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SLEEP AND THERMOREGULATION: A STUDY OF THE EFFECT OF AMBIENT TEMPERATURE MANIPULATION ON MOUSE SLEEP ARCHITECTURE

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Engineering at the University of Kentucky

By

Asma'a A. Ajwad

Lexington, KY

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Lexington, Kentucky

2018

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ABSTRACT OF DISSERTATION

SLEEP AND THERMOREGULATION: A STUDY OF THE EFFECT OF AMBIENT TEMPERATURE MANIPULATION ON MOUSE SLEEP ARCHITECTURE

Good quality sleep is essential for mental and physical health. Inadequate sleep impacts memory consolidation, learning and cognition, immune function, autonomic regulation, physical performance, and other vital functions. In many neurological disorders that are associated with sleep problems such as epilepsy and Alzheimer's disease, changes in brain circuitry affect sleep-wake regulation mechanisms; this is reflected in anomalous sleep-wake architecture and usually accompanied by poor sleep depth. Thus, over many years, many approaches have been tried in humans and animal models with the goal of improving sleep quality. Unfortunately, each of those approaches comes with limitations or side effects. Thus, there is a need for a natural, safe, and low cost approach that overcomes many limitations to improve sleep and eventually the lives of individuals with sleep problems.

Environmental temperature is one of the most important factors that affect sleep in humans and other animals. Studies have shown that the part of the brain governing thermoregulation is also involved in sleep-wake regulation. Even a mild change in environmental temperature can produce a significant effect on sleep. Thus, a better understanding of the sleep-thermoregulation interaction could lead to novel ways for treating many sleep disorders. As a first step on the translational pathway, experiments in animal models of disease conditions with disordered sleep are needed for investigating sleep–thermoregulation interactions and for devising and validating related approaches to enhance sleep quality before conducting them on humans.

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This dissertation explores and assesses the effect of changes in ambient temperature on sleep-wake architecture in control mice and epileptic mice, the latter from a model of temporal lobe epilepsy as an example of a disease model with disordered sleep. Then, based on the results of temperature effects on sleep in control and epileptic mice, different strategies are proposed and tested to modulate sleep through ambient temperature regulation in closed loop to improve sleep depth and regulate the timing of the sleep-wake cycle.

The results presented in this dissertation demonstrate the feasibility of sleep enhancement and regulation of its timing and duration through manipulation of ambient temperature using closed-loop control systems. Similar approaches could foreseeably be used as more natural means for enhancing deep sleep in patients with epilepsy, Alzheimer's, or Parkinson's disease in which poor sleep is common and associated with adverse outcomes.

Keywords: Sleep depth, Thermoregulation, Epilepsy, Closed-loop system, Mice.

Asma'a A. Ajwad

November 19, 2018

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November 19, 2018

Date

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DEDICATION

To

The memory of my father

My mother, sisters, brothers, and Aseel M. Ali

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First of all, I would like to express my sincere gratitude and thanks to my advisor Dr. Sridhar Sunderam for all continuous support he gave me, his patience, motivation, and enthusiastic spirit he lifted in me, and his outstanding guidance over the past five years. I am deeply indebted and grateful to him for all insightful discussions and suggestions. Completion of this dissertation would not have been possible without his advice and support. I do consider myself to be extremely privileged to have been his student.

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CHAPTER I INTRODUCTION

1.1 Significance

It is well known that sleep plays an important role in brain and body functions, thus its disruption may come with dangerous consequences to human health. Poor sleep is common in many neurological disorders including epilepsy and Alzheimer's disease. It may present as reduced sleep depth, fragmented sleep, altered proportions of different sleep stages, and other phenomena. In this dissertation, an animal model of temporal lobe epilepsy has been used as an example of neurological diseases that are associated with poor sleep.

Epilepsy is a serious neurological disorder that is characterized by recurrent spontaneous partial or generalized seizures. It is estimated that the proportion of the global population with active epilepsy (i.e. continuing seizures or with the need for treatment) is between 4 and 10 per 1000 people. This proportion is getting higher and higher in developing countries and can reach 6 to 10 per 1000 people (World Health Organization 2018). Epileptic seizures could happen at any age as well as at different levels of severity. They vary from brief twitches to prolonged and severe convulsions (Megiddo *et al.* 2016). Despite the fact that there is improvement in the types of available epilepsy treatment, about 30-40% of epilepsy patients fail to respond to antiepileptic drugs or other types of interventions (Laxer *et al*. 2014). Considering that even patients who respond to the available epilepsy treatments may experience complications and medical problems such as depression (Fisher *et al*. 2000), it is important to understand the complex mechanisms and factors that may have an effect on epilepsy. This understanding could lead to novel treatments that would modify its generation/progression and eventually control or reduce seizures in epilepsy patients, thus avoiding at least some of the medical complications associated with current available treatment.

The timing and structure of the sleep-wake cycle are mainly determined by the interaction of homeostatic and circadian drives. The homeostatic drive depends on the amount of preceding wakefulness: the longer the time spent awake, the greater the propensity to sleep.

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Circadian drive on the other hand is independent of the amount of preceding sleep or wakefulness, it promotes sleep during the night and wakefulness during the daylight hours (Hofstra and de Weerd 2009). The interactions between sleep and epilepsy are clinically relevant; a better understanding of these interactions will help in the development of therapeutic approaches for epilepsy and eventually improve the quality of life of epilepsy patients. Inadequate or fragmented sleep is common in epilepsy patients, as has been documented in several studies. Arielle Crespel (Crespel *et al*. 2000) described the effect of epilepsy and seizures on sleep at two levels :1) acute effects of seizures during sleep that result in non-continuous or fragmented sleep; and 2) chronic effects of epilepsy that impairs the whole organization and microstructure of sleep. These two effects depend on the type of seizures and the location of the epileptic focus. Arielle Crespel's study analyzed the occurrence of frontal and temporal lobe epileptic seizures in 30 patients using continues video and EEG monitoring. While patients with frontal lobe epilepsy have normal sleep organization in spite of the effect of seizures on sleep microstructure, patients with temporal lobe epilepsy experience significant sleep fragmentation, low sleep efficiency (defined by the ratio of total sleep time to total amount of time in bed (Reed and Sacco 2016)) , an increase in the number of wake episodes, and abnormalities in the architecture of their sleep. Patients with frontal lobe epilepsy have 61% of their seizures in sleep, whereas temporal lobe epilepsy patients have only 11% of their seizures in sleep (Crespel *et al*. 1998) . The effects of temporal lobe epilepsy on sleep architecture have been studied using polysomnographic recording (Bazil and Walczak 1997). Results showed that when seizures occur during the day, there is a significant reduction in REM (Rapid Eye Movement) sleep the following night, even in the absence of seizures that night, and without changing other sleep stages or efficiency. In contrast, when seizures occur early in night, there is a greater reduction in REM sleep with an increase in stage I of NREM (Non-Rapid Eye Movement) accompanied by a clear reduction in sleep efficiency (Bazil and Walczak 1997). Since epileptic seizures have a significant effect on sleep architecture and very likely other physiological systems, more attention should be paid to the type, time, and origin of seizure.

The sleep-epilepsy relationship is reciprocal, which means that not only do epileptic seizures affect sleep but different sleep stages also have an effect on seizure probability.

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NREM sleep, particularly stage II, could facilitate epileptic episodes and their spread due to the broadly synchronized nature of neural activity during NREM sleep. In contrast, REM sleep which is marked by desynchronized discharges could inhibit epileptic episodes (Shouse *et al*. 2000). In a study that was conducted on 188 patients with more than 1000 partial seizures, data showed that most seizures occurred during stage II of NREM sleep with few in stages III and IV and fewer or none in REM sleep (Bazil and Walczak 1997). Additionally, the results have shown that temporal lobe seizures are more likely to generalize when they occur in sleep while those of frontal lobe origin occur equally in sleep and wake states. In conclusion, the type of epileptic seizures influences sleep stages and sleep stages influence the facilitating or inhibiting mechanisms of sleep on epileptic seizures.

Thermoregulation is an important process that has been strongly linked to sleep. It is well known that thermoregulatory responses to change in ambient temperature (*Ta*) are highly related to sleep regulation mechanisms (Gilbert *et al.* 2004). Studies have shown that the preoptic area in the hypothalamus plays an important role in thermoregulation. It is also involved in sleep-wake regulation mechanisms (Romanovsky 2006 and Nakamura 2011). Though the neural pathways underlying the physiological interactions between thermoregulation and sleep regulation are still not very clear, studies have suggested that hypothalamic neurons that release hypocretin (HCRT) and receive projections from the preoptic area may have a major role in those pathways. The firing rate of HCRT neurons is state-dependent, decreasing from active wakefulness to quiet wakefulness, NREM sleep, and REM sleep (Sakurai *et al.* 2005 and Mileykovskiy *et al*. 2005).

It has been shown that changing the ambient temperature produces a significant effect on sleep (Jhaveri *et al*. 2007, Schmidek *et al.* 1972, Szymusiak and Satinoff 1984, Roussel *et al*. 1984). Given that temperature affects sleep and poor sleep is common in sleep-related disorders including epilepsy (St. Louis 2013) , here we hypothesize that manipulating *Ta* could be used as a noninvasive way to modulate sleep in a direction that enhances sleep quality and helps in the treatment of sleep disorders. Figure 1.1 shows a schematic diagram of an example of the study hypothesis of using temperature change as a simple means to enhance sleep in epilepsy patients and balance its effect on epileptic seizures.

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Reproducing human diseases in animal models has a great impact on developing the understanding of the disease and discovering different ways that can help in its diagnosis and treatment. After treatment with pilocarpine, mice can go on to display symptoms of temporal lobe epilepsy (TLE), the most common and difficult to treat type of epilepsy in adults (Lévesque *et al*. 2016). Those symptoms include status epilepticus (SE), a latent period, and recurring spontaneous seizures (Shibley and Smith 2002). Considering that studying the correlation between thermoregulation, sleep, and epilepsy (as an example of neurological disorders associated with poor sleep) is difficult in humans; and that mice offer convenient and suitable disease models in which brain circuitry and pathology is similar to humans (Carlos *et al.* 2016), a mouse model of TLE was used in this study to investigate the effect of temperature changes on sleep and epileptic seizures.

Closed- loop control systems have been applied to many neurophysiological problems. It has been used to alter alpha rhythm dynamics in human (Zhigalov *et al*. 2016), drive slow wave sleep through auditory stimulation (Ngo *et al.* 2015), and detect and control epileptic seizures through optogenetic inhibition of specific neuronal circuits in mice (Krook-Magnuson *et al.* 2013). Here, a closed loop temperature control system is employed to test the feasibility of: sleep modulation, sleep depth enhancement, and entrainment of the ultradian sleep-wake cycle to an externally imposed rhythm in mice.

This dissertation addresses three key aspects in studying the effects of thermal regulation on sleep in control and epileptic mice: *first*, the effect of an acute elevation in *Ta* on sleep structure and depth in control mice has been characterized; *second,* the effects of *Ta* elevation on sleep and epileptic seizures in the pilocarpine mouse model of temporal lobe epilepsy have been investigated; and *finally,* different strategies for dynamic sleep modulation by *Ta* regulation in control mice have been developed as a first step toward using them in future as a noninvasive route to treat sleep-related disorders. In summary, the main contribution of the work described in this dissertation is to demonstrate the feasibility of sleep enhancement through *Ta* regulation in mice.

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1.2 Specific aims

Aim I: Effect of an acute elevation in Ta on sleep in control mice

Changes in *Ta* elicit thermoregulatory responses that also influence the sleep-wake structure. Thus, this study aimed to evaluate the effect of *Ta* elevation on different vigilance states (Wake, NREM (slow wave sleep & light NREM sleep), and REM) in control mice with long term objective of using the findings of the study in modulating sleep and investigating the consequences in health and disease. Sleep quality is often identified by some conventional sleep metrics namely: proportion of time spent, mean bout duration, and mean number of bouts per state. As a first specific aim of this study, mice (*n=13*) were exposed to one of four temperatures (24, 27, 30, and 33 Celsius) in their thermoneutral zone on four consecutive days for several hours. Sleep metrics were computed and the effects of an acute elevation in *Ta* and the time were statistically evaluated. Sleep depth defined by more than one measure was the other sleep metric that has been used to characterize *Ta* effect on sleep quality. NREM sleep was further classified into light NREM sleep and slow wave sleep (deep sleep) using EEG zero-crossing criteria and the effect of *Ta* and time on both states was studied. Our data showed that an acute elevation in *Ta* promotes sleep depth as well as continuity significantly.

Aim II: Effect of Ta on sleep and epileptic seizures in a mouse model of temporal lobe epilepsy (TLE)

Seizures in individuals with epilepsy are often accompanied by poor sleep depth and intermittent arousals. Poor sleep quality in turn could precipitate seizures, thus sustaining a vicious cycle. Given that thermoregulation interacts with sleep and that sleep and epilepsy have mutual effects, it seems plausible that improvement in sleep quality - induced by deliberate changes in *Ta* – could possibly alleviate the seizure burden in patients with epilepsy if properly implemented. As a step toward this goal, we assessed the effect of elevated *Ta* on sleep dynamics and seizure yield in a chronic pilocarpine mouse model of TLE. Each epileptic mouse (*n=4*) was exposed to an elevated *Ta* of 30°C, the thermoneutral zone, every other day for up to two weeks, only during the 14-hour light period (7 a.m-9 p.m.). The results showed that temperature does in fact significantly affect

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the sleep-wake architecture. There was some variability in mean seizure rate across the animals but no significant effect of *Ta* on seizure rate or timing was observed.

Aim III: Sleep depth enhancement by dynamic Ta regulation

The third aim of this study is to demonstrate the feasibility of dynamic sleep enhancement by manipulating ambient temperature in closed loop; i.e. in response to observed behavior as a step toward using it to improve sleep in patients with sleep disorders. In this context, the feasibility of sleep modulation through *Ta* regulation has been tested. Using an automated EEG/EMG hidden Markov Model (HMM) classifier, the vigilance state of control animals was predicted in real time with 1-second resolution. A control policy was used to trigger *Ta* changes in a direction that reduced the error between an estimated sleep metric q (sleep/wake ratio) and pre-specified q^* (target sleep metric). The results suggested that it is feasible to modulate sleep through temperature regulation in control mice using any sleep quality metric.

Given that slow wave sleep (SWS), i.e., Deep NREM sleep (DS), is beneficial to healthy brain and body functions, a simple closed-loop strategy for sleep depth modulation (SDM) was developed to guide the EEG delta (0.5-4Hz)-theta (6-9Hz) power ratio Q — a measure of sleep depth that is typically high in NREM, low in Wake, and still lower in REM — towards a target value Q^* by manipulating *Ta*. *Ta* was changed by ± 1 ºC every 5 min in a direction that reduced the error between Q and Q^*. The results showed that mice ($n=5$) spent more time in SWS with some other subtle changes during dynamic manipulation of *Ta* compared to baseline recordings in which *Ta* was held constant at 25°C.

Aim IV: Sleep-wake induction by Ta regulation

Rodents exhibit polyphasic sleep in which multiple sleep and wake bouts are distributed over the 24-hour cycle and their timing and duration are modulated by the circadian rhythm. Results from static experiments (aim I) have shown that elevation in *Ta* influences the duration of those sleep and wake bouts. Thus, we tested whether this ultradian sleep-wake cycle can be entrained to an externally imposed rhythm by manipulating *Ta*. To

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achieve this aim, a sleep-wake induction (SWI) strategy was developed in which Q^* was programmed to follow exponential growth over 60 min and decay over 30 min between values of Q (EEG delta-theta power ratio) typical of NREM sleep and wake respectively. Trends in EEG measures Q and the Hi-Low bandpower ratio (8-30 Hz / 0.5-8 Hz) were compared against baseline days to determine the effect of the regulatory cycle. Results suggested that Q follows Q^* in sleep and wake with a finite delay in switching from sleep to wake phases.

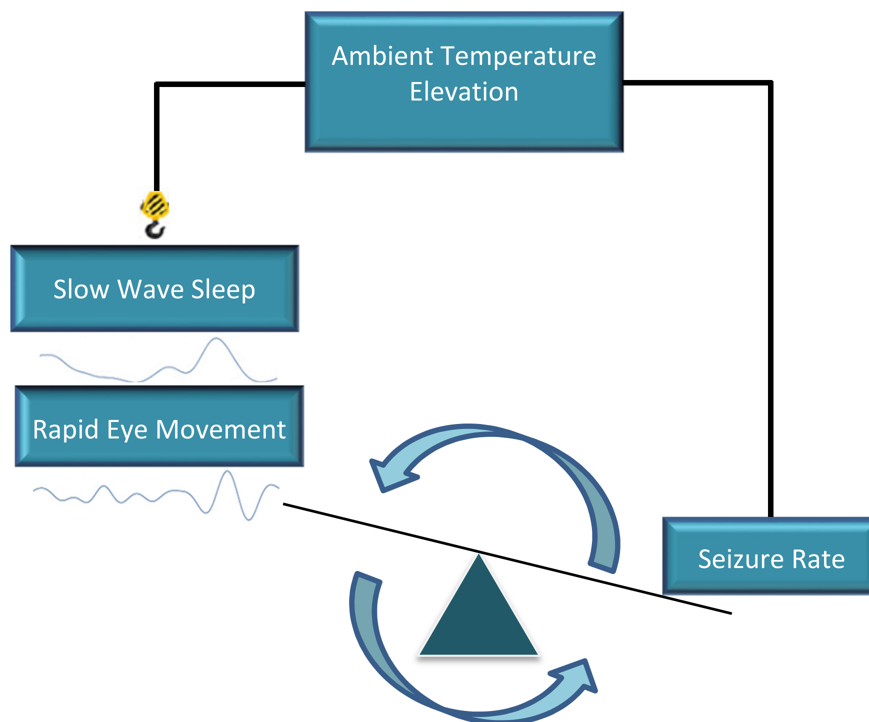

Figure 1.1. An illustration diagram of sleep-thermoregulation interaction in epilepsy patients as an example of diseases associated with poor sleep. The study hypothesis based on the assumption that an elevation in ambient temperature promotes slow-wave sleep and REM sleep in which seizures are less likely to happen (model and image proposed by S. Sunderam,2011).

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CHAPTER II EFFECTS OF ACUTE ELEVATION IN AMBIENT TEMPERATURE ON MOUSE SLEEP ARCHTECTURE

2.1 Introduction

The purpose of sleep and its underlying mechanisms has puzzled scientists for many years. Sleep helps both brain and body function correctly; thus, in recent years, many studies have been carried out in humans and animals to understand sleep mechanisms and devise possible approaches for improving its quality in health and disease.

Sleep structure generally cycles between two distinct states: Non-Rapid Eye Movement (NREM)/ and Rapid Eye Movement (REM) or Paradoxical sleep (PS) (Veasey *et al.* 2000). NREM itself may be subdivided into different stages : light sleep stages which characterized by the presence of sleep spindle and k-complex and deep sleep stages (stage 3 and stage 4) , also called slow wave sleep (SWS) (de Andrés and Reinoso-Suárez 2011). Both NREM and REM states play important roles in mental and physical activities. In the last two decades, the importance of sleep in memory consolidation (defines as a task by which the temporary memory is transferred to a long-lasting memory) has been extensively studied. In human and rodents, both NREM and REM sleep are involved in the memory consolidation process in different ways. Research has shown that while explicit (declarative) memories seem to be consolidated during NREM sleep, and SWS in particular, REM sleep plays an important role in consolidating procedural memories (Payne and Nadel 2004, Plihal and Born 1997). SWS also helps in improving some procedural skills (non-declarative memories) (Gais *et al.* 2007 and Aeschbach *et al.* 2008). SWS is important not only to the brain but to the body as well. SWS contributes in the regulation of glucose metabolism (Cautera *et al.* 2008) and some autonomic functions such heart rate and body temperature regulation (Bellesi *et al*. 2014). Thus, characterization

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of each sleep state and understanding how external stimulation (e.g., thermal stimulation) could affect each state are important in any sleep research investigating sleep quality improvement for better mental and physical health.

Sleep is a complex physiological process that is mainly regulated by homeostatic and circadian drives which combine together to determine sleep timing, duration, and depth. Homeostasis refers to the drive for sleep that accumulates during wake and dissipates during sleep. When sleep pressure increases above a certain threshold, sleep onset is triggered, and when sleep has been adequately recovered, wakefulness commences. The circadian rhythm on the other side regulates the body's internal biological processes. It has been suggested that the circadian rhythm promotes wakefulness more than sleep (Borbely and Achermann 1999, Edgar *et al.* 1993). Environmental temperature is an important factor that influences both circadian and homeostatic drives and thereby sleep. When ambient temperature (*Ta*) changes, this change is sensed by skin thermoreceptors which send this information to the preoptic area of the anterior hypothalamus (POAH), the thermoregulatory center of the brain. The information of ambient temperature change is then integrated with the activity of temperature-sensitive neurons in the POAH to initiate heat loss or heat gain, POAH neurons are also involved in sleep-wake regulation mechanisms (Lo Martire *et al.* 2012). Figure 2.1 shows a simple schematic illustration of the correlation between sleep and thermoregulation. The top part of the figure represents the circadian pacemaker that is located in the suprachiasmatic nuclei (SCN) of the hypothalamus and is responsible for regulating circadian rhythms of body temperature and the timing of the sleep-wake cycle. The left part is the control center of the thermoregulatory system that is located in the POAH. The thermal information about changes in external/internal temperature (that comes from thermal receptors distributed on the surface or core of the body) are integrated and sent by the POAH to effector organs as error signals to keep the core body temperature constant at a certain level: for example, in case of increasing ambient temperature the POAH will signal capillary blood vessels to vasodilate to increase heat loss and keep body temperature at constant level (Kräuchi, 2007). Comparing to thermoregulation system, sleep-wake regulation system is homostatically regulated. The interaction between sleep-wake regulation and thermoregulation system remains unclear. However, studies have shown that activation of

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temperature-sensitive neurons in POAH through peripheral thermal stimulation triggers NREM sleep (more sleep with an increase in ambient temperature (McGinty and Szymusiak, 2001). Obal explained that sleep promotion associated with an increase in ambient temperature is an active thermoregulatory response to prevent hypothermia (Obál *et al*. 1983).

Several studies have shown that sleep and thermoregulatory responses induced by changes in *Ta* are closely interrelated (thermoregulation is part of the homeostatic process). Those thermoregulatory responses are initiated by sleepiness and arousal neurons that are located in the brain, sensitive to even mild changes in environmental temperature, and involved in sleep-wake regulation (Gilbert *et al.* 2004 and Van Someren 2000). However, the central neural pathways that coordinate the control and interaction between thermoregulation and sleep-wake behavior are still inadequately understood and require more investigation (Lo Martire *et al.* 2012).

For each species, there is a temperature zone known as the thermoneutral zone (TNZ) in which the metabolic rate is independent of *Ta*, so the energy required to maintain body temperature at a constant level is at a minimum. TNZ is approximately 30°C for mice and reported values range from 26ºC to 34ºC (Harding *et al.* 2018, Cannon and Nedergaard 2011, Lodhi and Semenkovich 2009).

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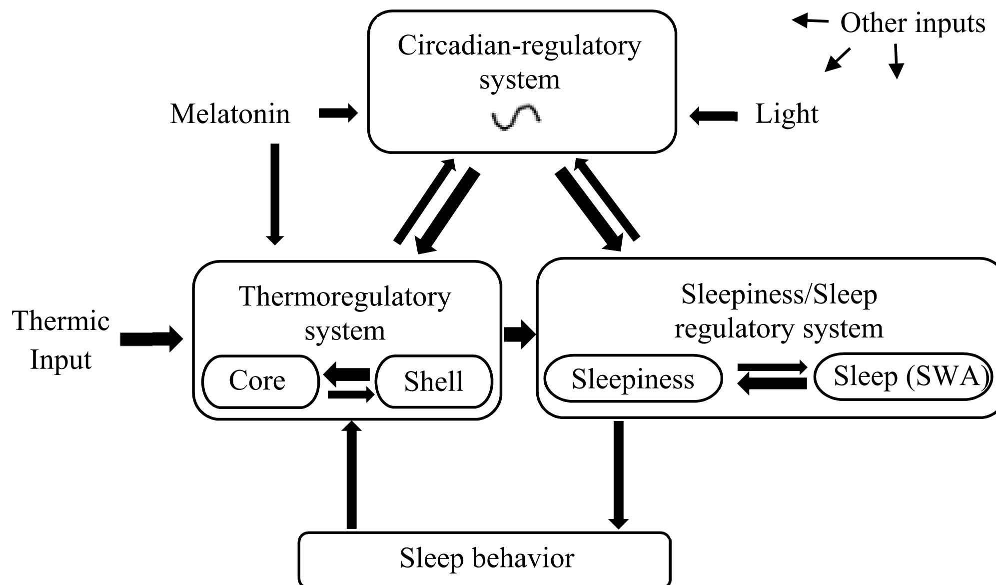

Figure 2.1. Sleep-thermoregulation interaction. Thermoregulatory responses induced by changes in external or internal body temperature affect sleep-wake structure. Both thermoregulatory and sleep/wake regulatory systems get feedback from the circadian regulatory system (SCN). POAH is involved in both thermoregulation and sleep/wake regulation (Kurt Kräuchi, 2007).

In spite of the fact that many sleep studies have been done in humans, animal models, mice and rats in particular, have been invaluable in studying and developing the understanding of sleep physiology and its underlying mechanisms. Because of the similarities in their brain circuitry to humans (Carlos *et al.* 2016), their availability, and ease of handling, mice have played a key role in sleep research and the preclinical validation of treatments for sleep-related disorders.

The normal sleep-wake cycle in mice is a well-orchestrated sequence of transitions between three distinct vigilance states: namely, wakefulness or "Wake", REM sleep, and NREM sleep. NREM sleep can be further classified into light and slow wave sleep. The three main vigilance states can be easily distinguished based on specific criteria applied to EEG/EMG signals (Veasey *et al.* 2000). Many studies in rodents have investigated the effect of ambient temperature changes on sleep-wake architecture. Compared to 22°C, mice that are exposed to higher temperature (26 and 30°C) for 24 hours have been reported

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to experience more NREM and REM sleep, along with a reduced EEG delta wave amplitude during NREM sleep which reflects a reduction in sleep depth (Jhaveri *et al.* 2007). Shifting *Ta* from baseline temperature (25°C) to higher temperature (34°C) is associated with more slow wave sleep and paradoxical (REM) sleep in mice accompanied with more REM bouts (Roussel *et al.* 1984). Chronic exposure to warm *Ta* (30°C) for four weeks increases both slow wave sleep and paradoxical sleep in rats (Mahapatra *et al.* 2005). The daily amounts of light NREM sleep and deep NREM sleep are increased by elevating *Ta* to 29°C in rats (Obál *et al.* 1983).

To our knowledge no previous study has characterized the acute effect (over several hours) of *Ta* elevation on mouse sleep. A couple of studies have investigated the acute effect on sleep in rats, specifically the effect of *Ta* on some sleep parameters over a few hours on alternate days at different temperatures. Both studies reported a significant effect of *Ta* on sleep (Gulia *et al.* 2005 and Kumar *et al.* 2009). In a different study, the effect of an elevated *Ta* (over two hours) on sleep was investigated in developing rats. Results showed that *Ta* significantly influenced the sleep-wake structure but no significant effect on sleep depth (defined by slow wave activity) was observed (Morrissette and Heller 1998).

Given that sleep and thermoregulation interact closely and that sleep promotion stimulated by an elevation in *Ta* may have some implications for sleep enhancement, the key goal of this chapter is to evaluate the effects of acute exposure to elevated *Ta* over a few hours on some conventional sleep metrics, namely percentage of time spent, mean number of bouts, and mean bout duration per vigilance state, to see if an acute elevation in *Ta* can produce the same effects seen in chronic experiments. *Ta* elevation effects on sleep depth and proportion of deep NREM sleep (SWS) are studied here using more than one measure since this effect has not been clarified sufficiently in previous chronic experiments. In addition to the temperature, time (circadian phase) is another factor that influences sleep; thus, the effects of time of day and its interaction with temperature on sleep are statistically evaluated.

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2.2 Animal care, housing conditions, and surgical procedure

All experimental work in this study were carried out in accordance with procedures approved by the Institutional Animal Care and Use Committee (IACUC) at University of Kentucky. All experiments were conducted on adult male wild type mice (C57BL/6; 6-12 weeks old; 24-29 g, Jackson Laboratory) that are widely used strain in biomedical researches. Before starting with any experimental procedure, mice were housed individually in their regular home cages in the animal room with free access to food and water. Animal room temperature during habituation, baseline and recovery was ~ 23°C with a relative humidity of (50 ± 10%). Mice were housed on a 14:10 hour Light/Dark cycle with light turned on from 7 a.m. to 9 p.m.

All mice were chronically implanted under 2.5% isoflurane anesthesia with Pinnacle's headmount with two EEG and EMG electrodes for brain and muscle activity recording. Anesthetized mice were placed on a stereotactic frame with access to inhalation tube. On the upper part of the skull a midline incision was made in the scalp, then skull was cleaned and sterilized with hydrogen peroxide solution. To record the frontal-parietal EEG, a pre-fabricated headmount (8202, Pinnacle Technology, Inc, Lawrence, KS) was placed on the skull so that the frontal border of the headmount located (3-3.5 mm) from Bregma. Four tiny stainless steel screws: 0.10" anterior and 0.12" posterior (8209 and 8212, Pinnacle Technology, Inc, Lawrence, KS) were placed in the four holes that were drilled in the frontal-parietal area corresponding to the existing holes within the headmount. For the purpose of conductivity enhancement, silver conductive epoxy was applied between screws and headmount. To record muscle activity, two stainless steel standard EMG electrodes were inserted bilaterally at the midline into dorsal nuchal muscle. After closing the incision with 2-3 stiches, a dental acrylic was applied around the headmount to seal/fix it. Immediately after surgery, mice were given an oral analgesic (Carprofen), returned to their cages, and allowed to recover for 7-10 days. Figure 2.2 shows the surgical procedure steps that were done on one of our mice.

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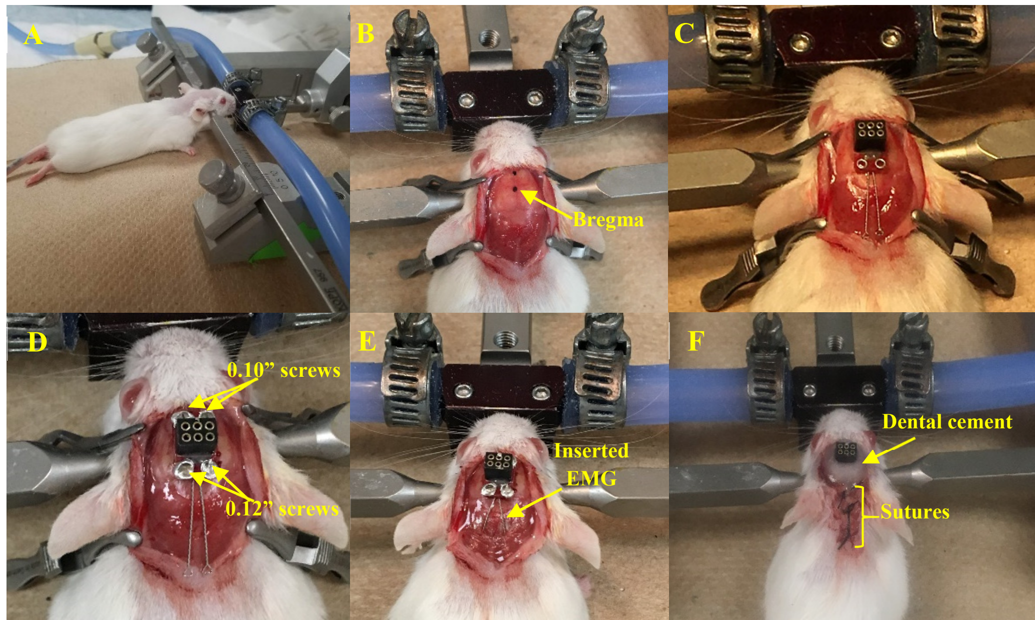

Figure 2.2. Chronic implantation of EEG/EMG electrodes in mouse skull. **(A)**Anesthetized mouse is fixed in the stereotactic frame. **(B)** Bregma and Lambda (3mm front to the Bregam) are marked to determine the head-mount position. **(C)**The headmount is glued to the mouse skull. **(D)** Screws (EEG electrodes) are implanted and epoxy is applied around them for conductivity. **(E)** EMG electrodes are inserted in the muscle. **(F)** Surgical incision is closed with three stitches and dental cement is placed around the headmount to fix it.

2.3 Data acquisition system and thermal chamber

EEG and EMG signals are amplified using Pinnacle's preamplifier (8202, Pinnacle Technology, Inc, Lawrence, KS) that goes into the headmount and does an amplification of 100x and high pass filtering with cut-off frequencies of 0.5Hz for EEG and 10Hz for EMG. Pinnacle's preamplifier is connected to data acquisition/conditioning (DACS, 8206, Pinnacle Technology, Inc, Lawrence, KS) system via a low torque commutator above the mouse cage. The DACS does a secondary amplification and filtering processes (50x amplification and low pass filtering: 100Hz for EEG and 100Hz for EMG) before sending the signals to Pinnacle Sirenia acquisition software for collection using a USB cable. The analog EEG/EMG signals are sent to a digital acquisition board (National Instrument USB -6210) with sampling rate of 400Hz. In addition to the EEG/EMG system, a piezoelectric "piezo" motion sensor is placed on the floor of the mouse cage to capture breathing and

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motion traces. A video monitoring system is set up to observe mouse behavior with an infrared (IR) LED illuminator to monitor the mouse during the Dark period.

A custom-built thermostatic control chamber has been built to maintain cage temperature as required. In that chamber, two infrared ceramic filament heating lamps (150W) were used to warm the mouse cage above the room temperature (~23°C) and create an even temperature field. A Vernier thermistor was suspended in the cage and the temperature (*Ta*) read into a computer as a calibrated voltage by a data acquisition board (NI USB-6211, National Instruments). A LabVIEW VI code was written to switch the heater on or off as needed to maintain cage temperature close to a desired set-point with a tolerance of 0.5°C. The cage was housed in a larger enclosure to slow heat loss to the surroundings. Figure 2.3 shows the setups of data acquisition system and thermal chamber that was built and used in this study.

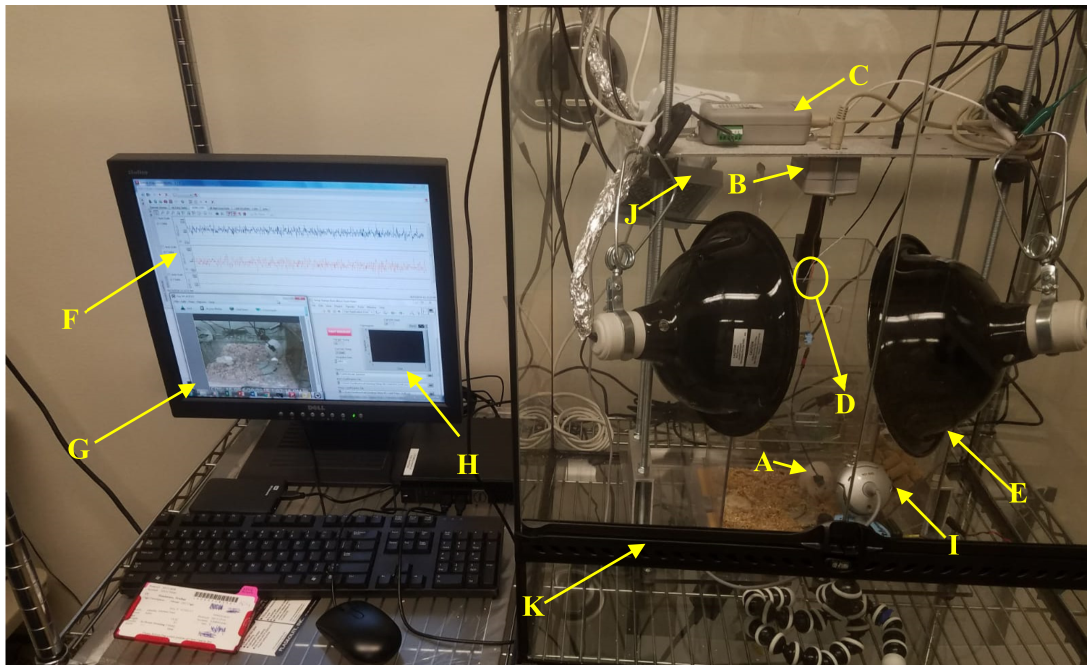

Figure 2.3. Experimental setup. An awake mouse sitting in a plexiglass cage with a head-mounted preamplifier **(A)**. Signals from the preamplifier are transmitted through the commutator **(B)** to the data acquisition system **(C)** that does another amplification to the signals. Temperature changes inside the cage are sensed by Vernier thermal sensor **(D),** two heating lamps were used as a source of heat **(E).** **F, G,** and **H** are the Sirenia acquisition, video, and LabVIEW recordings. Camera **(I)** was placed on tripod to capture all animal movements in the cage. Infrared LEDs illuminator **(J)** was placed at the top of the cage to provide an adequate illumination for continuous video monitoring. The whole setup was placed in a glassy enclosure (**K**) to minimize the heat loss from the cage to the surroundings.

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After recovery from surgery, each mouse (n = 13) was transferred to the thermal enclosure and exposed to one of four temperatures in the neighborhood of the TNZ of the mice: (24, 27, 30, and 33 Celsius) through four consecutive days for 8 hours (9 a.m. - 5 p.m.) for 9 mice, and room temperature (~23), 27, 30, and 33 Celsius through four consecutive days for 6 hours (11 a.m. -5 p.m.) for 4 mice (the first four mice that were exposed to changes in their cage temperature). The experimental period was selected to be during the Light period when mice are most somnolent. Thermal experiment was run with continuous recording of EEG, EMG, piezo, *Ta*, and video. After running the experiment on the 13 mice, data were collected and archived for analysis.

2.4 Manual scoring and statistical analysis

Sleep classification is a necessary and unavoidable step in any sleep research. Despite the fact that visual scoring depends on human bias and can be different between the scorers, it is still the "gold-standard" way for sleep classification. In this study, data were scored manually into NREM, REM, and wake using computer assisted software (Sirenia^{TM}, Pinnacle Tech.) in sequential of 4-second windows of each recording based on well-known changes in EEG and EMG criteria between vigilance states: (1) EMG activity level to differentiate sleep from wake (2) EEG delta (0.5-4Hz) band activity & (3) EEG theta (6-9Hz) band oscillations to differentiate NREM from REM sleep. When EMG activity is high, epochs were scored as a Wake. When EMG activity is low associated with predominant high amplitude/low frequency EEG delta oscillations, epochs were scored as NREM sleep. Epochs with low EMG amplitude and high theta rhythm were scored as REM sleep. Video recordings sometimes were used to help in scoring of vigilance states. Figure 2.4 shows samples from our data of EEG1/EEG2/EMG signals and EEG2 power spectral density during Wake, NREM, and REM. The figure demonstrates the changes in EEG and EMG characteristics during the three vigilance states.

Figure 2.5 shows sample features (EEG delta/theta power ratio and EMG power) computed in 4-sec epochs for one mouse at *Ta* =24°C along with four hypnograms (from manual scoring) at four different temperatures (24, 27, 30, 33°C) over six-hour period. The

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hypnograms structure suggests a general increase in NREM and REM sleep with *Ta* associated with a reduction in wake bout duration and number of bouts.

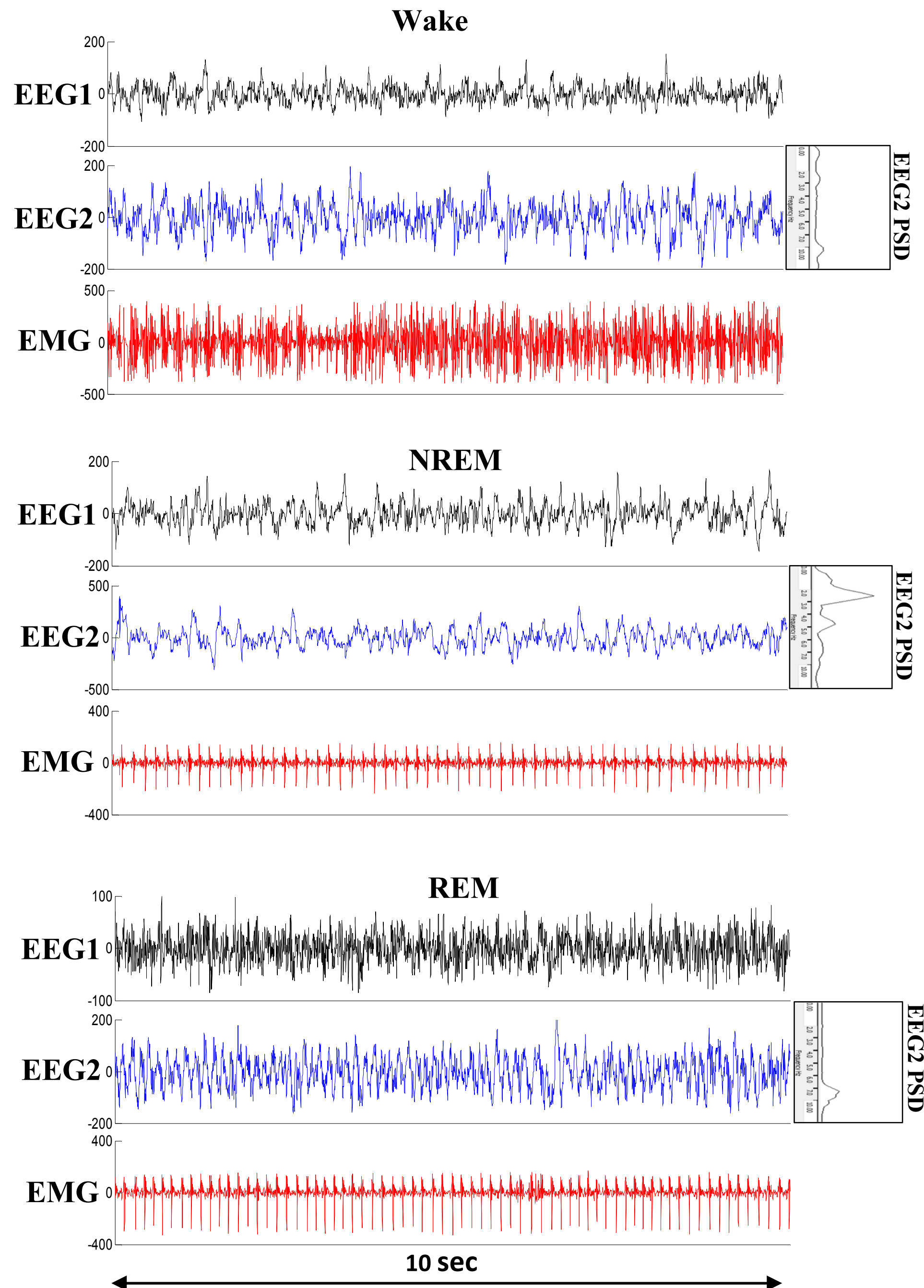

Figure 2.4. Typical EEG1/EEG2/and EMG signals with EEG2 power spectral density in a 10-second window during Wake, NREM, and REM. High muscle activity is a characteristic of wake. Low EMG and dominant EEG delta (0.5-4Hz) power are features of NREM. Low EMG and high EEG theta (6-9Hz) power are features of REM sleep. EEG and EMG amplitudes are in μv.

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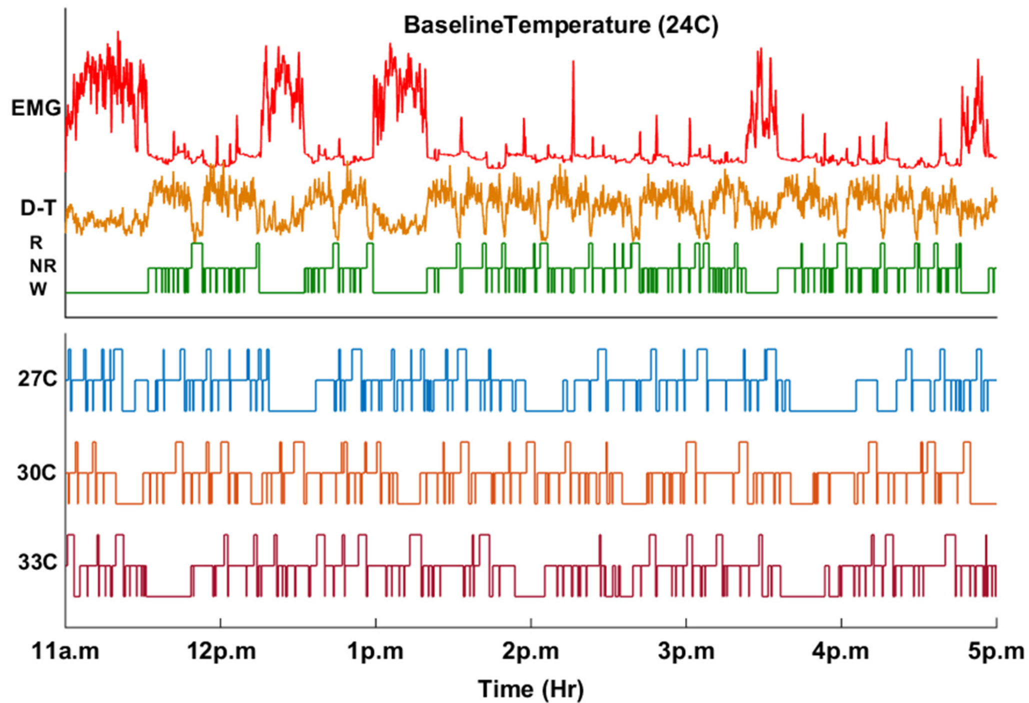

Figure 2.5. Six-hour recording features trend for EEG and EMG with the corresponding scores (Hypnogram) of vigilance states at baseline temperature (24)°C (***upper panel***). EMG power is high in wake (W) and low in sleep. The EEG delta/theta (D-T) power ratio is high in NREM (NR) and low in REM (R). Scores at higher temperatures (27, 30, and 33)°C (***lower panel***) showing that mouse tends to sleep more at higher temperature. A general increase in NREM and REM sleep with temperature is obvious.

To quantify the *Ta* effect on sleep clearly, three sleep metrics: time spent, number of bouts, and mean bout duration per state were computed from the manual scores and the effect of *Ta* was statistically evaluated using Wilcoxon signed rank test. Sleep depth has been characterized first by EEG delta (0.5-4Hz) power during NREM sleep as it has a good correlation with sleep duration and intensity (Borbely and Achermann 1999). The effect of *Ta* on the distribution of other measures of sleep depth namely: EEG delta to theta power ratio, EEG low delta power (0.5-2Hz), and EEG delta wave amplitude during NREM sleep was assessed using Kolmogrov-Smirnov (K-S) test.

We further computed the number of EEG zero-crossings in each NREM epoch to classify NREM sleep into light sleep (LS) and deep sleep (DS)/slow wave sleep (SWS). Any

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NREM epoch was assigned to SWS if the number of zero-crossings fell below a threshold defined as the lowest 30th percentile of zero-crossings in NREM epochs of the recordings at baseline *Ta* (Obál *et al*. 1983). Then the effect of *Ta* elevation on SWS time, duration, and number of episodes was assessed using Wilcoxon signed rank test. Since time of day is an important factor that influences sleep, sleep metrics were recomputed each hour with a two-hour moving window. An aligned rank transformation was applied to the data using the ARTOOL package (Wobbrock *et al.* 2011) to allow a non-parametric analysis of variance with repeated measures. Then, the non-parametric repeated measures analysis of variance (ANOVA) was applied to the aligned-rank-transformed data to evaluate the effect of *Ta*, time, and their interaction on sleep metrics. A false positive error probability *p* under 0.05 was considered significant.

2.5 Results and discussion

2.5.1 *Ta* effect on sleep metrics

The effects of *Ta* elevation on conventional sleep metrics (percent time spent, mean bout duration, and number of bouts) per state that are usually used to quantify sleep were assessed statistically using Wilcoxon signed rank test (Figure 2.6). Our data showed that while the time spent in NREM, REM, and SWS (overall and relative to NREM) increased significantly with *Ta*, wake time decreased ($p < 0.05$). Compared to 24ºC and 27ºC, wake time decreased significantly at 30ºC and 33ºC. It also decreased significantly at 33ºC compared to 30ºC. Time spent in NREM sleep increased significantly at 30ºC compared to 24 and 27ºC. It also increased significantly at 33ºC compared to 24, 27and 33ºC. REM sleep increased significantly at 30ºC compared to 24 & 27ºC and at 33ºC compared to 24 & 27ºC. Compared to 24 and 27ºC, time spent in SWS overall increased significantly at 30 and 33ºC. It is also significantly more at 33ºC compared to 30ºC. SWS as a proportion of NREM increased significantly at 30ºC compared to 27ºC and at 33ºC compared to 24, 27, and 30ºC. REM sleep relative to NREM did not change. The effect of *Ta* seemed to become significant upon entering the TNZ (30ºC) compared to being outside it (24 and 27ºC).

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While mean NREM bout duration increased with *Ta* ($p < 0.05$), the number of bouts decreased significantly which suggests less fragmented NREM sleep at higher temperatures. NREM bout duration increased significantly at: (a) 27 ºC versus 24ºC, (b) 30ºC versus 24 and 27ºC, (c) at 33ºC versus 24,27, and 30ºC. The number of NREM episodes, on the other hand, decreased significantly at 30ºC compared to 24ºC and at 33ºC compared to 24 and 30ºC. REM bout duration increased significantly with *Ta* but the number of bouts did not change. REM bout duration was significantly longer at 33ºC compared to 24 and 27ºC.

Both SWS bout duration and number of bouts increased significantly with *Ta*. Mean bout duration of SWS increased within TNZ (30 and 33ºC) compared to outside it (24 and 27ºC); no changes were observed between 24 and 27ºC or between 30 and 33ºC. The number of SWS bouts increased significantly at 30ºC compared to 27 and 24ºC and at 33ºC compared to 24, 27, and 30ºC. While wake time and number of bouts decreased significantly with *Ta*, mean bout duration decreased insignificantly within TNZ compared to outside TNZ.

Our results confirm the previous observations in chronic temperature elevation experiments in mice that reported an increase in NREM and REM sleep associated with a significant reduction in wake (Jhaveri *et al.* 2007 and Roussel *et al.* 1984). The SWS results are consistent with the observations in acute experiments in rats, i.e., more SWS at higher temperatures (Obál *et al*. 1983). However, our data contradicted the results for time in REM in an acute experiment in rats (Gulia *et al.* 2005). That study reported a reduction in REM at 33ºC compared to 30ºC which could be due to the study design or species differences (Gulia *et al*. 2005).

Wake bouts were then classified into prolonged wake (PW) bouts if more than 5 min in duration and brief arousal (BA) bouts otherwise. Results showed that while PW bout duration decreased progressively with *Ta* ($p < 0.05$, Kolmogorov-Smirnov test) but not the number of bouts, the number of BA bouts decreased with *Ta* ($p > 0.05$) but not the bout duration (Figure 2.7). Thus, the effects on both PW and BA contributed to the overall reduction in wake time but in different ways.

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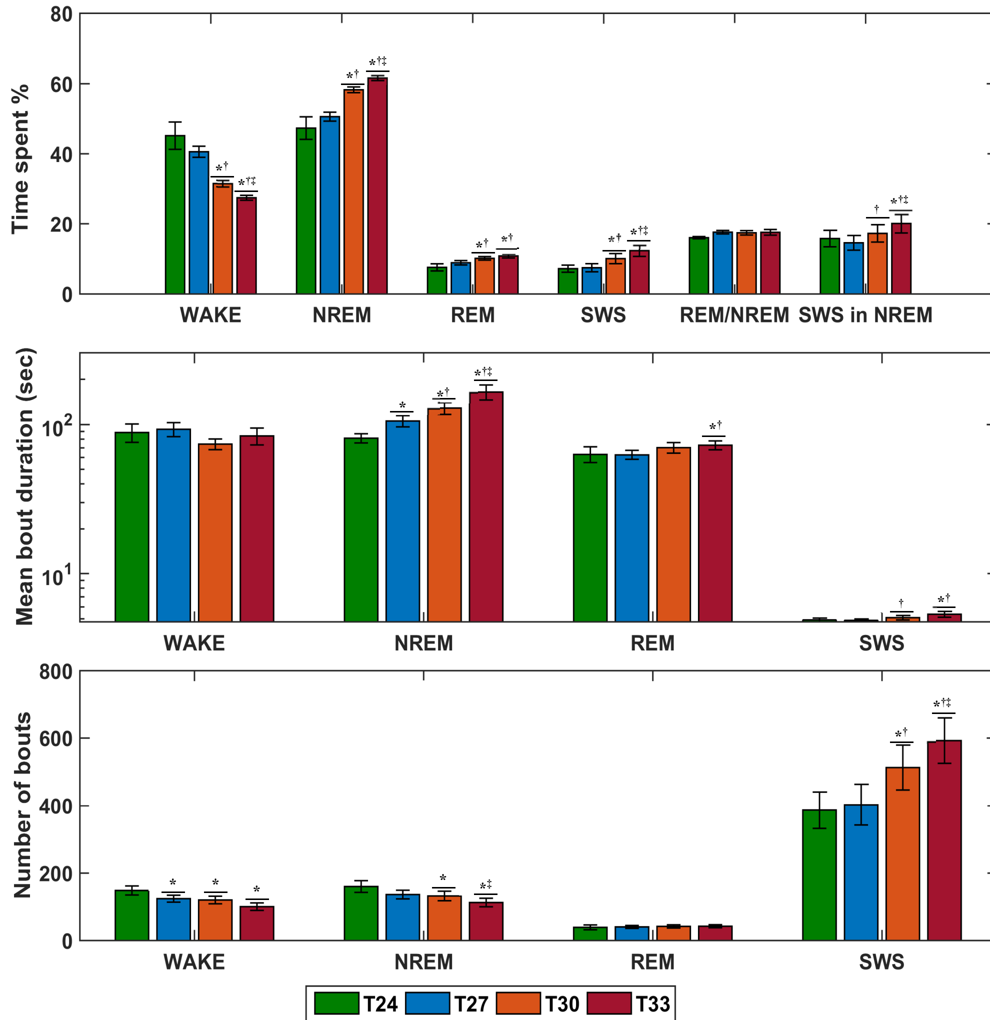

Figure 2.6. Temperature effect on sleep metrics. Data are expressed as *mean* ± *sem*. * indicates a significant difference relative to baseline *Ta*, † indicates a significant difference relative to 27°C, and ‡ indicates a significant difference relative to 30°C.

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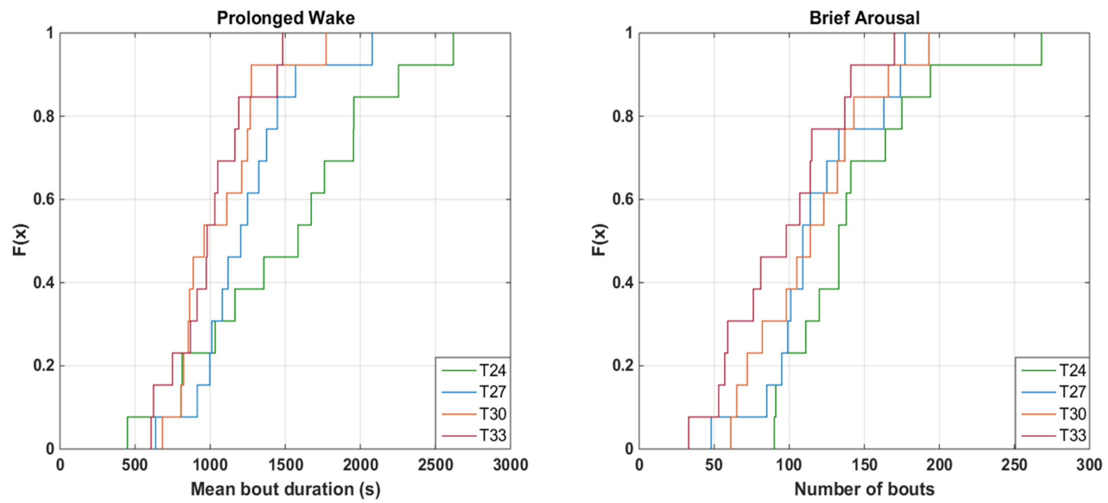

Figure 2.7. Effect of *Ta* on wake. ***Left:*** bout duration of prolonged wake (PW) decreased significantly with *Ta*. ***Right:*** though it did not reach the significant level, number of brief arousal (BA) decreased with *Ta*. Thus; the reduction in wake with *Ta* seems to come from a reduction in both of PW bout duration and BA number of bouts.

Some variation in sleep was observed across animals. The percentage of NREM sleep was in the expected 43-60% range for 10 out of 13 mice but abnormally low for the other three. Since the percentage of NREM saturates at ~60-67% with an increase in *Ta* to 33ºC in essentially all the mice, it appears to be a practical physiological limit, though the reason for this is unclear. In 9 out of 13 mice, there appears to be no significant change in NREM percentage as *Ta* goes from 24ºC to 27ºC (slight increase in some, decrease in others), but most experience a bigger jump on entry into the TNZ (~30-34ºC). In general, the increase in NREM percentage appears to come mostly from a steady increase in SWS relative to light NREM sleep and the net SWS also increases when this happens.

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2.5.2 Temperature and time effects on sleep metrics

As a second level of analysis in this study, we evaluated the effect of *Ta*, timing – which could be interpreted as time of day as well as time into the experiment – and their interaction on sleep metrics. Sleep metrics were recomputed in two-hour windows centered on every one hour and non-parametric repeated measures ANOVA applied on the aligned rank transformed data. Results showed that *Ta*, time, and their interaction all have a significant effect on sleep.

2.5.2.1 Effect of *Ta* and time on percent time spent in each state

Figure 2.8 shows the percent time spent in each vigilance state computed in each hour with a 2-hr overlapping window. Repeated measures ANOVA showed that both *Ta* and time but not their interaction have a significant effect on time spent in wake. Post-hoc analysis revealed that wake time decreased significantly at higher *Ta* (30 and 33ºC) compared to 24 and 27ºC, and was also less at 33ºC compared to 30ºC ($p < 0.05$). Regarding the time effect, results showed that time spent in wake in the first time segment (10 a.m.) was significantly different from all other segments. It was also significantly different in the second time segment (11 a.m.) compared to the last four segments. Wake % in the third time segment (12 p.m.) was significantly different from the fourth one. In general, there was a decreasing trend in wake % over time. No *Ta*-time interaction effect was observed.

Repeated measures ANOVA showed a significant effect of *Ta* and time on time spent in NREM. Post-hoc tests showed that both *Ta* and time affected NREM sleep in a similar way to wake except that it was not significantly different at the third time segment (noon) compared to the fourth one (1 p.m.). In contrast to wake, there was an increasing trend in NREM % over time.

REM percent at 30 and 33ºC was significantly different from 24 and 27ºC. It was almost significantly different at 27ºC compared to 24ºC ($p = 0.07$). Considering the time of day, it was significantly different in the first and second time segments compared to all other segments and at the third time segment compared to the fourth one. There was no interaction effect between *Ta* and time.

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While there was no significant effect of time or *Ta*-time interaction on SWS percent overall, *Ta* did have a significant effect. Post hoc analysis showed that SWS% increased significantly at: 30 and 33ºC compared to the 24 and 27 ºC, and at 33ºC compared to 30ºC. Temperature effect on SWS percent relative to NREM was significant. The effect was the same as on SWS percent overall. Time also had a significant effect on SWS%; it was significantly different in the first time segment compared to all others; in the second, third, and fourth segments compared to the fifth, sixth, and seventh; and in the fifth segment compared to the seventh. In general, the trend of SWS during NREM sleep is consistent with a previous study in rats (Obál *et al.* 1983) , which showed that the proportion of SWS during NREM sleep peaks in the first three hours of the Light period and then declines progressively. Since our experiment was conducted two or four hours after the onset of the Light period, the results suggest that the overall proportion of SWS is relatively low. With an increase in *Ta*, SWS% increased, which is shown in Figure 2.8 by upward shifts in the traces of SWS at elevated temperatures. Time trends seem to be consistent at elevated temperatures as in 24ºC (decline progressively over time). Finally, time has a significant effect on time spent in REM relative to NREM. It was different at the first time segment compared to all other segments, the second time segment compared to the last four time segments, and the third time segment compared to the fourth and seventh. Thus, in general there is a significant effect of temperature and time on time spent in each state but no *Ta*-time interaction effect. Temperature effects are consistent with those observed in the first level of analysis of *Ta* effect on gross or mean sleep metrics over the entire recording.

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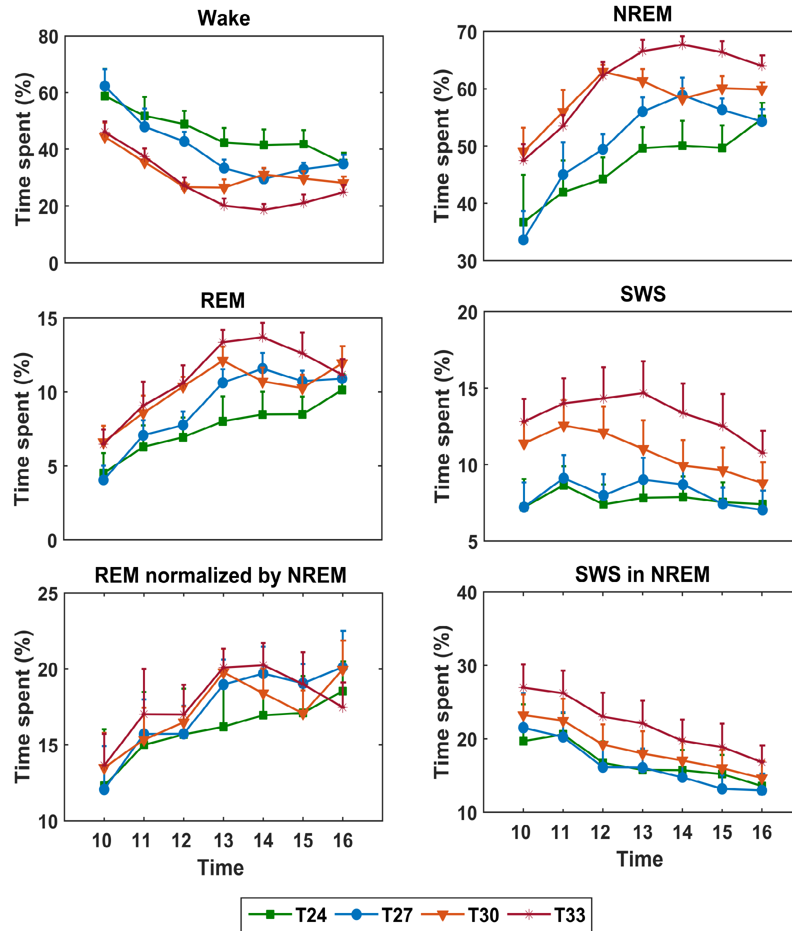

Figure 2.8. *Ta* effect on time spent in wake, NREM, REM, SWS, REM/NREM, and SWS during NREM in 13 control mice exposed to four different temperatures. Each data point (represented as *mean* ± *sem*) was computed hourly with a 2-hr moving window. Time starts at 10 a.m. and ends at 4 p.m. Repeated measures ANOVA showed that both *Ta* and time have a significant effect on the time spent, *p*-value of 0.05 was used as a level of significance. See text for details.

2.5.2.2 Effect of *Ta* and time on bout duration

Figure 2.9 shows the effect of *Ta* and time on mean bout duration of each state computed in each hour with a 2-hr moving window. Repeated measures ANOVA and post-hoc analyses showed a significant effect of temperature, time, and their interaction. The mean bout duration of wake was less at 30 and 33ºC compared to 24ºC (p = 0.0003) and at 30 and 33ºC compared to 27 ºC. Considering the time effect, it was significantly more at the first and second time segments compared to other segments and at the third time segment

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compared to the fourth. Results also showed that the interaction between time and *Ta* has a significant effect on wake bout duration. The interaction effect of the first time segment with 24ºC is significantly different from the interaction effect of the same time segment with 27ºC and the interaction effect of the first time segment with 27ºC is significantly more than the interaction with 30ºC. Third time segment and 33ºC interaction is significantly less than the interaction of the first time segment with 27ºC. For the fifth time segment, the interaction with 24ºC and with 27ºC is significantly different from the interaction with 33 and 30ºC respectively. The interaction effect of the sixth time segment with 24ºC is significantly less than the interaction of the fifth time segment with 30ºC and sixth time segment with 30 and 33ºC. Finally, the interaction of the seventh time segment with 24ºC is significantly less than the interaction with 27, 30, and 33ºC.

While time has no effect on mean NREM bout duration, *Ta* does have a significant effect. NREM bout duration increased significantly at 27,30, and 33ºC compared to 24ºC, at 30 and 33ºC compared to 27ºC, and at 33ºC compared to 30ºC. There was no significant effect of *Ta*-time interaction. The effect on REM bout duration was significant only from temperature. Mean bout duration of REM sleep increased at 30 and 33ºC compared to 24ºC and at 30ºC compared to 27ºC.

SWS bout duration increased significantly at: 30 and 33ºC compared to 24ºC, 30 and 33ºC compared to 27ºC, and at 33ºC compared to 30ºC. Time has a significant effect as well, SWS mean bout duration decreased with time (the general trend at each temperature). Post-hoc analysis showed that it is significantly different at the first time segment compared to all other segments, at second time segment compared to the seventh, at third and fourth time segments compared to the sixth and seventh segments, and at fifth time segment compared to the seventh.

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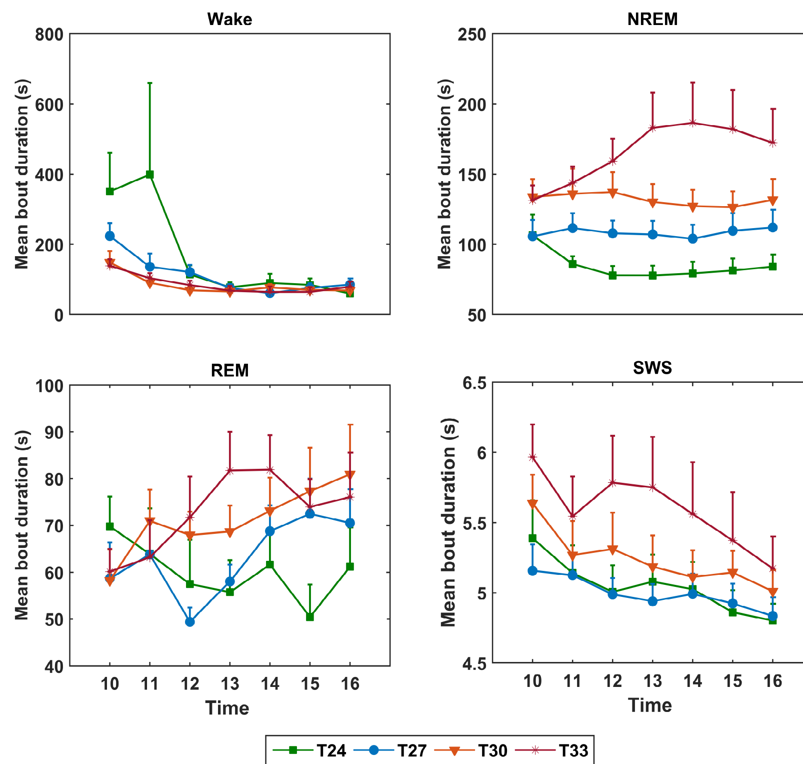

Figure 2.9. Effect of *Ta* on mean bout duration of wake, NREM, REM, and SWS. Each data point (expressed as *mean* ± *sem*) was computed hourly with a 2-hr moving window. Time starts at 10 a.m. and ends at 4 p.m. Repeated measures ANOVA was applied on aligned rank data to assess the effect. *Ta*, time, and their interaction had a significant effect. *p*-value of 0.05 was used as a level of significance. See text for details.

2.5.2.3 Effect of *Ta* and time on number of bouts

The last sleep metric is the number of bouts of each state, figure 2.10. For wake, repeated measures ANOVA showed that both *Ta* and time have a significant effect on number of bouts but not their interaction. Number of wake bouts decreased significantly at: 27, 30, and 33ºC compared to 24ºC, 33ºC compared to 27ºC, and 33ºC compared to 30ºC. Considering time factor, wake number of bouts increased with time. It was less at first and second time segments compared to the others ($p < 0.05$). The effect of *Ta*, time, and their interaction on NREM number of bouts was exactly the same as in wake.

Only time had a significant effect on REM number of bouts, it increased significantly at third to seventh time segments compared to the first and second. For SWS, number of bouts

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increased at: 30 and 33ºC compared to 24, 30 and 33ºC compared to 27 ºC, and at 33ºC compared to 30ºC. There was a significant difference in SWS number of bouts at the second time segment compared to the third, fourth, and fifth, and at third and fourth time segments compared to the seventh.

Considering PW and BA, both *Ta* and time had a significant effect on the time spent per state. While *Ta* and time had a significant effect on PW bout duration, only *Ta* had a significant effect on BA bout duration. For number of bouts, while both *Ta* and time had a significant effect on BA; only time had a significant effect on PW number of bouts (data not shown).

In conclusion, it is not only the *Ta* that exerts a considerable effect on mouse sleep, time also has a significant effect and should be considered in any research that investigates the effect of changes in external environment on sleep-wake architecture.

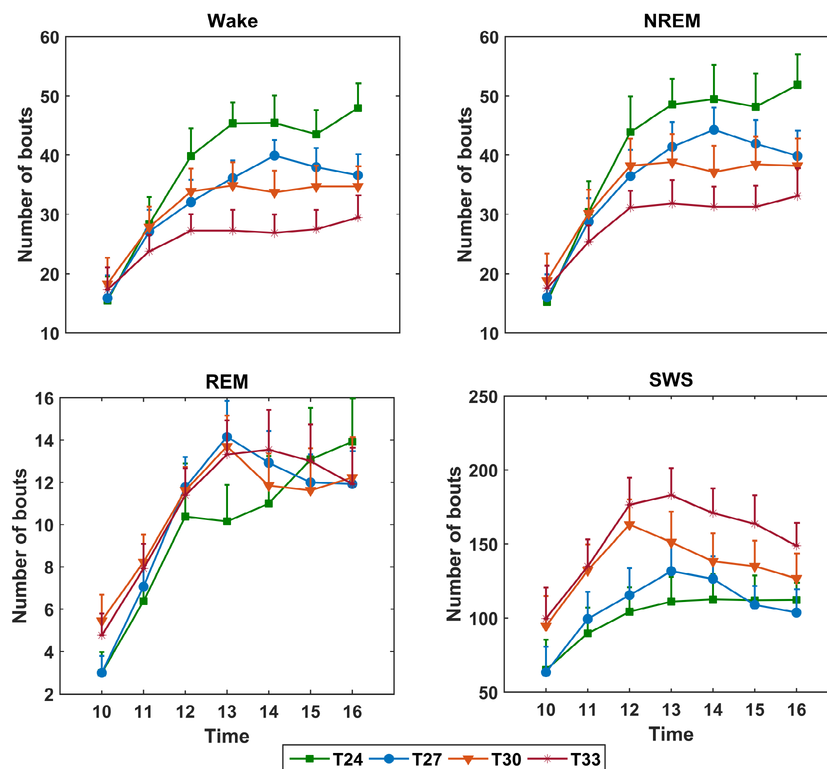

Figure 2.10. Effect of *Ta* on number of bouts of wake, NREM, REM, and SWS. Each data point (expressed as *mean* ± *sem*) was computed hourly with a 2-hr moving window. Time starts at 10 a.m. and ends at 4 p.m. Repeated measures ANOVA was applied on aligned rank data to assess the effect. *Ta* and time had a significant effect but not their interaction. *p-value* of 0.05 was used as a level of significance. See text for details.

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2.5.3 Temperature effect on sleep depth

In addition to the quantitative assessment on sleep metrics of SWS, the effect of *Ta* on qualitative measures of sleep depth was evaluated. EEG delta (0.5-4Hz) power and amplitude are well-known measures of sleep depth (Borbely and Achermann 1999, Jhaveri *et al*., 2007, and Dijk 2009). Figure 2.11 shows the distribution of EEG delta to theta power and EEG delta wave amplitude during NREM sleep at four different temperatures. Only two mice experienced a reduction in sleep depth when *Ta* changed from 24ºC to 27ºC, here the data from those two mice were excluded. Sleep depth increased significantly at 30 and 33ºC compared to 24ºC, at 30 and 33ºC compared to 27ºC, and at 33ºC compared to 30ºC ($p < 0.5$, Kolmogorov-Smirnov test). Thus, while temperature change outside the TNZ (24 to 27ºC) did not induce a significant effect on sleep depth, shifting in *Ta* within TNZ (from 30 to 33ºC) did. The effect was consistent for both of delta to theta power ratio and delta wave amplitude in all mice. Total EEG delta (0.5-4Hz) power and low delta (0.5-2Hz) power increased with *Ta* in the same way delta to theta and delta wave amplitude increased (data not shown).

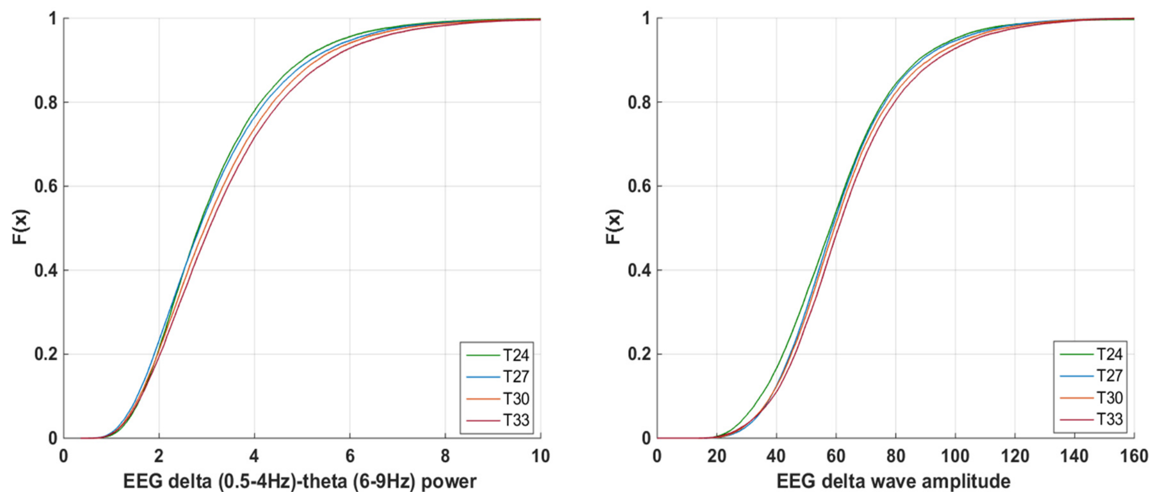

Figure 2.11. *Ta* effect on sleep depth during NREM sleep. ***Left:*** EEG delta (0.5-4Hz)/theta (6-9Hz) power during NREM. ***Right:*** EEG delta wave amplitude during NREM.Cumulative distribution function of both measures shifted rightward with *Ta* ; i.e. more sleep depth at higher *Ta*.

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2.6 Conclusions

More than one study has reported that an elevation in ambient temperature (*Ta*) produces a significant effect on sleep-wake architecture in mice (Roussel *et al*. 1984, Jhaveri *et al*. 2007, and Lo Martire *et al.* 2012). However, those studies were conducted on mice at least for 24 hr and typically for several days or weeks (chronic experiments). No previous study was done to evaluate the acute effect (over a few hours) of *Ta* elevation on sleep in mice. Here, we evaluated that effect to see if it is similar to the effect seen in chronic experiments in mice or acute experiments in rats. Our results showed that mice tend to sleep more and deeper at higher *Ta* and the sleep seems to be less fragmented; i.e. longer in bout duration and less in number of bouts. Our results were consistent with the results of chronic experiments in mice in term of time spent, bout duration, and number of bouts.

There is insufficient knowledge about how an elevation in *Ta* could affect sleep depth in mice. Results from one study showed that mice tend to have less sleep depth at higher *Ta* (Jhaveri *et al*. 2007) which is opposite to the results of another study in rats that showed rats sleep deeper at higher *Ta* (Obál *et al*. 1983). Using more than one measure to define sleep depth, our data clarified the effect of *Ta* on sleep depth and showed that mice sleep deeper at higher *Ta*.

Not only does *Ta* affect mouse sleep-wake architecture, but time has a significant effect as well and should be considered in the experiments that investigate the effect of external stimuli on sleep in mice.

The custom-built thermostatic control chamber used in this study was able increase cage temperature from room temperature (~23ºC) to the target *Ta* (24, 27, 30, and 33ºC) in a short time. Figure 2.13 shows the time needed by the chamber to get to target *Ta*. It took only few minutes to reach 24, 27, and 30ºC but longer time to get to 33ºC.

The present findings of this study add an important information to the literature on Sleep-*Ta* correlation and could have some important applications related to sleep depth enhancement in people with disordered sleep.

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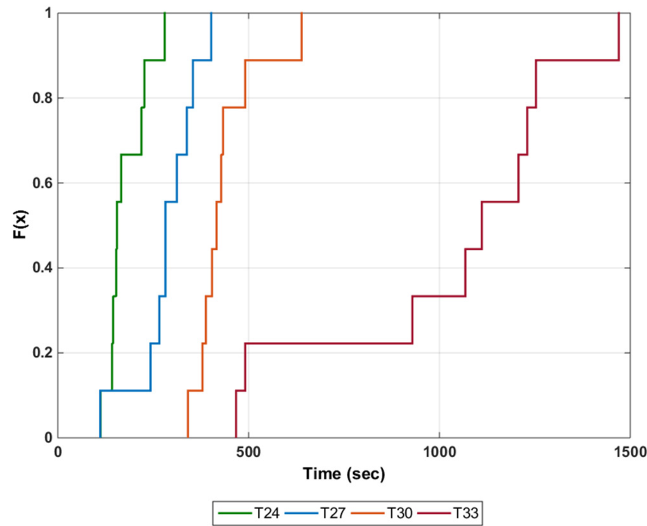

Figure 2.12. Time to get to target temperature from a "reference" point. The reference point defined by 95% of the difference between room (~23ºC) and target temperature (24, 27, 30, and 33ºC). The chamber took few minutes to get to 24, 27, and 30ºC but more time (median = 18 min.) to get to33ºC.

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CHAPTER III EFFECT OF DIURNAL AMBIENT TEMPERATURE ELEVATION ON SLEEP AND SEIZURES IN A MOUSE MODEL OF TEMPORAL LOBE EPILEPSY

3.1 Introduction

Epilepsy is one of the most common and serious neurological disorders, affecting around 1-3% of the world's population (World Health Organization 2005) and characterized by spontaneously recurring seizures. Epileptic seizures reflect abnormal electrophysiological activity in the brain and its physical manifestations can vary from the level of brief muscle twitches to severe and prolonged convulsions accompanied by loss of consciousness (World Health Organization 2018). In a complex and diverse field of study like epilepsy, there are many different questions researchers try to address depending on their study goals. Since sleep is correlated with seizure occurrence in many forms of epilepsy and we are interested in studying the effect of temperature on sleep, this chapter explores two important questions: how temperature change affects sleep in an animal model of epilepsy; and how the effect on different stages of sleep may contribute to epileptic seizure facilitation or inhibition.

The relationship between sleep and epilepsy has been recognized since ancient times by Hippocrates and Aristotle, as noted in Gowers' study, which revealed that around twenty percent of epilepsy patients had seizures during sleep and about one third of patients with epilepsy had diurnal seizures (Gowers 1885). From the time of the invention of EEG up to the present, many studies, taken together, show that epilepsy has a complicated and reciprocal relationship with sleep. Epileptic seizures interrupt sleep and therefore affect its quality, quantity, and structure. The quantitative and qualitative changes in sleep-wake structure is dependent on the type of epileptic seizures and their site of origin. Although their sleep is interrupted by the occurrence of seizures, patients with frontal lobe epilepsy usually have a sleep structure similar to that of control subjects; patients with temporal lobe epilepsy on the other hand experience fragmented sleep, reduction in total sleep time, and increase in the number and duration of wake episodes during sleep, reduced sleep quality

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and an increase in the time to the first REM bout (Crespel *et al*. 1998) and (Crespel *et al*. 2000).

The timing of seizure occurrence also affects sleep structure. Studies in patients with temporal lobe epilepsy showed that when seizures happened during the day, there was a significant reduction in time spent in REM sleep the following night without any significant changes in other sleep stages, while when they happened early in the night, a greater reduction in REM sleep but an increase in the first stage of NREM sleep have been observed (Crespel *et al*. 2000; Bazil and Walczak 1997).

Sleep in turn exerts an effect on the seizure occurrence. Sleep loss can precipitate seizures (Matos *et al.* 2010). Clinical and experimental studies have shown that different vigilance states influence seizure occurrence differently: empirical evidence suggests that NREM sleep, in which electrical activity is more synchronous across the cortex, may be much more conductive to seizure generation and spread than desynchronized REM sleep (Foldvary-Schaefer 2009). During NREM sleep, thalamic nuclei provide diffuse synchronized afferent inputs to the cortex and are responsible for the brief, rhythmic sleep spindles seen in stage II of NREM sleep. This diffuse cortical synchronization can lead to activation of ictal foci in susceptible individuals, which is perhaps why NREM sleep appears to facilitate convulsive activity. On the other hand, there is an inhibition of thalamocortical synchronization during REM sleep and also a reduction in interhemispheric transmission, which prevents generalization (spreading) of epileptiform discharges (C Quinto 2000). Interictal epileptiform discharges, not just seizures, are facilitated by NREM sleep. Beside the preferential occurrence of seizures in the lighter stages of NREM sleep (stage I and II), it has been observed that interictal epileptiform discharges are activated by deeper stages of NREM sleep (Minecan *et al.* 2002). Thus, besides our knowledge that epileptic seizures affect sleep-wake structure and contribute to sleep fragmentation, different sleep stages affect seizure generation in different ways. In the study described earlier in Chapter Two, ambient temperature elevation in mice increases slow wave sleep (i.e., deep NREM sleep) and REM sleep, the stages of sleep in which epileptic seizure probability is relatively low (Herman *et al*. 2001). To see if an increase in ambient temperature exerts the same effects on sleep in an animal model of

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epilepsy, we assessed the effect of temperature elevation on sleep and seizures in a mouse model of temporal lobe epilepsy (TLE). This is the most common and intractable form of epilepsy in humans. In addition, this serves as the first step toward eventually using temperature as the means for enhancing sleep in individuals with sleep problems including epilepsy patients.

Most of what is known about the sleep-epilepsy relationship is based on clinical studies in humans. However, understanding the complex mechanisms and correlation between different sleep stages and epileptic seizures from clinical studies is complicated by the effects of antiseizure medication, stress and anxiety due to the clinical environment (Matos *et al.* 2010), and the fact that most human studies are limited to surface EEG recordings. Animal models offer the opportunity to reduce the effects of these limitations and study the relationship between epilepsy and different vigilance states more clearly. There are several models of epilepsy that have been established in rodents. In the present study the pilocarpine mouse model of temporal lobe epilepsy (TLE) is used due to the fact that seizures in TLE are difficult to control using antiepileptic drugs (Lévesque *et al*. 2016) . Moreover, many aspects of human TLE are mirrored in the pilocarpine mouse model (Kandratavicius *et al.* 2014).

Not all epilepsy patients respond well to antiepileptic drugs or surgery; around a third of all cases remain unresolved. Even medications that help in seizure alleviation often affect sleep, mood, and perception negatively (St. Louis 2009, Mula and Monaco 2009, Park and Kwon 2008) . Alternative therapies have been developed such as electrical stimulation in response to developing or imminent seizure activity (Osorio *et al.* 2005). The timing and dose of the stimulation are critical factors for preventing or at least alleviating seizures, but some adverse effects on vigilance and autonomic function may occur (Garcia *et al.* 2008,Vyazovskiy *et al.* 2009, Ebben *et al.* 2008). Given that thermoregulation is strongly intertwined with sleep regulation (as discussed in Chapter Two), and that sleep and seizures have a bidirectional correlation, it appears plausible that improving sleep quality – by deliberately changing ambient temperature (*Ta*) – could serve as an unobtrusive way to alleviate the seizure burden in patients with epilepsy, if properly implemented. Thus, understanding the sleep-epilepsy interaction and the factors that may have an impact on it

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(including *Ta*) could lead to important insights toward developing therapies that could contribute to epileptic seizure control/reduction.

3.2 The pilocarpine mouse model of temporal lobe epilepsy

While studies in human subjects are desirable, they are often not feasible due to the unpredictability of seizure occurrence. Recordings can sometimes last on the order of weeks before capturing a seizure, consuming a great deal of monetary and time resources. Because of these limiting factors, animal models — and rodent models in particular — are widely used to investigate the neurophysiology of epilepsy and titrate experimental therapies. One such well-established animal model is the pilocarpine mouse model of TLE. Pilocarpine, a muscarinic receptor agonist, is widely used as a model of chronic TLE in rodents (Turski *et al.* 1989,Curia *et al.* 2008). The pilocarpine mouse shares many of the characteristics of human limbic epilepsy including morphological changes such as neuron loss in several hippocampal structures and reorganization of mossy fibers into the molecular layer of the fascia dentata, a latent period followed by intermittent and spontaneous seizures for life, and comorbid effects on sleep and cognition. Implementing this model allows for continuous recordings over several weeks if necessary and, are representative of electrophysiological dynamics that occur in human subjects (Leite *et al.* 2002). Pilocarpine induces status epilepticus (SE), which is a term used to define a series of uninterrupted seizures for an extended period of time (at least 30 min). After recovery from status epilepticus, pilocarpine-treated animals develop spontaneously recurring seizures (SRS) a few weeks later. During the silent period prior to the development of SRS, the brain, and especially the hippocampal formation, undergoes many changes including increased cell proliferation, cell death and mossy fiber sprouting (Mello *et al.* 1993, Parent *et al.* 1997). The main features of temporal lobe epilepsy can be summarized by: *(i)* the localization of seizure foci in the limbic system *(ii)* the frequent finding of an "initial precipitating injury" that precedes the appearance of TLE; *(iii)* a seizure-free time interval following the precipitating injury known as "latent period" (Curia *et al.* 2008).

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In this study, following the protocol described in Shibley and Smith (2002), SE was induced in mice by injecting them intraperitoneally (*ip*) with a pilocarpine dose of 290 mg/kg (near LD50). Fifteen minutes before pilocarpine injection, mice were injected with methylscopolamine (1mg/kg) *ip* to block peripheral cholinergic effects. Within two hours after pilocarpine injection, animals may develop SE, characterized by convulsive and intermittent seizures. The pilocarpine model of TLE is associated with high mortality (30-40%) during SE, and only about 50% of surviving animals that reach status epilepticus (SE) eventually develop SRS, the hallmark of chronic epilepsy. Thus, SE induction often gives only a small number of viable candidates (Shibley and Smith 2002).

According to the Racine scale, seizures are classified into five types based on the relative severity of observed behavior (Racine 1972):

Racine1: Mouth and facial movements, shivering.

Racine2: Head nodding, stiff tail.

Racine3: Forelimb clonus, chewing.

Racine4: Rearing with forelimb clonus, tonic immobility.

Racine5: Rearing and falling with forelimb clonus (tonic-clonic seizures).

Here, mice that survived SE (n = 4) were then given a sugar solution (to maintain glucose level in the blood) and monitored for signs of SRS over several weeks of latent period using piezoelectric ("piezo") motion sensing and digital video recordings. Epileptic seizures were detected using the line length (*LL*) and Teager energy (*TE*) features computed from the piezo signal (see next section for details). After verification of SRS, each epileptic mouse was surgically implanted under anesthesia with a three-channel headmount (Pinnacle Tech., Lawrence, Kansas) for continuous tethered recording of the frontal and parietal electroencephalogram (EEG) and nuchal electromyogram (EMG). The implantation procedure is described in detail in Chapter Two. Mice were treated with an oral analgesic (Carprofen) both before and after surgery and allowed to recover in their home cages before exposing them to an elevated temperature. Figure 3.1 shows the steps of SE induction and seizure detection (from piezo and video recordings) in the pilocarpine mouse model of TLE.

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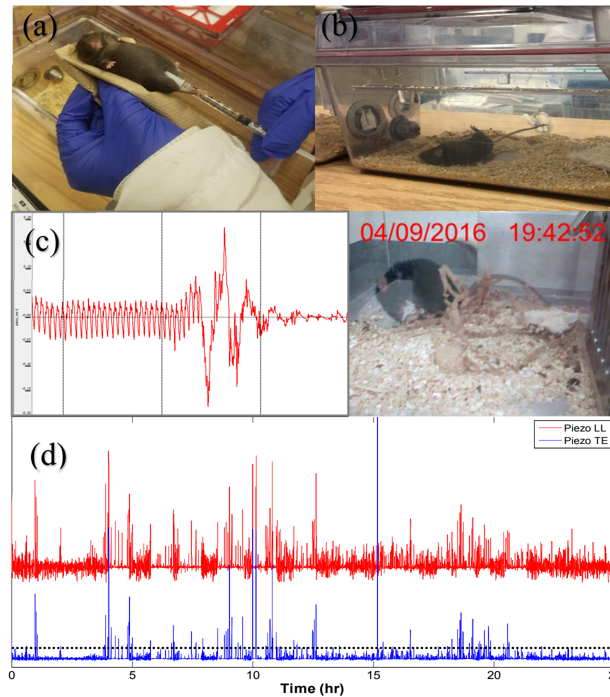

Figure 3.1. Pilocarpine model of TLE. Steps of SE induction and seizure detection in a mouse model of TLE. **(a)** Pilocapine injection; **(b)** Mouse develops SE for up to 2 hours; **(c)** Video/motion monitoring for 6-8 weeks (latent period); **(d)** Seizure detection using piezo line length (*LL*) and Teager energy (*TE*) features. The dashed black line is the threshold applied to identify seizure candidates before verifying them visually on video. Mice that developed SRS were then implanted with EEG/EMG electrodes.

3.3 Experimental procedures and methods

All procedures were performed in accordance with the prior approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky. Four adult male mice (C57BL/6, Harlan), 6-8 weeks of age, survived SE and developed chronic epileptic seizures. After spontaneous seizures were observed, each animal was surgically implanted with head-mounted EEG and EMG and allowed to recover for 7-10 days. Prior to the experiment, each mouse was housed in an individual cage under fixed environmental conditions (14h:10h Light: Dark cycle, ambient temperature, and humidity) for ten days with continuous recording of EEG, EMG, piezo, and video. Details of animal housing conditions, surgery and acquisition system and thermostatic control were described earlier

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in Chapter Two. After ten days of baseline recording at room temperature, each mouse was exposed to an elevated *Ta* of 30°C (in the thermoneutral zone) every other day for up to two weeks, but only during the 14-hour Light period (subjective day) with reversal to baseline room temperature (*Ta* ~ 23°C) at any other time. The protocol is therefore divided into "Prethermal" days at room temperature; "Off" days, again at room temperature; and "On" days, at elevated temperature in the Light period alone. After completion of this protocol, the experiment was terminated and the recordings archived for analysis.

3.3.1 Sleep analysis

Changes in vigilance state and the time of day can influence the occurrence of seizures. In order to track and characterize changes in vigilance dynamics at elevated and baseline *Ta*, the previously validated SegWay algorithm (Yaghouby and Sunderam 2016) was applied to automatically score each recording in sequential 4-s epochs into different vigilance states, namely wakefulness ("Wake"), NREM, and REM. Vigilance states are distinguished by the following EEG/EMG characteristics: muscle tone (EMG) is high in Wake and low in sleep, The EEG delta (0.5-4 Hz) to theta (6-9 Hz) power ratio is high in NREM sleep and low in REM sleep. Figure 3.2 shows sample features (EEG delta/theta power ratio and EMG power) with the corresponding sleep scores computed in 4-s epochs for a mouse exposed to baseline and elevated *Ta* over a 6-h period. The feature trends suggest a general increase in NREM with *Ta* and possibly other subtle changes.

To better quantify the effects of *Ta*, vigilance states (Wake, NREM, and REM) were scored, and three sleep metrics – the percent time, mean bout duration, and number of bouts of each state – were estimated from the sequence of vigilance scores. NREM sleep was further classified into light and deep sleep (or slow wave sleep: SWS) using an EEG zero-crossing criterion and SWS computed as a proportion of NREM. The sleep measures were compared statistically for the Light period during On days versus that of the intervening Off days, and of the Prethermal baseline. A false positive error probability $p \leq 0.05$ was considered statistically significant in all analyses.

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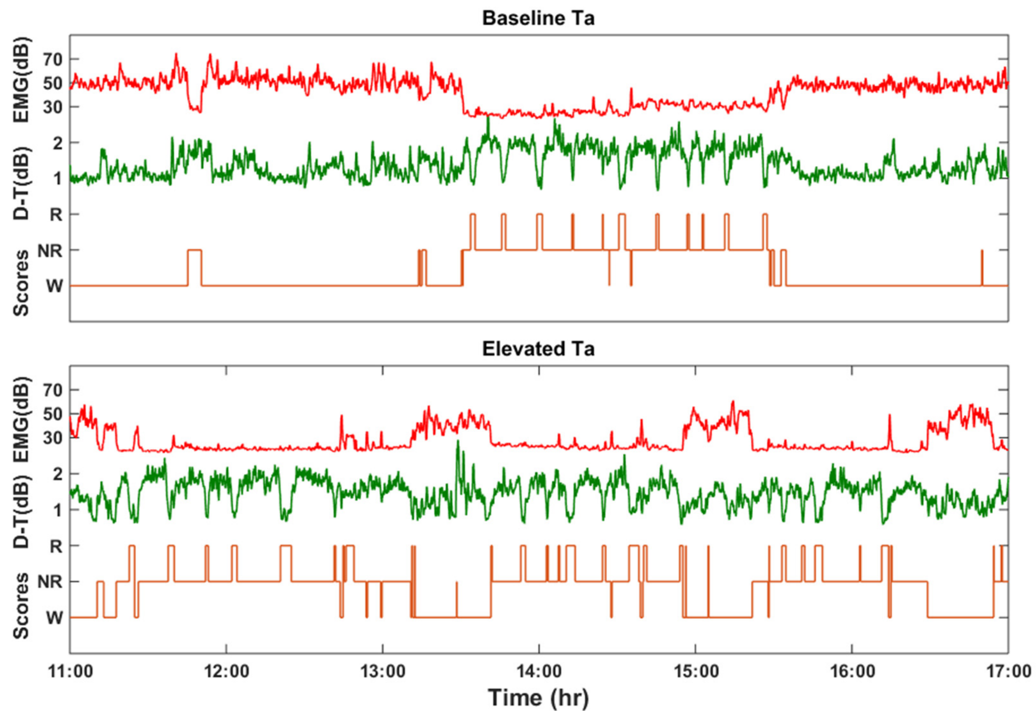

Figure 3.2. Six-hour recording showing feature trends for the EEG and EMG and the corresponding sequences of vigilance scores (hypnograms) at baseline and elevated *Ta*. EMG power is high in wakefulness (W) and low in sleep. The delta/theta power ratio (D-T) is high in NREM (NR) and low in REM (R). An epileptic mouse spent more time in NREM and REM sleep and less time in wake at higher *Ta*.

3.3.2 Seizure detection

Several automated algorithms have been developed for the purpose of seizure detection in humans and rodent models because reviewing EEG signal visually to detect seizures is a very difficult and time-consuming task. Those automated methods usually require two main steps: (1) selection and computation of appropriate signal features (e.g., line length, Teager energy) and (2) determination and application of a threshold or other statistical criteria to distinguish seizures from other normal behaviors like grooming, drinking, and eating (Ramgopal *et al.* 2014). Most of those methods are based on a computational analysis of EEG including line length, wavelet transforms, total variation of signal, and others (Bergstrom *et al.* 2013). The EEG during epileptic seizures is marked by spike waveforms of high amplitude and time-varying frequency; the electrical activity is

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accompanied by convulsive behaviors that are reflected in the EMG and piezo signals. Figure 3.3 shows a sample of electrophysiological recordings before, during, and after a seizure in a mouse model of TLE that illustrates these phenomena.

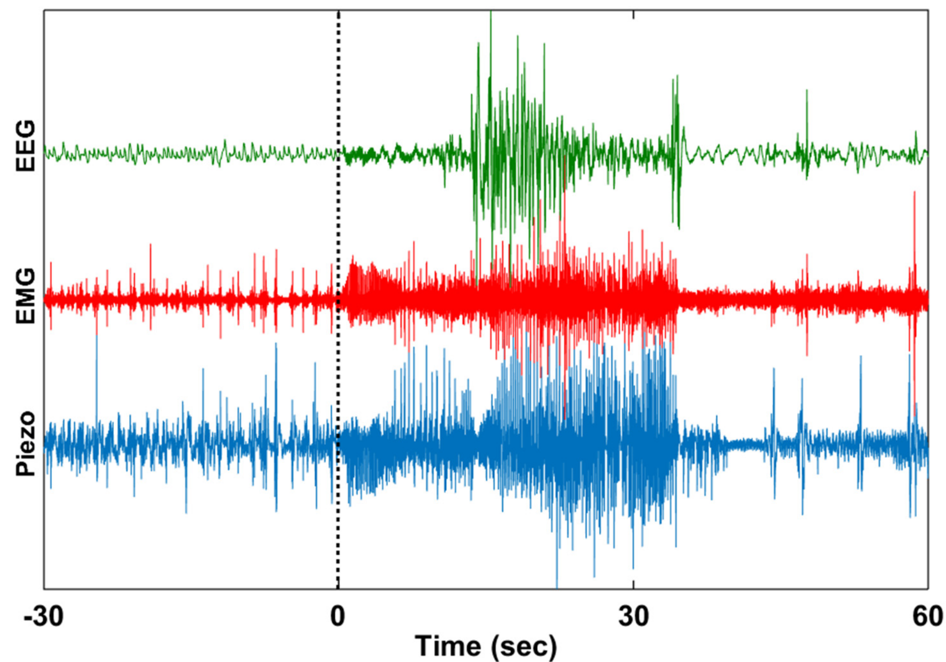

Figure 3.3. Sample of EEG, EMG, and piezo signals during a typical seizure in a mouse model of TLE. Time is shown relative to seizure onset. The seizure lasts about 35 s and was graded as a 5 on the Racine scale. High amplitude and frequency activity are observed on the EEG, EMG, and piezo traces during the seizure.

In this study, seizures were detected by computing the line length (*LL*) and Teager energy (*TE*) features in a 4-s moving window from the piezo signal before EEG/EMG implantation and from both EEG and piezo signals afterwards. Both features are sensitive to high energy activity, and depart from their baseline levels during seizures (Figure. 3.4). The values are compared to a threshold at each time instant to detect possible seizures. Briefly, *LL* is a simple computational measure that has been widely used before as an efficient feature for seizure detection as it goes up when signal magnitude and frequency increase. It is defined as the sum of the vertical distance between successive samples of the signal and is computed as (Esteller *et al.* 2001):

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$$LL(t) = \sum_{k=t-N+1}^{t} abs(x(k) - x(k-1))$$

where *x* is the sampled signal, *k* is the sample index in a window of length *N* ending at time *t*, and *abs* stands for absolute value. Here, *LL* was computed after filtering the EEG and piezo signals to within 0.5-45Hz and 0.5-20Hz respectively. Teager energy (*TE*) is a nonlinear operator that responds to rapid changes in the amplitude or frequency of the signal (Kaiser 1990). In a continuous time series $x(t)$, *TE* is computed as:

$$TE(t) = x^2(t) - x(t-1)x(t+1)$$

Both *LL* and *TE* features from EEG and *LL* from piezo were computed for each recording. Piezo *TE* seemed to be more variable than piezo *LL*. Figure 3.4 illustrates the changes in EEG *LL*, EEG *TE*, and piezo *LL* features along with the scores of different vigilance states (Wake, NREM, and REM) over four hours of recording in an epileptic mouse. All seizures are then verified by examining the video record and counting true detections separately for each day and experimental condition. Seizure rates were estimated separately for Light and Dark periods of Prethermal, Off, and On days in the protocol. Temperature effects on seizure frequency and timing were assessed statistically for On days versus Prethermal and Off days.

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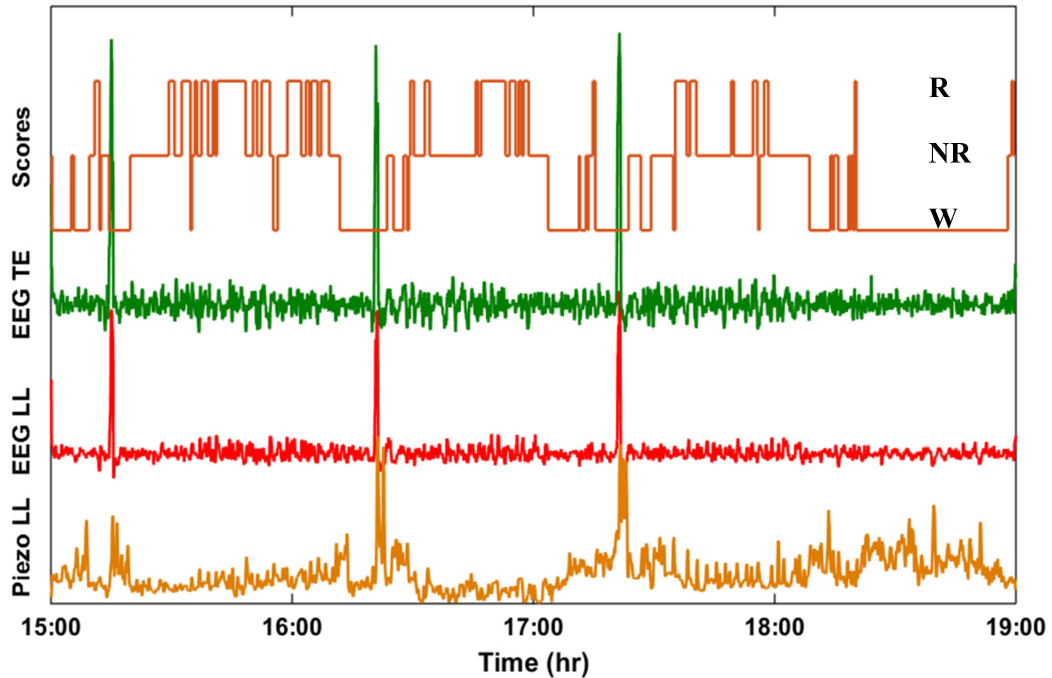

Figure 3.4. Four-hour recording showing sample features of the EEG line length (*LL*) and Teager energy (*TE*), and piezo line length (*LL*). *TE* and *LL* show rapid surges in value during seizures. This example shows three seizures during Wake. The piezo *LL* feature is presented here because it has been used in favor of piezo *TE* since it has a smaller dynamic range in the baseline and seems to be less influenced by other factors including circadian rhythms. W: Wake, NR: NREM, and R: REM.

3.4 Results and discussion

Though the correlation between sleep and epilepsy is well recognized by many studies in humans and rodents, sleep parameters have not been documented in the pilocarpine mouse model of TLE well enough to enable assessment of the effects of experimental intervention. Thus, before conducting any thermal manipulation on the epileptic mice and to get an idea about the sleep structure and seizure activity in this model under baseline conditions, the sleep metrics and seizure parameters, specifically seizure rate and timing, were characterized for data collected over 10 days from each epileptic mouse before running the temperature elevation protocol. Then, the *Ta* effect on sleep and seizure was statistically evaluated for On days versus Off days versus Prethermal baseline during the light period.

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3.4.1 Effect of *Ta* on sleep

The estimated sleep metrics during the light period for Prethermal baseline, Off, and On days are statistically compared in Figure 3.5 as daily averages pooled for all four animals. The Kruskal-Wallis test (with post hoc pairwise comparisons) was applied to assess the difference because the Prethermal baseline sample is unmatched with that of Off and On days; n equals 40 days for Prethermal baseline and 48 days each for Off and On samples. While time spent in wake increased significantly ($p < 0.05$) during Off days compared to Prethermal, time in NREM and REM decreased but not significantly. Compared to Off days, wake decreased and NREM increased (both significantly) during On days. Only NREM changed significantly during On days compared to Prethermal though there was a clear reduction in wake. An increase in wake time in Off days compared to Prethermal seems to come from a significant increase in bout duration associated with a non-significant reduction in number of bouts. There was a non-significant reduction in wake bout duration ($p > 0.05$) going from the Off to On condition that is reflected in the time spent in wake. Compared to Prethermal, there were no significant changes in wake bout duration or number of bouts during On days though the number of bouts decreased clearly. As in wake, NREM sleep seems to be more consolidated during Off days compared to Prethermal, i.e., a significant increase in bout duration associated with a significant reduction in number of bouts. Compared to Off days, an increase in NREM during On days appeared to come from a significant increase in number of bouts. No significant changes in NREM bout duration or number of bouts were observed in On days compared to Prethermal days. Finally, the reduction in REM sleep during Off days compared to Prethermal seemed to come from a non-significant reduction in the number of bouts rather than bout duration.

Next, a Wilcoxon signed rank test was used to assess the effect of *Ta* on sleep in the experimental period; i.e. On days versus preceding Off days (matched samples). These epileptic mice spent more time in NREM sleep and less time in Wake ($p < 0.05$) in On days compared to Off days. REM sleep increased at elevated *Ta* but not significantly. The number of NREM bouts increased significantly with *Ta* ($p = 0.0007$). The numbers of REM and Wake bouts both increased in On days but the effect was not significant. Looking at sleep changes with *Ta* in each animal, there was a significant increase in NREM sleep

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in two of the four mice (see Table 3.1), which coincidentally had significantly more NREM bouts as well. Time in Wake decreased with an increase in *Ta*; this was significant in three animals. The mean number of Wake bouts did not change significantly with *Ta*. Mean Wake bout duration decreased at elevated *Ta* in all animals, but significantly in only one of them (Ajwad *et al.* 2016). Time spent in REM sleep increased with *Ta* in three mice, but not significantly. The percent of REM sleep seemed to be high compared to that in nonepileptic controls (Jhaveri *et al*. 2007 and Yaghouby *et al*. 2016).

To summarize:

(a) **Off versus Prethermal:** Compared to Prethermal, time spent in wake in Off days increased significantly in bout duration not the number of bouts. NREM bout duration increased with a significant reduction in number of bouts. REM sleep decreased due to a non-significant reduction in number of bouts.

(b) **On versus Prethermal:** While wake in On days decreased insignificantly due to a reduction in number of bouts, NREM time increased along with non-significant increases in number of bouts and bout duration.

(c) **On versus Off:** there was some variability (as expected) in the effect of *Ta* on sleep metrics across the four mice. But in general, both NREM and REM sleep increased with *Ta* elevation due to an increase in their number of bouts. However, their mean bout duration did not change at elevated *Ta*. Time spent in Wake decreased significantly due to a reduction in its mean bout duration but not the number of bouts.

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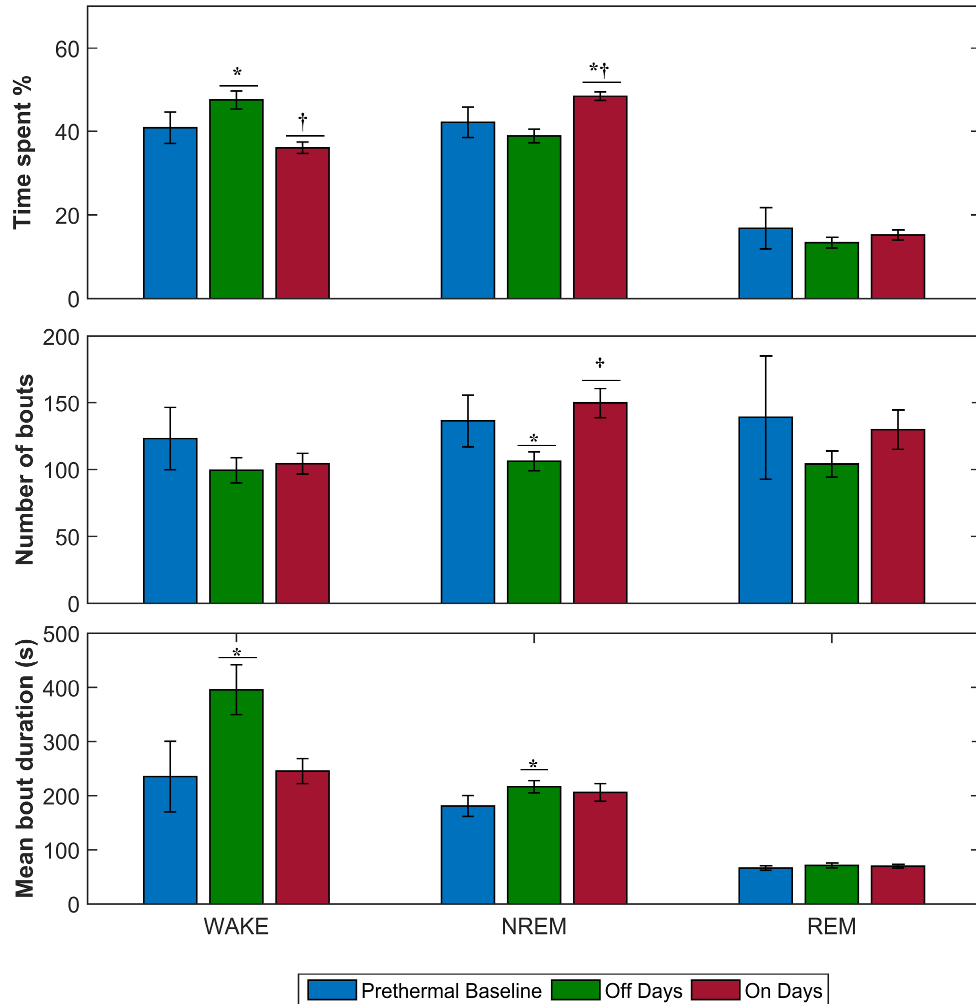

Figure 3.5. Different sleep-wake parameters are compared between vigilance states for Prethermal baseline versus Off days versus On days during the Light period. Compared to Prethermal, epileptic mice spent more time in wake during Off days ($p < 0.05$, Kruskal-Wallis test) and less time in NREM and REM ($p > 0.05$). Both wake and NREM bout duration increased significantly (i.e. more consolidated bouts). Compared to Off days, epileptic mice spent less time in Wake and more time in NREM ($p < 0.05$) in On days (Ajwad *et al*. 2016). Time spent in NREM during On days was significantly more than in Prethermal days. Data are expressed as *mean* ± *sem*. * indicates a significant difference relative to Prethermal baseline days and † indicates a significant difference relative to Off days.

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Wake bouts were further classified into: brief arousal (BA), defined as any wake bout with duration under 5 min, and prolonged wake (PW) otherwise. The effect of *Ta* elevation on BA and PW was statistically evaluated by comparing the distributions of their bout duration and number for Prethermal, On and Off samples using the Kolmogorov–Smirnov test. Mean bout duration and number of bouts were significantly different in Off days compared to Prethermal and in On days compared to Off and Prethermal days. While PW bout duration increased significantly in Off days compared to Prethermal, it decreased significantly in On days compared to Off/Prethermal days ($p < 0.05$). For BA, the mean bout duration was significantly lower for Prethermal and On days compared to Off days and for Prethermal compared to On days (Figure 3.6). There was a decreasing trend in the number of bouts for both PW and BA going from Prethermal to Off to On days; the effect was significant for both of them (data not shown). Thus, time spent in wake increased during Off days compared to Prethermal, an effect that came mainly from an increase in wake bout duration. A closer look revealed that the increase in bout duration originally came from an increase in the duration of PW and BA bouts, not their number of bouts. Going from Off to On days, time spent in wake reduced significantly, which seems to have come from a significant reduction in both PW and BA bout duration and their number of bouts. Finally, time spent in wake was slightly less for On days compared to Prethermal due to a reduction in number of bouts for both BA and PW (mainly BA).

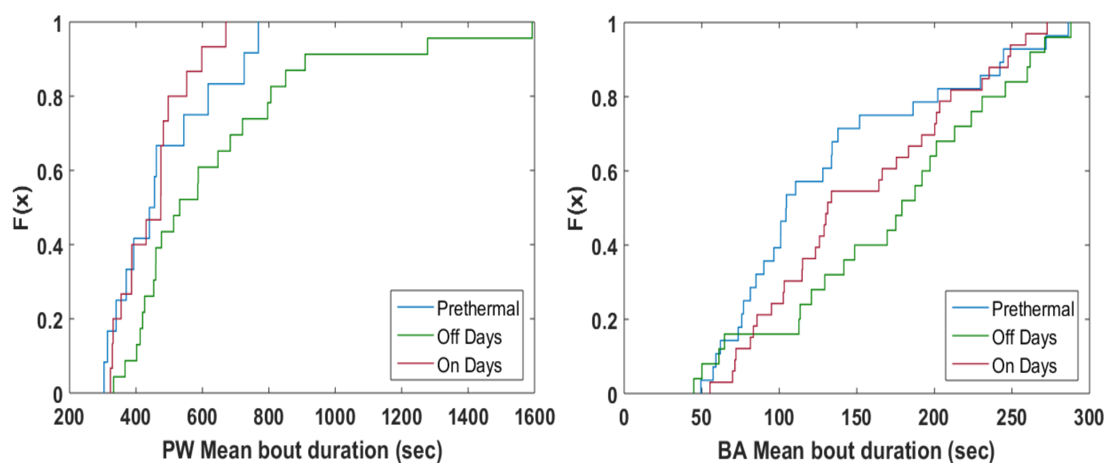

Figure 3.6. Effect of *Ta* on wake bout duration: ***(left)*** prolonged wake (PW), ***(right)*** brief arousal (BA) bouts. Both BA and PW bout duration decreased significantly with *Ta* ($p < 0.05$).

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Table 3.1. Seizure rate (no. /day), (% time, number of bouts, and mean bout duration) in Wake, NREM, and REM during the Light period (*n*: number of days, L: Off *Ta*, H: On *Ta*). All values are expressed as *mean* ± *sem*. * indicates significant difference between values in H and L (Wilcoxon signed rank test).

Outcome		***Ta***	**Mouse ID**			
			1 (*n*=11)	**2 (*n*=12)**	**3 (*n*=13)**	**4 (*n*=12)**
Seizure rate		L	1.4 ± 0.5	2.5 ± 1.1	0.7 ± 0.2	1.2 ± 0.6
		H	2.1 ± 0.7	1.8 ± 0.8	1.1 ± 0.3	0.4 ± 0.2
Percentage (%) of each vigilance state	Wake	L	53 ± 4	52 ± 2	35 ± 6	51 ± 2
		H	30 ± 2*	39 ± 2*	32 ± 3	44 ± 2*
	NREM	L	33 ± 3	35 ± 3	46 ± 4	40 ± 2
		H	53 ± 3*	46 ± 2*	52 ± 2	43 ± 1
	REM	L	14 ± 3	11 ± 2	19 ± 3	10 ± 2
		H	18 ± 3	14 ± 1	16 ± 2	13 ± 3
Number of bouts	Wake	L	11 ± 1	4 ± 1	9 ± 1	4 ± 1
		H	9 ± 1	6 ± 1	10 ± 1	4 ± 1
	NREM	L	6 ± 1	6 ± 1	11 ± 1	8 ± 1
		H	14 ± 3*	9 ± 1*	18 ± 7	9 ± 1
	REM	L	10 ± 2	4 ± 1	22 ± 12	6 ± 1
		H	16 ± 3	8 ± 1*	8 ± 1	6 ± 1
Mean bout duration (sec)	Wake	L	234 ± 35	640 ± 125	193 ± 55	519 ± 51
		H	157 ± 18	312 ± 53*	115 ± 12	401 ± 30
	NREM	L	252 ± 24	257 ± 22	165 ± 15	199 ± 20
		H	236 ± 59	224 ± 28	169 ± 11	201 ± 20
	REM	L	48 ± 8	98 ± 11	75 ± 6	60 ± 5
		H	44 ± 4	74 ± 6	80 ± 5	78 ± 6

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3.4.2 Effect of *Ta* on SWS and sleep depth

Using EEG zero-crossing criteria, any NREM epoch was classified as SWS if the number of EEG zero-crossings during NREM fell below the lowest 30th percentile of zero-crossing values in NREM epochs recorded at baseline *Ta*. Results showed that the time spent in SWS during the Light period is significantly higher in Prethermal compared to Off days but significantly lower than On days (Kruskal-Wallis test) (Figure 3.7). Epileptic mice spent more time in SWS in On days compared to Off days ($p < 0.05$).

Then Wilcoxon signed rank test was applied to test the effect of *Ta* elevation on the percent of SWS during On days versus Off days (matched data). An elevation in *Ta* increased SWS time significantly ($p < 0.05$). The changes in SWS % over time showed a different trend than the one seen in non-epileptic controls in which the proportion of SWS decreased gradually from 9 a.m. to 5 p.m. That gradual decrease in SWS % in controls has been supported by one study in rats (Obál *et al.* 1983). Looking at data pooled from four epileptic mice, SWS % increased gradually from 7 a.m. to around 4 p.m. before declining over the next five hours, the trend was the same at baseline and elevated *Ta*. However, the percent of SWS has increased with temperature each hour. The trend was consistent in three out of four epileptic mice; the fourth showed a decreasing trend in the first few hours and then increased over the rest of time. This needs to be clarified in a larger sample of data especially since it has not been examined before in a mouse model of TLE.

The reduced sleep depth is common in epilepsy patients. To our knowledge neither the measures of sleep depth nor the effect of *Ta* changes on sleep have been studied before in a pilocarpine mouse model of TLE or other epilepsy models. Here, EEG delta to theta power ratio (0.5-4Hz)/(6-9Hz) was defined to be the sleep depth measure. Figure 3.8 shows that the distribution of delta to theta power has shifted to the left in Off days comparing to Prethermal (less sleep depth) and then to the right in On days compared to Prethermal and Off days (more sleep depth). This is consistent with the SWS % differences between the three conditions.

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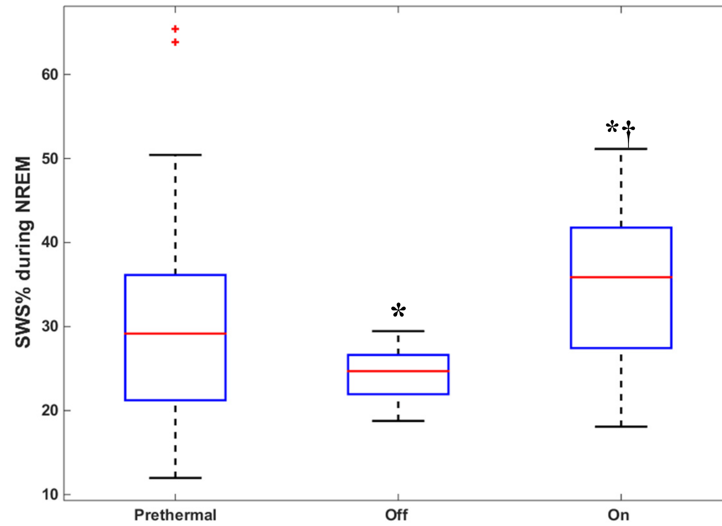

Figure 3.7. Effect of *Ta* on %SWS during NREM sleep. Epileptic mice tend to have less SWS in Off days compared to Prethermal and more SWS in On days compared to Off days and Prethermal. * and † indicate a significant difference relative to Prethermal and Off days respectively (*p*-value < 0.05, Kruskal-Wallis test).

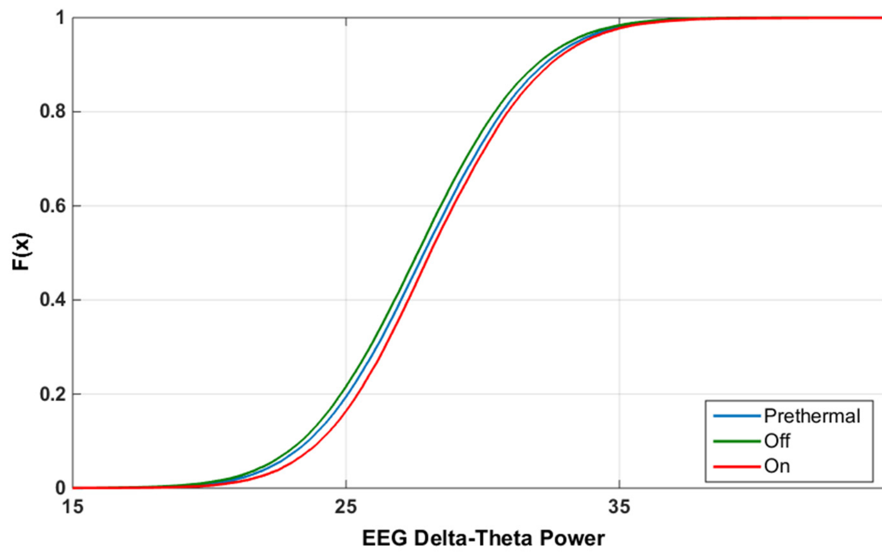

Figure 3.8. Effect of *Ta* on sleep depth. The distribution of Delta (0.5-4Hz) to theta (6-9Hz) power ratio shifted leftward in Off days compared to Prethermal and then to the right in On days compared to both Prethermal and Off days; i.e. greater sleep depth at higher temperature.

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3.4.3 Effect of *Ta* on seizure rate and timing

During Prethermal days, a total of 103 seizures were detected and verified using EEG, EMG, piezo, and video recordings in Light and Dark periods. There was some variability in seizure number across the four animals. More seizures occurred in the Light period (n = 64) than the Dark period (n = 39). While epileptic mice had 58 seizures during the Light period and 54 seizures during the Dark period in Off days, they had 53 seizures during the Light period and 79 seizures during the Dark period in On days. Figure 3.9 shows the daily seizure rate of data pooled from four mice in the Light and Dark periods for Prethermal, Off and On days. There was no significant difference in seizure rate between Prethermal, Off, and On days during Light or Dark phases ($p > 0.05$, Kruskal-Wallis test). No significant changes were observed in seizure rate between : (a) Off and On days during the Light and Dark periods (b) the Light and Dark periods of each condition ($p > 0.05$; Wilcoxon signed rank test).

A closer look at the seizure rate for On days versus Off days during the Light period revealed that there is some variability in mean seizure rate across animals. During On days, the rate increased in two animals and decreased for the other two; but neither effect was significant (Ajwad *et al.* 2016); see Table 3.1. The two animals (Mouse 2 and 4) in whom seizure rate was lower at elevated *Ta* were the same animals that demonstrated a significant reduction in Wake % coupled with an increase in REM % ($p < 0.05$; Wilcoxon signed rank test). This is consistent with the notion supported by a large body of literature that seizures are much less likely to occur in REM sleep than NREM (Foldvary-Schaefer 2009 and Herman *et al*. 2001).

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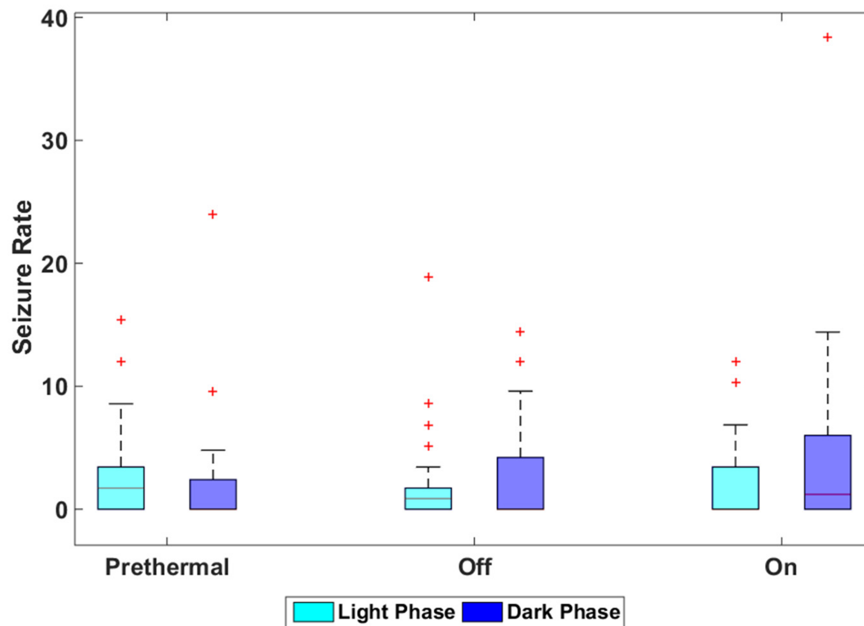

Figure 3.9. Effect of *Ta* on seizure rate during the Light and Dark periods. No significant effect was observed between the three conditions or between the Light and Dark periods of each condition ($p > 0.05$, Kruskal-Wallis test). Median of seizure rate is zero for Prethermal (Dark period), Off (Dark period), and On (Light period).

The seizure rate results suggest that an elevation in *Ta* (30ºC) during the Light period shifts the seizures toward the Dark period. Hence, we looked at the hourly seizure distribution for Prethermal, Off and On days during the Light and Dark periods. For Prethermal data; the hourly seizure rate varied with the time of day but interestingly (see Fig. 3.10.a) while it peaked at 20:00 (one hour before light off onset), no seizures were detected at 6 a.m. (one hour before light on onset). In three of four mice, seizures were clustered at 20:00 but the previous hour was seizure-free. Though the effect of the Light: Dark cycle on hourly seizure rate distribution is not very clear, there is an indication of this effect as shown in the figure below; but again this needs to be clarified in a larger sample. For Off days, seizure distribution seemed to be equal in the Light and Dark periods, Fig. 3.10.b. That was consistent for all four mice. Figure 3.10.c shows that more seizures happened in the Dark period for On days, especially in the first hour after the onset of the Dark period. Those findings support the observed *Ta* effects on seizure rate. To conclude: (a) seizures in the

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Dark period are less than the Light period for Prethermal, (b) they are equal in both periods for Off days, and (c) more in the Dark period than the Light period for On days.

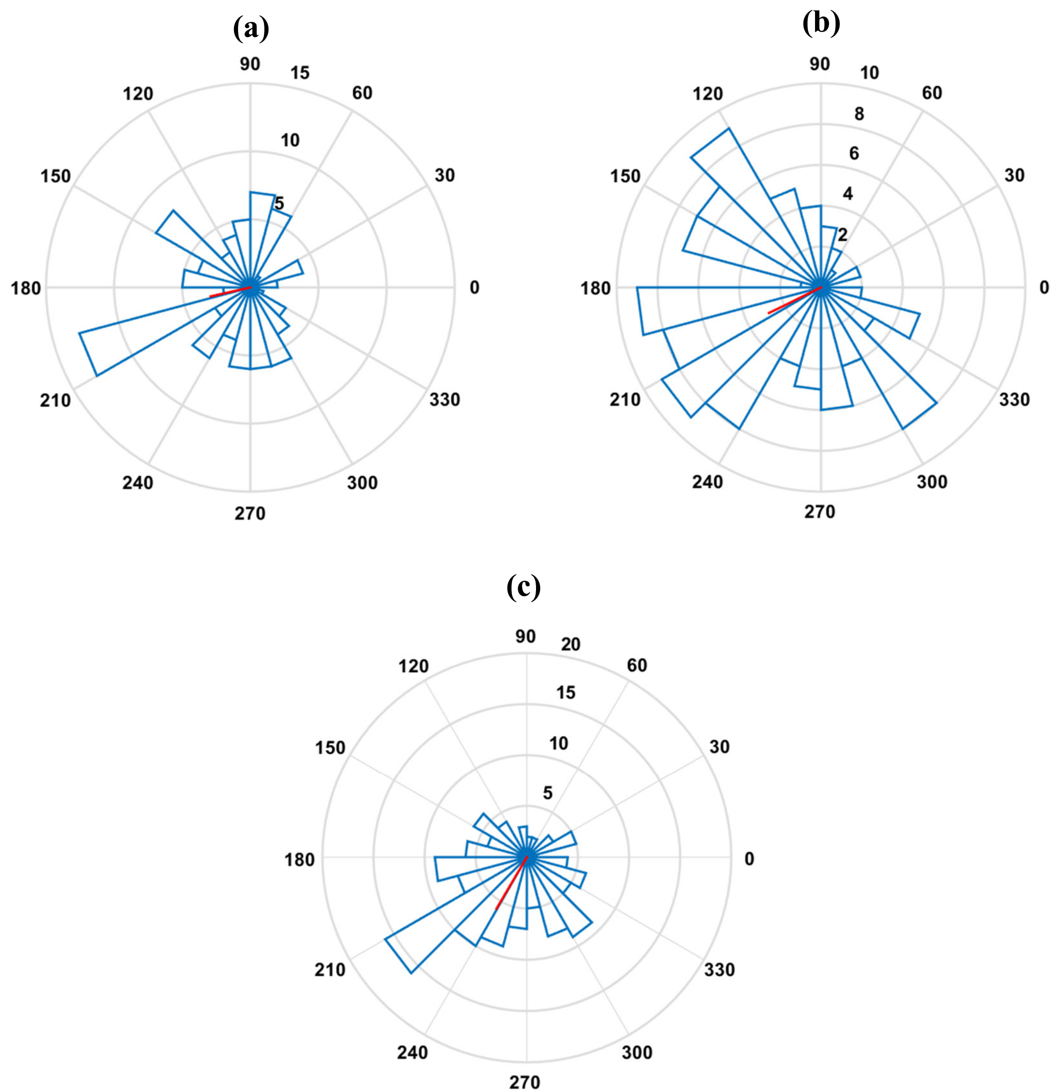

Figure 3.10. Effect of *Ta* on the hourly seizure rate (expressed in units per day) for data pooled from four epileptic mice. **(a) Prethermal:** fewer seizures occurred in the Dark period than the Light period, **(b) Off Days:** seizures were equally distributed in the Light and Dark periods, and (**c) On Days:** the seizures shifted toward the Dark period. Zero degrees on the polar histogram refers to the onset of the Light period (7 a.m.), and 210 degrees marks to the onset of the Dark period (9 p.m.). The red arrow points to the mean seizure phase (time of day) and is of length equal to the mean seizure rate.

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3.5 Conclusions

To our knowledge, the effect of *Ta* on sleep has not been investigated in this detail before in models of epilepsy. In general, the results of this study show that epileptic mice tend to have a reduction in Wake coupled with an increase in NREM, REM, and SWS at an elevated *Ta* compared to intervening days when *Ta* was kept at room temperature. The increase in NREM appears to come from an increase in the number of NREM bouts rather than the mean bout duration. Changes in time spent in REM appeared inconsistent, increasing in three animals and decreasing in the fourth. The increase in SWS and REM sleep hints at the possibility of using ambient temperature regulation as a simple and non-invasive way to modulate sleep toward the goal of reducing seizures since both SWS and REM sleep mechanisms work against generation and propagation of epileptic seizures (Herman *et al*. 2001). This study also shows that elevated *Ta* has similar effects on sleep in epileptic mice as in controls but with more NREM sleep bouts associated with no change in their mean duration.

All seizures in this study were classified in the range S3 to S5 on the Racine scale and they occurred during different vigilance states. A couple of other abnormal activities including absence seizures were captured on the EEG signal but with no sign of convulsion on EMG or piezo signals; these few events were neglected.

Age has an important effect on epileptic seizures (Karnam *et al.* 2009). Our epileptic mice at the start of thermal experiment were different in age, 13 to 24 week. This difference could be a factor in the variability seen in sleep and seizures outcomes across animals.

Due to the long latency to onset of chronic epilepsy in mice and the high mortality rate associated with pilocarpine SE induction, this study was severely handicapped by a small size. That was the major limitation of this study; but the number of days (minimum of 10 per animal for each condition (Prethermal, Off, and On)) is however large enough to suggest that the findings of this study may be more than a chance observation. The possibility of losing the animal due to seizure while running the experiment and having only one thermal chamber to run the experiment on one mouse for a month were the other limitations we had in this study.

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CHAPTER IV DYNAMIC SLEEP MODULATION IN CONTROL MICE THROUGH AMBIENT TEMPERATURE REGULATION

4.1 Introduction

Though its ultimate function is still unclear, sleep undoubtedly plays a very critical role in many fundamental processes associated with brain and body function such as memory consolidation and energy metabolism. In recent times, the importance of sleep in learning and memory consolidation has been extensively studied in human and rodents. Both NREM and REM sleep are involved in memory consolidation in different ways. While deep NREM sleep (also known as slow wave sleep, or SWS) enhances the consolidation of declarative memory by the hippocampus, REM sleep tends to support the memory consolidation that is not mediated by hippocampus (Giuditta et al. 1995). Other studies assert that while SWS helps in improving some procedural skills (also known as non-declarative memory) (Gais *et al.* 2007 and Aeschbach *et al*. 2008), REM sleep participates in enhancing declarative memory (Fogel *et al*. 2007 and Rauchs *et al.* 2004). Stage 2 of NREM sleep is found to contribute to the strengthening of memories in humans and rats (Rebecca *et al*. 2002 and Datta 2000). In addition to its beneficial role in brain function, SWS has been recognized for its importance in controlling glucose metabolism and regulating autonomic functions such as heart rate and body temperature (Cautera *et al.* 2008 and Bellesi *et al.* 2014). Thus, many studies have started investigating and trying different approaches to enhance SWS: some of these approaches involve pharmacological agents such as tiagabine and gaboxadol, which target an increase in deep sleep duration (Walsh 2009, Lundahl *et al.* 2007, Bazil *et al.* 2012). Other approaches use non-pharmacological interventions like sleep restriction as it is known to impair waking neurobehavioral function which in turn enhances SWS (Van Dongen *et al.* 2003), auditory stimulation (Garcia Molina *et al.* 2018) or transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) applied to the human cerebral cortex at

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specific frequencies (Marshall *et al.* 2006 and Massimini *et al.* 2007). However, some adverse effects such as nausea and vomiting in pharmacological approaches or limited sleep time in non-pharmacological approaches have been noted. Thus, there is a need for deep sleep enhancement approaches that are noninvasive, stress-free, low in cost, and with minimal adverse side effects.

Deep sleep is marked by the prevalence large amplitude slow oscillations (hence the name slow wave sleep, or SWS) in the delta band (0.5-4 Hz) of the EEG. Sleep depth is hence usually measured by the strength of EEG delta band power (as discussed in Chapter Two), which is easily detected in surface EEG or local field potential measurements during NREM sleep. SWS, which occurs mainly in stage 3 of NREM sleep in humans, increases with sleep depth and has been studied in many species; its correlation with prior wake duration has been well documented (Borbely *et al.* 1989, Lancel *et al*. 1992 , Huber *et al.* 2000). This chapter describes a simple non-invasive approach that has been proposed to test the feasibility of enhancing sleep depth and increasing the proportion of SWS in mice by using a closed-loop control system that manipulates ambient temperature to minimize the error between the actual sleep depth measure and a pre-specified target value. A successful outcome will provide a method of sleep modulation that avoids many limitations seen in other approaches and is amenable to clinical translation.

As in any closed-loop control system, error detection and compensation are the key elements of our proposed approach: the designed system tries to reach a desired or specified target value of sleep depth (defined here as a value typically seen in deep NREM sleep); the system's state (defined by the actual value of the sleep depth measure) is compared to the target value; and based on that error between actual and target values, the system output is corrected through ambient temperature regulation by either increasing or decreasing the mouse cage temperature. Closed-loop control theory has been applied in many areas of neuroscience: for instance, in optogenetic control for the purpose of stimulation, inhibition and modulation of neural activity in real time (Grosenick *et al*. 2015). EEG and transcranial magnetic stimulation (TMS) have been used in closed-loop protocols, to study the implications of this approach in neurophysiology (Zrenner *et al.* 2016). Auditory closed loop stimulation has been developed to drive sleep slow oscillations in humans (Ngo *et al.*

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2015). Closed-loop techniques in seizure detection and prevention have been demonstrated in several studies (Paz *et al.* 2013, Krook-Magnuson *et al.* 2013 , Salam *et al*. 2016).

The quantity and timing of sleep and wake are influenced by many external factors such as changes in environmental temperature as was explained in Chapter Two. Changes in ambient temperature (*Ta*) also have an effect on ultradian rhythms, which are biological rhythms that occur with periods less than 24 hours. In contrast to humans, rodents exhibit polyphasic (ultradian) sleep cycles, which makes their response to environmental changes more flexible (Stephenson *et al*. 2012). Our results from static experiments in Chapter 2 suggested that an elevation in *Ta* influences the duration of sleep and wake bouts. To see whether this ultradian sleep-wake cycle can be entrained to an externally imposed rhythm by manipulating *Ta*, a "sleep-wake induction" strategy was developed to control the timing and duration of sleep/wake bouts using a closed-loop control system.

Given that temperature changes have a significant effect on sleep in control and epileptic mice, this chapter covers aims III and IV of the dissertation which demonstrate the feasibility of sleep depth modulation and sleep-wake timing regulation in control mice in real time using closed-loop strategies. Here, three different strategies for sleep modulation were developed and tested:

1. *Ta* was manipulated to force the proportion of time spent in sleep to approach a target value using a closed-loop control system to test the feasibility of thermal sleep modulation (n = 2).

2. *Ta* was manipulated to enhance NREM sleep depth in real time based on the error between *Q*, the instantaneous EEG delta/theta bandpower ratio, and a preset target value typically seen in deep NREM sleep (n = 5).

3. The setpoint for *Q* was programmed to exponentially decay (over 30 min) or grow (over 60 min) to approach values typical of Wake and deep NREM sleep to see if the ultradian sleep-wake cycle could be entrained to an externally imposed rhythm (n = 11).

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4.2 Experimental procedure

All experimental procedures in this chapter were carried out with the prior approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. Mice were housed individually in 23cm × 15cm × 15cm plexiglass cages with food and water available ad libitum. Conditions in the animal facility during the study were maintained at a temperature of 20-23°C, relative humidity of about 50%, and a 14h/10h light/dark cycle with the lights turned on from 7 a.m. to 9 p.m. Each mouse was surgically implanted under anesthesia with a head-mounted preamplifier (Pinnacle Tech., Lawrence, KS, USA) for continuous tethered recording of frontal and parietal EEG and nuchal EMG (details of the surgical procedure were described in Chapter Two). For each sleep modulation strategy, a computer program was written in the LabVIEW environment to regulate *Ta* in the mouse cage, as measured by a suspended thermistor (details of the thermal system are described in Chapter Two). The system switched the heater on or off as needed to manipulate *Ta* and satisfy the objective of sleep modulation. After allowing about two weeks to recover from surgery, mice were transferred to the thermal chamber and allowed to acclimatize to the new cage for two days. Then, different protocols were applied to serve the purpose of each dynamic control strategy as described in the following sections.

4.3 Dynamic sleep modulation strategies

To test the feasibility of titrating sleep in mice by manipulating *Ta*, three dynamic strategies with different objectives were devised and tested.

4.3.1 Control of sleep proportion

After confirming that ambient temperature elevation has a significant effect on sleep in control and epileptic mice, the next logical step was to develop a simple strategy for dynamic sleep modulation by manipulating *Ta* in closed-loop, i.e., in response to observed changes in behavior. A sample trial on a control mouse (Fig. 4.1) demonstrates how dynamic sleep titration might be achieved. In this experiment, vigilance state labeled every one second in real time by an automated EEG/EMG classifier (Yaghouby *et al*. 2016) was

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used to estimate the ratio R of time in sleep (REM or NREM) relative to wake in a moving 5 min window. R was scaled to give a sleep quality metric $q \in [0, 1]$ using the transformation $(1-e^{-R}) / (1+e^{-R})$. The error ε between q and a target value q^* served as the basis for control of Ta (initially set to 22°C), which was raised or lowered by 1°C every 5 min to shift the sleep-wake proportion towards q^*. Here, q^* was set to 0.63 (i.e., $R = 2$, or twice as much sleep as wake) and the set point Ta changed by ±1°C—while remaining within the 22-30°C interval—every 5 min based on the following rule (Ajwad *et al.* 2016):

$$\boldsymbol{Ta(t) = Ta(t-1) - \Delta Ta(t-1)\, sgn(\varepsilon(t) - \varepsilon(t-1))},$$

where $\varepsilon(t) = |q(t)-q^*(t)|$. This control policy changes Ta at each time step in a direction that reduces the error in q. The system compares the error $\varepsilon(t)$ at each time with the previous error $\varepsilon(t\text{-}1)$. If $\varepsilon(t)$ is greater than $\varepsilon(t\text{-}1)$, then the system changes Ta in the opposite direction to the change that was made at $(t\text{-}1)$. If $\varepsilon(t)$ is less than $\varepsilon(t\text{-}1)$, the change in Ta will be in the same direction as in $(t\text{-}1)$. In effect, the algorithm follows a temperature profile in search of a target sleep quality. In the trial shown below, the net q over 9 hours is about 0.5, fairly close to the target value of 0.63; by comparison, a baseline recording in which Ta remained at ambient conditions gave a net q of about 0.3 (Figure 4.1). There is a clear increase in NREM and REM episodes during the dynamic control period compared to baseline. The increase in time spent in NREM and REM came mainly from an increase in the number of bouts and not their duration. Wake time decreased during dynamic control due to a reduction in bout duration though the number of wake bouts increased. A closer look at wake bouts showed that while the number of prolonged wake bouts decreased during dynamic control, the number of brief arousals increased but with a decrease in their duration. This is a naïve policy in which the controller has no prior knowledge of the effects of Ta on sleep but still manages to gradually increase Ta to drive q toward q^*. The results suggest the feasibility of sleep regulation in disease models such as epileptic animals using any sleep quality metric (e.g., REM/NREM ratio, REM bout duration), perhaps reducing seizure likelihood in the process.

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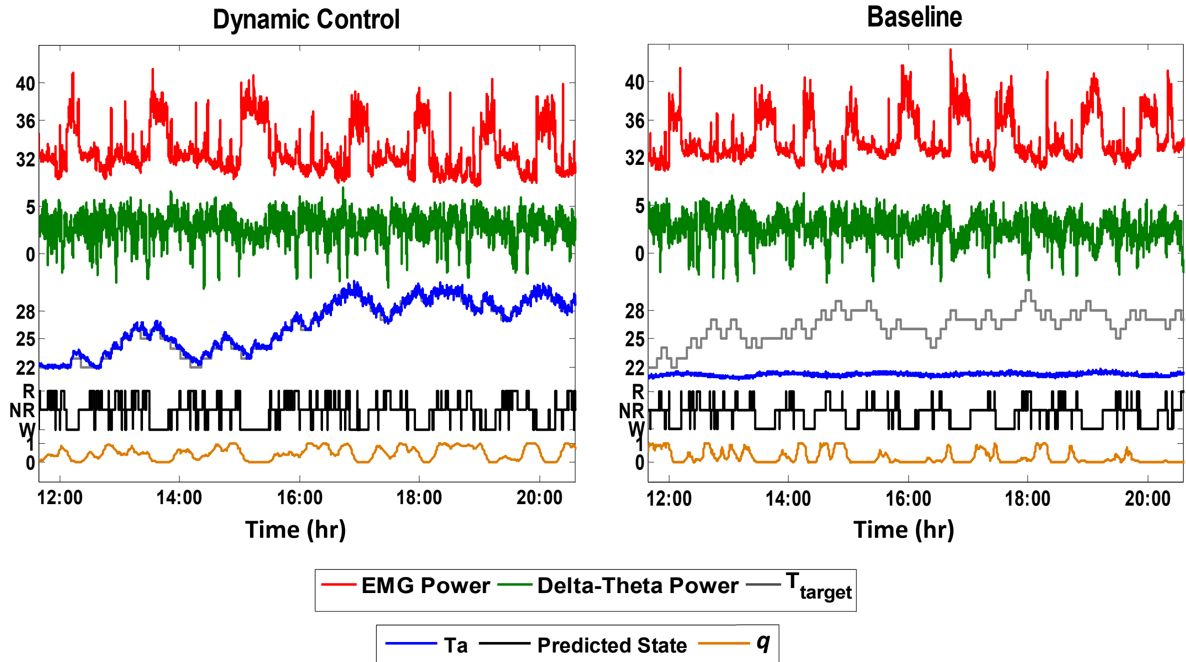

Figure 4.1. Dynamic thermal sleep modulation. ***Left:*** Demonstration of dynamic regulation of *Ta* to achieve a target sleep-wake ratio of 3:2 (q^*=0.63) over a 9-hour period. ***Right:*** A recording at baseline *Ta* from the same animal on a different day is shown for comparison purposes. Here, the setpoint is computed but not applied: i.e., *Ta* remains at baseline. Comparing to baseline, it is clear that under dynamic control condition the mouse had more NREM (NR) and REM (R) episodes and less wake (W).

Next, a random walk *Ta* control policy was applied to the same animal on a different day. In that trial, *Ta* started at room temperature (~22°C) and raised or lowered by 1°C at each step with 50% probability (but not to exceed the lower and upper limits of 22 and 30°C respectively. The purpose of this trial was to test whether it was the dynamic control of *Ta* which drove q toward q^* and not arbitrary changes in *Ta*. For the random walk policy, mean q was less than 0.1 (Figure 4.2) which is far removed from q^* (0.63). This confirms that the results of the control experiment did not occur by chance but as a consequence of the dynamic strategy, which changes *Ta* in a direction that minimizes the error between q and q^*. Thus, the main conclusion of the first dynamic strategy is that dynamic sleep modulation though ambient temperature manipulation is feasible.

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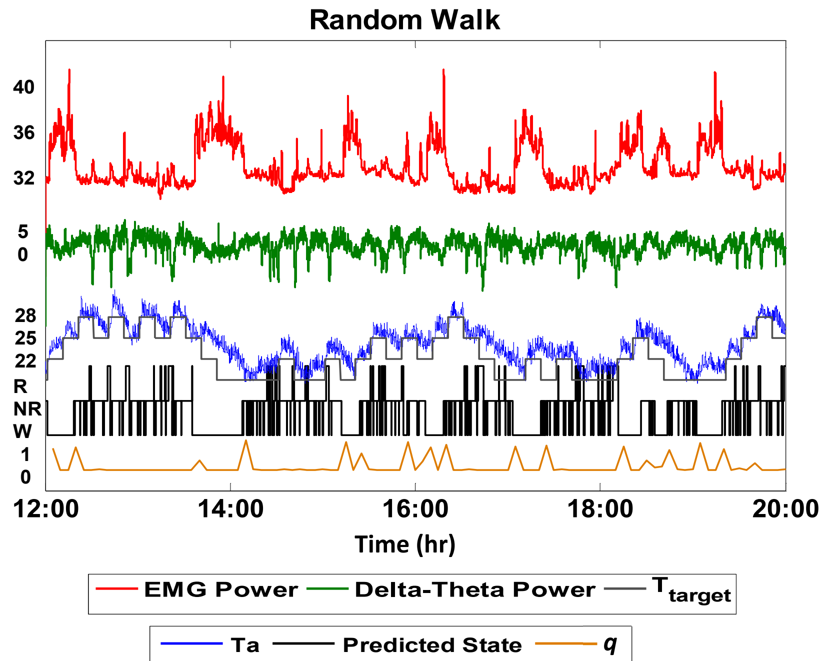

Figure 4.2. Random walk strategy. A recording from a mouse that received a random walk control policy over 8 hours. Temperature was incremented or decremented at random at 10-minute intervals regardless of the error between q and q^* to see if the animal would experience q close or far from q^*, where q is the sleep-to-wake ratio. q is zero most of the time with a mean less than 0.1.

4.3.2 Sleep depth enhancement

4.3.2.1 Sleep depth modulation (SDM) design

Five C57BL/6 mice (3 females, 2 males; Envigo-Harlan), 8-12 weeks of age, were surgically implanted with EEG/EMG electrodes for measuring brain and muscle activity. After adequate recovery, each mouse was exposed to dynamic changes in *Ta* within the thermoneutral zone (TNZ) to enhance deep sleep (slow wave sleep) in real time based on the error between Q, the ratio of instantaneous EEG power in the delta (0.5-4Hz) to the theta (6-9Hz) band of the EEG, and Q^*, a preset target value of Q typically observed in deep NREM sleep under baseline conditions. In this protocol, the EEG delta/theta power ratio Q was computed in real time in a moving 1-s window. The temperature setpoint *Ta*

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was changed by ±1°C every 5 min in inverse proportion to the error between Q and a pre-specified target value Q^* of 5 dB, which is typically observed in deep NREM sleep. With the assumption that an increase in Ta induces an increase in the EEG delta/theta power ratio (more sleep depth), Ta was regulated following the rule:

$$\boldsymbol{Ta(t) = Ta(t-1) - sgn(Q(t) - Q^*)}$$

However, the changes in Ta were restricted to the interval 22-30ºC during the SDM experiment. Deep NREM sleep in control mice is greatest at the onset of the light period and decreases steadily thereafter (Obál *et al*. 1983), even as total sleep increases over the next 12 hours. Hence, the SDM protocol was applied to each mouse from 12-7 p.m. when light NREM sleep usually dominates. This was done every other day over four consecutive days (i.e., two repeats). Ta was kept at 25ºC from 12-7 p.m. on intervening days and reverted to room temperature (~ 22°C) at other times with continuous recording of EEG, EMG, motion, Ta, and video. Data from two experiments each on five mice were collected to give a total of ten recordings for analysis. However, two recordings had to be excluded due to some recurring artifacts. Figure 4.3 shows a five-hour sample of recorded data from a preliminary experiment in which the controller was active for a three-hour period. A clear increase in Q and in NREM bout duration (defined by high Q) are observed while the controller was "ON".

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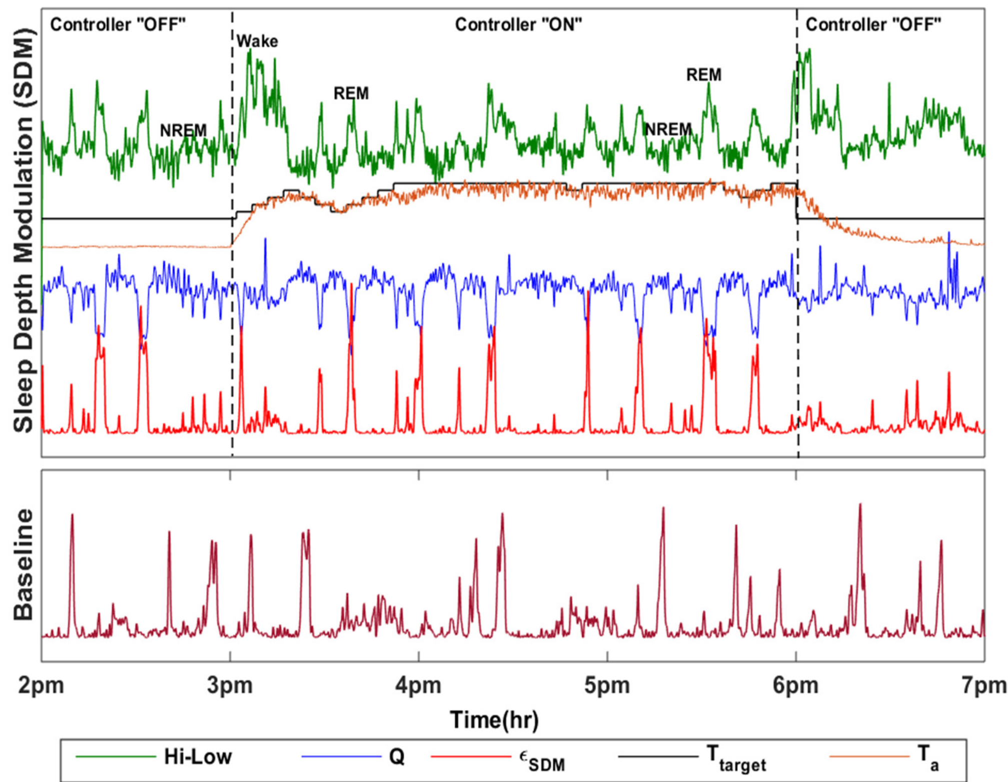

Figure 4.3. An SDM trial performed on a mouse over five hours. Except during episodes of REM sleep, marked by sharp dips in Q, the squared-error (ϵ_{SDM}) between Q and $Q*$ remains close to zero in the three-hour period 3-6 p.m. in which the controller was ON (***upper panel***) compared to a baseline (controller OFF) measured during the same period on the previous day (***lower panel***). *Ta* appears to be driven upward by the algorithm to achieve this effect. The Hi-Low EEG feature, i.e., (9-45 Hz)/ (0.5-9 Hz) band power ratio, is typically high in wake and low in NREM.

4.3.2.2 Scoring of vigilance state

To characterize changes in the sleep depth measure Q and other sleep parameters, data were scored offline into Wake, NREM, and REM in 1-sec windows using a previously validated hidden Markov model (HMM) algorithm (Yaghouby *et al*. 2016). Wakefulness is distinguished from sleep based on muscle activity (EMG) that is high in wake and low in sleep. EEG delta/theta power ratio is used to discriminate NREM from REM sleep; this is high in NREM and low in REM. The EEG Hi-Low ((9-45 Hz)/ (0.5-9 Hz)) helps in distinguishing sleep from wake. Figure 4.4 shows sample feature traces (EEG delta/theta power ratio, EEG Hi-Low power ratio, and EMG power) computed in 1-sec epochs over a 6-hr period for a mouse at baseline *Ta*, along with the vigilance state hypnogram scored by

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the HMM classifier. We further computed the number of EEG zero-crossings in each NREM epoch to classify NREM sleep into light NREM sleep (LS) and deep NREM sleep (denoted by DS or SWS). Any NREM epoch was assigned to DS if the number of zero-crossings fell below a threshold defined as the lowest 30th percentile of the number of zero-crossings in NREM epochs of any of the recordings at baseline *Ta* (Obál *et al*. 1983).

To quantify the effect of *Ta* manipulation on sleep depth, the EEG power in the low delta band (0.5-2Hz) and mean-squared error in *Q* with respect to *Q** during NREM sleep were computed and compared for controller ON and OFF days from 12-7 p.m. using the Kolmogorov–Smirnov (K–S) test. Using Wilcoxon signed-rank test, the effects of dynamic control on conventional sleep metrics, namely; the proportion of time, mean bout duration, and number of bouts of each state were evaluated for the period of *Ta* manipulation against the same period on intervening days in which *Ta* was set to 25ºC without variation from 12-7 p.m. and reverted to the room temperature (~22ºC) at other times; *p-value* under 0.05 was considered significant.

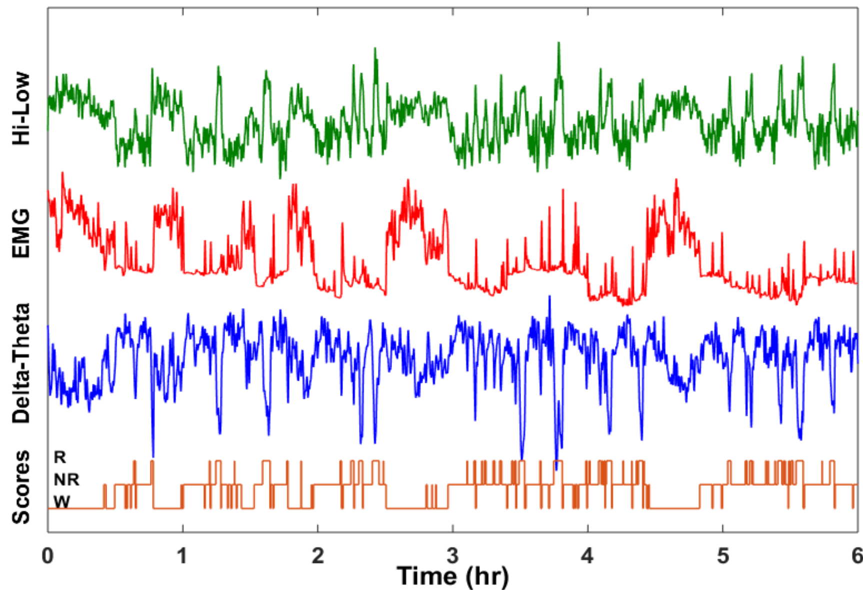

Figure 4.4. Six-hour snapshot showing EEG and EMG features used by the HMM classifier to track changes in vigilance states. Muscle tone (EMG) is high in wakefulness (W) and low in sleep. Within sleep, EEG Delta-Theta power ratio is high in NREM (NR) and low in REM (R). EEG Hi-Low power ratio is high in wake and low in NREM.

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4.3.2.3 Results and discussion

4.3.2.3.1 Effect of *Ta* manipulation on sleep depth

The cumulative distribution function of the squared error ϵ_{SDM} in Q with respect to Q^* during NREM sleep is compared for dynamic SDM versus baseline over all five mice in Figure 4.5. The error ϵ_{SDM} was significantly lower ($p < 0.05$) during dynamic regulation of *Ta* compared to the baseline, during which *Ta* was kept at 25ºC: this is evidence of a significant increase in sleep depth. Furthermore, EEG low delta (0.5-2Hz) power (a well-known marker of sleep depth (Dijk 2009)) was significantly greater for SDM versus baseline during NREM (see Figure 4.5). This verifies that mice experienced deeper sleep during periods of dynamic manipulation of *Ta* (Ajwad *et al.*, 2018) .

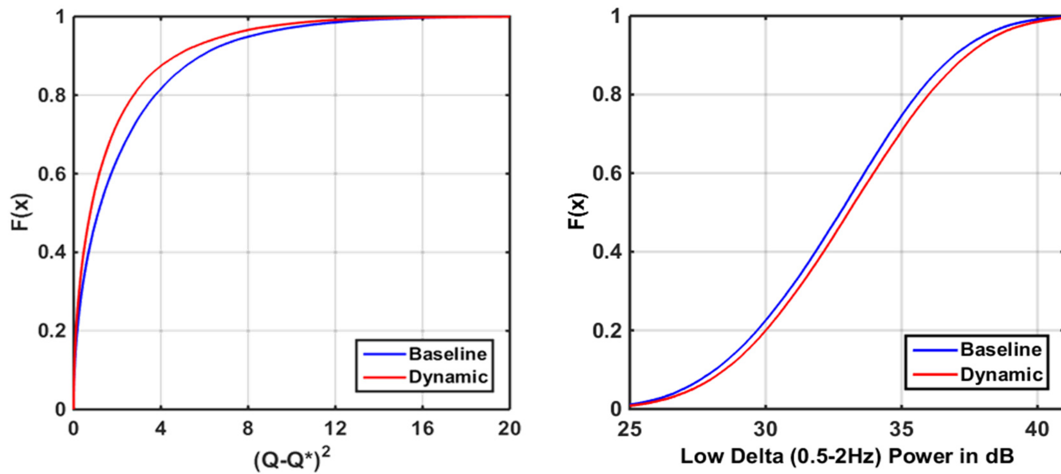

Figure 4.5. Sleep depth modulation. ***Left:*** Squared error in Q with respect to Q^* during NREM sleep shifts leftward during SDM (Dynamic); i.e. an increase in sleep depth. ***Right:*** Low delta power (logarithmic scale) during NREM shifts to the right in dynamic SDM period; i.e. more deep NREM sleep.

4.3.2.3.2 Effect of *Ta* manipulation on theta oscillations

REM sleep is controlled by homeostasis drive and can get affected by a mild change in *Ta*. Studies have shown that an elevation in *Ta* increases time spent in REM sleep (Jhaveri *et al*. 2007 and Roussel *et al*. 1984). EEG theta oscillations (6-9Hz) are predominant during REM sleep. Though there was no intention in this study to modulate REM sleep,

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interestingly a clear increase in theta power was observed during dynamic manipulation of *Ta*; which corresponds to an increase in REM sleep. Figure 4.6 shows the distribution of theta power during REM for SDM versus baseline. A clear shift in theta power to the right suggests REM sleep promotion.

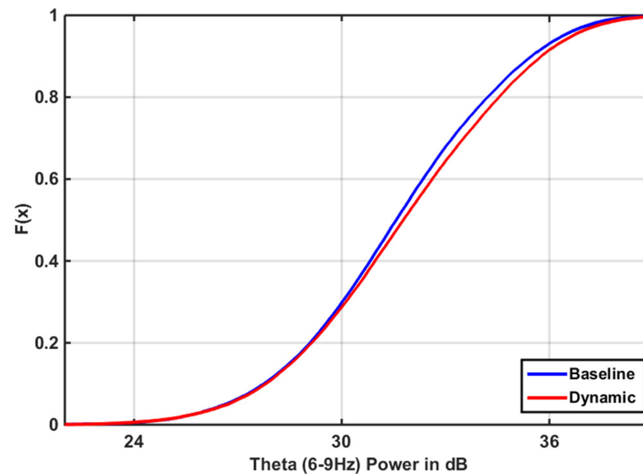

Figure 4.6. Effect of SDM on theta oscillations in REM sleep. EEG theta (6-9Hz) power during REM sleep shifts to the right in dynamic SDM period; i.e. more REM sleep. (*p-value* < 0.05, Kolmogorov–Smirnov test).

4.3.2.3.3 Effect of *Ta* manipulation on sleep metrics

Sleep metrics were estimated and compared statistically for dynamic SDM days against baseline days (Figure 4.7). There were no statistically significant effects of *Ta* manipulation on the time spent in each state ($p > 0.05$, Wilcoxon signed-rank test), but some subtle changes were observed. Though the time spent in NREM sleep did not change, the number of NREM bouts decreased ($p = 0.007$) while their mean bout duration increased ($p = 0.02$), which suggests that NREM sleep was less fragmented during SDM than in the baseline. This trend was consistent in all five mice. Mice spent more time in REM sleep. But while REM bout duration increased significantly ($p = 0.04$) in all mice, the number of bouts decreased (not significantly in all but one mouse, a male). Finally, time in Wake decreased during SDM, but there was some variability in both Wake bout duration and number of bouts across animals: Wake bout duration increased in three mice and decreased in two (one male and one female); and the mean number of Wake bouts decreased in all but one mouse (a female). The trends were similar when only data from the first day were

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included in the analysis to allow for the possibility of homeostatic carry-over effects on the second day. However, the effects will need to be clarified in a larger sample (Ajwad *et al.* 2018).

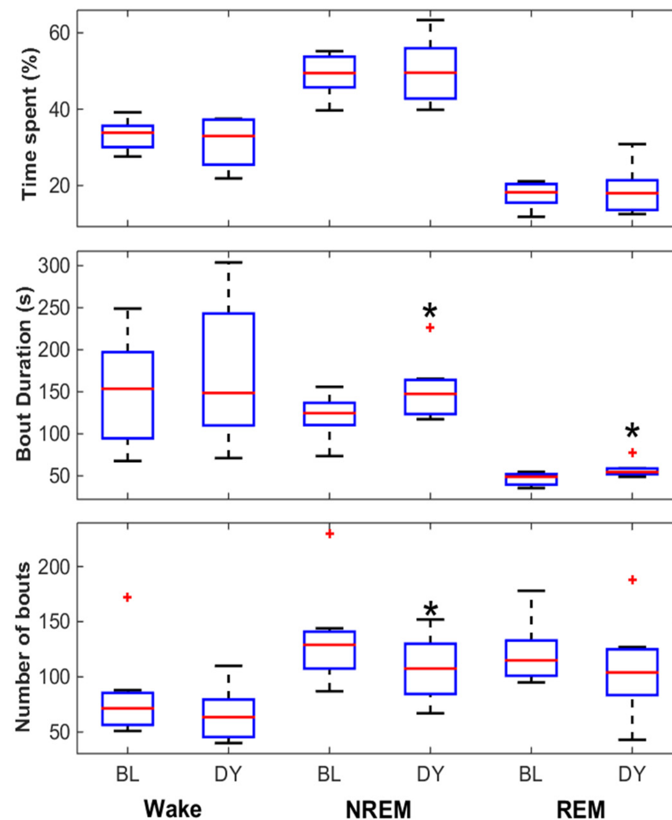

Figure 4.7. Effects of SDM on sleep metrics. BL: Baseline, DY: Dynamic. * indicates a significant difference ($p < 0.05$, Wilcoxon signed-rank test), $n = 8$ where n is the number of recordings from five mice. Though SDM has no significant effect on time spent in NREM, it does affect its mean bout duration and number of bouts in a direction that makes it less fragmented, i.e. longer bout duration and less number of bouts. While REM bout duration increases significantly during SDM, number of bouts decreases insignificantly.

4.3.2.3.4 Effect of *Ta* manipulation on deep sleep proportion

Manipulating *Ta* in closed loop induced a significant increase in the proportion of DS ($p = 0.02$; Wilcoxon signed-rank test). This effect was consistent in all five mice (Figure 4.8). It has been shown that DS peaks in the first three hours of the light period and then declines progressively (Obál *et al.* 1983). Since our experiment was conducted from 12-7

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p.m. (starting at zeitgeber time 5:00 or ZT5, i.e., five hours into the light period), this explains the relatively low overall proportion of DS (under 10%) observed in this study.

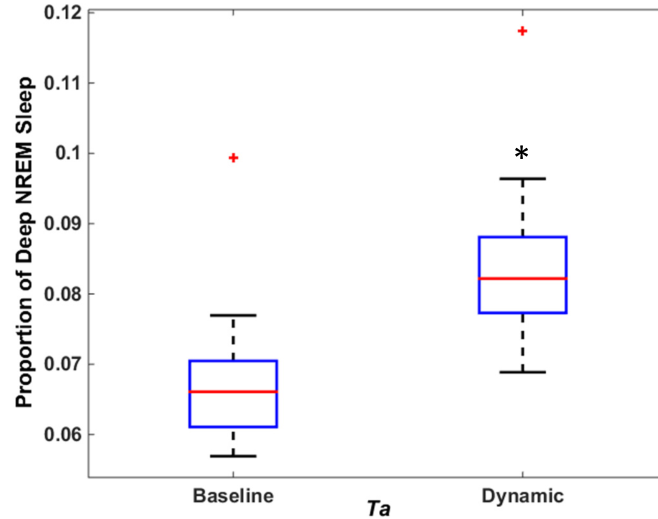

Figure 4.8. Effect of SDM on the proportion of deep NREM sleep. * indicates a significant difference (*p-value* < 0.05, *n*=8 where *n* is the number of recordings from five mice. All mice had more deep NREM sleep during SDM.

4.3.2.3.5 Effect of *Ta* manipulation on breathing regularity

The breathing trace is captured on piezo signal when the animal is relatively still or asleep. It changes during different vigilance states, and tends to be regular during NREM and irregular during REM (figure 4.9). It has been previously shown that breath rate and regularity measures can be estimated from piezo signal in control mice (Yaghouby *et al.* 2016), estimated based on Hilbert transform. Since breathing regularity is high in deep NREM sleep and low in light NREM and REM sleep, it could be used as a measure of sleep depth. Figure 4.10 shows the changes in the estimated breathing regularity index during NREM and REM in a 4-hr recording from a control mouse. It is not reliable during wake because the motion signal dominates over the breathing trace. Though there is some variability in breathing regularity within a state it is clearly high in NREM and low in REM. Breathing regularity was estimated and the effect of SDM on it during NREM and deep NREM sleep were statistically compared to baseline.

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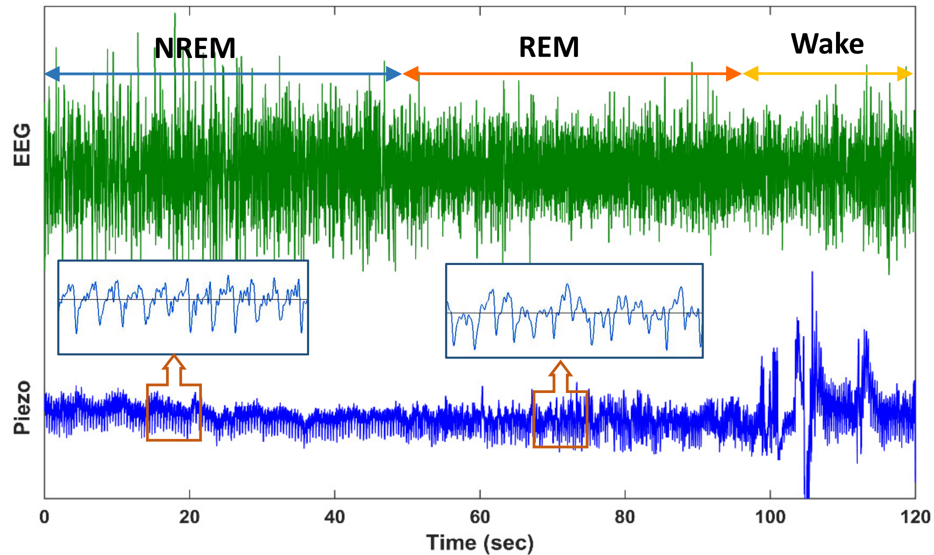

Figure 4.9. A two-minute sample showing the breathing pattern on piezo signal during NREM, REM, and wake. Breathing trace is regular during NREM and irregular during REM. EEG is presented to differentiate between vigilance states: high amplitude delta waves (0.5-4Hz) are predominant during NREM and low amplitude/ high frequency theta oscillations (6-9Hz) are predominant during REM.

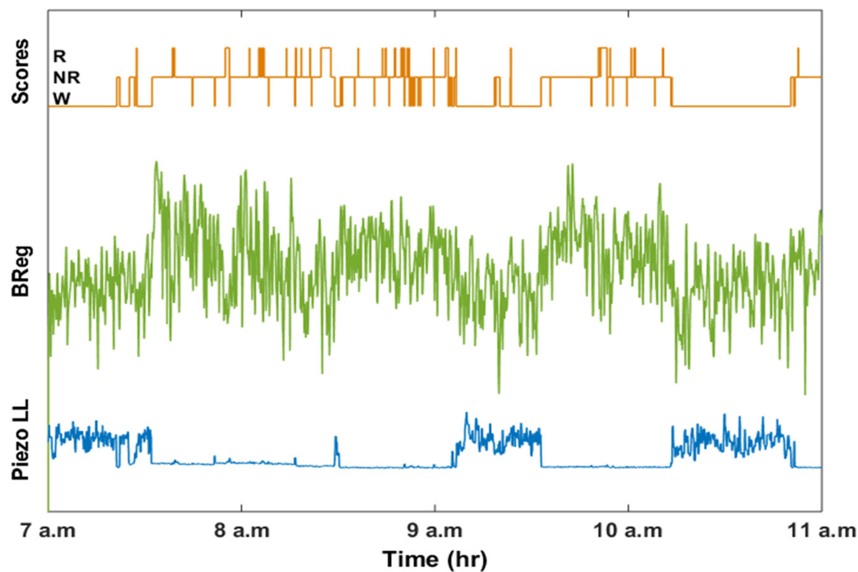

Figure 4.10. A four-hour sample of recording from a control mouse shows breathing regularity changes during different vigilance states: W= wake, NR= NREM, R= REM. Breathing regularity index (BReg) is high during NREM and low during REM. Piezo line length is presented here to reflect the animal's level of activity; it is low during sleep and high during wake. Some variability in breathing regularity could be observed within NREM sleep due to different stages going from light to deep sleep. The highest breathing regulatory occurs during deep NREM sleep.

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Figure 4.11 shows the effect of SDM on breathing regularity for data pooled from five mice. Breathing regularity during NREM sleep and SWS increased significantly ($p < 0.05$, rank sum test) during SDM compared to baseline, which add evidence that SDM increases SWS in mice. However, the values of breathing regularity during baseline and even during SDM seem low but again this can be due to the fact that SWS decreases gradually after light onset (Obál *et al*. 1983) and our SDM experiment was performed from 12 p.m-7 p.m. when SWS is low.

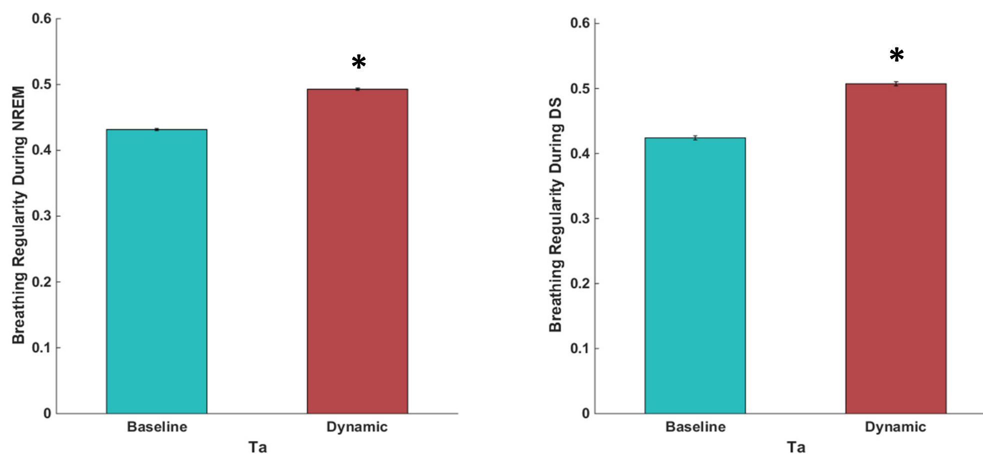

Figure 4.11. Effect of SDM on breathing regularity during sleep. Breathing regularity increased significantly in NREM (***left***) and in deep NREM during (***right***) SDM. * indicates a significant difference (*p-value* < 0.05). $n = 8$ where n is the number of recordings from five mice. Data are expressed as *mean* ± *sem*. All mice had more regular breathing in NREM and deep NREM sleep during SDM.

This study proposes a simple approach to enhance sleep depth in mice. Results from a small sample of five mice showed that the SDM protocol significantly increased sleep depth over a 7-hour period. Mice spent more time in SWS sleep during dynamic manipulation of *Ta* compared to baseline recordings in which *Ta* was held constant at 25ºC, which is higher than the room temperature in the animal facility and closer to the TNZ. Though it did not reach statistical significance, REM sleep increased along with DS during SDM. This was not unexpected since our results on temperature elevation effects on mouse sleep (Chapter Two) and some previous studies that have shown a significant increase in NREM sleep

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with an elevation of *Ta* in mice, also showed a corresponding increase in REM sleep (Roussel *et al*. 1984 and K. Jhaveri *et al*. 2007). The changes in *Ta* during SDM were limited to the neighborhood of the TNZ to avoid thermoregulatory stress associated with temperatures outside this range. To the best of our knowledge, no previous study has attempted sleep depth modulation in rodents through *Ta* manipulation using a closed-loop control system. One study in healthy and insomniac humans employed a thermosuit to produce mild changes in skin temperature using an open loop system (Raymann *et al*. 2008). The results of that experiment showed that an increase in skin temperature by 0.4ºC produces a significant suppression in wakefulness and a shift to deeper stages. We have previously shown that elevation of *Ta* significantly increases NREM sleep in a mouse model of temporal lobe epilepsy (Ajwad *et al.* 2016). More analysis of the data revealed that while the overall probability of seizures in deep NREM sleep and light sleep did not change significantly with *Ta*, the proportion of time spent in these stages of NREM sleep did change as we discussed in Chapter Three. Those findings suggest that SDM could perhaps serve as a way to alleviate epileptic seizures. Patients with insomnia who usually experience sleep fragmentation and poor sleep depth can benefit from this approach as well.

In conclusion, sleep quality can be improved by manipulating ambient temperature using a closed loop control system. The proposed approach can be implemented in human studies using state feedback from EEG monitoring to a programmable thermostat. It can be beneficial to individuals with disordered sleep. The main goal of this small sample trial was to test and establish the feasibility of SDM before conducting experiments in disease models (e.g., Alzheimer's disease and epilepsy). Two future goal are to : **(1)** perform SDM in a mouse model of temporal lobe epilepsy , and **(2)** track breathing regularity using a noncontact motion sensor (Yaghoub *et al.* 2016) and use it as a measure of sleep depth. The second goal will enable completely noninvasive sleep depth enhancement without the need for obtrusive and resource-intensive EEG/EMG monitoring.

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4.3.3 Regulation of the ultradian sleep-wake cycle

4.3.3.1 Sleep-wake induction (SWI) design

Following IACUC approval, eleven adult C57BL/6 mice (6F/5M) were instrumented for EEG/EMG monitoring. *Ta* was manipulated so that the error between *Q*, the ratio of instantaneous EEG power in delta (0.5-4Hz) to theta (6-9Hz) band, and a dynamically varying target value *Q**, was minimized. *Q** was programmed to exponentially decay and grow in 30: 60 min cycles to alternately approach values of *Q* typically observed in wakefulness and NREM sleep. For each time step *t*:

$$Q^*(\mathrm{t}) = k\, e^{(-\frac{t}{Tau})} \quad \text{for wake}$$

&

$$Q^*(\mathrm{t}) = k\,(1 - e^{(-\frac{t}{Tau})}) \quad \text{for sleep}$$

Where:

$k = 1/(Qmax\ -\ Qmin)$

$Qmax\ and\ Qmin$ are the values of *Q* typically seen in sleep and wake, 5dB for NREM sleep and 1dB for wake in mice.

Tau was calculated so that 95% of the response would be completed in each 30/60 min wake/sleep-promoting cycle.

This 30:60 min cycle was repeated for each mouse from 7 a.m. -9 p.m. every other day for six days (three repeats). We looked at the data from 8:30 a.m. – 7p.m. Changes in *Q* and EEG Hi-Low band power ratio (8-30Hz/0.5-8Hz) during those 30:60 min cycles were compared to sham control days when *Ta* was kept at room temperature (~22ºC). Then, the effects of *Ta* manipulation on sleep metrics and other parameters were evaluated for the period of manipulation against the same period on intervening days in which *Ta* was kept at room temperature. Figure 4.12 shows a sample of sleep-wake induction data in which *Q** was programmed in closed-loop mode to produce exponential decay and growth between typical values of *Q* in Wake and NREM sleep in a 30:60 cycle.

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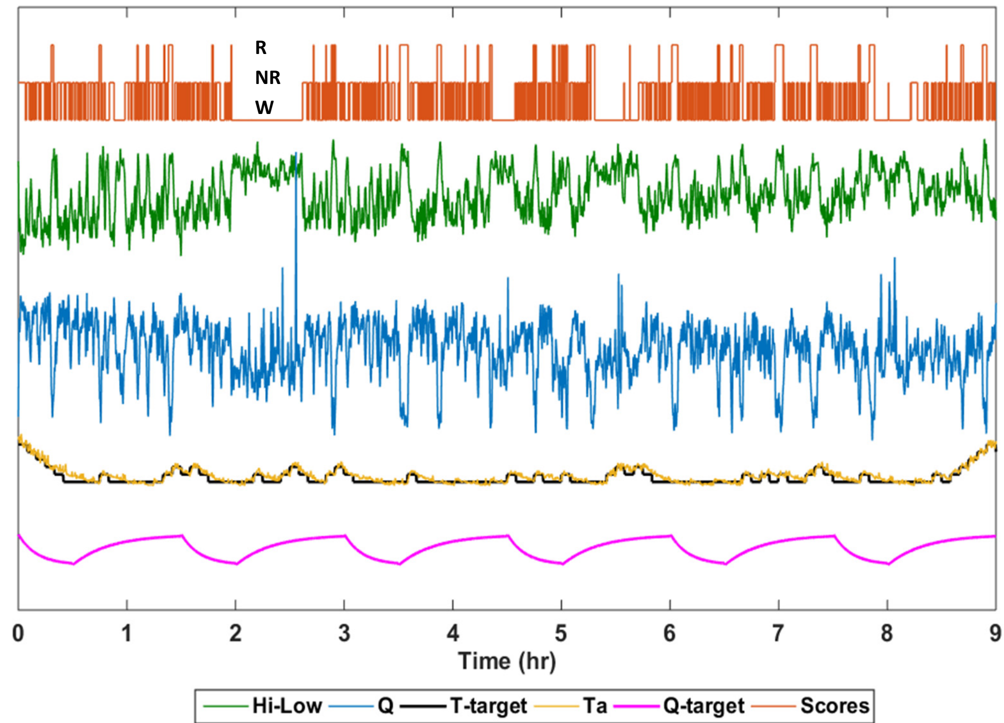

Figure 4.12. Sleep-wake induction (SWI) strategy. A 9-hour data sample from one mouse in which the 30:60 *Q** cycle was repeated six times. While *Ta* appears to increase slightly when *Q** approaches its peak, trends in *Q* and the EEG Hi-Low ratio (8-30Hz / 0.5-8Hz) are difficult to follow in each cycle and are brought out clearly only when averaged over multiple cycles, as will be shown in the results section. *Q* is high during NREM, low in wake, and even lower in REM. Hi-Low ratio is low in NREM and high in wake. R: REM, NR: NREM, W: Wake.

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4.3.3.2 Results and discussion

4.3.3.2.1 Regulation of the ultradian sleep-wake cycle

Delta to theta power ratio Q and Hi-Low ratio computed from the EEG were averaged over 225 cycles from 11 mice (6 cycles had to be excluded due to some recurring artifacts), figure 4.13. During the exponential drop in Q^*, *Ta* decreases gradually and induces a reduction in Q and an increase in Hi-Low, which are evidence of a wake-promoting effect. But when Q^* grows to a peak in the following hour, the trends are reversed; i.e. *Ta* increases gradually causing an increase in Q and a reduction in Hi-Low which are characteristics of sleep-promoting effect. Figure 4.13 (lower panel) shows trends of Q and EEG Hi-Low averaged over 225 cycles from the 11 mice at room temperature (baseline). Q does not follow Q^* as in the dynamic sleep-wake induction experiment (upper panel). Thus, it seems that the duration of sleep and wake bouts could be controlled, albeit with some delay in the response after switching between wake and sleep-promoting phases. Cross-correlation analysis showed that the response in Q lagged Q^* by about 12 min. The trends were consistent for male and female and for data pooled over three days from each mouse.

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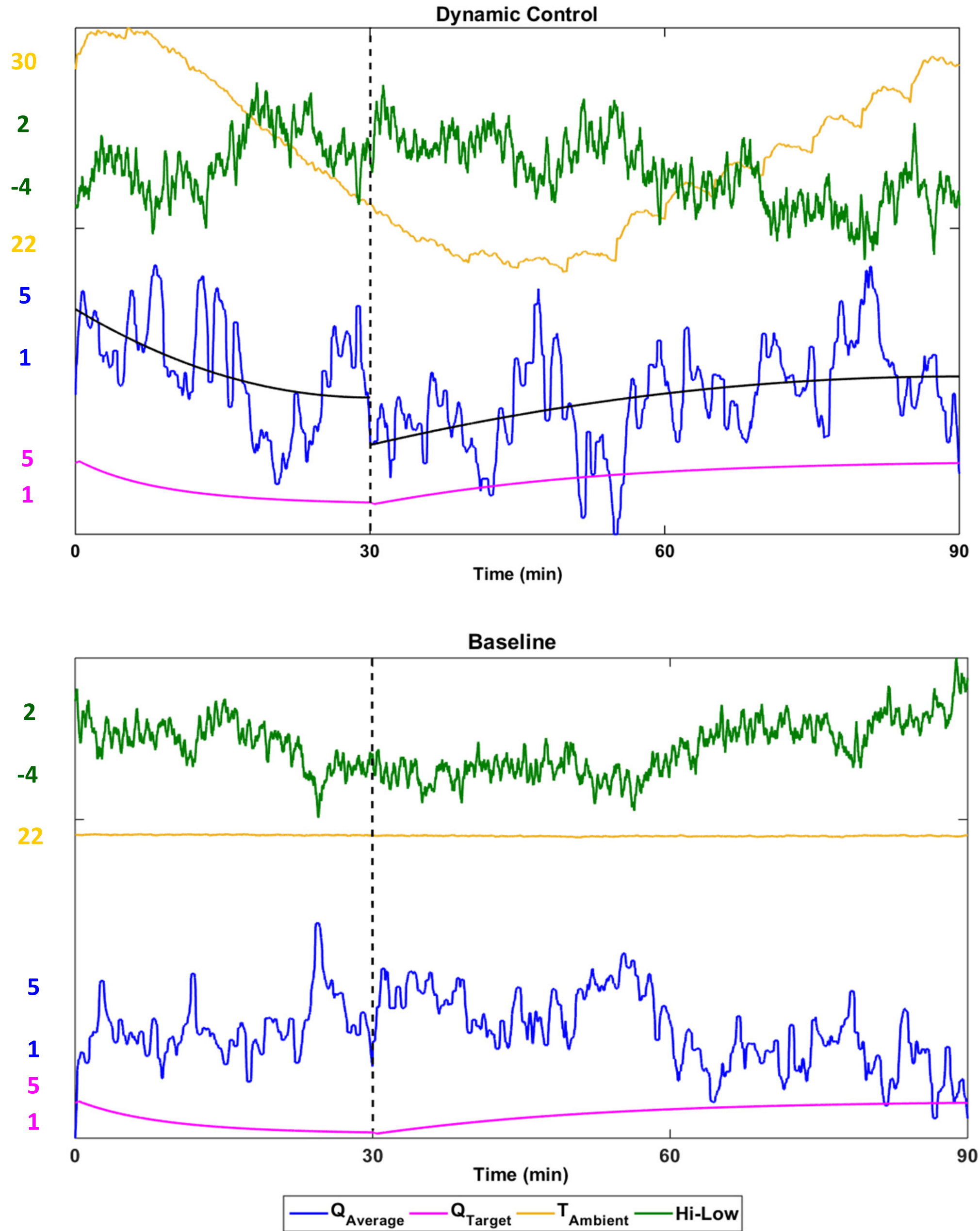

Figure 4.13. Triggered average response to the SWI protocol. ***Upper:*** *Q* and Hi-Low computed from the EEG were averaged over 225 cycles from 11 mice (6 cycles were excluded due to recurring artifacts). During the exponential drop in *Q**, *Ta* decreases gradually and induces a reduction in *Q* and an increase in Hi-Low, which are evidence of a wake-promoting effect. But when *Q** grows to a peak in the following hour, the trends are reversed, thereby promoting sleep, black trace shows the trending lines of *Q*. ***Lower:*** *Q* and EEG Hi-Low were averaged over 225 cycles from the 11 mice at room *Ta*. *Q* does not follow *Q** as in the dynamic SWI experiment (upper). This indicates that the sleep and wake duration could be controlled but with some delay in the response after switching between wake and sleep-promoting phases. Cross-correlation analysis showed that there is a lag of 12 min between *Q* and *Q**.

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4.3.3.2.2 Effect of SWI on probabilities of vigilance state

As in SDM , collected data from experiments of running SWI protocol were scored offline into Wake, NREM, and REM in 1-s windows using a validated hidden Markov model (HMM) algorithm (Yaghouby *et al.* 2016). Wakefulness is distinguished from sleep mainly based on muscle tone (EMG), which is high in wake and low in sleep. The EEG delta (0.5-4Hz) / theta (6-9Hz) power ratio discriminates NREM from REM sleep (high in NREM and low in REM). The EEG Hi-Low was used as an augmented feature to help differentiating sleep from wake. The probability of each vigilance state was computed over all 30:60 min cycles from 10 mice (data from one mouse had to be excluded due to bad quality EMG signal) to see if the trend is consistent with the trends of Q and Hi-Low during the 30:60 min cycles. The trends of wake and NREM sleep probabilities normalized by their baseline probabilities support the trends of Q and Hi-Low in wake and sleep phases, the probabilities were plotted as polar phases for clarity purpose (figure 4.14). Wake probability is high during wake-promoting phase (0 to 120º) and decreases in sleep promoting phase (120 º to 360 º) after some delay in switching to sleep phase. NREM sleep probability on other side is showing an opposite trend to wake probability, low in wake phase and high in sleep phase with some delay in switching to wake promoting phase. The plot also shows that there is a short sleep bout in the transition phase from wake to sleep promoting phase. The probability of REM sleep is variable because time spent in REM is relatively low comparing to wake or NREM. Recalling figure 4.13 the probabilities of wake and NREM sleep are consistent with the trends of Q and Hi-Low that have been seen during SWI days.

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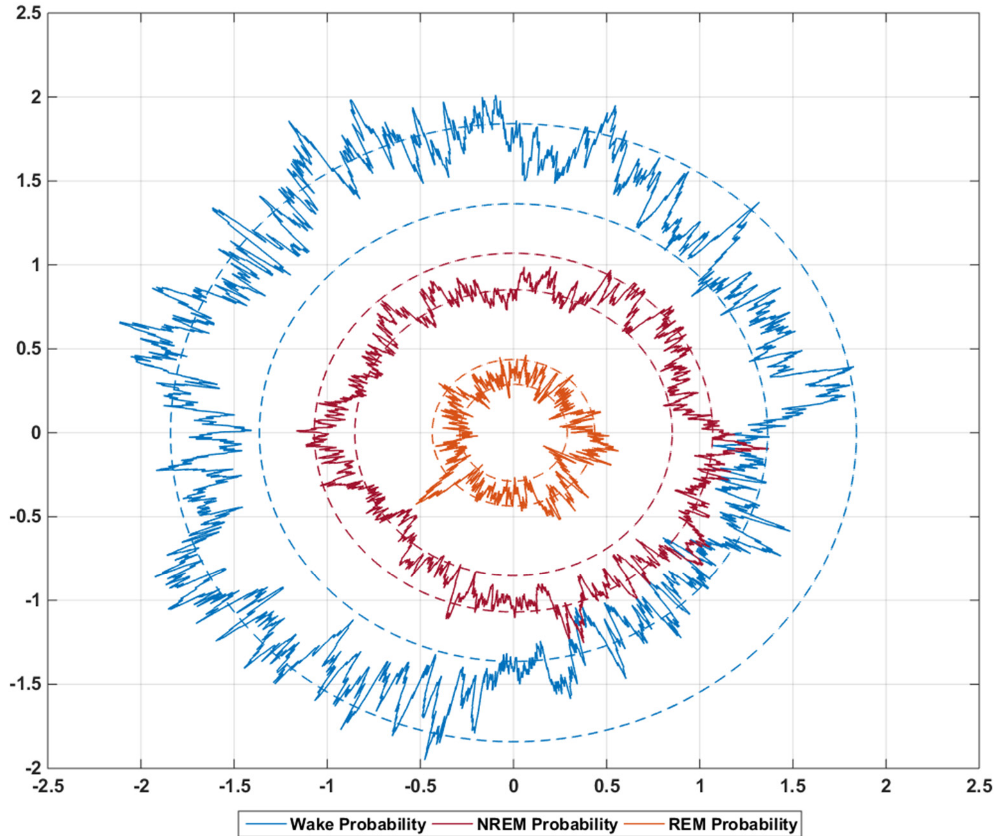

Figure 4.14. Probabilities of each vigilance state over 30:60 min cycles. All probabilities are normalized by their corresponding values during baseline. In general, wake probability is high in wake promoting phase (0-120º; 0-30 min) and NREM probability is high in sleep promoting phase (120 -360º; 30-90 min). However, wake probability starts low in the beginning of wake phase, then gets high and continues to be high even at the beginning of sleep phase due to the lag in switching from wake to sleep phase. NREM probability on the other side starts low in the beginning of sleep promoting phase (during the lag time) and gets clearly high in the fourth quarter. REM probability is quite variable due to its relatively low percentage but in general it is low in wake phase and high in sleep promoting phase, it has the same trend of NREM probability. Dashed lines represent the *mean* ± *standard deviation* of each probability (blue for wake, red for NREM, and brown for REM).

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4.3.3.2.3 Effect of SWI on sleep metrics

Sleep metrics were estimated from the scores obtained from running the HMM classifier and compared for SWI (dynamic) against baseline (Figure 4.15). Though there were no statistically significant differences ($p > 0.05$, Wilcoxon signed-rank test), some changes were observed. While the time spent in Wake increased, the time in NREM decreased. This could be due to the delay to sleep onset after the relatively short 30-min period of wake induction, which may also explain the non-significant reduction in NREM bout duration. The number of REM bouts may have also decreased with the available sleep time but with a slight increase in the bout duration.

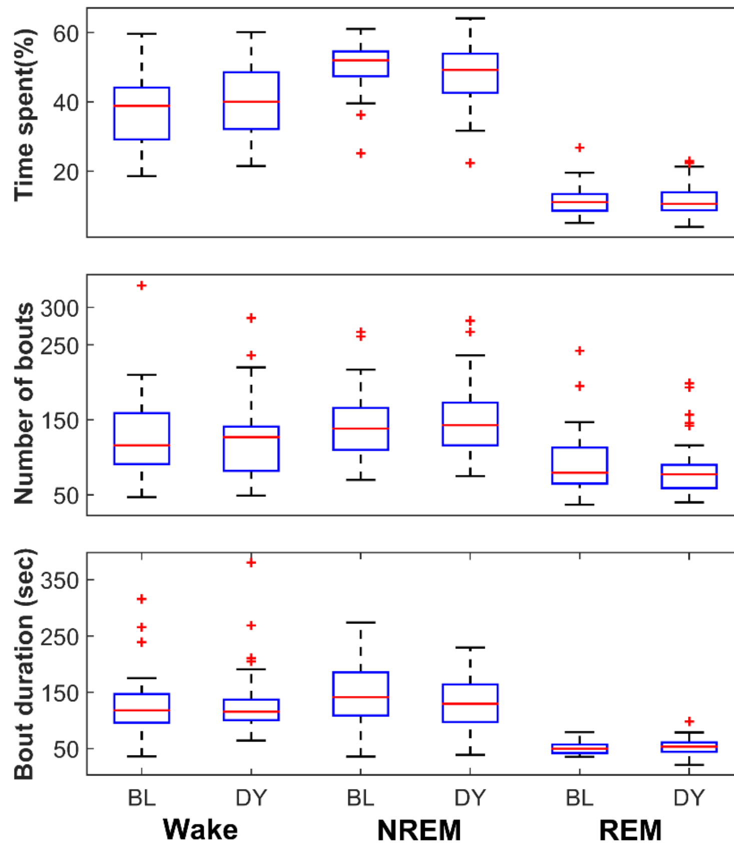

Figure 4.15. Effects of SWI on sleep metrics. **BL:** Baseline, **DY:** Dynamic SWI. No significant difference ($p > 0.05$, Wilcoxon signed-rank test) were observed. n=10, data from one mouse had to be excluded due to poor EMG signal quality that affects the ability to accurately determine vigilance states. In general, SWI resulted a slight increase in wake and decrease in NREM which could be due the delay in time that happened after switching from wake to sleep phases.

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4.3.3.2.4 Effect of SWI on the proportion of deep sleep

Using EEG zero crossing criteria, proportion of SWS was computed and the effect of SWI manipulation has been evaluated using Wilcoxon signed-rank test. Mice spent more time in SWS ($p < 0.05$) during the manipulation days comparing to the baseline, figure 4.16. Considering the fact that animals and even humans have more SWS in the first part of sleep phase and that in SWI experiment there is a reduction in NREM duration (due to the delay in switching from wake to sleep); which means that the animal has lost some of sleep in the late part of sleep phase (more likely light NREM sleep), those explain why our mice slept deeper during SWI experiment. The %SWS seems to be high comparing to the proportion observed in data of SDM experiments. This could be because the experimental period of SWI strategy included the times when SWS tends to be high, SWS is high at the onset of light phase and decreases progressively over time as we explained in Chapter Two.

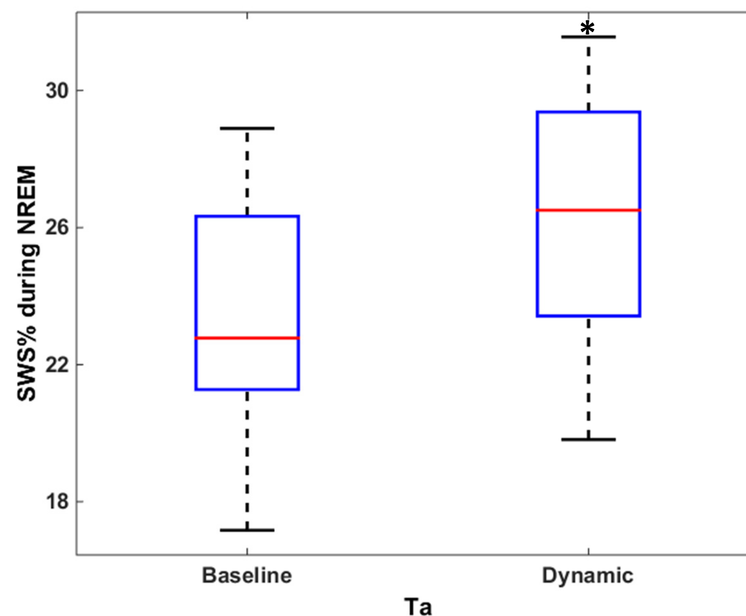

Figure 4.16. Effect of SWI on the proportion of SWS during NREM sleep. * indicates a significant difference (*p-value* < 0.05, $n = 29$ where n is the number of recordings from 10 mice. Mice had more SWS during SWI days.

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To conclude, since many factors (including changes in environmental temperature) influence the sleep regulation process, it is expected that the timing and duration of sleep bouts can be controlled using an appropriate protocol. Our results show that the timing and duration of the multiple sleep-wake bouts in mice can be modulated noninvasively through ambient temperature regulation. The sleep-wake induction study suggests that sleep and arousal can be induced by gentle manipulation of *Ta* in closed-loop, and that sleep and wake bout durations can be modulated by specifying the times of these changes, with some delay to be expected when switching between wake and sleep-promoting phases. The duration of sleep and wake bouts in this study was set at 60 min for sleep and 30 min for wake, which are in the neighborhood of durations typically seen in mice during the inactive light period under baseline conditions. Note that this refers to prolonged bouts that may contain multiple vigilance changes on much shorter timescales. The idea was to test the feasibility of controlling the timing of sleep and wake and observing the delay to sleep or wake onset associated with switching from one phase to the other. Increasing the duration of sleep and wake promoting phases should be considered in future to suit patients with sleep problems who may get inadequate or poor quality. Thus, the ability to control the timing of sleep and a better understanding sleep-thermoregulation interactions could help in developing more natural non-pharmacological ways to treat sleep-related disorders like insomnia.

4.4 Conclusions

In summary, the different strategies presented in this chapter demonstrate that it is feasible to enhance sleep and regulate its timing and duration non-pharmacologically by manipulating *Ta* using a closed loop control system. There are several types of sensory and neural stimulation that can be applied to change sleep-wake architecture but thermal stimulation is arguably the most non-invasive and perhaps effective way if we consider how even a small shift in *Ta* produces a significant effect on sleep. Our results add more significance to the literature about the *Ta*-sleep correlation and could be beneficial in developing a non-invasive approach for treating people with disordered sleep.

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CHAPTER V CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Overview

Although its underlying mechanisms and precise function remain as an enigma, sleep is essential to life and its disturbance affects brain and body functions negatively. Thus, it is very important to get sufficient sleep at an appropriate time. However, sleep disturbance is common in many neurologic disorders such as epilepsy, Alzheimer's, and Parkinson's. It is also common in the elderly. Pharmacological and non-pharmacological interventions to improve sleep are available and valuable but they are sometimes associated with adverse effects like nausea, vomiting, or limiting time of sleep. Thus, there is a need to put more effort into improving sleep quality with approaches that are non-invasive, less stressful, low in cost, and with minimal side effects than those currently available. In this research, we have proposed a simple and non-invasive approach to enhance sleep through ambient temperature (*Ta*) manipulation which was inspired by both the current body of scientific literature and our independent exploration of the interaction between sleep and *Ta*. Several studies have shown that changes in *Ta* within the thermoneutral zone exert a significant effect on sleep-wake architecture in humans and animals. However, few studies have investigated the acute effect of temperature elevation on sleep in rats and none have done so in mice. In this research, we have investigated that effect and compared it with the effects observed in chronic thermal experiments in mice.

The results presented in this dissertation demonstrate for the first time that: (1) An acute diurnal elevation in *Ta* produces significant effects on mouse sleep similar to those that have been observed in chronic experiments in which mice received thermal treatment for several days or weeks; (2) Diurnal elevation of *Ta* to the thermoneutral zone changes sleep-wake architecture in a mouse model of temporal lobe epilepsy as it does in controls but with more episodes of NREM; and (3) It is feasible to modulate sleep by dynamically manipulating *Ta* in closed-loop mode to improve sleep depth and regulate the timing and duration of sleep and arousal in control mice.

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The proposed approach of ambient temperature manipulation suggests a simple strategy for enhancing sleep in people with sleep problems including epilepsy patients and may even influence epileptic seizures as they are strongly correlated to the sleep dynamics. Considering that there are significant changes in sleep structure in response to a mild change in ambient temperature, and that there is continuous development in medical technology (e.g., wireless EEG, programmable thermostat), further research may make the proposed strategy practical in humans to improve sleep as it has very important implications in health and disease.

The main contribution of this dissertation is to highlight the importance of studying sleep-temperature correlation and using it to improve sleep in patients with disordered sleep. The findings of our research add valuable information to the sleep-thermoregulation field. That information could have very important implications in treating poor sleep that is common in many neural diseases including epilepsy with expectation of seizures control as an outcome of sleep improvement by ambient temperature regulation.

5.2 Effect of an acute elevation in *Ta* on mouse sleep architecture

Sleep is a complex and dynamic physiological process that is regulated mainly by homeostasis and circadian rhythms. Its importance to the human body (both physically and mentally) is clear and there is no doubt that its disturbance has a harmful impact on cognitive and physical performance. Insufficient sleep and poor sleep quality are common in many neurological disorders. Studying how changes in environmental factors could influence sleep would help gain a better understanding of its underlying mechanisms and develop approaches to improve its quality. Environmental temperature exerts a significant influence on sleep-wake behavior. As we explained in Chapter Two, research has shown that there is a strong relationship between sleep regulation and temperature regulation since the preoptic area of the hypothalamus in the brain is involved in regulating both systems. Even mild changes in *Ta* within the thermoneutral zone will induce a significant effect on sleep-wake architecture. Since no previous study has evaluated the effect of acute exposure to elevated *Ta* on mouse sleep, we have assessed that effect and compared it with the effect seen in rodents after exposure to chronically elevated *Ta*. This effort has produced three main findings: 1. An acute exposure to elevated *Ta* has the same effect on mouse sleep as

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in a chronic experiment, which is to promote sleep; 2. The intensity and proportion of deep NREM sleep, i.e., SWS, increase significantly with *Ta*; and 3. Sleep tends to be less fragmented at higher temperatures around the thermoneutral zone. All mice studied (n =13) spent more time in NREM, REM, and SWS at higher temperatures. Furthermore, NREM sleep appeared to be less fragmented by episodes of brief arousal and REM sleep. Though REM sleep changes significantly with *Ta*, it seemed to be less sensitive to changes in *Ta* than NREM and SWS, especially in term of bout length and the number of bouts. The most salient aspect of this study is the distinction made between light NREM sleep and SWS. Sleep in mice is usually only classified into NREM and REM sleep. However, we have used EEG zero crossing criteria previously used in rats to label NREM sub-stages. EEG low delta (0.5-2Hz) power fraction has been used as well to classify NREM sleep into light and deep stages. Good agreement was observed in sleep metrics using EEG zero-crossing criteria and low delta power fraction in term of time spent and bout duration of light NREM sleep and SWS; the number of SWS bouts was insignificantly less using low delta power fraction compared to the zero-crossing method. In general, results illustrate the possibility of improving sleep through *Ta* manipulation which may be beneficial to patients with sleep disorders such as epilepsy, Alzheimer, Parkinson, and insomnia. However, the effects of *Ta* change should be studied in animal models of these disorders to investigate the influence on sleep structure. In this research, we used a mouse model of temporal lobe of epilepsy as a disease model and studied the effect of *Ta* elevation on sleep-wake architecture, as discussed in the following section.

5.3 Effect of diurnal *Ta* elevation on sleep and seizures in a mouse model of temporal lobe epilepsy (TLE)

Many new ways of epilepsy treatment are being proposed, but many patients still do not derive benefit from them. Additionally, each type of treatment could be associated with some side effects. Deficits in sleep continuity and sleep depth are common in epilepsy patients. In many types of epilepsy, seizures and sleep are strongly influenced by each other. Epileptic seizures interrupt sleep and contribute to its fragmentation. While the

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synchronizing activity of low frequency/high amplitude oscillations during NREM sleep helps in seizures generation and propagation, the desynchronizing activity of high frequency/low amplitude oscillations during REM seems to discourage them (Foldvary-Schaefer 2014). Given that temperature changes influence sleep, sleep modulation through *Ta* regulation may offer a natural and non-invasive way that could also help in seizure control. Since no previous study has been conducted to evaluate the *Ta* effect on sleep in an animal model of epilepsy and as a first step toward our goal of modulating sleep in a disease model, we first assessed the effect of *Ta* elevation on sleep-wake architecture in a mouse model of TLE ($n = 4$) to see if the effect is similar to what has been seen in controls. Our data from chronically epileptic mice showed a general increase in NREM sleep (which came from an increase in the number of NREM bouts) coupled with a reduction in wake (which came from a reduction in the bout durations of both prolonged wake and brief arousal). Time in REM and number of REM bouts increased with *Ta* but not significantly (Ajwad *et al.* 2016). Sleep depth and the proportion of SWS increased significantly with *Ta*. In summary, the effect of *Ta* elevation on sleep structure in epileptic mice is similar to that observed in controls except that NREM number of bouts increased significantly with no change in bout duration. With more SWS and REM sleep expected at higher temperature, the chances of having epileptic seizures may be lowered because other studies have shown that seizure probability during those two stages of sleep is less than during other stages (Ng and Pavlova 2013 and Herman *et al*. 2001). In general, the seizure rate did not change significantly during days of elevated *Ta* compared to the baseline days; there was some variability in this effect across animals. However, the hourly seizure distribution over the 24-hr cycle suggests that the *Ta* elevation in the Light period pushed the seizures toward the Dark period, in which *Ta* returned to room temperature. Taken together, the results suggested that sleep-wake architecture changes in response to dynamic closed-loop manipulation of *Ta* could be a useful way to control seizures. However, this needs to be investigated in a larger sample; the *Ta* effect on seizure frequency is not clear enough in the small sample available in this study.

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5.4 Sleep depth enhancement through *Ta* manipulation in mice

Knowing that SWS improvement has very important applications, several experiments have been conducted in humans and animals to improve sleep depth and increase the time spent in SWS. Available approaches for SWS enhancement require either pharmacological intervention using drugs like Tiagabine (Walsh 2009, Lundahl *et al*. 2007, Bazil *et al*. 2012) , or non-pharmacological interventions like sleep restriction or brain stimulation with electrical/magnetic currents (Marshall *et al*. 2006 and Massimini *et al*. 2007). Though both approaches improve SWS notably they are usually associated with some side effects and limitations. To overcome those unwanted side effects, we have developed a simple and natural way to enhance sleep depth by manipulating *Ta* using a closed-loop control system. No previous study has attempted sleep depth modulation in rodents through closed-loop *Ta* manipulation. The proposed approach changes *Ta* dynamically in real time based on the error between *Q*, the ratio of instantaneous EEG power in the delta (0.5-4Hz) to the theta (6-9Hz) band, and *Q**, a preset target value of *Q* typically observed in deep NREM sleep. Data from five mice showed a consistent increase in SWS as estimated by: a reduction of error between *Q* and *Q**, an increase in delta power closer to our deep-sleep target, and an increase in the amount of time spent in SWS (computed via a threshold on the frequency of zero-crossings in the EEG). REM sleep duration increased along with SWS, which was not surprising since our data in control and epileptic mice have shown an increase in REM with *Ta* elevation, but interestingly REM episodes seemed to be more periodic during the days of sleep depth modulation compared to those in baseline days. All the results suggest that *Ta* manipulation is a good non-pharmacological approach to enhance sleep depth. This could have important applications in several disease models. For instance, given that sleep depth modulation results in more SWS and longer REM episodes and that seizures are less likely to happen in those two stages, it seems that our approach could be used as a non-invasive way to reduce seizures in epilepsy patients. Patients with Alzheimer's, Parkinson's, and insomnia can benefit from this approach as well. For practical application in humans, the proposed approach can be implemented using a surface EEG monitoring system and thermal sensors to assess the effects of *Ta* changes on sleep dynamics (which can be analyzed in real time), in particular SWS and REM sleep.

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To make the animal experiments completely non-invasive (i.e., without the need to implant the animal with EEG/EMG electrodes), breathing regularity computed from the piezo motion sensor could be used as a measure of sleep depth. Breathing regularity tends to be relatively high during NREM (reaching the highest values during SWS) and low during REM (Yaghouby *et al.* 2016). However, this brings another challenge to the study on how to validate the classification of NREM into light and deep sleep based on data collected only from the piezo motion sensor.

Without any doubt, the quest to enhance sleep quality will continue in both humans and animal models. The findings of this work can contribute to future studies of *Ta* manipulation within the TNZ to improve sleep in healthy subjects (young versus old) and in subjects with disordered sleep. Closed-loop *Ta* manipulation has not been conducted before in either humans or animals.

5.5 Non-pharmacological regulation of the ultradian sleep-wake Cycle in mice

Since our sleep habits are usually not in agreement with our circadian rhythm, the timing of sleep is no less important than getting a sufficient amount of it. Changes in ambient temperature influences both the timing and duration of sleep and arousal. In fact, considering that even a mild change in *Ta* produces a significant effect on sleep-wake architecture, *Ta* can be viewed as a strong driver of the sleep-wake cycle.

It is well known that rodents have a polyphasic sleep-wake structure in which multiple sleep and wake bouts are distributed over the 24-hour cycle, and that their timing and duration are modulated by circadian rhythm. Chapters II and III explained how an elevation in *Ta* influences the duration of sleep and wake bouts in control and epileptic mice. In this study, we have tested whether the ultradian sleep-wake cycle in control mice can be entrained to an externally imposed rhythm by manipulating *Ta*. A simple strategy that we label "sleep-wake induction" (SWI) was developed to alternately promote sleep and wake states using a closed-loop control system. In brief, this strategy manipulates *Ta* so that the error between *Q*, the ratio of instantaneous EEG power in the delta (0.5-4 Hz) and theta (6-

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9 Hz) bands, and a dynamically varying target value Q^*, was minimized. Q^* was programmed to exponentially decay (over 30 min) and grow (over 60 min) to alternately approach values of Q typically observed in wakefulness and NREM sleep. Results showed that Q follows Q^* in wake-promoting and sleep-promoting phases with some delay in switching from wake to sleep (and vice versa), which is reflected in a slight reduction in NREM time and bout duration coupled with an increase in wake time and number of bouts. During the exponential drop in Q^*, *Ta* decreases gradually and induces a reduction in Q and an increase in Hi-Low, which are evidence of a wake-promoting effect. But when Q^* grows to a peak in the following hour, the trends are reversed, thereby promoting sleep. Interestingly, regulating the timing of the sleep-wake cycle was associated with a significant increase in the proportion of SWS compared to the baseline. Implementation of SWI strategy to regulate sleep-wake timing combined with deeper sleep has very important implications in the sleep enhancement field. Thus, a better understanding of the sleep-thermoregulation interactions could help in regulating the timing of sleep-wake cycle. This in turn could provide insights useful for therapies of sleep-related disorders.

In summary, we have performed various experiments related to ambient temperature manipulation in mice that show the feasibility of non-pharmacological improvement of poor sleep in people with sleep disorders. However, these thermal control strategies must be validated in animal models of diseases like epilepsy and Alzheimer's before attempting them on human patients with those conditions.

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