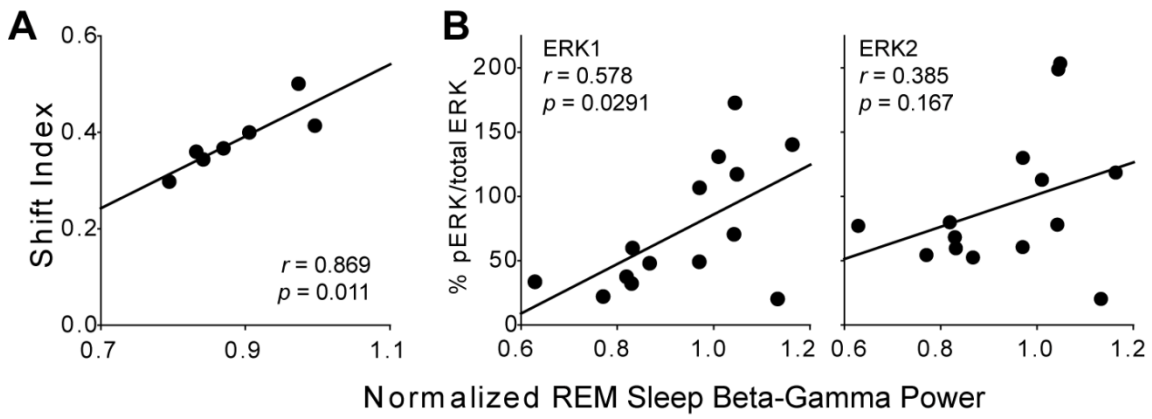

Figure 3.7. Beta-gamma EEG activity during REM sleep correlates with ODP and ERK phosphorylation. (A) In animals that received 1-2 hours of uninterrupted sleep, the shift index correlates with REM sleep beta-gamma power (displayed as a fraction of baseline power at the same recording site) during post-MD REM sleep. Pearson product-moment correlation, N=7 animals. (B) In hemispheres from animals that received 1-2h uninterrupted sleep, ERK1 phosphorylation correlates with REM sleep beta-gamma power. Spearman rank order correlation, n=14 hemispheres.

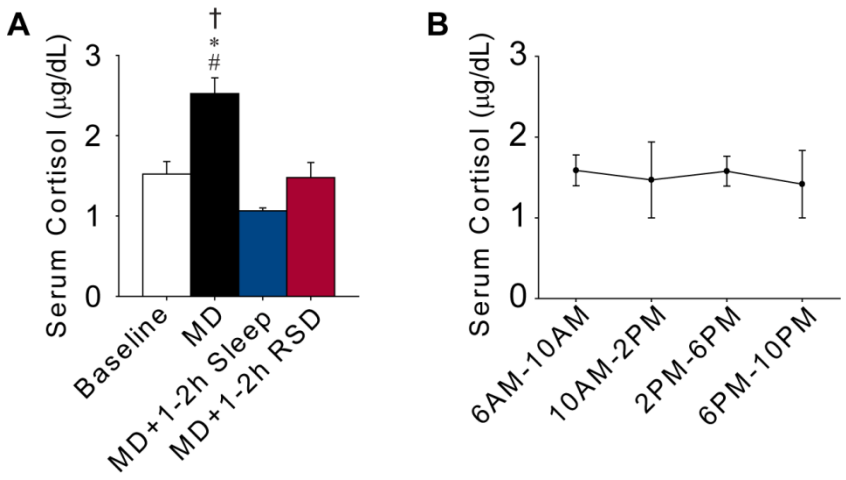

Figure 3.8. Serum cortisol is unaffected by RSD and circadian time. (A) Blood was obtained from a subset of animals at the end of the baseline, MD, and/or post-MD period for serum cortisol analysis. After MD, serum cortisol was elevated compared to all other groups. However, cortisol returned to baseline levels following 1-2 hours of uninterrupted or REM-deprived sleep. $p<0.001$ vs baseline (*), Sleep (†), and RSD (#); one-way ANOVA followed by Holm-Sidak post-hoc test; baseline N=10 animals; MD N=14; Sleep N=9; RSD N=8. (B) Serum cortisol in normal cats (with unmanipulated sleep and vision) did not exhibit overt circadian rhythms. 6-10AM: N=8 animals; 10AM-2PM: N=5; 2-6PM: N=8; 6-10PM: N=4; $p=0.58$; one-way ANOVA.

CHAPTER 4: CONCLUSIONS

An evolving view of cortical plasticity.

Many studies of learning, memory, and neuronal plasticity neglect to consider the role of sleep, let alone individual sleep states, in these processes. Investigating the role of sleep in ODP consolidation has shed light on how plasticity functions in the intact brain (Frank et al., 2001; Jha et al., 2005; Aton et al., 2009a; Seibt et al., 2012). The work presented in the previous chapters adds to our understanding of the mechanisms underlying sleep-dependent consolidation of plasticity (Figure 4.1). Evidence now suggests that, following the induction of ODP, NREM sleep promotes CaMKII phosphorylation and subsequent phosphorylation of the AMPA receptor subunit GluA1 at its CaMKII phosphorylation site, which may enhance synaptic currents by localizing AMPA receptors to the postsynaptic membrane and/or by increasing AMPA receptor currents and open probability (Correia et al., 2008; Kristensen et al., 2011). Subsequently during REM sleep, ERK is phosphorylated and activates Mnk1. Phosphorylated Mnk1 promotes the formation of translation initiation complexes and speeds translation via eIF4E phosphorylation (Scheper and Proud, 2002; Panja et al., 2009), indicating that ERK activation during REM sleep promotes the synthesis of plasticity-related proteins such as PSD-95. These events may stabilize the changes that occurred during the previous NREM sleep bout.

ERK activation during sleep is required for the potentiation of non-deprived eye responses during ODP. This suggests that, during sleep, ERK is activated in excitatory neurons within layer II/III of non-deprived eye columns. However, the use of whole-V1 homogenates and western blotting does not allow this assumption to be directly tested.

Immunohistochemical studies of ERK phosphorylation after normal and REM-deprived sleep are ongoing in our lab and will confirm whether ERK phosphorylation is restricted to the remodeling supra-granular layers; co-staining for cell-type specific markers will further reveal the identity of the cells that express increased ERK phosphorylation. In addition, injection of an anterograde tracer into one eye will allow us to distinguish between deprived- and non-deprived eye columns in order to confirm the identity of the cells in which ERK is activated.

Benefits of sleep or deficits due to sleep deprivation?

One problem commonly faced in studies of sleep function is determining the difference between the benefits of sleep and impairments due to sleep deprivation. The attenuation of ODP consolidation after a period of sleep deprivation could be ascribed to the stress of sleep deprivation, rather than the lack of sleep *per se*, although this explanation is unlikely (discussed in Frank et al., 2001). The current work directly addresses, for the first time, the question of stress in sleep-dependent ODP consolidation. As discussed in **Chapter 3**, serum cortisol is elevated by the end of the MD period. However, this elevation is well below levels that have been reported to have only minor effects on ODP when sustained over several days (Daw et al., 1991), and returns to baseline levels within two hours post-MD. This finding has implications for past and future studies, as it further validates ODP in the cat as a minimally stressful model for sleep-dependent consolidation.

Implications for systems consolidation.

Sleep has been proposed to play a role in the transfer of plasticity from the hippocampus to cortical regions, a process termed system consolidation (Rasch and Born, 2007). More specifically, activation of neuronal circuitry during NREM sleep may redistribute newly acquired information from sites of temporary storage (i.e. the hippocampus) to sites of long-term storage (the neocortex) where new and old information are integrated. Subsequently, REM sleep stabilizes these changes for long-term maintenance. Consistent with this hypothesis, Ribeiro and colleagues (2002, 2007) showed that hippocampal LTP induction and environmental enrichment promote waves of immediate early gene transcription that move from the hippocampus to the cortex with each bout of REM sleep.

In the current work, we found evidence supporting a role for REM sleep in translation, rather than transcription. Nonetheless, our studies are compatible with the system consolidation hypothesis. As discussed in **Chapter 3**, NREM sleep promotes temporary changes via post-translational modification of AMPA receptors, a mechanism that could also be engaged by activity in hippocampo-cortical circuitry to redistribute synaptic strengthening from the hippocampus to the cortex. Subsequently, REM sleep activates cascades that lead to long-term stabilization of plasticity within cortical circuitry via new protein synthesis.

Mechanisms of ERK activation during REM sleep.

Our results demonstrate that ERK is phosphorylated during REM sleep. However, we have not demonstrated the mechanism by which REM sleep activates ERK. There are two obvious candidate mechanisms: 1) elevated cholinergic tone during REM sleep may activate muscarinic acetylcholine receptors, leading to the activation of intracellular second-messenger cascades and ERK phosphorylation, or 2) patterns of neuronal firing in the cortex during REM sleep could activate ERK via activity-dependent NMDA receptor signaling.

Several studies have demonstrated a requirement for acetylcholine signaling in ODP. Combined lesions of noradrenergic and cholinergic input to the cortex impair ODP (Bear and Singer, 1986). Furthermore, pharmacological inhibition of specific cholinergic receptors showed that muscarinic M_1, but not nicotinic, acetylcholine receptors are required for ODP (Gu and Singer, 1989, 1993). However, whether M_1 receptor activation is required during waking, REM, or both cannot be discerned from these studies, since drugs were infused over a long period of MD regardless of arousal state. Infusion of a specific M_1 receptor antagonist during post-MD sleep would differentiate between these possibilities. If cholinergic signaling is required specifically during REM sleep, M_1 receptor antagonism would inhibit ODP consolidation (or, conversely, an M_1 agonist or acetylcholine reuptake inhibitor would enhance ODP). I began to investigate this question with a pilot experiment in which I infused the selective M_1 antagonist pirenzepine into V1 for one hour of post-MD sleep and measured ERK phosphorylation near to and far from the cannula site (following the design shown in Figure 2.2). I hypothesized that pirenzepine would mimic the effects of RSD by decreasing ERK phosphorylation; surprisingly, I found that pirenzepine *enhanced* ERK phosphorylation

near to the infusion site compared to regions of V1 far from the infusion site (Figure 4.2). This result was unexpected and inconsistent with a role for acetylcholine signaling during REM sleep in ERK-dependent non-deprived eye response potentiation. However, this finding is consistent with the idea that phospholipase C (PLC) activation through $G_{\alpha q}$-coupled receptors (including M_1 receptors) promotes LTD and suppresses LTP in the visual cortex and elsewhere (Kirkwood et al., 1999; Choi et al., 2005; Seol et al., 2007; Huang et al., 2012). Therefore, it appears that cholinergic signaling may influence ODP by promoting the depression of deprived eye responses during waking, or the maintenance of depression during sleep, through an ERK-independent mechanism. Single unit recordings following the intracortical infusion of pirenzepine during waking MD or post-MD sleep would reveal whether one or both of these scenarios is true. If cholinergic signaling is required for deprived eye response depression, we would expect pirenzepine to impair ODP when infused either during wake or sleep, and that deprived-eye response depression would be abolished. Conversely, infusion of M_1 receptor agonists or acetylcholinesterase inhibitors would be expected to enhance ODP and deprived eye response depression.

The second, and more likely, mechanism underlying REM-dependent ERK phosphorylation is neuronal activity in V1. In support of this hypothesis, NMDA receptor activation during post-MD sleep is required for ODP consolidation and ERK phosphorylation, and increases in neuronal firing rate in V1 during both REM and NREM sleep correlate with the OD shift (Aton et al., 2009a, 2013). Furthermore, REM sleep beta-gamma activity correlates with both ODP and ERK phosphorylation (**Chapter 3**).

The firing patterns of individual neurons during waking is replayed during REM sleep in the hippocampus (Louie and Wilson, 2001). If similar replay occurs during REM

sleep in the remodeling visual cortex, this may drive activity-dependent ERK activation and ODP consolidation. A preliminary analysis of firing across a tetrode array placed in V1 of a cat revealed that firing patterns during the last two hours of waking MD (driven by patterned visual input) correlated with firing patterns during the first two post-MD hours (in the absence of visual stimulation) (unpublished observations; data not shown). However, these correlations occurred across all arousal states, not just REM sleep. If neuronal activity does drive ERK phosphorylation during REM sleep, this raises additional questions. First, if replay occurs across all arousal states, what makes REM sleep a unique state for ERK activation? Second, could the coincidence of ERK activation during REM sleep and increased neuronal firing, which occur on the same timescale (within 2h post-MD), drive the consolidation of ODP? More detailed analysis of tetrode recordings is required to confirm this finding, and may reveal specific patterns of activation during REM sleep.

NREM sleep-dependent plasticity mechanisms.

The results presented in **Chapter 3** suggest a dual role for both NREM and REM sleep in ODP. However, the qualities of NREM sleep that promote plasticity mechanisms (i.e., CaMKII activation and AMPA receptor phosphorylation) are unknown. One possibility is that the oscillations of neocortical neurons may underlie these changes. T-type calcium channels contribute to multiple thalamocortical rhythms during NREM sleep (Crunelli et al., 2006), and cortical T-type calcium channels are required for ODP (Uebele et al., 2009), although their contribution to ODP during sleep has not been investigated. One possibility is that T-type calcium channel activation and rhythmic oscillations of cortical neurons during NREM sleep permits waves of calcium influx,

which then activate CaMKII. Direct infusion of the T-type calcium channel blocker TTA-I1 (Uebele et al., 2009) into the cortex specifically during post-MD sleep followed by measurements of CaMKII activation and ODP would provide insight into this possibility.

An additional scenario is that norepinephrine signaling may play a role in ODP consolidation during NREM sleep. Locus coeruleus (LC) firing is normally very low during NREM sleep (Aston-Jones and Bloom, 1981), but the LC firing that does occur is phase-locked to the cortical slow oscillation (Eschenko et al., 2012). Furthermore, LC firing during NREM sleep increases following an odor-reward learning task in rats, suggesting that LC activity during NREM sleep plays a role in consolidation (Eschenko and Sara, 2008). Infusion of noradrenergic β-receptor blockers into V1 over long periods of MD abolishes ODP (Shirokawa and Kasamatsu, 1986), but the contribution of NE signaling during sleep to ODP consolidation is unknown. In order to test the hypothesis that β-receptor activation during sleep is required for ODP, we conducted a pilot study in which three cats received bilateral intracortical infusions of the β-blocker propranolol during post-MD sleep, and collected tissue for western blot analysis. Propranolol-infused V1 tissue showed a nonsignificant trend towards decreased CaMKII phosphorylation when compared to levels in vehicle-infused V1 (72.1±11.3% of vehicle; p=0.066; n=6 hemispheres per group; t-test), whereas ERK1/2 phosphorylation was unaffected (ERK1: 101.65±21.7% of vehicle, p=0.48; ERK2: 84.51±25.5% of vehicle, p=0.34; n=6 hemispheres/group, t-test) (vehicle data shown in **Figure 2.2**). These results are suggestive of a role for NE signaling during NREM sleep in cortical consolidation, but ultimately more detailed studies of the biochemical events downstream of NE β-receptor activation, as well as the consequences of β-receptor blockade during sleep on ODP, are required.

Dual roles for REM sleep in development and experience-dependent plasticity.

Our finding that REM sleep is required for the consolidation of experience-dependent plasticity conflicts with the idea that REM sleep promotes the maturation of neuronal circuitry according to a developmental program. According to this idea, endogenous brain activity during REM sleep should promote the normal development of binocular vision within V1, rather than enhancing a shift towards monocular vision due to altered visual stimulation. However, the results presented in **Chapter 3** and previous studies suggest that these two ideas are not mutually exclusive. The current study provides evidence that REM sleep is required for ODP consolidation specifically within V1, as remodeling in the cat visual cortex occurs within layers II/III on a short (<24h) time scale. In contrast, REM sleep deprivation and PGO wave elimination over longer periods of MD, during which thalamocortical plasticity occurs, enhances an anatomical measure of ODP in the LGN (Oksenberg et al., 1996; Shaffery et al., 1998). Together, these studies suggest that different aspects of REM sleep contribute to developmental and experience-dependent plasticity: PGO waves during REM sleep promote the maturation of brain circuitry, whereas intracortical activity consolidates experience-dependent plasticity. This interpretation is consistent with the observation that REM sleep time is greatest during periods of brain development, but is still present in large amounts in the adult brain (Roffwarg et al., 1966).

Pharmacological agents, sleep, and plasticity.

Numerous pharmacological agents in common use can alter sleep. However, the effects of these drugs on the normal functions of sleep are largely unknown. For example, hypnotic drugs used to treat insomnia promote sleep, but is sleep able to

perform its required functions in the presence of these drugs? Furthermore, antidepressants can radically change sleep architecture, which may have consequences for cognitive function.

Insomnia can be defined as trouble initiating or maintaining restorative sleep, despite adequate sleep opportunity. Up to 22.1% of the American population can be classified as having insomnia, depending on the diagnostic criteria used (Roth et al., 2011). Prescription, over-the-counter, and herbal remedies are widely used to combat insomnia (reviewed in Gulyani et al., 2012), while little is known about the impact of these medications on brain plasticity. The effects of some of these drugs on ODP have been explored (Seibt et al., 2008; Aton et al., 2009b). Interestingly, both the non-benzodiazepine hypnotic zolpidem and the sedating antidepressant trazadone impair ODP consolidation, but have only minimal effects on REM sleep. It is unclear how these drugs impair plasticity, but one possibility is that they disrupt signaling pathways that are required for sleep-dependent consolidation, such as ERK activity, without altering sleep architecture. I conducted a pilot study in which cats received systemic vehicle or zolpidem injections (using a dose known to impair ODP (Seibt et al., 2008)) at the end of a six hour MD period and collected tissue after one hour of post-MD sleep, when ERK phosphorylation is normally high. Western blot analysis revealed that zolpidem does not impair ERK phosphorylation in V1 (Figure 4.3). This finding was unexpected, and we have yet to identify any mechanisms by which these hypnotic drugs impair plasticity. Further measurements of how zolpidem and trazadone affect the biochemical events that are known to consolidate plasticity during sleep (e.g. translation downstream of ERK and/or mTOR activation) would aid in the development of hypnotic agents that can promote normal sleep.

The current work also has implications for the use of antidepressants, which are widely prescribed and can profoundly reduce REM sleep time (reviewed in Holshoe, 2009). The findings in **Chapter 3** suggest that these drugs should cause cognitive deficits by impairing learning and memory. However, in addition to procedural memory, REM sleep has been associated with the strengthening of emotional memories in humans (Nishida et al., 2009; Groch et al., 2013). Therefore, it is possible that the REM-suppressing activity of these drugs reduces the consolidation of negative emotional memories, and this may, in part, underlie their therapeutic benefits. Consistent with this idea, REM sleep deprivation alone has been posited to have antidepressant effects in humans (Vogel et al., 1975).

Concluding remarks.

Studying the mechanisms involved in brain plasticity furthers our understanding of how learning and memory are achieved in the intact brain over the course of waking experience and subsequent sleep. A full understanding of plasticity mechanisms will create new therapeutic avenues, such as re-opening periods of heightened plasticity in the adult brain. Furthermore, investigating the role of sleep in consolidation aids our understanding of how sleep deprivation impairs cognitive function, and has implications for the development of pharmacological agents that do not interfere with normal sleep function.

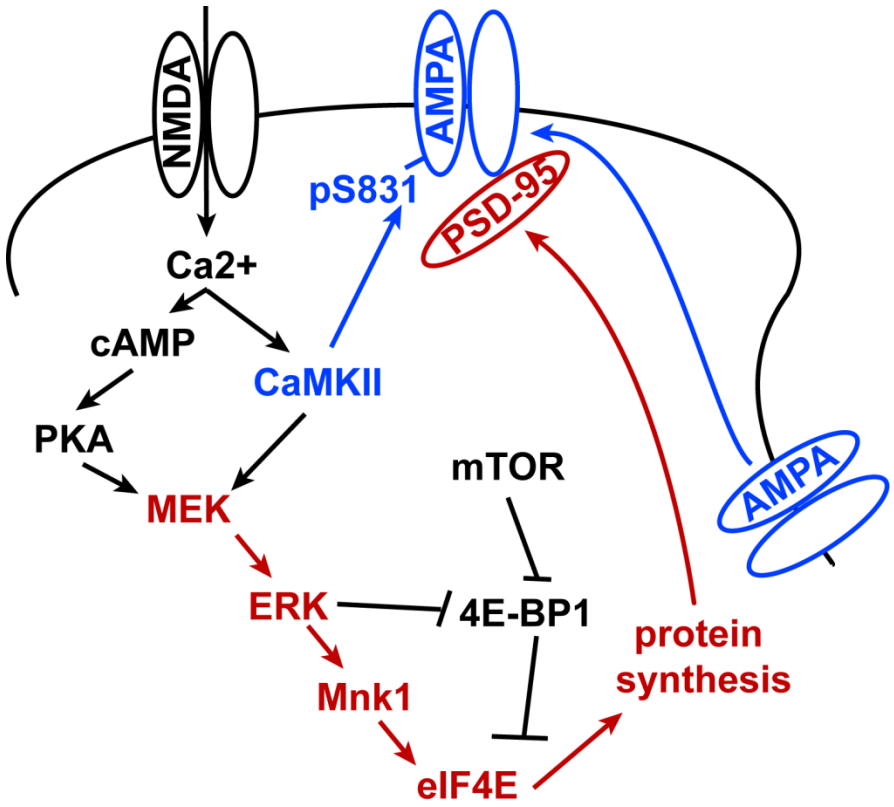

Figure 4.1. Mechanisms of sleep-dependent ocular dominance plasticity consolidation. The current studies provide evidence that CaMKII activation and AMPA receptor phosphorylation occur during NREM sleep (blue), whereas ERK and Mnk1 phosphorylation, as well as subsequent protein synthesis, are required during REM sleep (red), stabilizing the changes induced by NREM sleep. CaMKII activation during NREM sleep and ERK activation during REM sleep may occur downstream of NMDA receptor activation and/or other mechanisms that have not yet been identified (see text for details).

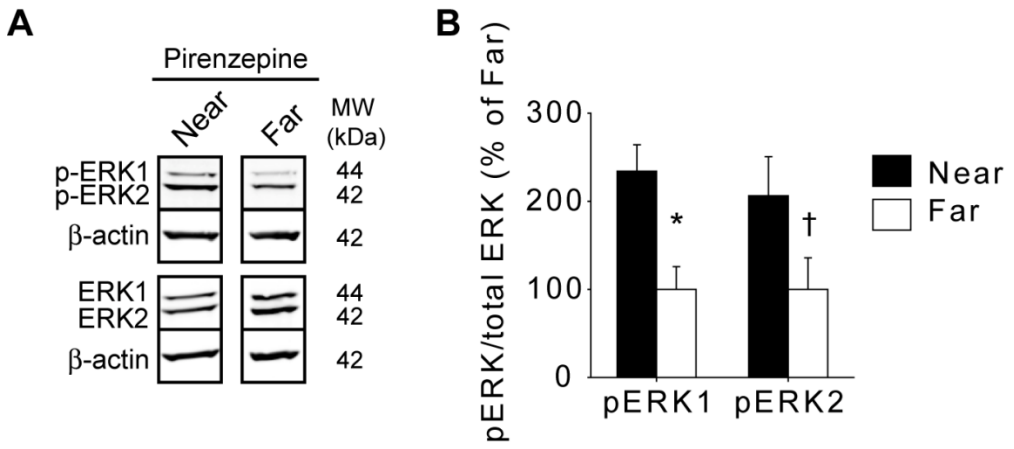

Figure 4.2. M_1 receptor blockade during post-MD sleep enhances ERK phosphorylation. Pirenzepine (1mg/mL in ACSF) was infused for one hour of post-MD sleep (following the experimental design presented in Figure 2.2) and tissue was collected for western blot analysis. (A) Representative western blots. (B) Pirenzepine infusion increased ERK1/2 phosphorylation near to the cannula site compared to tissue collected far from the cannula site. *p=≤0.001; †p=0.002; n=4 hemispheres; paired t-test.

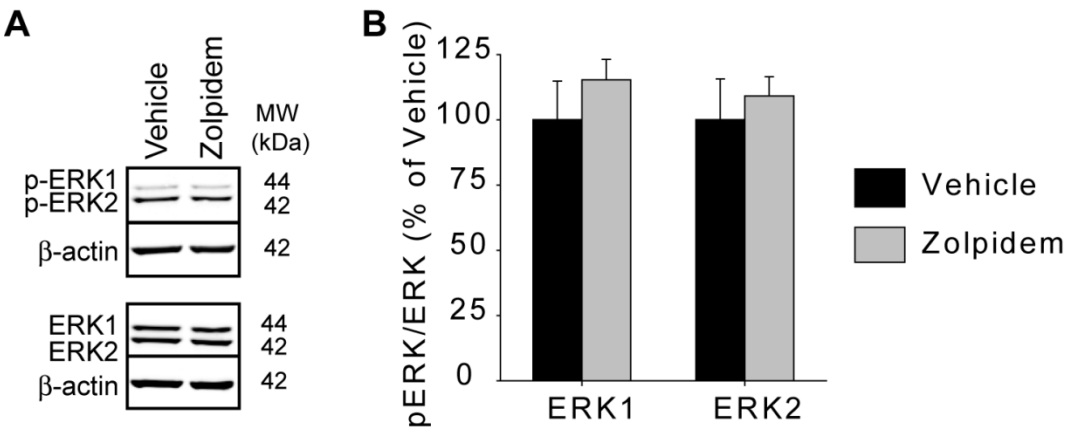

Figure 4.3. Systemic zolpidem administration does not affect ERK phosphorylation in V1. Zolpidem at a concentration known to impair ODP consolidation (10mg/kg (Seibt et al., 2008)) or its vehicle (DMSO) was administered IP after 6h MD, and animals were allowed 1h undisturbed sleep in complete darkness. V1 tissue was then collected for western blot analysis. (A) Representative western blots of V1 tissue. (B) ERK1/2 phosphorylation in V1 was unaffected by zolpidem. pERK1 p=0.397; pERK2 p=0.309; t-test; n=4 hemispheres/group.

APPENDIX I: ABBREVIATIONS

AMPA: 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid

Arc: Activity regulated cytoskeletal-associated protein

BDNF: Brain-derived neurotrophic factor

CaMKII: Calcium/calmodulin dependent kinase II

cAMP: Cyclic adenosine monophosphate

CBI: Contralateral eye bias index

CRE: cAMP response element

CREB: cAMP response element binding protein

CSPG: Chondroitin sulfate proteoglycan

EEG: Electroencephalogram

eIF4E: Eukaryotic initiation factor 4E

EMG: Electromyogram

ERK: Extracellular signal-regulated kinase

GABA: Gamma-aminobutyric acid

LC: Locus coeruleus

LGN: Lateral geniculate nucleus (of the thalamus)

LTD: Long-term depression

LTP: Long-term potentiation

MD: Monocular deprivation

MEK: Mitogen-activated protein kinase kinase

MI: Monocularity index

Mnk: MAP kinase-interacting kinase

MSK: Mitogen and stress activated protein kinase

NBI: Non-deprived eye bias index

NF: NREM fragmentation

NMDA: N-methyl-D-aspartate

NREM: Non-rapid eye movement

OD: Ocular dominance

ODP: Ocular dominance plasticity

PGO: Ponto-geniculo-occipital

PKA: Protein kinase A

PKC: Protein kinase C

PKG: Protein kinase G

PLC: Phospholipase C

PS: Paradoxical sleep (a.k.a. REM sleep)

PSD-95: Post-synaptic density protein 95

REM: Rapid eye movement

RSD: REM sleep deprivation

SI: Shift index

SRP: Stimulus-specific response potentiation

tPA: Tissue plasminogen activator

V1: Primary visual cortex

BIBLIOGRAPHY

Adams JP, Sweatt JD (2002) Molecular psychology: Roles for the ERK MAP kinase cascade in memory. Annu Rev Pharmacol Toxicol 42:135–163.

Aleisa AM, Helal G, Alhaider IA, Alzoubi KH, Srivareerat M, Tran TT, Al-Rejaie SS, Alkadhi KA (2011) Acute nicotine treatment prevents REM sleep deprivation-induced learning and memory impairment in rat. Hippocampus 21:899–909.

Anagnostaras SG, Maren S, Fanselow MS (1999) Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. J Neurosci 19:1106–1114.

Aserinsky E, Kleitman N (1953) Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science 118:273–274.

Aston-Jones G, Bloom F (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876–886.

Aton SJ, Broussard C, Dumoulin M, Seibt J, Watson A, Coleman T, Frank MG (2013) Visual experience and subsequent sleep induce sequential plastic changes in putative inhibitory and excitatory cortical neurons. Proc Natl Acad Sci USA 110:3101–3106.

Aton SJ, Seibt J, Dumoulin M, Jha SK, Steinmetz N, Coleman T, Naidoo N, Frank MG (2009a) Mechanisms of sleep-dependent consolidation of cortical plasticity. Neuron 61:454–466.

Aton SJ, Seibt J, Dumoulin MC, Coleman T, Shiraishi M, Frank MG (2009b) The sedating antidepressant trazodone impairs sleep- dependent cortical plasticity. PLoS ONE 4:e6078.

Backhaus J, Junghanns K (2006) Daytime naps improve procedural motor memory. Sleep Med 7:508–512.

Banko JL, Hou L, Poulin F, Sonenberg N, Klann E (2006) Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. J Neurosci 26:2167–2173.

Barria A, Muller D, Derkach V, Griffith LC, Soderling TR (1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science 276:2042–2045.

Bear M, Kleinschmidt A, Gu Q, Singer W (1990) Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J Neurosci 10:909–925.

Bear MF (2003) Bidirectional synaptic plasticity: from theory to reality. Phil Trans R Soc Lond B 358:649–655.

Bear MF, Cooper LN, Ebner FF (1987) A physiological basis for a theory of synapse modification. Science 237:42–48.

Bear MF, Singer W (1986) Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature 320:172–176.

Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, Malenka RC (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat Neurosci 3:1291–1300.

Beaver C, Ji Q, Fischer Q, Daw N (2001) Cyclic AMP-dependent protein kinase mediates ocular dominance shifts in cat visual cortex. Nat Neurosci 4:159–163.

Belelovsky K, Elkobi A, Kaphzan H, Nairn A, Rosenblum K (2005) A molecular switch for translational control in taste memory consolidation. Eur J Neurosci 22:2560–2568.

Berardi N, Pizzorusso T, Ratto GM, Maffei L (2003) Molecular basis of plasticity in the visual cortex. Trends Neurosci 26:369–378.

Berger H (1929) Über das elektrenkephalogramm des mensche. Arch Psychiat Nerven 87:527–570.

Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 2:32–48.

Binder S, Baier PC, Mölle M, Inostroza M, Born J, Marshall L (2012) Sleep enhances memory consolidation in the hippocampus-dependent object-place recognition task in rats. Neurobiol Learn Mem 97:213–219.

Bliss T, Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232:331–356.

Bliss TVP, Gardner-Medwin AR (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanesthetized rabbit following stimulation of the perforant path. J Physiol 232:357–374.

Boggio EM, Putignano E, Sassoè-Pognetto M, Pizzorusso T, Giustetto M (2007) Visual stimulation activates ERK in synaptic and somatic compartments of rat cortical neurons with parallel kinetics. PloS ONE 2:e604.

Bontempi B, Laurent-Demir C, Destrade C, Jaffard R (1999) Time-dependent reorganization of brain circuitry underlying long-term memory storage. Nature 400:671–675.

Brooks D, Bizzi E (1963) Brain stem electrical activity during sleep. Arch Ital Biol 101:648–665.

Buchegger J, Fritsch R, Meier-Koll A, Riehle H (1991) Does trampolining and anaerobic physical fitness affect sleep? Percept Motor Skill 73:243–252.

Buchegger J, Meier-Koll A (1988) Motor learning and ultradian sleep cycle: an electroencephalographic study of trampoliners. Percept Motor Skill 67:635–645.

Cai DJ, Shuman T, Gorman MR, Sage JR, Anagnostaras SG (2009) Sleep selectively enhances hippocampus-dependent memory in mice. Behav Neurosci 123:713–719.

Cancedda L, Putignano E, Impey S, Maffei L, Ratto GM, Pizzorusso T (2003) Patterned vision causes CRE-mediated gene expression in the visual cortex through PKA and ERK. Gene Expr 23:7012–7020.

Carr MF, Jadhav SP, Frank LM (2011) Hippocampal replay in the awake state: A potential substrate for memory consolidation and retrieval. Nat Neurosci 14:147–153.

Castaldo V, Krynicki V, Goldstein J (1974) Sleep stages and verbal memory. Percept Motor Skill 39:1023–1030.

Chernik DA (1972) Effect of REM sleep deprivation on learning and recall by humans. Percept Motor Skill 34:283–294.

Cho K, Khibnik L (2009) The ratio of NR2A/B NMDA receptor subunits determines the qualities of ocular dominance plasticity in visual cortex. Proc Natl Acad Sci USA 106:5377–5382.

Choi S-Y, Chang J, Jiang B, Seol G-H, Min S-S, Han J-S, Shin H-S, Gallagher M, Kirkwood A (2005) Multiple receptors coupled to phospholipase C gate long-term depression in visual cortex. J Neurosci 25:11433–11443.

Chowdhury S, Shepherd JD, Okuno H, Lyford G, Petralia RS, Plath N, Kuhl D, Huganir RL, Worley PF (2006) Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 52:445–459.

Coleman JE, Nahmani M, Gavornik JP, Haslinger R, Heynen AJ, Erisir A, Bear MF (2010) Rapid structural remodeling of thalamocortical synapses parallels experience-dependent functional plasticity in mouse primary visual cortex. J Neurosci 30:9670–9682.

Collingridge G, Kehl S, H M (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol 334:33–46.

Connor SA, Wang YT, Nguyen PV (2011) Activation of beta-adrenergic receptors facilitates heterosynaptic translation-dependent long-term potentiation. J Physiol 589:4321–4340.

Cooke SF, Bear MF (2010) Visual experience induces long-term potentiation in the primary visual cortex. J Neurosci 30:16304–16313.

Correia SS, Bassani S, Brown TC, Lisé M-F, Backos DS, El-Husseini A, Passafaro M, Esteban JA (2008) Motor protein-dependent transport of AMPA receptors into spines during long-term potentiation. Nat Neurosci 11:457–466.

Crozier RA, Wang Y, Liu C-H, Bear MF (2007) Deprivation-induced synaptic depression by distinct mechanisms in different layers of mouse visual cortex. Proc Natl Acad Sci USA 104:1383–1388.

Crunelli V, Cope DW, Hughes SW (2006) Thalamic T-type Ca2+ channels and NREM sleep. Cell Calcium 40:175–190.

Davenne D, Adrien J (1984) Suppression of PGO waves in the kitten: Anatomical effects on the lateral geniculate nucleus. Neurosci Lett 45:33–38.

Davenne D, Frégnac Y, Imbert M, Adrien J (1989) Lesion of the PGO pathways in the kitten. II. Impairment of physiological and morphological maturation of the lateral geniculate nucleus. Brain Res 485:267–277.

Davis CJ, Harding JW, Wright JW (2003) REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus. Brain Res 973:293–297.

Davis H, Davis P, Loomis A (1938) Human brain potentials during the onset of sleep. J Neurophysiol 1:24–38.

Davis H, Davis P, Loomis A, Harvey E, Hobart G (1937) Changes in human brain potentials during the onset of sleep. Science 86:448–450.

Davis S, Vanhoutte P (2000) MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus. J Neurosci 20:4563–4572.

Daw NW, Beaver CJ (2001) Developmental changes and ocular dominance plasticity in the visual cortex. Keio J Med 50:192–197.

Daw NW, Sato H, Fox K, Carmichael T, Gingerich R (1991) Cortisol reduces plasticity in the kitten visual cortex. J Neurobiol 22:158–168.

del Rio J, de Lecea L, Ferrer I, Soriano E (1994) The development of parvalbumin-immunoreactivity in the neocortex of the mouse. Dev Brain Res 81:247–259.

Dement W (1958) The occurence of low voltage, fast, electroencephalogram patterns during behavioral sleep in the cat. Electroencephalogr Clin Neurophysiol 10:291–296.

Dement W, Kleitman N (1957) Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. Electroencephalogr Clin Neurophysiol 9:673–690.

DiCristo G, Berardi N, Cancedda L, Pizzorusso T, Putignano E, Ratto GM, Maffei L (2001) Requirement of ERK activation for visual cortical plasticity. Science 292:2337–2340.

Diggle TA, Subkhankulova T, Lilley KS, Shikotra N, Willis AE, Redpath NT (2001) Phosphorylation of elongation factor-2 kinase on serine 499 by cAMP-dependent protein kinase induces Ca2+/calmodulin-independent activity. Biochem J 353:621–626.

Drosopoulos S, Wagner U, Born J (2005) Sleep enhances explicit recollection in recognition memory. Learn Mem 12:44–51.

Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci USA 89:4363–4367.

Dumoulin MC, Aton SJ, Watson AJ, Renouard L, Coleman T, Frank MG (2013) Extracellular signal-regulated kinase (ERK) activity during sleep consolidates cortical plasticity *in vivo*. Cereb Cortex. In Press.

Dunwiddie T, Lynch G (1978) Long-term potentiation and depression of synaptic responses in the rat hippocampus: Localization and frequency dependency. J Physiol 276:353–367.

Ehrlich I, Malinow R (2004) Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. J Neurosci 24:916–927.

Ekstrand B (1967) Effect of sleep on memory. J Exp Psychol 75:64–72.

Ekstrand BR, Sullivan MJ, Parker DF, West JN (1971) Spontaneous recovery and sleep. J Exp Psychol 88:142–144.

Ellenbogen JM, Hulbert JC, Jiang Y, Stickgold R (2009) The sleeping brain's influence on verbal memory: Boosting resistance to interference. PloS ONE 4:e4117.

Ellenbogen JM, Hulbert JC, Stickgold R, Dinges DF, Thompson-Schill SL (2006) Interfering with theories of sleep and memory: Sleep, declarative memory, and associative interference. Curr Biol 16:1290–1294.

Empson J, Clarke P (1970) Rapid eye movements and remembering. Nature 227:287–288.

Endo S, Launey T (2003) ERKs regulate PKC-dependent synaptic depression and declustering of glutamate receptors in cerebellar Purkinje cells. Neuropharmacology 45:863–872.

English JD, Sweatt JD (1997) A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. J Biol Chem 272:19103–19106.

Eschenko O, Magri C, Panzeri S, Sara S (2012) Noradrenergic neurons of the locus coeruleus are phase locked to cortical up-down states during sleep. Cereb Cortex 22:426–435.

Eschenko O, Sara SJ (2008) Learning-dependent, transient increase of activity in noradrenergic neurons of locus coeruleus during slow wave sleep in the rat: Brain stem-cortex interplay for memory consolidation? Cereb Cortex 18:2596–2603.

Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, Van Dyk DE, Pitts WJ, Earl RA, Hobbs F, Copeland RA, Magolda RL, Scherle PA, Trzaskos JM (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J Biol Chem 273:18623–18632.

Ferrara M, Iaria G, De Gennaro L, Guariglia C, Curcio G, Tempesta D, Bertini M (2006) The role of sleep in the consolidation of route learning in humans: A behavioural study. Brain Res Bull 71:4–9.

Fischer QS, Beaver CJ, Yang Y, Rao Y, Jakobsdottir KB, Storm DR, McKnight GS, Daw NW (2004) Requirement for the RIIbeta isoform of PKA, but not calcium-stimulated adenylyl cyclase, in visual cortical plasticity. J Neurosci 24:9049–9058.

Fischer S, Hallschmid M, Elsner AL, Born J (2002) Sleep forms memory for finger skills. Proc Natl Acad Sci USA 99:11987–11991.

Fishbein W (1971) Disruptive effects of rapid eye movement sleep deprivation on long-term memory. Physiol Behav 6:279–282.

Fogel SM, Smith CT, Beninger RJ (2010) Increased GABAergic activity in the region of the pedunculopontine and deep mesencephalic reticular nuclei reduces REM sleep and impairs learning in rats. Behav Neurosci 124:79–86.

Frank MG (2006) The mystery of sleep function: Current perspectives and future directions. Rev Neurosci 17:375–392.

Frank MG, Issa NP, Stryker MP (2001) Sleep enhances plasticity in the developing visual cortex. Neuron 30:275–287.

Frankland PW, Bontempi B (2005) The organization of recent and remote memories. Nat Rev Neurosci 6:119–130.

Frankland PW, Bontempi B, Talton LE, Kaczmarek L, Silva AJ (2004) The involvement of the anterior cingulate cortex in remote contextual fear memory. Science 304:881–883.

Frenkel MY, Bear MF (2004) How monocular deprivation shifts ocular dominance in visual cortex of young mice. Neuron 44:917–923.

Gais S, Köster S, Sprenger A, Bethke J, Heide W, Kimmig H (2008a) Sleep is required for improving reaction times after training on a procedural visuo-motor task. Neurobiol Learn Mem 90:610–615.

Gais S, Lucas B, Born J (2006) Sleep after learning aids memory recall. Learn Mem 13:259–262.

Gais S, Rasch B, Wagner U, Born J (2008b) Visual–procedural memory consolidation during sleep blocked by glutamatergic receptor antagonists. J Neurosci 28:5513–5518.

Gallagher SM, Daly CA, Bear MF, Huber KM (2004) Extracellular signal-regulated protein kinase activation is required for metabotropic glutamate receptor-dependent long-term depression in hippocampal area CA1. J Neurosci 24:4859–4864.

Gandhi SP, Yanagawa Y, Stryker MP (2008) Delayed plasticity of inhibitory neurons in developing visual cortex. Proc Natl Acad Sci USA 105:16797–16802.

Gassel M, Marchiafava P, Pompeiano O (1964) Phasic changes in muscular activity during desynchronized sleep in unrestrained cats. Arch Ital Biol 102:449–470.

Gelinas JN, Banko JL, Hou L, Sonenberg N, Weeber EJ, Klann E, Nguyen PV (2007) ERK and mTOR signaling couple β-adrenergic receptors to translation initiation machinery to gate induction of protein synthesis-dependent long-term potentiation. J Biol Chem 282:27527–27535.

Giese KP, Fedorov NB, Filipowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the calcium-calmodulin kinase II in LTP and learning. Science 279:870–873.

Graves LA, Heller EA, Pack AI, Abel T (2003) Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem 10:168–176.

Green JD, Arduini A (1954) Hippocampal electrical activity in arousal. J Neurophysiol 17:533–557.

Groch S, Wilhelm I, Diekelmann S, Born J (2013) The role of REM sleep in the processing of emotional memories: evidence from behavior and event-related potentials. Neurobiol Learn Mem 99:1–9.

Gu Q (2002) Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. Science 111:815–835.

Gu Q, Singer W (1989) The role of muscarinic acetylcholine receptors in ocular dominance plasticity. Exp Suppl 57:305–314.

Gu Q, Singer W (1993) Effects of intracortical infusion of anticholinergic drugs on neuronal plasticity in kitten striate cortex. Eur J Neurosci 5:475–485.

Gu Q, Singer W (1995) Involvement of serotonin in developmental plasticity of kitten visual cortex. Eur J Neurosci 7:1146–1153.

Gulyani S, Salas RE, Gamaldo CE (2012) Sleep medicine pharmacotherapeutics overview: Today, tomorrow, and the future (Part 1: insomnia and circadian rhythm disorders). Chest 142:1659–1668.

Habib D, Dringenberg HC (2010) Surprising similarity between mechanisms mediating low (1 Hz)-and high (100 Hz)-induced long-lasting synaptic potentiation in CA1 of the intact hippocampus. Neuroscience 170:489–496.

Habib D, Tsui CKY, Rosen LG, Dringenberg HC (2013) Occlusion of low-frequency-induced, heterosynaptic long-term potentiation in the rat hippocampus *in vivo* following spatial training. Cereb Cortex In Press.

Hagewoud R, Whitcomb SN, Heeringa AN, Havekes R, Koolhaas JM, Meerlo P (2010) A time for learning and a time for sleep: The effect of sleep deprivation on contextual fear conditioning at different times of the day. Sleep 33:1315–1322.

Haruta H, Kamishita T, Hicks T (1994) Induction of LTD but not LTP through metabotropic glutamate receptors in visual cortex. NeuroReport 5:1829–1832.

Hellman K, Abel T (2007) Fear conditioning increases NREM sleep. Behav Neurosci 121:310–323.

Hensch TK, Stryker MP (1996) Ocular dominance plasticity under metabotropic glutamate receptor blockade. Science 272:554–557.

Heynen AJ, Yoon B-J, Liu C-H, Chung HJ, Huganir RL, Bear MF (2003) Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nat Neurosci 6:854–862.

Hogan D, Roffwarg HP, Shaffery JP (2001) The effects of 1 week of REM sleep deprivation on parvalbumin and calbindin immunoreactive neurons in central visual pathways of kittens. J Sleep Res 10:285–296.

Holdstock T, Verschoor G (1974) Student sleep patterns before, during and after an examination period. S Afr J Psychol 4:16–24.

Holshoe JM (2009) Antidepressants and sleep: A review. Perspect Psychiatr C 45:191–197.

Hsu JC (1996) Multiple comparisons: Theory and methods. Boca Raton, FL: Chapman & Hall/CRC Press.

Huang S, Treviño M, He K, Ardiles A, Pasquale RD, Guo Y, Palacios A, Huganir R, Kirkwood A (2012) Pull-push neuromodulation of LTP and LTD enables bidirectional experience-induced synaptic scaling in visual cortex. Neuron 73:497–510.

Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98:739–755.

Hubel D, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol 206:419–436.

Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR (1998) Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 21:869–883.

Inostroza M, Binder S, Born J (2013) Sleep-dependency of episodic-like memory consolidation in rats. Behav Brain Res 237:15–22.

Issa NP, Trachtenberg JT, Chapman B, Zahs KR, Stryker MP (1999) The critical period for ocular dominance plasticity in the ferret's visual cortex. J Neurosci 19:6965–6978.

Jha SK, Jones BE, Coleman T, Steinmetz N, Law C-T, Griffin G, Hawk J, Dabbish N, Kalatsky VA, Frank MG (2005) Sleep-dependent plasticity requires cortical activity. J Neurosci 25:9266–9274.

Ji D, Wilson MA (2007) Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat Neurosci 10:100–107.

Jouvet D, Vimont P, Delorme F, Jouvet M (1964) Étude de la privation sélective de la phase paradoxale du sommeil chez le chat. C R Seances Soc Biol Fil 158:756–759.

Jouvet M, Michel F, Courjon J (1959) Sur un stade d'activité électrique cérébrale rapide au cours du sommeil physiologique. C R Seances Soc Biol Fil 153:1024–1028.

Kameyama K, Lee HK, Bear MF, Huganir RL (1998) Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. Neuron 21:1163–1175.

Kameyama K, Sohya K, Ebina T, Fukuda A, Yanagawa Y, Tsumoto T (2010) Difference in binocularity and ocular dominance plasticity between GABAergic and excitatory cortical neurons. J Neurosci 30:1551–1559.

Karni A, Tanne D, Rubenstein BS, Askenasy JJ, Sagi D (1994) Dependence on REM sleep of overnight improvement of a perceptual skill. Science 265:679–682.

Kato N (1993) Dependence of long-term depression on postsynaptic metabotropic glutamate receptors in visual cortex. Proc Natl Acad Sci USA 90:3650–3654.

Kawasaki H, Fujii H, Gotoh Y, Morooka T, Shimohama S, Nishida E, Hirano T (1999) Requirement for mitogen-activated protein kinase in cerebellar long term depression. J Biol Chem 274:13498–13502.

Kelleher RJ, Govindarajan A, Jung H-Y, Kang H, Tonegawa S (2004) Translational control by MAPK signaling in long-term synaptic plasticity and memory. Cell 116:467–479.

Khibnik LA, Cho KKA, Bear MF (2010) Relative contribution of feedforward excitatory connections to expression of ocular dominance plasticity in layer 4 of visual cortex. Neuron 66:493–500.

Kirkwood A, Lee H-K, Bear MF (1995) Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. Nature 375:328–331.

Kirkwood A, Rozas C, Kirkwood J, Perez F, Bear MF (1999) Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. J Neurosci 19:1599–1609.

Knauf U, Tschopp C, Gram H (2001) Negative regulation of protein translation by mitogen-activated protein kinase-interacting kinases 1 and 2. Mol Cell Biol 21:5500–5511.

Kristensen AS, Jenkins MA, Banke TG, Schousboe A, Makino Y, Johnson RC, Huganir R, Traynelis SF (2011) Mechanism of Ca2+/calmodulin-dependent kinase II regulation of AMPA receptor gating. Nat Neurosci 14:727–735.

Kumar T, Jha SK (2012) Sleep deprivation impairs consolidation of cued fear memory in rats. PloS ONE 7:e47042.

Kuriyama K, Stickgold R, Walker MP (2004) Sleep-dependent learning and motor-skill complexity. Learn Mem 11:705–713.

Leconte P, Hennevin E, Bloch V (1974) Duration of paradoxical sleep necessary for the acquisition of conditioned avoidance in the rat. Physiol Behav 13:675–681.

Lee AK, Wilson MA (2002) Memory of sequential experience in the hippocampus during slow wave sleep. Neuron 36:1183–1194.

Lee H-K, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405:955–959.

Lee H-K, Kameyama K, Huganir RL, Bear MF (1998) NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron 21:1151–1162.

Lee S, Kim W, Ham B-J, Chen W, Bear MF, Yoon B-J (2008) Activity-dependent NR2B expression is mediated by MeCP2-dependent epigenetic regulation. Biochem Biophys Res Commun 377:930–934.

Legault G, Delay S, Madore A (2010) Identification of a rapid eye movement sleep window for learning of the win-shift radial arm maze task for male Sprague-Dawley rats. J Sleep Res 19:508–515.

Legault G, Smith CT, Beninger RJ (2004) Scopolamine during the paradoxical sleep window impairs radial arm maze learning in rats. Pharmacol Biochem Behav 79:715–721.

Lewin I, Glaubman H (1975) The effect of REM deprivation: Is it detrimental, beneficial, or neutral? Psychophysiology 12:349–353.

Linden ML, Heynen AJ, Haslinger RH, Bear MF (2009) Thalamic activity that drives visual cortical plasticity. Nat Neurosci 12:390–392.

Liu C-H, Heynen AJ, Shuler MGH, Bear MF (2008) Cannabinoid receptor blockade reveals parallel plasticity mechanisms in different layers of mouse visual cortex. Neuron 58:340–345.

Loomis A, Harvey EN, Hobart G (1935a) Potential rhythms of the cerebral cortex during sleep. Science 81:597–598.

Loomis AL, Harvey EN, Hobart G (1935b) Further observations on the potential rhythms of the cerebral cortex during sleep. Science 82:198–200.

Lopez J, Roffwarg HP, Dreher A, Bissette G, Karolewicz B, Shaffery JP (2008) Rapid eye movement sleep deprivation decreases long-term potentiation stability and affects some glutamatergic signaling proteins during hippocampal development. Neuroscience 153:44–53.

Louie K, Wilson MA (2001) Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. Neuron 29:145–156.

Lovinger DM, Wong KL, Murakami K, Routtenberg A (1987) Protein kinase C inhibitors eliminate hippocampal long-term potentiation. Brain Res 436:177–183.

Lu W, Isozaki K, Roche KW, Nicoll RA (2010) Synaptic targeting of AMPA receptors is regulated by a CaMKII site in the first intracellular loop of GluA1. Proc Natl Acad Sci USA 107:22266–22271.

Luo J, Phan TX, Yang Y, Garelick MG, Storm DR (2013) Increases in cAMP, MAPK activity, and CREB phosphorylation during REM sleep: Implications for REM sleep and memory consolidation. J Neurosci 33:6460–6468.

Lynch G, Larson J, Kelso S (1983) Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature 305:719–721.

Malenka R, Kauer J, Perkel D, Mauk M (1989) An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. Nature 340:554–557.

Malinow R, Schulman H, Tsien RW (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science 245:862–866.

Martí-Nicolovius M, Portell-Cortés I, Morgado-Bernal I (1988) Improvement of shuttle-box avoidance following post-training treatment in paradoxical sleep deprivation platforms in rats. Physiol Behav 43:93–98.

Mataga N, Imamura K, Shiomitsu T (1996) Enhancement of mRNA expression of tissue-type plasminogen activator by l- threo-3, 4-dihydroxyphenylserine in association with ocular dominance plasticity. Neurosci Lett 218:149–152.

Mataga N, Nagai N, Hensch TK (2002) Permissive proteolytic activity for visual cortical plasticity. Proc Natl Acad Sci USA 99:7717–7721.

Matthies H, Reymann K (1993) Protein kinase A inhibitors prevent the maintenance of hippocampal long-term potentiation. Neuroreport 4:712–714.

McCarley RW, Winkelman JW, Duffy FH (1983) Human cerebral potentials associated with REM sleep rapid eye movements: Links to PGO waves and waking potentials. Brain Res 274:359–364.

McCoy P, Norton TT, McMahon LL (2008) Layer 2/3 synapses in monocular and binocular regions of tree shrew visual cortex express mAChR-dependent long-term depression and long-term potentiation. J Neurophysiol 100:336–345.

McCoy PA, McMahon LL (2007) Muscarinic receptor–dependent long-term depression in rat visual cortex is PKC independent but requires ERK1/2 activation and protein synthesis. J Neurophysiol 98:1862–1870.

McCurry CL, Shepherd JD, Tropea D, Wang KH, Bear MF, Sur M (2010) Loss of Arc renders the visual cortex impervious to the effects of sensory experience or deprivation. Nat Neurosci 13:450–457.

McDermott CM, Lahoste GJ, Chen C, Musto A, Bazan NG, Magee JC (2003) Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci 23:9687–9695.

Mednick S, Nakayama K, Stickgold R (2003) Sleep-dependent learning: A nap is as good as a night. Nat Neurosci 6:697–698.

Mednick SC, Nakayama K, Cantero JL, Atienza M, Levin A a, Pathak N, Stickgold R (2002) The restorative effect of naps on perceptual deterioration. Nat Neurosci 5:677–681.

Meienberg P (1977) The tonic aspects of human REM sleep during long-term intensive verbal learning. Physiol Psychol 5:250–256.

Miller L, Drew WG, Schwartz I (1971) Effect of REM sleep deprivation on retention of a one-trial passive avoidance response. Percept Motor Skill 33:118.

Morden B, Mitchell G, Dement W (1967) Selective REM sleep deprivation and compensation phenomena in the rat. Brain Res 5:339–349.

Morishita W, Connor JH, Xia H, Quinlan EM, Shenolikar S, Malenka RC (2001) Regulation of synaptic strength by protein phosphatase 1. Neuron 32:1133–1148.

Morris R, Garrud P, Rawlins J, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681–683.

Mouret J, Jeannerod M, Jouvet M (1963) L'activite electrique du systeme visuel au cours de la phase paradoxale du sommeil chez le chat. J Physiol (Paris) 55:305–306.

Mower AF, Liao DS, Nestler EJ, Neve RL, Ramoa AS (2002) cAMP/Ca 2+ response element-binding protein function is essential for ocular dominance plasticity. J Neurosci 22:2237–2245.

Mulkey R, Endo S, Shenolikar S, Malenka R (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369:486–488.

Mulkey RM, Herron CE, Malenka RC (1993) An essential role for protein phosphatases in hippocampal long-term depression. Science 261:1051–1055.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. Learn Mem 9:49–57.

Nguyen ND, Tucker MA, Stickgold R, Wamsley EJ (2013) Overnight sleep enhances hippocampus-dependent aspects of spatial memory. Sleep 36:1051–1057.

Nishida M, Pearsall J, Buckner RL, Walker MP (2009) REM sleep, prefrontal theta, and the consolidation of human emotional memory. Cereb Cortex 19:1158–1166.

Nádasdy Z, Hirase H, Czurkó A, Csicsvari J, Buzsáki G (1999) Replay and time compression of recurring spike sequences in the hippocampus. J Neurosci 19:9497–9507.

Oh MC, Derkach VA, Guire ES, Soderling TR (2006) Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. J Biol Chem 281:752–758.

Oksenberg A, Shaffery JP, Marks GA, Speciale SG, Mihailoff G, Roffwarg HP (1996) Rapid eye movement sleep deprivation in kittens amplifies LGN cell-size disparity induced by monocular deprivation. Dev Brain Res 97:51–61.

Palchykova S, Crestani F, Meerlo P, Tobler I (2006a) Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters. Physiol Behav 87:144–153.

Palchykova S, Winsky-Sommerer R, Meerlo P, Dürr R, Tobler I (2006b) Sleep deprivation impairs object recognition in mice. Neurobiol Learn Mem 85:263–271.

Panja D, Dagyte G, Bidinosti M, Wibrand K, Kristiansen A-M, Sonenberg N, Bramham CR (2009) Novel translational control in Arc-dependent long term potentiation consolidation *in vivo*. J Biol Chem 284:31498–31511.

Park S, Park JM, Kim S, Kim J-A, Shepherd JD, Smith-Hicks CL, Chowdhury S, Kaufmann W, Kuhl D, Ryazanov AG, Huganir RL, Linden DJ, Worley PF (2008) Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. Neuron 59:70–83.

Patti CL, Zanin KA, Sanday L, Kameda SR, Fernandes-Santos L, Fernandes HA, Andersen ML, Tufik S, Frussa-Filho R (2010) Effects of sleep deprivation on memory in mice: Role of state-dependent learning. Sleep 33:1669–1679.

Payne JD, Tucker MA, Ellenbogen JM, Wamsley EJ, Walker MP, Schacter DL, Stickgold R (2012) Memory for semantically related and unrelated declarative information: the benefit of sleep, the cost of wake. PloS ONE 7:e33079.

Pearlman C (1969) Effect of rapid eye movement (dreaming) sleep deprivation on retention of avoidance learning in rats. Rep No 563. Rep US Nav Submar Cent:1–4.

Peigneux P, Laureys S, Fuchs S, Delbeuck X, Degueldre C, Aerts J, Delfiore G, Luxen A, Maquet P (2001) Generation of rapid eye movements during paradoxical sleep in humans. NeuroImage 14:701–708.

Pham TA, Impey S, Storm DR, Stryker MP (1999) CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. Neuron 22:63–72.

Philpot BD, Cho KKA, Bear MF (2007) Obligatory role of NR2A for metaplasticity in visual cortex. Neuron 53:495–502.

Philpot BD, Espinosa JS, Bear MF (2003) Evidence for altered NMDA receptor function as a basis for metaplasticity in visual cortex. J Neurosci 23:5583–5588.

Plihal W, Born J (1997) Effects of early and late nocturnal sleep on declarative and procedural memory. J Cogn Neurosci 9:534–547.

Plihal W, Born J (1999) Effects of early and late nocturnal sleep on priming and spatial memory. Psychophysiology 36:571–582.

Putignano E, Lonetti G, Cancedda L, Ratto G, Costa M, Maffei L, Pizzorusso T (2007) Developmental downregulation of histone posttranslational modifications regulates visual cortical plasticity. Neuron 53:747–759.

Racz B, Weinberg RJ (2006) Spatial organization of cofilin in dendritic spines. Neuroscience 138:447–456.

Rasch B, Born J (2007) Maintaining memories by reactivation. Curr Opin Neurobiol 17:698–703.

Rasch B, Gais S, Born J (2009) Impaired off-line consolidation of motor memories after combined blockade of cholinergic receptors during REM sleep-rich sleep. Neuropsychopharmacol 34:1843–1853.

Ravassard P, Pachoud B, Comte J-C, Mejia-Perez C, Scoté-Blachon C, Gay N, Claustrat B, Touret M, Luppi P-H, Salin PA (2009) Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. Sleep 32:227–240.

Redpath NT, Proud CG (1993) Cyclic AMP-dependent protein kinase phosphorylates rabbit reticulocyte elongation factor-2 kinase and induces calcium-independent activity. Biochem J 293:31–34.

Ribeiro S, Goyal V, Mello CV, Pavlides C (1999) Brain gene expression during REM sleep depends on prior waking experience. Learn Mem 6:500–508.

Ribeiro S, Mello C, Velho T, Gardner TJ, Jarvis ED, Pavlides C (2002) Induction of hippocampal long-term potentiation during waking leads to increased extrahippocampal zif-268 expression during ensuing rapid-eye-movement sleep. J Neurosci 22:10914–10923.

Ribeiro S, Shi X, Engelhard M, Zhou Y, Zhang H, Gervasoni D, Lin S-C, Wada K, Lemos NAM, Nicolelis MAL (2007) Novel experience induces persistent sleep-dependent plasticity in the cortex but not in the hippocampus. Frontiers in Neuroscience 1:43–56.

Rideout BE (1979) Non-REM sleep as a source of learning deficits induced by REM sleep deprivation. Physiol Behav 22:1043–1047.

Rittenhouse CD, Siegler BA, Voelker CA, Shouval HZ, Paradiso MA, Bear MF (2006) Stimulus for rapid ocular dominance plasticity in visual cortex. J Neurophysiol 95:2947–2950.

Roberson ED, English JD, Adams JP, Selcher JC, Kondratick C, Sweatt JD (1999) The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J Neurosci 19:4337–4348.

Roffwarg HP, Muzio JN, Dement WC (1966) Ontogenetic development of the human sleep-dream cycle. Science 152:604–619.

Rolls A, Colas D, Adamantidis A, Carter M, Lanre-Amos T, Heller HC, de Lecea L (2011) Optogenetic disruption of sleep continuity impairs memory consolidation. Proc Natl Acad Sci USA 108:13305–13310.

Romcy-Pereira R, Pavlides C (2004) Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP. Eur J Neurosci 20:3453–3462.

Roth T, Coulouvrat C, Hajak G, Lakoma MD, Sampson NA, Shahly V, Shillington AC, Stephenson JJ, Walsh JK, Kessler RC (2011) Prevalence and perceived health associated with insomnia based on DSM-IV-TR; International Statistical Classification of Diseases and Related Health Problems, Tenth Revision; and Research Diagnostic Criteria/International Classification of Sleep Disorders. Biol Psychiatry 69:592–600.

Schafe GE, Atkins CM, Swank MW, Bauer EP, Sweatt JD, LeDoux JE (2000) Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of Pavlovian fear conditioning. J Neurosci 20:8177–8187.

Scheetz AJ, Nairn AC, Constantine-Paton M (2000) NMDA receptor-mediated control of protein synthesis at developing synapses. Nat Neurosci 3:211–216.

Scheper GC, Proud CG (2002) Does phosphorylation of the cap-binding protein eIF4E play a role in translation initiation? Eur J Biochem 269:5350–5359.

Scoville W, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiat 20:11–21.

Seibt J, Aton SJ, Jha SK, Coleman T, Dumoulin MC, Frank MG (2008) The non-benzodiazepine hypnotic zolpidem impairs sleep-dependent cortical plasticity. Sleep 31:1381–1391.

Seibt J, Dumoulin MC, Aton SJ, Coleman T, Watson A, Naidoo N, Frank MG (2012) Protein synthesis during sleep consolidates cortical plasticity *in vivo*. Curr Biol 22:676–682.

Seol G, Ziburkus J, Huang S, Song L, Kim T, Takamiya K, Huganir RL, Lee H-K, Kirkwood A (2007) Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. Neuron 55:919–929.

Shaffery J, Lopez J, Roffwarg H (2012) Brain-derived neurotrophic factor (BDNF) reverses the effects of rapid eye movement sleep deprivation (REMSD) on developmentally regulated, long-term potentiation. Neurosci Lett 513:84–88.

Shaffery J, Oksenberg A, Marks G, Speciale S, Mihailoff G, Roffwarg H (1998) REM sleep deprivation in monocularly occluded kittens reduces the size of cells in LGN monocular segment. Sleep 21:837–845.

Shaffery JP, Lopez J, Bissette G, Roffwarg HP (2006a) Rapid eye movement sleep deprivation revives a form of developmentally regulated synaptic plasticity in the visual cortex of post-critical period rats. Neurosci Lett 391:96–101.

Shaffery JP, Lopez J, Bissette G, Roffwarg HP (2006b) Rapid eye movement sleep deprivation in post-critical period, adolescent rats alters the balance between inhibitory and excitatory mechanisms in visual cortex. Neurosci Lett 393:131–135.

Shaffery JP, Roffwarg HP (2003) Rapid eye-movement sleep deprivation does not "rescue" developmentally regulated long-term potentiation in visual cortex of mature rats. Neurosci Lett 342:196–200.

Shaffery JP, Roffwarg HP, Speciale SG, Marks GA (1999) Ponto-geniculo-occipital-wave suppression amplifies lateral geniculate nucleus cell-size changes in monocularly deprived kittens. Dev Brain Res 114:109–119.

Shaffery JP, Sinton CM, Bissette G, Roffwarg HP, A. MG (2002) Rapid eye movement sleep deprivation modifies expression of long-term potentiation in visual cortex of immature rats. Neuroscience 110:431–443.

Shakespeare W (1998) Macbeth (Barnet S, ed). New York: Signet.

Shirokawa T, Kasamatsu T (1986) Concentration-dependent suppression by β-adrenergic antagonists of the shift in ocular dominance following monocular deprivation in kitten visual cortex. Neuroscience 18:1035–1046.

Shiromani P, Gutwein BM, Fishbein W (1979) Development of learning and memory in mice after brief paradoxical sleep deprivation. Physiol Behav 22:971–978.

Siegel JM (2008) Do all animals sleep? Trends Neurosci 31:208–213.

Silva A, Stevens C, Tonegawa S, Wang Y (1992) Deficient hippocampal long-term potentiation in α-calcium-calmodulin kinase II mutant mice. Science 257:201–206.

Smith C (1995) Sleep states and memory processes. Behav Brain Res 69:137–145.

Smith C, Butler S (1982) Paradoxical sleep at selective times following training is necessary for learning. Physiol Behav 29:469–473.

Smith C, Rose G (1996) Evidence for a paradoxical sleep window for place learning in the Morris water maze. Physiol Behav 59:93–97.

Smith CT, Nixon MR, Nader RS (2004) Posttraining increases in REM sleep intensity implicate REM sleep in memory processing and provide a biological marker of learning potential. Learn Mem 11:714–719.

Squire L, Zola S (1996) Structure and function of declarative and nondeclarative memory systems. Proc Natl Acad Sci USA 93:13515–13522.

Stanton P, Sarvey J (1984) Blockade of long-term potentiation in rat hippocampal CA1 region by inhibitors of protein synthesis. J Neurosci 4:3080–3088.

Steriade M, Nunez A, Amzica F (1993) A novel slow (< 1 Hz) oscillation of neocortical neurons *in vivo*: Depolarizing and hyperpolarizing components. J Neurosci 13:3252–3265.

Steriade M, Timofeev I, Grenier F (2001) Natural waking and sleep states: A view from inside neocortical neurons. J Neurophysiol 85:1969–1985.

Stickgold R, James L, Hobson JA (2000a) Visual discrimination learning requires sleep after training. Nat Neurosci 3:1237–1238.

Stickgold R, Whidbee D, Schirmer B, Patel V, Hobson JA (2000b) Visual discrimination task improvement: A multi-step process occurring during sleep. J Cogn Neurosci 12:246–254.

Suzuki H, Uchiyama M, Aritake S, Kuriyama K, Kuga R, Enomoto M, Mishima K (2012) Alpha activity during REM sleep contributes to overnight improvement in performance on a visual discrimination task. Percept Motor Skill 115:337–348.

Taha S, Hanover J, Silva A, Stryker M (2002) Autophosphorylation of αCaMKII is required for ocular dominance plasticity. Neuron 36:483–491.

Taha S, Stryker MP (2002) Rapid ocular dominance plasticity requires cortical but not geniculate protein synthesis. Neuron 34:425–436.

Takamura H, Ichisaka S, Hayashi C, Maki H, Hata Y (2007) Monocular deprivation enhances the nuclear signalling of extracellular signal-regulated kinase in the developing visual cortex. Eur J Neurosci 26:2884–2898.

Talamini LM, Nieuwenhuis ILC, Takashima A, Jensen O (2008) Sleep directly following learning benefits consolidation of spatial associative memory. Learn Mem 15:233–237.

Tartar JL, McKenna JT, Ward CP, McCarley RW, Strecker RE, Brown RE (2010) Sleep fragmentation reduces hippocampal CA1 pyramidal cell excitability and response to adenosine. Neurosci Lett 469:1–5.

Tartar JL, Ward CP, McKenna JT, Thakkar M, Arrigoni E, McCarley RW, Brown RE, Strecker RE (2006) Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. Eur J Neurosci 23:2739–2748.

Thiels E, Kanterewicz BI, Norman ED, Trzaskos JM, Klann E (2002) Long-term depression in the adult hippocampus *in vivo* involves activation of extracellular signal-regulated kinase and phosphorylation of Elk-1. J Neurosci 22:2054–2062.

Tieman SB (1984) Effects of monocular deprivation on geniculocortical synapses in the cat. J Comp Neurol 222:166–176.

Tilley A, Empson J (1978) REM sleep and memory consolidation. Biol Psychol 6:293–300.

Trachtenberg J, Trepel C, Stryker M (2000) Rapid extragranular plasticity in the absence of thalamocortical plasticity in the developing primary visual cortex. Science 287:2029–2032.

Tsokas P, Ma T, Iyengar R, Landau EM, Blitzer RD (2007) Mitogen-activated protein kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. J Neurosci 27:5885–5894.

Tucker M, McKinley S, Stickgold R (2011a) Sleep optimizes motor skill in older adults. J Am Geriatr Soc 59:603–609.

Tucker MA, Hirota Y, Wamsley EJ, Lau H, Chaklader A, Fishbein W (2006) A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. Neurobiol Learn Mem 86:241–247.

Tucker MA, Tang SX, Uzoh A, Morgan A, Stickgold R (2011b) To sleep, to strive, or both: How best to optimize memory. PloS ONE 6:e21737.

Uebele VN, Nuss CE, Santarelli VP, Garson SL, Barrow JC, Stauffer SR, Koblan KS, Renger JJ, Aton S, Seibt J, Dumoulin M, Jha SK, Coleman T, Frank MG (2009) T-type calcium channels regulate cortical plasticity in-vivo. NeuroReport:257–262.

Ueda T, Watanabe-Fukunaga R, Fukuyama H, Nagata S, Fukunaga R (2004) Mnk2 and Mnk1 are essential for constitutive and inducible phosphorylation of eukaryotic initiation factor 4E but not for cell growth or development. Mol Cell Biol 24:6539–6549.

Van Hulzen ZJ, Coenen AM (1979) Selective deprivation of paradoxical sleep and consolidation of shuttle-box avoidance. Physiol Behav 23:821–826.

Vecsey CG, Baillie GS, Jaganath D, Havekes R, Daniels A, Wimmer M, Huang T, Brown KM, Li X-Y, Descalzi G, Kim SS, Chen T, Shang Y-Z, Zhuo M, Houslay MD, Abel T (2009) Sleep deprivation impairs cAMP signalling in the hippocampus. Nature 461:1122–1125.

Vimont-Vicary P, Jouvet-Mounier D, Delorme F (1966) Effets EEG et comportementaux des privations de sommeil paradoxal chez le chat. Electroencephalogr Clin Neurophysiol 20:439–449.

Vogel G, Thurmond A, Gibbons P, Sloan K, Boyd M, Walker M (1975) REM sleep reduction effects on depression syndromes. Arch Gen Psychiatry 32:765–777.

Vorobyov V, Kwok JCF, Fawcett JW, Sengpiel F (2013) Effects of digesting chondroitin sulfate proteoglycans on plasticity in cat primary visual cortex. J Neurosci 33:234–243.

Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R (2002) Practice with sleep makes perfect: Sleep-dependent motor skill learning. Neuron 35:205–211.

Walker MP, Brakefield T, Seidman J, Morgan A, Hobson JA, Stickgold R (2003) Sleep and the time course of motor skill learning. Learn Mem 10:275–284.

Wamsley EJ, Tucker MA, Payne JD, Stickgold R (2010) A brief nap is beneficial for human route-learning: The role of navigation experience and EEG spectral power. Learn Mem 17:332–336.

Wang Y, Gu Q, Cynader MS (1997) Blockade of serotonin-2C receptors by mesulergine reduces ocular dominance plasticity in kitten visual cortex. Exp Brain Res 114:321–328.

Waskiewicz AJ, Flynn A, Proud CG, Cooper JA (1997) Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. EMBO J 16:1909–1920.

Wiesel TN, Hubel DH (1963a) Single-cell responses in striate cortex of kittens deprived of vision in one eye. J Neurophysiol 26:978–993.

Wiesel TN, Hubel DH (1963b) Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. J Neurophysiol 26:978–993.

Wilson MA, McNaughton BL (1994) Reactivation of hippocampal ensemble memories during sleep. Science 265:676–679.

Wong ST, Athos J, Figueroa XA, Pineda VV, Schaefer ML, Chavkin CC, Muglia LJ, Storm DR (1999) Calcium-stimulated adenylyl cyclase activity is critical for hippocampus-dependent long-term memory and late phase LTP. Neuron 23:787–798.

Yang R-H, Hu S-J, Wang Y, Zhang W-B, Luo W-J, Chen J-Y (2008) Paradoxical sleep deprivation impairs spatial learning and affects membrane excitability and mitochondrial protein in the hippocampus. Brain Res 1230:224–232.

Yazaki-Sugiyama Y, Kang S, Câteau H, Fukai T, Hensch TK (2009) Bidirectional plasticity in fast-spiking GABA circuits by visual experience. Nature 462:218–221.

Yoon B-J, Smith GB, Heynen AJ, Neve RL, Bear MF (2009) Essential role for a long-term depression mechanism in ocular dominance plasticity. Proc Natl Acad Sci USA 106:9860–9865.

Youngblood BD, Smagin GN, Elkins PD, Ryan DH, Harris RBS (1999) The effects of paradoxical sleep deprivation and valine on spatial learning and brain 5-HT metabolism. Physiol Behav 67:643–649.

Youngblood BD, Zhou J, Smagin GN, Ryan DH, Harris RB (1997) Sleep deprivation by the “flower pot” technique and spatial reference memory. Physiol Behav 61:249–256.

Zar JH (1999) Biostatistical analysis, 4th ed. Upper Saddle River, NJ: Prentice Hall.

Zhuo M, Hu Y, Schultz C, Kandel E, Hawkins R (1994) Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term potentiation. Nature 368:635–639.