STUDYING THE SPATIOTEMPORAL DYNAMICS OF CORTICAL ACTIVITY DURING REM-LIKE STATE IN URETHANE ANESTHETIZED MICE

MOJTABA NAZARIAHANGARKOLAEE
Bachelor of Science, Amirkabir University of Technology, 2011

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the Degree

MASTER OF SCIENCE

Department of Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

STUDYING THE SPATIOTEMPORAL DYNAMICS OF CORTICAL ACTIVITY DURING REM-LIKE STATE IN URETHANE ANESTHETIZED MICE

MOJTABA NAZARIAHANGARKOLAEE

Date of Defence: September 18, 2017

Dr. M. Mohajerani Supervisor	Assistant Professor	Ph.D.
Dr. M. Tatsuno Co-supervisor	Associate Professor	Ph.D.
Dr. D. Euston Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. A. Luczak Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. R. Gibb Chair, Thesis Examination Committee	Associate Professor	Ph.D.

Abstract

Sleep in mammals consists of two basic stages: non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM sleep can be distinguished by slow and high-amplitude cortical EEG signals while REM sleep is characterized by "desynchronized" low-amplitude fast cortical rhythms. While until recently it has been widely believed that cortical activity during REM sleep is globally desynchronized, using sparse electrophysiological recording a recent study showed local slow waves in primary cortical areas during REM sleep (Funk et al, 2016). However, the electrophysiological technique has been unable to resolve the regional structure and dynamics of these activities due to relatively sparse sampling. Here, we investigated local changes in neuronal activity during REM-like state using mesoscale imaging in the urethane model of sleep. The wide-field VSD imaging from neocortex was combined with LFP recording from hippocampus and motor/somatosensory cortex with a unilateral preparation in anesthetized mice. Generally, urethane anesthesia induced state alternation in VSD signal which was concurrent with LFP state changes. However, in REM-like state slow cortical fluctuations were not globally reduced; while midline and posterior areas typically showed desynchronized patterns, anterior and lateral regions showed synchronized activity. Our results also revealed that synchronized/ desynchronized regions during individual REM-like episodes can be dynamic.

Acknowledgment

I would like to thank my supervisor, Dr. Majid Mohajerani and my co-supervisor, Dr. Masami Tatsuno for their support and guidance during my graduate work. I would also like to thank the members of my supervisory committee, Dr. David Euston and Dr. Artur Luczak for their suggestions and support. I also want to thank members of the Mohajerani Lab, past and present. Without their help and inputs, I could have not completed my Master's degree.

Table of Contents

List of Figures

List of Abbreviations

REM	Rapid Eye Movement
NREM	Non-Rapid Eye Movement
EEG	Electroencephalogram
SWS	Slow Wave Sleep
SWA	Slow Wave Activity
SO	Slow Oscillation
IPSP	Inhibitory Postsynaptic Potential
EPSP	Excitatory Postsynaptic Potential
SWP-R	Sharp Wave-Ripple
CA	Cornu Ammonis
MS	Medial Septum
vDB	vertical limb of the Diagonal Band
PGO	Ponto-Geniculo-Occipital
LGN	Lateral Geniculate Nucleus
PB	Parabrachial Nucleus
5-HT	5-Hydroxytryptamine
IEG	Immediate Early Gene
POA	Preoptic Area
VLPO	Ventrolateral Preoptic Area
MnPO	Median Preoptic Nucleus
LC	Locus Coeruleus
TMN	Tuberomammillary Nucleus
MRN	Median Raphe Nucleus
DRN	Dorsal Raphe Nucleus
VPAG	Ventral Periaqueductal Grey matter
BF	Basal Forebrain
ACh	Acetylcholine
PV	Parvalbumin
SOM	Somatostatin
HA	Histamine

MCH	Melanin-concentrating Hormone
PPT	Pedunculopontine Tegmentum
LDT	Laterodorsal Tegmentum
SLD	Sublateral Dorsal Nucleus
vlPAG	Ventrolateral Periaqueductal Grey
SCP	Superior Cerebellar Peduncle
vM	Ventral Medulla
PZ	Parafacial Zone
NST	Nucleus of the Solitary Tract
VSDI	Voltage-sensitive Dye Imaging
ns	Not Significant
au	Arbitrary Unit
PDF	Probability Distribution Function
VSFP	Voltage-sensitive Fluorescent Protein

1. General Introduction

1.1 Sleep as a behavioral state

Sleep is a naturally recurring and reversible state of the body and mind, characterized by loss of consciousness, relatively reduced responsiveness to sensory stimuli and interaction with surroundings. Sleep is regulated so as to maintain homeostasis (Borbely & Achermann, 1999), i.e. deprivation from the regular hours of sleep creates a sleep debt which should be paid in a prolonged subsequent sleep. Sleep deprivation and disruption can have severe effects on attention, memory, decision making, emotional stability, and executive functions (L. K. Brown, 2012; Killgore, 2010). Rats continuously sleep deprived for several weeks showed deficits in body metabolism and thermoregulation, and consequently die from hyperthermia, catabolism, or systemic infection (Rechtschaffen & Bergmann, 1995).

Despite more than a century of research, the exact functions of sleep are still debatable. Sleep is usually regarded as a vulnerable state due to the loss of consciousness and the reduction of sensory awareness to potential dangers, which threaten survival. However, others suggest sleep is an adaptive state which helps the survival of animals by improving the efficiency of their activities (Siegel, 2009). Sleep does this by increasing animal activity when resources like food and pray are available or vital functions like mating need to be performed, and by minimizing the activity when survival dangers like predators are present or resources are scarce. During inactivity, sleep saves energy by reducing brain metabolism; but, the most energy is saved through reducing body metabolism and muscle tone, analogous to turning down the thermostat when you leave a room (Berger & Phillips, 1995).

Sleep has been proposed to restore the resource depletion and cell damage which occured during wakefulness. This includes depletion of energy compartments, downregulation of neuromodulatory receptors, cell death in cortical and subcortical regions, and oxidation stress produced by free radicals (Siegel, 2009). Sleep is also hypothesized to cool the brain and body down; providing several adaptations in the metabolism and timing of behaviour (McGinty & Szymusiak, 1990). One theory suggests sleep plays an important role in the maturation of neural circuits during development (Roffwarg, Muzio, & Dement, 1966). This idea stems from the observation that sleep is the predominant behavioral state in infant animals. Another theory holds that sleep is required, or important for, the consolidation of freshly acquired information in memory (Diekelmann & Born, 2010). Sleep is suggested to stabilize and enhance new memory representations and integrate them into the network of long-term memories. We will discuss this function of sleep more in section 1.3.

1.2 Sleep Stages

Sleep in mammals is divided into two basic stages: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep, each has specific physiological and biological characteristics. Below we describe these two stages and their associated electrical oscillations.

1.2.1 NREM sleep

NREM sleep can be distinguished from the waking and REM sleep by the occurrence of slow and high-amplitude activity in the cortical EEG signal and significant reduction of muscle tone. It is the predominant state in the beginning of human nocturnal sleep and its intensity and duration decrease over the course of sleep. NREM sleep in

humans is further divided into three sub-stages named N1, N2, and N3 in which sleep depth increases respectively. N3 is also called slow wave sleep (SWS) as slow wave activity (SWA) peaks during this stage. In rodents, NREM sleep is not usually divided into these sub-stages. During NREM sleep, physiological markers including heart rate, respiration rate, blood pressure, and metabolic levels all decrease compared to wakefulness and heart and breathing rates become regular (Shore, Millman, Silage, Chung, & Pack, 1985; Siegel, 2009; Zemaityte, Varoneckas, & Sokolov, 1984). This state is usually associated with three specific electrical oscillations:

Slow Oscillation

Slow oscillation (SO) refers to the <1-Hz activity which dominates the cortical EEG during slow wave sleep. In humans, SO peak frequency is around 0.8 Hz (Molle, Marshall, Gais, & Born, 2002). Slow oscillation comprises two phases of up and down state in which cortical neural populations show different activity patterns. During up state, cortical neurons depolarize accompanied by their sustained firing while in down state, they hyperpolarize and stop firing (Steriade, McCormick, & Sejnowski, 1993; Steriade, Timofeev, & Grenier, 2001). Mechanisms of SO generation are not fully understood. Up states are believed to be the result of a balance between excitation and inhibition (Haider, Duque, Hasenstaub, & McCormick, 2006; Shu, Hasenstaub, & McCormick, 2003). Down states, on the other hand, are suggested to be the result of the withdrawal of excitatory synaptic currents (disfacilitation) from the cortical and thalamic cells instead of active inhibition (Contreras, Timofeev, & Steriade, 1996; McVea, Murphy, & Mohajerani, 2016; Steriade et al., 2001).

Spindles

Spindles are waxing and waning oscillatory activity observed in cortical EEG which has the frequency range of ~10 to 15 Hz. In humans, they happen more frequently in the stage N2 of NREM sleep usually following a K-complex and last between 0.5 and 3 sec (De Gennaro & Ferrara, 2003). They are also present in SWS where they are superimposed on the slow oscillation to form less discrete spindles. Spindle activity is the result of interactions between the GABAergic neurons of thalamus reticular nucleus and thalamocortical glutamatergic neurons. During early NREM sleep, GABAergic neurons of reticular nucleus start to generate a burst of action potentials at spindle frequency due to activation of T-type (low-threshold) calcium channels (R. E. Brown, Basheer, McKenna, Strecker, & McCarley, 2012; Talley et al., 1999). This induces large IPSPs in the thalamocortical glutamatergic neurons which remove the inactivation gate of T-type calcium channels existing in these cells. Thus, at the end of these IPSPs, calcium enters the thalamocortical cells and causes a spike which triggers a short (5-15 ms) sodium-based burst of action potentials. This leads to a burst of EPSPs in the postsynaptic cortical neurons making them fire action potentials with spindle frequency which induces spindle activity in the EEG signal.

Sharp Wave-Ripple

Sharp waves are fast and large amplitude depolarizing events (40-100 msec) which can be recorded from hippocampus during NREM sleep of humans and rodents. They are usually, but not always, superimposed by faster short-lasting oscillatory activity (100–300 Hz), called ripple to form sharp wave-ripple (SPW-R) events (Bragin, Engel, Wilson, Fried, & Buzsaki, 1999; Buzsaki, 1986; Buzsaki, Leung, & Vanderwolf, 1983; O'Keefe, 1976). Sharp waves originate from CA3 of the hippocampus when subcortical

regions like MS/vDB release their suppressing control on the network of interconnected CA3 pyramidal cells (R. E. Brown et al., 2012). In fact, highly recurrent excitatory collateral network of CA3 pyramidal neurons is an optimum substrate for the synchronized population bursts occurring during sharp wave events (Buzsaki, 2015; Traub & Wong, 1982). Ripples, on the other hand, originate from CA1 and are induced by the strong excitation coming from the CA3-generated sharp waves while the interaction between PV-expressing interneurons and pyramidal cells helps set the ripple timing and support their spatial synchrony (Buzsaki, 2015).

1.2.2 REM sleep

REM sleep is characterized by fast and low amplitude cortical EEG, rapid and random movement of the eyes, and the loss of muscle tone (i.e. atonia). REM sleep is also associated with vivid dreaming in which sleepers can often provide a narrative description of what they were experiencing (Hobson, Pace-Schott, & Stickgold, 2000). This state is also known as paradoxical sleep because some of its physiological characteristics including cortical EEG resemble those of waking state. In fact, the wake-like low-amplitude fast cortical rhythms observed in this state is the result of “desynchronized” tonic firing of cortical neurons (Harris & Thiele, 2011). In this thesis, we use the term “desynchronized” to refer to the fast low-amplitude cortical activity which is one of the features of REM state. By contrast, we use “synchronized” to refer to slow and high-amplitude cortical activity.

REM sleep is less frequent in the early part of nocturnal sleep and its intensity and duration increase toward the end of the sleep period (Rasch & Born, 2013). In REM sleep, physiological markers, including heart rate, respiration rate, blood pressure, and

metabolic levels increase compared to NREM sleep to near waking levels (Brebbia & Altshuler, 1965; Madsen et al., 1991; Snyder, Hobson, Morrison, & Goldfrank, 1964; Zemaityte et al., 1984).

REM sleep can be subclassified into tonic and phasic events. Phasic REM is characterized by rapid eye movements and muscle twitches and constitutes about 5% of the entire REM sleep while tonic REM is characterized by steady muscle atonia and the absence of rapid eye movement (De Carli et al., 2016; Montgomery, Sirota, & Buzsaki, 2008). They can also be distinguished based on other electrophysiological activities which we will discuss about below.

Theta activity

Theta activity is referred to a very rhythmic field potential oscillation which is the hallmark of REM sleep in rodents and other lower mammals (Robinson, Kramis, & Vanderwolf, 1977). Its frequency varies from 4-8 Hz in tonic REM to 8-12 Hz in phasic REM events and its amplitude increases from tonic to phasic REM (R. E. Brown et al., 2012; Karashima, Nakao, Katayama, & Honda, 2005; Montgomery et al., 2008). In rodents, theta activity is prominent in the hippocampus where it can also be seen during active wakefulness. In addition to the hippocampus, theta field potential oscillations and entrainment of neuronal population by theta rhythm have been observed in other brain areas, including entorhinal and perirhinal cortex, subicular complex, cingulate cortex, and amygdala (Alonso & Garcia-Austt, 1987; Leung & Borst, 1987; Mitchell & Ranck, 1980; Pare & Collins, 2000). In humans, theta activity is less readily identified and usually limits to short epochs (Cantero et al., 2003).

In the classical theta model, medial septum-vertical limb of the diagonal band (MS/vDB) has been suggested to be the rhythm generator (pacemaker) of theta (Buzsaki, 2002). Removal of the MS/vDB inputs to hippocampus by pharmacological inactivation, transection or lesion completely abolishes theta waves (Andersen, Bland, Myhrer, & Schwartzkroin, 1979; Bland, Trepel, Oddie, & Kirk, 1996; Green & Arduini, 1954; Sainsbury & Bland, 1981). Two groups of cholinergic and GABAergic cells in the MS/vDB are postulated to regulate the hippocampal theta activity (Tsanov, 2015). Cholinergic neurons are slow firing and modulate the theta amplitude, but not the frequency, via the tonic excitation of MS/vDB GABAergic neurons as well as hippocampal interneurons and pyramidal cells (Frotscher & Leranth, 1985; Hangya, Borhegyi, Szilagyi, Freund, & Varga, 2009). GABAergic neurons of MS/vDB, on the other hand, fire bursts of action potentials which are phase-locked to theta activity (Borhegyi, Varga, Szilagyi, Fabo, & Freund, 2004). They selectively innervate hippocampal inhibitory interneurons and act as the theta pacemaker through periodic disinhibition of hippocampal pyramidal neurons (Buzsaki, 2002; Chamberland, Salesse, Topolnik, & Topolnik, 2010; Tsanov, 2015). In addition, in this classical model, rhythmic excitations from the entorhinal cortex, in which neurons also fire synchronized to theta, play an important role in the theta current generation (Buzsaki, 2002). Moreover, other structures, including CA3 recurrent network and dentate granular cells, may also contribute to the spatiotemporal profile of theta field potential in the hippocampus (Buzsaki, 2002).

PGO waves

Ponto-geniculo-occipital (PGO) waves are synchronized sharp field potentials that are typically identified as propagating activity from pons to lateral geniculate nucleus (LGN) and occipital cortex. They usually occur as high-amplitude single waves before the onset of REM sleep and as wave bursts of lower amplitude during phasic REM sleep, where they are time-locked to rapid eye movements (Brooks & Bizzi, 1963; Datta & Hobson, 1994; Jouvet, Jeannerod, & Delorme, 1965). Because of this co-occurrence, they are hypothesized as the source of dreaming and visual imagery in REM sleep (M. Steriade & R. W. McCarley, 2005).

PGO waves were initially and most extensively studied in cats, where the largest activities can be recorded from the LGN and visual cortex (R. E. Brown et al., 2012). However, recent studies in both humans and animals showed that PGO waves propagate far beyond the three key regions and can engage limbic, parahippocampal, and many thalamocortical systems (Amzica & Steriade, 1996; Datta, 1997; Wehrle et al., 2007). PGO waves in rodents are referred to as pontine waves (P waves) because only their pontine component has been recorded thus far (R. E. Brown et al., 2012). Their thalamic and cortical components proved to be hard to record, likely because rodents are not very visual animals. In humans (epilepsy and Parkinson's disease patients), phasic electrical activities were recorded in the pons, subthalamic nucleus, and medial temporal lobe during REM sleep (Andrillon, Nir, Cirelli, Tononi, & Fried, 2015; Fernandez-Mendoza et al., 2009; Lim et al., 2007). In addition, fMRI studies showed activation of the thalamus and occipital cortex together with the hippocampus and amygdala time-locked to REMs, which can be closely related to PGO potentials (Miyauchi, Misaki, Kan, Fukunaga, & Koike, 2009; Wehrle et al., 2007).

The neurons that play a role in generation, propagation, and regulation of PGO waves can be divided into two groups: executive neurons and modulatory neurons. Executive neurons are involved in generating and propagating the PGO waves across brain regions while modulatory neurons respond to fluctuations in neuromodulators and regulate how PGO waves are spread and maintained (Datta, 1997; Gott, Liley, & Hobson, 2017). Executive neurons are primarily located in the peribrachial area (PB) of the dorsolateral pons and receive inhibitory serotonergic (5-HT) inputs from raphe nucleus (Datta, 1997; Gott et al., 2017). These inputs justify why agents that deplete synaptic 5-HT such as reserpine and fenclonine enhance PGO waves (Brooks, Gershon, & Simon, 1972; Jacobs, Henriksen, & Dement, 1972). Modulatory neurons, on the other hand, are spread across multiple brain regions, including the prefrontal cortex, suprachiasmatic nucleus, amygdala, vestibular and brainstem auditory systems and consist of multiple cell types, including aminergic, cholinergic, nitroxergic, GABAergic, and glycinergic (Datta, 1997; Gott et al., 2017).

1.3 Memory function of sleep

While memory encoding and retention mainly take place during awake state, sleep is a brain state that favors consolidation of encoded memories. Consolidation refers to a process in which liable memory traces acquired during waking are stabilized and integrated to the pre-existing knowledge networks for a long period of time. A great number of behavioral studies have supported the helpful effect of sleep on declarative and non-declarative memory (Rasch & Born, 2013). A period of sleep after learning can improve declarative memory recall and enhance procedural skills (Barrett & Ekstrand, 1972; Jenkins & Dallenbach, 1924; Korman et al., 2007; Plihal & Born, 1997; Stickgold,

James, & Hobson, 2000). Sleep can also help the consolidation of emotional memories and its beneficial effect can be persistent even years after the initial encoding (Nishida, Pearsall, Buckner, & Walker, 2009; Wagner, Gais, & Born, 2001; Wagner, Hallschmid, Rasch, & Born, 2006). However, the consolidation effect of sleep usually takes place under certain conditions and favors explicitly encoded and behaviorally relevant memories (Diekelmann & Born, 2010).

Based on the Hebb's influential work, memory formation has been postulated as a mechanism in which neural circuits are activated repeatedly leading to lasting synaptic changes (Hebb, 1949). Building on this, consolidation of memory traces can be perceived as the reactivation of the neural circuits which were involved in the original encoding. Below we will discuss some evidence that support memory reactivation during sleep.

1.3.1 Reactivation of memory traces during sleep

Memory reactivation has been most extensively studied in the place cells of the rat hippocampus. Many studies have shown the firing patterns in the hippocampal cells of these animals are repeated in the same order during the sleep period after novel environment exploration or simple spatial tasks (Ji & Wilson, 2007; Nadasdy, Hirase, Czurko, Csicsvari, & Buzsaki, 1999; Pavlides & Winson, 1989; Wilson & McNaughton, 1994). These reactivations mostly occur during NREM sleep with a few studies suggesting they can also happen during REM sleep (Louie & Wilson, 2001; Poe, Nitz, McNaughton, & Barnes, 2000). The pattern reactivations are also most prominent within the first 20-40 minutes of sleep after learning and involve only a minority of recorded neurons (Battaglia, Sutherland, Cowen, Mc Naughton, & Harris, 2005; Rasch & Born, 2013). Moreover, compared with encoding patterns, reactivation patterns are noisier, less

accurate and happen at a much faster rate (Nadasdy et al., 1999). In addition to the hippocampus, replay activity during sleep has been reported in the thalamus, striatum, and neocortex (Euston, Tatsuno, & McNaughton, 2007; Ji & Wilson, 2007; Johnson, Euston, Tatsuno, & McNaughton, 2010; Lansink et al., 2008; Ribeiro et al., 2004).

Functional significance of sleep pattern reactivation was demonstrated in NREM sleep. In a study, human subjects learned spatial locations associated with an odor (Rasch, Buchel, Gais, & Born, 2007). Delivery of the odor as a cue during subsequent NREM sleep induced strong hippocampal activation and subsequently enhanced the memory retention. Thus, pattern reactivation during post-learning sleep indeed has a causal role in memory consolidation.

1.3.2 Mechanisms of memory consolidation in SWS and REM sleep

One of the debatable questions regarding the role of sleep in memory consolidation is whether the mechanism of memory consolidation is different between slow-wave sleep and REM sleep. It has been suggested that SWS supports system consolidation in which newly encoded memory traces in the hippocampus (temporary memory store) get repeatedly reactivated which itself drives concurrent reactivation of corresponding memory representations in the cortex (long-term memory store). This process enhances reorganization and redistribution of newly encoded memory traces and promotes their integration into the network of pre-existing long-term memories in the cortex (Diekelmann & Born, 2010). SWS specific field potential activities including neocortical slow oscillation, spindle and hippocampal sharp-wave ripple are shown to support this system consolidation process. The depolarizing cortical up phases of SO drive the hippocampal replay which leads the reactivation in the cortical circuits and other

brain regions (Ji & Wilson, 2007; Lansink, Goltstein, Lankelma, McNaughton, & Pennartz, 2009; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009). Cortical SO also drives hippocampal SPW-R in parallel with thalamo-cortical spindles allowing for the formation of spindle-ripple events which might support the transfer of information from hippocampus to neocortex (Buzsaki, 1998; Molle & Born, 2009; Sirota, Csicsvari, Buhl, & Buzsaki, 2003).

REM sleep, on the other hand, has been shown by many studies as a state that favors synaptic consolidation. Unlike SWS, post-learning REM sleep accompanies enhanced level of plasticity-related immediate early gene (IEG) activity which is localized to the brain regions involved in the encoding phase (Ribeiro et al., 2002; Ribeiro et al., 2007; Ulloor & Datta, 2005). The activity of plasticity-related IEG depends on the cholinergic activity which increases to near waking level during REM sleep (Teber, Kohling, Speckmann, Barnekow, & Kremerskothen, 2004; von der Kammer et al., 1998). Regarding the electrophysiological signatures of REM sleep, studies showed that the density of PGO waves increases robustly after training on an active avoidance task which promotes the expression of plasticity-related genes and subsequently memory consolidation (Datta, Li, & Auerbach, 2008; Ulloor & Datta, 2005). Moreover, desynchronized EEG oscillations of REM sleep are suggested to promote local synaptic consolidation in a disengaged memory system of cortex (Diekelmann & Born, 2010).

In conclusion, for an effective consolidation of memory traces, complementary sequences of SWS and REM sleep that provide both system and synaptic consolidation would probably be necessary. However, because declarative memory needs transfer and integration of invariant characteristics from different memories in different memory

systems, it benefits more from SWS-dependent system consolidation while procedural memory, due to its discrete and independent nature, benefits more from local synaptic consolidation during REM sleep. After all, it is worth mentioning that it is not a specific sleep stage per se that promotes memory consolidation, but rather associated neuronal mechanisms that provide proper electrophysiological, neurochemical, and genetic conditions for long-term memory storage (Diekelmann & Born, 2010).

1.4 Neuromodulation of sleep/wake

Up until the 20th century, it was believed that sleep is controlled by passive mechanisms in which reduced sensory stimulations prime the body and brain to reduce their normal physical and cognitive activities. However, we now know that there are circuits in the brain which actively control sleep and also wakefulness. Technological advances over the past decades, including optogenetics, pharmacogenetics, virus-based tracing, and imaging with genetically encoded indicators has helped us to better dissect and understand these neural circuits and the mechanisms through which they control behavioral states (Weber & Dan, 2016). Below, we will discuss some of these circuits and their interactions with each other.

1.4.1 Preoptic hypothalamus

Preoptic area (POA) of the anterior hypothalamus has been suggested to contain neurons which are important for sleep generation. C-Fos immunohistochemistry has shown the presence of GABAergic neurons in the ventrolateral preoptic area (VLPO) and the median preoptic nucleus (MnPO) which are active during NREM and REM sleep (Gong et al., 2004; Sherin, Shiromani, McCarley, & Saper, 1996). Lesion of cell clusters in the VLPO reduces sleep and in severe case cause insomnia (Lu, Greco, Shiromani, &

Saper, 2000; Von Economo, 1930) while activation of c-Fos labeled neurons using chemogenetics in VLPO can induce sleep (Zhang et al., 2015), suggesting a causal role for VLPO in sleep regulation.

VLPO and MnPO induce sleep by sending inhibitory projections, mainly GABAergic, to the wake-promoting regions of the brain (Figure 1). These regions include noradrenergic locus coeruleus (LC), histaminergic tuberomammillary nucleus (TMN), serotoninergic median and dorsal raphe nuclei (MRN and DRN), and orexinergic lateral hypothalamus (Sherin, Elmquist, Torrealba, & Saper, 1998; Steininger, Gong, McGinty, & Szymusiak, 2001; Yoshida, McCormack, Espana, Crocker, & Scammell, 2006). The ventral periaqueductal gray matter (VPAG) and the parabrachial nucleus (PB) which are important for wakefulness and arousal also receive inhibitory inputs from POA (Lu, Jhou, & Saper, 2006; Sherin et al., 1998).

On the other hand, POA receives projections from different neuromodulatory systems (Figure 1.B). Among them are wake-associated neuromodulators such as noradrenaline, acetylcholine, histamine, and serotonin which directly or indirectly inhibit POA neurons (Gallopin et al., 2000; Liu, Li, & Ye, 2010). This has led to the idea of a flip-flop for the regulation of sleep-wake cycle in which mutual inhibition between POA and arousal system can switch the state from sleep to waking and vice versa (Saper, Fuller, Pedersen, Lu, & Scammell, 2010). POA neurons are also shown to be excited by high concentrations of glucose (Varin et al., 2015). This finding together with the inhibitory effect of sucrose on the wake-promoting orexin neurons (Yamanaka et al., 2003) might explain the sleepiness after eating a sugar-rich meal. Moreover, there are some thermosensitive neurons in the POA that increase their activity by higher

temperature, the majority of them sleep-active (Alam, McGinty, & Szymusiak, 1995). This may provide a link between the body temperature and control of sleep.

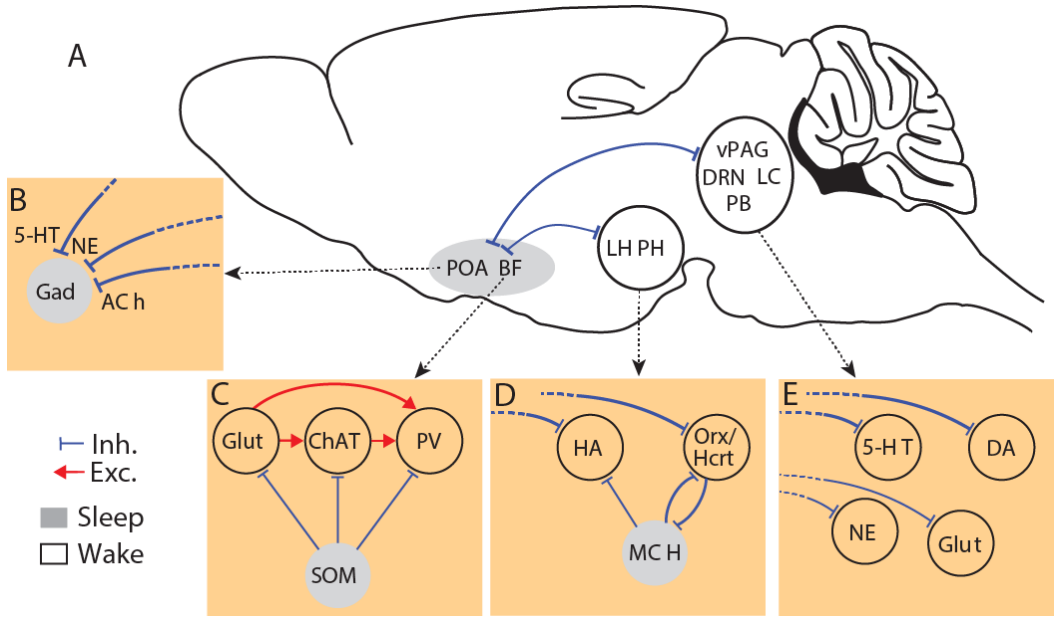

Figure 1. Circuit diagram of sleep-promoting mechanisms in forebrain. (A) Inhibitory inputs of POA to multiple brain regions. (B) Neuromodulatory inputs to POA GABAergic cells. (C, D) interconnections between basal forebrain (BF) and lateral and posterior hypothalamus (LH/PH) cell groups. (E) Inhibition of POA on wake-promoting cell groups in the brainstem.

1.4.2 Basal forebrain

The basal forebrain (BF) is the main source of acetylcholine (ACh) to the cortex and plays an important role in sleep and wakefulness regulation (S. H. Lee & Dan, 2012). There are three major types of neurons in the basal forebrain: cholinergic, GABAergic, and glutamatergic. Cholinergic and glutamatergic cells are wake- and REM-active and selective stimulation of these neurons promotes wakefulness (M. G. Lee, Hassani, Alonso, & Jones, 2005; Xu et al., 2015). Parvalbumin (PV)-expressing GABAergic neurons are similarly wake- and REM-active while somatostatin (SOM)-expressing ones

are NREM-active and their activation promotes NREM sleep (Xu et al., 2015). Mapping the connections between these intermingled populations of neurons using optogenetics revealed that SOM GABAergic neurons strongly inhibit wake-promoting cholinergic, glutamatergic, and PV neurons (Figure 1.C) (Xu et al., 2015). It also provided information on the excitatory local connections between the wake-promoting cells (Figure 1.C). At the cellular level, acetylcholine release from the basal forebrain into the neocortex is known to induce desynchronization of cortical LFP which depends on both muscarinic and nicotinic ACh receptors (Kalmbach, Hedrick, & Waters, 2012). This desynchronization is suggested to be caused by the excitation of neocortical pyramidal neurons and suppression of intracortical synapses (Bazhenov, Timofeev, Steriade, & Sejnowski, 2002).

1.4.3 Lateral and posterior hypothalamus

Posterior hypothalamus contains histaminergic (HA) neurons clustered in the tuberomammillary nucleus. Histaminergic activity is important for maintaining vigilance and wakefulness which explain why antihistamine drugs promote sleep (Thakkar, 2011). Lateral hypothalamus contains orexin/hypocretin neurons which are active during wakefulness and silent during sleep (Mileykovskiy, Kiyashchenko, & Siegel, 2005). These neurons are important for stabilizing the wake-sleep cycle and their loss or loss of orexin receptors cause narcolepsy in which the patient have severe sleepiness and can briefly lose muscle tone (cataplexy) (Chemelli et al., 1999). Orexin neurons send projections to multiple brain regions including the cortex, basal forebrain, and brainstem where they reinforce the brain on-state by exciting wake-promoting neurons (Sutcliffe & de Lecea, 2002).

Melanin-concentrating hormone (MCH) neurons are another subtype of lateral hypothalamic neuron which are sleep- and more specifically REM-active (Hassani, Lee, & Jones, 2009). These neurons are suggested to be sufficient but not necessary for the initiation and maintenance of REM sleep; and, their chronic activity is also important for NREM sleep (Jego et al., 2013; Tsunematsu et al., 2014). Optogenetic approaches have revealed that MCH neurons induce sleep by inhibiting neighboring orexin neurons (Rao et al., 2008) which themselves indirectly inhibit MCH neurons (Figure 1.D) (Apergis-Schoute et al., 2015). MCH neurons have also been shown to have an indirect inhibitory influence on the histaminergic cells in the lateral and posterior hypothalamus (Jego et al., 2013).

1.4.4 Brainstem

After the suppression of waking state by forebrain sleep-active neurons, different neuronal populations in the brainstem would change the brain state between REM and NREM sleep. This so-called ultradian cycle is tuned in a way that creates REM/NREM antagonism in which at any time point, one state can be maintained. Up until now, different cell types including cholinergic, glutamatergic and GABAergic, which may regulate the ultradian cycle, have been identified in the brainstem (Weber & Dan, 2016).

Brainstem cholinergic neurons, concentrated in the pedunculopontine tegmentum (PPT) and laterodorsal tegmentum (LDT), are wake- and REM-active (Boucetta, Cisse, Mainville, Morales, & Jones, 2014) and they extensively innervate the thalamus, hypothalamus, and basal forebrain (S. H. Lee & Dan, 2012). Activation of these cells increases the occurrence of REM sleep but not its duration (Van Dort et al., 2015), indicating that they are important for the initiation but perhaps not the maintenance of

REM sleep. C-Fos immunohistochemistry following sustained REM-sleep also revealed REM-active glutamatergic neurons in the sublateral dorsal nucleus (SLD) of the pons (Lu, Sherman, Devor, & Saper, 2006). Pharmacological activation of SLD caused continuous muscle atonia and low voltage EEG (Boissard et al., 2002), while a glutamate-transporter-knockout mouse showed disrupted REM sleep (Krenzer et al., 2011), suggesting the important contribution of glutamatergic SLD neurons to REM sleep.

SLD glutamatergic neurons are inhibited by the NREM-active GABAergic cells in the ventrolateral periaqueductal gray (vlPAG) (Figure 2.A) (Lu, Sherman, et al., 2006). In fact, it has been demonstrated that activation of vlPAG GABAergic neurons increases NREM sleep while suppressing REM sleep and wakefulness (Sastre, Buda, Kitahama, & Jouvet, 1996; Weber et al., 2015). A recent study also suggested a group of NREM-active glutamatergic neurons located ventromedial to the superior cerebellar peduncle (SCP) which could excite GABAergic vlPAG cells (Hayashi et al., 2015), reinforcing the NREM-promoting circuit (Figure 2.B). vlPAG GABAergic neurons which inhibit SLD glutamatergic cells are themselves inhibited by another group of REM-active GABAergic neurons located in the ventral medulla (vM) (Figure 2.A), supporting the antagonistic relationship between REM and NREM sleep (Weber et al., 2015). Optogenetic activation of these cells could increase the transition from NREM to REM sleep and prolong REM

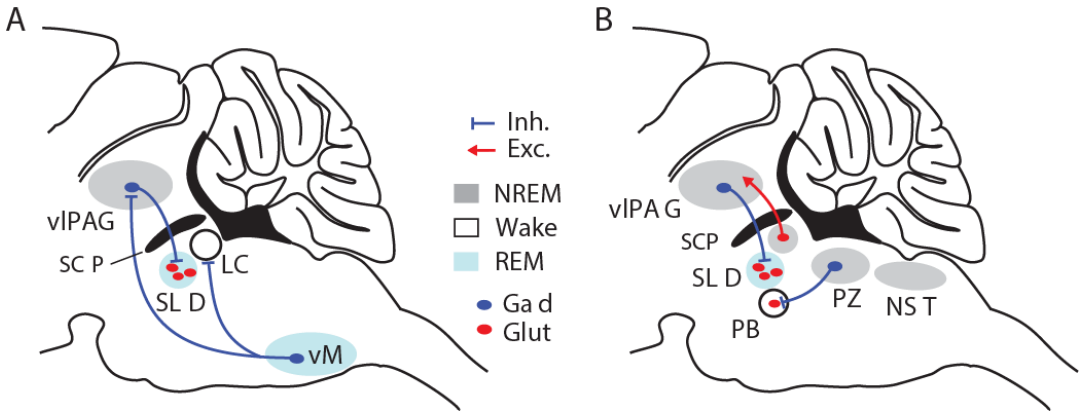

Figure 2. Brainstem circuits controlling sleep stages (A) REM promoting circuit in the brainstem. (B) Brainstem circuit promoting NREM sleep.
Reprinted by permission from Macmillan Publishers Ltd: *Nature* (Weber & Dan), copyright (2016).

episodes while silencing them, shorten REM bouts; suggesting that they are important for initiation and maintenance of REM sleep. Moreover, they maintain REM sleep and delay awakening by suppressing the wake-active REM-off noradrenergic neurons in LC (Aston-Jones, Ennis, Pieribone, Nickell, & Shipley, 1986; Weber et al., 2015).

There is another population of sleep-active GABAergic neurons located in the parafacial zone (PZ) in medulla which specifically promotes NREM sleep (Anaclet et al., 2012). While activating these cells strongly initiated NREM sleep, lesion to PZ neurons or preventing GABA release from them increased wakefulness (Anaclet et al., 2014; Anaclet et al., 2012). This effect could be mediated by their inhibitory influences on the glutamatergic neurons of medial parabrachial nucleus (PB), which are important for wakefulness and arousal (Figure 2.B) (Fuller, Sherman, Pedersen, Saper, & Lu, 2011). Moreover, the nucleus of the solitary tract (NTS) in hindbrain has been shown to contain NREM-active neurons which synchronize EEG activity and increase sleep (Eguchi &

Satoh, 1980; Magnes, Moruzzi, & Pompeiano, 1960), although the exact cell types mediating this effect are yet to be understood.

In summary, several neuronal populations in the brain actively control sleep and wakefulness and also the ultradian cycle regulating NREM and REM sleep. These neuronal clusters interact with each other through excitatory and inhibitory connections to regulate different behavioral states. Identification of other pieces of this puzzle and their organization would help to further clarify the mechanism of sleep-wake control in the brain.

2. Local nature of cortical desynchronization of REM state

2.1 Introduction

As was discussed in the previous chapter, the electrophysiological activity associated with each sleep stage plays an important role in priming the cortical and hippocampal memory networks for consolidation of memories. Cortical EEG activity, which transitions from high-amplitude slow oscillations in NREM sleep to fast low-amplitude rhythms in REM sleep, is of particular importance. While it has been widely held that cortical activity patterns are homogenous and global in different behavioral states, recent studies have shown they can be local. Local slow waves that occur in some cortical regions but not in others were reported in awake humans and rodents and they appear more often after a period of sleep deprivation (Bennett, Arroyo, & Hestrin, 2013; Bernardi et al., 2015; Vyazovskiy et al., 2011). Depth EEG recoding in humans and rodents have also demonstrated that during NREM sleep, slow waves most often engage a limited number of cortical regions and do not necessarily propagate across the entire cortex (Nir et al., 2011; Vyazovskiy et al., 2011). Moreover, some parts of the cortex can transiently be activated, exhibiting wake-like high frequency EEG patterns in a sleeping brain (Nobili et al., 2011).

More interestingly and relevant to this thesis, while it was believed that cortical desynchronization during REM sleep is a global phenomenon, a recent study by Funk et al. showed that cortical desynchronization can happen locally (Funk, Honjoh, Rodriguez, Cirelli, & Tononi, 2016). Using laminar electrophysiological recording in naturally sleeping mice, they showed that desynchronized cortical activity in REM sleep is confined to the secondary cortices as well as deep layers of primary cortices. In other

words, superficial/intermediate layers of primary cortical regions show slow wave activity during REM sleep. However, the electrophysiological technique used in this study has been unable to resolve the regional structure and dynamics of these activities due to relatively sparse sampling. Moreover, because partial results from multiple mice were combined to produce the final result in the laminar electrode study, it is not clear whether the local activity in different cortical regions happened simultaneously during REM sleep.

Here, we investigate the spatial profile of cortical desynchronization during REM state in mice by taking advantage of a mesoscale imaging technique called voltage-sensitive dye imaging (VSDI). VSD imaging can capture neural activity in a large field of view with high spatial and temporal resolution and is a method appropriate to study population activity over multiple cortical regions (Grinvald & Hildesheim, 2004).

2.2 Voltage sensitive dye imaging

VSD imaging uses organic dye molecules that bind to cell membranes and change their fluorescent properties in response to a change in membrane potential. Thus, by exciting these molecules and collecting the emitted fluorescent, they can be employed to transduce electrical activity to an optical signal (Grinvald & Hildesheim, 2004). They were first used to record the activity of individual neurons and nerves and then for monitoring the neuronal population activity in invertebrates and mammalian neocortex (Cohen et al., 1974; Grinvald, Cohen, Lesher, & Boyle, 1981; Orbach, Cohen, & Grinvald, 1985; Salzberg, Davila, & Cohen, 1973; Tasaki & Warashina, 1976). A major breakthrough in the field happened by the development of a red-shifted dye which

worked beyond the absorption band of hemoglobin, dramatically reducing the pulsation artifacts and hemodynamic noise (Shoham et al., 1999).

VSD imaging measures the subthreshold and suprathreshold neuronal activity with millisecond temporal and micron spatial resolution (Shoham et al., 1999) and has been previously used to study the structure of spontaneous activity during anesthesia in a large portion of mouse cortex (Mohajerani et al., 2013; Mohajerani, McVea, Fingas, & Murphy, 2010). In vivo electrophysiological recording combined with simultaneous VSD imaging in rodent cortex indicated that LFP and VSD signals are correlated closely with each other, suggesting the VSDI as a proper candidate for studying the cortical activity across different brain states (Ferezou et al., 2007; Lippert, Takagaki, Xu, Huang, & Wu, 2007). There are some weaknesses and limitations associated with VSD imaging which need to be taken into consideration. VSD stains glia cells along with neurons which increases the unwanted background signal. VSD imaging cannot resolve vertical profile of the emitted signal, and for applying the dye, cortex needs to be exposed. Nevertheless, considering all its pros and cons, it is still an optimum choice for this study.

2.3 Urethane as a model of sleep

Performing VSD imaging, especially in an extended unilateral configuration, requires extensive removal of muscles and bones which makes it very difficult to have the animal naturally sleep in the setup. It also requires a complex experimental setup for VSD imaging which minimizes any perturbation or heat stress caused by the light application. Therefore, we decided to use a sleep model which can mimic the characteristics of brain activity present during natural REM sleep and SWS. It has been shown in mice and rats that urethane anesthesia induces spontaneous alternation of brain state between REM-like

and SWS-like states in which cortical and hippocampal electrophysiological activity resemble those of natural sleep (Clement et al., 2008; Pagliardini, Gosgnach, & Dickson, 2013). Urethane anesthesia also modulates other physiological markers including respiration rate, heart rate, and temperature in a fashion that is similar to natural sleep states (Clement et al., 2008; Pagliardini, Funk, & Dickson, 2013).

Despite these similarities, there are some differences between natural sleep and urethane anesthesia. Firstly, while sleep is homeostatically regulated, urethane anesthesia relies on the circulating level of urethane. Moreover, phasic REM sleep events, including rapid eye movements and muscle twitches, are absent under urethane anesthesia and the occurrence of P waves has not been reported in the anesthetized condition (Clement et al., 2008; Robinson et al., 1977). Nonetheless, the integrity of tonic REM-like events including cortical desynchronized activity under urethane anesthesia justifies this model to be used along with VSD imaging for this study.

2.4 Method

2.4.1 Subjects

7 adult C57 mice, aged 2-4 months and weighed ~30 g, were used for experiments. They were housed under 12:12 h dark/light cycle and had ad libitum access to water and food at all time. Animal protocols were approved by the University of Lethbridge Animal Care Committee and followed the guidelines issued by the Canadian Council for Animal Care.

2.4.2 Surgery

Mice were anesthetized with urethane (0.12% wt/wt) and a large unilateral craniotomy (6.5x6 mm; bregma 2.8 to -3.7 mm and lateral 0 to 6 mm) was made on top of

the right cortical hemisphere. Underlying dura matter was removed. Body temperature was kept at 37±0.5 C using feedback loop heating pad. For assistance to animal breathing, a tracheotomy was performed. Additional doses of urethane (10% of initial dose) were administered when necessary to keep the surgical plane of anesthesia.

2.4.3 Voltage-Sensitive Dye Imaging

RH1692 (Optical Imaging) was dissolved in HEPES-buffered saline solution (0.5 mg/ml) and applied to the cortex exposed by the craniotomy for 40-60 min. Unbounded dye molecules were washed out afterward. For minimizing respiration artifact, 1.5% agarose made with a HEPES-buffered saline was spread over the cortex and sealed by the coverslip. VSD image stacks were collected in 12-bit format at 100 Hz frame rate using CCD camera (1M60 Pantera, Dalsa) and XCAP 3.8 imaging software (EPIX, Inc.). Images were taken through a microscope composed of front-to-front video lenses (8.6×8.6 mm field of view, 67 μm per pixel). VSD was excited by a red LED (627-nm center, Luxeon K2) and emitted fluorescence passed through a 673- to 703-nm bandpass emission filter. Each VSD stack of spontaneous activity usually included 90,000 frames.

2.4.4 Electrophysiological recording

The hippocampal electrode was implanted outside the planed cranial window prior to performing the craniotomy. For that, a Teflon-coated stainless-steel wire (bare diameter 50.8 μm) was placed in pyramidal layer of the right dorsal hippocampus. It was inserted posterior to the occipital suture with 33-degree angle (with respect to the vertical axis) according to the following coordinates relative to bregma: mediolateral (ML): 2.3 mm; dorsoventral (DV): 1.6 to 1.9 mm. The position of the electrode tip was confirmed using an audio monitor (Grass Instrument Co.). For cortical recording, a bipolar electrode

made with Formvar-coated nichrome wire (50.8 µm) was inserted into the primary motor, lip, or auditory sensory cortex; the tip separation was 0.5 mm and the upper tip located in layer 2/3. For EMG recording, a monopolar Teflon-coated stainless-steel wire (127 µm) was inserted into the neck musculature using a 27 gauge needle as described elsewhere (Pearson, Acharya, & Fouad, 2005). The reference and ground electrodes were placed on the cerebellum. The LFP and EMG signals were amplified (x1000) and filtered (0.1-10,000 Hz) using a Grass P5 Series AC amplifier (Grass Instrument Co.) and were sampled at 20 kHz using a data acquisition system (Axon Instruments). After data collection, 100 µA current was injected into the hippocampal electrode for 10 seconds. Animals were sacrificed and brains were extracted, sectioned and mounted. Location of the hippocampal electrode was further confirmed using cresyl violet staining (Figure 3).Figure 3

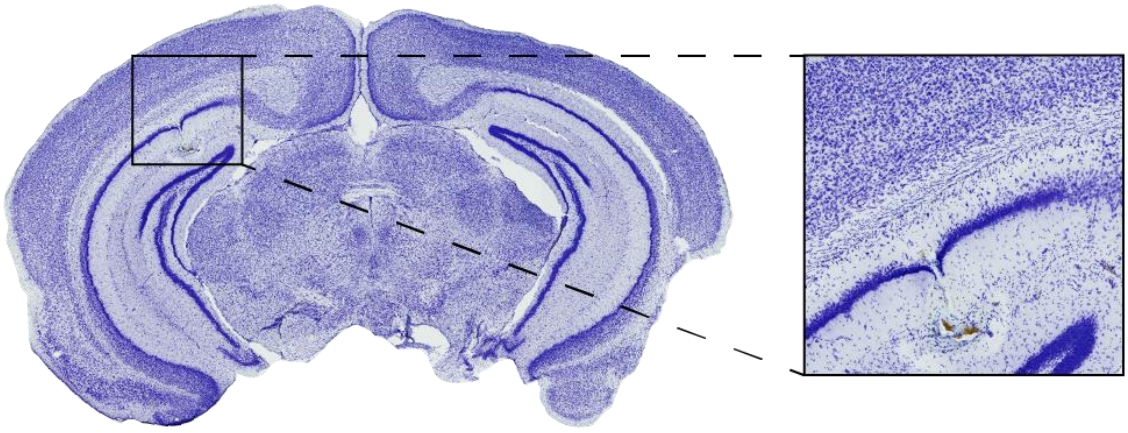

Figure 3. Brain section stained with cresyl violet showing the location of hippocampal electrode. In the magnified image on right, trace of the electrode pierced the pyramidal layer of CA1 can be clearly seen.

2.4.5 Data analysis

LFP and EMG signals were downsampled to 2 kHz offline and analyzed using custom-written codes in MATLAB (MathWorks). REM-like, NREM-like and transition states were scored based on the hippocampal LFP. The ratio of theta (3-5 Hz) power and slow wave (0.2-1.2 Hz) power to the total power was calculated in a 4-sec window and the threshold of mean plus one standard deviation was used to differentiate the states. The hippocampal LFP was scored as a REM-like state when the theta ratio was above the threshold for 10 consecutive seconds and the slow wave ratio was below the threshold simultaneously. A NREM-like state was defined as an opposite case of the REM-like state, that is, when the slow wave ratio was above the threshold for 10 consecutive seconds and the theta ratio was below the threshold simultaneously. A period that did not meet these conditions was classified as a transition state.

Raw EMG activity was filtered between 50 Hz and 1000 Hz and normalized by subtracting the mean and dividing the result by one standard deviation. The normalized signals were rectified and integrated using 4-sec moving windows. Distributions of the integrated EMG signal for REM-like and NREM-like states were then calculated for each animal based on the timing determined from the hippocampal LFP. Medians of these distributions were calculated for REM- and NREM-like states in each animal and were compared pair-wise across the two states using paired-sample t-test (α=0.05).

VSD spontaneous raw data was preprocessed based on the following steps: First, VSD time series of each pixel were filtered using a zero-phase highpass Chebyshev filter above 0.1 Hz. Then a baseline signal (F_0) was calculated by averaging all the frames and the fluorescence changes were quantified as $(F - F_0)/ F_0 \times 100$ where F is the filtered

signal. PCA was subsequently performed on the VSD stacks and the first 40 principal components were kept and the rest were discarded. To further reduce a spatial noise, images were filtered by a Gaussian kernel (5x5 pixels, sigma=1).

To identify cortical desynchronization of VSD signal in each REM-like event, first VSD stacks during all NREM-like episodes (scored based on hippocampal LFP) of one continuous recording were concatenated in time. Then 0.5- to 7-Hz power of VSD signal for each pixel was calculated using a sliding window (3-sec long, 2-sec overlap) which spanned over the concatenated NREM-like data and also over individual REM-like events. This generates a distribution of power values for the concatenated NREM-like data and the individual REM-like events (for each pixel). The frequency range of 0.5 to 7 Hz was chosen because most of the VSD signal power concentrated in this range and the possible involvement of heartbeat artifact could be eliminated. To determine if VSD activity of a specific pixel during a chosen REM-like episode undergoes state transition, the left-tailed test of significance was performed against the null hypothesis that median of the REM-like distribution is equal to that of NREM-like distribution (The Wilcoxon rank-sum test, α=0.05). The VSD activity was labeled as desynchronized if the calculated p-value was significant. Repeating this procedure for all the pixels inside the mask generated the spatial map of desynchronization.

To calculate the dynamic maps of desynchronization, each REM-like bout was divided into multiple 8-sec long epochs. Then, 0.5- to 7-Hz VSD power with a sliding window (3-sec long, 2-sec overlap) was calculated for each 8-sec epoch. Distribution of power values for these REM-like epochs was compared with the distribution of total NREM-like power values (pixel wise, the Wilcoxon rank-sum test). At the end, dynamic

p-value maps were converted to binary maps by thresholding (above α=0.05). The grand average map in each animal was generated by averaging these binary maps calculated for all REM-like events.

2.5 Results

We sought to study the local spatial changes in cortical neural activities in the REM-like state. To that end, we simultaneously recorded spontaneous cortical VSD activity and hippocampal and cortical LFP from 7 mice under urethane anesthesia. These resting-state activities were acquired in the absence of external sensory stimuli using a custom-built experimental setup that did not produce significant vibration or auditory stimulation (Figure 4.A). VSD images were captured with 100 Hz frame rate at which we were able to resolve multiple time signatures of ongoing resting-state activity. Spontaneous electrophysiological and imaging data were recorded in continuous 15-minute epochs sufficient to capture multiple forebrain state transitions. Performed craniotomy was large enough to include multiple cortical regions in the imaging window (Figure 4.B). These regions included: primary and secondary motor cortex (M1, M2), upper lip area of the primary somatosensory cortex (LPS1), hindlimb area of the primary and secondary somatosensory cortex (HLS1, HLS2), forelimb area of the primary and secondary somatosensory cortex (FLS1, FLS2), primary and secondary barrel cortex (BCS1, BCS2), parietal association cortex (ptA), retrosplenial cortex (RS), primary and secondary visual cortex (V1, V2), and primary auditory cortex (A1).

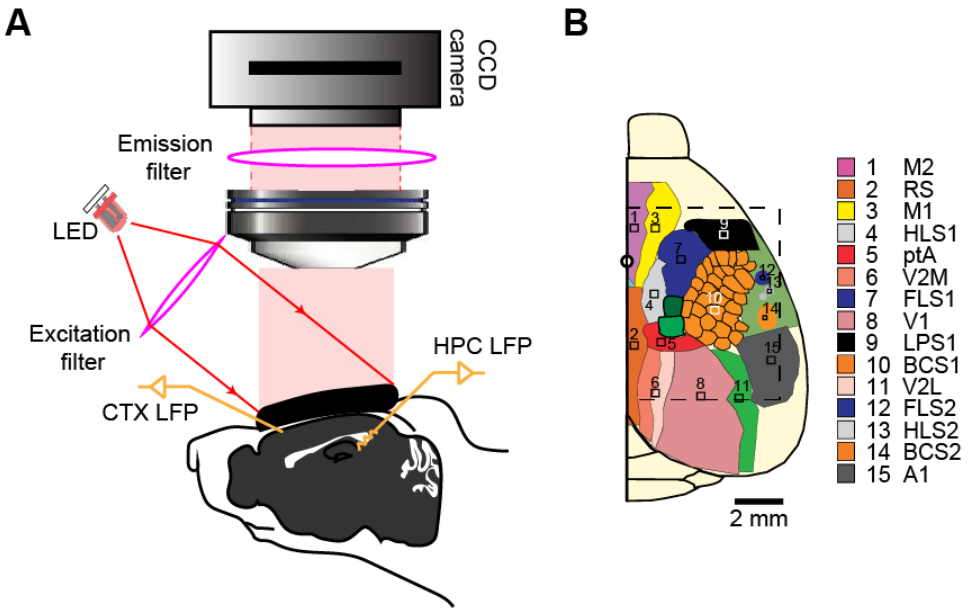

Figure 4. Experimental paradigm and surgical preparation for studying forebrain activity using VSD imaging and LFP recording (A) Experimental setup used for simultaneous wide-field VSD imaging and cortical and hippocampal LFP recording. (B) Cartoon of unilateral preparation showing the location of imaged cortical regions. Dotted rectangular shows the boundary of craniotomy.

2.5.1 Spontaneous state transition in LFP/VSD under urethane anesthesia

We first investigated if urethane anesthesia could induce brain state alternation by changing the characteristics of the electrophysiological and more importantly optical signal. As documented previously (Clement et al., 2008; Pagliardini, Gosgnach, et al., 2013), indeed urethane anesthesia caused cyclic evolution of brain state between NREM-like and REM-like state, each of them having specific physiological signatures. Expectedly, hippocampal and cortical field potentials showed slow-frequency (~ 1 Hz) activity during NREM-like state whereas REM-like state consisted of high-frequency low-amplitude cortical activity and hippocampal theta (~ 4 Hz) (Figure 5). The observed hippocampal theta was ~2 Hz slower than theta usually seen during natural REM sleep in rodents which also matches previous studies (Clement et al., 2008; Pagliardini, Gosgnach,

et al., 2013; Robinson et al., 1977). The transition state was identified in between NREM-like and REM-like states and had intermediate electrographic characteristics.

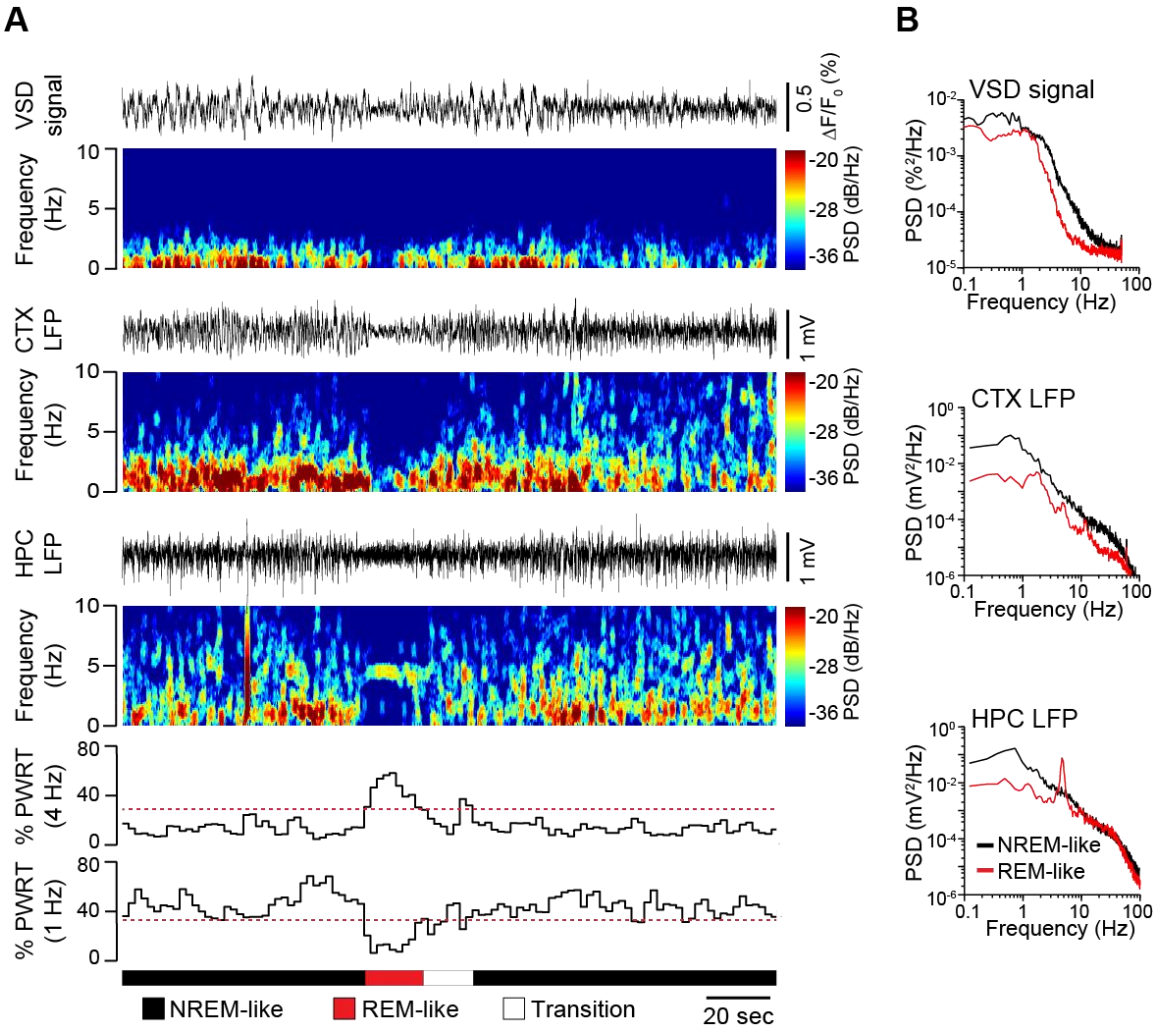

Figure 5. Spontaneous state alternation of brain activity in urethane anesthetized mice. (A) Continuous VSD and LFP traces and corresponding spectrograms show spontaneous changes in the brain state of urethane-anesthetized mouse. VSD signal was derived from an ROI (0.112 mm^2) around the cortical electrode in primary motor cortex. Below, the percentage of the power in the 3-5 Hz and 0.2-1.2 Hz bands to the total power of hippocampal LFP are displayed. These ratios are used to score the recording into NREM-like, REM-like and transition states. (B) Power spectral analysis of VSD and EEG traces indicates a reduction of ~ 1 Hz power in VSD, CTX and HPC and an increase of ~ 4 Hz power in HPC during REM-like state.

Hippocampal and cortical LFP state alternations coincided with a general reduction of VSD signal power when comparing NREM- to REM-like states (Figure 5). This was in line with the fact that neural population activity modulating fluorescence signal transitions from synchronous firing in the NREM-like state to asynchronous tonic firing in the REM-like state (Harris & Thiele, 2011; M. Steriade & R. W. McCarley, 2005). Power spectrum of VSD signal shows the reduction of power in almost all the frequency bands from 0.1 to 50 Hz when comparing NREM- to REM-like states; however, most of the power and also the power reduction concentrated in the frequencies less than 10 Hz (Figure 5.B).

EMG activity recorded from the neck muscle failed to show any significant change across two states, despite the observations in natural sleep that have reported a significant reduction of muscle tone across NREM to REM transitions (Figure 6). This can be due to the fact that urethane is known to cause muscle atonia, suppressing the muscle tone in both NREM- and REM-like states (Robinson et al., 1977). Phasic muscular twitches as one of the signatures of phasic REM sleep were absent in our EMG recording, supporting the previous report on the selective suppression of phasic REM by urethane (Clement et al., 2008). However, we did not assess the presence of other phasic events including P waves and rapid eye movements.

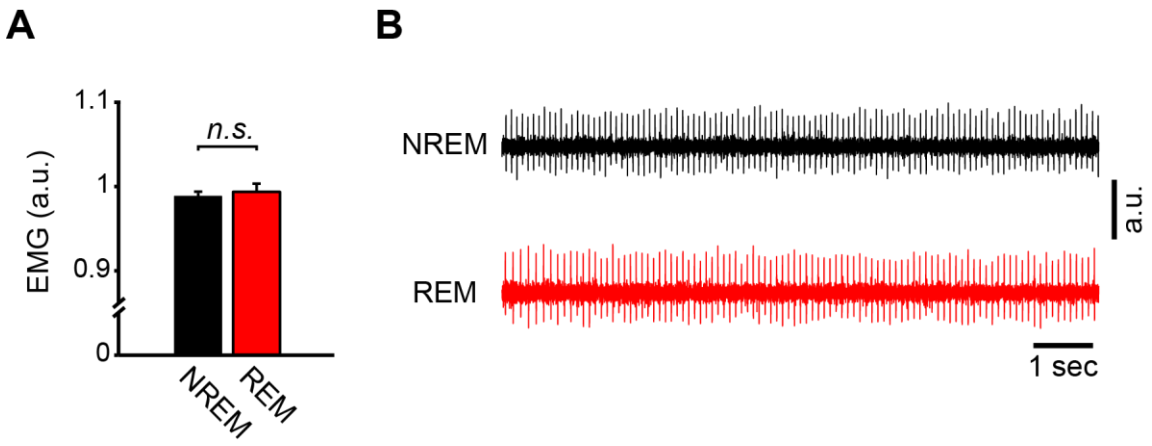

Figure 6. Comparison of neck muscle activity between NREM- and REM-like states (A) Average neck EMG content across two states does not show any significant change ($p>0.05$, n=7 mice, error bars, s.e.m) (B) Example of EMG traces in two states. Periodic spikes are heart beat artifacts.

Figure 7.A and B display a magnified representative LFP and VSD traces in the two states wherein the different characteristics of the signals can be clearly differentiated. Cross-correlation of the cortical LFP and the VSD signal collected from the pixels surrounding the cortical electrode exhibits a high level of covariation (Figure 7.C). Because VSD signal is modulated positively by the membrane potential and LFP is dependent on the extracellular currents and negatively correlated with membrane potential, they are anti-correlated. Montage of VSD activity over time further characterizes the up/down phases and propagating waves of depolarization in NREM-like state and the downscaling of cortical activity during REM-like state (Figure 7.D). However, as it can be seen there are some activities occurring in the local cortical circuits during REM-like state. These activities are studied in detail in the next sections.

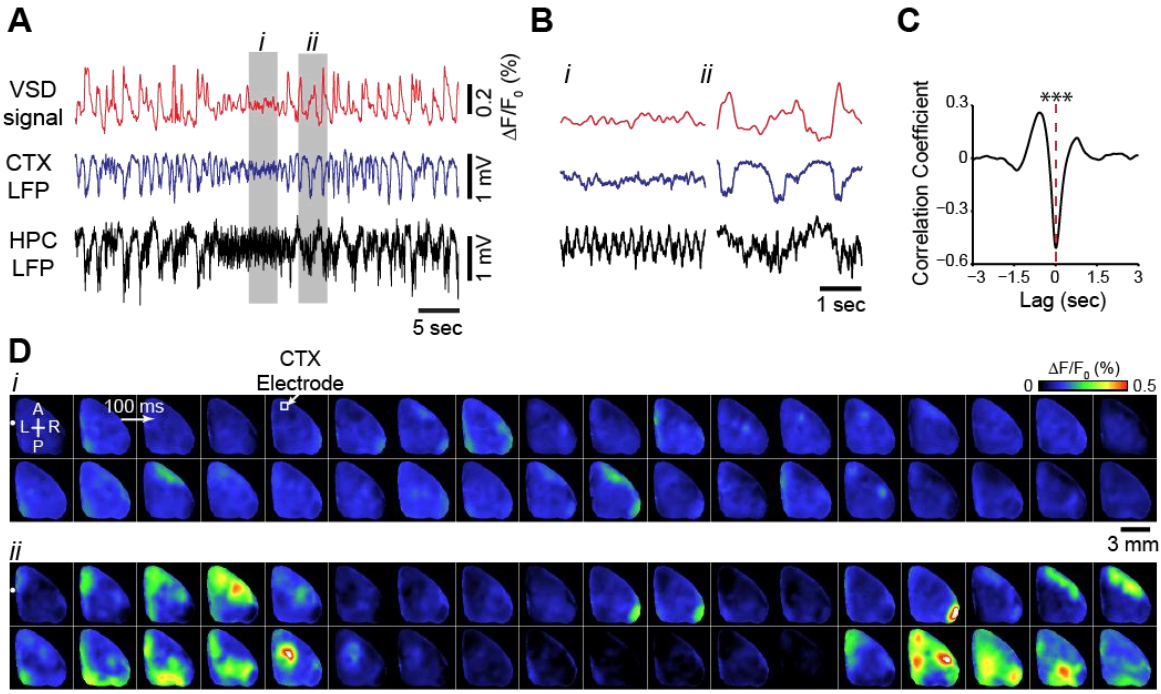

Figure 7. Spatiotemporal characteristics of spontaneous cortical activity during NREM/REM states. (A, B) A representative epoch of VSD (0.2-7 Hz) and EEG traces and magnification in time of two segments *i* and *ii* selected from REM and NREM states respectively. Vertical scale bars are the same for two panels. (C) Cross-correlogram displaying the correlation between VSD signal around the cortical electrode (M1) and cortical LFP. Significance of the correlation value in zero lag was measured using a Student's t-distribution (*** $p<0.001$). (D) Montage of spontaneous VSD activity (0.2-7 Hz) corresponding to the epoch *i* and *ii* further depicting the spatiotemporal characteristics of cortical fluctuations during two states.

2.5.2 Cortical desynchronization of REM-like state is local

Next, we investigated whether the spontaneous activity of all cortical regions reported by VSD signal undergoes desynchronization in REM-like episodes or not. We found that there was a portion of REM-like bouts in which we did not observe a distinct reduction of VSD fluctuations in all cortical regions (Figure 8). In other words, some cortical areas showed large-amplitude slow wave type of activity in ~1 Hz frequency band during REM-like state similar to what has been reported previously in natural REM sleep (Funk et al., 2016). To characterize cortical desynchronization of VSD signal for

each cortical region, we compared its 0.5- to 7-Hz power across two states (see method). If the p-value calculated from this comparison was statistically significant, that region was classified as desynchronized and if it was not, it was classified as synchronized. Example of p-value maps generated for representative animals (Figure 9) showed that indeed cortical desynchronization of REM-like state could be confined to local regions. In the desynchronized regions, VSD power distribution of the REM-like episode could be differentiated from the NREM-like one whereas it is not possible for a synchronized region (Figure 9.A). Interestingly, these local patterns of desynchronization could be absent (global desynchronization) in some REM-like bouts and when they were not, they were located in the anterior and lateral part of the cortical hemisphere (Figure 9.B and Figure 11.E).

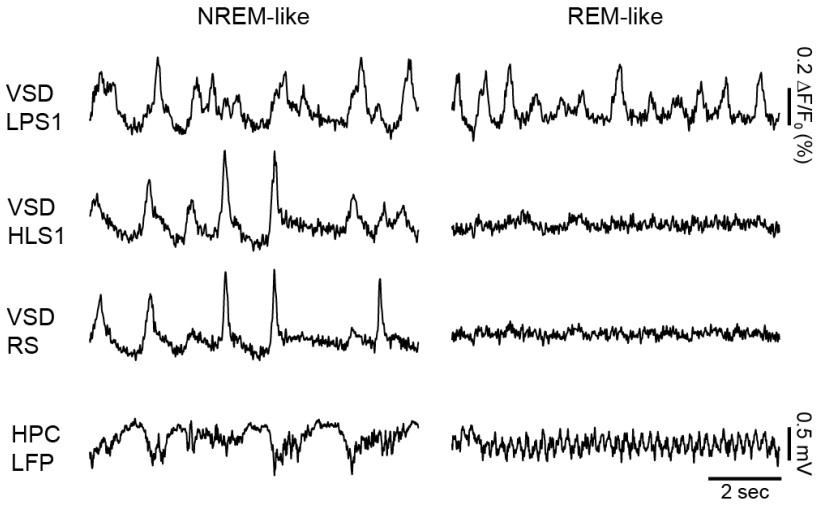

Figure 8. Local desynchronized cortical patterns in REM-like state. Representative VSD signals (top three rows) selected from three different cortical regions together with the simultaneous hippocampal LFP traces during NREM-like and REM-like states. LPS1 shows slow wave activity during REM-like state while HLS1 and RS showing desynchronized rhythms.

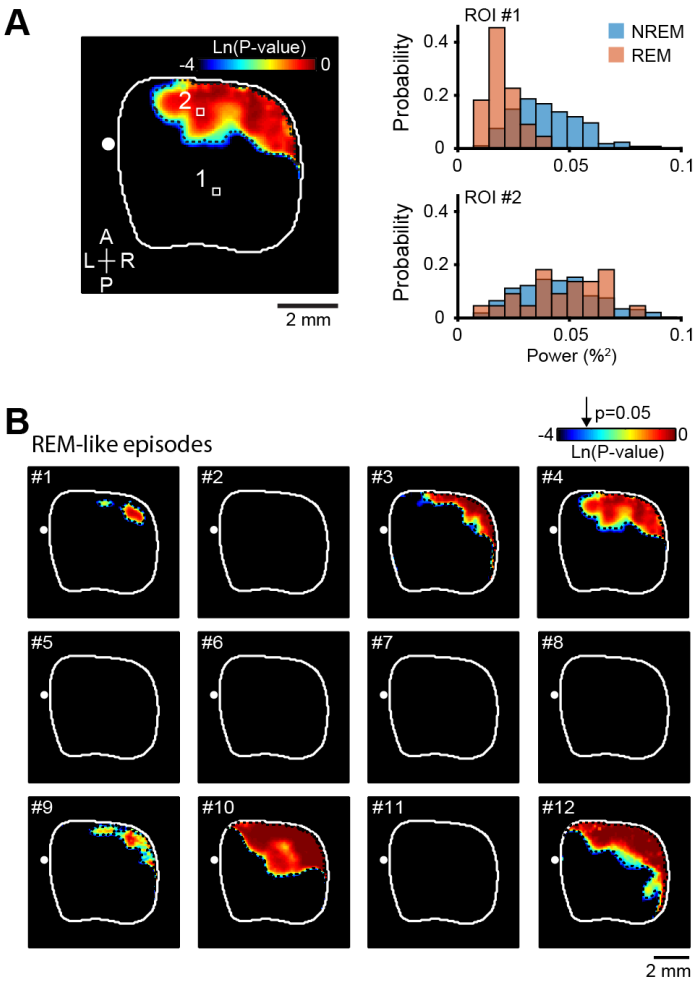

Figure 9. Cortical extent of desynchronization in REM-like state. (A, Left) A representative p-value map showing cortical areas in which VSD signal undergoes desynchronization during a REM-like event. Dark areas are desynchronized (significant change based on α=0.05) and areas inside the black dotted line are synchronized. (A, Right) Histograms showing the VSD power distribution during REM-like and NREM-like states for points 1 (desynchronized) and 2 (synchronized). It can be seen that while REM/NREM distributions are quite separated for ROI#1, they have a significant overlap for ROI#2. (B) P-value maps for other REM-like events from the same animal.

Concurrent cortical LFP which was recorded from the synchronized regions also showed similar patterns to VSD signal. While the LFP signal had a good correlation with the surrounding VSD activity, it showed slow wave activity during the REM-like episodes in which p-value maps had local patterns (*ii* in Figure 10) and it displayed desynchronized activity during the REM-like episodes in which p-value maps showed a

global desynchronization (*i* in Figure 10). This would further support the validity of p-value maps of desynchronization calculated based on the VSD signal and suggests a transient nature for the high-amplitude slow wave activity occurring in the REM-like state.

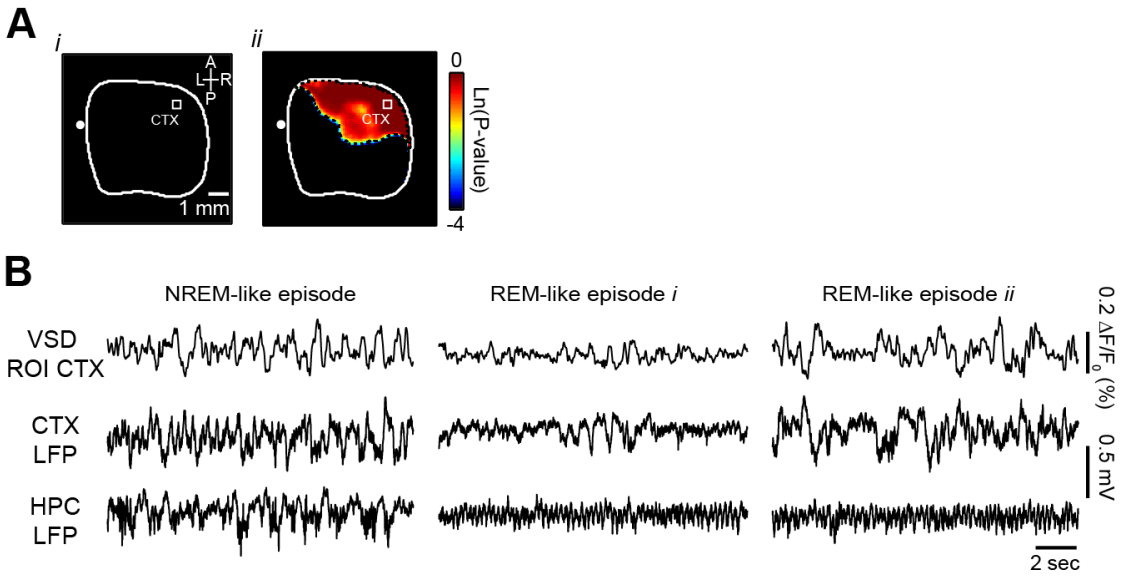

Figure 10. Cortical LFP further supports local desynchronization of VSD signal. (A) P-value map of desynchronization for two distinct REM-like episodes *i*, *ii*. (B) Traces show VSD signal (around cortical electrode) and cortical and hippocampal LFP during a NREM-like episode (left) and REM-like episodes *i* (middle) and *ii* (right). As it is clear, cortical LFP along with neighboring VSD signal became desynchronized during REM-like episode *i* while they did not during episode *ii*.

2.5.3 Locality of cortical desynchronization is temporally dynamic in the individual REM-like episodes

We then investigated the presence of local cortical desynchronization in shorter time windows through REM-like episodes; especially in the episodes which had given global desynchronization patterns (dark maps). We found cortical regions could transiently show synchronized or desynchronized activity independent of each other in the REM-like state (Figure 11.A, B). In other words, dynamic p-value maps of cortical

desynchronization were not necessarily constant over the time course of a REM-like event and synchronized/desynchronized patterns could move from one cortical region to another. However, the dynamic maps for a REM-like event followed to some extent the general pattern of the desynchronization map for that event (Figure 11.C). We measured the similarity of these dynamic maps with each other using Pearson correlation coefficient and found a non-normal distribution (p-value<0.001, Lillieforc test) for correlation values with a mean of 0.29 and standard deviation of 0.07 (Figure 11.D). Moreover, based on this distribution ~14 percent of the dynamic maps not only aren't similar but also are anticorrelated.

Lastly, we averaged these dynamic maps for each animal to find the spatial extent of cortical desynchronization in REM-like state (Figure 11.E). Although there were slight differences between the patterns across animals, some conclusions could be made based on these maps. Generally, midline and posterior regions including secondary motor/cingulate, retroslpenial, and visual cortices tended to remain desynchronized throughout REM-like state. Anterior and lateral areas, on the other hand, including upper lip somatosensory, primary motor, and barrel cortices showed a mixture of synchronized and desynchronized activity during REM-like states. A firm conclusion could not be made for auditory cortex as the results were variable across animals.

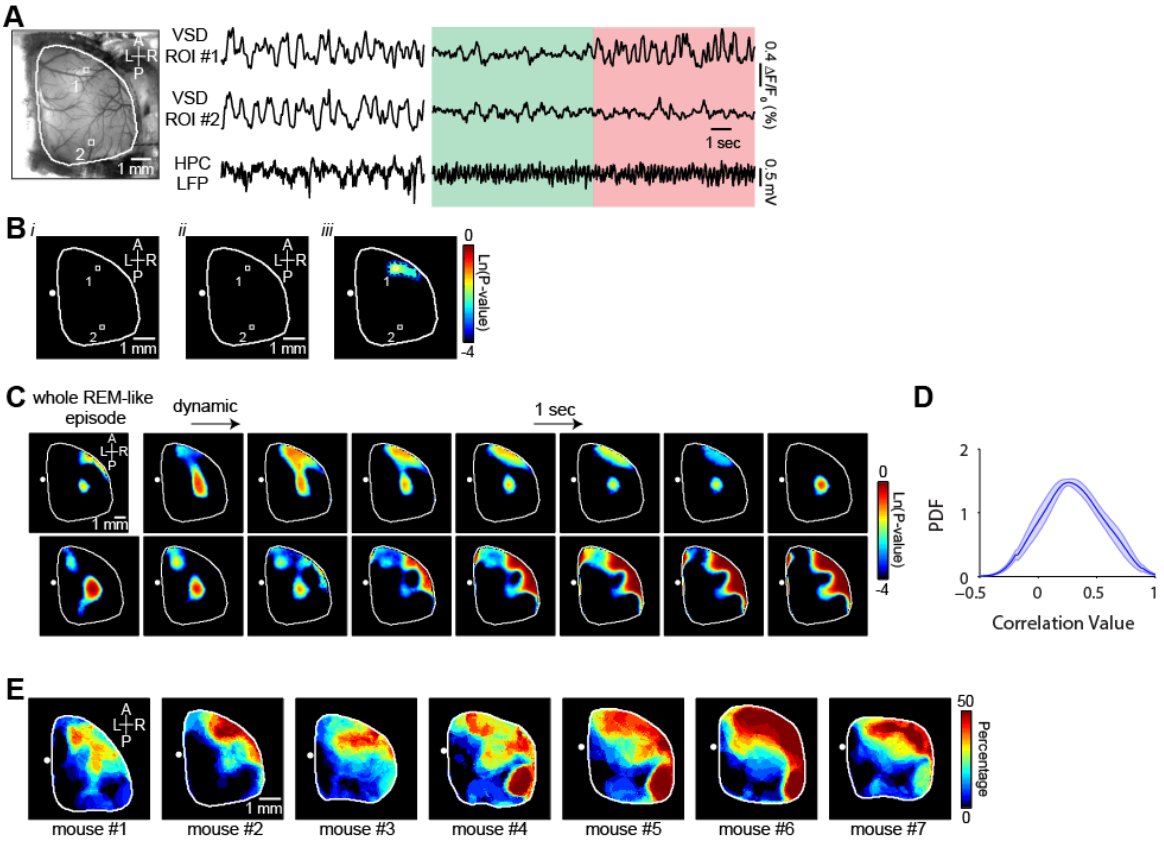

Figure 11. Dynamic of cortical desynchronization in REM-like state. (A, left) Position of ROI#1 and ROI#2 in the cranial window. Traces show VSD and hippocampal activity during preceding NREM-like (middle) and REM-like (right) episodes. (B) P-value maps of desynchronization for the whole (*i*, combined green and pink in A), first half (*ii*, green in A) and second half (*iii*, pink in A) of REM-like episode. It is clear that while cortical activity at ROI#2 remained desynchronized during this REM-like event, ROI#1 was desynchronized at the beginning but not for the rest of the event. (C) Another example of p-value map of desynchronization for a REM-like episode and its corresponding dynamic maps. Dynamic maps calculated for 8 sec window and have 7 sec overlap. (D) Average power distribution function (PDF) of the correlation coefficient between dynamic maps of desynchronization (n=7). Shaded areas show standard error across animals. Only non-dark maps were included. (E) Grand average of thresholded p-value maps for 7 animals. Warmer colors mean the areas that more often show synchronized activity during REM-like events.

3. Discussion

We aimed to study the activity of a large network of cortical circuits in the REM-like state under urethane anesthesia using wide-filed VSD imaging. We reported here that alternation of brain state between NREM-like and REM-like states induced by urethane anesthesia could modulate spontaneous activity reported by field potential and, more importantly, VSD signal. We demonstrated that in REM-like state, some cortical regions deviate from the global activity patterns and show large slow wave fluctuations. In other words, the activity of all cortical regions did not become homogeneously desynchronized in REM-like state. Surprisingly, we also found these synchronized cortical areas do not continuously remain in the same state but rather transiently switch to a desynchronized state. This contrasts with a previous study by Funk et al. (2016) which claimed REM sleep slow waves as ever present phenomena; however, they overlook to address this temporal dynamic feature of slow waves in REM state.

In general, based on our results, regions located in the anterior and lateral part of one hemisphere of the neocortex tended to show recurring synchronized activities and regions in the medial and posterior part had a propensity towards desynchronized activity during REM-like state. Although this would not completely match with the previous results (Funk et al., 2016) indicated on the synchronized primary and desynchronized secondary/association cortices, there are some similarities. For example, in both studies, secondary motor and retrosplenial cortices showed desynchronized activity while primary somatosensory and motor cortices showed synchronized patterns. One difference in our results compared to Funk et al. (2016) is that we showed desynchronized activity for primary visual cortex. These discordances might stem from the differences between

desynchronization induced by urethane anesthesia versus natural sleep or the different nature of fluorescence versus electrophysiological signal.

Mechanisms for the locality of cortical desynchronization are yet to be understood. It is known that acetylcholine level is high during REM/REM-like state, activating the neocortex and inducing the tonic firing of cortical neurons (Clement et al., 2008; Hasselmo & McGaughy, 2004; Krnjevic, 2004). The basal forebrain, as the main source of cholinergic input to the cortex, is suggested to have a topographic organization for its cortical projections (Kim et al., 2016; Zaborszky, 2002). This would provide the platform for the selective neuromodulation and activation of cortical regions. Low levels of noradrenaline in REM/REM-like state can also contribute to the observed local desynchronization because noradrenergic projections, originating mainly from the locus coeruleus, can desynchronize multiple sensory cortices (Kim et al., 2016). However, how neuromodulatory systems can facilitate dynamic characteristics of desynchronization in individual REM-like events is another question that needs to be addressed.

What can be the possible functions of local slow waves in REM state? Occurrence of slow waves in other behavioral states has physiological and behavioral effects. In sleep-deprived awake rats, slow activity occurring during a reaching task impair their performance (Vyazovskiy et al., 2011). Slow wave activity in NREM sleep is suggested to impair cortical effective connectivity and be responsible for sensory disconnection (Funk et al., 2016; Massimini et al., 2005). Similarly, local slow waves in REM state can contribute to sensory disconnection and behavioral unresponsiveness by gating the cortical information transmission mainly from primary to secondary cortices. This would solve the paradox of deep sensory disconnection of REM sleep while considering a global

wake-like cortical activity as previously thought. Occurrence of slow waves in REM-like state and possibly in REM sleep might also explain the surprising results of some studies reporting the beneficial effect of REM sleep on declarative memory; a type of memory that has been considered to be primarily consolidated by the slow wave sleep (Fogel, Smith, & Cote, 2007; Rauchs et al., 2004).

Considering the similarity between urethane induced REM state and natural REM sleep, we expect our results can be extended to natural sleep. Nevertheless, replicating the same results under natural REM sleep in an intact brain would be of great value. Recent development of a voltage-sensitive fluorescent protein (VSFP) called Butterfly can help us to achieve this goal (Akemann et al., 2012). Butterfly has been used in wide-field imaging as a voltage indicator to report evoked and spontaneous neuronal population activity in vivo with high spatiotemporal resolution (Akemann et al., 2012; Shimaoka, Song, & Knopfel, 2017). Replacing VSD imaging with this technique in our study would eliminate the requirement for skull removal for dye application and enable us to record brain activity over several days, facilitating a reliable natural sleep recording in an intact mouse brain. In addition, this strategy has the potential to tag specific cell types which can be used to elucidate the role of different neuronal classes in generation of local slow wave activity of REM sleep.

4. Conclusion

Sleep and its two core stages, NREM and REM, change the ongoing brain oscillations that are important for communications within the neuronal networks. Some of these changes are the defining features of NREM and REM sleeps. REM sleep for example is characterized by fast and low-amplitude desynchronized cortical oscillations and hippocampal theta activity in rodents. In this study, we demonstrated that cortical areas can show a mixture of desynchronized and slow wave activity during REM state under urethane model of sleep; the slow waves that are the common cortical rhythms of NREM state. Taking the advantage of an imaging technique with a large spatial coverage, we also showed for the first time that these large slow waves usually occur in the anterior and lateral regions of one cortical hemisphere.

These results together with the previous studies challenge the classical views, considering the cortical activity patterns as homogeneous and global in different behavioral states (Bernardi et al., 2015; Funk et al., 2016; Nobili et al., 2011; Vyazovskiy et al., 2011). Our findings may also be used to understand how the memory traces in different cortical circuits undergo system and synaptic consolidation during sleep stages.

5. Reference

Akemann, W., Mutoh, H., Perron, A., Park, Y. K., Iwamoto, Y., & Knopfel, T. (2012). Imaging neural circuit dynamics with a voltage-sensitive fluorescent protein. *J Neurophysiol, 108*(8), 2323-2337. doi:10.1152/jn.00452.2012

Alam, M. N., McGinty, D., & Szymusiak, R. (1995). Neuronal discharge of preoptic/anterior hypothalamic thermosensitive neurons: relation to NREM sleep. *Am J Physiol, 269*(5 Pt 2), R1240-1249.

Alonso, A., & Garcia-Austt, E. (1987). Neuronal sources of theta rhythm in the entorhinal cortex of the rat. I. Laminar distribution of theta field potentials. *Exp Brain Res, 67*(3), 493-501.

Amzica, F., & Steriade, M. (1996). Progressive cortical synchronization of ponto-geniculo-occipital potentials during rapid eye movement sleep. *Neuroscience, 72*(2), 309-314.

Anaclet, C., Ferrari, L., Arrigoni, E., Bass, C. E., Saper, C. B., Lu, J., & Fuller, P. M. (2014). The GABAergic parafacial zone is a medullary slow wave sleep-promoting center. *Nat Neurosci, 17*(9), 1217-1224. doi:10.1038/nn.3789

Anaclet, C., Lin, J. S., Vetrivelan, R., Krenzer, M., Vong, L., Fuller, P. M., & Lu, J. (2012). Identification and characterization of a sleep-active cell group in the rostral medullary brainstem. *J Neurosci, 32*(50), 17970-17976. doi:10.1523/JNEUROSCI.0620-12.2012

Andersen, P., Bland, H. B., Myhrer, T., & Schwartzkroin, P. A. (1979). Septo-hippocampal pathway necessary for dentate theta production. *Brain Res, 165*(1), 13-22.

Andrillon, T., Nir, Y., Cirelli, C., Tononi, G., & Fried, I. (2015). Single-neuron activity and eye movements during human REM sleep and awake vision. *Nat Commun, 6*, 7884. doi:10.1038/ncomms8884

Apergis-Schoute, J., Iordanidou, P., Faure, C., Jego, S., Schone, C., Aitta-Aho, T., . . . Burdakov, D. (2015). Optogenetic evidence for inhibitory signaling from orexin to MCH neurons via local microcircuits. *J Neurosci, 35*(14), 5435-5441. doi:10.1523/JNEUROSCI.5269-14.2015

Aston-Jones, G., Ennis, M., Pieribone, V. A., Nickell, W. T., & Shipley, M. T. (1986). The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. *Science, 234*(4777), 734-737.

Barrett, T. R., & Ekstrand, B. R. (1972). Effect of sleep on memory. 3. Controlling for time-of-day effects. *J Exp Psychol, 96*(2), 321-327.

Battaglia, F. P., Sutherland, G. R., Cowen, S. L., Mc Naughton, B. L., & Harris, K. D. (2005). Firing rate modulation: a simple statistical view of memory trace reactivation. *Neural Netw, 18*(9), 1280-1291. doi:10.1016/j.neunet.2005.08.011

Bazhenov, M., Timofeev, I., Steriade, M., & Sejnowski, T. J. (2002). Model of thalamocortical slow-wave sleep oscillations and transitions to activated States. *J Neurosci, 22*(19), 8691-8704.

Bennett, C., Arroyo, S., & Hestrin, S. (2013). Subthreshold mechanisms underlying state-dependent modulation of visual responses. *Neuron, 80*(2), 350-357. doi:10.1016/j.neuron.2013.08.007

Berger, R. J., & Phillips, N. H. (1995). Energy conservation and sleep. *Behav Brain Res, 69*(1-2), 65-73.

Bernardi, G., Siclari, F., Yu, X., Zennig, C., Bellesi, M., Ricciardi, E., . . . Tononi, G. (2015). Neural and behavioral correlates of extended training during sleep deprivation in humans: evidence for local, task-specific effects. *J Neurosci, 35*(11), 4487-4500. doi:10.1523/JNEUROSCI.4567-14.2015

Bland, B. H., Trepel, C., Oddie, S. D., & Kirk, I. J. (1996). Intraseptal microinfusion of muscimol: effects on hippocampal formation theta field activity and phasic theta-ON cell discharges. *Exp Neurol, 138*(2), 286-297. doi:10.1006/exnr.1996.0067

Boissard, R., Gervasoni, D., Schmidt, M. H., Barbagli, B., Fort, P., & Luppi, P. H. (2002). The rat ponto-medullary network responsible for paradoxical sleep onset and maintenance: a combined microinjection and functional neuroanatomical study. *Eur J Neurosci, 16*(10), 1959-1973.

Borbely, A. A., & Achermann, P. (1999). Sleep homeostasis and models of sleep regulation. *J Biol Rhythms, 14*(6), 557-568.

Borhegyi, Z., Varga, V., Szilagyi, N., Fabo, D., & Freund, T. F. (2004). Phase segregation of medial septal GABAergic neurons during hippocampal theta activity. *J Neurosci, 24*(39), 8470-8479. doi:10.1523/JNEUROSCI.1413-04.2004

Boucetta, S., Cisse, Y., Mainville, L., Morales, M., & Jones, B. E. (2014). Discharge profiles across the sleep-waking cycle of identified cholinergic, GABAergic, and glutamatergic neurons in the pontomesencephalic tegmentum of the rat. *J Neurosci, 34*(13), 4708-4727. doi:10.1523/JNEUROSCI.2617-13.2014

Bragin, A., Engel, J., Jr., Wilson, C. L., Fried, I., & Buzsaki, G. (1999). High-frequency oscillations in human brain. *Hippocampus, 9*(2), 137-142. doi:10.1002/(SICI)1098-1063(1999)9:2<137::AID-HIPO5>3.0.CO;2-0

Brebbia, D. R., & Altshuler, K. Z. (1965). Oxygen consumption rate and electroencephalographic stage of sleep. *Science, 150*(3703), 1621-1623.

Brooks, D. C., & Bizzi, E. (1963). Brain Stem Electrical Activity during Deep Sleep. *Arch Ital Biol, 101*, 648-665.

Brooks, D. C., Gershon, M. D., & Simon, R. P. (1972). Brain stem serotonin depletion and ponto-geniculo-occipital wave activity in the cat treated with reserpine. *Neuropharmacology, 11*(4), 511-520.

Brown, L. K. (2012). Can sleep deprivation studies explain why human adults sleep? *Curr Opin Pulm Med, 18*(6), 541-545. doi:10.1097/MCP.0b013e3283596740

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E., & McCarley, R. W. (2012). Control of sleep and wakefulness. *Physiol Rev, 92*(3), 1087-1187. doi:10.1152/physrev.00032.2011

Buzsaki, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Res, 398*(2), 242-252.

Buzsaki, G. (1998). Memory consolidation during sleep: a neurophysiological perspective. *J Sleep Res, 7 Suppl 1*, 17-23.

Buzsaki, G. (2002). Theta oscillations in the hippocampus. *Neuron, 33*(3), 325-340.

Buzsaki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus, 25*(10), 1073-1188. doi:10.1002/hipo.22488

Buzsaki, G., Leung, L. W., & Vanderwolf, C. H. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Res, 287*(2), 139-171.

Cantero, J. L., Atienza, M., Stickgold, R., Kahana, M. J., Madsen, J. R., & Kocsis, B. (2003). Sleep-dependent theta oscillations in the human hippocampus and neocortex. *J Neurosci, 23*(34), 10897-10903.

Chamberland, S., Salesse, C., Topolnik, D., & Topolnik, L. (2010). Synapse-specific inhibitory control of hippocampal feedback inhibitory circuit. *Front Cell Neurosci, 4*, 130. doi:10.3389/fncel.2010.00130

Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., . . . Yanagisawa, M. (1999). Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell, 98*(4), 437-451.

Clement, E. A., Richard, A., Thwaites, M., Ailon, J., Peters, S., & Dickson, C. T. (2008). Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. *PLoS One, 3*(4), e2004. doi:10.1371/journal.pone.0002004

Cohen, L. B., Salzberg, B. M., Davila, H. V., Ross, W. N., Landowne, D., Waggoner, A. S., & Wang, C. H. (1974). Changes in axon fluorescence during activity: molecular probes of membrane potential. *J Membr Biol, 19*(1), 1-36.

Contreras, D., Timofeev, I., & Steriade, M. (1996). Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *J Physiol, 494 (Pt 1)*, 251-264.

Datta, S. (1997). Cellular basis of pontine ponto-geniculo-occipital wave generation and modulation. *Cell Mol Neurobiol, 17*(3), 341-365.

Datta, S., & Hobson, J. A. (1994). Neuronal activity in the caudolateral peribrachial pons: relationship to PGO waves and rapid eye movements. *J Neurophysiol, 71*(1), 95-109.

Datta, S., Li, G., & Auerbach, S. (2008). Activation of phasic pontine-wave generator in the rat: a mechanism for expression of plasticity-related genes and proteins in the dorsal hippocampus and amygdala. *Eur J Neurosci, 27*(7), 1876-1892. doi:10.1111/j.1460-9568.2008.06166.x

De Carli, F., Proserpio, P., Morrone, E., Sartori, I., Ferrara, M., Gibbs, S. A., . . . Nobili, L. (2016). Activation of the motor cortex during phasic rapid eye movement sleep. *Ann Neurol, 79*(2), 326-330. doi:10.1002/ana.24556

De Gennaro, L., & Ferrara, M. (2003). Sleep spindles: an overview. *Sleep Med Rev, 7*(5), 423-440.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nat Rev Neurosci, 11*(2), 114-126. doi:10.1038/nrn2762

Eguchi, K., & Satoh, T. (1980). Characterization of the neurons in the region of solitary tract nucleus during sleep. *Physiol Behav, 24*(1), 99-102.

Euston, D. R., Tatsuno, M., & McNaughton, B. L. (2007). Fast-forward playback of recent memory sequences in prefrontal cortex during sleep. *Science, 318*(5853), 1147-1150. doi:10.1126/science.1148979

Ferezou, I., Haiss, F., Gentet, L. J., Aronoff, R., Weber, B., & Petersen, C. C. (2007). Spatiotemporal dynamics of cortical sensorimotor integration in behaving mice. *Neuron, 56*(5), 907-923. doi:10.1016/j.neuron.2007.10.007

Fernandez-Mendoza, J., Lozano, B., Seijo, F., Santamarta-Liebana, E., Ramos-Platon, M. J., Vela-Bueno, A., & Fernandez-Gonzalez, F. (2009). Evidence of subthalamic PGO-like waves during REM sleep in humans: a deep brain polysomnographic study. *Sleep, 32*(9), 1117-1126.

Fogel, S. M., Smith, C. T., & Cote, K. A. (2007). Dissociable learning-dependent changes in REM and non-REM sleep in declarative and procedural memory systems. *Behav Brain Res, 180*(1), 48-61. doi:10.1016/j.bbr.2007.02.037

Frotscher, M., & Leranth, C. (1985). Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study. *J Comp Neurol, 239*(2), 237-246. doi:10.1002/cne.902390210

Fuller, P. M., Sherman, D., Pedersen, N. P., Saper, C. B., & Lu, J. (2011). Reassessment of the structural basis of the ascending arousal system. *J Comp Neurol, 519*(5), 933-956. doi:10.1002/cne.22559

Funk, C. M., Honjoh, S., Rodriguez, A. V., Cirelli, C., & Tononi, G. (2016). Local Slow Waves in Superficial Layers of Primary Cortical Areas during REM Sleep. *Curr Biol, 26*(3), 396-403. doi:10.1016/j.cub.2015.11.062

Gallopin, T., Fort, P., Eggermann, E., Cauli, B., Luppi, P. H., Rossier, J., . . . Serafin, M. (2000). Identification of sleep-promoting neurons in vitro. *Nature, 404*(6781), 992-995. doi:10.1038/35010109

Gong, H., McGinty, D., Guzman-Marin, R., Chew, K. T., Stewart, D., & Szymusiak, R. (2004). Activation of c-fos in GABAergic neurones in the preoptic area during sleep and in response to sleep deprivation. *J Physiol, 556*(Pt 3), 935-946. doi:10.1113/jphysiol.2003.056622

Gott, J. A., Liley, D. T., & Hobson, J. A. (2017). Towards a Functional Understanding of PGO Waves. *Front Hum Neurosci, 11*, 89. doi:10.3389/fnhum.2017.00089

Green, J. D., & Arduini, A. A. (1954). Hippocampal electrical activity in arousal. *J Neurophysiol, 17*(6), 533-557.

Grinvald, A., Cohen, L. B., Lesher, S., & Boyle, M. B. (1981). Simultaneous optical monitoring of activity of many neurons in invertebrate ganglia using a 124-element photodiode array. *J Neurophysiol, 45*(5), 829-840.

Grinvald, A., & Hildesheim, R. (2004). VSDI: A new era in functional imaging of cortical dynamics. *Nature Reviews Neuroscience, 5*(11), 874-885. doi:10.1038/nrn1536

Haider, B., Duque, A., Hasenstaub, A. R., & McCormick, D. A. (2006). Neocortical network activity in vivo is generated through a dynamic balance of excitation and inhibition. *J Neurosci, 26*(17), 4535-4545. doi:10.1523/JNEUROSCI.5297-05.2006

Hangya, B., Borhegyi, Z., Szilagyi, N., Freund, T. F., & Varga, V. (2009). GABAergic neurons of the medial septum lead the hippocampal network during theta activity. *J Neurosci, 29*(25), 8094-8102. doi:10.1523/JNEUROSCI.5665-08.2009

Harris, K. D., & Thiele, A. (2011). Cortical state and attention. *Nat Rev Neurosci, 12*(9), 509-523. doi:10.1038/nrn3084

Hassani, O. K., Lee, M. G., & Jones, B. E. (2009). Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep-wake cycle. *Proc Natl Acad Sci U S A, 106*(7), 2418-2422. doi:10.1073/pnas.0811400106

Hasselmo, M. E., & McGaughy, J. (2004). High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation. *Prog Brain Res, 145*, 207-231. doi:10.1016/S0079-6123(03)45015-2

Hayashi, Y., Kashiwagi, M., Yasuda, K., Ando, R., Kanuka, M., Sakai, K., & Itohara, S. (2015). Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice. *Science, 350*(6263), 957-961. doi:10.1126/science.aad1023

Hebb, D. O. (1949). *The organization of behavior: A neuropsychological theory*. New York: John Wiley & Sons.

Hobson, J. A., Pace-Schott, E. F., & Stickgold, R. (2000). Dreaming and the brain: toward a cognitive neuroscience of conscious states. *Behav Brain Sci, 23*(6), 793-842; discussion 904-1121.

Jacobs, B. L., Henriksen, S. J., & Dement, W. C. (1972). Neurochemical bases of the PGO wave. *Brain Res, 48*, 406-411.

Jego, S., Glasgow, S. D., Herrera, C. G., Ekstrand, M., Reed, S. J., Boyce, R., . . . Adamantidis, A. R. (2013). Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. *Nat Neurosci, 16*(11), 1637-1643. doi:10.1038/nn.3522

Jenkins, J. G., & Dallenbach, K. M. (1924). Obliviscence during sleep and waking. *Am J Psychol, 35*, 605-612.

Ji, D., & Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nat Neurosci, 10*(1), 100-107. doi:10.1038/nn1825

Johnson, L. A., Euston, D. R., Tatsuno, M., & McNaughton, B. L. (2010). Stored-trace reactivation in rat prefrontal cortex is correlated with down-to-up state fluctuation density. *J Neurosci, 30*(7), 2650-2661. doi:10.1523/JNEUROSCI.1617-09.2010

Jouvet, M., Jeannerod, M., & Delorme, F. (1965). [Organization of the system responsible for phase activity during paradoxal sleep]. *C R Seances Soc Biol Fil, 159*(7), 1599-1604.

Kalmbach, A., Hedrick, T., & Waters, J. (2012). Selective optogenetic stimulation of cholinergic axons in neocortex. *J Neurophysiol, 107*(7), 2008-2019. doi:10.1152/jn.00870.2011

Karashima, A., Nakao, M., Katayama, N., & Honda, K. (2005). Instantaneous acceleration and amplification of hippocampal theta wave coincident with phasic pontine activities during REM sleep. *Brain Res, 1051*(1-2), 50-56. doi:10.1016/j.brainres.2005.05.055

Killgore, W. D. (2010). Effects of sleep deprivation on cognition. *Prog Brain Res, 185*, 105-129. doi:10.1016/B978-0-444-53702-7.00007-5

Kim, J. H., Jung, A. H., Jeong, D., Choi, I., Kim, K., Shin, S., . . . Lee, S. H. (2016). Selectivity of Neuromodulatory Projections from the Basal Forebrain and Locus Ceruleus to Primary Sensory Cortices. *J Neurosci, 36*(19), 5314-5327. doi:10.1523/JNEUROSCI.4333-15.2016

Korman, M., Doyon, J., Doljansky, J., Carrier, J., Dagan, Y., & Karni, A. (2007). Daytime sleep condenses the time course of motor memory consolidation. *Nat Neurosci, 10*(9), 1206-1213. doi:10.1038/nn1959

Krenzer, M., Anaclet, C., Vetrivelan, R., Wang, N., Vong, L., Lowell, B. B., . . . Lu, J. (2011). Brainstem and spinal cord circuitry regulating REM sleep and muscle atonia. *PLoS One, 6*(10), e24998. doi:10.1371/journal.pone.0024998

Krnjevic, K. (2004). Synaptic mechanisms modulated by acetylcholine in cerebral cortex. *Prog Brain Res, 145*, 81-93.

Lansink, C. S., Goltstein, P. M., Lankelma, J. V., Joosten, R. N., McNaughton, B. L., & Pennartz, C. M. (2008). Preferential reactivation of motivationally relevant information in the ventral striatum. *J Neurosci, 28*(25), 6372-6382. doi:10.1523/JNEUROSCI.1054-08.2008

Lansink, C. S., Goltstein, P. M., Lankelma, J. V., McNaughton, B. L., & Pennartz, C. M. (2009). Hippocampus leads ventral striatum in replay of place-reward information. *PLoS Biol, 7*(8), e1000173. doi:10.1371/journal.pbio.1000173

Lee, M. G., Hassani, O. K., Alonso, A., & Jones, B. E. (2005). Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. *J Neurosci, 25*(17), 4365-4369. doi:10.1523/JNEUROSCI.0178-05.2005

Lee, S. H., & Dan, Y. (2012). Neuromodulation of brain states. *Neuron, 76*(1), 209-222. doi:10.1016/j.neuron.2012.09.012

Leung, L. W., & Borst, J. G. (1987). Electrical activity of the cingulate cortex. I. Generating mechanisms and relations to behavior. *Brain Res, 407*(1), 68-80.

Lim, A. S., Lozano, A. M., Moro, E., Hamani, C., Hutchison, W. D., Dostrovsky, J. O., . . . Murray, B. J. (2007). Characterization of REM-sleep associated ponto-geniculo-occipital waves in the human pons. *Sleep, 30*(7), 823-827.

Lippert, M. T., Takagaki, K., Xu, W., Huang, X., & Wu, J. Y. (2007). Methods for voltage-sensitive dye imaging of rat cortical activity with high signal-to-noise ratio. *J Neurophysiol, 98*(1), 502-512. doi:10.1152/jn.01169.2006

Liu, Y. W., Li, J., & Ye, J. H. (2010). Histamine regulates activities of neurons in the ventrolateral preoptic nucleus. *J Physiol, 588*(Pt 21), 4103-4116. doi:10.1113/jphysiol.2010.193904

Louie, K., & Wilson, M. A. (2001). Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron, 29*(1), 145-156.

Lu, J., Greco, M. A., Shiromani, P., & Saper, C. B. (2000). Effect of lesions of the ventrolateral preoptic nucleus on NREM and REM sleep. *J Neurosci, 20*(10), 3830-3842.

Lu, J., Jhou, T. C., & Saper, C. B. (2006). Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *J Neurosci, 26*(1), 193-202. doi:10.1523/JNEUROSCI.2244-05.2006

Lu, J., Sherman, D., Devor, M., & Saper, C. B. (2006). A putative flip-flop switch for control of REM sleep. *Nature, 441*(7093), 589-594. doi:10.1038/nature04767

Madsen, P. L., Schmidt, J. F., Wildschiodtz, G., Friberg, L., Holm, S., Vorstrup, S., & Lassen, N. A. (1991). Cerebral O2 metabolism and cerebral blood flow in humans during deep and rapid-eye-movement sleep. *J Appl Physiol (1985), 70*(6), 2597-2601.

Magnes, J., Moruzzi, G., & Pompeiano, O. (1960). Synchronization of the EEG produced by low-frequency electrical stimulation of the region of the solitary tract. *Arch Ital Biol, 99*, 33-67.

Massimini, M., Ferrarelli, F., Huber, R., Esser, S. K., Singh, H., & Tononi, G. (2005). Breakdown of cortical effective connectivity during sleep. *Science, 309*(5744), 2228-2232. doi:10.1126/science.1117256

McGinty, D., & Szymusiak, R. (1990). Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends Neurosci, 13*(12), 480-487.

McVea, D. A., Murphy, T. H., & Mohajerani, M. H. (2016). Large Scale Cortical Functional Networks Associated with Slow-Wave and Spindle-Burst-Related Spontaneous Activity. *Front Neural Circuits, 10*, 103. doi:10.3389/fncir.2016.00103

Mileykovskiy, B. Y., Kiyashchenko, L. I., & Siegel, J. M. (2005). Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron, 46*(5), 787-798. doi:10.1016/j.neuron.2005.04.035

Mitchell, S. J., & Ranck, J. B., Jr. (1980). Generation of theta rhythm in medial entorhinal cortex of freely moving rats. *Brain Res, 189*(1), 49-66.

Miyauchi, S., Misaki, M., Kan, S., Fukunaga, T., & Koike, T. (2009). Human brain activity time-locked to rapid eye movements during REM sleep. *Exp Brain Res, 192*(4), 657-667. doi:10.1007/s00221-008-1579-2

Mohajerani, M. H., Chan, A. W., Mohsenvand, M., LeDue, J., Liu, R., McVea, D. A., . . . Murphy, T. H. (2013). Spontaneous cortical activity alternates between motifs defined by regional axonal projections. *Nature Neuroscience, 16*(10), 1426-+. doi:10.1038/nn.3499

Mohajerani, M. H., McVea, D. A., Fingas, M., & Murphy, T. H. (2010). Mirrored bilateral slow-wave cortical activity within local circuits revealed by fast bihemispheric voltage-sensitive dye imaging in anesthetized and awake mice. *J Neurosci, 30*(10), 3745-3751. doi:10.1523/JNEUROSCI.6437-09.2010

Molle, M., & Born, J. (2009). Hippocampus whispering in deep sleep to prefrontal cortex--for good memories? *Neuron, 61*(4), 496-498. doi:10.1016/j.neuron.2009.02.002

Molle, M., Marshall, L., Gais, S., & Born, J. (2002). Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. *J Neurosci, 22*(24), 10941-10947.

Montgomery, S. M., Sirota, A., & Buzsaki, G. (2008). Theta and gamma coordination of hippocampal networks during waking and rapid eye movement sleep. *J Neurosci, 28*(26), 6731-6741. doi:10.1523/JNEUROSCI.1227-08.2008

Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J., & Buzsaki, G. (1999). Replay and time compression of recurring spike sequences in the hippocampus. *J Neurosci, 19*(21), 9497-9507.

Nir, Y., Staba, R. J., Andrillon, T., Vyazovskiy, V. V., Cirelli, C., Fried, I., & Tononi, G. (2011). Regional Slow Waves and Spindles in Human Sleep. *Neuron, 70*(1), 153-169. doi:10.1016/j.neuron.2011.02.043

Nishida, M., Pearsall, J., Buckner, R. L., & Walker, M. P. (2009). REM sleep, prefrontal theta, and the consolidation of human emotional memory. *Cereb Cortex, 19*(5), 1158-1166. doi:10.1093/cercor/bhn155

Nobili, L., Ferrara, M., Moroni, F., De Gennaro, L., Russo, G. L., Campus, C., . . . De Carli, F. (2011). Dissociated wake-like and sleep-like electro-cortical activity during sleep. *Neuroimage, 58*(2), 612-619. doi:10.1016/j.neuroimage.2011.06.032

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. *Exp Neurol, 51*(1), 78-109.

Orbach, H. S., Cohen, L. B., & Grinvald, A. (1985). Optical mapping of electrical activity in rat somatosensory and visual cortex. *J Neurosci, 5*(7), 1886-1895.

Pagliardini, S., Funk, G. D., & Dickson, C. T. (2013). Breathing and brain state: urethane anesthesia as a model for natural sleep. *Respir Physiol Neurobiol, 188*(3), 324-332. doi:10.1016/j.resp.2013.05.035

Pagliardini, S., Gosgnach, S., & Dickson, C. T. (2013). Spontaneous sleep-like brain state alternations and breathing characteristics in urethane anesthetized mice. *PLoS One, 8*(7), e70411. doi:10.1371/journal.pone.0070411

Pare, D., & Collins, D. R. (2000). Neuronal correlates of fear in the lateral amygdala: multiple extracellular recordings in conscious cats. *J Neurosci, 20*(7), 2701-2710.

Pavlides, C., & Winson, J. (1989). Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. *J Neurosci, 9*(8), 2907-2918.

Pearson, K. G., Acharya, H., & Fouad, K. (2005). A new electrode configuration for recording electromyographic activity in behaving mice. *J Neurosci Methods, 148*(1), 36-42. doi:10.1016/j.jneumeth.2005.04.006

Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nat Neurosci, 12*(7), 919-926. doi:10.1038/nn.2337

Plihal, W., & Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *J Cogn Neurosci, 9*(4), 534-547. doi:10.1162/jocn.1997.9.4.534

Poe, G. R., Nitz, D. A., McNaughton, B. L., & Barnes, C. A. (2000). Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. *Brain Res, 855*(1), 176-180.

Rao, Y., Lu, M., Ge, F., Marsh, D. J., Qian, S., Wang, A. H., . . . Gao, X. B. (2008). Regulation of synaptic efficacy in hypocretin/orexin-containing neurons by melanin concentrating hormone in the lateral hypothalamus. *J Neurosci, 28*(37), 9101-9110. doi:10.1523/JNEUROSCI.1766-08.2008

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiol Rev, 93*(2), 681-766. doi:10.1152/physrev.00032.2012

Rasch, B., Buchel, C., Gais, S., & Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science, 315*(5817), 1426-1429. doi:10.1126/science.1138581

Rauchs, G., Bertran, F., Guillery-Girard, B., Desgranges, B., Kerrouche, N., Denise, P., . . . Eustache, F. (2004). Consolidation of strictly episodic memories mainly requires rapid eye movement sleep. *Sleep, 27*(3), 395-401.

Rechtschaffen, A., & Bergmann, B. M. (1995). Sleep deprivation in the rat by the disk-over-water method. *Behav Brain Res, 69*(1-2), 55-63.

Ribeiro, S., Gervasoni, D., Soares, E. S., Zhou, Y., Lin, S. C., Pantoja, J., . . . Nicolelis, M. A. (2004). Long-lasting novelty-induced neuronal reverberation during slow-wave sleep in multiple forebrain areas. *PLoS Biol, 2*(1), E24. doi:10.1371/journal.pbio.0020024

Ribeiro, S., Mello, C. V., Velho, T., Gardner, T. J., Jarvis, E. D., & 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*(24), 10914-10923.

Ribeiro, S., Shi, X., Engelhard, M., Zhou, Y., Zhang, H., Gervasoni, D., . . . Nicolelis, M. A. (2007). Novel experience induces persistent sleep-dependent plasticity in the cortex but not in the hippocampus. *Front Neurosci, 1*(1), 43-55. doi:10.3389/neuro.01.1.1.003.2007

Robinson, T. E., Kramis, R. C., & Vanderwolf, C. H. (1977). Two types of cerebral activation during active sleep: relations to behavior. *Brain Res, 124*(3), 544-549.

Roffwarg, H. P., Muzio, J. N., & Dement, W. C. (1966). Ontogenetic development of the human sleep-dream cycle. *Science, 152*(3722), 604-619. doi:10.1126/science.152.3722.604

Sainsbury, R. S., & Bland, B. H. (1981). The effects of selective septal lesions on theta production in CA1 and the dentate gyrus of the hippocampus. *Physiol Behav, 26*(6), 1097-1101.

Salzberg, B. M., Davila, H. V., & Cohen, L. B. (1973). Optical recording of impulses in individual neurones of an invertebrate central nervous system. *Nature, 246*(5434), 508-509.

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J., & Scammell, T. E. (2010). Sleep state switching. *Neuron, 68*(6), 1023-1042. doi:10.1016/j.neuron.2010.11.032

Sastre, J. P., Buda, C., Kitahama, K., & Jouvet, M. (1996). Importance of the ventrolateral region of the periaqueductal gray and adjacent tegmentum in the control of paradoxical sleep as studied by muscimol microinjections in the cat. *Neuroscience, 74*(2), 415-426.

Sherin, J. E., Elmquist, J. K., Torrealba, F., & Saper, C. B. (1998). Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J Neurosci, 18*(12), 4705-4721.

Sherin, J. E., Shiromani, P. J., McCarley, R. W., & Saper, C. B. (1996). Activation of ventrolateral preoptic neurons during sleep. *Science, 271*(5246), 216-219.

Shimaoka, D., Song, C., & Knopfel, T. (2017). State-Dependent Modulation of Slow Wave Motifs towards Awakening. *Front Cell Neurosci, 11*, 108. doi:10.3389/fncel.2017.00108

Shoham, D., Glaser, D. E., Arieli, A., Kenet, T., Wijnbergen, C., Toledo, Y., . . . Grinvald, A. (1999). Imaging cortical dynamics at high spatial and temporal resolution with novel blue voltage-sensitive dyes. *Neuron, 24*(4), 791-802. doi:Doi 10.1016/S0896-6273(00)81027-2

Shore, E. T., Millman, R. P., Silage, D. A., Chung, D. C., & Pack, A. I. (1985). Ventilatory and arousal patterns during sleep in normal young and elderly subjects. *J Appl Physiol (1985), 59*(5), 1607-1615.

Shu, Y., Hasenstaub, A., & McCormick, D. A. (2003). Turning on and off recurrent balanced cortical activity. *Nature, 423*(6937), 288-293. doi:10.1038/nature01616

Siegel, J. M. (2009). Sleep viewed as a state of adaptive inactivity. *Nat Rev Neurosci, 10*(10), 747-753. doi:10.1038/nrn2697

Sirota, A., Csicsvari, J., Buhl, D., & Buzsaki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proc Natl Acad Sci U S A, 100*(4), 2065-2069. doi:10.1073/pnas.0437938100

Snyder, F., Hobson, J. A., Morrison, D. F., & Goldfrank, F. (1964). Changes in Respiration, Heart Rate, and Systolic Blood Pressure in Human Sleep. *J Appl Physiol, 19*, 417-422.

Steininger, T. L., Gong, H., McGinty, D., & Szymusiak, R. (2001). Subregional organization of preoptic area/anterior hypothalamic projections to arousal-related monoaminergic cell groups. *J Comp Neurol, 429*(4), 638-653.

Steriade, M., & McCarley, R. W. (2005). *Brainstem Control of Wakefulness and Sleep*. New York: Plenum Press.

Steriade, M., McCormick, D. A., & Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science, 262*(5134), 679-685.

Steriade, M., Timofeev, I., & Grenier, F. (2001). Natural waking and sleep states: a view from inside neocortical neurons. *J Neurophysiol, 85*(5), 1969-1985.

Stickgold, R., James, L., & Hobson, J. A. (2000). Visual discrimination learning requires sleep after training. *Nat Neurosci, 3*(12), 1237-1238. doi:10.1038/81756

Sutcliffe, J. G., & de Lecea, L. (2002). The hypocretins: setting the arousal threshold. *Nat Rev Neurosci, 3*(5), 339-349. doi:10.1038/nrn808

Talley, E. M., Cribbs, L. L., Lee, J. H., Daud, A., Perez-Reyes, E., & Bayliss, D. A. (1999). Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. *J Neurosci, 19*(6), 1895-1911.

Tasaki, I., & Warashina, A. (1976). Dye-membrane interaction and its changes during nerve excitation. *Photochem Photobiol, 24*(2), 191-207.

Teber, I., Kohling, R., Speckmann, E. J., Barnekow, A., & Kremerskothen, J. (2004). Muscarinic acetylcholine receptor stimulation induces expression of the activity-regulated cytoskeleton-associated gene (ARC). *Brain Res Mol Brain Res, 121*(1-2), 131-136. doi:10.1016/j.molbrainres.2003.11.017

Thakkar, M. M. (2011). Histamine in the regulation of wakefulness. *Sleep Med Rev, 15*(1), 65-74. doi:10.1016/j.smrv.2010.06.004

Traub, R. D., & Wong, R. K. (1982). Cellular mechanism of neuronal synchronization in epilepsy. *Science, 216*(4547), 745-747.

Tsanov, M. (2015). Septo-hippocampal signal processing: breaking the code. *Prog Brain Res, 219*, 103-120. doi:10.1016/bs.pbr.2015.04.002

Tsunematsu, T., Ueno, T., Tabuchi, S., Inutsuka, A., Tanaka, K. F., Hasuwa, H., . . . Yamanaka, A. (2014). Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. *J Neurosci, 34*(20), 6896-6909. doi:10.1523/JNEUROSCI.5344-13.2014

Ulloor, J., & Datta, S. (2005). Spatio-temporal activation of cyclic AMP response element-binding protein, activity-regulated cytoskeletal-associated protein and brain-derived nerve growth factor: a mechanism for pontine-wave generator activation-dependent two-way

active-avoidance memory processing in the rat. *J Neurochem, 95*(2), 418-428. doi:10.1111/j.1471-4159.2005.03378.x

Van Dort, C. J., Zachs, D. P., Kenny, J. D., Zheng, S., Goldblum, R. R., Gelwan, N. A., . . . Brown, E. N. (2015). Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. *Proc Natl Acad Sci U S A, 112*(2), 584-589. doi:10.1073/pnas.1423136112

Varin, C., Rancillac, A., Geoffroy, H., Arthaud, S., Fort, P., & Gallopin, T. (2015). Glucose Induces Slow-Wave Sleep by Exciting the Sleep-Promoting Neurons in the Ventrolateral Preoptic Nucleus: A New Link between Sleep and Metabolism. *J Neurosci, 35*(27), 9900-9911. doi:10.1523/JNEUROSCI.0609-15.2015

von der Kammer, H., Mayhaus, M., Albrecht, C., Enderich, J., Wegner, M., & Nitsch, R. M. (1998). Muscarinic acetylcholine receptors activate expression of the EGR gene family of transcription factors. *J Biol Chem, 273*(23), 14538-14544.

Von Economo, C. (1930). Sleep as a problem of localization. *J Nerv Ment Dis, 71*(3), 249-259.

Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011). Local sleep in awake rats. *Nature, 472*(7344), 443-447. doi:10.1038/nature10009

Wagner, U., Gais, S., & Born, J. (2001). Emotional memory formation is enhanced across sleep intervals with high amounts of rapid eye movement sleep. *Learn Mem, 8*(2), 112-119. doi:10.1101/lm.36801

Wagner, U., Hallschmid, M., Rasch, B., & Born, J. (2006). Brief sleep after learning keeps emotional memories alive for years. *Biol Psychiatry, 60*(7), 788-790. doi:10.1016/j.biopsych.2006.03.061

Weber, F., Chung, S., Beier, K. T., Xu, M., Luo, L., & Dan, Y. (2015). Control of REM sleep by ventral medulla GABAergic neurons. *Nature, 526*(7573), 435-438. doi:10.1038/nature14979

Weber, F., & Dan, Y. (2016). Circuit-based interrogation of sleep control. *Nature, 538*(7623), 51-59. doi:10.1038/nature19773

Wehrle, R., Kaufmann, C., Wetter, T. C., Holsboer, F., Auer, D. P., Pollmacher, T., & Czisch, M. (2007). Functional microstates within human REM sleep: first evidence from fMRI of a thalamocortical network specific for phasic REM periods. *Eur J Neurosci, 25*(3), 863-871. doi:10.1111/j.1460-9568.2007.05314.x

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science, 265*(5172), 676-679.

Xu, M., Chung, S., Zhang, S., Zhong, P., Ma, C., Chang, W. C., . . . Dan, Y. (2015). Basal forebrain circuit for sleep-wake control. *Nat Neurosci, 18*(11), 1641-1647. doi:10.1038/nn.4143

Yamanaka, A., Beuckmann, C. T., Willie, J. T., Hara, J., Tsujino, N., Mieda, M., . . . Sakurai, T. (2003). Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron, 38*(5), 701-713.

Yoshida, K., McCormack, S., Espana, R. A., Crocker, A., & Scammell, T. E. (2006). Afferents to the orexin neurons of the rat brain. *J Comp Neurol, 494*(5), 845-861. doi:10.1002/cne.20859

Zaborszky, L. (2002). The modular organization of brain systems. Basal forebrain: the last frontier. *Prog Brain Res, 136*, 359-372.

Zemaityte, D., Varoneckas, G., & Sokolov, E. (1984). Heart rhythm control during sleep. *Psychophysiology, 21*(3), 279-289.

Zhang, Z., Ferretti, V., Guntan, I., Moro, A., Steinberg, E. A., Ye, Z., . . . Franks, N. P. (2015). Neuronal ensembles sufficient for recovery sleep and the sedative actions of alpha2 adrenergic agonists. *Nat Neurosci, 18*(4), 553-561. doi:10.1038/nn.3957