Investigating the Homeostatic Regulation of Sleep in Response to Anesthetic Agents and Cortical Development

by

Aaron Bruce Nelson

A dissertation submitted in partial fulfillment of

The requirements for the degree of

Doctor of Philosophy

(Neuroscience)

at the

UNIVERSITY OF WISCONSIN- MADISON

2013

Date of final oral examination: 4/2/13

The dissertation is approved by the following members of the Final Oral Committee:

Chiara Cirelli, Professor, Psychiatry
Giulio Tononi, Professor, Psychiatry
Ronald E. Kalil, Professor, Opthalmology and Visual Sciences
Matt I. Banks, Associate Professor, Anesthesiology
Avtar Roopra., Associate Professor, Neuroscience

Thanks and Acknowledgements

I am grateful for all the help I have received from my colleagues, classmates and family. The shear amount of assistance I have received prevents me from addressing each individually. Although I will not be able to repay all this generosity, I intend to pass these gifts forward, helping others as I have been helped. I will specifically acknowledge Dr. Ruth Benca and Dr. William Obermeyer for providing my first opportunity to study sleep and encouraging me at that early stage of my career. Next, I thank Dr. Chiara Cirelli and Dr. Giulio Tononi for including me as a part of their research team. I directly benefited from their unmatched dedication to the field and was truly fortunate to have a position with such easy access to both resources and expertise. Dr. Ugo Faraguna gave me extensive training prior to and during my graduate school years. He has been extremely generous helping me professionally and personally. Dr. Vladyslav Vyazovskiy was a constant source of advice and was especially helpful providing encouragement and guidance for troubleshooting when I was discouraged. Finally, I thank Emily for being my most trusted confidant and primary source encouragement.

Table of Contents

Abstract:

Sleep's function remains a mystery. However, we know that in birds and mammals the homeostatic regulation of sleep is related to the spectral power between 0.5 and 4.0 Hz (SWA) measured during non-rapid eye movement sleep (NREM). NREM SWA is the best characterized marker of sleep's homeostatic regulation; it increases as an inverse exponential function with time spent awake and decreases as exponential function of time spent asleep. The work presented together in this dissertation advances our understanding of how NREM SWA is linked to sleep need by testing predictions of the synaptic homeostasis hypothesis (SHY). In SHY it is posited that the essential function of sleep is to downscale the overall strength of synapses in order to counterbalance a dynamic waking state during which there is an unsustainable net increase in synaptic strength. The return to sustainable levels of lowered overall synaptic strength may explain both why NREM SWA decreases as a function of time spent asleep and why sleep is beneficial for memory and performance. The two most prominent corollaries of this hypothesis are: 1) synaptic number/strength have a positive relationship with the slope and amplitude of NREM sleep slow waves, 2) NREM sleep slow waves play an active role in synaptic homeostasis, decreasing synaptic strength in proportion to their magnitude. Thus, central to the hypothesis is a stabilizing mechanism in which NREM slow waves complete a negative feedback loop with synaptic strength.

An interesting question raised by this hypothesis is whether non-sleep states that produce slow waves similar to those occurring during NREM sleep interact with sleep's homeostatic regulation. Here we titrated volatile anesthetics to produce a NREM sleep mimicking state that was followed by a reduction in NREM SWA, suggesting anesthesia slow waves may be performing the same role as natural slow waves [**Chapter 2: Effects of anesthesia on the response to sleep deprivation**]. However, a similar decrease in NREM SWA occurred following the higher dose, which produced a near isolectric EEG trace and no slow waves, suggesting that the crucial factor may not be the up and down states associated with slow waves, but perhaps a state of prolonged hyperpolarization.

Next we investigated the widely held notion that the decline in NREM SWA occurring in many species during adolescence is primarily a reflection of synaptic pruning; a direct comparison has yet to be performed. Here we used a transgenic mouse model that expresses yellow fluorescent protein (YFP) in cortical neurons and allows us to

directly evaluate anatomical, molecular and electrophysiological markers of synaptic strength. We first characterized sleep/wake dependent changes in synaptic density using in vivo two-photon (2P) microscopy. Consistent with SHY, we found in adolescent mice that when animals were allowed to sleep there was a net decrease in dendritic spines, and a net increase in spines after animals were kept awake (Maret et al., 2011). Here in the same strain we characterize in detail the EEG measured changes in response to sleep deprivation at ages spanning rodent adolescence, an analysis that had not been done before [**Chapter 3: Sleep Patterns and Homeostatic Mechanisms in Adolescent Mice.**]. Similar to what has previously been reported in rats, young mice did not reliably show an increase in SWA in response to sleep deprivation. Based on these young mice having higher SWA in general, showing the expected SWA increase across normal baseline waking periods, and having relatively elevated SWA prior to the sleep deprivation, we determined that the lack of an increase in SWA after sleep deprivation is likely due to a ceiling effect.

Adolescence is a time when biological and societal factors combine to shift bed times later without a commensurate shift in rise times. These dual factors precipitate a state of chronic sleep deprivation. The blunted response in adolescent mice to an acute sleep deprivation raises the question of what happens when sleep is chronically restricted during this period thought critical for synaptic pruning. If even a brief 4 hour deprivation places young mice near their physiological limit for SWA, what happens when sleep is chronically restricted as is often the case for human adolescents? Here we looked at the normal maturational trajectory of NREM SWA in adolescent mice and characterized the effect of chronic sleep deprivation on its developmental time course [**Chapter 4: The Response to Chronic Sleep Deprivation in Adolescent Mice**]. As has been shown in other mammals our mice showed a decrease in SWA across adolescence. When adolescent mice (P20,P25) experienced 4 days of restricted sleep they had a profound but reversible decrease in NREM SWA that was not observed in age-matched controls allowed to sleep *ad libitum*. Future molecular and imaging work in the same mouse model will investigate the mechanism for this profound decrease.

Prologue:

Life on earth has, for the most part, become entrained to a persistent 24 hour cycle characterized by roughly 12 hours of bright light and high temperatures alternating with 12 hours of lower temperatures and darkness. Because this light dark transition is both pervasive and predictable, the majority of animals have become specialized for activity during only a portion of the day. This affords each species a period of quiescence during which the organism may engage in a species specific behavior that typically includes retiring to a safe location, assuming a typical position, and then curiously accepting the enormous risk of having a greatly diminished responsiveness to the environment. This behavior is tightly regulated, conserved across phylogeny, and there are serious negative consequences when it is postponed. We call this behavior sleep and it apparently serves an essential evolutionarily conserved function.

Chapter I:

Introduction

Sleep factors and homeostatic sleep regulation

For over a century researchers have attempted to explain the restorative benefit of sleep by characterizing sleep factors underlying its homeostatic regulation. During the first half of the 20th century sleep was widely considered to be a passive homogeneous state entered by the brain when the intensity of external stimuli was too weak to maintain wakefulness (Dement, 1998). Consistent with this perspective, early hypotheses of the homeostatic mechanism proposed that a circulating hypnogenic endotoxin accumulated during wake and that the sleep's restorative benefits were associated with the dissipation of such a substance. Early protocols injected extracts from sleep deprived animals into fully rested animals and seemed to be remarkably successful at transferring sleep need; injected animals fell asleep as if they had been kept awake for many hours before the injection (Legendre and Pieron, 1907; Ishimori, 1909). These early experiments likely activated an inflammatory signaling cascade that precipitated neural inflammation. More recent studies have shown that many pro-inflammatory signaling molecules are sleep factors (tumor necrosis factor alpha (TNF), prostaglandin D2, interleukin-1). They locally increase in the cortex during wakefulness, decrease during sleep, and can locally increase NREM SWA (Krueger et al., 2008). The high level of neuronal activity associated with sustained wakefulness is pro-inflammatory while a decrease in firing rates during NREM down states is anti-inflammatory. Thus, one perspective on the restorative benefit of sleep is that it locally reduces inflammation (Krueger et al., 2008).

A related and highly investigated benefit of sleep is that it offers a less demanding metabolic state that allows restoration of the brain's energy reserves (Benington and Heller, 1995). Consistent with this benefit, a group of putative sleep factors related to energy metabolism has been proposed, with the most intensively studied factor being adenosine (Benington and Heller, 1995). Adenosine increases extracellularly as a function of neuronal activity but especially following activity patterns related to long-term potentiation (Wigren et al., 2007). Because Process S is defined by a correlate of neuronal firing, NREM SWA, any proposed homeostatic regulator should mediate a feedback mechanism that alters SWA. Adenosine partially meets this criterion as it is tightly linked to the energy status of the brain and is capable of inhibiting overall neuronal excitability. This inhibitory effect could explain entry into the phasic firing pattern that produces NREM sleep but does not provide a good mechanism for how slow wave

amplitude would dynamically decrease with time spent asleep; the hallmark of the homeostatic process. Specifically, there is no explanation for why slow waves would be largest and when adenosine is at its maximum concentration at sleep onset. So far much of the work with adenosine has focused on its ability to alter global sleep regulation through the inhibition of wake promoting neurons in the basal forebrain (Basheer et al., 2004). This focus on adenosine concentrations in the forebrain may have been due to the conflation of the concepts of "triggering sleep" with that of homeostatic regulation (Blanco-Centurion et al., 2006; Heller, 2006). Aside from its presence and activity in the forebrain, adenosine also responds to local cortical energy levels and therefore should mediate local changes in NREM SWA (Benington and Heller, 1995; Porkka-Heiskanen et al., 2000). More recent work has blocked the neural activity dependent build-up of adenosine in mice using an inducible knockout of gliotransmission and in so doing prevented a homeostatic increase in NREM SWA (Halassa 2009). However, the prolonged loss of such an integral inhibitory drive may have significantly increased the baseline NREM SWA (all spectra were normalized to baseline) such that the lack of an increase might be explained as a ceiling effect (Halassa et al., 2009). Considering the inducible knockouts were activated at the beginning of rodent adolescence, a beneficial study might look at the effect of the knockout on cortical maturation and normal sleep patterns across adolescence.

Another proposed benefit of sleep is that of facilitating learning and memory. A promising plasticity-related sleep factor studied in our laboratory is brain derived neurotrophic factor (BDNF), which increases with time spent awake. We have shown that the amount of exploratory behavior was correlated with the extent of cortical BDNF induction and subsequent SWA (Huber et al., 2007). A causal relationship between BDNF and SWA was suggested when unihemispheric cortical microinjections of BDNF locally increased SWA, while two different antagonists of BDNF locally suppressed SWA (Faraguna et al., 2008). This causal relationship positions BDNF as a key regulatory sleep factor connecting the local homeostatic regulation of sleep with the amount of synaptic plasticity that occurred locally during a waking period.

With so many sleep factors mediating multiple beneficial processes it is likely that the homeostatic regulation of sleep is more robust than can be explained be a solitary sleep factor. A cytokine such as TNF is related to inflammation, metabolism and neuronal plasticity; meanwhile the energy related molecule adenosine is clearly also

related to plasticity and inflammation. Consistent with the idea that sleep serves multiple inter-related functions, a micro-array study that looked at state dependent cortical gene expression revealed that genes up regulated during waking are associated with high energy demand, excitatory transmission, cellular stress and synaptic potentiation (Cirelli et al., 2004). Genes preferentially activated during sleep were related to protein synthesis, membrane trafficking and synaptic depression (Cirelli et al., 2004). Identifying individual molecules that underlie sleep's homeostatic regulation remains informative. However, if there is a fundamental function of sleep, it is much broader than can be explained by any one sleep factor.

Homeostatic sleep regulation: understanding sleep's restorative process

Currently the best established model of sleep regulation is the so-called "two process model" in which mammalian sleep regulation emerges from a homeostatic process "Process S" (S stands for Sleep) and a circadian process "Process C" (Borbely, 1982). The molecular basis of the circadian Process C is well characterized and the function is clear, to encourage sleep to occur at an ecologically appropriate period, the day for nocturnal animals (most rodents) and the night for diurnal animals (humans). Process S measures the need for sleep, and thus increases with time spent awake. More precisely, the time course of the Process S is derived from an easy to measure continuously sampled electrophysiological variable, NREM SWA. Indeed SWA increases as an inverse exponential function with time spent awake and decreases as an exponential function with time spent asleep. This physiologically based indicator of sleep need has allowed for robust quantitative elaborations of Process S that accurately model sleep regulation across species (from rodents to humans), development, and cortical topography (Achermann and Borbely, 1990; Franken et al., 1991; Huber et al., 2000; Jenni et al., 2005; Rusterholz and Achermann, 2011). In the two process model sleep need is formally related to changes in NREM SWA. Thus, it is implicit that these state dependent changes in EEG reflect the essential restorative process that occurs during sleep. However, what that restorative process is remains unclear. Here we tested corollaries of the synaptic homeostasis hypothesis which states that the fundamental restorative function of sleep is to allow a generalized, net decrease in synaptic strength in many brain circuits, which balances and facilitates a waking state during which there is a net increase in synaptic strength due to learning (Tononi and Cirelli, 2003). According to this hypothesis, the well documented decrease in SWA in the course of sleep is a direct consequence of the overall decrease in synaptic strength that occurs during

sleep (Tononi and Cirelli, 2003). There is mounting evidence supporting this "synaptic homeostasis hypothesis" (SHY).

NREM SWA: a measure of synaptic strength

EEG is a non-invasive, straightforward technique that measures brain generated voltage changes at the surface of the scalp with high temporal resolution. These surface measured voltages result from cortical extracellular current sources and sinks that reflect the sum of many post-synaptic (primarily excitatory) potentials (Mitzdorf, 1985). The prominent slow waves that occur during NREM sleep result from an intrinsic cortical slow (<1 Hz) oscillation characterized by a depolarized up state and hyperpolarized down state, which alternate due to intrinsic channel properties (Steriade et al., 1993; Steriade et al., 2001). During the depolarized up state of this slow oscillation nearly all neurons are active and the firing rate of cortical neurons is similar to that observed during waking and REM sleep, while during the down states nearly all neurons are silent (Steriade et al., 2001; Vyazovskiy et al., 2009). EEG slow waves are a reflection of the transitions between up and down states in large populations of cortical neurons. Large scale computer simulations along with studies performed in humans and rodents indicate that the amplitude and slope of a slow wave is related to the number of neurons synchronously entering an up state (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2009). Furthermore, the synchrony of neuronal firing is related to the number, strength and efficacy of the connections among them (Esser et al., 2007). In other words, the larger the number of neurons simultaneously entering an up state, the larger the slow waves.

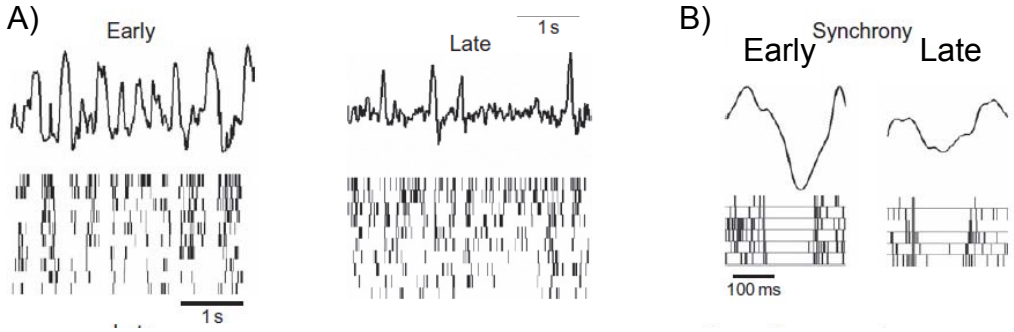


Figure 1. Sleep-wake history affects cortical neuronal firing patterns. (A) 4-s frontal local field potential (LFP) records for NREM early in the sleep period and late in the sleep period. Corresponding raster plots of spike activity (10 units are shown below the LFP traces). Note the close temporal relationship between silent (OFF periods) positive phases of LFP slow waves in NREM sleep. (B) Representative example of a high-amplitude slow EEG wave with steep slopes typical of NREM early in the sleep period (left) and a slow wave typical of NREM late in the sleep period. Note that neurons are more synchronous during the high amplitude slow wave. Figure from ((Vyazovskiy et al., 2011)) with permission from Vyazovskiy VV.

Recent animal studies have shown that molecular and electrophysiological markers of increased synaptic strength, the frequency and amplitude of miniature postsynaptic potentials and the ability to learn a reaching task, are all related to increased NREM SWA. (Vyazovskiy et al., 2008; Hanlon et al., 2009; Liu et al., 2010). In humans, synaptic potentiation and depression can be experimentally induced locally based on the timing of a transcranial magnetic stimulation protocol; with this method potentiation leads to an increase in SWA while depression leads to a decrease (Huber et al., 2008). Altogether this work provides evidence for a strong relationship between NREM SWA and overall synaptic strength (Tononi and Cirelli, 2006). Based on this link EEG could prove to be an ideal non-invasive biomarker for measuring changes related to both short term plasticity and longer term developmental changes occurring during the critical period of adolescence. However, a direct comparison of developmental changes in synaptic density, maturation of EEG and changes in molecular markers of synaptic strength has yet to be performed.

Evidence for synaptic downscaling during sleep

A study with rats *in vivo* shows that both electrophysiological and molecular markers of synaptic strength decrease during sleep and increase with time spent awake (Vyazovskiy et al., 2008). The slope and amplitude of electrically evoked potentials, an indicator of synaptic strength, were correlated with the duration of NREM sleep and the homeostatic decline in SWA (Vyazovskiy et al., 2008). Molecular correlates of synaptic strength were measured in synaptoneurosomes, a preparation that specifically purifies for synaptic proteins (Whitlock et al., 2006). Using this preparation there was an increase in GluA1 containing AMPA receptors, an indicator of long term potentiation, after waking conditions relative to after sleep (Vyazovskiy et al., 2008). Additionally, phosphorylation at a site associated with AMPA insertion was higher after waking while dephosphorylation at a site associated with long term depression was apparent after sleep (Vyazovskiy et al., 2008). A recent complementary study in the rat somatosensory cortex demonstrated that after waking there was an increase in the excitatory post synaptic potential (EPSP) amplitude mediated by AMPA receptors lacking the GluA2 subunit (these receptors likely contained GluA1 subunits) and after sleep there was a decrease in these putative GluA1 receptors (Lante et al., 2011). It was further demonstrated that a burst firing pattern similar to what occurs during NREM sleep administered after a period of waking decreased the contribution of GluA1 containing AMPA receptors, while after sleep this firing pattern had no effect on the EPSP (Lante et al., 2011). Direct evidence for a decrease in synaptic strength after sleep and an increase after wake comes from measuring miniature EPSCs *in vitro*. A study performed in frontal cortical slices found that both frequency and amplitude of miniature EPSCs increased after periods of wake and decreased after periods of sleep in rats (Liu et al., 2010). Finally, in humans an increase in cortical excitability across wake and decrease across sleep was demonstrated using transcranial magnetic stimulation and simultaneously recording EEG (Huber et al., 2012).

In addition to molecular and electrophysiological markers of synaptic strength, the synaptic homeostasis hypothesis is also supported by studies looking at vigilance state dependent structural modifications. Recent work in our laboratory with *Drosophila* shows that both the number/ size of synapses and the level of pre- and postsynaptic proteins increase with wake, decrease with sleep and that this decrease is prevented by sleep deprivation (Gilestro et al., 2009; Bushey et al., 2011). This finding corresponds well with a growing body of evidence from mice. First, in

the mouse olfactory bulb there is a high turnover of granule cells with neurogenesis and apoptosis continuing into adulthood. Interestingly, a recent study in mice found that apoptosis of newly formed bulbar neurons was not uniform across the day but doubled during the sleep period after eating (Yokoyama et al., 2011). Further investigation of this postprandial period revealed that the best predictor of cell death was the time spent in slow wave sleep (Yokoyama et al., 2011). This work is in line with recent work in our laboratory that found that during adolescence the balance between synaptogenesis and synaptic pruning is vigilance state dependent (Maret et al., 2011). We found that after 6-8 hours of sleep there was net decrease of roughly 2% of spine, while after a waking period (independent of circadian time) there was a net increase (Maret et al., 2011). Another group working in the same strain found a similar pattern in younger mice (P21) and additionally found that significant changes occurred after a shorter two hour period of either wake or sleep (Yang and Gan, 2011). Thus, recent evidence supports the hypothesis that synaptic strength and number decrease after sleep and increase after wake.

If, as suggested by this body of evidence, periods of sleep and wake have differential effects on overall synaptic strength, it would follow that manipulating the balance of time spent in wake or sleep during the critical period of adolescence would impact the ongoing remodeling of cortical circuits, possibly with long-term consequences.

NREM SWA: Developmental changes

In humans, NREM SWA follows a developmental pattern similar to the suspected curve for synaptic density, increasing until early adolescence and decreasing during late adolescence. For this reason, SWA has long been thought to be an indicator of the adolescent decrease in synaptic density (Feinberg, 1982). Recent evidence in humans supports this proposition. A large longitudinal EEG sleep study that spans adolescence confirmed that within subjects there is a decrease in NREM SWA beginning at age 10 and leveling off by age 16 (Feinberg and Campbell, 2009). A topographical analysis of this same cohort revealed that the regional period of peak decline occurred first in the occipital cortex and progressed to the frontal cortex (Feinberg et al., 2011). This pattern of maturation has a behavioral correlate, as the occipital cortex and visual acuity develop early in childhood while executive function, the purview of the frontal cortex, does not develop until early adulthood (Ringli and Huber, 2011). A cross sectional study using high density EEG again showed that there was a progressive decrease in the

lower EEG frequencies during adolescence and that the region of highest SWA moves from the occipital to frontal cortex (Kurth et al., 2010). Interestingly, even with the limited samples, EM studies in humans show a similar topographical pattern with the synaptic density of posterior primary sensory areas peaking prior to anterior higher order processing regions (Huttenlocher and Dabholkar, 1997). A physiological and structural correlate for the adolescent decrease in SWA was also suggested by a large cross-sectional study (n=1861; 6-86 years) of waking EEG, which demonstrated that spectral power across age could be explained by an estimate of cortical metabolic rate refined by cortical grey matter volume (Boord et al., 2007). A relationship between grey matter volume and EEG maturation was further shown in a more recent sleep study that correlated the developmental decrease in regional grey matter volume with a decrease across the EEG spectrum, but especially in the SWA band (Buchmann et al., 2011). Altogether these studies suggest that NREM SWA, MRI measured gray matter volume and synaptic density all display a similar developmental pattern that corresponds with cognitive development.

While there is a large body of correlative evidence in humans suggesting that EEG can be used to track cortical synaptic pruning, this proposition has not been tested directly. The main difficulty is that in humans there is currently no practical method to systematically track developmental changes in synaptic density, much less to relate these changes to EEG parameters. EM studies so far have very sparse sampling, especially during the critical adolescent years and thus the developmental time course is not known with any degree of precision. MRI scans can be performed repeatedly at any age however they measure cortical grey matter volume, which changes independent of synaptic density (Rakic et al., 1994; Paus et al., 2008). As synapses represent a very small portion of cortical volume, decreases in grey matter volume primarily reflect complementary changes in the neuropil, glia and myelination rather than synaptic number (Paus et al., 2008). In fact, while grey matter volume generally follows the same pattern as synaptic density and NREM SWA, peaking in early adolescence and then decreasing into adulthood, depending on the region the overall pattern and the magnitude of the decrease are very different; during adolescence grey matter decreases by ~10%, EM measured synaptic density decreases by ~40%, and NREM SWA decreases by 60 to 80% (Huttenlocher and Dabholkar, 1997; Paus et al., 2008; Feinberg et al., 2011). Thus, it remains unclear to what extent changes in synaptic density can account for changes in SWA.

Maturation of sleep in Rodents

In order to directly characterize changes in synaptic density and NREM SWA we have utilized a transgenic mouse model such that EEG and *in vivo* 2P data can be acquired across adolescence. As in humans, adolescence in rodents is a transitional time during which the physical and behavioral traits of adulthood develop (Spear, 2000; Laviola et al., 2003). Following infancy and preceding adulthood, rodent adolescence is demarcated by weaning (~P21) and sexual maturity (P50-60). In rodents the early sleep stages, quiet sleep and active sleep, are distinguishable almost immediately after birth and are the precursors of NREM and REM sleep (Blumberg et al., 2005; Blumberg et al., 2007). Even if eye opening does not occur until ~P12 a component of circadian regulation of sleep is present in the first week as P6 mouse pups have melanopsin mediated photosensitivity, which facilitates entrainment to the light dark cycle (Johnson et al., 2010). The homeostatic regulation of sleep, characterized by a compensatory increase in the intensity and/or duration of sleep in response to sleep loss is present within the first week. In rats as early as P5, pups exhibit an increase in sensory threshold, indicative of enhanced sleep intensity, after 30 minutes of sleep deprivation (Todd et al., 2010). Additionally an increase in sleep duration following deprivation has been shown in rats as early as P12, but increases in SWA following sleep deprivation, the best established marker of sleep need in adults curiously does not manifest until after P20 (Alfoldi et al., 1990; Frank et al., 1998). Although between the first week and adulthood mice have not been investigated as thoroughly as rats, the early sleep-wake patterns follow a similar developmental profile as rats with discernable adult like EEG NREM and REM traces similarly appearing around ~P12 (Castellano and Oliverio, 1976; Daszuta and Gambarelli, 1985; Blumberg et al., 2007). Thus, by the time adolescence begins in rodents (>P20), normal sleep wake patterns and many of the hallmarks of sleep regulation have developed. However there is not yet a systematic study in rats or mice that tracks the developmental changes in NREM SWA during adolescence.

Chronic Sleep Deprivation during Adolescence

The modern adolescent is chronically sleep deprived. According to a 2006 United States poll, from age 13 to age 18 the amount of sleep on week nights is reduced from 8.5 to less than 7 hours a night (Sleep in America poll (2006)). Interestingly a similar study published in 1913 reports that 13 year olds achieved 9.5 hours while 18 year olds slept well over 8.5 hours (Terman and Hocking, 1913). This phenomenon is not restricted to the United States. Multiple

studies across cultures reached the same conclusion: adolescents live under chronic sleep restriction (Carskadon et al., 1998; Gibson et al., 2006; Yu et al., 2007; Liu et al., 2008; Loessl et al., 2008). Recent evidence shows that this proclivity for limiting sleep is not simply due to mounting social and academic pressures but also arises from a combination of biological changes to the homeostatic and circadian regulation of sleep that permit and encourage adolescents to delay bed time (Carskadon, 2011). As children mature, sleep pressure accumulates more slowly across the day, allowing postpubertal children to stay up later than their prepubertal counterparts (Jenni et al., 2005). Meanwhile even when light/dark cycle is held constant for a week there is an age related delay in circadian phase; meaning, adolescents have an increased circadian drive to stay up late even after controlling for social influences (Carskadon et al., 2004).

Any school child knows that the more sleep is restricted the harder it is to perform complex cognitive tasks. Interestingly, in 1913 no relationship was found between school performance and time in bed (Terman and Hocking, 1913). An inability to statistically cope with the greatly differing home environments (electrification, shared-bed sleeping) may explain the lack of an effect of sleep time on aptitude. Alternatively, given that Progressive Era American students slept 10-20% more per night than their early 21st century counterparts, it is likely that the vast majority of students actually received their required level of sleep, removing any possible effect of sleep restriction. Interestingly, now that children sleep on average one hour less per night, multiple studies have demonstrated that children who sleep less perform worse in school (Carskadon et al., 1998; Epstein et al., 1998; Giannotti et al., 2002; Gibson et al., 2006; Meijer, 2008). Furthermore, studies have moved beyond correlation and found that experimentally reducing sleep time reduces cognitive performance while extending time in bed is beneficial for performance (Sadeh et al., 2003; Fallone et al., 2005; Beebe et al., 2008; Jiang et al., 2011). These results in adolescents are consistent with more extensive work in adults demonstrating that sleep deprivation impairs attention, learning and memory (Van Dongen et al., 2004; Tucker et al., 2007; Yoo et al., 2007; Van Der Werf et al., 2009). Work in adults further shows that impairments from chronic sleep restriction is cumulative, such that after 2 weeks of sleep restricted to 6 hours/night performance is as impaired as after one night of total sleep deprivation (Van Dongen et al., 2003).

In response to sleep loss rodents show similar behavioral results as humans but additionally allow for examination of the molecular and anatomical correlates of learning and memory (Tartar et al., 2006; Christie et al., 2008). For example, in adult rats, induction and maintenance of cortical and hippocampal LTP is reduced after sleep deprivation and sleep fragmentation (McDermott et al., 2003; Marks and Wayner, 2005; Kopp et al., 2006; Tartar et al., 2006; Vyazovskiy et al., 2008; Ravassard et al., 2009). Recent 2P studies in adolescent mice demonstrate that acute sleep deprivation leads to an increase in spine formation during a period that is normally associated with a net decrease (Maret et al., 2011; Yang and Gan, 2011). It is unknown if such an alteration of spine dynamics during this critical period for synaptic pruning has a persistent effect. More importantly it is unknown whether chronic sleep deprivation, a condition common in adolescents, has additional pernicious effects on cortical development.

Chronic Sleep Deprivation

Due to the demanding nature of studies of chronic sleep deprivation for both the animal and experimenter, they are far less common than acute deprivation studies. A pioneering sleep study in puppies (aged from 2 to 4 months) showed that complete sleep deprivation by forced locomotion for just a few days proved fatal with postmortem histology indicating that the brain was the site of the "most severe" and "most irreparable changes" (De Manaceine, 1894). Thus, from the very first experimental sleep deprivation ever performed there was evidence that sleep is primarily for the brain and performed a vital function. Interestingly it was also noted that older dogs were more resistant to sleep deprivation than younger puppies (De Manaceine, 1894). A few rodent studies have measured SWA after total and partial deprivations in rats lasting longer than 1 day. Unlike following acute deprivation, chronic sleep deprivations do not produce an easily measured compensatory increase in NREM SWA. However, historically these studies have had limited ability to assess the EEG during waking due to frequent movement artifacts (Lancel and Kerkhof, 1989; Rechtschaffen et al., 1999; Kim et al., 2007; Akerstedt et al., 2009). Meanwhile, a more recent study from our lab was able to record almost artifact free waking EEG and showed that in adult rats chronic sleep restriction qualitatively altered waking such that there was a substantial increase in waking SWA that could account for missing SWA during sleep (Leemburg et al., 2010). Despite the prevalence of chronic sleep restriction during adolescence no study has yet looked at the electrophysiological, anatomical or molecular effects of chronic sleep deprivation during this critical period.

Summary:

The work presented in this dissertation advances our understanding of how NREM SWA may be linked to sleep need by testing predictions of the synaptic homeostasis hypothesis (SHY). In SHY it is posited that the essential function of sleep is to downscale the overall strength of synapses in order to counterbalance a dynamic waking state during which there is an unsustainable net increase in synaptic strength. The return to sustainable levels of lowered overall synaptic strength may explain both why NREM SWA decreases as a function of time spent asleep and why sleep is beneficial for memory and performance. The two most prominent corollaries of this hypothesis are: 1) synaptic number/strength have a positive relationship with the slope and amplitude of NREM sleep slow waves, 2) NREM sleep slow waves play an active role in synaptic homeostasis, decreasing synaptic strength in proportion to their magnitude. Thus, central to the hypothesis is a stabilizing mechanism in which NREM slow waves complete a negative feedback loop with synaptic strength.

Chapter 2: Effects of anesthesia on the response to sleep deprivation

Here we investigated whether slow waves, independent of whether they occur during physiological NREM sleep or in other conditions, will also lead to a NREM sleep like decrease in SWA. This would suggest an active role for slow waves in reducing sleep pressure. The concentration of volatile anesthetic agents can dynamically be controlled and titrated by an experimenter monitoring the EEG signal. We took advantage of this level of control to force two very different EEG patterns while using the same anesthetic agent. By comparing the SWA response to an anesthetic state predominated by slow waves and an induced isoelectric state with very few slow waves, we hoped to disentangle the effect of the slow waves from that of the anesthetic agent.

Chapter 3: Sleep Patterns and Homeostatic Mechanisms in Adolescent Mice.

Here we investigated age dependent effects of sleep/wake on NREM SWA. Consistent with SHY we have recently shown that adolescent mice undergo a net increase in synaptic density during wake and a decrease during sleep (Maret 2011). In order to characterize the homeostatic regulation in this same strain, we recorded EEG in mice spanning adolescence and looked at how the sleep deprivation induced increase in NREM SWA develops during adolescence.

Chapter 4: The Response to Chronic Sleep Deprivation in Adolescent Mice.

Here we performed a chronic sleep deprivation and compared the change in NREM SWA to the developmental time course of non-deprived animals. The blunted response in adolescent mice to an acute sleep deprivation shown in chapter 3 raises the question, of what happens when sleep is chronically restricted during this period thought critical for synaptic pruning. This work is a first step in directly testing the notion that the decrease in NREM SWA during adolescence reflects a decrease in synaptic density. Previous work has relied on inferences made from studies which either offer only indirect measures of synaptic density or suffer from sparse sampling during the critical adolescent period. In an effort to fill this gap we utilized chronic EEG recording which will in the future be combined with *in vivo* 2P, and molecular markers of synaptic strength to establish which neurodevelopmental changes underlie the developmental decrease in NREM SWA.

References:

Achermann P, Borbely AA (1990) Simulation of human sleep: ultradian dynamics of electroencephalographic slow-wave activity. J Biol Rhythms 5:141-157.

Akerstedt T, Kecklund G, Ingre M, Lekander M, Axelsson J (2009) Sleep homeostasis during repeated sleep restriction and recovery: support from EEG dynamics. Sleep 32:217-222.

Alfoldi P, Tobler I, Borbely AA (1990) Sleep regulation in rats during early development. Am J Physiol 258:R634-644.

America Si (2006) Summary of findings: 2006 Sleep in America Poll. In. Washington D.C.: National Sleep Foundation.

Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep-wake regulation. Prog Neurobiol 73:379-396.

Beebe DW, Fallone G, Godiwala N, Flanigan M, Martin D, Schaffner L, Amin R (2008) Feasibility and behavioral effects of an at-home multi-night sleep restriction protocol for adolescents. J Child Psychol Psychiatry 49:915-923.

Benington JH, Heller HC (1995) Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol 45:347-360.

Blanco-Centurion C, Xu M, Murillo-Rodriguez E, Gerashchenko D, Shiromani AM, Salin-Pascual RJ, Hof PR, Shiromani PJ (2006) Adenosine and sleep homeostasis in the Basal forebrain. J Neurosci 26:8092-8100.

Blumberg MS, Seelke AM, Lowen SB, Karlsson KA (2005) Dynamics of sleep-wake cyclicity in developing rats. Proc Natl Acad Sci U S A 102:14860-14864.

Blumberg MS, Coleman CM, Johnson ED, Shaw C (2007) Developmental divergence of sleep-wake patterns in orexin knockout and wild-type mice. Eur J Neurosci 25:512-518.

Boord PR, Rennie CJ, Williams LM (2007) Integrating "brain" and "body" measures: correlations between EEG and metabolic changes over the human lifespan. J Integr Neurosci 6:205-218.

Borbely AA (1982) A two process model of sleep regulation. Hum Neurobiol 1:195-204.

Buchmann A, Ringli M, Kurth S, Schaerer M, Geiger A, Jenni OG, Huber R (2011) EEG sleep slow-wave activity as a mirror of cortical maturation. Cereb Cortex 21:607-615.

Bushey D, Tononi G, Cirelli C (2011) Sleep and synaptic homeostasis: structural evidence in Drosophila. Science 332:1576-1581.

Carskadon MA (2011) Sleep's effects on cognition and learning in adolescence. Prog Brain Res 190:137-143.

Carskadon MA, Acebo C, Jenni OG (2004) Regulation of adolescent sleep: implications for behavior. Ann N Y Acad Sci 1021:276-291.

Carskadon MA, Wolfson AR, Acebo C, Tzischinsky O, Seifer R (1998) Adolescent sleep patterns, circadian timing, and sleepiness at a transition to early school days. Sleep 21:871-881.

Castellano C, Oliverio A (1976) Early malnutrition and postnatal changes in brain and behavior in the mouse. Brain Res 101:317-325.

Christie MA, McKenna JT, Connolly NP, McCarley RW, Strecker RE (2008) 24 hours of sleep deprivation in the rat increases sleepiness and decreases vigilance: introduction of the rat-psychomotor vigilance task. J Sleep Res 17:376-384.

Cirelli C, Gutierrez CM, Tononi G (2004) Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron 41:35-43.

Daszuta A, Gambarelli F (1985) Early postnatal development of EEG and sleep-waking cycle in two inbred mouse strains. Brain Res 354:39-47.

De Manaceine M (1894) Quelques observations experimentales sur l'infulence de l'insomnie absolue. Arch Ital Biol 21:322-325.

Dement WC (1998) The study of human sleep: a historical perspective. Thorax 53 Suppl 3:S2-7.

Epstein R, Chillag N, Lavie P (1998) Starting times of school: effects on daytime functioning of fifth-grade children in Israel. Sleep 21:250-256.

Esser SK, Hill SL, Tononi G (2007) Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. Sleep 30:1617-1630.

Fallone G, Acebo C, Seifer R, Carskadon MA (2005) Experimental restriction of sleep opportunity in children: effects on teacher ratings. Sleep 28:1561-1567.

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J Neurosci 28:4088-4095.
Feinberg I (1982) Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? J Psychiatr Res 17:319-334.
Feinberg I, Campbell IG (2009) Sleep EEG changes during adolescence: an index of a fundamental brain reorganization. Brain Cogn 72:56-65.
Feinberg I, de Bie E, Davis NM, Campbell IG (2011) Topographic differences in the adolescent maturation of the slow wave EEG during NREM sleep. Sleep 34:325-333.
Frank MG, Morrissette R, Heller HC (1998) Effects of sleep deprivation in neonatal rats. Am J Physiol 275:R148-157.
Franken P, Tobler I, Borbely AA (1991) Sleep homeostasis in the rat: simulation of the time course of EEG slow-wave activity. Neurosci Lett 130:141-144.
Giannotti F, Cortesi F, Sebastiani T, Ottaviano S (2002) Circadian preference, sleep and daytime behaviour in adolescence. J Sleep Res 11:191-199.
Gibson ES, Powles AC, Thabane L, O'Brien S, Molnar DS, Trajanovic N, Ogilvie R, Shapiro C, Yan M, Chilcott-Tanser L (2006) "Sleepiness" is serious in adolescence: two surveys of 3235 Canadian students. BMC Public Health 6:116.
Gilestro GF, Tononi G, Cirelli C (2009) Widespread changes in synaptic markers as a function of sleep and wakefulness in Drosophila. Science 324:109-112.
Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, Haydon PG, Frank MG (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron 61:213-219.
Hanlon EC, Faraguna U, Vyazovskiy VV, Tononi G, Cirelli C (2009) Effects of skilled training on sleep slow wave activity and cortical gene expression in the rat. Sleep 32:719-729.
Heller HC (2006) A global rather than local role for adenosine in sleep homeostasis. Sleep 29:1382-1383; discussion 1387-1389.
Huber R, Deboer T, Tobler I (2000) Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. Brain Res 857:8-19.
Huber R, Tononi G, Cirelli C (2007) Exploratory behavior, cortical BDNF expression, and sleep homeostasis. Sleep 30:129-139.
Huber R, Maki H, Rosanova M, Casarotto S, Canali P, Casali AG, Tononi G, Massimini M (2012) Human cortical excitability increases with time awake. Cereb Cortex 23:332-338.
Huber R, Maatta S, Esser SK, Sarasso S, Ferrarelli F, Watson A, Ferreri F, Peterson MJ, Tononi G (2008) Measures of cortical plasticity after transcranial paired associative stimulation predict changes in electroencephalogram slow-wave activity during subsequent sleep. J Neurosci 28:7911-7918.
Huttenlocher PR, Dabholkar AS (1997) Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 387:167-178.
Ishimori K (1909) the demonstration of intracerebral substances in sleep-deprived animals: sleep-inducing substances as the true cause of sleep. Chuo Igakkai Zasshi 84:1-47.
Jenni OG, Achermann P, Carskadon MA (2005) Homeostatic sleep regulation in adolescents. Sleep 28:1446-1454.
Jiang F, VanDyke RD, Zhang J, Li F, Gozal D, Shen X (2011) Effect of chronic sleep restriction on sleepiness and working memory in adolescents and young adults. J Clin Exp Neuropsychol 33:892-900.
Johnson J, Wu V, Donovan M, Majumdar S, Renteria RC, Porco T, Van Gelder RN, Copenhagen DR (2010) Melanopsin-dependent light avoidance in neonatal mice. Proc Natl Acad Sci U S A 107:17374-17378.
Kim Y, Laposky AD, Bergmann BM, Turek FW (2007) Repeated sleep restriction in rats leads to homeostatic and allostatic responses during recovery sleep. Proc Natl Acad Sci U S A 104:10697-10702.
Kopp C, Longordo F, Nicholson JR, Luthi A (2006) Insufficient sleep reversibly alters bidirectional synaptic plasticity and NMDA receptor function. J Neurosci 26:12456-12465.
Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J (2008) Sleep as a fundamental property of neuronal assemblies. Nat Rev Neurosci 9:910-919.
Kurth S, Ringli M, Geiger A, LeBourgeois M, Jenni OG, Huber R (2010) Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. J Neurosci 30:13211-13219.
Lancel M, Kerkhof GA (1989) Effects of repeated sleep deprivation in the dark- or light-period on sleep in rats. Physiol Behav 45:289-297.

Lante F, Toledo-Salas JC, Ondrejcak T, Rowan MJ, Ulrich D (2011) Removal of synaptic Ca(2)+-permeable AMPA receptors during sleep. J Neurosci 31:3953-3961.

Laviola G, Macri S, Morley-Fletcher S, Adriani W (2003) Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. Neurosci Biobehav Rev 27:19-31.

Leemburg S, Vyazovskiy VV, Olcese U, Bassetti CL, Tononi G, Cirelli C (2010) Sleep homeostasis in the rat is preserved during chronic sleep restriction. Proc Natl Acad Sci U S A 107:15939-15944.

Legendre R, Pieron H (1907) Les rapports entre les physiologiques et les modifications histologiques des cellules cerebrales dans l'insomnie experimentale. C R Soc Biol 62:312-314.

Liu X, Zhao Z, Jia C, Buysse DJ (2008) Sleep patterns and problems among chinese adolescents. Pediatrics 121:1165-1173.

Liu ZW, Faraguna U, Cirelli C, Tononi G, Gao XB (2010) Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. J Neurosci 30:8671-8675.

Loessl B, Valerius G, Kopasz M, Hornyak M, Riemann D, Voderholzer U (2008) Are adolescents chronically sleep-deprived? An investigation of sleep habits of adolescents in the Southwest of Germany. Child Care Health Dev 34:549-556.

Maret S, Faraguna U, Nelson AB, Cirelli C, Tononi G (2011) Sleep and waking modulate spine turnover in the adolescent mouse cortex. Nat Neurosci 14:1418-1420.

Marks CA, Wayner MJ (2005) Effects of sleep disruption on rat dentate granule cell LTP in vivo. Brain Res Bull 66:114-119.

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

Meijer AM (2008) Chronic sleep reduction, functioning at school and school achievement in preadolescents. J Sleep Res 17:395-405.

Mitzdorf U (1985) Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. Physiol Rev 65:37-100.

Paus T, Keshavan M, Giedd JN (2008) Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 9:947-957.

Porkka-Heiskanen T, Strecker RE, McCarley RW (2000) Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. Neuroscience 99:507-517.

Rakic P, Bourgeois JP, Goldman-Rakic PS (1994) Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. Prog Brain Res 102:227-243.

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

Rechtschaffen A, Bergmann BM, Gilliland MA, Bauer K (1999) Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat. Sleep 22:11-31.

Riedner BA, Vyazovskiy VV, Huber R, Massimini M, Esser S, Murphy M, Tononi G (2007) Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. Sleep 30:1643-1657.

Ringli M, Huber R (2011) Developmental aspects of sleep slow waves: linking sleep, brain maturation and behavior. Prog Brain Res 193:63-82.

Rusterholz T, Achermann P (2011) Topographical aspects in the dynamics of sleep homeostasis in young men: individual patterns. BMC Neurosci 12:84.

Sadeh A, Gruber R, Raviv A (2003) The effects of sleep restriction and extension on school-age children: what a difference an hour makes. Child Dev 74:444-455.

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417-463.

Steriade M, McCormick DA, Sejnowski TJ (1993) Thalamocortical oscillations in the sleeping and aroused brain. Science 262:679-685.

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

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

Terman LM, Hocking A (1913) The sleep of school children: Its distribution according to age, and its relation to physical and mental efficiency. Journal of Educational Psychology 4:138-147.

Todd WD, Gibson JL, Shaw CS, Blumberg MS (2010) Brainstem and hypothalamic regulation of sleep pressure and rebound in newborn rats. Behav Neurosci 124:69-78.

Tononi G, Cirelli C (2003) Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull 62:143-150.

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10:49-62.

Tucker AM, Dinges DF, Van Dongen HP (2007) Trait interindividual differences in the sleep physiology of healthy young adults. J Sleep Res 16:170-180.

Van Der Werf YD, Van Der Helm E, Schoonheim MM, Ridderikhoff A, Van Someren EJ (2009) Learning by observation requires an early sleep window. Proc Natl Acad Sci U S A 106:18926-18930.

Van Dongen HP, Maislin G, Mullington JM, Dinges DF (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 26:117-126.

Van Dongen HP, Baynard MD, Maislin G, Dinges DF (2004) Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. Sleep 27:423-433.

Vyazovskiy VV, Cirelli C, Tononi G (2011) Electrophysiological correlates of sleep homeostasis in freely behaving rats. Prog Brain Res 193:17-38.

Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G (2008) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 11:200-208.

Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G (2009) Cortical firing and sleep homeostasis. Neuron 63:865-878.

Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093-1097.

Wigren HK, Schepens M, Matto V, Stenberg D, Porkka-Heiskanen T (2007) Glutamatergic stimulation of the basal forebrain elevates extracellular adenosine and increases the subsequent sleep. Neuroscience 147:811-823.

Yang G, Gan WB (2011) Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. Dev Neurobiol.

Yokoyama TK, Mochimaru D, Murata K, Manabe H, Kobayakawa K, Kobayakawa R, Sakano H, Mori K, Yamaguchi M (2011) Elimination of adult-born neurons in the olfactory bulb is promoted during the postprandial period. Neuron 71:883-897.

Yoo SS, Hu PT, Gujar N, Jolesz FA, Walker MP (2007) A deficit in the ability to form new human memories without sleep. Nat Neurosci 10:385-392.

Yu Y, Lu BS, Wang B, Wang H, Yang J, Li Z, Wang L, Liu X, Tang G, Xing H, Xu X, Zee PC, Wang X (2007) Short sleep duration and adiposity in Chinese adolescents. Sleep 30:1688-1697.

Chapter II:

Effects of anesthesia on the response to sleep deprivation

Aaron B. Nelson, Ugo Faraguna, Giulio Tononi, and Chiara Cirelli

Published in:
Sleep, Vol. 33, No 12, 2010 1659-67

Abstract:

Study Objective: Slow wave activity (SWA) during NREM sleep is the best characterized marker of sleep homeostasis, and the occurrence of sleep slow waves is necessary to reduce sleep need. Recent evidence suggests that sleep slow waves may mediate several beneficial effects of sleep on performance, from the prevention of cognitive impairments to memory consolidation. However, slow waves are also triggered by low doses of many anesthetics, but very few reports have examined whether anesthesia-mediated slow waves affect the homeostatic regulation of sleep. Moreover, no study has examined how sleep is affected by higher doses of anesthetics, which lead to a predominantly "isoelectric" EEG tracing without slow waves.

Design: We studied in rats whether 1 hour of a dose of isoflurane or desflurane able to induce almost continuous slow waves (ISO-sw, DES-sw), and of a dose of desflurane resulting in a predominantly isoelectric EEG (DES-iso) reduces the sleep pressure caused by 4 hours of sleep deprivation. Anesthesia was compared to a mock condition in which rats were only anesthetized for 2-3 min.

Setting: Basic sleep research laboratory.

Patients or Participants: Male WKY rats (n=31).

Interventions: Total sleep deprivation by exposure to novel objects starting at light onset, followed by one hour of anesthesia or mock anesthesia.

Measurements and Results: One hour of anesthesia (sw or iso) did not affect either sleep duration or the overall sleep pattern. Anesthesia with ISO-sw or DES-sw, both associated with the occurrence of almost continuous slow waves, reduced the SWA rebound expected following 4 hours of sleep deprivation. One hour of anesthesia with DES-iso, associated with isoelectric EEG and few slow waves, also reduced the SWA rebound after sleep deprivation, and did so to an extent similar to that observed after DES-sw. However, in contrast to DES-sw, SWA after DES-iso remained chronically lower than in baseline, resulting in reduced slow wave energy (SWE, SWA x time) for at least 2 days.

Conclusion: The blunted SWA rebound after ISO-sw and DES-sw suggests that anesthesia slow waves may substitute for sleep slow waves. The reduced SWA rebound after DES-iso may reflect a pathological condition that results in a chronic decrease in SWA, or may suggest that anesthesia slow waves are not an absolute requirement to discharge sleep pressure.

Introduction

Sleep is homeostatically regulated in that sleep need increases with time spent awake and decreases during sleep. The best characterized marker of sleep homeostasis is SWA, the EEG power between 0.5-4.0 Hz during NREM sleep. In birds and mammals NREM SWA reaches an apex at the beginning of the sleep period and decreases with time spent asleep [1-3]. Moreover, staying awake from ~3 to ~24 hours results in progressively higher SWA levels at sleep onset, while naps during the day reduce SWA the following night [4, 5]. Usually recorded from the scalp via the EEG, SWA reflects synchronous firing of large neuronal populations that are coordinated by an underlying slow oscillation, the fundamental cellular phenomenon of NREM sleep [6, 7]. Increasing evidence suggests that slow waves are more than just an epiphenomenon of NREM sleep. In fact, they can mediate some of sleep's beneficial effects. For instance, intermittent transcranial direct current stimulation during early NREM sleep, which temporarily increased SWA, was found to enhance retention in a paired-associate memory task [8]. Moreover, pharmacological enhancement of slow wave sleep reduces cognitive impairments associated with sleep restriction [9, 10]. Finally, two recent studies showed that the selective suppression of slow waves using acoustic stimuli (without waking up the subjects) prevents the post-sleep improvement in performance after visual texture discrimination learning [11] and visuomotor learning [12]. Thus, it seems that slow waves may benefit several aspects of performance, from the prevention of cognitive impairments to memory consolidation.

Much work has been done to characterize the role of slow waves in sleep homeostasis, and it has been demonstrated that the occurrence of slow waves during NREM sleep is necessary to reduce sleep need [13]. However, slow waves are not exclusive to sleep states and, in fact, the slow oscillation was first reported simultaneously in anesthetized cats and sleeping humans [14]. Indeed, many anesthetics used in the clinic, including the volatile anesthetics isoflurane (ISO) and desflurane (DES) and the intravenous agent propofol produce, at relatively low concentrations, a NREM-like EEG activity dominated by slow waves, while higher concentrations lead to an isoelectric tracing which lacks slow waves [15]. However, whether slow waves as induced by anesthetics affect the homeostatic regulation of sleep remains largely unexplored. In humans a 3-hour period of low concentration ISO in the morning was found to reduce the percentage of time spent in slow wave sleep during the subsequent night [16], suggesting that sleep pressure may have been reduced by anesthesia. However, the EEG was not recorded during anesthesia, and thus whether continuous slow waves were indeed present was not confirmed. Moreover, ISO was

administered in the morning, when the sleep pressure was presumably low, and 75% of the subjects were allowed to take naps, making the interpretation of the subsequent reduction in slow wave sleep quite difficult. In a more recent study rats were sleep deprived for 24 hours and then anesthetized with propofol for 6 hours [17]. After recovery from anesthesia the duration of NREM and REM sleep decreased, and SWA did not show the rebound normally expected after prolonged sleep loss. However, rats were allowed to recover during the dark phase, when they are normally active. Moreover, because of filter settings, only the high range (2-4 Hz) SWA was analyzed. Several studies found that the SWA rebound after sleep deprivation is either most prominent [18], longer-lasting [19, 20], or even limited to its lowest frequency range [21].Thus, it is important to assess whether anesthesia affects low range sleep SWA. Finally, a recent study found that 4 hours of ISO anesthesia (presumably with slow waves) does not affect the REM sleep rebound after selective REM sleep deprivation for 24 hours, but NREM sleep and SWA were not studied [22].

The goal of this study was to determine whether 1 hour of anesthesia characterized by continuous slow waves reduces the sleep pressure previously created by a period of total sleep deprivation. To this end rats were sleep deprived for 4 hours starting at light onset, anesthetized for 1 hour, and then allowed to recover during the light phase, when they normally sleep. Moreover, we wanted to determine whether the effects on recovery sleep differ when the dose of anesthetic is increased to induce isoelectric EEG with few slow waves.

Materials and Methods

Animals, surgery, and recordings.

Male WKY rats (n=31; Charles River Laboratories, Wilmington, MA; 250-300 g at time of surgery) were maintained on a 12 h light / 12 h dark cycle (lights on at 10:00 AM; room temperature 23 ± 1° C). Under deep ISO anesthesia (1.5-2% volume), rats were implanted bilaterally for chronic polysomnographic recordings in the frontal cortex (B, + 2-3 mm; L 2-3 mm) and parietal cortex (B - 3-4 mm; L 3-4 mm). For experiments using ISO (n=14 rats) bipolar local field potential electrodes (PlasticsOne Inc, Roanoke, VA; Rhodes Medical Instruments, Summerland, CA) were used, while for experiments with DES (n=8 rats) gold screws were used for EEG recording. Nine additional rats implanted with bipolar local field potential electrodes were used for sleep deprivation experiments without any anesthesia (see Results). LFP bipolar wire electrodes had 1 mm vertical and 0.8 mm horizontal separation between electrode tips, with the shallow lead aimed at cortical layers I–II. Electrodes were fixed to the skull with dental cement. All animals used for the experiments showed opposite polarity in the EEG signal when superficial and deep electrodes were independently referenced to the cerebellar screw. Gold screws (instead of LFP electrodes) were used for the DES experiments because they provide a signal that is more stable over time, and the study did not require the increased spatial resolution provided by LFP electrodes. The differences in signal between the LFP electrodes and gold screws are not relevant for the findings of this paper and therefore from here onward both LFP and gold screw recordings will be referred to simply as EEG signals. Two stainless-steel wires (diameter, 0.4 mm) inserted into the neck muscles were used to record the electromyogram (EMG). Immediately after surgery, the animals were individually placed in transparent Plexiglas cages (36.5 x 25 x 46 cm), and kept in sound-attenuating recording boxes for the duration of the experiment. At least 8 days were allowed for recovery after surgery, and experiments were started only after the sleep/wake cycle had fully normalized. The rats were connected by means of a flexible cable to a commutator (Airflyte, Bayonne, NJ) and recorded continuously. To habituate the animals to the sleep deprivation procedure (see below), every day beginning the day after surgery, rats were handled and exposed to a novel object between 10:00 and 10:30 AM. Video recordings were performed continuously with infrared cameras (OptiView Technologies, Potomac Falls, VA) and stored in real-time (AVerMedia Technologies, Milpitas, CA; 24 Seven Surveillance Inc, Alhambra, CA). To verify that the animals

were fully entrained to the light/dark cycle, cages were equipped with Chronokit activity monitor infrared sensors (Stanford Chronokit; Stanford Software Systems, Santa Cruz, CA). All animal procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and facilities were reviewed and approved by the IACUC of the University of Wisconsin-Madison, and were inspected and accredited by AAALAC.

Data acquisition.

Rats were connected by means of a flexible cable and a commutator to a Grass mod. 8 polygraph (Grass Instruments, West Warick, RI). EEG and EMG signals were conditioned by analog filters (EEG = high pass, 0.1 Hz; low pass, 35 Hz; EMG = high pass, 5 Hz; low pass, 70 Hz), digitalized at 128 Hz (Kissei America, Irvine, CA) and stored on a computer. Wake, NREM sleep and REM sleep were manually scored off-line (Sleep Sign; Kissei COMTEC, Matsumoto, Japan) in 4-s epochs according to standard criteria. Artifacts were always removed simultaneously from all derivations and vigilance states could always be determined. EEG power spectra (fast Fourier transform routine, Hanning window) were calculated for consecutive 4-s epochs within the 0.25–20.0 Hz frequency range. In each rat a frontal and parietal channel showing a stable signal across all conditions were used for final analysis. Since results were similar in the two derivations, only data from the frontal channels are presented. SWA was computed for each epoch as the mean power from 0.5 to 4 Hz. SWE for each 24-hour period was computed as the cumulative sum of SWA over time (equivalent to computing the product of NREM duration and 24-hour mean SWA).

Sleep deprivation.

Total sleep deprivation began at light onset and was performed for 4 hours by exposing the animals to a variety of novel objects and by transferring bedding material between cages. If rats became inactive and began to exhibit slow waves visible via real-time EEG monitoring, a new object was inserted into the cage, the cage was tapped, or the rat was gently prodded. Rats were never disturbed when they were spontaneously awake and active.

Anesthesia.

Anesthesia was delivered via a mobile anesthesia apparatus (Vaporstick; Surgivet, Waukesha, Wisconsin) fitted with the appropriate vaporizer (ISO: tech 4; DES: tech 6, Ohmeda, Madison, Wisconsin). In all cases anesthesia was induced in a chamber placed inside the home cage. Following loss of righting reflex and the appearance of EEG slow waves, rats were removed from the chamber and transferred to an anesthesia mask and placed on a heat blanket. Cortical temperature was measured in 2 animals receiving DES using chronically implanted thermistors. In both rats the decrease in temperature during anesthesia (both sw and iso) relative to wake was ~ 1°C, similar or smaller than the decrease during NREM sleep relative to wake in the mock condition, suggesting that the use of the heat blanket prevented changes in brain temperature in our experimental conditions. A pulse oximeter (V3402; Surgivet, Waukesha, Wisconsin) was used to monitor heart rate and O_2 saturation from a clamp on the hind foot. O_2 saturation remained > 95% throughout the duration of anesthesia in all experimental groups. Heart rate during both ISO-sw and DES-sw was close to published values during sleep [23] at the beginning of the experiment (~ 330 beats/min), and then gradually and slightly decreased during the hour of anesthesia (Supplementary Fig. 2), while during DES-iso it remained steady across the hour (334 $\pm$ 4.3).

The burst suppression ratio (BSR), i.e. the % of time spent in isoelectric EEG (suppression), was calculated for each 4-s epoch. For ISO, the amplitude threshold to define suppression was conservatively set at ±15 uV as in [24], and the minimum duration for an isoelectric event was set to 0.5 sec as in [25]. For DES, the threshold was determined for each rat individually as $\pm$3 standard deviations of the mean voltage of clearly non-bursting EEG activity (mean 23.6326 $\pm$1.8 uV; method for threshold used in [26]). BSR calculation was verified with visual EEG scoring for each rat: epochs scored as "isoelectric EEG" had BSR close to 100%, while epochs dominated by slow waves had BSR close to 0%.

All rats were visually monitored during the recovery from anesthesia, and actively prevented from going to sleep until they exhibited coordinated and purposeful movements (exploration of the cage), which in all cases occurred within 10 min. Wake behavior (eating, drinking, grooming, exploring) and sleep posture post-anesthesia were also monitored via video, and the time of the first grooming period was recorded.

Statistical analysis.

All statistics were performed using the MATLAB 2007, statistics toolbox. Since the normalized SWA and SWE were normally distributed on the experiment day, comparisons were done via paired t-test or, for multiple comparisons, two-way ANOVA (Rat x Condition) followed by post-hoc paired t-tests with Bonferroni correction. Behavioral measures and frequency bins (bin size 0.25 Hz, no correction for bin number) were assessed by a paired Wilcoxon test or, for multiple comparisons, a Friedman test followed by post-hoc paired Wilcoxon with Bonferroni correction.

Results

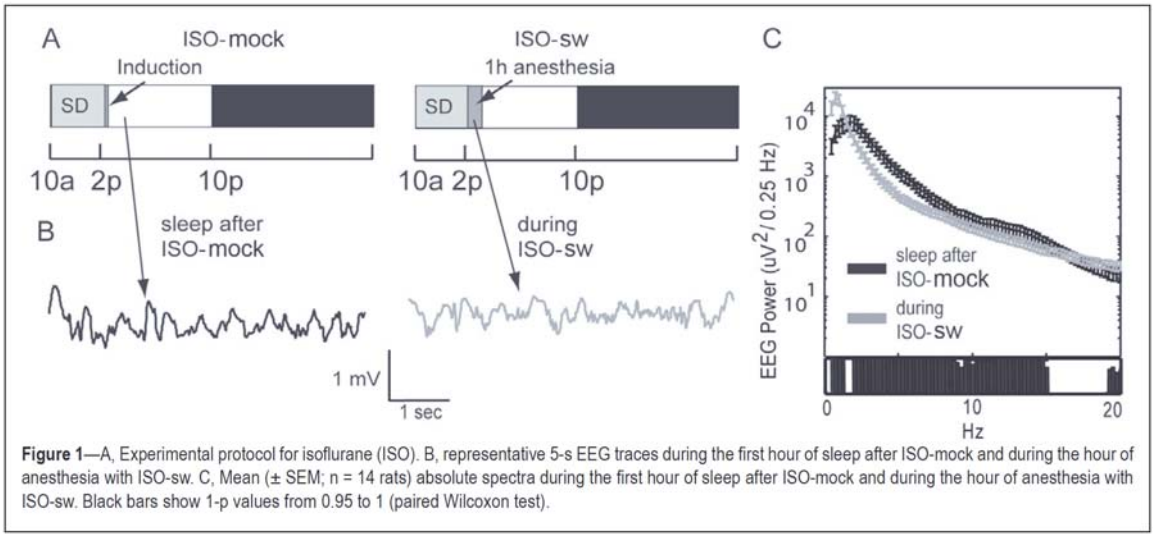


Figure 1—A, Experimental protocol for isoflurane (ISO). B, representative 5-s EEG traces during the first hour of sleep after ISO-mock and during the hour of anesthesia with ISO-sw. C, Mean (± SEM; n = 14 rats) absolute spectra during the first hour of sleep after ISO-mock and during the hour of anesthesia with ISO-sw. Black bars show 1-p values from 0.95 to 1 (paired Wilcoxon test).

Isoflurane experiments

Each rat was used in 2 experiments, both preceded by an undisturbed baseline recording (Fig. 1A). Each experiment started with 4 hours of sleep deprivation (beginning at light onset), followed by either ISO-mock or ISO-sw in a counterbalanced design, with one week between each condition. In ISO-mock animals were immediately allowed to recover after anesthesia induction (2-3 min). In ISO-sw instead, anesthesia lasted for ~ 1 hour, and the concentration of the anesthetic (0.5-1.5%) was closely checked to maintain an EEG tracing with slow waves as similar as possible to the slow waves of NREM sleep (Fig. 1B). Indeed, the BSR during ISO-sw was very low (mean ± SEM, 3.5% ± 0.9, n = 14 rats), indicating that very few epochs of isoelectric EEG occurred throughout the hour of anesthesia (see also Supplementary Table 1 and Supplementary Fig. 1), and the EEG power spectrum during the hour of anesthesia was dominated by large slow waves, similar to those observed during the first hour of sleep after ISO-mock. However, during anesthesia there was more EEG power in the lowest frequencies of the spectrum, corresponding to the low range SWA (0.5-1.5 Hz), and less power in the 2-15 Hz range (Fig. 1C).

All rats recovered coordinated purposeful movements soon after anesthesia, but the latency to the first grooming episode was ~ 11 min longer after ISO-sw relative to ISO-mock (Table 1A). On the other hand, latency to sleep, sleep amount, and other sleep parameters calculated from sleep onset to the beginning of the dark phase (from

~ 3 PM to 10 PM) did not differ between the 2 experimental conditions (Table 1A). Thus, one hour of anesthesia did not seem to affect either sleep duration or the overall sleep pattern.

Table 1—Sleep parameters after isoflurane (A) and desflurane (B) anesthesia

	A		B		
	ISO-mock	ISO-sw	DES-mock	DES-sw	DES-iso
Sleep Latency	9.6 (2.0)	12.9 (2.1)	14.9 (4.8)	16.5 (3.0)	11.2 (4.0)
REM Latency	46.4 (3.1)	40.2 (2.9)	46.6 (3.9)	42.6 (4.8)	40.3 (5.2)
1st Grooming	14.3 (2.4)	25.5 (3.0)*	7.9 (1.2)	13.0 (2.8)	25.0 (4.3)*
NREM sleep (min)	461.2 (7.2)	471.7 (7.6)	447.0 (9.9)	458.8 (11.5)	466.6 (9.5)
REM sleep (min)	122.7 (5.2)	128.2 (4.6)	126.9 (5.4)	129.4 (3.9)	130.2 (7.0)
Wakefulness (min)	529.9 (10.4)	514.0 (9.8)	547.5 (13.0)	533.1 (14.0)	528.2 (6.5)
Brief awakenings	31.2 (2.0)	31.3 (2.2)	27.9 (0.9)	29.3 (2.0)	26.1 (1.5)

Mean sleep values (± SEM in parenthesis) refer to the period (~15:00-10:00) from the onset of consolidated sleep until the end of the dark period. The number of brief awakenings (< 16 sec) is expressed per hour of sleep. Paired Wilcoxon test, *P < 0.05 (mock vs low).

After ISO-mock the relative EEG power spectrum during the first 4 hours of NREM sleep was increased relative to baseline over most of the SWA range (0.5-3 Hz, Fig. 2A), consistent with the changes observed in sleep deprived rats [18]. SWA as a whole (0.5-4.0 Hz) was also significantly increased in ISO-mock compared to baseline for the first 4 hours after sleep onset (+ 27.2% ± 6.3, p=0.022, paired t-test), and a negative rebound was observed afterwards (Fig. 2B), again consistent with the SWA response after sleep deprivation [27]. In a separate group of 9 rats that underwent 4 hours of sleep deprivation starting at light onset (without any anesthesia) the SWA increase during the first 4 hours of recovery sleep was + 30.5% ± 3.8 (p=0.027, paired t-test), very similar to that observed after ISO-mock, strongly suggesting that anesthesia induction per se does not affect the SWA rebound. Analysis of the cumulative changes in SWA (SWE) during ISO-mock showed that the SWA lost during the 4 hours of sleep deprivation was fully recovered before the end of the light period, with no changes during the following dark period (Fig. 2C).

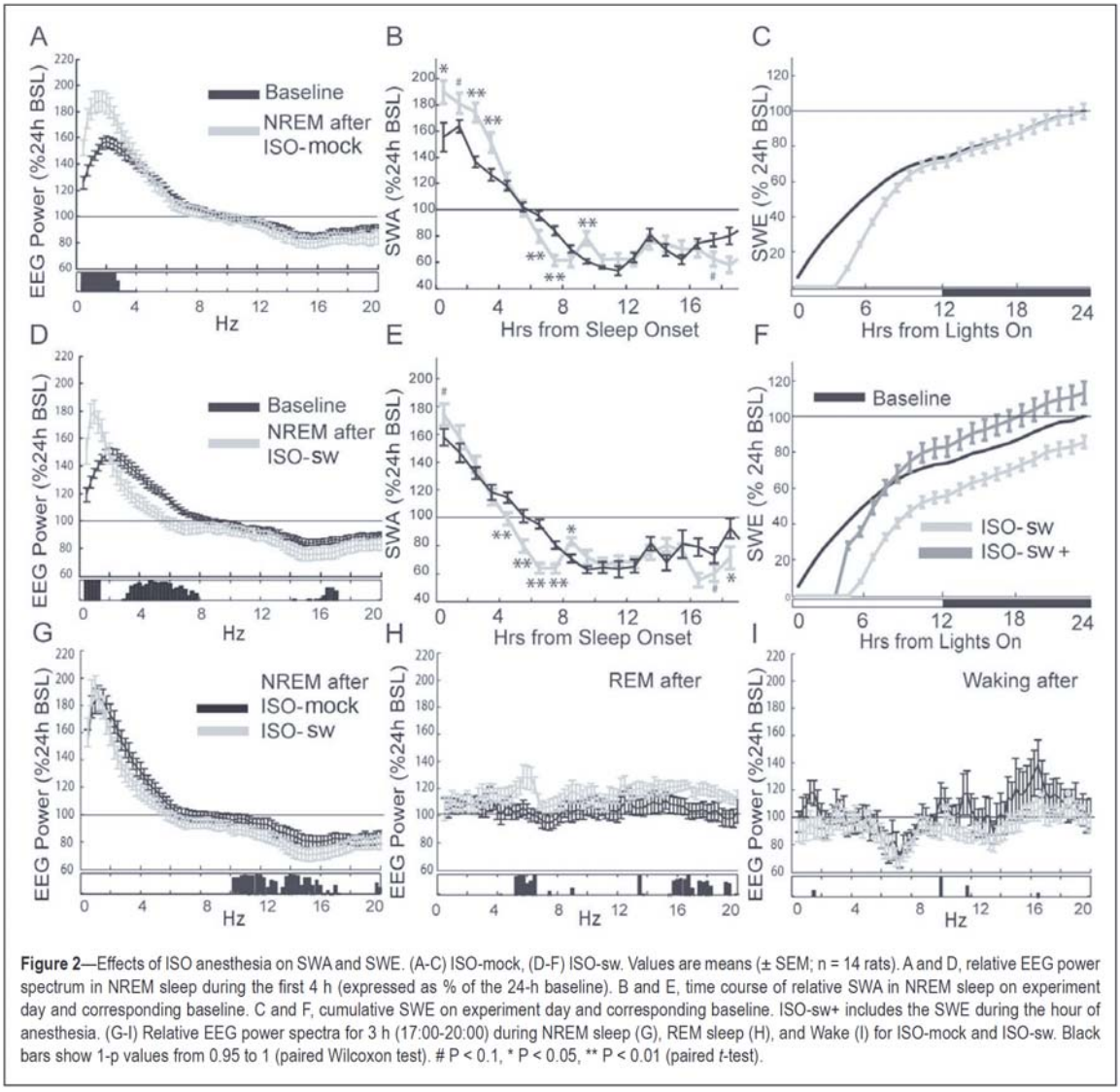


Figure 2—Effects of ISO anesthesia on SWA and SWE. (A-C) ISO-mock, (D-F) ISO-sw. Values are means (± SEM; n = 14 rats). A and D, relative EEG power spectrum in NREM sleep during the first 4 h (expressed as % of the 24-h baseline). B and E, time course of relative SWA in NREM sleep on experiment day and corresponding baseline. C and F, cumulative SWE on experiment day and corresponding baseline. ISO-sw+ includes the SWE during the hour of anesthesia. (G-I) Relative EEG power spectra for 3 h (17:00-20:00) during NREM sleep (G), REM sleep (H), and Wake (I) for ISO-mock and ISO-sw. Black bars show 1-p values from 0.95 to 1 (paired Wilcoxon test). # P < 0.1, * P < 0.05, ** P < 0.01 (paired *t*-test).

After ISO-sw the EEG power spectrum during the first 4 hours of NREM sleep was also increased relative to baseline in the low range SWA (Fig. 2D). In contrast to ISO-mock however, there was a decrease in the higher frequencies that spanned from 2.75 to 8 Hz, including the upper end of SWA (Fig. 2D). SWA as a whole (0.5-4.0 Hz) was increased only at sleep onset; across the first 4 hours of sleep there was a non-significant increase (+ 8.6 ± 7.7, p=0.276, paired t-test) compared to baseline (Fig. 2E). Moreover, the SWA increase from baseline was significantly smaller than the increase observed after ISO-mock (p=0.0006, paired t-test). As for ISO-mock, SWA showed a negative rebound starting 4 hours after sleep onset. By the end of the dark period SWE after ISO-sw was still lower (86.6%) than in baseline (Fig. 2F, light grey line), and lower than after ISO-mock (p=0.018, paired t-test).

When SWE was calculated including the SWA that occurred during the hour of anesthesia this was no longer the case: the SWA lost during sleep deprivation was quickly "recovered" within a few hours after the end of anesthesia (Fig. 2F, dark grey line), and SWE after ISO-sw and after ISO-mock no longer differed from each other.

We compared the relative spectra after ISO-sw and after ISO-mock during the first 3 hours after the end of anesthesia. During NREM sleep we found no differences in SWA but a decrease after ISO-sw in the 10-16 Hz frequency range, which encompasses sleep spindles (Fig. 2G). Furthermore, the relative spectra during REM sleep differed around 6 Hz and above 16 Hz (Fig. 2H), but were very similar during wake (Fig. 2I).

Desflurane experiments

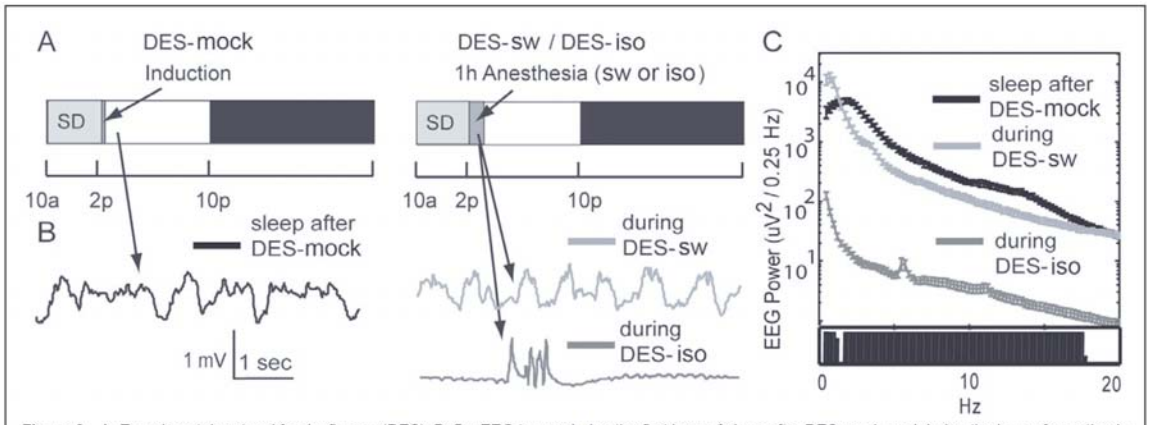


Figure 3—A, Experimental protocol for desflurane (DES). B, 5-s EEG traces during the first hour of sleep after DES-mock, and during the hour of anesthesia with DES-sw and DES-iso. During DES-iso bursts like the one shown were rare, and the EEG was isoelectric 91% of the time (mean BSR 90.9% ± 0.7, n = 8 rats). C, Mean (± SEM; n = 8 rats) absolute spectra during the first hour of sleep after DES-mock and during the hour of anesthesia with DES-sw and DES-iso. Black bars refer to DES-mock vs DES-sw (paired Wilcoxon test).

Each rat was used in 3 experiments, all preceded by an undisturbed baseline recording (Fig. 3A). DES-mock and DES-sw replicated the corresponding ISO conditions, except that a higher concentration of DES (4-12%) was used. For DES-iso rats were exposed to DES for 1 hour at a constant concentration of 12%. As expected, DES-sw had low BSR (mean ± SEM, 5.8% ± 1.1, n = 8 rats; see also Supplementary Table 1 and Supplementary Fig. 1), and the EEG power spectrum during DES-sw was dominated by large amplitude slow waves (Fig. 3B). DES-iso, on the other hand, was characterized by a predominantly flat EEG signal (mean BSR 90.9% ± 0.7, n = 8 rats; Supplementary Table 1 and Supplementary Fig. 1), rarely interrupted by burst firing (Fig. 3B). Similar to what was observed with

ISO, the absolute EEG power during DES-sw was dominated by very low frequencies (0.5-1.5 Hz), while the power in most other frequencies (1.75-18 Hz) was decreased relative to the first hours of sleep after DES-mock (Fig. 3C).

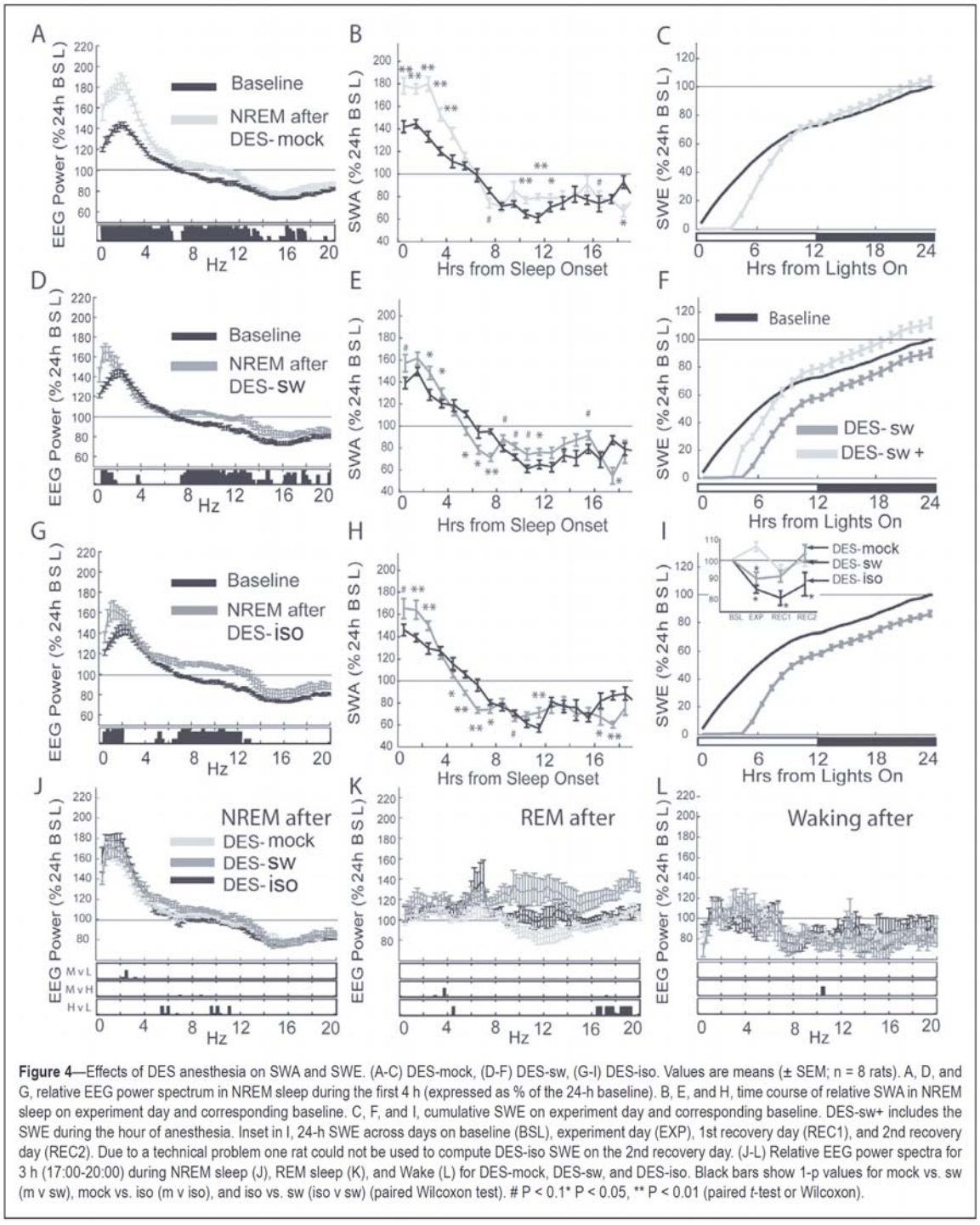


Figure 4—Effects of DES anesthesia on SWA and SWE. (A-C) DES-mock, (D-F) DES-sw, (G-I) DES-iso. Values are means (± SEM; n = 8 rats). A, D, and G, relative EEG power spectrum in NREM sleep during the first 4 h (expressed as % of the 24-h baseline). B, E, and H, time course of relative SWA in NREM sleep on experiment day and corresponding baseline. C, F, and I, cumulative SWE on experiment day and corresponding baseline. DES-sw+ includes the SWE during the hour of anesthesia. Inset in I, 24-h SWE across days on baseline (BSL), experiment day (EXP), 1st recovery day (REC1), and 2nd recovery day (REC2). Due to a technical problem one rat could not be used to compute DES-iso SWE on the 2nd recovery day. (J-L) Relative EEG power spectra for 3 h (17:00-20:00) during NREM sleep (J), REM sleep (K), and Wake (L) for DES-mock, DES-sw, and DES-iso. Black bars show 1-p values for mock vs. sw (m v sw), mock vs. iso (m v iso), and iso vs. sw (iso v sw) (paired Wilcoxon test). # $P < 0.1$* $P < 0.05$, ** $P < 0.01$ (paired *t*-test or Wilcoxon).

DES has a much lower solubility in fat than ISO, and thus leaves the tissue more quickly [28]. Consistent with a faster recovery time, the first grooming episode occurred 13 min earlier after DES-sw than after ISO-sw ($p=0.02$, Wilcoxon), while the timing was similar for DES-iso and ISO-sw ($p=0.6$, Wilcoxon; Table 1B). Sleep architecture was largely unchanged after DES-sw and DES-iso relative to DES-mock, as detailed in Table 1B.

After DES-mock the relative EEG power spectrum during the first 4 hours of NREM sleep was increased relative to baseline for most frequency bins, with the most prominent increase occurring in a cluster that spans SWA (Fig. 4A). Relative SWA indeed increased compared to baseline during the first 5 hours after sleep onset (Fig. 4B), and as with ISO-mock the SWA of the first 4 hours after sleep onset was higher than baseline ($+ 37.08 \pm 3.9$, $p<0.001$, paired t-test). The analysis of SWE showed that the SWA lost during the 4 hours of sleep deprivation was fully recovered before the end of the light period, with no further changes during the following dark period (Fig. 4C).

After DES-sw the relative EEG power spectrum during the first 4 hours of NREM sleep showed a modest increase within the low-range SWA band (0.5-1.75) and an increase above 8 Hz (Fig. 4D). SWA as a whole (0.5-4.0 Hz) was significantly increased during the 3rd, 4th and 11th hours after sleep onset, and decreased during hours 6-8 (Fig. 4E). Unlike after ISO-sw, SWA after DES-sw was higher compared to baseline during the first 4 hours after sleep onset ($+14.04 \pm 4.7$, $p=0.042$, paired t-test). Still, the SWA increase was significantly lower after DES-sw as compared to DES-mock ($p=0.0399$, paired t-test). By the end of the dark period SWE after DES-sw was still lower (91%) than in baseline (Fig. 4F, light grey line). As was the case for ISO however, when the SWA that occurred during anesthesia was included SWE surpassed baseline levels a few hours after the end of anesthesia (Fig. 4F, dark grey line). By the end of the day SWE after DES-mock and after DES-sw no longer differed from each other.

After DES-iso the relative EEG spectrum during the first 4 hours of NREM sleep showed a modest increase within the low-range SWA band (0.5-2 Hz), and an increase in the 4.5-13 Hz range (Fig. 4G). SWA as a whole (0.5-4.0 Hz) increased during the first 3 hours of sleep, and decreased after the 4th hour (Fig. 4H); when calculated across the first 4 hours after sleep onset, SWA after DES-iso increased compared to baseline ($+15.67 \pm 3.3$, $p=0.044$, paired t-test), but this SWA rebound was significantly lower than after DES-mock ($p=0.0465$, paired t-test) and not different from DES-sw. SWE analysis showed that the SWA lost during the 4 hours of sleep deprivation was not

recovered by the end of the following dark period (Fig. 4I), when SWE was still 86% of baseline. SWE calculated separately for each of the following 2 recovery days remained lower than baseline (Fig. 4I, inset). SWE after DES-iso also remained significantly lower than after DES-mock on both the first and second recovery day (Rec 1: p=0.0391, Rec 2: p=0.0313, paired-Wilcoxon), while SWE after DES-sw did not differ from SWE after DES-mock on either day. Finally, we compared the relative spectra after all 3 conditions during the first 3 hours after the end of anesthesia. Relative EEG spectra in all behavioral states were similar, except for a difference between DES-iso and DES-sw above 16 Hz in REM sleep (Fig. 4 J-L).

Discussion

SWA is an established marker of sleep pressure that is high at sleep onset and low after sleep. A seminal study demonstrated that the occurrence of slow waves during NREM sleep is necessary to reduce sleep need [13]. Specifically, a deprivation of slow waves during the first part of the night resulted in a subsequent increase in SWA, demonstrating that the occurrence of slow waves, not simply the time spent asleep, decreases SWA. Thus, we reasoned that if anesthesia slow waves count as sleep slow waves, then anesthesia slow waves should cause a homeostatic decrease in SWA. In agreement with this hypothesis we found that one hour of ISO or DES anesthesia associated with the occurrence of large, almost continuous slow waves reduced the SWA rebound that was expected following 4 hours of sleep deprivation. Moreover, when anesthesia slow waves were included in calculating cumulative SWA (SWE), the later returned to baseline levels within a few hours after the end of anesthesia. Thus, anesthesia slow waves may substitute for sleep slow waves.

These findings are consistent with previous studies in rats that showed that 12 hours of propofol anesthesia during the normal sleep period do not increase subsequent sleep pressure [29], and that 6 hours of either propofol anesthesia or undisturbed sleep allow for a similar recovery from 24 hours of sleep deprivation[17]. Our ISO-sw and DES-sw conditions are likely comparable to the propofol condition in these reports (described as producing continuous high-amplitude tracing), and propofol, ISO and DES have similar mechanisms of action, in that they are primarily GABA(A) agonists (reviewed in [30]). In contrast to the propofol studies, however, we could measure the effects of anesthesia on the lowest EEG frequencies, and found that the EEG power spectrum during ISO-sw and DES-sw was skewed towards frequencies below 1.5 Hz, which correspond to the slow oscillation. The link between

slow oscillation and SWA is complex. The former is the cellular phenomenon that underlies the occurrence of NREM slow waves, and thus SWA. Yet, several reports [31-34] have suggested a possible dissociation between the homeostatic behavior of frequencies below 1 Hz and the remaining SWA frequencies. More recent studies in rodents and humans, however, as well as computer simulations, show that the apparent dissociation may simply reflect homeostatic changes in the slope of slow waves [35, 36]. Specifically, the decrease of homeostatic sleep pressure in the course of sleep is associated not only with an overall decrease in SWA, but also with a redistribution of the EEG power spectrum towards lower frequencies, due to a decrease in the slope of slow waves [35]. In this respect, the EEG during anesthesia is more similar to the EEG during late sleep, when sleep pressure is low, than to the EEG during early sleep, when sleep pressure peaks. This observation is consistent with the notion that anesthesia can in itself discharge previous sleep pressure due to sleep deprivation.

One hour of anesthesia with high concentration of DES, associated with isoelectric EEG and few slow waves, also reduced the SWA rebound following 4 hours of sleep deprivation. This finding was unexpected, since the results with ISO-sw and DES-sw, as well as a previous study [13], were consistent with the idea that slow waves are necessary to discharge sleep pressure. One possible explanation is that the blunted "SWA rebound" after DES-iso reflects not a physiological decrease in sleep need, but rather a pathological condition that may last long after the end of anesthesia. Consistent with this interpretation, the SWA lost during sleep deprivation and DES-iso was not recovered even 2 days after the end of anesthesia, when SWE was still ~ 85% of baseline. It is possible that the exposure to 12% DES in the DES-iso condition may have caused hypotension, acidemia, and possibly ischemia, and that had these effects been prevented, the chronic decrease in SWE would not have occurred. Of note, however, there were no obvious abnormalities in the EEG signal after DES-iso, and the chronic decrease in the EEG power spectrum was specific for SWA. Another possibility, therefore, is that what counts to discharge sleep pressure is not the presence of slow waves per se, but some other cellular phenomena that normally are associated with them. These phenomena may include the repeated alternation between up and down states, as during the slow oscillation, and/or the occurrence of relatively long periods of neuronal silence (as in the down states of the slow oscillation). Interestingly, recent intracellular recordings were performed in cats anesthetized with high doses of ISO, which produced a burst-suppression pattern (Fig. 3B). As expected, it was found that during the suppression phase with isoelectric EEG neurons were chronically hyperpolarized and silent. Surprisingly, however, it was also found that

during the burst phases neurons were not tonically depolarized, but oscillated between up and down states as during normal sleep or during low doses of anesthesia [37]. Thus, the DES-iso condition that we used in this study, in which bursts were present although rare, shares at least some of the features of the DES-sw condition. It remains to be explained, however, why the reduced sleep need presumably caused by DES-iso continues for at least 2 days.

Whether NREM sleep and anesthesia have similar cellular consequences is unclear. Few transcriptomic and proteomic studies have examined changes in brain gene expression after anesthesia, and direct comparisons with similar studies that compared sleep and wake states are difficult, due to major differences in experimental design and statistical analysis. On one hand, sleep seems to be associated with increased expression of genes related to synaptic depression, protein synthesis, cholesterol synthesis, and membrane trafficking [38]. On the other hand, one report found hardly any changes at the mRNA or protein level following clinically relevant and repeated doses of ISO [39, 40], while other studies found significant changes in gene expression long after the end of anesthesia [41, 42], and yet another study found changes in membrane proteins, but only 3-72 hours after anesthesia [43].

The mechanisms of action of volatile anesthetics remain poorly understood, and most likely include pre- and postsynaptic effects at both subcortical and cortical level. The slow oscillation of NREM sleep occurs in most thalamocortical neurons, and intracellular recordings have shown that during the down state of this oscillation cortical neurons are disfacilitated, rather than inhibited; in other words, they stop firing due to the absence of excitatory synaptic activity, and not because of the activation of GABA(A) inhibitory currents [44]. Disfacilitation, and not direct inhibition, also occurs under anesthesia with ketamine-xylazine or urethane [45], but unfortunately volatile anesthetics have not been studied. Interestingly, however, a recent study found that the cerebral cortex is a major target of volatile anesthetics, and that these agents decrease cortical firing by enhancing neocortical GABA(A) receptor-mediated inhibition [46]. Assuming that this is the predominant mechanism by which ISO and DES affect cortical activity, it would follow that ISO and DES slow waves and sleep slow waves are caused by different cellular mechanisms, involving inhibition for the former and disfacilitation for the latter.

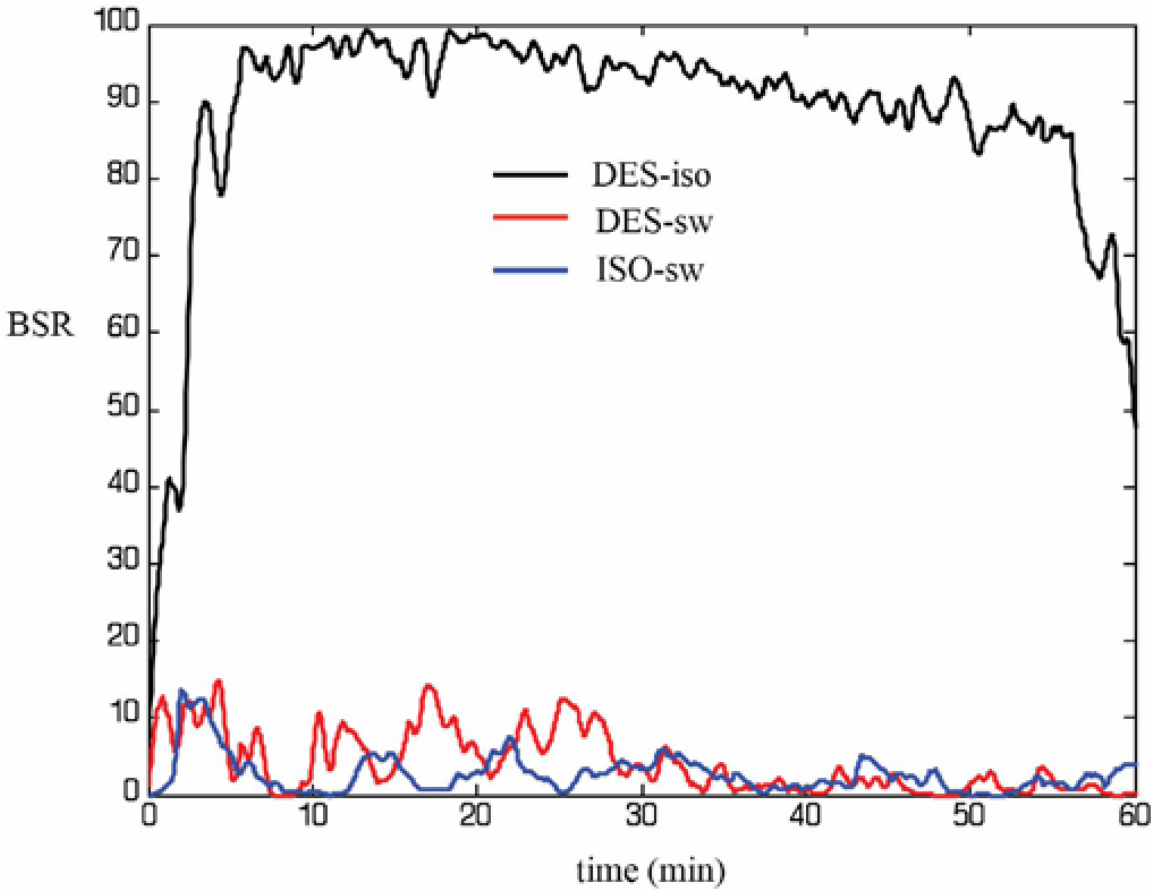
Although its functions remain elusive, there is compelling evidence that sleep benefits the brain and improves vigilance, attention, learning, and memory. The underlying mechanisms are unclear, and may involve overall synaptic renormalization in most brain regions [47] and/or synaptic strengthening of restricted neural circuits [48]. Our results based on SWA analysis suggest that, as far as the homeostatic regulation of sleep is concerned,

anesthesia may "count" as NREM sleep. One recent study in mice also suggested that anesthesia may counterbalance some of the negative effects of sleep loss on performance and synaptic plasticity. Specifically, it was found that a day after 2 hours of ISO anesthesia (presumably with slow waves) mice showed improved visuospatial learning and enhanced hippocampal LTP, and both effects were mediated by the increased hippocampal expression of the NR2B subunit of NMDA receptors [49]. Of note, sleep restriction and total sleep deprivation impair spatial learning and the induction of hippocampal LTP, and one of the underlying mechanisms seems to be a decrease in NMDA currents, associated with an increase in the NR2A/NR2B ratio [50-54]. These results, however, do not mean that anesthesia should be considered as an option to counteract the effects of sleep deprivation, since most available evidence suggests that it cannot provide the restorative functions of sleep. For instance, a study in rats found that 2 hours of anesthesia (ISO / NO mixture) impairs the acquisition of a spatial memory task for several weeks [55]. Most importantly, post-operative cognitive dysfunction is a well described decline in cognitive performance observed at all ages after a surgery performed under anesthesia (reviewed in [56, 57]), and growing evidence suggests that anesthesia may contribute to the development of Alzheimer's disease [58].

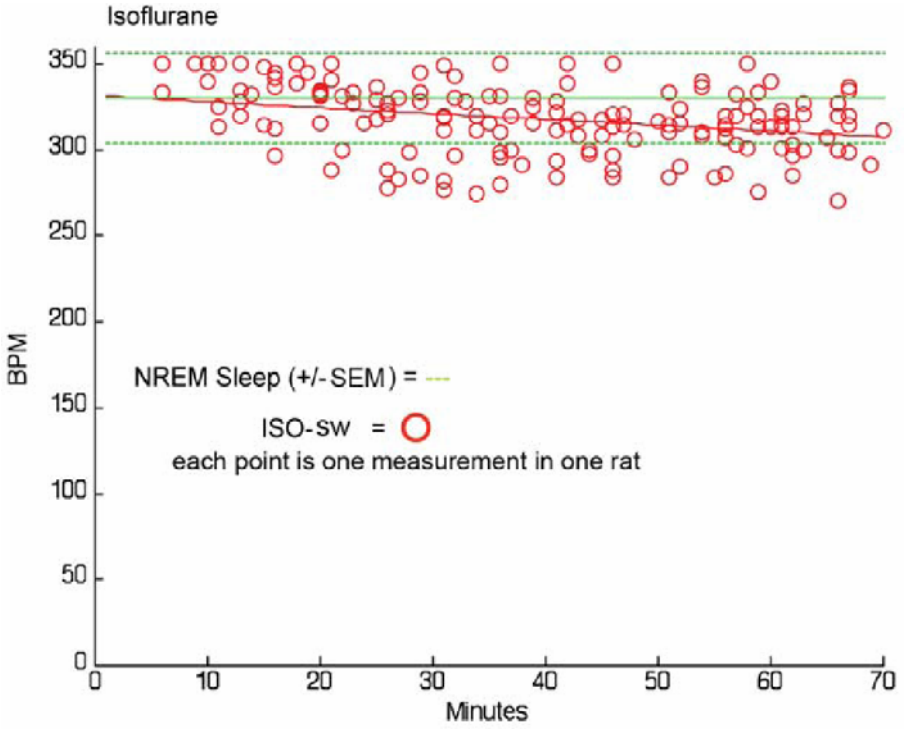
In summary, we find that two different doses of volatile anesthetics can blunt the SWA response to sleep deprivation. Slow waves were present only with the lower doses, suggesting that their presence may not be necessary to decrease the sleep pressure accumulated during sleep deprivation. The repeated occurrence of slow oscillations in corticothalamic neurons, and/or multiple, sustained periods of hyperpolarization and neuronal silence in these cells may be required to decrease sleep pressure. Unfortunately, these two possibilities are difficult to distinguish, because in our experience it is not possible to maintain isoelectric EEG without any burst activity for an entire hour.

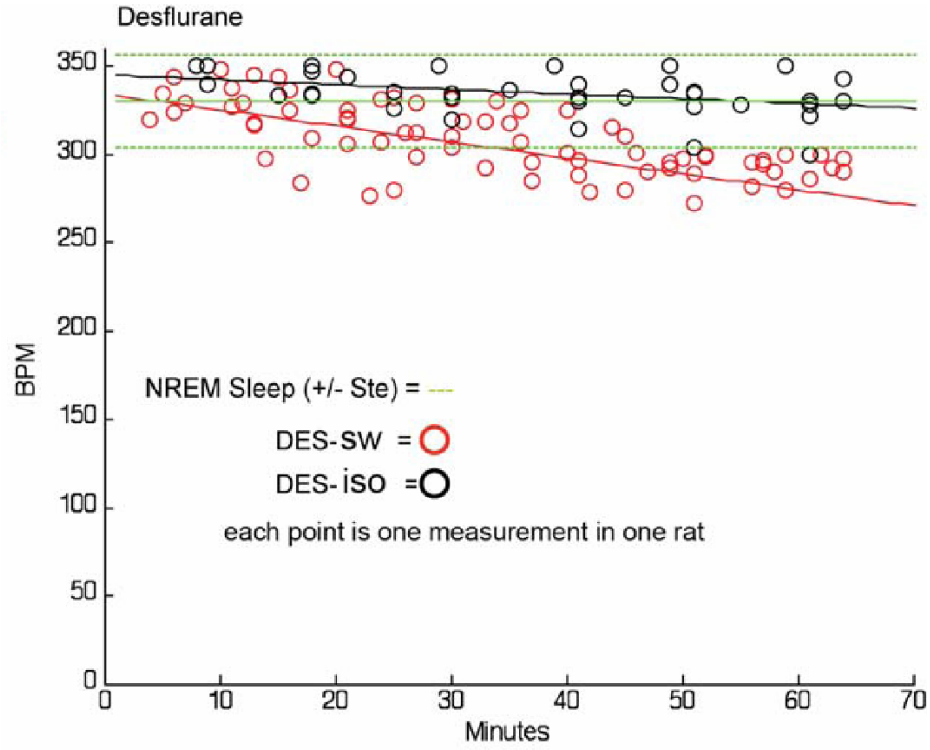
This study has several limitations. We cannot rule out that anesthesia, especially at high doses, affected cardiovascular and respiratory functions. However, heart rate and oxygen saturation were monitored and remained within normal levels for all rats. We used only one hour of anesthesia, following 4 hours of sleep deprivation in order to produce a reliable sleep rebound and a detectable anesthetic effect while still allowing recovery during the light period, when rats normally sleep. Longer periods of anesthesia may produce different results. Finally, sleep pressure was measured using SWA; functional studies that examine the recovery of attention and performance after sleep loss followed by anesthesia should also be performed.

Supplementary Figure 1. Time course of the mean burst suppression ratio (BSR) for all experimental conditions.



Supplementary Figure 2. Heart rate (beats per min, BPM) during the hour of anesthesia. NREM BPM from Meunier et al.[23]





Supplementary Table 1. Burst suppression ratio (BSR), i.e. the % of time spent in isoelectric EEG for all experimental conditions.

ISOFLURANE BSR (per rat)	ISO-Mock	ISO-sw	DESFLURANE BSR (per rat)	DES-Mock	DES-sw	DES-iso
1	0	0.82	1	0.002	2.32	89.77
2	0.002	0.65	2	0.003	6.54	88.97
3	0.003	2.55	3	0.002	1.67	88.98
4	0.002	4.92	4	0.002	5.86	92.99
5	0.001	9.31	5	0	2.50	90.83
6	0	1.25	6	0.002	9.35	91.18
7	0.0005	7.24	7	0	8.76	94.54
8	0	0.19	8	0	9.00	93.03
9	0	9.15				
10	0	4.62				
11	0	0.38				
12	0.0008	0.84				
13	0.02	2.54				
14	0.001	3.93				
Mean (SEM)	**0**	**3.46 (± 0.86)**	**Mean (SEM)**	**0**	**5.75 (± 1.14)**	**90.92 (± 0.73)**

References

1. Jones SG, Vyazovskiy VV, Cirelli C, Tononi G, Benca RM. Homeostatic regulation of sleep in the white-crowned sparrow (Zonotrichia leucophrys gambelii). BMC Neurosci 2008;9:47.

2. Martinez-Gonzalez D, Lesku JA, Rattenborg NC. Increased EEG spectral power density during sleep following short-term sleep deprivation in pigeons (Columba livia): evidence for avian sleep homeostasis. J Sleep Res 2008;17:140-53.

3. Tobler I. Phylogeny of sleep regulation. In: MH K, T R, WC D, eds. Principles and Practice of Sleep Medicine. . Philadelphia W. B. Saunders, 2005.

4. Tobler I, Borbely AA. Sleep EEG in the rat as a function of prior waking. Electroencephalogr Clin Neurophysiol 1986;64:74-6.

5. Werth E, Dijk DJ, Achermann P, Borbely AA. Dynamics of the sleep EEG after an early evening nap: experimental data and simulations. Am J Physiol 1996;271:R501-10.

6. Steriade M, Nunez A, Amzica F. Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci 1993;13:3266-83.

7. Steriade M, Timofeev I, Grenier F. Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol 2001;85:1969-85.

8. Marshall L, Helgadottir H, Molle M, Born J. Boosting slow oscillations during sleep potentiates memory. Nature 2006;444:610-3.

9. Walsh JK, Randazzo AC, Stone K, et al. Tiagabine is associated with sustained attention during sleep restriction: evidence for the value of slow-wave sleep enhancement? Sleep 2006;29:433-43.

10. Walsh JK, Krystal AD, Amato DA, et al. Nightly treatment of primary insomnia with eszopiclone for six months: effect on sleep, quality of life, and work limitations. Sleep 2007;30:959-68.

11. Aeschbach D, Cutler AJ, Ronda JM. A role for non-rapid-eye-movement sleep homeostasis in perceptual learning. J Neurosci 2008;28:2766-72.

12. Landsness EC, Crupi D, Hulse BK, et al. Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. Sleep 2009;32:1273-84.

13. Dijk DJ, Beersma DG, Daan S, Bloem GM, Van den Hoofdakker RH. Quantitative analysis of the effects of slow wave sleep deprivation during the first 3 h of sleep on subsequent EEG power density. Eur Arch Psychiatry Neurol Sci 1987;236:323-8.

14. Steriade M, Nunez A, Amzica F. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J Neurosci 1993;13:3252-65.

15. Sloan TB. Anesthetic effects on electrophysiologic recordings. J Clin Neurophysiol 1998;15:217-26.

16. Moote CA, Knill RL. Isoflurane anesthesia causes a transient alteration in nocturnal sleep. Anesthesiology 1988;69:327-31.

17. Tung A, Bergmann BM, Herrera S, Cao D, Mendelson WB. Recovery from sleep deprivation occurs during propofol anesthesia. Anesthesiology 2004;100:1419-26.

18. Tobler I, Borbely AA. The effect of 3-h and 6-h sleep deprivation on sleep and EEG spectra of the rat. Behav Brain Res 1990;36:73-8.

19. Tobler I, Deboer T, Fischer M. Sleep and sleep regulation in normal and prion protein-deficient mice. J Neurosci 1997;17:1869-79.

20. Huber R, Deboer T, Tobler I. Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. Brain Res 2000;857:8-19.

21. Franken P, Dijk DJ, Tobler I, Borbely AA. Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature. Am J Physiol 1991;261:R198-208.

22. Mashour GA, Lipinski WJ, Matlen LB, et al. Isoflurane anesthesia does not satisfy the homeostatic need for rapid eye movement sleep. Anesth Analg 2010;110:1283-9.

23. Meunier JM, Nosjean A, Lacombe J, Laguzzi R. Cardiovascular changes during the sleep-wake cycle in spontaneous hypertensive rats and their genetically normotensive precursors. Pflugers Arch 1988;411:195-9.

24. Vijn PC, Sneyd JR. I.v. anaesthesia and EEG burst suppression in rats: bolus injections and closed-loop infusions. Br J Anaesth 1998;81:415-21.

25. Rampil IJ, Laster MJ. No correlation between quantitative electroencephalographic measurements and movement response to noxious stimuli during isoflurane anesthesia in rats. Anesthesiology 1992;77:920-5.

26. Luo T, Leung LS. Basal forebrain histaminergic transmission modulates electroencephalographic activity and emergence from isoflurane anesthesia. Anesthesiology 2009;111:725-33.

27. Franken P, Tobler I, Borbely AA. Sleep homeostasis in the rat: simulation of the time course of EEG slow-wave activity. Neurosci Lett 1991;130:141-4.

28. Yasuda N, Lockhart SH, Eger EI, 2nd, et al. Kinetics of desflurane, isoflurane, and halothane in humans. Anesthesiology 1991;74:489-98.

29. Tung A, Lynch JP, Mendelson WB. Prolonged sedation with propofol in the rat does not result in sleep deprivation. Anesth Analg 2001;92:1232-6.

30. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994;367:607-14.

31. Achermann P, Borbely AA. Low-frequency (< 1 Hz) oscillations in the human sleep electroencephalogram. Neuroscience 1997;81:213-22.

32. Achermann P, Borbely AA. Temporal evolution of coherence and power in the human sleep electroencephalogram. J Sleep Res 1998;7 Suppl 1:36-41.

33. Campbell IG, Higgins LM, Darchia N, Feinberg I. Homeostatic behavior of fast Fourier transform power in very low frequency non-rapid eye movement human electroencephalogram. Neuroscience 2006;140:1395-9.

34. Dijk DJ, Beersma DG, Daan S. EEG power density during nap sleep: reflection of an hourglass measuring the duration of prior wakefulness. J Biol Rhythms 1987;2:207-19.

35. Vyazovskiy VV, Riedner BA, Cirelli C, Tononi G. Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. Sleep 2007;30:1631-42.

36. Bersagliere A, Achermann P. Slow oscillations in human non-rapid eye movement sleep electroencephalogram: effects of increased sleep pressure. J Sleep Res 2009.

37. Kroeger D, Amzica F. Hypersensitivity of the anesthesia-induced comatose brain. J Neurosci 2007;27:10597-607.

38. Cirelli C. The genetic and molecular regulation of sleep: from fruit flies to humans. Nat Rev Neurosci 2009;10:549-60.

39. Pan JZ, Wei H, Hecker JG, Tobias JW, Eckenhoff RG, Eckenhoff MF. Rat brain DNA transcript profile of halothane and isoflurane exposure. Pharmacogenet Genomics 2006;16:171-82.

40. Pan JZ, Xi J, Eckenhoff MF, Eckenhoff RG. Inhaled anesthetics elicit region-specific changes in protein expression in mammalian brain. Proteomics 2008;8:2983-92.

41. Culley DJ, Yukhananov RY, Xie Z, Gali RR, Tanzi RE, Crosby G. Altered hippocampal gene expression 2 days after general anesthesia in rats. Eur J Pharmacol 2006;549:71-8.

42. Rampil IJ, Moller DH, Bell AH. Isoflurane modulates genomic expression in rat amygdala. Anesth Analg 2006;102:1431-8.

43. Futterer CD, Maurer MH, Schmitt A, Feldmann RE, Jr., Kuschinsky W, Waschke KF. Alterations in rat brain proteins after desflurane anesthesia. Anesthesiology 2004;100:302-8.

44. Timofeev I, Grenier F, Steriade M. Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. Proc Natl Acad Sci U S A 2001;98:1924-9.

45. Contreras D, Steriade M. Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. J Physiol 1996;490 (Pt 1):159-79.

46. Hentschke H, Schwarz C, Antkowiak B. Neocortex is the major target of sedative concentrations of volatile anaesthetics: strong depression of firing rates and increase of GABAA receptor-mediated inhibition. Eur J Neurosci 2005;21:93-102.

47. Tononi G, Cirelli C. Sleep function and synaptic homeostasis. Sleep Med Rev 2006;10:49-62.

48. Diekelmann S, Wilhelm I, Born J. The whats and whens of sleep-dependent memory consolidation. Sleep Med Rev 2009;13:309-21.

49. Rammes G, Starker LK, Haseneder R, et al. Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B subunit expression. Neuropharmacology 2009;56:626-36.

50. McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci 2003;23:9687-95.

51. Chen C, Hardy M, Zhang J, LaHoste GJ, Bazan NG. Altered NMDA receptor trafficking contributes to sleep deprivation-induced hippocampal synaptic and cognitive impairments. Biochem Biophys Res Commun 2006;340:435-40.

52. Kopp C, Longordo F, Nicholson JR, Luthi A. Insufficient sleep reversibly alters bidirectional synaptic plasticity and NMDA receptor function. J Neurosci 2006;26:12456-65.

53. Tartar JL, Ward CP, McKenna JT, et al. Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. Eur J Neurosci 2006;23:2739-48.

54. Longordo F, Kopp C, Mishina M, Lujan R, Luthi A. NR2A at CA1 synapses is obligatory for the susceptibility of hippocampal plasticity to sleep loss. J Neurosci 2009;29:9026-41.

55. Culley DJ, Baxter MG, Yukhananov R, Crosby G. Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. Anesthesiology 2004;100:309-14.

56. Newman S, Stygall J, Hirani S, Shaefi S, Maze M. Postoperative cognitive dysfunction after noncardiac surgery: a systematic review. Anesthesiology 2007;106:572-90.

57. Monk TG, Weldon BC, Garvan CW, et al. Predictors of cognitive dysfunction after major noncardiac surgery. Anesthesiology 2008;108:18-30.

58. Baranov D, Bickler PE, Crosby GJ, et al. Consensus statement: First International Workshop on Anesthetics and Alzheimer's disease. Anesth Analg 2009;108:1627-30.

Chapter III:

Sleep Patterns and Homeostatic Mechanisms in Adolescent Mice

Aaron B. Nelson, Ugo Faraguna, Jeffrey T. Zoltan, Giulio Tononi, and Chiara Cirelli

Accepted in:
Brain Sciences, 3, 2013

Received: 30 December 2012; in revised form: 30 January 2013 / Accepted: 27February 2013 / Published

Abstract: Sleep changes were studied in mice (n = 59) from early adolescence to adulthood (postnatal days P19–111). REM sleep declined steeply in early adolescence, while total sleep remained constant and NREM sleep increased slightly. Four hours of sleep deprivation starting at light onset were performed from ages P26 through adulthood (>P60). Following this acute sleep deprivation all mice slept longer and with more consolidated sleep bouts, while NREM slow wave activity (SWA) showed high interindividual variability in the younger groups, and increased consistently only after P42. Three parameters together explained up to 67% of the variance in SWA rebound in frontal cortex, including weight-adjusted age and increase in alpha power during sleep deprivation, both of which positively correlated with the SWA response. The third, and strongest predictor was the SWA decline during the light phase in baseline: mice with high peak SWA at light onset, resulting in a large SWA decline, were more likely to show no SWA rebound after sleep deprivation, a result that was also confirmed in parietal cortex. During baseline, however, SWA showed the same homeostatic changes in adolescents and adults, declining in the course of sleep and increasing across periods of spontaneous wake. Thus, we hypothesize that, in young adolescent mice, a ceiling effect and not the immaturity of the cellular mechanisms underlying sleep homeostasis may prevent the SWA rebound when wake is extended beyond its physiological duration.

Keywords: adolescence; cerebral cortex; sleep deprivation; slow wave activity

1. Introduction

As in humans, adolescence in rodents is a transitional time during which the physical and behavioral traits of adulthood develop [1,2]. In mice, adolescence spans the period from weaning (~P21) to sexual maturity (P50–60), and can be further subdivided into a "periadolescence" period (P37–48) around the onset of puberty (at 5–7 weeks of age in most mouse strains), preceded by early adolescence (from weaning to P36), and followed by late adolescence (young adults, P49–60) [3]. This age classification, and the overall strength of rodent models including mice for the purpose of comparison or extrapolation to human development, have been validated extensively [1–5].

At time of weaning, most of the growth in the rodent cerebral cortex has been completed. For instance, in the rat mature cortical lamination is reached by P8 [6], myelination starts and most blood vessels grow rapidly and become patent after P10, when explosive synaptic growth occurs [7,8], and by P20 extracellular space has decreased significantly [9,10]. Consistent with major maturational changes occurring during the second week, the total power in the EEG signal in all behavioral states increases after P9 [11], and cortical activity increases sharply after P11 [12]. By the time adolescence starts, at the end of the third week, the EEG signals during NREM sleep, REM sleep and wake are similar to from those in adults [11,13].

While structural and electrophysiological aspects of cortical maturation are reasonably well understood, a complete quantitative description of sleep patterns during the entire adolescence period is missing in rodents. Previous studies recorded sleep at a few select times during adolescence, and in some cases only during part of the light phase [11,13–18]. Even less characterized is how sleep homeostatic mechanisms develop and change during adolescence. Four studies have been conducted in rats sleep deprived for 2–6 h during adolescence [17,19–21]. Consistently, these experiments found evidence of sleep homeostasis well before weaning (as early as P12), as indexed by changes in sleep amount (e.g., increased duration of NREM sleep) and in sleep consolidation (e.g., decreased number of brief arousals) [17,19]. The same studies, however, found that only some time between the third and the fourth week of age NREM slow wave activity, the EEG power in the 0.5–4.5 Hz range during NREM sleep, becomes a reliable marker of sleep homeostasis as described in adults, increasing with time spent awake and declining with time spent asleep. The reasons for this delay remain unclear. Here we performed in mice a

comprehensive study of the changes in sleep quantity and quality, as well as in the response to sleep deprivation, from early adolescence to adulthood.

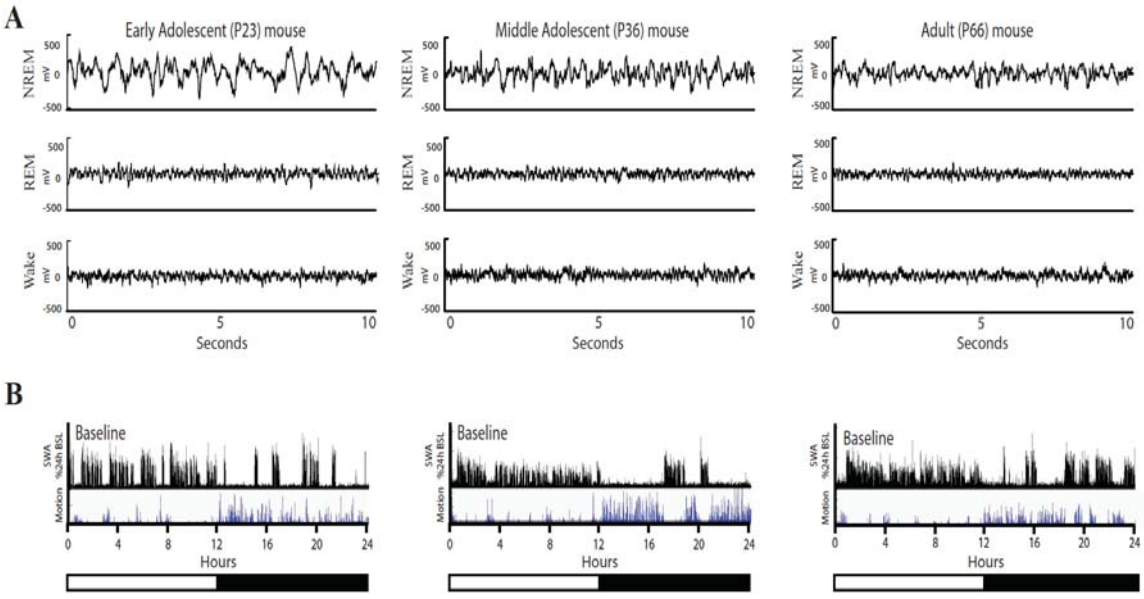
2. Results

2.1. Sleep in Baseline

2.1.1. Sleep-Wake Patterns during Baseline

Fifty-nine male C57/BL-6, yellow fluorescent protein (YFP)-H expressing mice, ages ranging from P15 to P87, were implanted and continuously recorded for 1–3 weeks. Raw EEG traces looked similar in adolescent and adult mice across all behavioral states, except for the greater amplitude of the EEG signals in the younger mice (Figure 1).

Figure 1. Representative examples of EEG traces in adolescent and adult mice. (**A**) EEG traces (10 s) for NREM sleep, REM sleep, and wake in an early adolescent (P23), middle adolescent (P36) and adult (P66) mouse are shown. (**B**) The bottom panels show the time course of NREM slow wave activity (SWA, expressed as % of 24-h mean SWA, 4-s epochs) and locomotor activity (video-based motion detection, 4-s epochs) for the same animals during 24 h of baseline. Motion is measured in arbitrary units and values cannot be compared across animals.



In the following figures, all sleep and wake parameters are shown for each individual mouse to show interindividual differences, as well as averaged across age groups spanning 3–7 days (except the adult cluster) and "centered" around the day of the sleep deprivation experiment (P<25 n = 8, P26–29 n = 14, P34–36 n = 8, P41–44 n = 8, P50–56 n = 9, P>60 n = 10, no group n = 2). The age groups were chosen to span the entire adolescent period as comprehensively as possible, but with an emphasis placed on early and middle (peri)adolescence until around puberty (P26–44). All mice older than P25 underwent an acute 4-h sleep deprivation by exposure to novel objects beginning at lights-on, and then were left undisturbed for the next 20 h. The day of the sleep deprivation and the preceding baseline day were used for the statistical analysis to characterize maturational changes in sleep homeostasis. Additional days (n = 47) that did not qualify as "baseline" (*i.e.*, they occurred >1 day before sleep deprivation, or at least 3 days afterwards) were scored for a subset of mice. These days are shown in some of the figures (indicated by X) for reference, but were not included in the statistical analysis. In total, sleep staging was performed for 106 baseline days spanning the ages from P19 to P111. To avoid an unnecessarily stressful extended deprivation, mice under P25 underwent a shorter (2 h) sleep deprivation. Individual data points from these mice are shown in the figures but were not included in the ANOVA groups or in the regression analysis; this is why there are six groups for ANOVAs concerning changes in baseline sleep across adolescence, but only five groups for the analysis of the response to 4 h of sleep deprivation. The EEG signal became unusable following the sleep deprivation for three mice under P25 and 1 mouse in the P26–29 age group. Only baseline sleep data for these mice are included (Figure 2, Figure 3 top). Due to poor quality of the signal, only eight mice contributed data from the frontal derivation (P<25 n = 2, P26–29 n = 1, P34–36 n = 2, P41–44 n = 1, P>60 n = 1), and one mouse (P50–56) is included with data only from the parietal derivation.

Figure 2. Daily sleep and wake amounts (min/24 h) during baseline for each mouse. In all figures, symbols show values for each mouse, with P and R corresponding to the regression line computed from all animals except those denoted by a faint "X". Insets depict group means ± SEM, with bars indicating significant differences between groups, and asterisks indicating one group different from all others ($p < 0.05$, Tukey's HSD). Age groups in all figures: 1 ≤ P25; 2 = P26–29; 3 = P34–36; 4 = P41–44; 5 = P50–56; 6 ≥ P60. Age ranges correspond to those chosen for the acute sleep deprivation experiment. The bold "**X**" are baseline days, *i.e.*, the days just before the sleep deprivation day, and are included in the regression analysis. The faint X denotes additional scored days from the same mice that did not qualify as "baseline" (*i.e.*, they occurred >1 day before sleep deprivation, or at least 3 days afterwards). The *x* days are included for reference only but were not used for the ANOVA. In this figure, NREM sleep is fit to age with a linear relationship, $f = y_0 + a \times x$, (f = min NREM, y_0 = 429.98, a = 1.08, *x* = Postnatal day) while REM sleep is fit to age with an exponential decay, $f = y_0 + a \times \exp(-b \times x)$, (f = min REM, y_0 = 98.80, a = 2012.99, b = 0.17, *x* = Postnatal day).

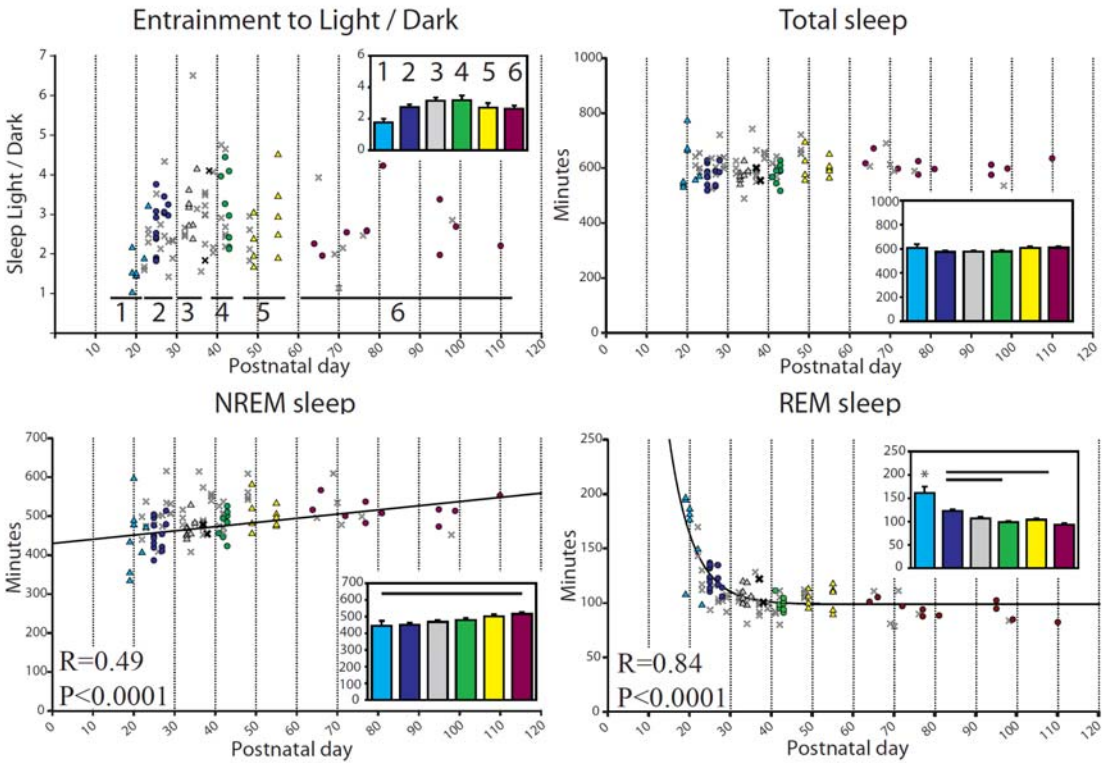
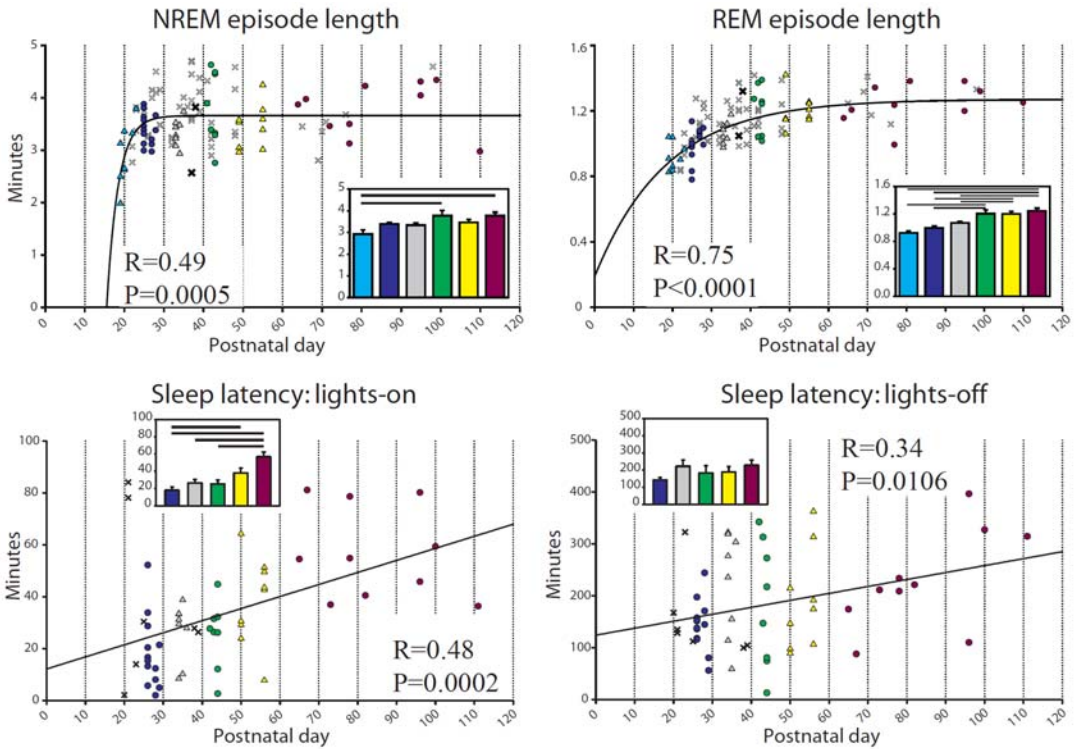


Figure 3. Episode duration and sleep latency during baseline. For episode length, *p* and *R* are from the regression for an exponential equation increasing to a maximum, $f = y_0 + a \times [1 - \exp(-b \times x)]$ (top left: f = min, $y_0 = -407.10$, a = 410.67, b = 0.32, *x* = Postnatal day), (top right: f = min, $y_0 = 0.19$, a = 1.08, b = 0.054, *x* = Postnatal day); for sleep latency, *p* and *R* are from the linear regression ($f = y_0 + a \times x$) (bottom left: f = min, $y_0 = 12.12$, a = 0.47, *x*= Postnatal day; bottom right: f = min, $y_0 = 120.45$, a = 1.41, *x* = Postnatal day).



Mice were entrained to the light/dark cycle early in adolescence, with all age groups showing an average ~2:1 ratio of sleep during the light period relative to the dark period (Figure 2, top left). There was, however, a significant interindividual variability in this ratio, from 1:1 up to 6:1. The total amount of sleep during 24 h did not change from early adolescence to adulthood (599.9 ±4.76 min, mean ± SEM), while NREM sleep increased slightly across adolescence, showing a weak linear relationship with age that resulted in the youngest mice having significantly less NREM than the adults (Figure 2). Meanwhile, REM sleep decreased steeply during early adolescence, a pattern that was fit well by an exponential decay function ($p < 0.0001$, Figure 2). The amount of REM sleep differed across

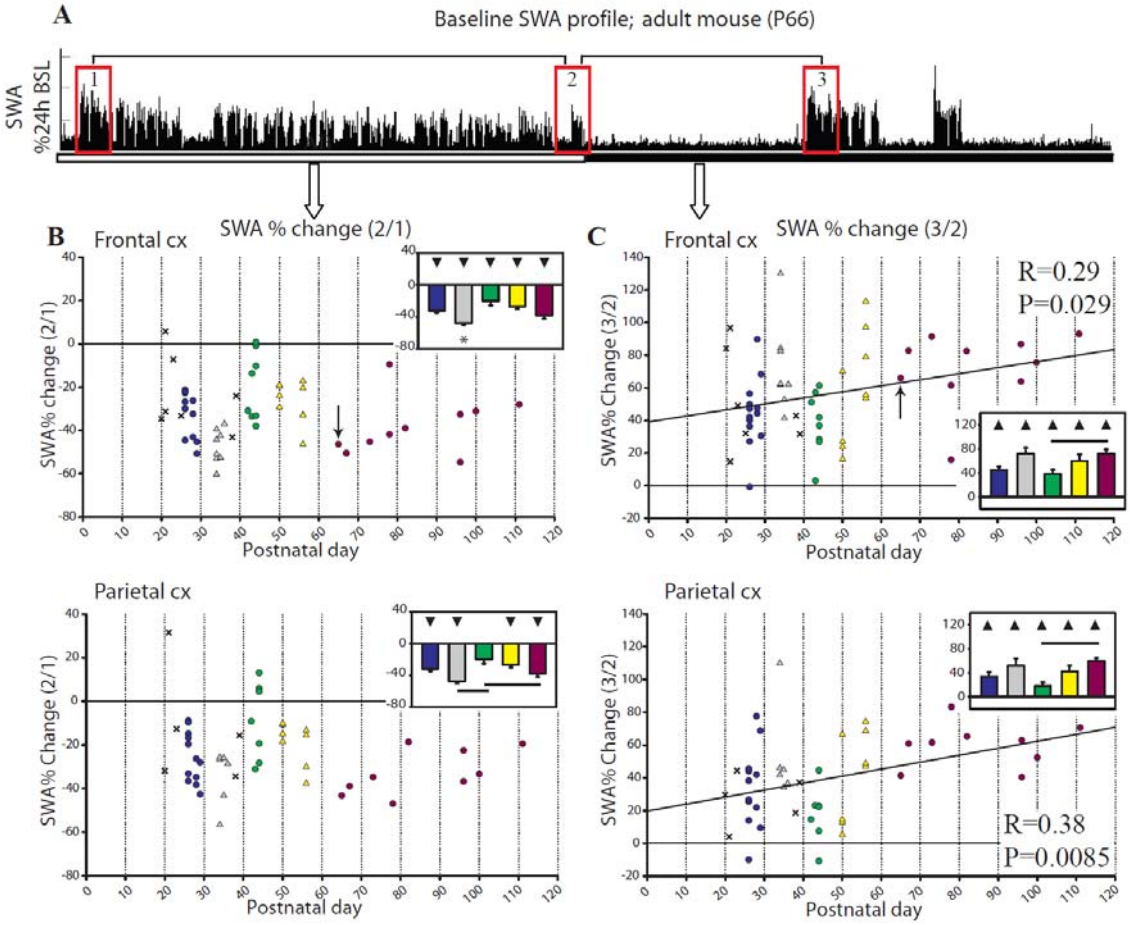
groups, with the P<25 mice having significantly more REM sleep than all other groups, and the P26–29 mice having more REM sleep than the adult and P41–44 groups (Figure 2).

The length of NREM and REM sleep episodes increased steeply in the youngest animals before leveling off, a pattern fit by an exponential function increasing to a maximum (Figure 3). Group comparisons showed that the adult level of NREM episode length was reached quickly, with only the <P25 group having shorter bouts than the adults, while adult-like levels for REM episode length were first reached in the P41–44 group (Figure 3). Sleep latency, defined as the time in minutes between lights-on or lights-off and the first episode of consolidated sleep (>1 min), showed a progressive increase with age (Figure 3).

2.1.2. Changes in EEG Power Spectrum during Baseline

In adult mice NREM SWA is a reliable marker of sleep homeostasis: it declines during sleep and increases with time spent awake, although "intensity" of wake and genotype also affect SWA (e.g., [22–24]). To determine whether the SWA time course described in adults is also present during adolescence we first quantified the baseline decrease in SWA during the day, the major sleep phase in mice. Specifically, we measured the ratio of NREM SWA in the last hour relative to the first hour of the light period (ratio 2/1 in Figure 4A). In the frontal derivation all groups showed a similar and significant SWA decline during the day (ratio 2/1), with P34–36 mice having a significantly larger decrease than all other groups (Figure 4B, top). Similar changes were present in the parietal derivation, where all groups had a significant decrease except for the P41–44 group, which had a smaller decline than the adult and P34–36 groups (Figure 4B, bottom).

Figure 4. Baseline changes in relative NREM SWA. (**A**) shows changes in relative SWA for the frontal derivation in a representative adult (P66) mouse (indicated by an arrow in **B** and **C**). Boxes 1,2,3 indicate first and last hour of consolidated sleep during the light period, and first hour of consolidated sleep at night, respectively. The ratios 2/1 and 3/2 were used for the analysis shown in (**B**) and (**C**). Bars show differences across groups (Tukey's HSD), and triangles indicate group means different from 0 (*t*-test; filled symbols $p < 0.05$).



To further assess SWA changes during baseline we calculated the increase in SWA across the extended spontaneous wake period that typically occurs at the light/dark transition; to do so we measured the ratio of NREM SWA in the first hour of consolidated sleep after lights-off relative to the last hour of the light period (ratio 3/2 in Figure 4A). Both in frontal and parietal cortex all groups showed a significant SWA increase, although the P41–44

group had a blunted increase compared to adults (Figure 4C). As was the case for sleep latency, the change in SWA increased linearly with age in both frontal and parietal derivations (Figure 4C).

2.2. Sleep after Sleep Deprivation

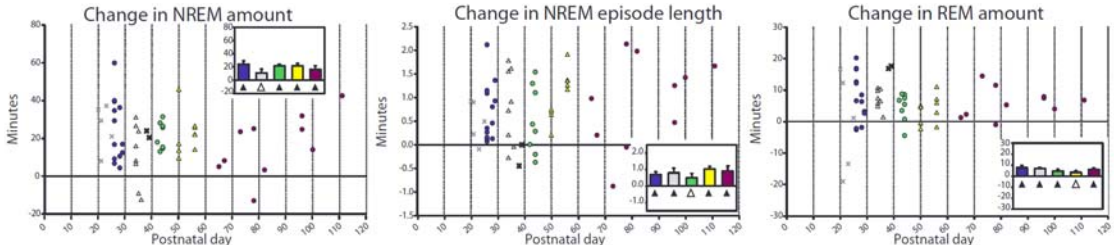
2.2.1. Changes in Sleep duration after Sleep Deprivation

To further assess SWA changes across adolescence all mice older than P25 were deprived of sleep by exposing them to novel objects for 4 h. During sleep deprivation, which started at light onset, time spent in NREM sleep was in all cases less than 4%, although there was a difference across groups, with the P26−29 group sleeping more than the adults (1.5% ± 0.16% *vs.* 0.2% ± 0.16%). REM sleep never occurred during sleep deprivation. In the first 4 h following sleep deprivation (noon-4pm), NREM sleep amount was significantly increased compared to baseline in all groups except in the P33-36 mice, which only showed a trend ($p = 0.09$); there was no difference across groups ($p = 0.30$, Figure 5, left). Similarly, NREM sleep bout duration increased following sleep deprivation except in the P41–44 mice, which only showed a trend ($p = 0.054$); there was no difference across groups ($p = 0.68$, Figure 5, middle). By the end of the night after sleep deprivation all groups showed a significant NREM deficit (*i.e.*, not all NREM sleep lost during deprivation was recovered), with no difference among them ($p = 0.42$, Table 1). During the light phase (noon – 8 p.m.), REM sleep was increased relative to the corresponding time period at baseline in all groups except in the P50–56 mice, which only showed a trend ($p = 0.052$); again there was no difference across groups ($p = 0.42$, Figure 5, right). As was the case for NREM sleep, there was a REM sleep deficit by the end of the dark period following sleep deprivation, with no difference among groups ($p = 0.24$, Table 1). Several other sleep parameters, including number of brief awakenings and duration of REM sleep bouts showed either no changes or small changes in the same direction in all groups (Table 1). In summary, the homeostatic response to sleep deprivation, as measured by increases in sleep amount and sleep bout duration, was present in all groups, with no differences between adolescent and adult mice.

Table 1. Changes in sleep parameters following 4 h of sleep deprivation (8 a.m.–12 p.m.). Mean values and standard error (in parenthesis) for each age group are displayed. The deficit in NREM or REM sleep is the difference in minutes between the amount of each sleep phase during the recovery phase (the 20 h of recovery that follow 4 h of sleep deprivation), minus the amount during the 24 h baseline. During the first 20 h following sleep deprivation mice never recovered all the sleep that they lost during sleep deprivation, resulting in a negative value (deficit). The change in REM episode length is the difference in REM episode length for each mouse, as above calculated by subtracting the 20 h mean value during recovery minus the 24 h amount during baseline. The change in brief arousals is the difference in the number of short wake periods (<16 s) per minute of sleep during the first 4 h of recovery sleep after sleep deprivation, minus the number during the first 4 h of baseline sleep. Sleep during the deprivation is the total amount of sleep achieved during the 4 h of sleep deprivation. Sleep attempts during the deprivation is the total number of sleep attempts during the 4 h of sleep deprivation.

Age Group	**P26–29**	**P34–36**	**P41–44**	**P50–56**	**Adults**
NREM Deficit (min)	−38.13	−44.60	−43.00	−59.36	−66.91
	(11.11)	(13.96)	(15.77)	(10.66)	(11.09)
REM Deficit (min)	−11.68	−8.32	−12.75	−12.58	−8.72
	(3.04)	(2.56)	(3.80)	(3.26)	(3.68)
Change in REM episode	0.077	0.097	0.056	0.23	0.29
length (min)	(0.059)	(0.044)	(0.068)	(0.081)	(0.069)
Change in Brief Arousals	−0.0043	−0.0046	−0.0017	−0.0020	−0.0054
(number per min sleep 1st 4 h)	(0.0014)	(0.0023)	(0.0031)	(0.0021)	(0.0018)
Sleep during deprivation (min)	1.46	0.64	0.70	0.85	0.20
	(0.16)	(0.26)	(0.29)	(0.34)	(0.16)
Sleep attempts (number during	21.77	7.13	9.13	6.78	2.00
deprivation)	(2.33)	(3.78)	(3.34)	(1.76)	(1.57)

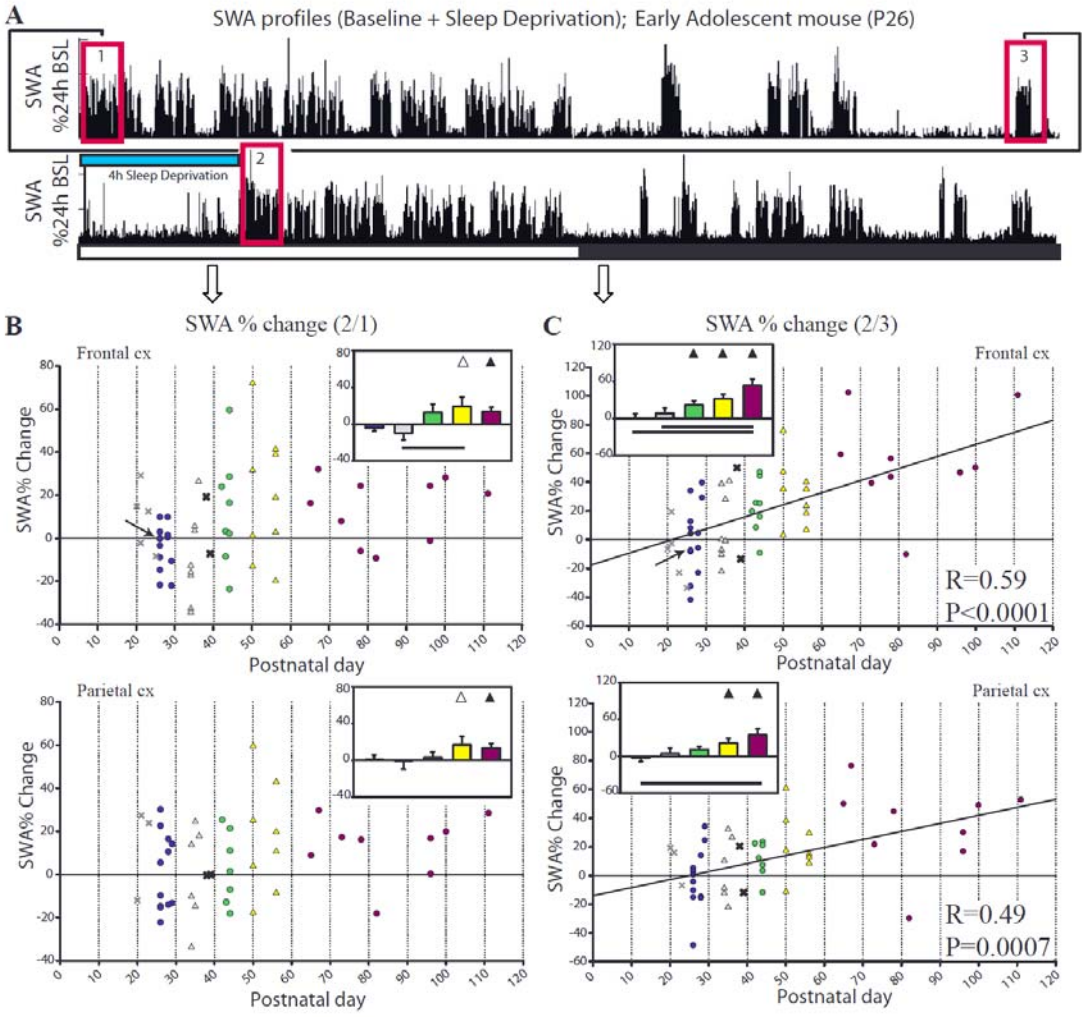
Figure 5. Changes in sleep duration after sleep deprivation. Each faint "X" denotes a mouse that underwent a 2 h deprivation and is included only as a reference. Left and middle panels show the difference (in min) in NREM amount and NREM episode length during the first 4 h of recovery sleep (starting at noon) relative to the first 4 h of baseline sleep (starting at 8 a.m.), while the right panel shows changes in REM amount during the first 8 h of recovery sleep (from noon until lights-off at 8 p.m.) relative to the first 8 h of baseline sleep. Triangles indicate group mean different from 0 (paired 2-tailed *t*-test; filled symbols $p < 0.05$, empty symbols $p < 0.1$).



2.2.2. Changes in SWA after Sleep Deprivation

In adults, as expected, SWA in both frontal and parietal cortex increased during the first hour of recovery sleep relative to the first hour of the baseline light period (ratio 2/1, Figure 6A,B). By contrast, the youngest groups of adolescent mice (P26–29, P34–36) showed no group average SWA increase, and several individual animals actually showed a negative SWA rebound (Figure 6A,B). To confirm the lack of a consistent SWA rebound in the youngest mice we also compared SWA levels immediately before and after the period of sleep deprivation (ratio 2/3, Figure 6A,C). Consistent with the previous finding, group average SWA increased after sleep deprivation in adult and older adolescent mice (starting at P41) but not in the younger groups. This change in SWA showed a linear relationship with age for both the frontal ($R = 0.59$, $p < 0.0001$) and parietal ($R = 0.49$, $p = 0.0007$) derivations, such that the older groups (P41-adults) had robust increases in SWA while in the younger mice (P26–29, P34–36) SWA did not significantly increase across at least 4 consecutive hours of wake (Figure 6).

Figure 6. Change in NREM SWA after sleep deprivation. Each faint "X" denotes a mouse that underwent a 2 h deprivation and is included only as a reference. *p* and *R* correspond to the regression line computed from all animals except those denoted by a faint "X". (**A**) shows changes in relative SWA for the frontal derivation in a representative adolescent (P26) mouse (indicated by an arrow in **B** and **C**). Boxes 1,2,3 indicate the first hour of baseline sleep during the light period, the first hour of recovery sleep after sleep deprivation, and the last hour of baseline sleep at night, respectively. The ratios 2/1 and 2/3 were used for the analysis shown in (**B**) and (**C**). Bars show differences between groups (Tukey's HSD), and triangles indicate group means different from 0 (one sample, 2-tailed *t*-test; filled symbols $p < 0.05$, empty symbols $p < 0.1$).



2.2.3. Parameters Affecting SWA Changes after Sleep Deprivation

By examining SWA values for individual animals in Figure 6, it becomes apparent that a large interindividual variability contributes to the lack of a SWA rebound in the younger groups. To understand the source of this

variability and tease apart the contribution of multiple parameters, we performed a regression analysis of the percent change in SWA during the first hour after sleep deprivation (Table 2). We first performed this analysis on the frontal derivation, which in adult mice shows the largest SWA rebound after sleep loss [25]. We considered a number of parameters known to affect the SWA rebound, at least in adults, including time spent awake and sleep deprivation efficiency [26,27], REM sleep pressure [28], and changes in the wake spectra (e.g., [29–39]). Because of the inherent variability in maturity levels for individual mice of the same age, we used *weight-adjusted age* (see Experimental Section), which adjusts the age by a factor computed from the weight measured at surgery. Our models focused on the mice spanning adolescence (P26–56, Frontal: $n = 40$ Parietal: $n = 35$). Adult mice exhibited the expected post-deprivation increase in SWA, which did not change as a function of age.

Table 2. Multiple predictor linear regression for the SWA rebound after sleep deprivation (**A**, **D**), the SWA changes from immediately before to after sleep deprivation (**B**, **E**), and the SWA changes during spontaneous periods of wake (**C**, **F**). **A**–**C**, frontal cortex; **D**–**F**, parietal cortex. The appropriateness of models to explain the variability in SWA changes was assessed using the Akaike's information criteria (AIC). For models including multiple predictors, *p*-values indicate the probability that the corresponding parameter has predictive value when all other parameters are included. AIC and R^2 are given for each model; R^2 indicates the portion of variance explained by the model and will increase when additional variables are added to a model. AIC is a measure of the relative goodness of fit for a model. More adequately fitting models have lower AIC, but adding poor predictors will increase AIC (indicating a less appropriate model) even if the R^2 increases.

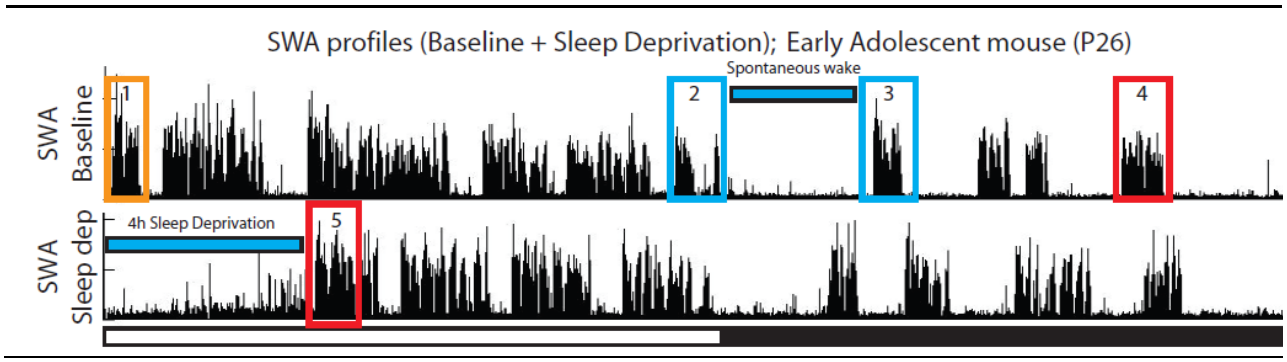


Table 2. *Cont.*

A. Frontal: Predictors for SWA rebound (5/1); % change SWA (5/1) = B_0 + (predictors)	***p***	**AIC**	R^2
Weight-adjusted age (using Gompertz fit)	0.00507	362	0.189
Recovery days from surgery	0.43	370	0.0164
REM / NREM amount (12 h of recovery)	0.0071	363	0.175
n of sleep attempts during sleep deprivation	0.053	367	0.0953
Sleep during sleep deprivation (min/4 h)	0.0582	367	0.0913
Brief arousals (*n*/min of sleep, first 4 h of recovery)	0.31	369	0.0273
Increase in wake SWA (1–4 Hz) during sleep deprivation	0.76	370	0.0249
Slow wave energy (SWE = SWA × time) during deprivation (wake and NREM)	0.84	371	0.0011
Time spent awake since last sleep	0.91	371	0.00033
NREM SWA decline in baseline (1/2)	0.000154	355	0.317
NREM SWA decline in baseline (1/2) + Increase in wake alpha (8–12 Hz)	<0.0001 0.0352	352	0.395
NREM SWA decline in baseline (1/2) + Weight-adjusted age	0.0009 0.0297	352	0.400
NREM SWA decline in baseline (1/2) + Increase in wake alpha (8–12 Hz) + Weight-adjusted age	0.00043 0.17293 0.14348	352	0.431
B. Frontal: Predictors for SWA change from before to after sleep deprivation (5/4); % change SWA (5/4) = B_0 + (parameters)	***P***	**AIC**	R^2
Weight-adjusted age (using Gompertz fit)	0.00025	364	0.30
Recovery days from surgery	0.64	378	0.00595
REM / NREM amount (12 h of recovery)	0.0036	369	0.20
Length of time since last sleep	0.00462	369	0.19
Increase in wake alpha (8–12 Hz)	0.00013	362	0.324
NREM SWA increase in baseline (4/2)	<0.0001	355	0.44
NREM SWA (4/2) + Weight-adjusted age	0.00017 0.01467	350	0.53
NREM SWA (4/2) + Increase in wake alpha (8–12 Hz)	0.000037 0.0015	346	0.575
NREM SWA (4/2) + Increase in wake alpha (8–12 Hz) + Weight-adjusted age	0.00044 0.00618 0.06158	344	0.615
NR SWA (4/2) × Increase wake alpha (8–12 Hz) + Weight-adjusted age	0.022 0.071	340	0.669

Table 2. *Cont.*

C. Frontal: Models Predicting SWA % change (3/2); % change SWA (3/2) = B_0 + (parameters)	***p***	**AIC**	R^2
Weight-adjusted age (using Gompertz fit)	0.913	383	0.0003
Recovery days from surgery	0.39	382	0.0197
Length of time since last sleep	0.016	376	0.14
Increase in wake alpha (8–12 Hz)	0.018	377	0.139
NREM SWA decline in baseline (1/2)	0.000047	365	0.357
NREM SWA decline in baseline (1/2) + Increase in wake alpha	0.00039 0.1654	365	0.390
NREM SWA decline in baseline (1/2) + Length of time since last sleep	0.000095 0.029	362	0.435
NREM SWA (1/2) × Length of time since last sleep	0.029	358	0.506
D. Parietal: Predictors for SWA rebound (5/1); % change SWA (5/1) = B_0 + (predictors)	***P***	**AIC**	R^2
Weight-adjusted age (using Gompertz fit)	0.0045	317	0.219
Recovery days from surgery	0.46	325	0.0165
REM / NREM amount (12 h of recovery)	0.0024	315	0.246
N of sleep attempts during sleep deprivation	0.0100	318	0.185
Sleep during sleep deprivation (min/4 h)	0.011	318	0.180
Brief arousals (*N*/min of sleep, first 4 h of recovery)	0.62	325	0.0076
Increase in wake SWA (1–4 Hz) during sleep deprivation	0.581	325	0.0093
Slow wave energy (SWE = SWA × time) during deprivation (wake and NREM)	0.801	325	0.0020
Time spent awake since last sleep	0.967	325	0.0000
NREM SWA decline in baseline (1/2)	0.0015	314	0.266
NREM SWA decline in baseline (1/2) + *N* of sleep attempts during sleep deprivation	0.00048 0.00292	307	0.446
NREM SWA decline in baseline (1/2) + Weight-adjusted age	0.012 0.038	312	0.360
NREM SWA decline in baseline (1/2) + *N* of sleep attempts during sleep deprivation + Weight-adjusted age	0.0033 0.0164 0.2466	307	0.470

Table 2. *Cont.*

E. Parietal: Predictors for SWA change from before to after sleep deprivation (5/4); % change SWA (5/4) = B_0 + (parameters)	***p***	**AIC**	R^2
Weight-adjusted age (using Gompertz fit)	0.0004	319	0.322
Recovery days from surgery	0.51	332	0.0135
REM / NREM amount (12 h of recovery)	0.0004	319	0.320
Length of time since last sleep	0.0046	324	0.219
Increase in wake alpha (8–12 Hz)	0.00059	320	0.304
NREM SWA increase in baseline (4/2)	0.00056	320	0.306
NREM SWA (4/2) + Weight-adjusted age	0.034 0.023	316	0.412
NREM SWA (4/2) + Increase in wake alpha (8–12 Hz)	0.0039 0.0041	313	0.466
NREM SWA (4/2) + Increase in wake alpha (8–12 Hz) + Weight-adjusted age	0.063 0.010 0.057	311	0.526
NR SWA (4/2) × Increase wake alpha (8-12 Hz) + Weight-adjusted age	0.100 0.034	311	0.567
F. Parietal: Models Predicting SWA % change (3/2); % change SWA (3/2) = B_0 + (parameters)	*P*	**AIC**	R^2
Weight-adjusted age (using Gompertz fit)	0.953	334	0.000
Recovery days from surgery	0.47	334	0.016
Length of time since last sleep	0.0060	326	0.207
Increase in wake alpha (8–12 Hz)	0.133	332	0.067
NREM SWA decline in baseline (1/2)	0.0018	324	0.258
NREM SWA decline in baseline (1/2) + Increase in wake alpha	0.0061 0.5839	326	0.265
NREM SWA decline in baseline (1/2) + Length of time since last sleep	0.00084 0.00260	316	0.444
NREM SWA (1/2) × Length of time since last sleep	0.026	312	0.527

First, we considered the parameters affecting the SWA rebound as classically defined—the increase in SWA during the first hour of recovery sleep relative to the first hour of the baseline light period (ratio 5/1, Table 2, top). The single best predictor of SWA rebound was the ratio between "peak" and "trough" SWA during baseline, i.e. the ratio between SWA during the first hour of the light period, when sleep pressures peaks, and SWA at the end of the major, consolidated sleep period during the light phase (ratio 1/2, Table 2A): animals with a large peak–to-trough

ratio were less likely to show a SWA rebound after sleep deprivation. This model was further refined by including the change in the wake alpha EEG power (8–12 Hz) during sleep deprivation, and weight-adjusted age; as both wake alpha and age increased, so did the SWA rebound. Together, these three parameters (decline in baseline SWA, wake alpha, weight-adjusted age), accounted for 43.1% of the variability in SWA rebound. Other parameters, including the efficiency of sleep deprivation and REM sleep amount during recovery sleep, had some value when considered alone but did not significantly improve the model in the presence of stronger parameters. We also tested this model in the two youngest age groups (P26–29, P34–36, $n = 21$), who showed the least SWA rebound. Within this subset of mice the model provided an even better fit, accounting for 56.9% of the variance, and becoming significantly worse if any one of the 3 parameters was removed.

Next, we considered the parameters affecting the increase in SWA from immediately before to after the period of sleep deprivation (ratio 5/4, Table 2, top). The best predictor for this SWA increase, accounting for 44% of the variance, was the ratio between the SWA immediately preceding the onset of sleep deprivation, at the end of the dark period during baseline, and the SWA at the end of the major sleep period during the light phase (ratio 4/2, Table 2B); in other words, in the mice with a large increase in SWA during the baseline dark phase, SWA was less likely to increase from immediately before to after the period of sleep deprivation. As before, other relevant predictors included the increase in wake alpha EEG power across the sleep deprivation and weight-adjusted age. Together, these 3 parameters accounted for 61.5% of the variance, which increased to 66.9% when an interaction was allowed to occur between the increase in wake alpha and the SWA decline in baseline: the larger was the increase in wake alpha, the less negative was the effect of the baseline SWA decline (Table 2B).

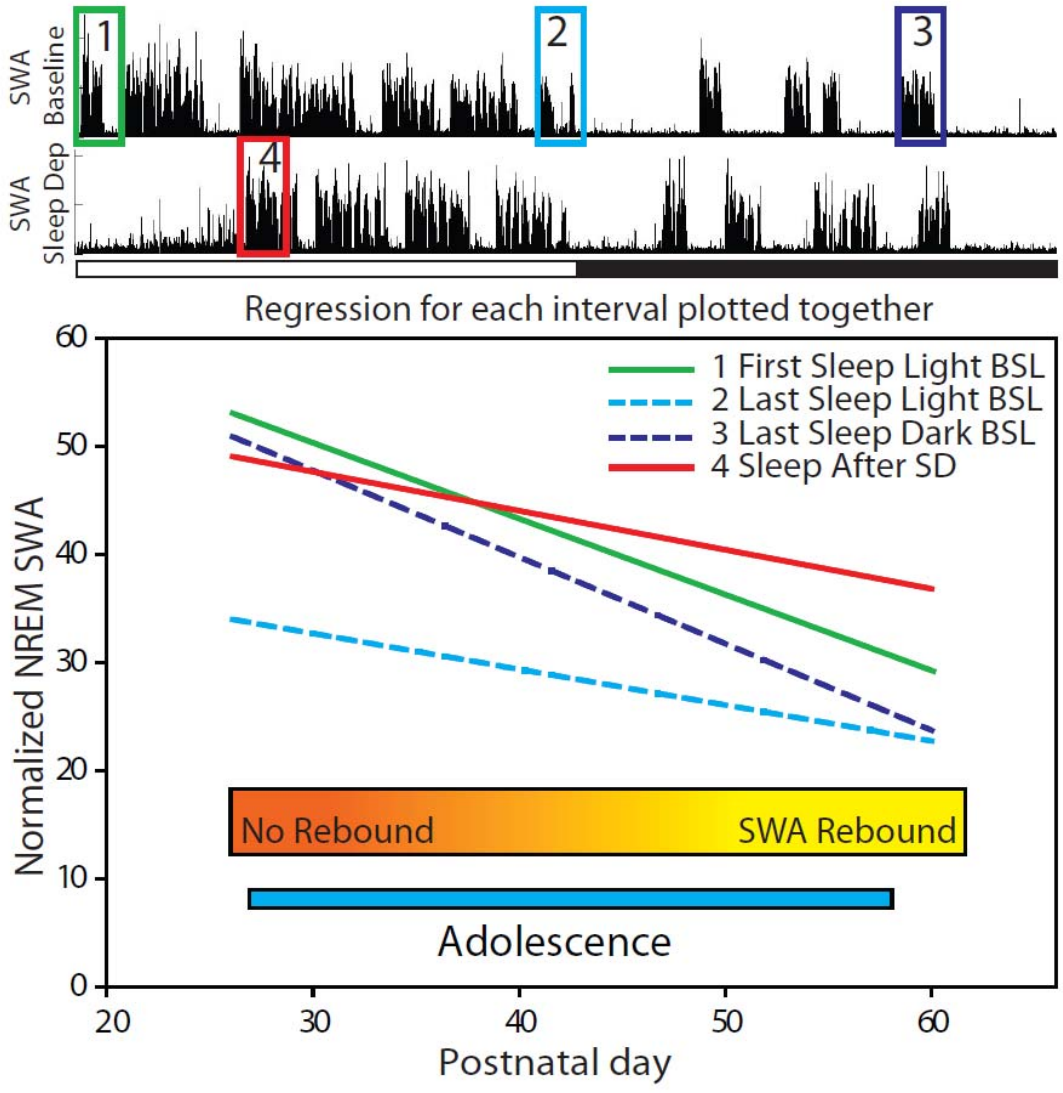
Finally, we considered the change in SWA across the consolidated periods of spontaneous wake that typically occur at the light/dark transition during baseline (ratio 3/2, Table 2, top). Again, the single best predictor was the peak–to-trough SWA ratio during the baseline light phase (ratio 1/2) but, in this case, the larger was the decrease in NREM SWA across the light period, the *larger* was the increase across the following wake period (Table 2C). The time spent awake at the light/dark transition (between 2 and 3 in Table 2, top; see also Figure 3 bottom, sleep latency: lights-off) was significantly related to the increase in SWA and accounted for 14% of the variance. The SWA decline during the light phase combined with time spent awake explained 44% of the variance. Weight-

adjusted age had no predictive value, and the increase in alpha during spontaneous wake had some value (R^2 = 0.139) as a single predictor.

Then, we examined whether the same parameters affect SWA in the parietal cortex (Table 2D–F). As was the case for the frontal derivation, the single best predictor of the SWA rebound was the ratio between "peak" and "trough" SWA during baseline (Table 2D). Unlike for the frontal derivation, however, the change in alpha EEG power was not a significant predictor (p = 0.44), and the most appropriate parietal model, which explained 47% of the variability in the SWA rebound, included SWA decline, weight-adjusted age, and number of sleep attempts rather than wake alpha power (Table 2D). The significant predictors for the increase in SWA across sleep deprivation (Table 2E), and those for the change in SWA across spontaneous wake (Table 2F), were exactly the same as in the frontal derivation.

Overall, this analysis revealed that the most important predictor of the change in SWA after sleep deprivation is the extent of SWA changes during baseline. Specifically, whether or not a mouse had an increase in SWA in response to a sleep deprivation was primarily determined by the magnitude of the decline in SWA during the light period, as well as by increase in SWA during the dark period. To understand why, we measured how the difference in SWA amplitude between younger and older mice varied as a function of time of day, and found that such difference varied significantly across the 24-h cycle, as shown in Figure 7. Specifically, the high pressure SWA values, reached at the onset of the light phase, differed much more across age groups than the low pressure SWA values, reached at the end of the major sleep phase. Indeed, considering the lines of best fit for each interval, the expected initial value for NREM SWA on the baseline day decreased from 53.9 for P25 mice to 29.3 for adult mice at P60, which represents a decrease of 0.703 per day across the entire adolescent period. Meanwhile at low sleep pressure, NREM SWA decreased by only 0.332 per day, from 34.3 at P25 to 22.7 in adult mice. Thus, younger mice can reach higher peak SWA values during baseline than adults, but after the sleep pressure is discharged their SWA reaches low levels not very different from those of adults. Of note, Figure 7 also shows that during the last sleep episode of the dark period (box 3), older mice have SWA that is near their trough value (box 2), while younger mice are already near their peak value (box 1).

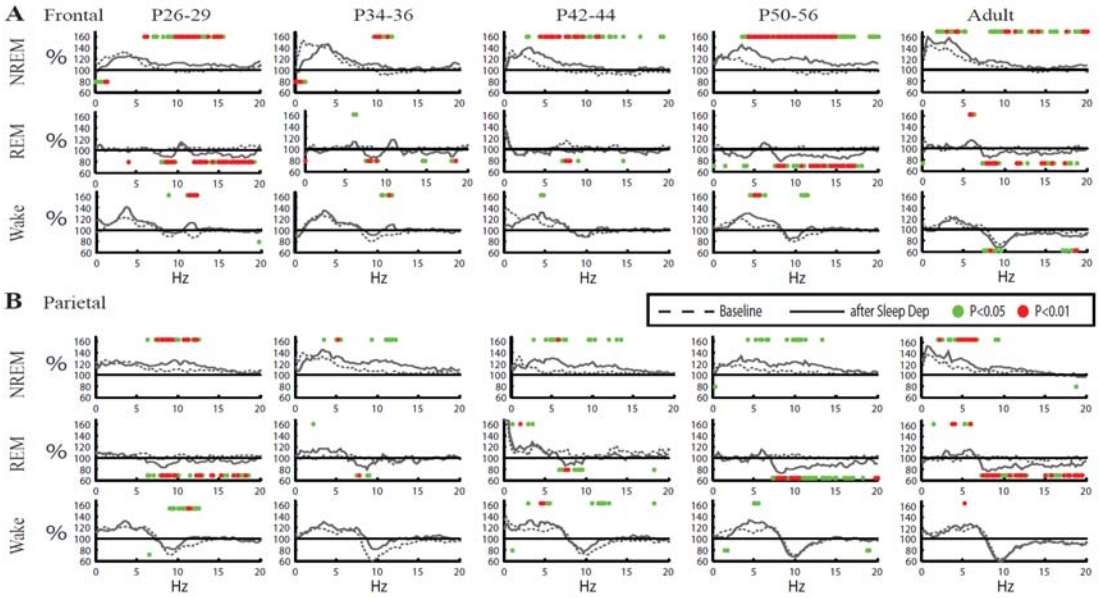
Figure 7. Relationship between age and mean frontal SWA values for 4 daily time points. Plotted is the line of best fit ($f = y_0 + a \times x$) (f = normalized SWA, y_0 = intercept, a = slope (SWA/ postnatal day), x = Postnatal day) for each specified interval (Age *vs.* Normalized NREM SWA) for adolescent mice (P26–56, $n = 40$). NREM SWA was normalized for the mean power density in the 15–30 Hz range for each day. All lines represent statistically significantly relationships ($p < 0.05$).



2.2.3. Other Changes in the EEG Power Spectrum after Sleep Deprivation

To determine whether the differences between adult and adolescent mice described above were specific for NREM SWA we examined the whole spectra (0.1–30 Hz) in all three behavioral states, NREM sleep, REM sleep, and wake (Figure 8). In both frontal and parietal cortex of adults and older adolescent mice the increase in NREM SWA after sleep deprivation was accompanied by significant increases in a broad range of higher frequencies. By contrast, during NREM sleep the youngest mice (P26–29, P34–36) showed either a significant decrease (frontal) or no change (parietal) in the SWA range after sleep deprivation, despite still showing increases in higher EEG frequencies, usually centered around 10 Hz (Figure 8). Neither frontal nor parietal cortex showed changes in the SWA range during wake or REM sleep, in any age group. Instead, at all ages the post-deprivation REM sleep spectra showed a decrease at around 10 Hz, while there was no clear pattern during wake. Overall, these results suggest that the post-deprivation lack of an SWA rebound in some adolescent mice is restricted to the lowest EEG frequencies and specific for NREM sleep.

Figure 8. Overall changes in EEG power spectra after sleep deprivation for all vigilance states. Mean EEG spectra for NREM sleep, REM sleep and wake during baseline (dotted line) and sleep deprivation (solid line) are plotted in 0.25 Hz frequency bins. The mean of each frequency bin is expressed relative to its 24-h baseline mean. Spectra for NREM sleep refer to the first hour from sleep onset, when most SWA rebound occurs. Since little REM sleep occurs during the first hour of recovery, spectra for REM sleep and wake are instead mean of the first 4 h from sleep onset. Dots above and below traces indicate significant increases and decreases, respectively, after sleep deprivation *vs.* baseline ($p < 0.05$ red, $p < 0.01$ green; one sample, 2-tailed *t*-test).



3. Discussion

The main goal of this study was to provide a comprehensive analysis of the changes in sleep pattern in adolescent mice, and to try to clarify previously unanswered questions relative to the development of sleep homeostasis as measured by SWA. We found few changes in sleep pattern across adolescence, the major one being a significant decline in the amount of REM sleep, while the duration of total sleep remained constant and NREM sleep increased slightly. Human studies have generally reported no change in total sleep time across adolescence when subjects were not forced to anticipate wake-up time due to school schedules, and significant declines in total

sleep time and NREM sleep time only during schooldays [40–42]. This suggests that psychosocial factors are a major determinant of sleep time in adolescents, and may affect NREM sleep especially. The lack of such psychosocial factors in our mice, recorded in isolation in a laboratory cage, may explain why total time spent asleep remained constant in our study from early adolescence to adulthood, and NREM sleep showed a small trend to increase with age. Several human studies have also reported a decrease in the deepest stages of NREM sleep (slow wave sleep, stages 3 and 4, N3) and an increase in stage 2 (N2) (e.g., [43–46]). Since NREM sleep in rodents is not traditionally subdivided in substages, we do not know whether a similar switch occurred in our mice throughout adolescence. REM sleep, on the other hand, showed no change throughout adolescence in some reports [43,44,47], and an increase during both school days and weekends in a recent longitudinal study [42]. In our experimental conditions, in which mice could fall asleep whenever they wanted, there was a clear developmental decline in REM duration. This decline, however, was not gradual, but happened quickly within a few days during early adolescence, well before the onset of puberty (at ~P40), and afterwards REM sleep amounts were stable. This early steep decline in REM sleep may be similar to the decrease in REM sleep occurring in children before 8 years of age [47]. Of note, in our mice the steep decline in REM sleep occurred while total time spent asleep remained constant, suggesting that even as there was a relative shift from REM to NREM sleep need, the overall sleep requirement remained relatively constant across early adolescence. We also found that the duration of NREM sleep episodes showed a steep and early increase, reaching adult-like levels already during early adolescence, while REM episode length showed a slower, more progressive increase (Figure 3). Feinberg [48] observed that the duration of slow wave sleep episodes in humans decreases from 7–13 years to 21 years of age and then remains stable, while the duration of REM sleep episodes does not change from childhood to adulthood. It is difficult, however, to directly compare the human data with ours, because they are strictly dependent on the minimum duration criteria used to define a single episode; moreover, in humans only slow wave sleep was considered, rather than the entire NREM sleep episode as in our case.

Previous studies found that throughout adolescence, and in some cases even well before weaning [49], rats respond to short periods of sleep deprivation by increasing the duration of sleep and decreasing sleep fragmentation [17,19–21]. The same studies, however, found that NREM SWA shows different, if not opposite, temporal profiles before and after P24 [17,19,20]. Specifically, one study found that SWA already shows an adult-like profile at P24,

increasing during the major wake period (dark phase) and declining during the major sleep period (light phase), while it progressively increases during both the light and dark phase at P12 and P16, and at P20 the 24-h SWA time course is almost flat [13]. Another study found rapid, ultradian declines in SWA across a few hours of sleep at P23, but no progressive decrease during the light phase, and very high SWA levels at night [17]. Moreover, at P24 SWA was found to strongly increase after sleep deprivation, to an extent even larger than at P30 [17], while the SWA rebound was absent at P12 and P16 and small at P20 [19,20]. Finally, more recent studies found evidence for a homeostatic SWA response after sleep deprivation at both P22 and P30 [21] and, in response to mild chronic sleep restriction (4 h/day), in both adult (P65–72) and adolescent (P29–34) rats [50]. Thus, it seems that, in rats, it is only some time between the third and the fourth week of age that SWA reliably increases across extended periods of wake. Why this does not occur before P24 is unclear, since, as mentioned above, most maturational changes in rodent cortex are completed by the end of the second week of age. It has been proposed that developmental changes in brain metabolism and adenosinergic signaling could underlie the differential response to sleep deprivation before and after P24, while early cortical synaptogenesis could account for some of the progressive SWA increase at P12 and P16 [13,19]. The studies in rats discussed above reported only average group data. Our experiments in mice show that there is high interindividual variability in the response to sleep deprivation as measured by both frontal and parietal SWA, and identified three parameters that together could explain from 43% to 67% of the variance in the SWA response in the frontal derivation, depending on how the SWA change was calculated: the ratio between "peak" and "trough" SWA during baseline, the increase in the wake alpha EEG power (8–12 Hz) during sleep deprivation, and the weight-adjusted age. While the first parameter was negatively correlated with the SWA rebound, the last two were positively correlated with the SWA response. Thus, the SWA rebound in frontal cortex is more likely in older mice, with a small decline in frontal SWA during baseline sleep, and a large increase in alpha activity during sleep deprivation. The SWA changes measured in the parietal derivation were affected by almost all the same parameters described for the frontal cortex, the only exception being that the number of sleep attempts during deprivation, rather than the wake alpha power, was a significant predictor in parietal cortex for the SWA rebound as classically defined (the first hour of recovery sleep relative to the first hour of the baseline light period). Wake alpha power, however, continued to be a significant predictor for the other two SWA changes that we examined, across sleep deprivation and across spontaneous wake. Previous work in humans found that

increased alpha activity in the wake EEG with eyes open may be associated with higher subjective sleepiness and reduced alertness [51], and a more recent study showed that an adenosine deaminase polymorphism that increases slow wave sleep also results in reduced attention and higher wake EEG alpha activity during sleep deprivation [52]. Thus, wake alpha power and number of sleep attempts during deprivation may reflect the same phenomenon, an increase in sleep pressure.

The single best predictor of SWA rebound in adolescent mice was the decline in frontal SWA during the major sleep phase at baseline. To understand why, it is important to remember that our youngest mice have the highest absolute values of SWA (Figure 1), consistent with many studies in human adolescents [53–56]. Moreover, as summarized in Figure 7, we found that the SWA values at the peak of sleep pressure (at the onset of the light phase) differed much more across age groups than the SWA values after sleep pressure was discharged at the end of the light phase, or at the end of the dark period. Overall, these findings suggest that a ceiling effect may explain, at least in part, the lack of SWA rebound after sleep loss in many adolescent mice. This working hypothesis, however, will need to be tested by other experimental studies.

The few studies in humans that assessed the response to sleep deprivation during adolescence found that relative to older adolescents, prepubertal children show (1) higher absolute values of SWA or other related parameters, such as the slope of slow waves, during baseline, and (2) similar absolute increases but smaller relative increases of these parameters after sleep deprivation [57,58]. For instance, despite similar absolute changes, the average relative increase in NREM SWA after 36 h of sleep deprivation was 39% of mean all-night SWA in Tanner 5 adolescents, compared to 18% in Tanner 1 and 2 adolescents [57]. Similarly, the mean relative increase in the slow wave slope after sleep deprivation was 131% in mature adolescents, relative to 117% in prepubertal children [58]. Of note, Kurth and colleagues [58] show slope values for the first 5 sleep cycles in their Figure 3, from which it is apparent that the difference between younger and older subjects is most obvious in the peak values of the slope reached during the first sleep cycle, and much less prominent, although still significant, in the low pressure values reached at the end of the night. The smaller relative changes in SWA and slope in younger subjects, combined with faster SWA dynamics during wake as estimated by simulations, have prompted the suggestion that children live under higher sleep pressure and closer to “saturation” [57], a conclusion consistent with our findings in mice. Older subjects, on

the other hand, could have more headroom for SWA to grow, and thus may afford longer sustained wake and bigger relative SWA increases before reaching saturation.

To understand the mechanisms underlying SWA changes, and how they are affected by age, it is important to distinguish between fast, daily changes in SWA, those that have been classically linked to sleep homeostasis, and slower, developmental changes. In adult mice of the same strain used in this study, we found that daily changes in SWA are not reflected in changes in the number of cortical synapses, although the analysis so far has been limited to the apical dendrites of layer V neurons in one cortical area [59]. In adolescent mice, the same layer V neurons show a net decrease in synapse number after several hours of sleep, and a net increase after several hours of wake [59,60]. Even in adolescents, however, it is likely that most of the daily changes in SWA are driven by changes in synaptic efficacy, rather than synaptic number. Indeed, converging evidence from experiments in both animals and humans show that, at least in adults, high SWA values at sleep onset reflect rapid increases in synaptic efficacy occurring in a matter of few hours due to experience and learning during wake, and that the progressive decline in SWA during sleep reflects a process of synaptic down regulation in many synapses [61–63]. Thus, one could speculate that the large absolute difference in SWA that distinguishes younger from older mice during early, high pressure sleep is driven mainly by difference in synaptic efficacy, while the much smaller difference that remains after sleep pressure has been discharged may be due to differences in synaptic density. Testing this hypothesis requires a systematic analysis of changes in SWA, synaptic efficacy, and synapse number across adolescence, and is the subject of a future study.

Despite the lack of SWA increase, the youngest groups of mice (P26–29, P34–36) showed an increase of frequencies around 10 Hz after sleep deprivation, as did all the other experimental groups. In adults, it is well established that the increase in EEG frequencies after sleep deprivation is not confined to the SWA range, but often extends to the theta and alpha band (~0.5–11 Hz; e.g., [64–66]). Classically, theta (5–9 Hz) activity has been described as the wake EEG marker of sleep need [30,32,36], but recent studies show that local changes in wake theta activity correlate with local changes in sleep SWA, and suggest that the same cellular mechanisms may underlie both slow waves and theta waves [67,68]. In human infants (2–9 months old), low range (<1.75 Hz) SWA does not decline monotonically during the night, while theta activity (6.5–9 Hz) does, prompting the suggestion that during early development theta activity during sleep may be able to reflect sleep homeostasis before SWA [69]. Our

data are consistent with this interpretation, but also suggest that SWA fails as a homeostatic marker because of saturation.

4. Experimental Section

4.1. Recordings of Sleep and Locomotor Activity and Sleep Deprivation

Male YFP-H mice (Jackson Laboratory, Bar Harbor, Maine) were maintained in a colony room on a 12 h light/12 h dark cycle (lights on at 8 a.m.) with food and water available *ad libitum*. Mice (P15–P87) were implanted for chronic polysomnographic recordings under isoflurane anesthesia (1%–2% in 100% O_2). Prior to surgery, all electrodes were directly soldered to flexible wires (#NUF30-4046, Cooner Wire, Chatsworth, CA, USA). Gold plated miniature screw electrodes (0.7 mm diameter) were placed over the right and left frontal (anteroposterior, AP, +1 mm from bregma; mediolateral, ML, 1 mm), and parietal (AP −2 mm; ML 2 mm) cortices and one over cerebellum (AP −1 mm from lambda) as reference. During placement special care was made to advance the screws the minimum amount to remain fixed (typically <1 turn). Two vinyl-coated braided stainless steel wire electrodes (#AS636, Cooner Wire) were placed in the nuchal muscle for electromyogram (EMG) recording. Electrodes were insulated and affixed to the skull using dental cement. Following surgery, mice were housed individually in sound-attenuating, environmentally controlled recording chambers (12:12 LD, lights on at 8 a.m., 25 °C ± 1 °C, food and water *ad libitum*). All electrodes were gathered into a flexible cable and connected to the Multichannel Neurophysiology Recording system (Tucker-Davis Technologies, TDT, Alachua, FL, USA). EEG and EMG signals were collected continuously at a sampling rate of 256 Hz (digitally filtered between 0.1 and 100 Hz). For sleep staging, signals were processed by custom-made Matlab scripts (Mathworks, Natick, MA) using standard TDT routines and subsequently converted into European Data Format (EDF) with Neurotraces software (Fort Lauderdale, FL, USA). Twenty-four hour polygraphic recordings were scored offline for NREM sleep, REM sleep, and wake by visual inspection of 4-s epochs (SleepSign; Kissei Comtec, Irvine, CA, USA) according to standard criteria. Wake was characterized by low voltage, high frequency EEG pattern and phasic EMG activity. NREM sleep was characterized by the occurrence of high amplitude slow waves and low tonic EMG. During REM sleep the EEG was similar to that during wake, but only heart beats and occasional twitches were

evident in the EMG signal. NREM and REM episodes were defined according to criteria that allow brief interruptions (≤16 s) but require that the vigilance state accounts for >80% of the episode length [70,71]. The average EEG power spectra for the fronto-cerebellar and parieto-cerebellar derivations (0.0−30 Hz) was computed using averaged periodograms from an FFT routine using consecutive 4-s Hanning windows. Absolute SWA (0.5−4.0 Hz) and SWA relative to NREM high frequencies (>15 Hz), a previously described normalization [72], were computed. The normalization to high frequencies was used to control for possible daily changes in signal strength due to technical issues (progressive deterioration of the signal, skull growth, *etc*.). Motor activity was quantified by custom-made video-based motion detection algorithms with a time resolution of 1 s (Matlab) [59]. Acute sleep deprivation started at light onset, and lasted 2 (at ages <P25) or 4 h (all other ages). Mice were kept awake for up to 4 h by introducing novel objects into their cages.

For all mice having surgery before P20 special care was taken to make sure they received proper nutrition, following the recommendation of the veterinary staff. Specifically, in addition to the normal food pellets (given to allow chewing/gnawing), these mice received the breeder diet (8626 Teklad Mouse breeder diet, Harlan laboratories, Madison, WI, USA), which is softer and has a higher fat content. Additionally, 2 pellets of breeder diet were softened in water and replaced daily with fresh softened pellets until there was clear visual evidence of the solid pellets being consumed. Each mouse was given special bedding material made from pulped cotton fiber for maintaining warmth when single housed (NestlestsT, Ancare, Bellmore, NY, USA). For the youngest animals a portion of the bedding from the home cage was transferred to the recording cage so that a nest was available immediately after surgery.

All animal procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and facilities were reviewed and approved by the IACUC of the University of Wisconsin-Madison, and were inspected and accredited by AAALAC.

4.2. Weight-Adjusted Age

During the early postnatal period in mice recording the age in days is only an approximate indicator of maturation due to a number of difficult to control variables including, health of the mother, litter size and competition for maternal nourishment between siblings [73]. Thus, even if the exact moment of birth is known, age

in days becomes only an approximation of actual maturity. In order to remove some of the variability associated with this uncertainty we transformed our approximate measurement of age into a more continuous and accurate indicator of maturity by combining the age at sleep deprivation and the weight recorded at the time of surgery. The surgery weight was satisfactorily fit to a Gompertz curve ($R^2 = 0.9420$, $p < 0.0001$) giving the expected maturational curve for the mice in this study. We chose to fit surgery weight to a Gompertz curve because it is an asymmetrical sigmoid curve that allows for the higher valued asymptote to be reached more slowly than the lower valued asymptote. This pattern is also clearly beneficial for weight across adolescence since the mice initially grow quickly but then continue to gain weight at a slower rate into adulthood. Additionally, a recent study in humans extensively used the inverse of a Gompertz curve to describe the maturational change in SWA during adolescence [74]. From this expected curve we estimated the actual age at surgery by solving the Gompertz equation for age.

$$\text{Surgery Weight Age} = -9.98 \times -\ln\{\ln[(\text{Surgery Weight} - 4.12)/20.17)]\} + 21.68$$

A weight adjusted age for the day of sleep deprivation was determined by adjusting the sleep deprivation age by the number of days each mouse was ahead or behind the Gompertz growth curve at surgery. This weight-adjusted age was used in the models shown in Table 2 to explain the variability in the % increase SWA, because it was a better predictor than either weight or age alone, and because it simplified the models by condensing two parameters of maturity (weight and age) into a single value. For the sake of clarity only models with weight-adjusted age have been included in Table 2. In the figures and corresponding statistical analyses, instead, age indicates chronological age.

4.3. Statistical Analysis.

Regression analysis was performed using R (R Foundation for Statistical Computing, Vienna, Austria); all other statistics were performed using Matlab 2007 statistics toolbox (Mathworks, Natick, MA, USA). Figures depicting changes in sleep parameters *vs.* age were created using SigmaPlot (Systat Software, Inc, San Jose, CA, USA) and were fit to linear models except when exponential functions were superior at reducing the sum of squares and producing an even residual distribution. Baseline sleep parameters and the response to sleep deprivations were analyzed both using age as continuous variable for regression and as a grouping criterion for one-way ANOVA

when sleep deprivations occurred. Scored days that did not fit within an age cluster were included in the regression but not the ANOVA. If significance $p < 0.05$ was reached the ANOVA was followed by post-hoc Tukey's honestly significant difference test. Differences between baseline and sleep deprivation were assessed by paired 2-tailed t-tests. Whether the % change in SWA differed from 0 was assessed by one-sample 2-tailed t-tests.

5. Conclusions

Our results show that adolescent mice respond to sleep deprivation in a way very similar to adults, by sleeping longer and with more consolidated sleep bouts. The ability of adolescent mice to show a significant rebound in SWA after sustained periods of wake is strongly and negatively affected by their high absolute values of SWA at sleep onset. Thus, the absence of a SWA rebound after sleep deprivation most likely reflects a ceiling effect rather than the immaturity of the cellular mechanisms—whether they be of metabolic, hormonal, and/or plastic nature—that underlie sleep homeostasis. This conclusion is supported by the fact that as long as wake duration remains within the physiological limits, as during baseline, SWA shows the same expected homeostatic changes in adolescents and adults, declining in the course of sleep and increasing across periods of spontaneous wake.

Acknowledgments

The study was supported by NIH Director's Pioneer award (to GT) and NIMH (1R01MH091326 to GT and CC).

Conflict of Interest

The authors declare no conflict of interest.

References

1. Spear, L.P. The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* **2000**, *24*, 417–463.
2. Laviola, G.; Macri, S.; Morley-Fletcher, S.; Adriani, W. Risk-taking behavior in adolescent mice: Psychobiological determinants and early epigenetic influence. *Neurosci. Biobehav. Rev.* **2003**, *27*, 19–31.
3. Adriani, W.; Macri, S.; Pacifici, R.; Laviola, G. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. *Neuropsychopharmacology* **2002**, *27*, 212–224.
4. Spear, L.P.; Brake, S.C. Periadolescence: Age-dependent behavior and psychopharmacological responsivity in rats. *Dev. Psychobiol.* **1983**, *16*, 83–109.
5. Kellogg, C.K.; Awatramani, G.B.; Piekut, D.T. Adolescent development alters stressor-induced Fos immunoreactivity in rat brain. *Neuroscience* **1998**, *83*, 681–689.
6. Molnar, Z.; Adams, R.; Blakemore, C. Mechanisms underlying the early establishment of thalamocortical connections in the rat. *J. Neurosci.* **1998**, *18*, 5723–5745.
7. Aghajanian, G.K.; Bloom, F.E. The formation of synaptic junctions in developing rat brain: A quantitative electron microscopic study. *Brain Res.* **1967**, *6*, 716–727.
8. Ashby, M.C.; Isaac, J.T. Maturation of a recurrent excitatory neocortical circuit by experience-dependent unsilencing of newly formed dendritic spines. *Neuron* **2011**, *70*, 510–521.
9. Caley, D.W.; Maxwell, D.S. An electron microscopic study of neurons during postnatal development of the rat cerebral cortex. *J. Comp. Neurol.* **1968**, *133*, 17–44.
10. Caley, D.W.; Maxwell, D.S. Development of the blood vessels and extracellular spaces during postnatal maturation of rat cerebral cortex. *J. Comp. Neurol.* **1970**, *138*, 31–47.
11. Gramsbergen, A. The development of the EEG in the rat. *Dev. Psychobiol.* **1976**, *9*, 501–515.
12. Mirmiran, M.; Corner, M. Neuronal discharge patterns in the occipital cortex of developing rats during active and quiet sleep. *Brain Res.* **1982**, *255*, 37–48.
13. Frank, M.G.; Heller, H.C. Development of diurnal organization of EEG slow-wave activity and slow-wave sleep in the rat. *Am. J. Physiol.* **1997**, *273*, R472–R478.
14. Jouvet-Mounier, D.; Astic, L.; Lacote, D. Ontogenesis of the states of sleep in rat, cat, and guinea pig during the first postnatal month. *Dev. Psychobiol.* **1970**, *2*, 216–239.
15. Gramsbergen, A.; Schwartze, P.; Prechtl, H.F. The postnatal development of behavioral states in the rat. *Dev. Psychobiol.* **1970**, *3*, 267–280.
16. Daszuta, A.; Gambarelli, F. Early postnatal development of EEG and sleep-waking cycle in two inbred mouse strains. *Brain Res.* **1985**, *354*, 39–47.
17. Alfoldi, P.; Tobler, I.; Borbely, A.A. Sleep regulation in rats during early development. *Am. J. Physiol.* **1990**, *258*, R634–R644.

18. Vogel, G.W.; Feng, P.; Kinney, G.G. Ontogeny of REM sleep in rats: Possible implications for endogenous depression. *Physiol. Behav.* **2000**, *68*, 453–461.
19. Frank, M.G.; Morrissette, R.; Heller, H.C. Effects of sleep deprivation in neonatal rats. *Am. J. Physiol.* **1998**, *275*, R148–R157.
20. Hairston, I.S.; Peyron, C.; Denning, D.P.; Ruby, N.F.; Flores, J.; Sapolsky, R.M.; Heller, H.C.; O'Hara, B.F. Sleep deprivation effects on growth factor expression in neonatal rats: A potential role for BDNF in the mediation of delta power. *J. Neurophysiol.* **2004**, *91*, 1586–1595.
21. Gvilia, I.; Suntsova, N.; Angara, B.; McGinty, D.; Szymusiak, R. Maturation of sleep homeostasis in developing rats: A role for preoptic area neurons. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *300*, R885–R894.
22. Huber, R.; Deboer, T.; Tobler, I. Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: Empirical data and simulations. *Brain Res.* **2000**, *857*, 8–19.
23. Vyazovskiy, V.V.; Ruijgrok, G.; Deboer, T.; Tobler, I. Running wheel accessibility affects the regional electroencephalogram during sleep in mice. *Cereb. Cortex* **2006**, *16*, 328–336.
24. Franken, P.; Chollet, D.; Tafti, M. The homeostatic regulation of sleep need is under genetic control. *J. Neurosci.* **2001**, *21*, 2610–2621.
25. Huber, R.; Deboer, T.; Tobler, I. Topography of EEG dynamics after sleep deprivation in mice. *J. Neurophysiol* **2000**, *84*, 1888–1893.
26. Tobler, I.; Borbély, A.A. Sleep EEG in the rat as a function of prior waking. *Electroencephalogr. Clin. Neurophysiol.* **1986**, *64*, 74–76.
27. Deboer, T.; Tobler, I. Sleep regulation in the Djungarian hamster: comparison of the dynamics leading to the slow-wave activity increase after sleep deprivation and daily torpor. *Sleep* **2003**, *26*, 567–572.
28. Borbely, A.A.; Neuhaus, H.U. Sleep deprivation: effects on sleep and EEG in the rat. *J. Comp. Physiol.* **1979**, *133*, 71–87.
29. Torsvall, L.; Akerstedt, T. Sleepiness on the job: Continuously measured EEG changes in train drivers. *Electroencephalogr. Clin. Neurophysiol.* **1987**, *66*, 502–511.
30. Cajochen, C.; Brunner, D.P.; Krauchi, K.; Graw, P.; Wirz-Justice, A. Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. *Sleep* **1995**, *18*, 890–894.
31. Aeschbach, D.; Matthews, J.R.; Postolache, T.T.; Jackson, M.A.; Giesen, H.A.; Wehr, T.A. Dynamics of the human EEG during prolonged wakefulness: Evidence for frequency-specific circadian and homeostatic influences. *Neurosci. Lett.* **1997**, *239*, 121–124.
32. Finelli, L.A.; Baumann, H.; Borbely, A.A.; Achermann, P. Dual electroencephalogram markers of human sleep homeostasis: Correlation between theta activity in waking and slow-wave activity in sleep. *Neuroscience* **2000**, *101*, 523–529.

33. Aeschbach, D.; Matthews, J.R.; Postolache, T.T.; Jackson, M.A.; Giesen, H.A.; Wehr, T.A. Two circadian rhythms in the human electroencephalogram during wakefulness. *Am. J. Physiol.* **1999**, 277, R1771–R1779.
34. Cajochen, C.; Khalsa, S.B.; Wyatt, J.K.; Czeisler, C.A.; Dijk, D.J. EEG and ocular correlates of circadian melatonin phase and human performance decrements during sleep loss. *Am. J. Physiol.* **1999**, 277, R640–R649.
35. Cajochen, C.; Wyatt, J.K.; Czeisler, C.A.; Dijk, D.J. Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. *Neuroscience* **2002**, *114*, 1047–1060.
36. Vyazovskiy, V.V.; Tobler, I. Theta-activity in the waking EEG is a marker of sleep propensity in the rat. *Brain Res.* **2005**, *1050*, 64–71.
37. Retey, J.V.; Adam, M.; Gottselig, J.M.; Khatami, R.; Durr, R.; Achermann, P.; Landolt, H.P. Adenosinergic mechanisms contribute to individual differences in sleep deprivation-induced changes in neurobehavioral function and brain rhythmic activity. *J. Neurosci.* **2006**, *26*, 10472–10479.
38. Tinguely, G.; Finelli, L.A.; Landolt, H.P.; Borbely, A.A.; Achermann, P. Functional EEG topography in sleep and waking: state-dependent and state-independent features. *Neuroimage* **2006**, *32*, 283–292.
39. Leemburg, S.; Vyazovskiy, V.V.; Olcese, U.; Bassetti, C.L.; Tononi, G.; Cirelli, C. Sleep homeostasis in the rat is preserved during chronic sleep restriction. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 15939–15944.
40. Ohayon, M.M.; Carskadon, M.A.; Guilleminault, C.; Vitiello, M.V. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: Developing normative sleep values across the human lifespan. *Sleep* **2004**, *27*, 1255–1273.
41. Carskadon, M.A. Sleep in adolescents: The perfect storm. *Pediatr. Clin. North. Am.* **2011**, *58*, 637–647.
42. Feinberg, I.; Davis, N.M.; de Bie, E.; Grimm, K.J.; Campbell, I.G. The maturational trajectories of NREM and REM sleep durations differ across adolescence on both school-night and extended sleep. *Am. J. Physiol. Regul Integr. Comp. Physiol.* **2012**, *302*, R533–R540.
43. Carskadon, M.A.; Harvey, K.; Duke, P.; Anders, T.F.; Litt, I.F.; Dement, W.C. Pubertal changes in daytime sleepiness. *Sleep* **1980**, *2*, 453–460.
44. Gaudreau, H.; Carrier, J.; Montplaisir, J. Age-related modifications of NREM sleep EEG: From childhood to middle age. *J. Sleep Res.* **2001**, *10*, 165–172.
45. Tarokh, L.; Carskadon, M.A. Developmental changes in the human sleep EEG during early adolescence. *Sleep* **2010**, *33*, 801–809.
46. Tarokh, L.; van Reen, E.; LeBourgeois, M.; Seifer, R.; Carskadon, M.A. Sleep EEG provides evidence that cortical changes persist into late adolescence. *Sleep* **2011**, *34*, 1385–1393.
47. Kurth, S.; Ringli, M.; Geiger, A.; LeBourgeois, M.; Jenni, O.G.; Huber, R. Mapping of cortical activity in the first two decades of life: A high-density sleep electroencephalogram study. *J. Neurosci.* **2010**, *30*, 13211–13219.
48. Feinberg, I. Changes in sleep cycle patterns with age. *J. Psychiatric. Res.* **1974**, *10*, 283–306.

49. Todd, W.D.; Gibson, J.L.; Shaw, C.S.; Blumberg, M.S. Brainstem and hypothalamic regulation of sleep pressure and rebound in newborn rats. *Behav. Neurosci.* **2010**, *124*, 69–78.
50. Yang, S.R.; Sun, H.; Huang, Z.L.; Yao, M.H.; Qu, W.M. Repeated sleep restriction in adolescent rats altered sleep patterns and impaired spatial learning/memory ability. *Sleep* **2012**, *35*, 849–859.
51. Oken, B.S.; Salinsky, M.C.; Elsas, S.M. Vigilance, alertness, or sustained attention: physiological basis and measurement. *Clin. Neurophysiol.* **2006**, *117*, 1885–1901.
52. Bachmann, V.; Klaus, F.; Bodenmann, S.; Schafer, N.; Brugger, P.; Huber, S.; Berger, W.; Landolt, H.P. Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. *Cereb. Cortex* **2012**, *22*, 962–970.
53. Ringli, M.; Huber, R. Developmental aspects of sleep slow waves: Linking sleep, brain maturation and behavior. *Prog. Brain Res.* **2011**, *193*, 63–82.
54. Tarokh, L.; Raffray, T.; van Reen, E.; Carskadon, M.A. Physiology of normal sleep in adolescents. *Adolesc. Med. State Art Rev.* **2010**, *21*, 401–417.
55. Feinberg, I.; Campbell, I.G. Sleep EEG changes during adolescence: An index of a fundamental brain reorganization. *Brain Cogn.* **2010**, *72*, 56–65.
56. Colrain, I.M.; Baker, F.C. Changes in sleep as a function of adolescent development. *Neuropsychol. Rev.* **2011**, *21*, 5–21.
57. Jenni, O.G.; Achermann, P.; Carskadon, M.A. Homeostatic sleep regulation in adolescents. *Sleep* **2005**, *28*, 1446–1454.
58. Kurth, S.; Jenni, O.G.; Riedner, B.A.; Tononi, G.; Carskadon, M.A.; Huber, R. Characteristics of sleep slow waves in children and adolescents. *Sleep* **2010**, *33*, 475–480.
59. Maret, S.; Faraguna, U.; Nelson, A.; Cirelli, C.; Tononi, G. Sleep and wake modulate spine turnover in the adolescent mouse cortex. *Nat. Neurosci.* **2011**, *14*, 1418–1420.
60. Yang, G.; Gan, W.B. Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. *Dev. Neurobiol.* **2011**, *72*, 1391–1398.
61. Tononi, G.; Cirelli, C. Sleep and synaptic homeostasis: A hypothesis. *Brain Res. Bull.* **2003**, *62*, 143–150.
62. Tononi, G.; Cirelli, C. Sleep function and synaptic homeostasis. *Sleep Med. Rev.* **2006**, *10*, 49–62.
63. Tononi, G.; Cirelli, C. Time to be SHY? Some comments on sleep and synaptic homeostasis. *Neural Plast.* **2012**, *2012*, 415250.
64. Borbely, A.A.; Baumann, F.; Brandeis, D.; Strauch, I.; Lehmann, D. Sleep deprivation: Effect on sleep stages and EEG power density in man. *Electroencephalogr. Clin. Neurophysiol.* **1981**, *51*, 483–495.
65. Dijk, D.J.; Brunner, D.P.; Borbely, A.A. Time course of EEG power density during long sleep in humans. *Am. J. Physiol.* **1990**, *258*, R650–R661.
66. Cajochen, C.; Foy, R.; Dijk, D.J. Frontal predominance of a relative increase in sleep delta and theta eeg activity after sleep loss in humans. *Sleep Res. Online* **1999**, *2*, 65–69.

67. Vyazovskiy, V.V.; Olcese, U.; Hanlon, E.C.; Nir, Y.; Cirelli, C.; Tononi, G. Local sleep in awake rats. *Nature* **2011**, *472*, 443–447.
68. Hung, C.S.; Sarasso, S.; Ferrarelli, F.; Riedner, B.; Ghilardi, M.F.; Cirelli, C.; Tononi, G. Local, experience-dependent changes in the wake EEG after prolonged wakefulness. *Sleep* **2013**, *36*, 59–72.
69. Jenni, O.G.; Borbely, A.A.; Achermann, P. Development of the nocturnal sleep electroencephalogram in human infants. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2004**, *286*, R528–R538.
70. Franken, P.; Dijk, D.J.; Tobler, I.; Borbely, A.A. Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature. *Am. J. Physiol.* **1991**, *261*, R198–R208.
71. Deboer, T.; Franken, P.; Tobler, I. Sleep and cortical temperature in the Djungarian hamster under baseline conditions and after sleep deprivation. *J. Comp. Physiol A* **1994**, *174*, 145–155.
72. Faraguna, U.; Vyazovskiy, V.V.; Nelson, A.B.; Tononi, G.; Cirelli, C. A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. *J. Neurosci.* **2008**, *28*, 4088–4095.
73. Castellano, C.; Oliverio, A. Early malnutrition and postnatal changes in brain and behavior in the mouse. *Brain Res.* **1976**, *101*, 317–325.
74. Feinberg, I.; de Bie, E.; Davis, N.M.; Campbell, I.G. Topographic differences in the adolescent maturation of the slow wave EEG during NREM sleep. *Sleep* **2011**, *34*, 325–333.

Chapter IV:

The Response to Chronic Sleep Deprivation in Adolescent Mice

Aaron B. Nelson, Luisa De Vivo, Giulio Tononi, and Chiara Cirelli

In preparation:

Abstract

Chronic sleep restriction is especially common during adolescence. A profound decrease in synaptic density is thought to occur during this period, but whether this process is affected by sleep loss is unknown. Although the response to chronic sleep restriction of varying durations and intensities has been studied extensively in rats, much less work has so far been performed in mice and never during the critical period of adolescence. Yet, the transgenic mouse offers a set of tools particularly useful for investigating both adolescent synaptic pruning and the underlying physiological changes associated with the chronic sleep restriction. Here YFP-H mice (P15-P87, n=100) were implanted for chronic EEG recording in order to track developmental changes in the power spectra under normal conditions and in response to a chronic sleep restriction. In line with findings in humans, EEG power between 0.5 and 4.0 Hz during NREM sleep (SWA, 24 hour mean high frequency normalized) progressively declined during adolescence (P25 to >P60). A subset of mice additionally underwent 4 days of sleep restriction starting at two ages in early adolescence (P21 and P26). Sleep restriction was performed with 12 hours of manual deprivation during the light period and 12 hours of automated deprivation using forced locomotion during the dark period. These mice were given caffeinated water both to aid in the deprivation efficiency and to better model the behaviour of sleep restricted humans. The most striking change in response to the sleep restriction was a profound but reversible decrease in NREM SWA. This pattern may indicate a disruption in the ongoing cortical maturation during the adolescent period. This study facilitates future work that will systematically compare EEG power spectra changes with *in vivo* measures of spine density using two-photon microscopy as well as ex vivo analyses of molecular markers of synaptic strength.

Introduction

As children reach the later stages of adolescence a progressive change in intrinsic biological factors combined with increased salience of psychosocial factors combine to make adolescents particularly susceptible to living under chronic sleep restriction (Hagenauer et al., 2009). Recent large scale surveys of sleep patterns as well as longitudinal studies with at home EEG recordings indicate that the typical practice among adolescents is to operate on limited sleep during the normal week and then extend sleep on the weekend (America, 2006; Feinberg et al., 2012; Williams et al., 2013).Whether or not this practice of extended sleep on the weekend is sufficient to make up for lost sleep opportunities is unknown, but studies in adults suggest that some detriments associated with chronic sleep loss are cumulative and may not be fully offset by 2 days of recovery (Van Dongen et al., 2003; Banks et al., 2010). The negative effects of sleep restriction may be more serious for the adolescent brain, which is thought to undergo large scale synaptic pruning well into early adulthood, especially in the prefrontal cortex (Huttenlocher and Dabholkar, 1997; Feinberg and Campbell, 2009; Petanjek et al., 2011). Furthermore, it is believed that the development of several psychiatric disorders, particularly those with etiologies of decreased prefrontal grey matter volume, stems from a disregulation of cortical maturation and especially synaptic pruning during this sensitive period (Paus et al., 2008). Thus, considering a diathesis-stress model for the development of psychiatric disorders, it is imperative that we understand the long term neurological consequences associated with a chronic stressor that is now endemic in our society.

During much of sleep, the cerebral cortex generates slow waves, one every second or so, which travel along the cortical surface and are especially prominent over frontal regions (Werth et al., 1996; Huber et al., 2000; Massimini et al., 2004; Vyazovskiy et al., 2009). SWA, defined as the EEG power between 0.5 and 4 Hz during non-rapid eye movement (NREM) sleep, conveniently and quantitatively captures most of this slow activity. In birds and mammals NREM SWA acutely increases in response to time spent awake and decreases during sleep and is the best established indicator of the homeostatic regulation of sleep (Borbély and Achermann, 2005; Jones et al., 2008). A growing body of evidence in multiple model systems suggests that slow waves during NREM sleep, in addition to being indicative of the level of sleep need, also play an active role in mediating sleep's restorative function, which may be of particular importance during development (Tononi and Cirelli 2010).

Intracellular recordings have shown that SWA in the EEG is generated by virtually all cortical neurons engaging in a slow (<1 Hz) oscillation, consisting of a depolarized up state, when neurons show sustained firing, and a hyperpolarized down state, characterized by neuronal silence (Steriade et al., 1993; Amzica and Steriade, 1998; Steriade et al., 2001). There is a close temporal relationship between these cellular phenomena and simultaneously recorded slow (or delta) waves, which are defined as surface-negative EEG events that fall in the SWA frequency range (Contreras and Steriade, 1995; Amzica and Steriade, 1998). Specifically, the surface negativity in the EEG signal (or depth positivity in the local field potential, LFP) corresponds to the down state of cortical neurons as recorded intracellularly and to the suppression of spiking activity as recorded extracellularly, suggesting that EEG or LFP slow waves are a reflection of near-synchronous transitions between up and down states in large populations of cortical neurons (Steriade et al., 1993; Steriade et al., 2001). Both theoretical considerations (Lopes da Silva et al., 1991), large-scale simulations (Esser et al., 2007) and empirical studies (Riedner et al., 2007; Vyazovskiy et al., 2009) indicate that the amplitude and slope of EEG slow waves is related to the number of neurons that enter an up state or a down state near-synchronously, and that synchrony is directly related to the number and efficacy of synaptic connections among them.

Several recent studies have shown that in humans absolute values of sleep SWA follow an inverted U curve during development, with a progressive increase during childhood, a peak around 8 years of age, and a rapid decline during adolescence. For instance, a longitudinal study documented a steep decline in sleep SWA (1-4 Hz) and theta (4-8 Hz) activity, especially between age 11 and 16.5 years (Feinberg and Campbell, 2009). The authors compared their EEG data with published longitudinal declines in MRI-estimated cortical thickness, and found that changes in SWA tracked the thinning of 5-layer cortex, while theta activity paralleled the earlier maturational thinning in 3-layer cortex. High-density EEG recordings also indicate that the location of maximal SWA undergoes a shift in topography, from a peak in posterior regions to a peak in frontal regions, which seems to parallel brain maturation (Kurth et al., 2010). Moreover, during adolescence sleep SWA decreases the most over the brain regions that show the largest decrease in gray matter (Buchmann et al., 2011). Changes in cortical synaptic density as revealed by a few ultrastructural studies (Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997; Petanjek et al., 2011) also

follow an inverted U curve, with a peak before adolescence followed by a decline. Finally, developmental changes in brain glucose consumption (Chugani et al., 1987; Chugani, 1998), which is a function of synaptic density/strength (Attwell and Laughlin, 2001), also follow an inverted U curve. Thus, the hypothesis has been put forward that developmental changes in the sleep EEG may reflect changes in synaptic density (Feinberg and Campbell, 2009), and more specifically, that the decline in sleep SWA during adolescence may reflect the elimination (pruning) of cortical synapses (Campbell and Feinberg, 2009). However, MRI cannot assess synapses directly, and reduced cortical thickness could be due to many other factors (Rakic et al., 1994; Paus et al., 2008). Moreover, electron microscopy data in humans have been obtained in a limited number of samples. An animal model is required to directly link developmental changes in sleep EEG and adolescent synaptic pruning.

It was recently demonstrated by us and others that adolescent transgenic mice (YFP-line H), which express yellow fluorescence protein in a subset of layer V cortical pyramidal neurons, undergo a small but positive net increase in spine density during normal waking and after an acute sleep deprivation (Maret et al., 2011; Yang and Gan, 2012). Yet, in this same strain mice younger than postnatal day 40 did not have an increase in SWA following 4-hours sleep deprivation (Nelson et., al 2013). Here, to directly and systematically compare changes in synaptic number and strength with changes in EEG power spectra, we collected longitudinal EEG data from early adolescence (postnatal day P21) to adulthood (>P60) of sleep in YFP-H transgenic mice under normal baseline conditions and following a chronic sleep restriction.

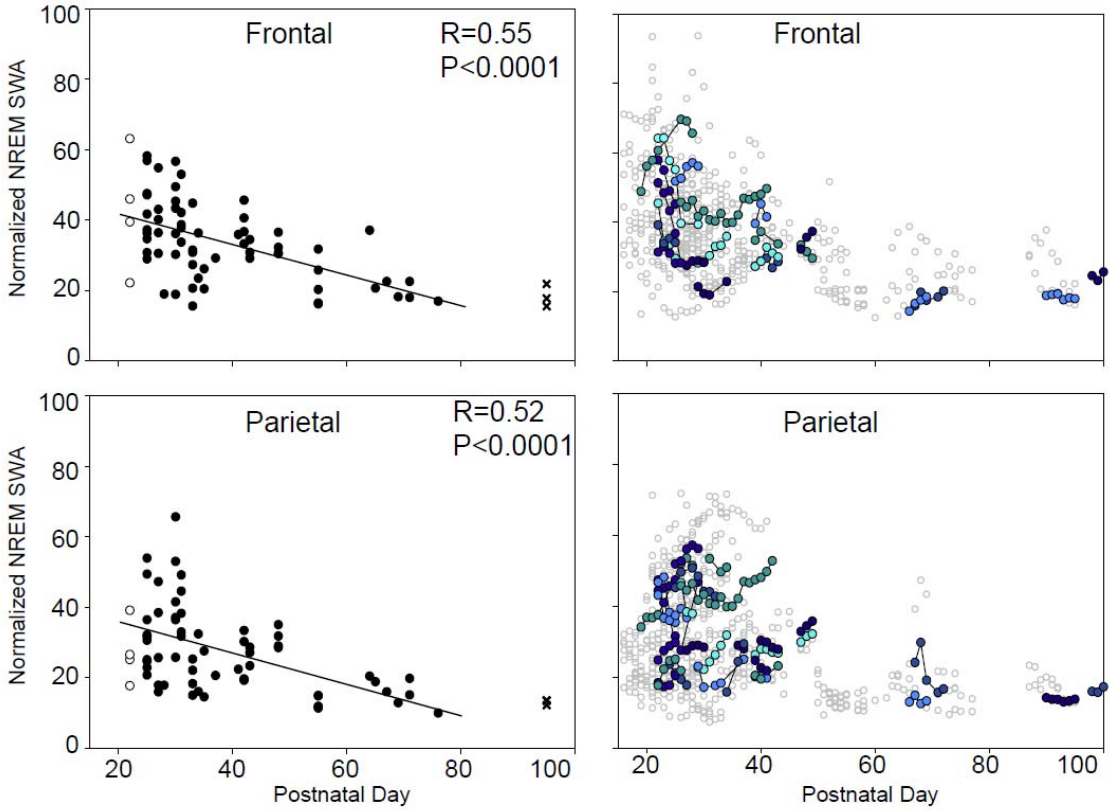
Results

NREM SWA decrease in mice across adolescence

88 male C57/BL-6, yellow fluorescent protein (YFP)-H expressing mice, ages ranging from P15-P87, were implanted and continuously recorded for 1-3 weeks. The baseline sleep-wake patterns and within day homeostatic changes in SWA have been described previously (Nelson et al 2013). Here for each mouse 1 scored baseline day that is at least 5 days post surgery is used to show the change in the NREM SWA across age (Figure 1 left). The daily NREM SWA mean decreased as a function of age in both the frontal (-0.4341 per day, R^2=0.30) and parietal derivation (-0.4452 per day, R^2=0.27) decreasing by approximately 50% in both derivations across the period of adolescence.

The recovery days from surgery and additional days were scored with an automated scoring system for the purpose of providing a comprehensive view of NREM SWA changes across age. The algorithm described in the methods section assigns vigilance states based on the motion detection, SWA and Theta to SWA ratio per epoch. For the purpose of this study the algorithm accuracy was verified by correlating the mean 24 hour NREM SWA determined by the algorithm with 153 manual scored days (R^2=0.9936) (Figure 8). The algorithm is not meant to replace manual scoring but can be relied on for the purpose of reporting a value as robust as is mean 24 hour NREM SWA on baseline days. Auto-scored days are shown for every full clean recording day, representing 580 days in total (Figure 1, right). A portion of the mice included in this study underwent an acute sleep deprivation; these days are excluded from the analysis. In order to include as many scored days as possible, especially in early adolescence, we display all recorded days including those that are within the week of recovery from surgery. The EEG implant surgery is typically less than 1 hour and mice recover very quickly, walking around and exploring within 5 minutes after the end of anesthesia. Since the skull is still growing, the chronic EEG implant is stable only for 10-14 days in adolescent mice.

Figure 1. NREM SWA on baseline days across adolescence. **Left** shows 1 manual scored baseline day per mouse for the Frontal (top) and Parietal (bottom). Regression includes only solid black dots. *P* and *R* are from the regression *f*=y0+*a***x*. Frontal slope= -0.4341 (± 0.0843) per day, Parietal slope= -0.4452 (± 0.0952) per day. **X** denotes adult animals, O denotes mice weaned prior to P17. **Right** is every recorded day auto-scored for all mice. Representative mice are set apart in color for emphasis to show the within animal change in SWA with age. These emphasized days are at least 4 days post surgery.



Sleep restriction experiment

Mice in two groups [P21 (5 mice), P26 (7 mice)] underwent chronic sleep restriction for 4 consecutive days as described in detail in the methods section. EEG and motion detection were recorded continuously during the deprivation. An example of the time course of NREM SWA and corresponding motion during the experiment are displayed for a P26 mouse (Figure 2). Each of the 4 days of sleep restriction included 12 hours of manual deprivation during the light period and 12 hours of automated deprivation by forced locomotion during the dark

period. During the light period if mice no longer reacted to the various stimuli they were allowed to sleep for 5-10 minutes. During the dark period the belt turned at a slow enough speed to allow mice to eat, drink and have brief sleep episodes. Following the 4 days of sleep restriction most mice had noticeably reduced NREM SWA (Figure 2 Recovery 1) but NREM SWA increased in the following days and returned to baseline levels after several recovery days (Figure 2, Recovery 4).

Figure 2. SWA and motion for a representative P26 sleep restriction mouse. Shown are the days P25-30, P33. Each panel shows the time course of SWA across each 24 hour period (SWA per 4 second epoch) and motion detection for 6 consecutive days including the undisturbed baseline day and 4 days of chronic sleep restriction consisting of alternating 12 hours manual deprivation and 12 hours of forced locomotion.

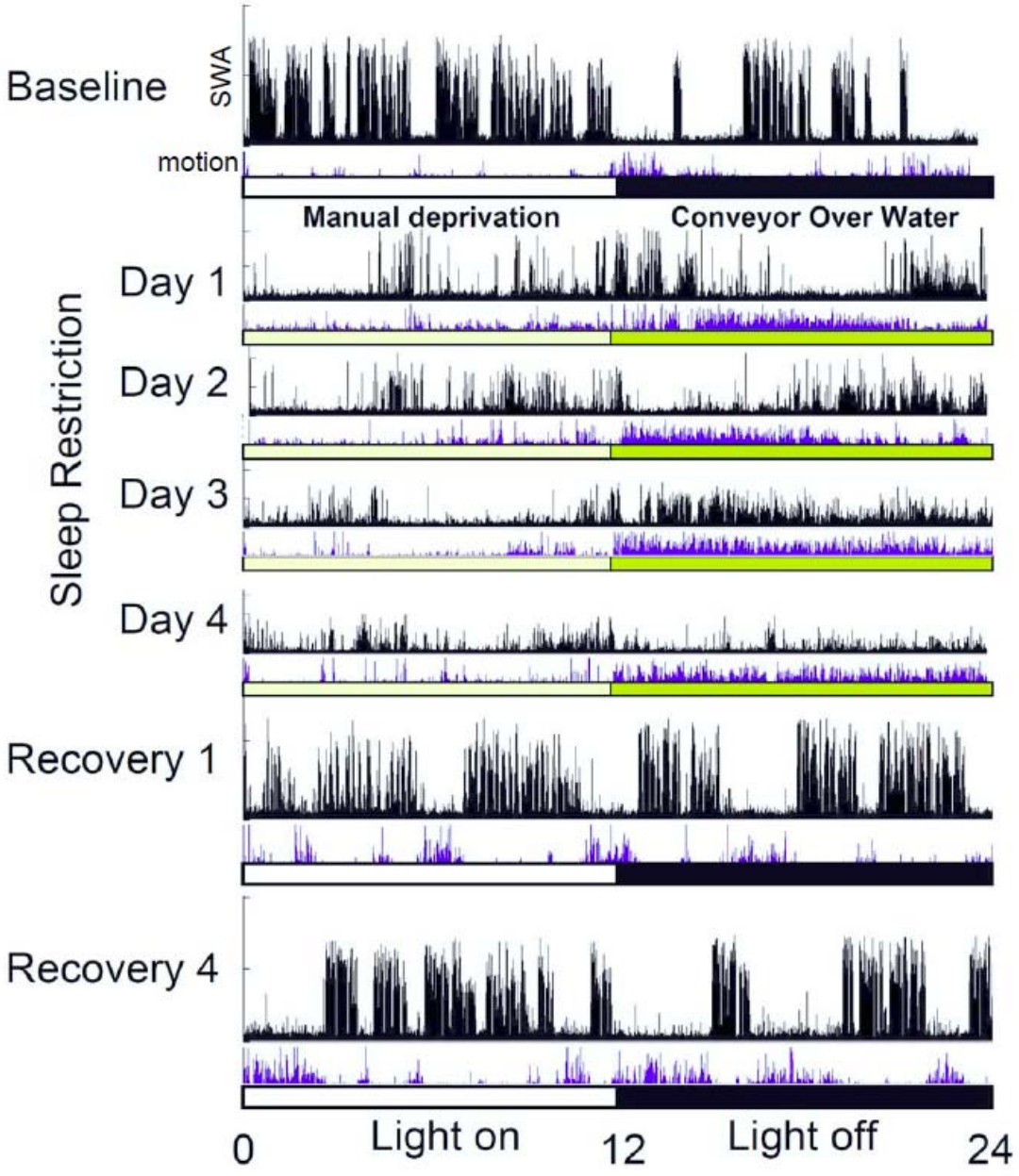


Figure 3. Mean daily vigilance states. 24 hour time course of time per day spent in wake, NREM and REM. States stacked are with a y-axis that is 1440 minutes = 24 hours to display the portion of each day in a given state. **Top** mean values from mice undergoing sleep restriction from P21-24 (n=5). **Bottom** mice undergoing restriction from P26-29 (n=7).

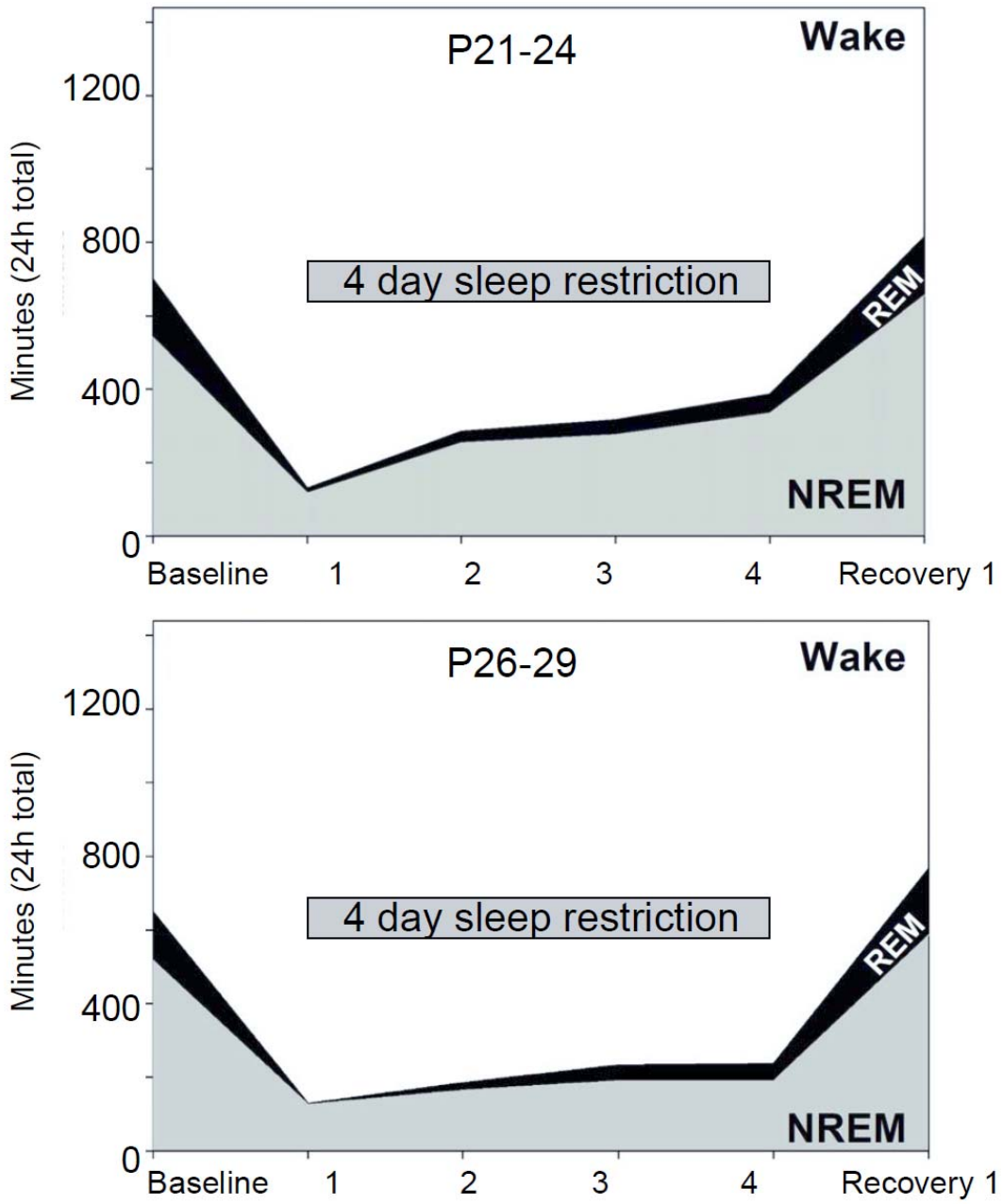
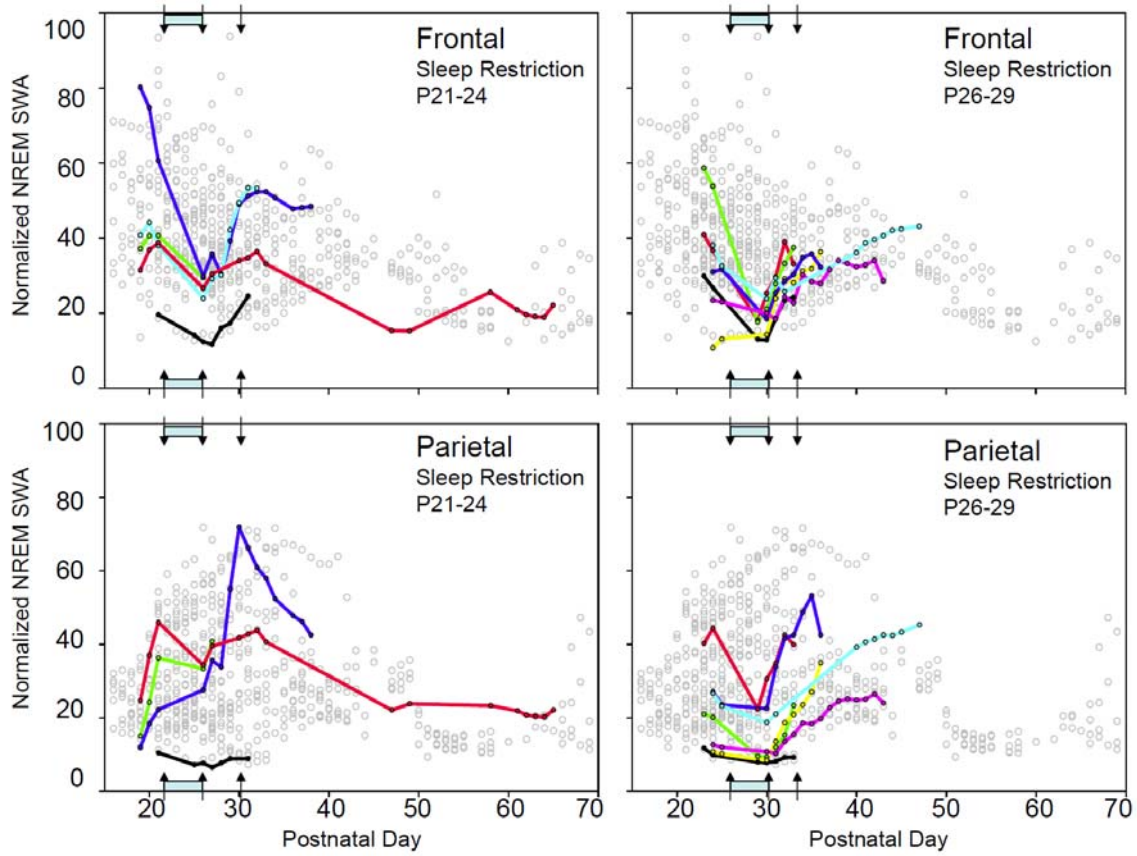


Table 1. Mean change in sleep states relative to baseline. Values given are the mean of % change relative to the baseline day for each mouse on sleep restriction day 1-4 (SR 1-4) and the first day of recovery (Rec1).

	SR1	SR2	SR3	SR4	Rec1
P21 NREM	**- 74.43**	**- 42.50**	**- 47.70**	**- 34.42**	**+ 34.64**
P21 REM	**- 91.06**	**- 75.56**	**- 73.35**	**- 66.31**	**+ 11.34**
P21 Sleep	**- 81.06**	**- 58.56**	**- 54.63**	**- 44.15**	**+ 17.87**
P26 NREM	**- 74.94**	**- 68.78**	**- 63.54**	**- 62.53**	**+ 13.52**
P26 REM	**- 97.71**	**- 84.30**	**- 67.00**	**- 64.94**	**+ 38.63**
P26 Sleep	**- 79.73**	**- 71.82**	**- 64.19**	**- 63.08**	**+ 18.26**

The baseline day, each day of deprivation and the first day of recovery were manually scored for all mice to determine the efficiency of the deprivation. Total sleep was reduced for the duration of the restriction such that mice were far behind the total amount of sleep expected based on the baseline value (P21=1666 minutes, P25=1809 minutes, Figure 3). On the first day of the sleep restriction the total time slept was reduced by 80 percent for both age groups (Table 1). The periods of sleep occurring on the first day were not long enough to allow consolidated REM periods to occur. Thus, REM sleep was almost entirely prevented except in one P21 mouse that was able to achieve nearly 60 minutes of REM sleep during the course of the first day. By the last day of the deprivation REM occurred more frequently and sleep onset REM bouts were common in both age groups. The accumulated sleep debt for NREM across this study was 1099.32 minutes per animal for the P21 mice and 1396.18 minutes for P26 mice. On the first day of recovery mice had only a modest increase in NREM sleep compared to baseline (P21=+161.8, P26=+69.3 minutes, Figure 3), which returned to baseline levels by Recovery 4. Thus, it is unlikely that the lost sleep would ever be recovered in terms of duration, as full recovery would require 6-20 days similar to Recovery 1. As was the case with NREM sleep, there was a substantial REM sleep debt accrued (P21=494.06, P26=412.93 minutes). Based on the duration of the REM sleep rebound during Recovery 1, it is unlikely that the REM sleep debt could be repaid in full in subsequent days.

Figure 4. Change in NREM SWA after chronic sleep restriction. Each gray circle is an auto-scored day from non-deprived mice (as in figure 1). **Top** Frontal NREM SWA **Bottom** Parietal NREM SWA. Shown with color lines is the mean 24 hour NREM SWA for non-sleep restriction days for each individual mouse that underwent chronic sleep restriction (**Left** P21-24, **Right** P26-29). The period of sleep restriction is denoted with a colored light blue bar and arrows mark 3 days compared in subsequent figures (Baseline, Recovery 1 and Recovery [4/5]). The mean NREM SWA during the restriction is not shown.



Change in NREM SWA after chronic sleep restriction

As was done with the baseline days of non-sleep restricted mice, all good non-sleep restriction days for the P21 and P26 mice were auto-scored and the NREM SWA for each day is shown in Figure 4, with the baseline days from non-deprived mice shown in light grey for reference. In several animals we were able to obtain an EEG recording for a week or more following the last day of restriction. The frontal derivation for both groups shows a consistent pattern across animals, with a decrease across the sleep restriction period followed by a steady and reliable increase in NREM SWA during the recovery days (Figure 4). In the parietal derivation the P26 group showed the same pattern as in the frontal derivation, while there is no clear pattern in the P21 group, likely because of the smaller sample size (n=4 recovery 1, n=3 recovery 5).

The sleep restricted mice were compared to a cohort of non-deprived animals (n=5 for each group) that had surgeries between P18 and P22 and were left to record undisturbed for the same duration as the sleep deprived animals. Note that one age-matched animal was dropped prior to the oldest recovery date (P26, Recovery 5, P33), so there are only four controls for that day. The baseline NREM SWA was significantly lower for the parietal derivation baseline days at P25 for the older deprivation group, otherwise baseline days were similar between groups. Following sleep deprivation, the frontal derivation for the P21 and P26 group was significantly lower and nearly half the value of the control group at the same age. By Recovery 4/5 NREM SWA values returned to near baseline values and there was no significant difference between the sleep restricted mice and the control group (Figure 5). There is still a trend difference ($p=0.057$) at Recovery day 4 for the P26 group but this is largely attributable to a significantly lower initial baseline NREM SWA (Figure 5,6 bottom right).

To remove variability associated with different initial baseline values all days were normalized to the day prior to sleep restriction (Figure 6). Similar to what was shown in Figure 5, in the frontal derivation there was a significant difference between Recovery 1 and the control day in both age groups. Control mice had a moderate increase while deprived mice had a profound 40% decrease in NREM SWA (Figure 6, top). By contrast, there was no difference at recovery day 4/5. After normalizing to the baseline day the parietal derivations show a pattern similar to that described for the frontal derivations (Figure 6, bottom).

Figure 5. Comparison of NREM SWA to normal maturation. For each deprivation group animals are compared to a cohort of age-matched controls that were implanted near the same age and left undisturbed for the entire period of recording. Values are the 24 hour normalized NREM SWA determined from the auto-scoring algorithm. The days compared are noted as arrows in figure 4. Significance is for two-tailed t-tests comparing the sleep restriction day with the age-matched corresponding day. * = $p<0.05$, # = $p<0.10$

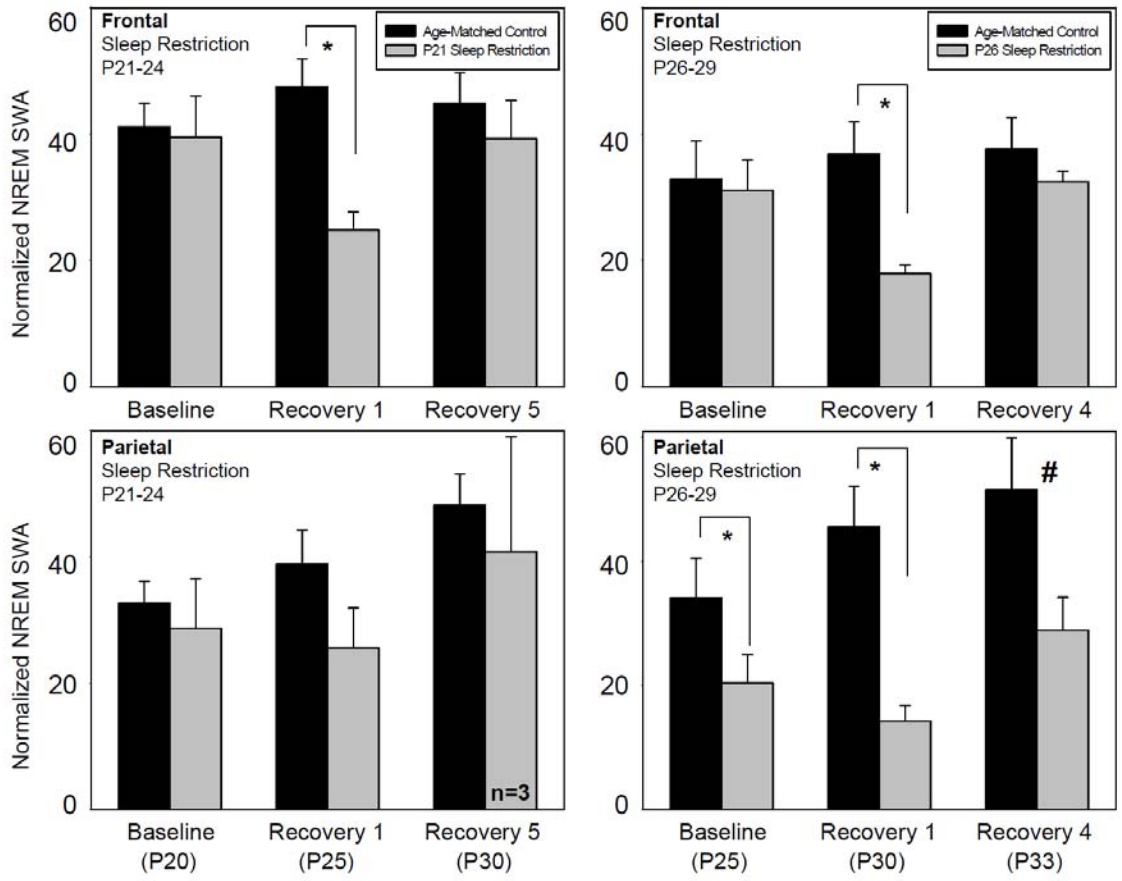
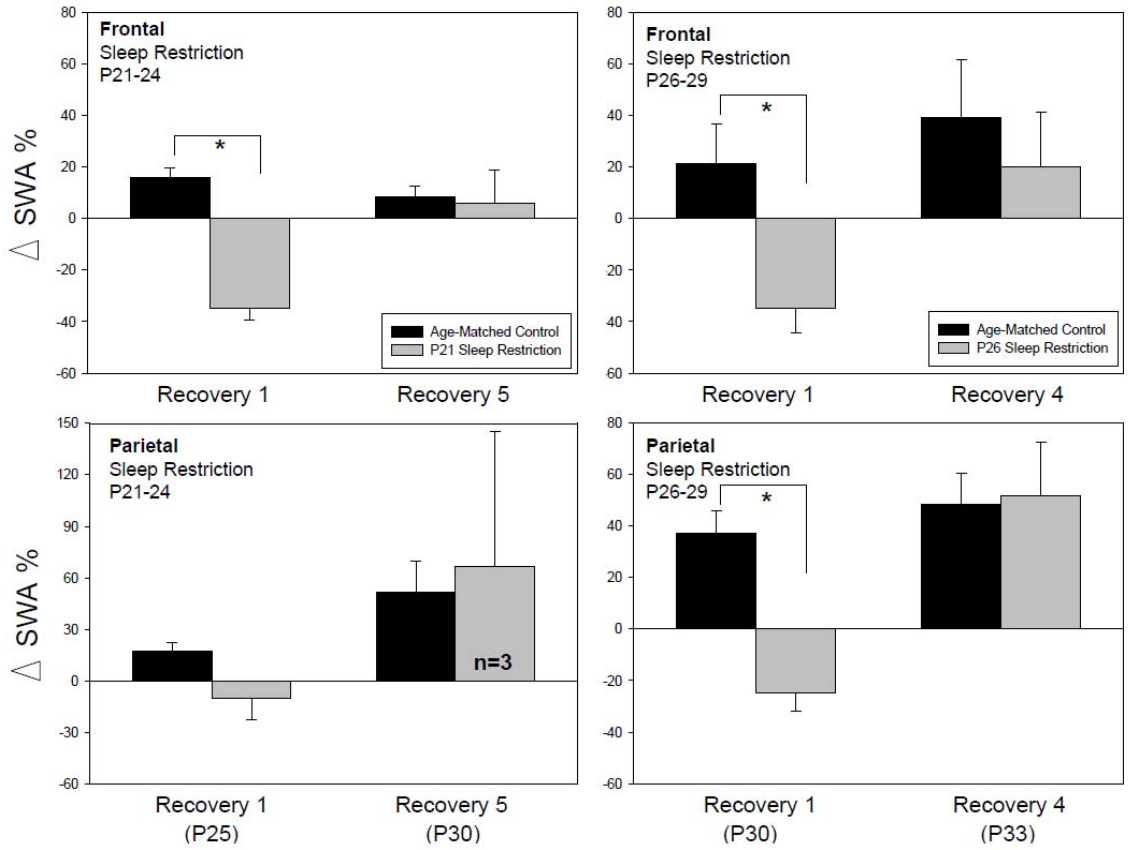


Figure 6. Comparison of change in 24 hour NREM SWA relative to baseline day. The mean 24 hour NREM SWA (auto-scored) for two recovery days (noted as arrows in figure 4) for mice undergoing chronic sleep restriction are shown as a percent change from the baseline day, for age-matched controls the corresponding days are referenced similarly. Significance displayed is for two tailed t-test comparing the % change from baseline for sleep restricted mice to that of the age-matched controls. * = $p<0.05$.



Methods

Recordings of Sleep and Locomotor Activity and Sleep Deprivation

Male YFP-H mice (Jackson Laboratory, Bar Harbor, Maine) were maintained in a colony room on a 12 h light/12 h dark cycle (lights on at 8 a.m.) with food and water available *ad libitum*. Mice (P15–P87) were implanted for

chronic polysomnographic recordings under isoflurane anesthesia (1%–2% in 100% O_2). Prior to surgery, all electrodes were directly soldered to flexible wires (#NUF30-4046, Cooner Wire, Chatsworth, CA, USA). Gold plated miniature screw electrodes (0.7 mm diameter) were placed over the right and left frontal (anteroposterior, AP, +1 mm from bregma; mediolateral, ML, 1 mm), and parietal (AP −2 mm; ML 2 mm) cortices and one over cerebellum (AP −1 mm from lambda) as reference. During placement special care was made to advance the screws the minimum amount to remain fixed (typically <1 turn). Two vinyl-coated braided stainless steel wire electrodes (#AS636, Cooner Wire) were placed in the nuchal muscle for electromyogram (EMG) recording. Electrodes were insulated and affixed to the skull using dental cement. Following surgery, mice were housed individually in sound-attenuating, environmentally controlled recording chambers (12:12 LD, lights on at 8 a.m., 25 °C ± 1 °C, food and water *ad libitum*). All electrodes were gathered into a flexible cable and connected to the Multichannel Neurophysiology Recording system (Tucker-Davis Technologies, TDT, Alachua, FL, USA). EEG and EMG signals were collected continuously at a sampling rate of 256 Hz (digitally filtered between 0.1 and 100 Hz). For sleep staging, signals were processed by custom-made Matlab scripts (Mathworks, Natick, MA) using standard TDT routines and subsequently converted into European Data Format (EDF) with Neurotraces software (Fort Lauderdale, FL, USA). Twenty-four hour polygraphic recordings were scored offline for NREM sleep, REM sleep, and wake by visual inspection of 4-s epochs (SleepSign; Kissei Comtec, Irvine, CA, USA) according to standard criteria. Wake was characterized by low voltage, high frequency EEG pattern and phasic EMG activity. NREM sleep was characterized by the occurrence of high amplitude slow waves and low tonic EMG. During REM sleep the EEG was similar to that during wake, but only heart beats and occasional twitches were evident in the EMG signal. The average EEG power spectra for the fronto-cerebellar and parieto-cerebellar derivations (0.0−30 Hz) was computed using averaged periodograms from an FFT routine using consecutive 4-s Hanning windows. Absolute SWA (0.5−4.0 Hz) and SWA relative to NREM high frequencies (15-30 Hz), a previously described normalization were computed. The normalization to high frequencies was used to control for possible daily changes in signal strength due to technical issues (progressive deterioration of the signal, skull growth, *etc*.). Motor activity was quantified by custom-made video-based motion detection algorithms with a time resolution of 1 sec (Matlab) (Maret et al., 2011).

Chronic sleep restriction

The chronic sleep restriction lasted in total 96 hours starting at lights-on for the first day and ending at lights-on at the end of day 4. Each day consisted of a period of manual deprivation and automated deprivation (Figure 2, Figure 7). During the 12 hours of the light period, during which rodents typically sleep, mice were kept awake by presenting them with novel objects, running wheel access, social interaction with un-implanted litter mates and by changing the cage and bedding. When mice were unable to stay awake in response to the above techniques they were allowed a brief nap (5-15 minutes), after which the deprivation resumed. Sleep deprivation was aided by giving mice caffeinated water (0.1 mg/ml). The room lights were turned off for portions of the first 6 hours of the light period to provide an additional stimulus to stay awake. Dosages of caffeine are not easily translated from humans to mice based on the differences in the percentage of metabolically active tissue and the shorter half life of caffeine in rodents compared to humans. A caffeine concentration of 0.1 mg/ml in the drinking water was chosen since this dose results in plasma concentrations of caffeine in mice similar to those reported in most coffee drinkers (Lu et al., 2007).

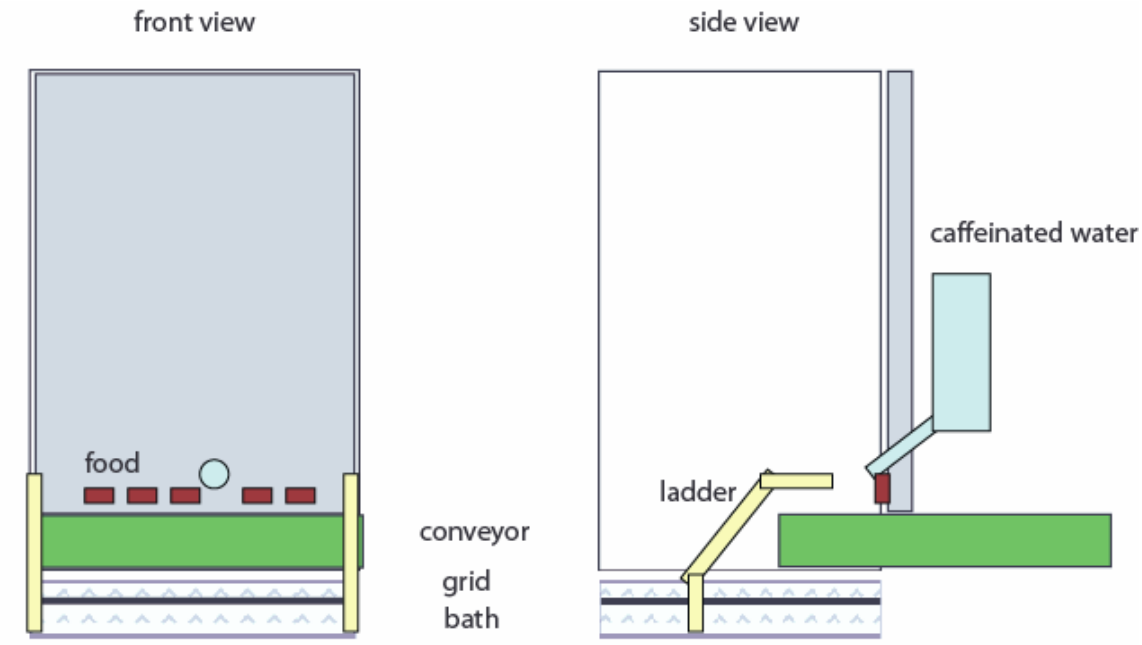


Figure 7

Diagram of the conveyor-over-water (COW) apparatus modified from the device described in (Newman et al 2009). Cameras were mounted above the apparatus and an IR light source provided heat to help maintain body temperature. Food pellets were individually attached to the far wall with wire. The surface provided by the ladder, water spout and food pellets offered perches that did not move but were not large enough that mice could comfortably sleep.

During the 12 hours of the dark period mice were transferred from their recording cages to a conveyer over water (COW) deprivation apparatus. This device is described in detail in Newman et al 2009 and was chosen because it is similar to the disk-over-water apparatus used in numerous studies (Rechtschaffen et al., 1999; Newman et al., 2008; Leemburg et al., 2010), but was adapted to meet the challenges associated with depriving EEG implanted immature mice. The conveyer was run continually at a slow enough speed that mice were able to sleep for brief periods (<8 seconds) before being forced to move, or fall in the water. Two key modifications insured the safety of the mouse: 1) a grid was placed under the surface of the water such that a mouse could stand without having to swim 2) custom-made mouse ladders were fixed to the side walls of the apparatus, so that after falling in the water mice could return

to the moving platform easily even with the belt in motion. Additionally, mice were remotely monitored while on the COW; if a mouse appeared unable to continue with the automated deprivation an experimenter would intervene, allowing the mouse a period of recovery sleep prior to continuing the deprivation. All mice survived the deprivation procedure, for one mouse (P21 group) the procedure was ended after 3 days because it was apparent it could not safely continue the deprivation.

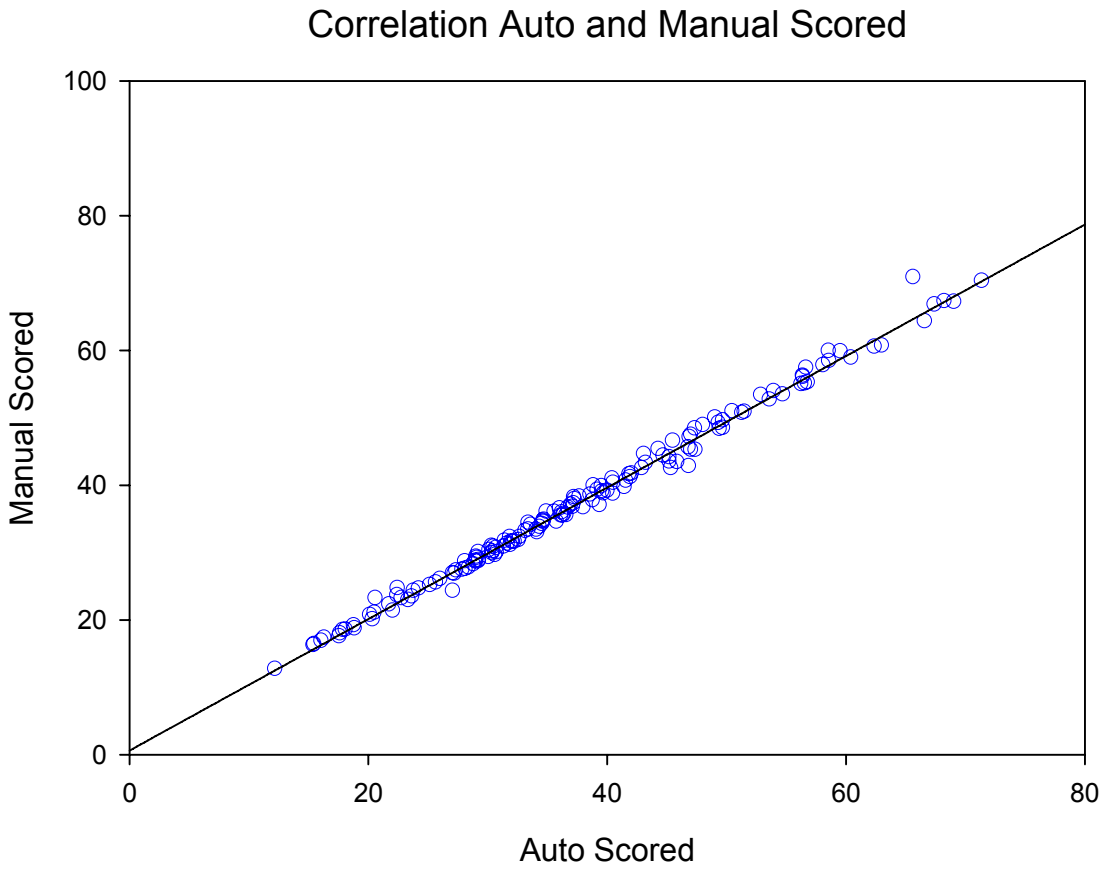
For all mice having surgery before P20 special care was taken to make sure they received proper nutrition, following the recommendation of the veterinary staff. Specifically, in addition to the normal food pellets (given to allow chewing/gnawing), these mice received the breeder diet (8626 Teklad Mouse breeder diet, Harlan laboratories, Madison, WI, USA), which is softer and has a higher fat content. Additionally, 2 pellets of breeder diet were softened in water and replaced daily with fresh softened pellets until there was clear visual evidence of the solid pellets being consumed. Each mouse was given special bedding material made from pulped cotton fiber for maintaining warmth when single housed (NestlestsT, Ancare, Bellmore, NY, USA). For the youngest animals a portion of the bedding from the home cage was transferred to the recording cage so that a nest was available immediately after surgery.

All animal procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and facilities were reviewed and approved by the IACUC of the University of Wisconsin-Madison, and were inspected and accredited by AAALAC.

Vigilance State Auto-Scoring Algorithm

The auto-scoring algorithm is a modified version of the motion based sleep detection algorithm used in (Maret et al., 2011). Sleep was scored in 4 second epochs and vigilances states determination was applied to every epoch. The majority of wake epochs are determined by the simple criteria that a moving mouse, determined by video based motion detection, is not a sleeping mouse. Sleep states are then assigned based on minimum thresholds for: 1) delta band power (0.5-4.0 Hz) from the frontal lead and 2) the ratio of theta band power (5.0-7.5) and delta band power in the parietal lead. Aside from replacing EMG with motion detection this method is similar to a semi-automated

system used in seminal rodent sleep papers (Trachsel et al., 1988). For each day a first pass identified NREM and REM epochs during periods of inactivity using restrictive criteria for each state. Epochs not initially passing stringent thresholds to match with a vigilance state were subsequently assigned states using an iterative process which relaxed criteria based on the nearest previously assigned epoch. We verified the accuracy of the script by comparing the correspondence with 156 manual scored baseline days. Of particular importance for this study, the automated scoring provides a near perfect match to manual scoring for the normalized mean 24 hour NREM SWA, which is the focus of the study.



.

Figure 8

A comparison of the frontal mean 24 hour NREM SWA (high frequency normalized 0.5-4.0 / 15-30 hz). There is a near perfect relationship between manual SWA and auto-scored SWA. (R=0.9968, comparison of 156 scored days)

Statistical Analysis.

All statistics were performed using Matlab 2010 statistics toolbox (Mathworks, Natick, MA, USA). Figures were produced using SigmaPlot (Systat Software, Inc, San Jose, CA, USA)

Discussion

SWA decreases with age

We show here that NREM SWA in mice decreases during adolescence. This decrease is similar in its scale and timing to what has been shown previously in humans (Feinberg and Campbell, 2009; Kurth et al., 2010; Buchmann et al., 2011). The earliest reliable EEG recordings were collected at P23, after allowing 5 days of recovery from surgery. From this point, which corresponds to the beginning of adolescence in mice, both frontal and parietal derivations showed a progressive SWA decrease that continued beyond P60. We additionally show the NREM SWA values for additional days during the recovery period from implant surgery, including days prior to P20. In the frontal derivation there is considerable variability in SWA levels during this early period, but the overall declining trend is the same as occurs at later ages. In the parietal derivation there is less variability in these early days, such that there appears to be an initial increase in SWA prior to P20, before a progressive decrease occurs. However, interpretation of these data collected close to surgery is difficult.

Our results show that during adolescence mice undergo a long term decrease in NREM SWA across age. In humans this decrease has been proposed to be accounted for by developmental synaptic pruning. Future work will systematically investigate within the same mouse model, EEG power spectra changes and changes in *in vivo* 2p microscopy measured spine density as well as molecular markers of synaptic strength. Utilizing these three complementary methods we will be able to directly test to what extent synaptic pruning underlies maturational changes in NREM SWA.

Daily changes in SWA

The trend across age is for a decrease but looking at the within animal changes in NREM SWA from 1 day to the next reveals that temporally local changes in SWA follow a more complicated pattern than of a linear decrease with age. This more complicated pattern is also suggested by the uneven distribution of residuals to the linear fit for both the frontal and parietal derivation in (Figure 1 left). In the frontal derivation early implanted mice are variable with different animals having both steep increases and decreases at the same age (Figure 1 right). Generally, for the

frontal derivation mice have a steep decrease around P23 before levelling off prior to a period of increase from P30 to P40. Then just prior to the adulthood (>P60) there appears to be a final period of decreasing SWA. In the parietal derivation despite a long term trend for a decrease, within each mouse there is paradoxically often an increase in mean SWA. What can explain this variability? One possible explanation is that mice will move and explore less following surgery, which may locally decrease cortical activity and therefore locally decrease NREM SWA (Huber et al., 2006; Huber et al., 2007; Hanlon et al., 2009). As mice recover they increase locomotion and exploration. Such a pattern would lead to each day having a local increase in use dependent SWA even as there might be a very slow long term trend for a decrease in SWA (Huber et al., 2007; Hanlon et al., 2009).

Reversible Change in SWA following Sleep Restriction

Chronic sleep restriction during early adolescence resulted first in a profound decrease in NREM SWA followed by a recovery period that brought SWA back to the values observed in non-deprived mice. Thus, young adolescent mice respond to chronic sleep loss in a manner that differs greatly from that seen after a few hours of sleep deprivation, after which there was no SWA decline (Nelson 2013). In the same strain of mice, we have shown that during adolescence a few hours of spontaneous or forced waking result in a small net increase in synaptic density, while sleep leads to a net decrease (Maret et al., 2011). The extent to which changes in synaptic density explain the changes in the EEG signal observed after chronic sleep loss will be addressed in future experiments in which spine density and EEG are recorded within the same mouse. Of note, the SWA decline after chronic sleep restriction is reversible, suggesting that even if synaptic changes occur and underlie the SWA changes, they are not permanent.

The adolescent mice also respond to chronic sleep loss in a manner that differs greatly from that seen in adult rats (Leemburg et al., 2010). That study found that the established measures of sleep pressure and specifically NREM SWA are maintained during the chronic sleep deprivation. This maintenance occurs in large part due to an increase in waking SWA that makes up for and explains much of the “missing” SWA during NREM sleep (Leemburg et al., 2010). These adult rats, which were allowed 4 consolidated hours of sleep per day, had no long term changes in SWA. Meanwhile, our adolescent mice, which were allowed short and fragmented sleep opportunities throughout the 24-hour period that amounted to roughly 4 hours/day, experienced a profound decrease in NREM SWA. Aside

from the different age and species, differences in the deprivation procedure may explain this difference. In the Leemburg study rats were allowed normal extended sleep, which included an alternating period of NREM and REM sleep. In the current study mice had disrupted sleep for the full 96 hours of the restriction that prevented normal cycling of NREM and REM sleep. Thus, chronic fragmentation of sleep seems more disruptive for long term SWA dynamics than chronic sleep loss that maintains some consolidated sleep.

References

America Si (2006) Summary of findings: 2006 Sleep in America Poll. In. Washington D.C.: National Sleep Foundation.

Amzica F, Steriade M (1998) Electrophysiological correlates of sleep delta waves. Electroencephalogr Clin Neurophysiol 107:69-83.

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21:1133-1145.

Banks S, Van Dongen HP, Maislin G, Dinges DF (2010) Neurobehavioral dynamics following chronic sleep restriction: dose-response effects of one night for recovery. Sleep 33:1013-1026.

Borbély AA, Achermann P (2005) Sleep homeostasis and models of sleep regulation. . In: Principles and Practice of Sleep Medicine. (MH K, T R, WC D, eds), pp 405-417. Philadelphia W. B. Saunders.

Buchmann A, Ringli M, Kurth S, Schaerer M, Geiger A, Jenni OG, Huber R (2011) EEG sleep slow-wave activity as a mirror of cortical maturation. Cereb Cortex 21:607-615.

Campbell IG, Feinberg I (2009) Longitudinal trajectories of non-rapid eye movement delta and theta EEG as indicators of adolescent brain maturation. Proc Natl Acad Sci U S A 106:5177-5180.

Chugani HT (1998) A critical period of brain development: studies of cerebral glucose utilization with PET. Prev Med 27:184-188.

Chugani HT, Phelps ME, Mazziotta JC (1987) Positron emission tomography study of human brain functional development. Ann Neurol 22:487-497.

Contreras D, Steriade M (1995) Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. J Neurosci 15:604-622.

Esser SK, Hill SL, Tononi G (2007) Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. Sleep 30:1617-1630.

Feinberg I, Campbell IG (2009) Sleep EEG changes during adolescence: an index of a fundamental brain reorganization. Brain Cogn 72:56-65.

Feinberg I, Davis NM, de Bie E, Grimm KJ, Campbell IG (2012) The maturational trajectories of NREM and REM sleep durations differ across adolescence on both school-night and extended sleep. Am J Physiol Regul Integr Comp Physiol 302:R533-540.

Hagenauer MH, Perryman JI, Lee TM, Carskadon MA (2009) Adolescent changes in the homeostatic and circadian regulation of sleep. Dev Neurosci 31:276-284.

Hanlon EC, Faraguna U, Vyazovskiy VV, Tononi G, Cirelli C (2009) Effects of skilled training on sleep slow wave activity and cortical gene expression in the rat. Sleep 32:719-729.

Huber R, Deboer T, Tobler I (2000) Topography of EEG dynamics after sleep deprivation in mice. J Neurophysiol 84:1888-1893.

Huber R, Tononi G, Cirelli C (2007) Exploratory behavior, cortical BDNF expression, and sleep homeostasis. Sleep 30:129-139.

Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G (2006) Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat Neurosci 9:1169-1176.

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195-205.

Huttenlocher PR, Dabholkar AS (1997) Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 387:167-178.

Jones SG, Vyazovskiy VV, Cirelli C, Tononi G, Benca RM (2008) Homeostatic regulation of sleep in the white-crowned sparrow (Zonotrichia leucophrys gambelii). BMC Neurosci 9:47.

Kurth S, Ringli M, Geiger A, LeBourgeois M, Jenni OG, Huber R (2010) Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. J Neurosci 30:13211-13219.

Leemburg S, Vyazovskiy VV, Olcese U, Bassetti CL, Tononi G, Cirelli C (2010) Sleep homeostasis in the rat is preserved during chronic sleep restriction. Proc Natl Acad Sci U S A 107:15939-15944.

Lopes da Silva FH, Wieringa HJ, Peters MJ (1991) Source localization of EEG versus MEG: empirical comparison using visually evoked responses and theoretical considerations. Brain Topogr 4:133-142.

Lu YP, Nolan B, Lou YR, Peng QY, Wagner GC, Conney AH (2007) Voluntary exercise together with oral caffeine markedly stimulates UVB light-induced apoptosis and decreases tissue fat in SKH-1 mice. Proc Natl Acad Sci U S A 104:12936-12941.

Maret S, Faraguna U, Nelson AB, Cirelli C, Tononi G (2011) Sleep and waking modulate spine turnover in the adolescent mouse cortex. Nat Neurosci 14:1418-1420.

Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G (2004) The sleep slow oscillation as a traveling wave. J Neurosci 24:6862-6870.

Newman SM, Paletz EM, Rattenborg NC, Obermeyer WH, Benca RM (2008) Sleep deprivation in the pigeon using the Disk-Over-Water method. Physiol Behav 93:50-58.

Paus T, Keshavan M, Giedd JN (2008) Why do many psychiatric disorders emerge during adolescence? Nat Rev Neurosci 9:947-957.

Petanjek Z, Judas M, Simic G, Rasin MR, Uylings HB, Rakic P, Kostovic I (2011) Extraordinary neoteny of synaptic spines in the human prefrontal cortex. Proc Natl Acad Sci U S A 108:13281-13286.

Rakic P, Bourgeois JP, Goldman-Rakic PS (1994) Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. Prog Brain Res 102:227-243.

Rechtschaffen A, Bergmann BM, Gilliland MA, Bauer K (1999) Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat. Sleep 22:11-31.

Riedner BA, Vyazovskiy VV, Huber R, Massimini M, Esser S, Murphy M, Tononi G (2007) Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. Sleep 30:1643-1657.

Steriade M, Nunez A, Amzica F (1993) Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci 13:3266-3283.

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

Tononi G, Cirelli C Time to be SHY? Some comments on sleep and synaptic homeostasis. Neural Plast 2012:415250.

Trachsel L, Tobler I, Borbely AA (1988) Electroencephalogram analysis of non-rapid eye movement sleep in rats. Am J Physiol 255:R27-37.

Van Dongen HP, Maislin G, Mullington JM, Dinges DF (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 26:117-126.

Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G (2009) Cortical firing and sleep homeostasis. Neuron 63:865-878.

Werth E, Achermann P, Borbely AA (1996) Brain topography of the human sleep EEG: antero-posterior shifts of spectral power. Neuroreport 8:123-127.

Williams JA, Zimmerman FJ, Bell JF (2013) Norms and trends of sleep time among US children and adolescents. JAMA Pediatr 167:55-60.

Yang G, Gan WB (2012) Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. Dev Neurobiol 72:1391-1398.