Center for Sleep & Respiratory Neurobiology

Inaugural Research Retreat

June 17, 2004

Levy Conference Center at the
University of Pennsylvania Law School

University of Pennsylvania

Program and Abstracts

PROGRAM

8:30-9:00	Poster Mounting
9:00-11:00	Morning Symposium *New Findings on Monoaminergic Transmitter Pathways and Regulation of Behavioral State*
Speakers:	Amita Sehgal, Ph.D.: *The Role of Serotonin in Regulating Circadian Rhythms*
	Gary Aston-Jones, Ph.D.: *Brain Mechanisms for Circadian Regulation of Arousal and Performance*
	Steven A. Thomas, M.D., Ph.D.: *Adrenergic Signaling Plays a Critical Role in the Maintenance of Waking and in the Regulation of REM Sleep*
	Sigrid Veasey, M.D.: *Sleep Apnea: Oxidative Injury to Monoaminergic Wake-Active Neuron Groups*
11:00-11:15	Coffee Break
11:15-12:30	Data blitz

Carole Marcus, M.D.
Lan Chen, M.D.
David Raizen, M.D., Ph.D.
Denys Volgin, Ph.D.
Darshil Amin
Julie Williams, Ph.D.
Jini Naidoo, Ph.D.

12:30-1:30	Lunch and poster previewing
1:30-2:30	Keynote speaker: Masashi Yanagisawa, M.D., Ph.D. *"Sleep/Wake, Energy Homeostasis, and Orexin Neuropeptides"*
2:30-4:00	Afternoon Symposium
Speakers:	Allan Pack, M.B., Ch.B., Ph.D.: *Genetic/Genomic Approaches to Sleep Apnea*
	David Dinges, Ph.D.: *Neurobehavioral Function and Elevated Sleep Pressure*
	Leszek Kubin, Ph.D.: *Regulation of Sleep by Posterior Hypothalamic GABAA Receptors*
4:00-6:00	Poster viewing and Reception
5:00	Trainee Award Presentation

FROM THE DIRECTOR:

This is the first full-day Research Retreat of the Center for Sleep and Respiratory Neurobiology (CSRN). The idea for this arose from the Executive Committee of the Center that includes Dr. David Dinges, Dr. Leszek Kubin, Dr. Samuel Kuna, Dr. Richard Schwab and Dr. Amita Sehgal. We decided that the best approach to organizing this was to invite our junior faculty and trainees to take ownership and responsibility for this. We are extremely grateful to the organizing committee of Dr. Marcos Frank, Dr. Grace Pien, Dr. Hans Van Dongen and Dr. Julie Williams, who have done an outstanding job. This promises to be an outstanding event and we are particularly delighted that we have received 50 abstracts. We are fortunate, at the University of Pennsylvania, to have a relatively large and very collegial group of faculty pursuing research in sleep and its disorders. It is a diverse group and this Retreat will further contribute to ensuring that we have a community of scholars and will be an important part of the "glue" that the CSRN fosters.

Allan I. Pack, M.B., Ch.B., Ph.D.
Professor of Medicine and
Director, Center for Sleep and Respiratory Neurobiology

Keynote Speaker:
Masashi Yanagisawa, M.D., Ph.D.

We are delighted to have as our guest and keynote speaker Masashi Yanagisawa, M.D., Ph.D., professor and holder of the Patrick E. Haggerty Distinguished Chair in Basic Biomedical Science at the University of Texas Southwestern Medical Center at Dallas. Born in Tokyo, Japan, Dr. Yanagisawa received his medical degree in 1985 and his doctorate in 1988 from the University of Tsukuba, Japan. Dr. Yanagisawa was recruited to the University of Texas Southwestern as an associate professor of molecular genetics in 1991 and was promoted to professor in 1996.

Although the sleep research community is most familiar with Dr. Yanagisawa's work on the discovery of orexin, Dr. Yanagisawa has received significant attention since the late 1980's with his discovery of endothelins, vasoconstrictive peptides derived from vascular endothelial cells that participate in the development of hypertension and atherosclerosis and mediate cardiac hypertrophy and remodeling in congestive heart failure. Although endothelins were initially identified for their vasoactive role, they are now known to exert diverse biological effects on a wide variety of tissues and cell types, with effects on regulation of airway tone, modulation of renal acid-base balance and the CNS cardiorespiratory centers.

In 1998, the journal Cell published the work of Dr. Yanagisawa and his collaborators on 2 novel neuropeptides, orexin-A and –B. The initial description of the function of these peptides in the integrated control of feeding and energy homeostasis was quickly followed by recognition of their role in the regulation of sleep and wakefulness states. Dr. Yanagisawa's work using the orexin knockout mice, with a phenotype strikingly similar to both canine and human narcolepsy, has been followed by further work on the relationship between sleep, wakefulness, energy homeostasis and orexin neuropeptides, which forms the basis of his keynote presentation.

Among Dr. Yanagisawa's many honors are his election to the National Academy of Sciences, the J.J. Abel Award from the American Society of Pharmacology and Experimental Therapeutics, the Novartis Award from the American Heart Association, the Kilby Award from the Kilby Awards Foundation, the Amgen Award from the American Society of Biochemistry and Molecular Biology, and the Special Recognition Award from The Sleep Research Society.

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Investigating the Relationship between Subjective Assessment of Sleep & Mood

McGlinchey E, Rogers N, Rider R, Dorrian, J, Dinges D.
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Introduction: Alterations in subjective assessments of sleep quality and quantity have been associated with changes in mood, with decreased sleep quality typically associated with increased mood disturbance. This relationship is thought to be present particularly in individuals suffering from insomnia, but has not been examined widely in normal sleepers. Typically this relationship is examined via alterations in sleep duration and not manipulations of mood. The aim of the current analysis was to examine the relationship between subjective assessments of sleep and mood in a population of healthy subjects undergoing a stress induction protocol.

Methods: Sixty healthy subjects (29m; 31f; aged 22-45y) who reported no problems with their sleep completed a single-day in-laboratory protocol. Subjects completed a range of psychometric and sleep questionnaires, including the Pittsburgh Sleep Quality Index (PSQI) and self reported sleep duration on the previous night. During the testing session subjects completed neurobehavioral assessment batteries (NAB) that involved both low stressor and high stressor components. Stress levels were altered by varying task difficulty, task duration and feedback provided to the subjects via the computer and experimenter. Following completion of each component of the test batteries, subjective ratings of mood were assessed using the Profile of Mood States (POMS). Relationships between sleep duration, PSQI variables and mood variables were analyzed using Pearson's correlations.

Results: We expected that subjects who reported sleeping poorly at home would have greater mood disturbance when provoked by a stressful situation. Poor sleep, as reflected by the PSQI global scale, was correlated with POMS total mood disturbance following both the low stressor ($r=0.414$, $p=0.003$) and high stressor ($r=0.348$, $p=0.006$) test batteries. There was a significant correlation between POMS vigor and both self reported sleep duration ($r=0.401$, $p<0.001$) and PSQI sleep quality ($r=0.509$, $p<0.001$) following the high stressor test battery, such that increased levels of vigor were associated with increased sleep duration and better sleep quality. In addition, longer self-reported sleep latencies were correlated with POMS measures of increased depression-dejection ($r=0.576$, $p<0.001$), increased anger-hostility ($r=0.507$, $p<0.001$), increased fatigue ($r=0.446$, $p<0.001$), increased confusion-bewilderment ($r=0.461$, $p<0.001$), and increased total mood disturbance ($r=0.396$, $p=0.002$).

Conclusion: These findings illustrate that the relationship between decreased sleep quality and decreased mood previously described in insomniacs, is also present in healthy individuals with no evidence of any sleep disorder. Further, expression of this relationship is amplified following exposure to stressful stimuli.

Supported by: NASA cooperative agreement NCC 9-58 with NSBRI

Fear Conditioning Increases NREM Sleep and NREM Delta Oscillations

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To understand the role that sleep may play in memory storage, our work investigates how learning affects sleep. Our previous experiments have revealed that sleep deprivation selectively impairs contextual fear conditioning. Although many behavioral experiments demonstrate the necessity for sleep after learning, the molecular relationship between sleep and memory remains unexamined. To investigate the influence of sleep on memory, we have examined mice using fear conditioning--a task that is beginning to be understood in molecular terms.

Adult C57BL/6J mice (12-24 weeks of age) were implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes under isofluorane anesthesia. Mice were allowed two weeks recovery before recording. After allowing two weeks recovery from surgical implantation of EEG and EMG electrodes, mice were given 3 days to habituate to being handled for 5 minutes per day. On training day, mice either received context exposure for two minutes followed by a 30 second tone (sham group, n=15), context exposure followed by a 30 second tone and foot-shock at two and half minutes (fear conditioned group, n=16), or contextual exposure followed immediately by shock and tone (immediate shock group, n=12). 24 hours after training, mice were tested in the same context by evaluating the amount of freezing over a five minute period.

Mice receiving fear conditioning froze 20 +/- 2 % during the five minute testing period, but mice receiving immediate shock treatment froze 4 +/- 3 % and mice receiving sham treatment froze 2 +/- 1 %. During the first 24 hours after training fear conditioned mice had 626 +/- 24 minutes of NREM sleep, immediate shock mice had 562 +/- 23 minutes, and sham treated mice had 546 +/- 22 minutes. In contrast, no differences were seen in REM sleep during the 24 hour period. Fear conditioned mice had 65 +/- 5 minutes of REM sleep, immediate shocked mice had 64 +/- 6 minutes, and sham treated mice had 62 +/- 5 minutes of REM sleep. Increased NREM sleep in fear conditioned mice was due to a decrease in wakefulness. Fear conditioned mice were awake 745 +/- 24 minutes, immediate shock mice were awake 811 +/- 21 minutes, and sham treated mice were awake 826 +/- 20 minutes. Because fear conditioned mice sleep longer than both sham treated mice and immediate shock mice ($p < 0.05$), our results suggest that the learning experience of fear conditioning, but not context exposure or shock experience alter sleep. Fear conditioned mice had more delta (1-4 Hz) after the training experience ($p < 0.01$), and increases in NREM sleep were correlated with increase in delta power ($p < 0.05$).

Single trial training for fear conditioning in C57BL/6J increases NREM sleep, but has no effect on REM sleep during the 24 hour period following training.

Changes in delta power suggest that sleep homeostasis has been altered after the training experience.
This work was supported by grants from NIA (AG-18199), NIMH (MH-60244), and NHLBI (HL-60287) (T.A.), and NIMH (MH-64329) (K.H.).

Genetic Evidence that Protein Kinase A Regulates Thalamocortical Oscillations during NREM Sleep

Hellman K, Young A, Park A, Abel T.
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Computer models have suggested that activity during wakefulness could affect sleep and its associated oscillations via the cAMP/protein kinase A (PKA) pathway. PKA has been suggested to modulate thalamocortical synaptic plasticity during spindle oscillations during NREM sleep. Although several neurotransmitters that regulated sleep/wake states are known to increase levels of cAMP, the role of this signaling pathway in the regulation of sleep and its associated oscillations have not yet been characterized. To assess the role of PKA, we have characterized sleep/wake states using EEG/EMG recordings in R(AB) transgenic mice, which express a dominant negative form of the regulatory subunit of PKA in neurons within cortex, hippocampus, striatum and amygdala. These mice have reduced PKA activity in cortex and hippocampus, which results in the impairment of hippocampus-dependent long-term memory and long-lasting forms of hippocampal LTP.

Transgenic R(AB) and wild-type littermate mice were backcrossed in the hemizygous state to C57BL/6J for 11-13 generations, and bred in our colony under standard conditions. Adult mice (12-24 weeks of age) were implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes under isofluorane anesthesia. Mice were allowed two weeks recovery before recording. After baseline recording, R(AB) and wild-type mice were subjected to sleep deprivation using gentle handling.

We have found that R(AB) transgenic mice exhibit normal amounts of NREM sleep and wakefulness, but exhibit 17 % more REM sleep per day than wild-type mice. R(AB) transgenic mice have more delta power (1-4Hz) during NREM sleep throughout the entire light-dark cycle, as well as less sigma power (10-14 Hz) during NREM sleep than wild-type mice. Sleep deprivation revealed that homeostatic regulation in R(AB) transgenic mice was comparable to wild-type mice, suggesting that an alteration in a mechanism unrelated to sleep homeostasis underlies the delta power phenotype. The sigma power rebound that lasted for many hours after sleep deprivation in wild-type mice was reduced in R(AB) transgenic mice. The decrease in sigma power in R(AB) transgenic mice is due to lower amplitude spindles. Differences in delta power and spindle oscillations could either be due to increased hyperpolarization of R(AB) cortical neurons or alterations in synaptic connections between cortical and thalamic regions. Our results suggest a role for PKA in sleep oscillations independent of homeostatic sleep or circadian regulation that may be responsible for modulation of delta spindle amplitude during NREM sleep.

PKA in a brain region outside of the medial pontine reticular formation activity can decrease REM sleep.

Delta oscillations can be modulated by mechanisms independent of the sleep homeostat.

A reduction PKA activity can result in the increased amplitude of delta oscillations reduced amplitude spindle oscillations.

This work was supported by grants from NIA (AG-18199), NIMH (MH-60244), NHLBI (HL-60287) (T.A.), and NIMH (MH-64329) (K.H.).

Mood States in Obstructive Sleep Apnea: Comparisons among Severity Levels of Disease & Normal Controls, Before and After CPAP Treatment

Reishtein JL, Pack AI, Maislin G, Dinges DF, Bloxham TJ, George CFP, Greenberg H, Kader GH, Mahowald MW, Younger JB, Weaver TE.
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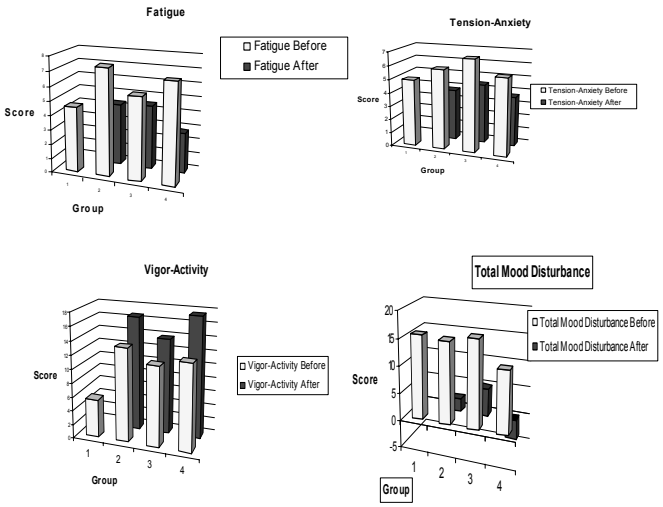
There is little evidence of the degree of impairment in mood among levels of OSA compared to normal functioning, or the magnitude of post-treatment improvement related to disease severity. One aim of this multicenter study was to describe OSA disease severity deficits in mood compared to normal controls before and after CPAP treatment.

Methods: Clinical sample was 166 OSA patients (*M* age = 46.53, 83% male, *M* AHI = 65.46). Patients were grouped by disease severity (milder [M] AHI 20 to < 40, n = 42, moderate [MOD], AHI 40 to < 60, severe [S], AHI ≥ 60, n = 95). 21 interview and PSG-screened controls were recruited from the community (*M* AHI 0.55, *M* age 42.58, 67% male). Compared to the OSA patients, the normal group was significantly younger and less overweight. All participants completed the Profile of Mood States (POMS). After 3 months monitored CPAP treatment, patients again completed the POMS. T-tests with Bonferroni correction were used to analyze differences between groups, pre and post treatment.



Results (see figure): At baseline, Mild and Severe patients differed from normals on anger/hostility subscale and on total mood disturbance. Mean CPAP use did not differ among the three patient groups. After 3 months CPAP, all three patient groups improved significantly on vigor/vitality and total mood disturbance, the Moderate and Severe groups improved on tension/anxiety, and the Mild and Severe groups improved on fatigue, confusion/ bewilderment, and on depression/ dejection. At this time, significant differences existed between Normals and all three patient groups on vigor and anger, and between the normals and both the Mild and Severe groups on fatigue and total mood disturbance (with the patients better than the normals). Additionally, there were differences between the Mild and Severe groups on fatigue and vigor, and between the Moderate and Severe groups on confusion/bewilderment and on vigor.

Conclusion: OSA does not appear to affect many mood states in mild disease. CPAP treatment improves some mood states in OSA, with larger improvement seen in more severe disease.

Daytime Sleepiness and Function in OSA Pre- and Post-CPAP Treatment: Comparison of Levels of Disease with Normal Controls

Reishtein, JL, Pack AI, Maislin G, Dinges DF, Bloxham TJ, George, CFP, Greenberg H, Kader GH, Mahowald MW, Younger JB, Weaver TE.
School of Nursing and Departments of Medicine and Psychiatry, University of Pennsylvania

Introduction: Clinical trials have demonstrated that CPAP is efficacious in improving daytime sleepiness and functional status in OSA. However, there is little evidence of the degree of impairment compared to normal function and the magnitude of post-treatment improvement related to disease severity. Aims of this multicenter study were to 1) describe OSA-related deficits in sleepiness and functional status compared to normal controls and 2) compare post-treatment improvement among 3 levels of disease severity and normal controls.

Methods: 159 OSA patients (mean age 46.53; 83% male) at 7 sites in US and Canada were recruited from clinical practice. Inclusion criteria were age 21 to 60 years, excessive daytime sleepiness, and candidate for CPAP treatment. Those with another co-existing sleep disorder, history of sedative-hypnotic use, CVA, COPD or other pulmonary disease, or CHF were excluded from the study. OSA patients were grouped by disease severity (AHI 20 to <40; AHI 40 to <60; and AHI >60). Twenty-one interview and PSG-screened (AHI < 5) normal controls (mean age 42.58 years; 67% male) were recruited from the community. During one day of testing in the laboratory, all participants completed the Epworth Sleepiness Scale (ESS), Multiple Sleep Latency Test (MSLT), Functional Outcomes of Sleep Questionnaire (FOSQ) (to measure disease-specific functional status), and Sickness Impact Profile (SIP) (to measure generic functional status). After 3 mo of monitored CPAP treatment, patients again completed the same measures.

Results: At baseline, all three patient groups were significantly more impaired on the MSLT, ESS, and FOSQ than the normal group ($p < .05$). There were no differences between the normal controls and the three groups on the SIP. Mean CPAP mask-on use was 4.93 + 2.05 hours, with no significant differences among the three groups. Following treatment, patients improved on all measures (except the MSLT), with robust effect sizes (ESS: .72-1.43, FOSQ: .64-1.13, SIP: .74-.95). Only the most severely affected had a significant change post treatment on the MSLT (effect size .73). For all outcomes, the most severe patient group experienced the greatest change. At this time, the three patient groups no longer significantly differed from each other or from the normal controls on the ESS and MSLT, but on the FOSQ total scores, the mildest group was significantly worse than the normal controls and the two worse AHI patient groups. On the SIP, all three patient groups had better scores following treatment than the normal controls, but this difference was not significant.

Conclusions: OSA substantially affects patients' daytime daily functioning. CPAP treatment mitigates this impairment, alleviating sleepiness and restoring daily functioning to levels similar to normal individuals, with greater effects in the more severely affected.

Seeking the Mechanisms of Action of Modafinil in *Drosophila*

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The mechanism of action of the drug modafinil, a psychostimulant commonly prescribed for treatment of excessive daytime sleepiness, is not well understood. Pharmacological studies in mammals indicate that modafinil most likely promotes wakefulness by affecting catecholamine neurotransmission in sleep-relevant areas such as the ventrolateral preoptic nucleus, but a direct mechanism has not yet been determined. In *Drosophila*, modafinil disrupts rest consolidation and increases wakefulness, as well as disrupting circadian rhythmicity at higher doses. These behavioral phenotypes allow us to propose genetic screens for mutants unresponsive to modafinil. Our lab has generated hundreds of mutant lines through both chemical and P-element mediated mutagenesis. We have used a variety of experimental protocols for screening, seeking flies with phenotypes such as continued maintenance of circadian rhythms or reduction of rest-associated locomotor patterns while on modafinil. Using these protocols, we are also screening known mutations in *Drosophila* affecting catecholamine levels. Here we present the results of several ongoing screens to demonstrate various methods of data analysis, which attempt to cope with the high variability of rest behavior and modafinil response within groups and between different genetic backgrounds. We hope that our work will eventually help to both elucidate the mechanism of action of modafinil, as well as shed light on the control of rest in flies.

Gender Differences in Response to Sleep Loss

Rogers NL, Coble M, Maislin G, Gordon E, Grunstein RR, Dinges DF.
Center for Sleep & Respiratory Neurobiology and Department of Psychiatry, University of Pennsylvania

Several studies have described gender differences in sleep physiology, sleep quality and the incidence of insomnia and other sleep disorders, particularly in older subjects. A number of cognitive and neurobehavioral functions have also been reported to display gender differences. Possible gender differences in sleep physiology and neurobehavioral functioning in response to sleep loss, however, have not been widely studied. Recently, gender differences in hormonal and immunological responses to one week of sleep restriction were described (Vgontzas et al., Endocrine Society Abstracts, 2003). Female subjects were also reported to show a greater degree of sleep consolidation during the sleep restriction period, compared to males. In our laboratory, we have been investigating the neurobehavioral and physiological effects of chronic sleep restriction in healthy male and female subjects (aged 22-50 years), in a series of controlled laboratory experiments. Preliminary analyses have demonstrated significant differences in the accumulation of cognitive impairments on some performance measures between male and female subjects, (e.g., number of correct responses on the digit symbol substitution task, $p=0.01$) and a trend for differences on subjective sleepiness scales (e.g., Karolinska Sleepiness Scale, $p=0.08$). Consistent with the Vgontzas et al. report, we also find differences in sleep physiology in response to sleep restriction, with significantly increased sleep efficiency in female versus male subjects ($p=0.006$). Differences in sleep and the response to sleep loss may potentially be mediated via changes in hormone levels, for example oestrogen, as suggested by Vgontzas et al (Endocrine Society Abstracts, 2003) or testosterone, as demonstrated by Liu et al (JCEM 2003; 88:3605-3613). Additional analyses of other cognitive performance tasks and subjective scales, as well as sleep architecture and hormonal responses to sleep loss are currently underway. Further, in another set of studies using an international standardized database of more than 1,000 healthy subjects (aged 5-95 years) we have assessed a range of psychometric and psychophysiological variables in both male and female subjects. Male subjects were found to have enhanced speed of cognitive processing to novel stimuli, while female subjects demonstrated increased performance on language tasks. The gender differences in neurobehavioral variables were shown to co-vary with the level of subjective sleep disturbance, as assessed by the multivariate apnea (MAP) risk index.

Research supported by NIH grants NR04281 and RR00040; and NASA cooperative agreement NCC 9-58 with NSBRI.

Compliance With CPAP vs. Bilevel Pressure in Children

Marcus C, Rosen G, Davidson Ward S, Halbower A, Sterni L, Lutz J, Bolduc D, Gordon N.
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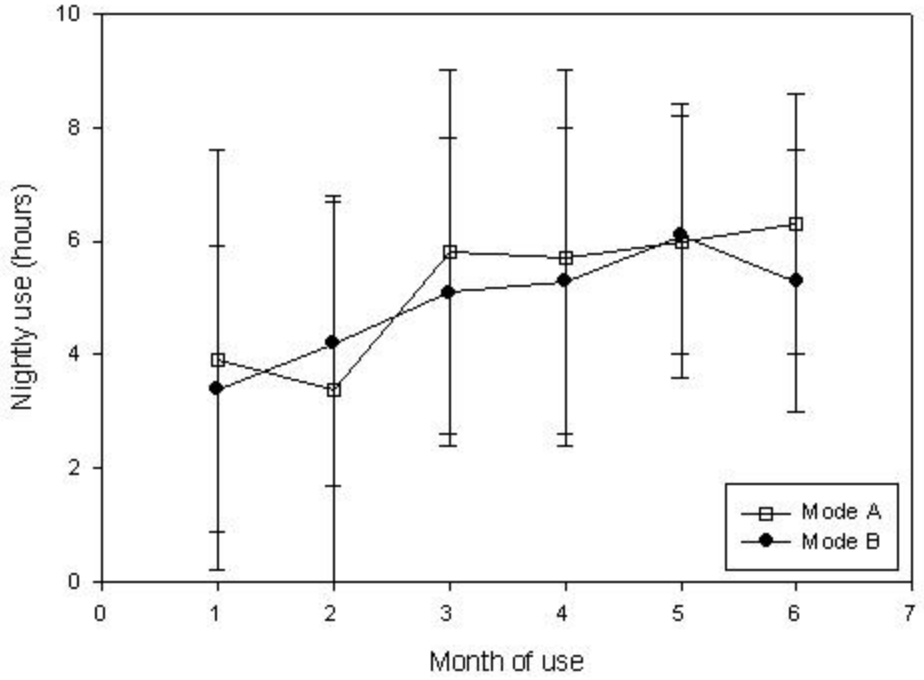
Introduction: CPAP and bilevel pressure (BPAP) are frequently used to treat children with the obstructive sleep apnea syndrome (OSAS) who do not respond to adenotonsillectomy. However, the efficacy and compliance with these modes have not been compared in the pediatric population. We hypothesized that BPAP would result in improved compliance and similar efficacy in children.

Methods: A multicenter, double-blind study was performed. Children with OSAS, aged 2-18 years, were randomized to 6 months of CPAP *vs* BPAP therapy. Children with respiratory failure or central hypoventilation were excluded. Efficacy was evaluated using polysomnography. Compliance was measured objectively using the equipment's computerized output.

Results: The ongoing study remains blinded and is reported as modes A (13 children, aged 9 $\pm$ 4 [SD]years) *vs* B (10 children, aged 11 $\pm$ 4 years; NS). The mean use was 4.7 $\pm$ 3.0 *vs.* 4.6 $\pm$ 2.3 hours/night, respectively (NS). Hours of use in both modes improved over time (P = 0.008, figure). Both modes were efficacious in overcoming OSAS.

Conclusion: Children are equally compliant with both CPAP and BPAP, with nightly usage similar to that reported in adults. Both modes are efficacious. We speculate that either mode is an option in pediatric OSAS; however, more patients need to be studied.



Time Effect of Lippopolysaccharide and Interferon Gamma Stimulation on iNOS by Raw 264.7 Macrophages

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Introduction: Nitric oxide production by macrophages is primarily regulated by inducible nitric oxide synthase (iNOS). Both lipopolysaccharide (LPS) and interferon-gamma (IFN-) synergistically act to stimulate expression of iNOS with subsequent production of nitric oxide. Previous in vitro studies have relied on static continuous exposure to LPS/IFN. LPS/IFN exposure and subsequent iNOS response are more likely to be a dynamic process. We investigated the effect of duration of LPS/IFN exposure on iNOS protein and iNOS activity in macrophages using a forced convection culture system.

Methods: RAW 264.7 cells (a mouse MΦ cell line) were cultured using a culture system designed to produce forced convection. Using capillary columns and a peristaltic pump, cells were cultured then allowed to adhere to the columns for 4 hours with a continuous flow of media equilibrated with room air and 5% CO2 at 37° C. All experiments lasted 18 hours. Cells were randomly assigned to receive an initial 1-hour, 4 hour, or 18 hours of stimulation with continuous flow media containing LPS (100ng/ml) and IFN- (100U/ml). Negative controls consisted of cells exposed to media for 18 hours without stimulation. All experiments were performed four times. Following the 18 hours, cells were rinsed in 1X PBS and immediately frozen at –70ºC for batch analysis. Total protein concentration, iNOS western blots and iNOS activity was determined.

Results: Following stimulation for an initial 1 and 4 hours of an 18 hr experiment, limited iNOS activity was detectable. Accumulation of iNOS protein increased with duration of LPS/IFN exposure.

Conclusions: Brief exposure of murine macrophages to LPS and IFN- does not result in persistent iNOS expression and activity**.**

Distinct Components of Sleep Structure at Baseline and Following 36 Hours of Sleep Deprivation

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Introduction: Previous studies revealed trait-like inter-individual variability in the structure of sleep. It has also been demonstrated that different aspects of sleep (e.g., REM, non-REM sleep) respond differentially to sleep deprivation. Here we distinguished independent components of sleep structure on the basis of trait inter-individual differences in sleep parameters at baseline and upon recovery after 36 hours of sleep deprivation.

Methods: As part of a larger study, n=21 subjects (age 29.5±5.3; 9 females) underwent two laboratory-based 36-hour total sleep deprivations at intervals of at least 2 weeks. Preceding each laboratory sleep deprivation, subjects were required to spend 12h TIB (22:00–10:00) per day for 6 days. The night before sleep deprivation was spent in the laboratory, and also involved 12h TIB (22:00–10:00). After this baseline sleep period, subjects were kept awake for 36h, performing a 60min neurobehavioral test bout every 2h. Following deprivation, subjects were given recovery sleep with 12h TIB (22:00–10:00). The baseline and recovery sleep periods were recorded polysomnographically. Sleep records were scored fully blinded and according to conventional criteria. For every sleep record, we determined TST, S1–S4, REM, WASO, movement time (MT), sleep latency, REM latency (REML), and average sleep cycle duration (ASCD). We considered only sleep parameters that were stable between the two baseline records and the two recovery records, as evaluated by the intraclass correlation coefficient (ICC>0.5). WASO and sleep latency did not meet this criterion. The other sleep parameters were averaged over the two baseline records and separately over the two recovery records for each subject. Two subjects' data could not be used due to equipment problems.

Results: Principal factor analysis was performed to assess independent components of sleep structure in baseline sleep as well as recovery sleep. For baseline sleep, inspection of the scree plot of eigenvalues revealed that 4 factors explained the dominant part of the variance, with high factor loadings (absolute values >0.7) found as follows. First factor: REM (+0.93), TST (+0.74), REML (–0.76); second factor: S1 (+0.99); third factor: S2 (+0.96); and fourth factor: S4 (+0.96). For recovery sleep, 5 factors explained most of the variance, with high factor loadings found as follows. First factor: S2 (+0.94), S3 (–0.78); second factor: S1 (+0.90), MT (+0.75); third factor: REM (+0.87), TST (+0.86); fourth factor: ASCD (+0.85), REML (+0.78); and fifth factor: S4 (+0.94).

Conclusions: In the context of trait-like inter-individual variability, baseline and recovery sleep appeared to have four distinct components in common. These corresponded to REM sleep, slow wave sleep, stage 2 sleep, and sleep consolidation (with MT and S1 as inverse markers). In addition, recovery sleep showed a fifth dimension related to sleep cycle duration, which may reflect (inter-individual differences in) increased expression of both non-REM and REM sleep following sleep deprivation. These findings suggest that sleep structure is more complex than just sleep duration or a mere distinction between REM sleep and non-REM sleep, as is often assumed in conceptual and mathematical models of sleep architecture.

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Polysomnograms in Children 2 – 9 Years Old.

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Background: There is little published data regarding normative polysomnography in the pediatric age group despite its importance for pediatric sleep medicine research and clinical care.

Aims: (1) To describe the respiratory events and sleep architecture of normal children at the age of peak incidence of obstructive sleep apnea syndrome, with emphasis on the occurrence of arousals and periodic limb movements in sleep (PLMS). (2) To compare the appearance of paradoxical breathing when using different technologies for detection of movement of the chest and abdomen.

Population: Sixty-six children, mean age of 6.6 ± 1.9, with normal growth and development that did not meet exclusion criteria.

Methods: Subjects that passed a screening questionnaire then underwent a brain MRI scan and a standard overnight polysomnogram.

Results: The percent of total sleep time spent in sleep stages 1, 2, 3, 4, and REM were 4 ± 3%, 44 ± 10%, 10 ± 6%, 22 ± 8%, and 21 ± 6%, respectively. The Arousal and Awakening index (N/h) was 11.2 ± 4.3. The frequency of respiratory events included a central apnea index (N/h) of 0.08 ± 0.14, obstructive apnea index (N/h) of 0.01 ± 0.03, and obstructive hypopnea index of (N/h) 0.3 ± 0.5. The baseline SpO_2 was 97 ± 1%, with a nadir of 92 ± 3%. The baseline end-tidal PCO_2 ($ETCO_2$) was 42.5 ± 3.6 torr, with a peak of 50.9 ± 4.5 torr. The PLMS index (N/h) was 1.3 ± 2.2. The appearance of paradoxical breathing was more frequent with piezo crystal technology (40.0 ± 24.2% of epochs) than with respiratory inductive plethysmography (RIP) (1.5 ± 3.3% of epochs).

Conclusions: We add to the current knowledge regarding the occurrences of hypopneas during sleep, arousals and awakenings, and PLMS. We illustrate how the apparent amount of paradoxical breathing can vary due to different technologies. We also confirm previous data on the frequency distribution of the various sleep stages, normal oxyhemoglobin saturations, and the relative rarity of respiratory events in this age group.

Increased Incidence of Sleep Apnea in Fibromyalgia Patients

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Purpose: To study the structure and pattern of sleep disturbance in patients with fibromyalgia (FM) by polysomnogram. Alpha wave intrusion has been well documented in FM but there is controversy regarding any association with sleep apnea.

Methods: 15 patients with fibromyalgia were referred from rheumatologist in Penn Health System and Veteran Medical Center at Philadelphia. Subjects must have had a diagnosis of fibromyalgia by criteria for classification of fibromyalgia established by The American College of Rheumatology 1990. Exclusion criteria include congestive heart failure, chronic obstructive pulmonary disease, malignancy, active thyroid disorders, hepatitis C or B infection, RA, SLE, seronegative spondyloarthropathies, Sjogren's syndrome and other auto immune disease. Overnight polysomnograms was performed in the sleep laboratory. Analyses of alpha activity in non-rapid eye movement (non-REM) sleep were performed using time domain, frequency domain, and visual analysis techniques. Using standard techniques, a computer data acquisition and analysis system recorded the following signals: electroencephalogram (C3A2, O2A1); bilateral electrooculograms; submental and bilateral tibialis anterior electromyograms; impedance plethysmography of the rib cage and abdomen, airflow at the nose and mouth (nares pressure), body position, pulse oximetry, and tracheal breath sounds.

Results: Patient's ages ranged from 35-58 years old. There were 8 females and 7 males. 7 patients (47%) including of 4 female and 3 male patients had an apneas and hypopnea index > 5 and were found to fulfill diagnostic criteria for obstructive sleep apnea. In these 7 patients, 5 of them also showed alpha wave intrusion in non-REM sleep. A total of 10 patients (67%) showed alpha wave intrusion in non-REM sleep.

Conclusion: Sleep apnea occurs more often than generally recognized in men and women with primary FM. This is amenable to different interventions than for alpha wave intrusion. In most of these patients, obstructive sleep apnea was unappreciated prior to our study. Untreated obstructive sleep apnea has been implicated in pathogenesis of hypertension, heart failure. Further studies are needed to establish the relationship between sleep apnea and symptoms of FM.

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Adjusting Process C of the Two-Process Model in Response to Light

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Introduction: The two-process model of sleep regulation, which consists of a homeostatic process S and a circadian process C, successfully predicts sleep timing and duration in a variety of experimental scenarios. The published equation of process C reflects a static process that, unlike the actual circadian pacemaker, cannot shift phase or change amplitude in response to light. This limits the use of the two-process model to experiments in which circadian rhythmicity is either known or assumed to be stable. Consequently, relevant operational scenarios like shiftwork cannot be readily modeled. This restriction can be overcome with the limit cycle oscillator model for the circadian pacemaker, which has a provision for light input as part of the differential equations that drive it. However, even under stable entrained circumstances with approximately 8 hours daily sleep, substituting the limit cycle oscillator in lieu of process C does not properly integrate the two models, for the limit cycle oscillator has a different phase, amplitude, and shape than process C. We derived a transformation that can morph the limit cycle oscillator into the profile of process C, so that the limit cycle oscillator can be employed as a component of the two-process model to improve predictions of sleep timing and duration when the circadian pacemaker changes dynamically.

Methods: First we ran a 22-day simulation with the limit cycle oscillator model, starting at midnight and taking steps of 0.01 hours, using the Runge-Kutta method for numerically solving differential equations. For every simulation day, we implemented 8-hour sleep periods with 0 lux of light input (midnight to 08:00), and 16-hour wake periods with 150 lux of light exposure (08:00 to midnight). We sampled the predictions for the oscillator state variables x and x_c from the last 2 days of the simulation, at intervals of 0.1 hours. We then calculated amplitude $A=\sqrt{(x^2+x_c^2)}$ and phase $\varphi=\text{atan}(x_c/x)$ across all time points. The transformation equation was posited as $C = A \sum_k \beta_k \sin[k(\varphi+\Delta\varphi)]$, where k=1,...,m denotes the harmonics in the oscillatory profile, and C is the value of process C as per the published equation. Using non-linear multiple regression, we estimated the parameters β_k and $\Delta\varphi$ for up to 6 harmonics (m=1,...,6).

Results: Evaluated by explained variance, the optimal transformation equation was found to contain m=5 harmonics (just like process C). The parameter values were β_1=0.1065, β_2=–0.0243, β_3=0.0100, β_4=–0.0029, β_5=–0.0013, and $\Delta\varphi$=0.8731. This transformation of the limit cycle oscillator captured 99.4% of the variance in process C.

Conclusions: We successfully replaced process C of the two-process model of sleep regulation by the limit cycle oscillator model, offering flexibility of circadian phase and amplitude adjustment to the two-process model in response to light without adversely affecting the model under normal, steady state conditions. This modification to the two-process model may expand its range of applicability to situation of, for instance, shift work and jet lag. The predictive potential of the modified model is investigated in a companion abstract (J. Xiao *et al.*).

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Respiratory-Gated MRI Shows Regional Pharyngeal Airway Size is Reduced in Obese Zucker Rats Compared to Their Lean Littermates

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There is good evidence that obesity is the most important predictor for OSA and patients with OSA have decreased airway size and increased airway collapsibility. However, we have almost no understanding of how obesity alters the mechanical properties in regions of the pharynx to increase the risk for OSA. To address this gap in our knowledge, we have used MRI to compare the changes in pharyngeal airway size in obese Zucker rats to those of their lean age-matched littermates. We hypothesized that obesity compromises patency in pharyngeal regions through airway narrowing. An intra-pleural pressure transducer in anesthetized spontaneously breathing rats triggered a spoiled gradient recalled echo, gated MRI sequence wherein two images were acquired, the first in late inspiration, and the second in mid expiration. A 128x128 matrix on a 4 cm2 field of view (Tr/Te: 87/2.2 msec) was used to acquire 1 mm thick contiguous axial images encompassing the pharyngeal airway in 5 obese and 5 lean Zucker rats. Airway cross-sectional area was measured in rostral to caudal levels beginning 1 mm caudal to the junction of the hard and soft palate. At most airway levels, in both obese and lean rats, airway size increased during inspiration. Pharyngeal airway area was decreased in both inspiration and expiration in obese compared to lean rats and this reduction was significant at 4, 8 and 9 mm caudal to the junction of the hard to soft palate (t test, $p < 0.05$). The change in airway size across all levels between expiration and inspiration was reduced in obese compared to lean littermates ($p<0.01$). The results indicate that the pharyngeal airway is narrower in spontaneously breathing obese Zucker rats compared to their lean littermates. A decrease in pharyngeal airway size due to obesity could help explain why obese humans are at increased risk of obstructive sleep apnea.

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Opposite Effects of Two Shock Training Regimens on Rapid Eye Movement (REM) Sleep in Rats

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To examine the influence of conditioned fear stimuli on sleep-wake (S-W) states, we recorded sleep in rats after exposure to tones previously paired with footshock. After habituation to a recording chamber and the recording procedure, a baseline sleep recording was obtained the next day. One day later, experimental animals were exposed to cued fear conditioning (FC), consisting of 5 tone-footshock pairings. The 5-sec tones (conditioned stimuli; CS) co-terminated with 1-sec footshocks (unconditioned stimuli; US). Controls received the identical procedures except that the tones and shocks were explicitly unpaired (UP). The next day sleep was recorded for 4 hr in the recording chamber after presentation of 5 CS's alone. Sleep efficiency and REM sleep (REM) and non-REM (NREM) measures were calculated. While sleep efficiency was not significantly changed after CS presentation, the percent of total sleep time spent in REM (REM percent) was reduced in the FC animals. REM percent *increased* in the UP group. The reduction in REM percent in the FC animals was due to both an increased latency to enter REM and a decrease in the number of REM bouts. The increase in REM percent in the UP group was due to an increase in the number of sequential REM episodes, defined as those separated by intervals of less than three minutes. Results are discussed in terms of the decreases in REM as a response to conditioned fear, and the relevance of these findings to the sleep changes seen in post-traumatic stress disorder (PTSD).

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Dose Response Effects of Short Duration Naps During Extended Wakefulness

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Introduction: Previous research has demonstrated significant deficits in neurobehavioral functioning during periods of extended wakefulness. Strategic use of napping may be a countermeasure to reduce these deficits, but studies have not established the dose-response effects of increasing nap opportunities. This study examined the dose response effects on neurocognitive function and subjective assessments for two 2h naps per 24h, versus one 2h nap per 24h, versus no naps, during 88h of sleep deprivation.

Methods: Forty-one healthy subjects (37m, 4f, aged 21-47y) completed a 10-day laboratory protocol. After 3 nights of baseline sleep, subjects remained awake for 88h (3.7 days), followed by 3 recovery sleep periods. Subjects were randomized to receive either two naps of 2h TIB per 24h (02:45-04:45, 14:45-16:45, n=15), one nap of 2h TIB per 24h (02:45-04:45, n=13), or no nap sleep (n=13). Subjects remained in the laboratory throughout the protocol, with light levels <50lx (<lx during sleep) and ambient temperature at 24±1C°. Every 2h during wakefulness, subjects completed a 35min computerized neurobehavioral assessment battery containing both objective tests of neurocognitive performance and subjective measures of fatigue and sleepiness. Performance on the psychomotor vigilance task (PVT), digit symbol substitution task (DSST) and probed recall memory task (PRM), as well as the KSS and POMS were compared among the three groups using repeated-measures analysis of variance (ANOVA) with baseline as a covariate. For the present report, performance bouts immediately following the nap sleep periods (which could be affected by sleep inertia) were not included in the analyses.

Results: Significant dose-related effects were evident for a number of neurobehavioral performance measures, with higher levels of performance observed in subjects allocated to two naps per 24h, followed by subjects allocated to one nap per 24h, and with the lowest levels of performance found in subjects receiving no sleep during the 88h sleep deprivation. There were main effects of time for PVT lapses ($F[36,1332]=6.33$, $p<0.001$), fatigue on the POMS ($F[36,1260]=5.86$, $p<0.001$), and sleepiness on the KSS ($F[36,1332]=2.49$, $p<0.001$). There were main effects of condition for PVT lapses ($F[2,37]=7.21$, $p=0.002$), DSST number correct ($F[2,37]=7.57$, $p=0.002$), and PRM number correct ($F[2,37]=3.07$, $p=0.058$). Significant interactions of condition by time were found for PVT lapses ($F[72,1332]=1.68$, $p=0.022$), DSST number correct ($F[72,1332]=2.77$, $p<0.001$), PRM number correct ($F[72,1332]=1.52$, $p=0.013$), and KSS sleepiness ($F[36,1332]=2.49$, $p<0.001$). There was a trend for an interaction for POMS fatigue ($F[72,1260]=1.38$, $p=0.095$).

Conclusions: Dose-response effects of even short duration (2h) naps were observed during extended sleep loss, with the greatest amount of sleep allowed per 24h producing the greatest attenuation of sleep loss-induced decreases in neurobehavioral outcomes. Although subjective assessments of fatigue did not exhibit statistically significant effects of nap sleep, measures of sleepiness and cognitive performance clearly showed beneficial effects of increasing nap frequency. These findings suggest that strategic use of naps may be an efficient countermeasure to reduce the detrimental effects of extended sleep deprivation on cognitive functions.

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Predictor Variables of Vulnerability to Sleep Loss

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Introduction: Individuals have recently been documented to systematically vary in their response to sleep deprivation. This project aimed to identify psychological, neuroendocrine and physiological predictors for these trait-like inter-individual differences in vulnerability to neurobehavioral deficits from sleep loss.

Methods: 21 healthy subjects (age 29.5±5.3; 9 females) underwent 36h of laboratory-based sleep deprivation on two occasions separated by at least 2 weeks. Subjects were required to maintain a regular schedule of 12h time in bed (22:00–10:00) in the 7 days before each sleep deprivation, so as to satiate prior sleep need. Every 2h during the 36h sleep deprivation periods, subjects completed a 1h neurobehavioral test bout which included, among other tasks, the Karolinska Sleepiness Scale (administered twice), serial addition/subtraction task (SAST), digit symbol substitution task (DSST), word detection task (WDT), repeated acquisition of response sequences task (RARST), and psychomotor vigilance task (PVT). To yield a single measure of impairment, neurobehavioral outcomes were averaged over the last 24h of sleep deprivation as well as the two sleep deprivation sessions.

Before the first sleep deprivation, subjects completed 11 questionnaires measuring psychological and sleep physiological characteristics and underwent a blood screening. The concentrations of 41 different hormones, cells, enzymes and ions were measured. Core (rectal) body temperature was recorded at 6min intervals across the 36h of sleep deprivation. The phase and amplitude of the circadian temperature rhythm were determined by fitting a two-harmonic sinusoidal curve. The circadian phase and amplitude, the 46 questionnaire subscales, the blood results, and various demographic variables were all considered as candidate predictors of individual subjects' vulnerability to sleep loss.

The 92 candidate predictor variables were grouped into 11 separate domains (e.g. psychological traits, immune system). All candidate predictor variables in a given domain were entered into a forward stepwise regression analysis. For each domain, only the predictor variable that explained the most variance (if any significant predictor variables emerged) was considered for further analysis. A cross-domain linear regression analysis was then performed using the remaining predictors. This analysis was carried out for every neurobehavioral outcome measure.

Results: For each neurobehavioral outcome measure, with the exception of the SAST, there were predictor variables that could explain significant amounts of variance. For the WDT, 68% of the variance could be explained by considering plasma dehydroepiandrosterone-sulfate, plasma total bilirubin, body mass index and red blood cell distribution width as predictors. For the RARST, 54% of the variance was explained by a sleep disorders questionnaire, plasma total bilirubin, and age. For the DSST, 48% of the variance was explained by weight, plasma hematocrit and plasma creatinine. For the PVT, levels of urea nitrogen and handedness accounted for 12% of the variance.

The predictive model developed for the first administration of the KSS explained 9% of the variance with testosterone, transferrin and DHEA-sulfate. For the second administration, 51% of the variance could be explained by considering potassium, transferrin and thyroid stimulating hormone. Since both administrations of the Karolinska Sleepiness Scale are highly correlated, the analysis of the first administration was repeated using the candidate predictors identified for the second. In this analysis, 63% of the variance was explained.

Conclusions: The extent to which people's responses to sleep deprivation can be predicted appeared to vary according to the type of neurobehavioral outcome considered. With the set of candidate predictor variables evaluated in this study, the largest percentage of variance could be explained cognitive performance measures. The inconsistent results from the KSS suggest a need for more robust procedures for identifying the most promising predictor variables, such as computer-intensive model selection by bootstrap and jack-knife methods.

The most predictive domain of vulnerability to sleep loss was the kidney function domain (including levels of potassium, urea nitrogen, creatinine, and total bilirubin. Although the extent to which these variables stay predictive across different samples remains to be established, this project provided some guidance as to which potential markers of vulnerability to sleep loss may be focused on in future investigations.

Altered Discharge Properties of VTA Dopamine Neurons in the Active Circadian Period

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Although a great deal of research has focused on input pathways to the suprachiasmatic nucleus (SCN) and on the central clock itself, relatively little is known about SCN output signaling pathways. The ventral tegmental area (VTA) contains dopaminergic (DAergic) and GABAergic neurons which are involved in motivated behaviors and, more recently, implicated in behavioral state regulation. Both these phenomena are influenced by circadian rhythms. To examine possible circadian regulation in VTA neuron impulse activity, extracellular recordings of VTA neurons were obtained in halothane-anesthetized rats taken from either their rest (light) or active (dark) circadian period. Results revealed a subpopulation of neurons preferentially active during the active period that exhibited a high mean firing rate of 20.8 ± 1.67 Hz. The high firing subpopulation were termed 'novel cells' as their topographical location, waveform shape and duration were consistent with that of dopaminergic cells, but their mean firing rate was significantly higher than that of classically defined DA cells. Tests on a few 'novel' cells indicated that apomorphine, a mixed D1-D2 agonist, and quinpirole, a specific D2 agonist, inhibited the firing rate of these 'novel' cells, as typically found in DA neurons. Subsequent application of the D2 antagonist eticlopride reversed the quinpirole-induced inhibition. However, juxtacellular labeling of a few 'novel' neurons indicated that they are neither TH (+) or GAD (+). These results suggest that the activity of a previously undescribed VTA subpopulation is sensitive to the circadian period. In addition, there were significant changes in the mean firing rates of 'classic' DA cells within the circadian cycle, and during the transition from rest to active periods. VTA GABAergic neurons also displayed circadian fluctuations, as the mean firing rates of GABA neurons were significantly higher during the active cycle as compared to the rest cycle. Collectively, these results indicate that VTA DA, GABA and a previously undescribed 'novel' population of neurons exhibit circadian-like fluctuations in impulse activity. These circadian fluctuations in the VTA may be important in the temporal expression of behaviors, such as drug addiction and sleep/wake.

The Molecular Basis of the Interaction between Sleep and Feeding Behaviors in *Drosophila melanogaster*

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Although the function of sleep remains a mystery, many possible roles have been postulated for its purpose, including tissue restoration and healing, thermal and energy regulation, immune system function, and memory consolidation. A wide body of evidence indicates that sleep is influenced by food intake, supporting the idea that sleep has a role in tissue restoration and/or energy regulation. As recent advances have demonstrated that rest in D. melanogaster meets the behavioral requirements of mammalian sleep, we are using this genetically tractable organism to explore the molecular basis of this interaction. We have measured sleep phenotypes in a panel of 20 introgression lines derived from the isogenic parental lines Oregon-R and 2b in order to map quantitative trait loci (QTL) affecting variation in sleep. Preliminary results indicate that baseline sleep times exhibit high levels of sex-specific genetic variation in these lines. We have also subjected the lines to sleep deprivation using light as the depriving stimulus and measured the amount of sleep lost during the deprivation period as well as the subsequent sleep rebound time. The preliminary results of this mapping procedure will be presented, as well as a comparison of sleep QTL locations to those previously identified for starvation resistance using recombinant inbred lines derived from the same two parental strains.

Bayesian Individualization of Biomathematical Predictions of Cognitive Performance Impairment from Sleep Loss

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Introduction: Considerable trait-like inter-individual differences in cognitive impairment from sleep loss have been documented in our laboratory for healthy young adults. Current biomathematical models of sleep/wake and performance regulation do not take these differences into account. The existing population-average models might be tailored to individuals by modifying the model parameters, but this requires prior knowledge of the individuals' vulnerability to sleep loss. We investigated a different approach to individualization of biomathematical models, relying on the distribution of model parameters in the population at large (i.e., a population-distribution model) without requiring prior knowledge about the subjects at hand.

Methods: As part of a larger study, n=13 subjects (ages 27.7±5.4y) spent 20 days inside a laboratory. After two adaptation days and one baseline day with 8h TIB (23:30–07:30), subjects' sleep was restricted to 4h TIB (03:30–07:30) for 14 days. Performance on a psychomotor vigilance test (PVT) was tested every 2h during wakefulness. Daily averages (09:30–23:30) were computed for PVT performance lapses (RT≥500ms). These data were described with a mixed-effects model: $y(t)=\alpha+\beta t^{\theta}+\varepsilon$, where y stands for performance lapses, t denotes time (baseline day 0, restriction days 1–14), and ε is normally distributed within-subject noise. Baseline and slope parameters α and β were assigned lognormal distributions, and non-linearity parameter θ was assigned a normal distribution over subjects. The distribution parameters of these three parameters were estimated by fitting the mixed-effects model to the data of ten of the available subjects (randomly selected). This established the population-distribution model underlying the present individualization approach. The remaining three subjects were set aside as "previously unstudied individuals." We employed Bayesian forecasting to predict the performance of these test subjects. This procedure uses the parameter distributions in the population-distribution model to make likelihood-based adjustments to the individual subjects' model parameters.

Results and Discussion: Using the data acquired in our sleep restriction study, we simulated sampling the data for the three test individuals on a day-by-day basis. The model parameters were adjusted each day by means of the Bayesian forecasting procedure. One-day-ahead predictions were then made daily, using the adjusted model parameters. These individualized one-day-ahead predictions were compared to the subjects' overall data sets, which showed the typical large inter-individual differences. We found that the predictions adapted readily to the profiles that later turned out to best characterize the individual test subjects across the 14 days of sleep restriction. Out of 42 one-day-ahead predictions (14 predictions for each test subject), the individualized predictions were more accurate 38 times (i.e., >90%) compared to predictions that would have been made by the corresponding population-average model. Assuming an a-priori 50% chance of outperforming the population-average model, we thus achieved statistically significant improvement ($P<0.001$) of performance predictions with the novel Bayesian forecasting approach. Thus, Bayesian forecasting is a promising technique for tailoring biomathematical model predictions of performance impairment to individual subjects. This is important considering the existence of substantial trait-like inter-individual differences in vulnerability to sleep loss.

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Microarray Study of the Brain of *Drosophila melanogaster* During Rest Deprivation

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As a complimentary study to our mouse microarray project, we have examined the effects of rest deprivation on the global RNA expression patterns of the Drosophila brain. We have identified genes that show modified expression due to sleep deprivation and due to the dynamic nature of this experiment were able to ascertain the time course of these changes.

Based on the rest/activity cycle of the *Drosophila* wild type strain Canton S (see figure 1), we chose 3 durations of sleep deprivation at 2 hour intervals (ZT16, ZT18 and ZT20). Time matched controls (ZT16, ZT18 and ZT20) and a 0 hour control (ZT14) were used for comparison to the deprived tissue samples (150-180 fly brains). In addition, to identify genes that are differentially regulated due to manipulation of the flies and not sleep deprivation, a 4 hour stimulation (ZT14) during their active period was performed with time matched controls (ZT14) and a 0 hour active period control (ZT10)(Figure 1).

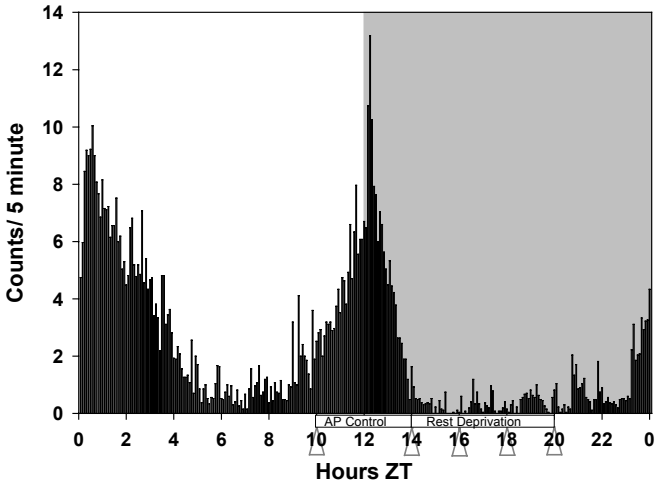

Figure 1. Rest deprivation was performed during the consolidated rest period of Canton-S Females from ZT14 to ZT20 and the active period control was performed during the second peak of activity from ZT10 to ZT14. Average activity of 50 female flies over a 24-hour period is shown with the time course of the experiments designated below the graph by open bars. Arrows designate collection times for rest deprivation experiments and active period (AP) control. Rest deprivation began at ZT14 and the 3 durations were 2, 4 and 6 hours (ZT16, ZT18 and ZT20 respectively). AP control started at ZT10 and ended after 4 hours (ZT14).

The microarray data was analyzed using the Statistical Analysis of Microarray (SAM) program. Using three levels of stringency as determined by the percentage of genes that are expected by SAM to be false positives, we have determined that 2 hours of rest deprivation does not significantly change overall gene expression (1 gene) and that with increasing deprivation one finds increasing alteration of gene expression (see Table 1). We are currently discerning the function of the identified genes using a battery of databases.

FDR	2C vs 2SD Up	2C vs 2SD Down	4Cvs 4SD Up	4Cvs 4SD Down	6Cvs 6SD Up	6Cvs 6SD Down
5%	1	0	6	1	34	3
10%	1	0	8	1	63	75
20%	1	0	33	1	139	389

Table 1. **The number of genes that show significant changes of expression increases with increasing duration of deprivation**. The number of significant genes depends on the False Discovery Rates [FDR] as determined by SAM analysis. C= control; SD=sleep deprived at 2, 4 and 6 hours of deprivation. Up= genes that SD signal>C signal. Down= genes that SD signal< C signal.

Modulation of Circadian Light Sensitivity in *Drosophila* by Serotonin Signaling

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Serotonin (5-hydroxytryptamine, 5-HT) plays important roles in the regulation of different behaviors, including control of appetite, sleep, mood, depression, memory and learning. With respect to sleep : wake cycles, sertonin signaling is involved in non-photic phase shifts, photo sensitivity and the regulation of paradoxical sleep in mammals. However, the physiological relevance and underlying molecular mechanisms of these effects are yet to be established. We studied the modification of circadian light responses by serotonin in *Drosophila*. As in mammals, pharmacological treatments that increase extracellular serotonin, inhibit light responses in flies. The spatial expression profile of the *Drosophila* serotonin receptor 1B (d5-HT1B) indicated a possible role of this receptor subtype in circadian regulation. Indeed, overexpression of the d5-HT1B receptor in clock neurons reduced the light sensitivity of circadian behavioral rhythms and the effect was synergistic with a mutation in the circadian photoreceptor, cryptochrome. Conversely, decreased levels of d5-HT1B resulted in increased circadian light sensitivity. Consistent with these behavioral observations, light induced TIM degradation was reduced in d5-HT1B overexpressing flies. These findings demonstrate that the inhibitory effect of serotonin on circadian photosensitivity is conserved in mammals and *Drosophila*. We propose that the modulation of light sensitivity by serotonin constitutes a homeostatic mechanism that regulates the circadian system.

Sleep Deprivation Induces the Unfolded Protein Response in Mouse Cortex

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Little is known about the molecular mechanisms underlying sleep. Data from a recently completed microarray study in our laboratory, to identify candidate genes involved in sleep homeostasis, indicated that increasing durations of wakefulness lead to up-regulation of the endoplasmic reticulum resident chaperone BiP/GRP78, and down regulation of protein synthesis factors and ribosomal proteins in mouse cortex. This increase in molecular chaperone expression, coupled with a decrease in protein synthesis capability, suggested a phenomenon similar to that observed during the unfolded protein response (UPR) triggered when proteins are misfolded. We therefore investigated whether the UPR was induced in mouse cortex during prolonged wakefulness. Using C57/B6 male mice maintained on a 12:12 light-dark cycle, we examined in frontal cortex the effect of different durations of prolonged wakefulness (0, 3, 6, 9 and 12 hours) in sleep deprived as compared to unhandled controls from the beginning of the lights on period, on protein levels and post-translational modifications. Protein levels of BiP/GRP78, a chaperone and classical UPR marker, is increased with increasing durations of sleep deprivation (6, 9 and 12 hours) (Western blots), and PERK, the transmembrane kinase responsible for attenuating protein synthesis becomes separated from BiP (as revealed by immunoprecipitation studies) and activated by phosphorylation (phospho-antibodies). There is also phosphorylation of the elongation initiation factor (eIF2α) that will reduce protein translation; compatible changes in ribosomal function are found. These changes are first observed after 6 hours of induced wakefulness. All of the key events of the UPR occur during wakefulness longer than 6 hours. Thus, prolonging wakefulness induces the unfolded protein response and may be an important determinant of the physiological limit to wakefulness.

REM Sleep-Suppressing Effect of Hypothalamic Perifornical Microperfusion with $GABA_A$ Receptor Antagonist, Bicuculline, Increases with the Duration of Sleep

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Manipulations of GABAergic transmission in the hypothalamic perifornical (PF) region have profound effects on sleep-wake behavior. We previously reported that mRNA levels for á1, á2 and â2 subunits of the GABAA receptor increase in the PF region during the afternoon when compared to the morning (Volgin and Kubin, Sleep 26 (Suppl.): A38, 2003). The goal of the present study was to assess whether these transcriptional changes can be related to circadian time-dependent, endogenous GABAergic inhibitory effects on sleep exerted in the PF region. In seven chronically instrumented (cortical and hippocampal EEG, nuchal EMG) rats, the hypothalamic PF region was unilaterally microperfused with the GABAA receptor antagonist, bicuculline (BIC; 20 μM). Sleep-wake and motor activity were recorded for 4 hrs of continuous perfusion with artificial cerebrospinal fluid (CSF) or with BIC substituting for CSF during the middle three hours. Recording sessions with CSF or CSF-BIC-CSF were made in a random order from either 9:00 AM to 1:00 PM (AM sessions) or 12:01 PM to 4:00 PM (PM sessions). As expected, the amounts of both slow-wave sleep (SWS) and rapid eye movement sleep (REMS) were significantly higher during the PM sessions. The average percentages of SWS and REMS during perfusion with CFS were 41.8±5.2 (SE) and 11.0±2.6 at 10-12 AM. At 1-3 PM, they were 52.3±6.0 ($p<0.01$ re. the AM sessions) and 17.3±2.2 ($p<0.02$ re. the AM sessions), respectively. The main finding was that, during the PM sessions, BIC nearly eliminated the afternoon increase in REMS, whereas its effect on SWS was not significant. At 10-12 AM, the average percentages of SWS and REMS during perfusion with BIC were 26.1±4.8 ($p<0.007$ re. CSF-AM) and 4.0±1.8 ($p<0.04$ re. CSF-AM). At 1-3 PM, they were 31.5±10.4 (not different from CSF-PM) and 5.1±1.7 ($p<0.004$ re. CSF-PM), respectively. The effects of BIC on sleep were not correlated with its effects on motor activity; in the AM sessions, BIC tended to increase the total number of movements during wakefulness, whereas in the PM sessions motor activity tended to decrease. The results suggest that the magnitude of GABAergic inhibition increases in the PF region with the duration of sleep, and that this process may contribute to the characteristic increase in the relative amount of REMS in the second half of the sleep period. *(HL-071097, MH-42903, Dept. of Veterans Affairs)*

[These results were previously presented at the Annual Meeting of the Associated Professional Sleep Societies: Lu *et al.*, Sleep 27 (Suppl.), A14 (Abstr. 031).]

Adenosine and Sleep in *Drosophila* melanogaster..

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Adenosine is a neuromodulator that plays an important role in mammalian sleep regulation. Pharmacological evidence suggests that adenosine promotes slow wave sleep (SWS), especially during the recovery from sleep deprivation. Endogenous levels of extracellular adenosine rise in the mammalian basal forebrain in response to sleep deprivation, and returns to baseline levels with the dissipation of sleep need. To determine if adenosine also regulates sleep in the fruit fly *Drosophila melanogaster*, we have used pharmacological as well as genetic approaches to assess the importance of adenosine receptor function on fly locomotor rhythms and sleep behaviors. The results of these studies will be presented.

Correlations Between Sleep Physiology and Neurobehavioral Performance Following Recovery from 88h Total Sleep Deprivation.

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Introduction: Previous work has indicated that subjects who received 1 night of 14h TIB for recovery sleep had a greater improvement in neurobehavioral performance and subjective alertness than subjects who received 1 night of 7h TIB, following 88h total sleep-deprivation (TSD). In addition, providing 14h TIB relative to 7h TIB for recovery resulted in reduced homeostatic drive for sleep on subsequent recovery nights. This study examined the relationship between recovery sleep physiology and neurobehavioral performance after one night of recovery sleep (7h vs. 14h TIB) following 88h TSD.

Methods: Twenty-five adult males (mean 28.2y) lived in the laboratory for 10-days. Following 3 baseline days and nights (8h TIB), subjects remained awake for 88h, followed by 3 recovery days and nights. During the 88h TSD period n=13 were randomized to a caffeine intervention, and n=12 to placebo. Subjects were further randomized to two 7h TIB recovery nights followed by one night of 14h TIB (n=14) or three 14h TIB recovery nights (n=11). Lights-out for all sleep periods was 2330h. Subjects were tested on a 30min computerized neurobehavioral assessment battery every 2h of wakefulness throughout the study. Psychomotor vigilance task (PVT) number of lapses and Karolinska Sleepiness Scale (KSS) ratings after the first night of recovery sleep were evaluated for two periods of the day: 1000-1600h and 1600-2200h. Data were analyzed using a Pearson correlation coefficient.

Results: On recovery night 1, subjects in the 7h TIB condition averaged 6.68h TST, while those in the 14h TIB condition averaged 12.48h TST ($p<0.0001$). Paired t-tests revealed that subjects receiving one night of 7h TIB recovery reported feeling significantly sleepier and had significantly more lapses on recovery day 1 compared to baseline and recovery day 3 ($p<0.001$), whereas there was no difference between baseline and recovery days 1 and 3 for subjects receiving one night of 14h TIB. Across both 7h and 14h recovery conditions, there was only one reliably significant correlation: PVT lapses after recovery night 1 were inversely correlated with the amount of SWS ($r=-0.605$, $p<0.002$). When analyses were performed separately within each recovery condition (7h and 14h), PVT lapses were inversely correlated with SWS ($r=-0.600$, $p<0.05$), and KSS ratings were positively correlated with stage 2 sleep ($r=0.691$, $p<0.01$) for subjects receiving 7h TIB recovery, but not for subjects receiving 14h TIB recovery. Results did not change when partial correlations were performed to remove the influence of age on SWS.

Conclusions: Following 88h TSD, PVT lapses after the first recovery night of sleep were predicted by the amount of SWS obtained during the recovery sleep period, particularly when the recovery sleep period was limited to 7h TIB which was inadequate for full recovery. KSS ratings were predicted by the amount of stage 2 sleep obtained, but again only when sleep was restricted to 7h TIB. When TIB was 14h, which was adequate for recovery, no one aspect of sleep physiology was correlated with PVT or KSS markers of recovery. Further analyses are underway to examine other measures of performance and the effects of additional recovery sleep periods.

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Effect of Extended Wakefulness and Recovery Sleep on Thyroid Axis Activity

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Introduction: Thyrotropin stimulating hormone (TSH) secretion reaches a nocturnal peak shortly after the onset of sleep, and sleep loss is reported to have an attenuating effect on TSH levels. T_3 levels have been reported to increase rapidly during sleep deprivation, and remained elevated, while T_4 levels have been found to increase more gradually. We sought to investigate the effects of 3 nights of total sleep loss and partial sleep loss on thyroid hormones, as well as the nature of nocturnal and diurnal thyroid responses to recovery sleep following deprivation.

Methods: Twenty-two healthy, male adults (m=28.5y, range=21-47y) completed a 10-day laboratory protocol. Following 3 nights of baseline sleep, subjects were randomized to either 88h total sleep deprivation (TSD) or two 2h naps (NAP) per day (02:45-04:45 and 14:45-16:45) for 88h, followed by 3 recovery nights for sleep. Blood samples were collected at 90 minute intervals via an indwelling venous canula from the final baseline night, through the 88h sleep deprivation period, and to the end of the first recovery day. Plasma levels of TSH, T_4, and T_3 (via RIA) were then determined at 3h intervals. Differences in the plasma thyroid hormone levels during nocturnal periods and diurnal periods across the 5 days of the protocol (baseline 3 through recovery 1) were compared using paired t-tests and ANOVA.

Results: There were few differences in levels of TSH, T_3 and T_4 between the NAP and TSD conditions, hence analyses are reported for pooled data. As expected, sleep loss elevated plasma levels of TSH, T_3 and T_4 (all $p<0.05$). The first night of sleep loss resulted in elevated plasma levels of TSH (all $p <0.001$), but as has been reported elsewhere, these levels were reduced on the second and third nights of sleep loss relative to the first night of sleep loss ($p=0.029$ and $p =0.005$, respectively), with no difference between the TSH levels on latter nights (both of which remained above baseline; $p<0.057$). In contrast, T_3 and T_4 remained elevated across all nights of sleep loss. TSH and T_3 levels (but not T_4 levels) during the first recovery night of sleep were significantly below all 3 deprivation nights ($p<0.018$), but only TSH was below the baseline night during recovery sleep ($p=0.041$). Diurnal levels of TSH, T_3 and T_4 were slightly but significantly increased on the diurnal portion of deprivation days relative to the baseline day (all $p<0.05$).

Conclusions: The thyroid axis responded in a similar manner to 88h of total sleep loss and 88h of partial sleep loss in which 2h nap opportunities were possible every 12h, suggesting that the well known inhibitory effects of sleep on thyroid hormone measures require more than 2h of sleep to be evident. Consistent with previous studies, sleep loss resulted in elevated TSH, T_3 and T_4, but this effect was attenuated on subsequent sleep loss nights for TSH (but not T_3 and T_4). TSH was also markedly suppressed below baseline sleep levels during recovery sleep following sleep deprivation, consistent with recovery sleep being more hypometabolic than daily nocturnal sleep.

Research supported by: AFOSR grant F49620-95-1-0388, and NIH grant RR00040.

Nitric Oxide Production by Activated Hypoxic Macrophages

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Introduction: Activated macrophages upregulate inducible nitric oxide synthase (iNOS) mRNA and protein, and produce large amounts of nitric oxide (NO). Excessive production of NO is thought to contribute to tissue injury in many disease states. However, several of these diseases, such as sepsis and acute respiratory distress syndrome, also result in tissue hypoxia. Molecular oxygen is a required substrate for NO production. Therefore, we hypothesized that hypoxia limits NO production due to oxygen substrate limitation.

Methods: To test this hypothesis, the iNOS Km for oxygen was determined in lipopolysaccharide (LPS) and interferonγ (IFNγ) stimulated macrophages (RAW 264.7) cultured with a forced convection cell culture system.

Results: We found that the apparent iNOS Km was approximately 5 Torr.

Conclusions: Tissue oxygen levels are between 5 and 70 Torr in healthy animals, so it is conceivable that oxygen levels may fall below this Km during hypoxia-associated diseases. Therefore, further investigations are needed to fully understand the role NO may play in the pathogenesis of these disease states.

The Evolution of Sleep Deprivation, Depression, Burnout and Empathy During Medical Internship

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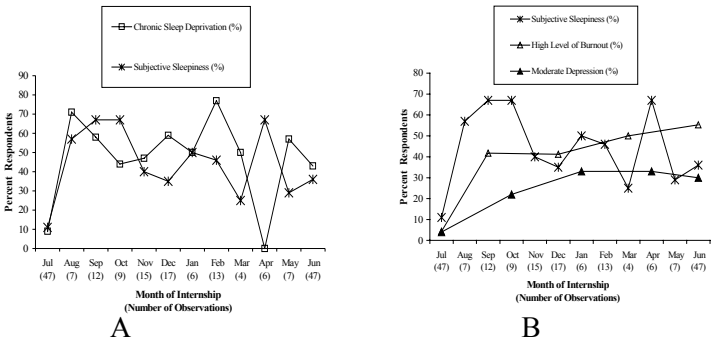
Problem Statement and Background:

Despite the high prevalence of sleep deprivation among physicians-in-training, recent mandates that limit work hours do not offer specific recommendations regarding sleep quantity. This study characterized the relationships between sleep deprivation and the evolution of mood disturbances, empathy and burnout among a cohort of interns.

Figure 1. Trends in prevalence of acute and chronic sleep deprivation(A), and sleepiness, burnout, and depression(B). *p < .001, †p=.004



Methods: Forty-seven interns completed baseline and year end instruments. Additionally, general trends in scores over the year were explored monthly.

Results: The prevalence of chronic sleep deprivation, depression, burnout and empathy all significantly increased from baseline to year end (Table 1). There was an association between becoming chronically sleep deprived and becoming depressed (OR = 7, p = .014). Many unfavorable changes in scores were identifiable by the fourth month of internship (Figure 1).

Conclusions: Given the association between chronic sleep deprivation and mood disturbances during internship, outcome assessment is warranted to see if duty hour reform will translate into more hours slept and if patient care is ultimately affected.

Table 1. Changes in Sleep Quantity, Subjective Sleepiness and Depression, Burnout and Empathy Scores from Baseline to the End of Internship.

Measurement	Beginning of the Year (No. = 47)	End of the Year (No. = 47)	p- value
Sleep Quantity			
Prior 24 hours	7.10	6.98	.76
Prior 7 days	50.34	42.95	< .001
Epworth Sleepiness Scale*	6.55	9.19	.001
Beck Depression Inventory†	1.30	4.96	< .001
Interpersonal Reactivity Index‡			
Empathic Concern	22.64	19.51	< .001
Perspective Taking	20.45	18.66	.004
Fantasy Scale	16.74	14.45	.005
Personal Distress	8.45	8.68	.71
Maslach Burnout Inventory§			
Depersonalization	6.87	14.15	< .001
Emotional Exhaustion	16.11	32.15	< .001
Personal Accomplishment	5.94	13.26	< .001

* Participants were asked to circle 0 = would never doze; 1 = slight chance of dozing; 2 = moderate chance of dozing; 3 = high chance of dozing.
† Participants were asked to check the statement that best described how they had been feeling over the past week.
‡ Participants were asked to indicate how well the statement described their thoughts and feelings by writing the appropriate letter from A-E based on the specified scale printed on the instrument.
§ Participants were asked to indicate how often the statement described their feelings about their job by writing the appropriate number from 0-6 based on the specified scale printed on the instrument.

Quiescence in *C. Elegans* is a Reversible Behavioral State Whose Duration is Altered by a cGMP-dependent Protein Kinase Mutation

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In mammals, periods of behavioral quiescence usually correspond to sleep, and are controlled by circadian and homeostatic processes. Behavioral quiescence in *Drosophila* is also controlled by these two processes and by some of the same neurochemicals that function in mammalian sleep (1,2), suggesting that the genetic control of quiescence is phylogenetically ancient. To complement genetic studies of quiescence in *Drosophila*, I have been developing *C. elegans* as a model system to study quiescence. In addition to powerful genetic tools, *C. elegans* offers the advantage of a small and anatomically well-characterized nervous system.

Locomotion of *C. elegans* has been observed to stop for prolonged periods during the lethargus periods, immediately prior to the molts (3). The first lethargus period has been shown to be a time of extensive nervous system synaptic changes (4). I have been measuring the quiescence associated with the first lethargus by imaging single worms from the time of hatching.

I found that in *wild type* worms grown at 22 deg, the longest consolidated quiescent period begins at 11.5+/-0.2, consistent with the reported time of onset of the first lethargus period. When mechanically stimulated during its consolidated quiescent period, the animal is able to move normally, indicating that this quiescent state is reversible. Perturbing the quiescence does not eliminate it; it only delays it. This result suggests that quiescence in worms, as in flies and mammals, is under homeostatic regulation and must serve an important function. I propose that one function of lethargus is to promote nervous system change.

In *eat-7*, a mutant that was isolated based on decreased movement and eating when not stimulated (5), the duration of the L1 quiescent period is prolonged while the time of onset of this quiescent period is unchanged. In addition to this L1 quiescent phenotype, I have found that adult *eat-7* mutants, when left unperturbed, make fewer tracks than *wild type* worms on a lawn of bacteria. When mechanically stimulated however, *eat-7* mutants are capable of rapid and coordinated movement, indicating that the increased behavioral quiescence cannot be explained simply by an inability to move well.

eat-7 is defined by a single dominant allele which maps to a genetic interval in which the cGMP-dependent protein kinase gene *egl-4* is found. Body size, life span, and roaming behaviors are increased in *egl-4* mutants while these three phenotypes are decreased in *eat-7* mutants, suggesting that *eat-7* is a gain-of-function *egl-4* allele. This suggestion is supported by the finding that two *egl-4* recessive alleles, *n477* and *n479*, dominantly suppress the small body phenotype of *eat-7*/+. I found a Glycine to Arginine mutation in the EGL-4a cDNA in *eat-7* mutants. This Glycine, located in the second cGMP-binding domain, is conserved in all proteins containing a cGMP- or cAMP-binding domain, suggesting that the identified mutation will have major consequences to enzyme function.

(1) Hendricks et al, Neuron 2000. (2) Shaw et al, Science 2000. (3) Singh and Sulston, Nematologica 1978. (4) Hallam and Jin, '98. (5) Avery, Genetics 1993.

Upper Airway Dynamic Responses During Development and Disease

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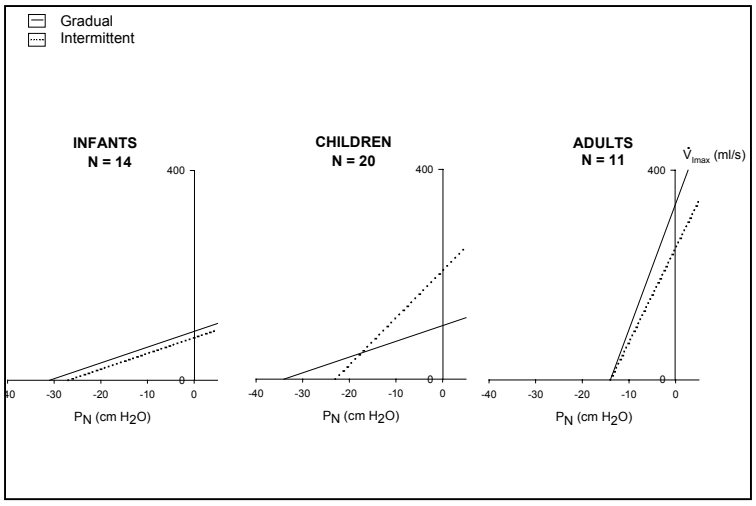
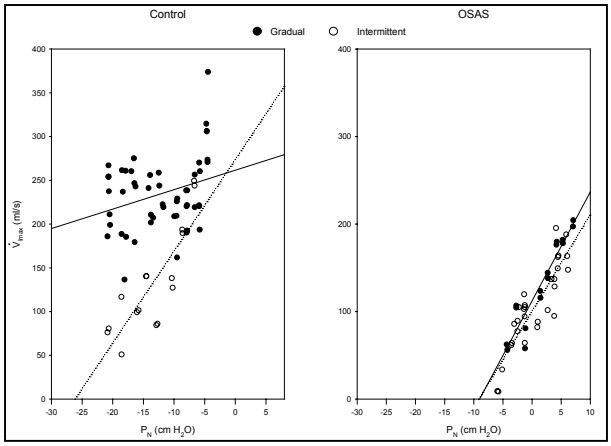
Normal children have a less collapsible upper airway in response to subatmospheric pressure administration (P_{NEG}) during sleep than normal adults, and this upper airway response appears to be modulated by the central ventilatory drive. Children have a greater ventilatory drive than adults. We therefore hypothesized that children have increased neuromotor activation of their pharyngeal airway during sleep compared to adults, and that this response is deficient in children with the obstructive sleep apnea syndrome (OSAS). As infants have few obstructive apneas during sleep, we hypothesized that infants would have an upper airway that was resistant to collapse. We therefore compared the upper airway pressure-flow relationship during sleep between normal infants, prepubertal children and adults; as well as children with OSAS compared to controls. We evaluated the upper airway response to (i) intermittent, acute P_{NEG} (infants, children and adults), and (ii) hypercapnia (children and adults). We found that normal adults had a more collapsible upper airway during sleep than either infants or children. The children exhibited a vigorous response to both P_{NEG} and hypercapnia during sleep ($P < 0.01$), whereas adults had no significant change. Infants had an airway that was resistant to collapse, and showed a very rapid response to P_{NEG}. Compared to normal children, children with OSAS had no significant response to either hypercapnia or negative pressure during sleep. Following treatment of OSAS by tonsillectomy and adenoidectomy, there was a trend for normalization of upper airway responses. We conclude that the upper airway is resistant to collapse during sleep in normal infants and children. Normal children have preservation of upper airway responses to P_{NEG} and hypercapnia during sleep, whereas responses are diminished in adults. Infants appear to have a different pattern of upper airway activation than older children. Upper airway dynamic responses are decreased in children with OSAS, but recover following treatment. We speculate that the pharyngeal airway neuromotor responses present in normal children are a compensatory response for a relatively narrow upper airway. Further, we speculate that this compensatory response is lacking in children with OSAS, most likely due to either habituation to chronic respiratory abnormalities during sleep, or to mechanical damage to the upper airway.





The Role of Context in Differential Sleep Architecture Changes Following Footshock

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Fearful stressors affect sleep in humans and animals. We, and others, have previously demonstrated that both the presentation of mild footshocks and reexposure to the context in which footshocks were presented suppress rapid eye movement sleep (REMS) selectively over several subsequent hours. In this study, we examined rats' sleep-wake response to footshock 24 and 48 h after a single training session. To our knowledge, this represents the first time sleep architecture changes resulting from a single session of shock training have been examined in a neutral context beyond 24 h in the rat. We also studied how reexposure to the context in which footshocks were presented modulates the sleep-wake response to a fearful stressor at these time points. The group that was shock trained and studied in the neutral context (ST/NC; n=8) displayed a REMS-selective increase in sleep on the day following training. Interestingly, a greater, and relatively non-selective increase in sleep was seen on the second post-training day. As expected, the group that was shock trained and studied in the presence of situational reminders of the training context (ST/TC; n=7) showed a REMS-selective decrease in sleep on the first post-training day. Second post-training day studies in a small subset (n=3) of the ST/TC group suggested that the REMS-selective decrease persisted. The suppression of REMS observed in the presence of situational reminders of the training context 24 h after exposure to footshock demonstrates rats' ability to differentiate between the two contexts and is evidence of contextual fear conditioning. Fear conditioning in animals has been viewed as a means of modeling anxiety disorders in humans. Thus, the alteration of the normal rodent sleep-wake response to footshock presentation by reminders of the aversive stimulus may provide a window into the pathogenesis of prolonged sleep disturbances associated with anxiety disorders, including posttraumatic stress disorder, in humans.

Does Time of Day Affect Simple Arithmetic Skills of Teenagers?

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Background: The delay of teenagers' circadian rhythms is a topic of current investigation and discussion. Teens have a biologic tendency to fall asleep at a later time and to wake up at a later time. Although both teens and adults may be sleep deprived, teens are especially sleepy in the morning because of their delayed circadian rhythm. Because sleepiness may affect learning, several high schools have changed their school times in response to this problem, for example, the Minnesota state school system.

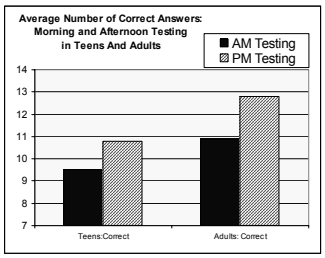
Purpose: The primary purpose of this project was to see how the time of day may affect simple arithmetic skills of teenagers. The numbers of correct and incorrect answers were compared for morning and afternoon simple arithmetic tests. Previous sleep time before testing, subjective alertness or sleepiness at the time of testing, and test scores were analyzed for morning testing and for afternoon testing.

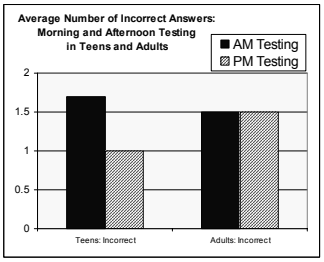
Materials and Methods: Eighth grade students and (adult) teachers from a Philadelphia school were recruited to participate. Participants were asked to complete a questionnaire and to take a two-minute timed, simple arithmetic test during homeroom or first period and to take a second two minute timed, simple arithmetic test in the afternoon. If the students completed both tests, they were given Jolly Rancher lollipops. The test was taken in either order to minimize an experience or learning effect.

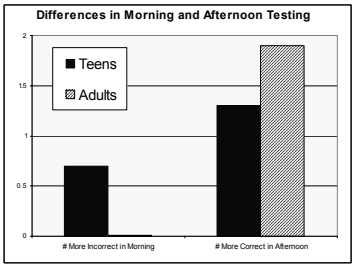
Results: Twenty-three eighth graders (fourteen males, nine females, mean age 13.7 years) and ten adults (seven males, three females, mean age 36.7 years) completed questionnaires and timed (two-minutes) arithmetic tests in the morning and in the afternoon.

Both teens and adults appeared sleep deprived. Teens slept an average of 8.0 +/- 0.9 hours. Adults slept and average of 6.9 +/- 1.3 hours. Both teens and adults felt more alert in the afternoon. Teens rated alertness as 5.7 (scale 0 (very sleepy) to 10 (very alert) in the morning compared to 6.7 in the afternoon. Adults rate alertness as 5.5 in the morning and 6.5 in the afternoon.

Both teens and adults had more correct answers on the afternoon test. Teens had 10.8 correct answers on the afternoon test and 9.5 correct answers on the morning test. Adults had 12.8 correct answers on the afternoon test and 10.9 correct on the morning test. Teens made fewer mistakes in the afternoon while there was no difference in the number of incorrect answers for adults. Teens had 1.0 incorrect answer on the afternoon test and 1.7 correct answers on the morning test. Adults had 1.5 incorrect answers on the afternoon test and 1.5 incorrect on the morning test. To summarize, teens and adults had more correct on afternoon testing. Teens had more incorrect answers on morning testing.







Conclusions: Both teens and adults were sleep deprived. Both teens and adults felt more alert in the afternoon. There was no correlation with subjective alertness and the amount of previous sleep. Both teens and adults got more correct answers on afternoon testing. Only teens had more incorrect answers on morning testing. Time of day does affect testing results on a simple two-minute arithmetic test.

If correct answers show the degree of alertness and incorrect answers shows the degree of inattentiveness, then teens are both less alert and more inattentive in the morning. This may have implications on learning and safety (driving to school) in the morning for teens.

Serotonergic and Noradrenergic Antagonists Combined Abolish the REM Sleep-Like Atonia of Hypoglossal Motoneurons

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It is hypothesized that REM sleep-related motor atonia is caused by glycine- and/or GABA-mediated inhibition of motoneurons combined with a withdrawal of excitation mediated by serotonin (5-HT) and norepinephrine (NE). However, the relative contributions of these mechanisms have not been determined for any motoneuronal pool. In urethane-anesthetized, paralyzed and artificially ventilated rats, injections of carbachol into the dorsal pontine tegmentum elicit short REM sleep-like effects that comprise profound depression of hypoglossal (XII) nerve activity, cortical activation, hippocampal theta, and silencing of NE neurons (J.Appl.Physiol. 93:1448, 2002). We used this model to identify the receptors that mediate the depression of XII motoneurons. REM sleep-like episodes were elicited by carbachol (10 nl, 10 mM) prior to, and at different times after, the injection of 5-HT, adrenergic, GABAA, and glycine receptor antagonists into the XII nucleus (1 mM methysergide, 0.2 mM prazosin, 1 mM bicuculline and 1 mM strychnine, respectively). In separate experimental groups (5-7 rats), two or more antagonists were injected together in a manner ensuring that all XII motoneurons were affected. About 50 min after the combined antagonist injections that included methysergide and prazosin but did not require either bicuculline or strychnine, XII nerve activity was reduced to 20-30% of the control. At this time, carbachol triggered no further depression of XII nerve activity, whereas all the other REM sleep-like effects were intact. The abolition of the depressant effect of carbachol on XII nerve activity partially recovered after 1-2 hours. We conclude that the REM sleep-like depression of XII motoneurons is caused by a combined withdrawal of excitatory effects of 5-HT and NE, whereas the contribution of active inhibition is minimal. From this it also follows that postsynaptic inhibitory events that occur in XII motoneurons during REM sleep and its models are not the main cause of the atonia. *(HL-60287, HL-47600)*

[These results were previously presented at the International Symposium "*The Paradox of Sleep: an Unfinished Story," Lyon, France,* Sept. 2-3, 2003, and at the Annual Meeting of the Society for Neuroscience: Fenik et al., Soc. Neurosci. Abstr. 29, Abstr. 769.9; see also Fenik et al., *Arch. Ital. Biol.* 143, 2004, in press.]

Single-Cell Gene Expression Profiling of Acutely Dissociated and Immunocytochemically Identified Central Neurons

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Single-cell gene expression profiling allows one to analyze molecular regulatory mechanisms of individual cells, thus helping understand how gene expression affects the cellular phenotype and function. To apply the technique to the mammalian brain, a highly heterogeneous tissue, its cells need to be pre-selected using criteria such as anatomic location, cellular morphology, projections, and/or expression of neurotransmitters or other markers. Our aim was to develop a technique that would combine acute dissociation of central neurons needed for mRNA recovery from single neurons with immunocytochemical cell phenotype identification.

Hypothalamic orexin (ORX) and melanin-concentrating hormone (MCH) neurons were investigated because they are distinct and have different functions but similar anatomic distribution and appearance. These two types of cells are located almost exclusively within the perifornical (PF) region of the posterior hypothalamus where multiple neuronal populations are distributed with limited or no segregation. To validate the technique, we compared the expression of two genes important for both sleep and metabolic regulation, the adrenergic á2A receptor (á2Ar) and type 2 orexin receptor (ORX2r), in cells identified as ORX- or MCH-containing by vital immunocytochemistry.

Cells acutely dissociated from the PF region of juvenile or adult rats were incubated with anti-MCH or anti-prepro-ORX primary antibodies, followed by washout and incubation with fluorescein-tagged secondary antibodies. Portions of the material collected from individual labeled cells were subjected to reverse transcription followed by semi-nested polymerase chain reaction with primers for prepro-ORX and MCH. MCH mRNA expression was detected in 26 out of 38 successfully reverse-transcribed cells obtained from seven rats an immunoctochemically identified as MCH-containing. Prepro-ORX mRNA was detected in 28 out of 42 successfully reverse-transcribed neurons dissociated from six rats and identified as prepro-ORX-positive. None of the MCH cells expressed prepro-ORX mRNA and none of the prepro-ORX-immunopositive cells expressed MCH mRNA. Most MCH neurons tested (5 out of 6) expressed á2Ar mRNA, whereas none out of 7 preproORX neurons tested had á2Ar mRNA ($p<0.0005$). ORX and MCH cells were similar in that none of the 11 prepro-ORX mRNA-expressing cells nor any of the six MCH mRNA-expressing cells tested expressed ORX2r mRNA.

These results show that immunocytochemical identification of acutely dissociated neurons can be successfully combined with cell harvesting and mRNA detection at the single-cell level. We estimate that the yield of verified MCH or ORX cells collected following their respective immunocytochemical labeling was at least ten-fold better than expected from a random harvesting of cells dissociated from the PF region. Thus, the method allows one to collect satisfactory numbers of immunolabeled neurons expressing a marker of interest from the hypothalamus, or any other non-homogenous brain region, for the purpose of mRNA expression analysis at the single-cell level. *(HL-071097, HL-47600, and the University of Pennsylvania Research Foundation)*

[These results were previously presented at the Annual Meeting of the Society for Neuroscience: Volgin et al., Soc. Neurosci. Abstr. 29, Abstr. 81.2; see also Volgin et al., J. Neurosci. Meth., 136, 2004, in press.]

Effects of Sleep Deprivation on Gene Expression and the Immune Response in *Drosophila* Melanogaster

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Rest behavior in flies is a sleep like state. Recent studies have implicated a role of components of cAMP-signaling, heat-shock proteins, and the clock gene *cycle* in sleep homeostasis. To identify other molecular candidates involved in sleep regulation, using microarray analysis we performed a genome-wide screen for changes in gene expression associated with sleep deprivation in flies. We found that three major classes of genes change expression with sleep deprivation. These include genes involved in the immune response, energy and metabolism, and cell signaling, particularly those related to synaptic plasticity.

We further analyzed the role of immune related genes in sleep homeostasis. Genetic manipulation of the NF kappa B transcription factor, *relish*, did not alter rest behavior. However, consistent with the increase in expression of immune related genes, we found that sleep deprivation increased resistance to bacterial infection following an immune challenge. These results demonstrate a correlation between acute sleep deprivation and the immune system indicating that increased expression of genes underlying the immune response is an output of the sleep homeostat.

Modulating the Two-Process Model

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Introduction: The two-process model of sleep regulation, as described by Borbély and Achermann,[1] consists of a process representing homeostatic sleep drive (process S) and a process representing circadian rhythmicity (process C). This model has been used successfully to describe cognitive performance under normal sleep cycles as well as acute total sleep deprivation.[2] The model is inaccurate, however, when predicting performance under chronic sleep restriction and subsequent recovery.[3] Johnson and colleagues[4] developed a performance model that appears to more accurately describe performance under chronic sleep restriction as well as recovery.[5] Their model, however, is not based on the standard two processes S and C.[6] In order to develop a model that preserves the predictive capabilities of the two-process model but also accurately predicts performance during chronic sleep restriction, we sought to combine the essential features of both models.

Methods: Our key modification of the two-process model was in altering the asymptotes toward which process S trends during extended sleep and during extended wakefulness. In the original two-process model, S is assumed to always have an upper asymptote (UA) of 1 and a lower asymptote (LA) of 0. We modulated these asymptotes by means of the "chronic modulating process" postulated by Johnson et al.[4] The revised model equations are:

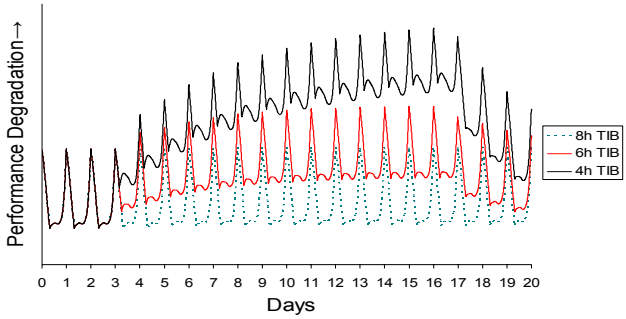
During Sleep: $S_t = e^{-\Delta t/Td}(S_{t-1} - LA) + LA$ $\quad UA_t = UA_{t-1} + (1 - UA_{t-1})(1 - e^{-Mr\,\Delta t})$

During Wake: $S_t = UA - e^{-\Delta t/Tr}(UA - S_{t-1})$ $\quad UA_t = UA_{t-1} + M_d\,\Delta t$

During Sleep/Wake: $A_t = S_t - C_t$ $\quad LA_t = UA_t - 1$

where A_t represents alertness as a function of time t, Δt is the time step, and T_r, T_d, M_r, and M_d are model parameters.

Results: We ran simulations for a scenario of 3 baseline days with 8h time in bed (TIB), 14 days of chronic sleep restriction at 4h, 6h, or 8h nocturnal TIB, and 3 days of recovery with 8h TIB. We observed that the revised model yielded performance profiles similar to earlier experimental observations[3]—see figure.



Conclusion: The revised two-process model successfully captured the pattern of performance under chronic sleep restriction and subsequent recovery. One issue still under consideration is defining the lower asymptote relative to the upper asymptote, as there are multiple possibilities for implementing this. Finally, laboratory data will need to be employed to calibrate the model parameters—this is work in progress.

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Effects of Chronic Sleep Restriction and Circadian Phase on Sleep Physiology

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Introduction: Changes in sleep physiology due to chronic sleep restriction and circadian disruption have been reported. The majority of these studies have been relatively short (<4 days) investigating either the effects of nocturnal sleep restriction or the effects of circadian disruption independent of sleep restriction. The aim of the present study was to investigate changes in sleep physiology during chronic sleep restriction with sleep periods placed at different circadian phases.

Methods: A total of N=27 subjects (age 30.5±7.3; 10 females) completed 1 of 3 sleep restriction protocols. In group A, following 3 baseline sleep periods (8h TIB), subjects were allowed 14 nights of 6h TIB from 01:30 to 07:30. In group B, subjects were allowed 2 baseline sleep periods (8h TIB), separated by 20h of TSD and followed by 10 nights of 6h TIB from 05:30 to 11:30. In group C, subjects were allowed 2 baseline sleep periods (8h TIB) separated by 28h of TSD and followed by 10 nights of 6h TIB from 13:30 to 19:30. Subjects remained in the laboratory throughout the protocol, with light levels <50lx (<1lx during scheduled sleep) and ambient temperature at 24±1˚C. Polysomnographic recordings were made on all baseline days, and on 2 out of every 3 days throughout the restriction period. Records were scored using standard criteria. Differences in sleep variables across days and among conditions were assessed using repeated-measures ANOVA.

Results: For total sleep time (TST), there was a main effect of day ($F[5, 100]=4.2$, $p<0.01$), and a day by condition interaction ($F[10, 100]=2.7$, $p<0.01$). Post-hoc analyses (independent samples t-test for equality of means, equal variances not assumed) revealed that group C slept less than group A on restriction days 1 and 4 ($p<0.05$), and less than group B on restriction days 1 and 2 ($p<0.05$). Group A slept less than group B on the second night of restriction ($p=0.01$). There was a day by condition interaction for slow wave sleep (SWS) ($F[10, 110]=3.3$, $p<0.01$), with group B having less SWS than group C on restriction day 4 ($p<0.05$). There was a trend for a main effect of day for REM ($F[5, 105]=2.1$, $p=0.09$).

Conclusion: These results suggest that despite increased homeostatic pressure for sleep due to the acute period of TSD (28h) in condition C, subjects experienced a reduced TST during the first restriction day compared to the nocturnally placed (condition A) and delayed (condition B) sleep periods. The decreased SWS in condition C and increased REM in condition B relative to condition A may reflect a circadian pressure for REM competing with homeostatic pressure for SWS. The results of this study must be interpreted in the context of potential changes of circadian phase over days of sleep restriction, which are reported elsewhere (Starzyk *et al*). These investigations are part of a larger study involving chronic restriction of sleep to 4h, 6h and 8h TIB at various circadian phases; analyses of these conditions are underway to further assess the effect of chronic sleep restriction and circadian phase on sleep physiology.

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Screening for Obstructive Sleep Apnea in Primary Care

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Introduction: While obstructive sleep apnea (OSA) is common among middle-aged adults (NEJM 1993;328:1230), its occurrence among individuals with hypertension is still greater (NEJM 2000;342:1378). OSA may be particularly important in individuals with hypertension that is refractory to traditional treatment approaches (Sleep 2001;24:721). We did a prospective comparison of four methods that could signal the presence of sleep apnea among patients with diagnosed hypertension. All were methods that could be performed effectively at the bedside by the primary care provider. These included a composite score that assessed frequency of apnea-related symptoms; body mass index (BMI); oral cavity measurements, and symptom score taken together with BMI.

We scored responses to three questions related to apnea symptom-frequency on a Likert scale, and averaged the responses to obtain our composite symptom score. We chose BMI as a second strategy because it is a surrogate for obesity, which is strongly associated with apnea. We also chose the multivariable apnea prediction (MAP) questionnaire (Sleep 1995;18:158), which assesses relative risk for apnea, by combining the composite symptom frequency score described above with other apnea-related variables -- BMI, age, and gender. Historically, the advantage of this MAP score has been its usefulness in settings where BMI is low. We chose oral measurements, because this was a previously validated measure among general populations (Ann Int Med 1997;127:581) outside of sleep referral settings. However, application of this method requires that subjects have adequate dentition.

Methods: We recruited subjects from primary-care outpatient settings at the Philadelphia Veterans Affairs Medical Center and from the University of Pennsylvania Hypertension outpatient practices. Subjects' ages ranged from 30-65 years. All had hypertension diagnosed by a health care provider, or were taking antihypertensive medications. Anyone with known OSA, or any condition associated with nocturnal hypoxia, or those using supplemental oxygen were excluded. We used the apnea-hypopnea index on 12-channel, in-laboratory polysomnography as the criterion standard, and compared it against each of the predictors - symptom score, BMI, oral cavity measurements, and symptoms together with BMI (MAP). We assessed apnea as present when the apnea-hypopnea index was >= 15 events/hour. We did area-under-curve analysis for receiver operating characteristic curves using the c-statistic on a logistic regression using SAS.

Results: We enrolled 230 subjects, and received usable symptom data on 213 (93%), BMI data on 229 (99.6%), oral measurements on 161 (70%), and calculated MAP scores on 225 (98%). Our analysis revealed AUC values as follows: 0.626 for symptoms alone, 0.658 for BMI, 0.684 for oral measurements, and 0.734 for symptoms together with BMI.

Conclusions: Data regarding symptoms, and data to compute BMI and the multivariable prediction index could be obtained on the majority of subjects. On the other hand, the oral measurement approach was less useful because it could not be obtained in the largest number of subjects. Symptoms taken alone were least useful for predicting the presence of sleep apnea. BMI was a stronger predictor, and oral measurements performed slightly better. Symptoms combined with BMI had the strongest discriminatory power in our study, and may represent a potential means of risk-stratification of subjects presenting to an ambulatory practice setting with OSA.

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Development of a Measure of Knowledge and Attitudes about Obstructive Sleep Apnea in Children (OSAKA-KIDS)

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Introduction: Untreated Obstructive sleep apnea syndrome (OSAS) can result in serious complications. In order to appropriately screen children for OSAS, primary care providers require adequate core knowledge about childhood OSAS. This study describes the development of the Obstructive Sleep Apnea Knowledge and Attitudes in Children (OSAKA-KIDS) questionnaire and characteristics of the measure.

Method: The 23-item OSAKA-KIDS questionnaire was mailed to 1195 community and academic-based physicians in KY, PA and MO. Data were tabulated and assessed using descriptive statistics. All tests were two-tailed.

Results: Of 576 surveys returned, 476 (309 community and 167 academic-based) had complete data. The sample was 47 % female, with mean age of 45.6 years (+/- 10.4) and mean number of years since medical school graduation at 18.5 (+/- 10.9) years. 73% of respondents completed residency training in pediatrics; 25% in family practice. The mean total knowledge score out of 18 possible was 12.5 (+/- 2.7). Six knowledge items were answered correctly only by < 60% of respondents. Only 15% of respondents knew that sickle cell disease is an associated risk for OSAS. A significant correlation occurred between number of years since medical school graduation and knowledge ($r = -0.121$, $p=.008$) and between knowledge and confidence in diagnosing and managing children with OSAS ($r = .270$, $p<.001$). Knowledge (12.3 vs. 12.8; $p=.046$) and confidence (2.7 vs. 3.0; $p<.001$) differed significantly between community- and academic-based physicians, respectively.

Conclusion: In spite of recent advances in pediatric sleep medicine, this study showed deficits in basic knowledge about childhood OSAS regardless of physician practice setting and specialty training. More education is needed regarding the risk factors, diagnosis and management of childhood OSAS.

Sleep Duration, Fatigue, and Work Performance among Hospital Staff Nurses with Multiple Caregiving Roles

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Introduction: Irregular sleep/wake patterns, inadequate rest, and poor sleep hygiene occur frequently among family caregivers. Feelings of mental and physical fatigue often escalate when family caregivers are employed outside the home, and must manage multiple responsibilities. However little attention has been given to understanding the effects on caregiver well-being when individuals combine family caregiving with professional caregiving. Therefore, this study a) compares fatigue among full-time hospital staff nurses who provide care for aging family members, children under 18 years of age, and those with no dependent care responsibilities; b) examines differences in sleep duration; and c) explores the effects on nurse work performance by caregiving status.

Methods: A random sample of hospital staff nurses (n=392) recorded information about their sleep/wake patterns, work hours, level of alertness on duty, and errors daily in a logbook for 28 days. Participants also rated their daily fatigue levels. Of the 392 nurse participants, 391 identified their informal caregiving status as: 1) not caregiving (n=197), 2) caring for children (n=133), caring for elders (n=32), or 3) caring for both children and elders (n=29). Although 38.2% (n=149) of the nurses were employed in 8-hour shift patterns, mean work hours ranged from 10.96 (no care dependents) to 11.54 (dual dependent care duties) hours per shift.

Results: The total amount of sleep obtained across all groups was 7.42+ 2.09 hours, with participants averaging fewer hours of sleep on work days (6.77 hours) than non-work days (8.17 hours). On work days mean total sleep durations for the four caregiving groups ranged from 6.43 hours to 6.83 (SD=1.75) hours (no care dependents). Nurses who were providing elder care obtained the least amount of sleep, averaging 6.43 hours (SD + 1.74) of sleep on work days, with one quarter of the nurses in this group sleeping less than 5.75 hours/day. Nurses providing care to elderly relatives were also more than twice as likely to make and error at work (OR =2.38; p = .005). However, elder care was only marginally associated with the prevalence of near misses (OR = 1.33; p =. 33). No other associations between errors and near misses with caregiving status were apparent despite the fact that fatigue scores were significantly higher among nurses who were caring for both children and elders. Additionally nurses providing concurrent elder and child care were also more fatigued than the caregiving other groups on non-work days. On average, this group of nurses worked more hours per shift than their other colleagues in the remaining caregiving groups.

Conclusion: Fatigue and inadequate sleep are issues for nurses in personal and professional caregiving roles. Nurses providing elder care at home obtained less sleep, were more fatigued even on non-work days, and likely to make errors at work. Fatigue levels among nurses providing concurrent elder and child care may be attributed to insufficient sleep combined with the burden associated with multiple caregiving responsibilities.

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Self-Treatment of Sleep Disorders in Older Adults

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Sleep disorders are common in older adults, with the prevalence rate for insomnia in particular ranging from 19-30% in this population. However, little is known about the different treatment options that elders elect to use. We conducted a study to determine the prevalence of the various types of treatments that older adults use for their insomnia and gauge their relative effectiveness based on self-report.

Methods: A 21-item questionnaire was developed which listed various treatment modalities for insomnia based on a review of the literature and interviews. Subjects were also asked to rate the relative effectiveness of that activity on a scale from 0 (not effective at all) to 4 (completely effective). It was mailed to a population-based cohort of older adults (age>65) living in the greater Philadelphia metropolitan area. Subjects with insomnia complaints were asked to fill out the questionnaire.

Results: 686 subjects were sent the questionnaire, of whom 460 responded (67% response rate). Of these 460, 247 (53.7%) complained of sleep difficulties and completed the treatment questionnaire. The most common treatment options were non-pharmacologic ones, including using the radio/TV or reading (59.1% and 52.6% respectively). Medications were also commonly used, with 35.6% taking pain medication, 27.1% using over the counter sleep aids, and 19.8% using prescription sleeping pills. Alcohol was used by 12.1% to help with their sleep. Vitamins or herbal supplements were used by 10.9%. Subjects used or had used an average of 4.7 different modalities (+/- 3.22), with 26.3% having used seven or more modalities in an effort to improve their sleep. Self-report of effectiveness was highest for prescription sleeping pills (2.18 +/- 1.31), with over-the-counter sleeping pills rated at 1.77 (+/-1.32) and pain medications at 1.71 (+/-1.17). Other strategies that were viewed as highly effective included reading (2.06 +/- 1.15) and using the radio/TV (1.84 +/- 0.97). When comparing treatment choices by gender, we noted that reading was preferred by women (63.9% of women vs 42.0% of men, chi-sq p=0.002). Patients who perceived their health as poor were more likely to take a pain medication (chi-sq p=0.005), use an over-the-counter sleeping pill (chi-sq p=0.02) or take a nap the following day (chi-sq p=0.06).

Conclusion: Our study has found that older adults engage in a variety of practices to help treat their sleep problems. Many of these practices center around non-pharmacologic interventions such as reading. Of the pharmacologic treatments, pain-relieving medications are the most commonly used; this is in accord with other research showing that pain is a common cause of insomnia. In general, elders felt that they received only moderate or little benefit from the different modalities. A significant proportion of elders used alcohol in an attempt to improve their sleep, a practice that in reality can worsen sleep quality and carries the risk of abuse. Our findings suggest that health care providers should ask detailed and direct questions regarding self-treatment of sleep problems as part of an evaluation of insomnia in order to counsel patients regarding potentially unhealthy activities.

Kinase-Phosphatase Balance in the *Drosophila* Circadian Clock

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Drosophila has emerged as an excellent model system to study circadian rhythm. Components of a transcriptional auto-regulatory feedback loop, important for generating rhythmic expression of clock genes have been identified. Recent studies have shown that post-translational mechanisms are important for generating a functional clock. Since phosphorylation of Drosophila PERIOD (PER) cycles robustly over the course of a day, rhythmic phosphorylation is a likely mechanism for controlling cycling at a post-translational level. Although the kinases that phosphorylate PER have been identified, the mechanisms that drive cycling are not known. Here we demonstrate a role for Protein Phosphatase 2A (PP2A) in regulating PER protein stability and nuclear entry. Inhibitors of PP2A destabilize complexes containing PER and its partner clock protein, TIMELESS (TIM). We identified two regulatory subunits, TWINS (TWS/PR55) and WIDERBORST (WDB/B56-2), that target PP2A to the PER-TIM complex in S2 cells. In the adult fly head, expression of *tws* cycles robustly under control of the circadian clock. Levels of PP2A are important for maintaining behavioral and molecular oscillations. Flies expressing a dominant negative PP2A catalytic subunit display long periods and arrhythmia, which is associated with a decrease in PER expression. In addition, over-expression of the PP2A catalytic subunit in clock neurons results in loss of behavioral rhythms and constitutive nuclear expression of PER. Also levels of PP2A regulatory subunits TWS and WDB affect PER stability and circadian behavior. PP2A also affects PER phosphorylation *in vitro* and *in vivo*. These results suggest that the post-translational mechanisms which drive the cycling of PER levels require the rhythmic expression of PP2A. The effect of phosphatases on PER function is being investigated.

The Effect of Work Breaks on Alertness and Performance

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Introduction: Research has shown that short breaks can improve performance and decrease subjective fatigue during sleep deprivation and other laboratory protocols. Since recent studies have shown that hospital staff nurses frequently work long hours, often with inadequate sleep, the goal of this sub-study is to determine if nurses who were able to take a break during their shift would report fewer difficulties remaining alert and make fewer errors than those who were not able to take a break during their shift.

Methods: Three hundred ninety-three randomly selected nurses participated in a larger study of staff nurse fatigue and patient safety. Data collected for 28 consecutive days included information about the participants' sleep, mood, scheduled work hours, actual work hours, errors, episodes of drowsiness and actual sleep on duty, and difficulties driving home due to drowsiness. Participants were also asked if they were able to take a break or sit down for a meal during their shift, to indicate the total duration of breaks taken during the shift and if they were relieved of patient care responsibilities during their meals and break periods.

Results: There were 534 shifts (10.2%) where participants reported having no chance to take a break or sit down for a meal during their shift, and 2248 shifts (43.1%) where they reported getting some time for a break or meal period, but were not relieved of patient care responsibilities. Nurses reported having a break or meal period free of patient care responsibilities less than half of the shifts they worked (2429/5211 shifts).

Breaks and meal periods averaged only 23.8 ± 30.1 minutes, even though 39% of the shifts exceeded 12.5 hours. Shift duration did not affect duration of breaks and meal periods, with mean durations for break and meal periods averaging 23.4 ± 28.5 minutes for shifts </= 8.5 hr, 22.3 ± 27.9 minutes for shifts >8.5 but < 12.5 hr, and 25.5 ± 33.0 hr for shifts >/= 12.5 hr.

There were no differences in the number of errors or episodes of drowsiness reported by nurses who were able to take a break free of patient care responsibilities when compared to those who were unable to take a break during their shift. However, night shift nurses who were able to take a break reported significantly more episodes of falling asleep during their shift than night shift nurses who did not have a break or meal period free of patient care responsibilities (OR = 0.46, p=0.02),, suggesting the possibility that at least some of the night shift nurses may have napped during their breaks.

Conclusions: These findings support earlier reports that nurses frequently skip their breaks and meal periods to provide patient care. Although the practice is undesirable, it was not associated with an increased risk of errors or greater numbers of drowsy episodes.

Operating Characteristics of the Multivariable Apnea Prediction Index in Non-Clinic Populations

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Introduction: The multivariable apnea prediction (MAP) index is a simple relative risk measure for apnea validated in multi-center clinical settings in the early 1990's. The MAP includes self-responses to three apnea symptom-frequency questions producing a summary index, obesity (BMI), age, and gender. Symptom-frequency has greater predictive value in the MAP when BMI is small. The MAP has been employed in research and in clinical populations outside of sleep disorders referral centers. We report on MAP indices in two such populations, each consisting of clinical research subjects at the University of Pennsylvania. In our recently completed study of performance impairment in commercial drivers (Study 1), the MAP facilitated stratified sampling enriching the laboratory cohort with regard to apnea. Currently (Study 2), we are evaluating the identification of elders with excessive sleepiness who have sleep apnea syndrome and determining whether sleepiness improves with treatment. We examined screening operating characteristics of the MAP in these two large, non-sleep-clinic cohorts.

Methods: Study 1 included MAP survey respondents among commercial drivers contained in a randomized list provided by local authorities. The cohort comprised 247 higher (MAP>0.44) and 159 lower risk drivers; with oversampling of higher risk drivers (weighted mean age = 45.4 y; BMI 29.9 kg/m^2). We measured apnea hypopnea index (AHI) in events/hour using in-laboratory polysomnography. The weighted prevalence of apnea (events/hr) was 17.6% (5-<15), 5.8% (15-<30), and 4.7% (>=30). Study 2 includes 296 elders (85 M, 211 F) with subjectively reported excessive daytime sleepiness (mean age = 70.6 y; BMI 29.5 kg/m^2) and with apnea prevalence of 30.4% (5-<15), 24.0%, (15-<30), and 25.7% (>=30). Receiver operating characteristic (ROC) curve analyses were performed for the MAP, BMI, and the apnea symptom-frequency index. The area under the ROC curve (AUC) was used to summarize predictive value. Results were compared to AUC's we reported previously in a sleep disorders clinic population for AHI>==10 (AUC MAP=0.786, BMI=0.734, Symptoms=0.695).

Figure 1
Percentages with AHI>=15/hr in Two Populations

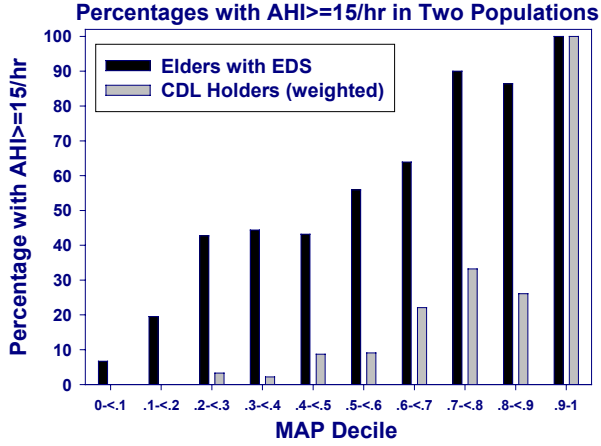

Table 1

Elderly With EDS (N=296)						
	MAP				BMI	Symptoms
	AUC	OR	LB	UB	AUC	AUC
AHI>=5	0.763	2.6	1.9	3.7	0.707	0.587
AHI>=10	0.720	2.1	1.6	2.7	0.633	0.608
AHI>=15	0.749	2.3	1.8	2.9	0.646	0.636
AHI>=20	0.747	2.2	1.8	2.9	0.659	0.617
AHI>=30	0.742	2.1	1.7	2.8	0.671	0.628
Commercial Drivers (N=406)						
	MAP				BMI	Symptoms
	AUC	OR	LB	UB	AUC	AUC
AHI>=5	0.781	3.2	2.5	4.2	0.744	0.624
AHI>=10	0.793	3.4	2.5	4.8	0.766	0.603
AHI>=15	0.759	2.9	2.1	4.1	0.757	0.559
AHI>=20	0.773	3.2	2.2	4.8	0.765	0.576
AHI>=30	0.796	3.7	2.3	6.5	0.777	0.622
OR - Odds ratio for a 0.20 increase in MAP						

Results: For Study 1, AUC values for AHI>=10 were MAP=0.793, BMI=0.766, Symptoms=0.603. For Study 2, AUC values for AHI>=10 were MAP=0.720, BMI=0.633, Symptoms=0.608. Similar AUC values were found for other cutpoints (e.g., AHI>=5 (AHI>=30), AUC's were 0.781 (0.796) and 0.763 (0.742) for Studies 1 and 2, respectively).

Conclusions: MAP summary predictive values, which integrate symptom-frequency scores and BMI, were very similar among general populations compared to patients from the sleep disorders clinics. BMI alone was a stronger predictor among commercial drivers. In contrast, both symptoms and BMI were required to achieve adequate levels of predictive value among elderly individuals with EDS. No commercial driver with BMI<25 had AHI>=30 and only 2.4% had AHI>=15. In contrast, these percentages were 16.7% and 40.0% in the elderly with EDS population. Overall, MAP operating characteristics do not appear to vary substantially between clinical and diverse non-clinical populations.

Mammalian Brain Cytochrome C Oxidase in Sleep and Sleep Deprivation

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An increase in the catalytic subunit 1 mRNA of cytochrome c oxidase (COX) has been reported after short-term sleep deprivation and spontaneous wakefulness by using microarray, differential display, Rnase protection assay and *in situ* hybridization. This finding was demonstrated on rat and mouse cortex and in Drosophila heads. But whether this change in the mRNA of one subunit of the enzyme leads to an increase in the overall enzyme activity was not known. We hypothesized that there will be increase in the enzyme's activity likely aiming to adjust for the increased cellular needs for ATP during active period.

To address this, we measured COX activity on isolated mitochondria from five brain regions (frontal cortex, parietal cortex, occipital cortex, thalamus and hypothalamus) on 3 groups of male Fisher 344 rats implanted with EEG/EMG electrodes: 3 hrs spontaneous sleep, 3 hrs spontaneous wake, and 3 hrs of sleep deprivation. Similar studies had been replicated on same groups of C57B6 mice. Additionally, in rats Northern analyses on frontal cortex were performed to characterize the levels of mRNA expression of two COX subunits: Cox1 (mitochondrial) and Cox4 (nuclear). In mice, we assessed mRNA for several subunits of the enzyme both nuclear- and mitochondrially encoded by qRT-PCR, and initiated studies of protein expression by Western blotting.

Our results show that (1) there is an increase in COX activity after 3 hrs wake and 3 hrs sleep deprivation as compared to 3 hrs sleep throughout rat and mouse brain, (2) in rats by Northern blot analysis, there is an up-regulation of both Cox1 and Cox4 mRNAs after 3hrs sleep deprivation and after 3 hrs spontaneous wake as compared to 3 hrs spontaneous sleep; (3) in mice by qRT-PCR, there is an up-regulation of mitochondrial (Cox1, Cox2, and Cox3) as well as nuclear (Cox4, Cox5b, Cox6aL, Cox6c, but not Cox5a and Cox8a) subunits of the enzyme after 3hrs of sleep deprivation; (4) in mice by Western, COXI protein expression pattern follows that of the mRNA expression (preliminary data).

We conclude that there is an up-regulation of at least one of the enzymes of oxidative phosphorylation crucial for ATP production after both 3 hrs wake and 3 hrs sleep deprivation as compared to 3 hrs spontaneous sleep which will adjust for the increasing neuronal demands for ATP. This up-regulation is in part transcriptionally regulated with increased mRNA levels for both nuclear and mitochondrial subunits of the enzyme.

This work was supported by:
Sleep Medicine Education and Research Foundation provided to Dr. Nikonova.

Sleep Disordered Breathing In Obese Type 2 Diabetics

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Introduction:

Weight loss is frequently recommended for obese patients with obstructive sleep apnea, but the empirical evidence for this recommendation is not well substantiated. The purpose of the ongoing research project Sleep AHEAD (*Action for Health in Diabetes*) is to determine the effect of weight loss on sleep disordered breathing in obese adults with type 2 diabetes mellitus randomized to a formal weight loss program versus usual care. Home-unattended polysomnograms are being performed prior to treatment and at 1- and 2-year intervals to determine the effect of weight loss on the apnea-hypopnea index (AHI) in individuals with moderate to severe OSA (AHI > 15). Sleep AHEAD is an ancillary study of Look AHEAD and Sleep AHEAD participants have been recruited from that larger clinical trial. This abstract reports our preliminary findings from the baseline assessments.

Methods:

Subjects were recruited from 4 clinical sites: University of Pennsylvania, University of Pittsburgh, Brown University, and St. Luke's-Roosevelt Medical Center in NYC. The following measurements were obtained at baseline: overnight polysomnography (Compumedics PS2), upper airway morphometrics, waist circumference, blood pressure, Epworth Sleepiness scale (ESS), and FOSQ (Functional Outcome of Sleep Questionnaire). Overnight polysomnography was performed in the participant's home. The following parameters were recorded during the overnight sleep study: EEG (C3A2, C4A1), bilateral EOG, submental EMG, thoracic and abdominal effort (piezo-electric belts), nasal pressure, pulse oximetry, EKG, body position, and snoring. The studies were all scored at a centralized Reading Lab at the University of Pennsylvania by registered sleep technologists. An obstructive respiratory event (apnea/hypopnea) was defined as an event of 10 seconds or longer with either of the following: 1) a > 50% decrease from baseline in the amplitude of a valid measure of breathing during sleep, or 2) a clear amplitude reduction of a validated measure of breathing during sleep that does not reach 50% but is associated with either an oxygen desaturation of > 3% or an arousal. An event was called central when there was no associated chest wall movement. AHI was calculated as the mean number of apnea/hypopnea events per hour of sleep.

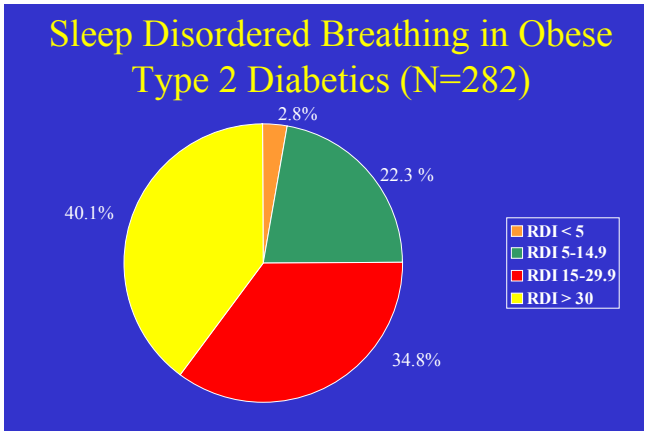
Results:

282 subjects have been evaluated at baseline (mean age 62.9 ± 6.4 years, mean BMI (Body Mass Index) 37.0 ± 13.7 kg/m^2 and 59.2% female).

The following graph summarizes the overall findings of the AHI for the group at baseline. Mild sleep apnea (AHI 5 - 14.9) was present in 63 participants (22.3%), moderate sleep apnea (AHI 15 – 29.9) was present in 98 participants (34.8%), and severe sleep apnea (AHI > 30) was present in 113 participants (40.1%). Only 8 of the 282 participants had an AHI < 5.

Conclusion:

The results indicate a high prevalence of obstructive sleep apnea in obese individuals with type 2 diabetes mellitus. The majority of individuals have moderate to severe sleep apnea. 1- and 2-year follow-up studies will be completed over the next 2 years.



This study was funded by NIH grant #HL70301.

Changes In Plasma Melatonin Profiles During Chronic Nocturnal Sleep Restriction

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Sleep deprivation has been reported to have no effect on melatonin secretion, while shifting the timing of sleep-wake behaviour can produce alterations in the phase of secretion. In the present study we examined the effects of shortened nocturnal sleep, with and without daytime naps, on melatonin. N=19 subjects (14m; 5f; aged 21-38y) completed a 14-day in-laboratory study. Following 2 baseline nights (8.2h TIB: 2154h-0606h) subjects were randomly assigned to a 10d chronic sleep restriction condition: 8.2h TIB (2154h-0606h; n=5); 4.2h TIB (2354h-0406h; n=14) with and without a daytime nap (between 0.0h and 2.4h). On baseline day 1 and restriction day 10, subjects were maintained in a constant posture paradigm for 26h, with sleep at the allocated times, and blood samples collected via an indwelling catheter. Plasma melatonin concentrations were determined hourly using RIA. Melatonin profiles were analysed using within-subjects repeated-measures ANOVA comparing day 1 with day 10 across conditions. All subjects demonstrated a circadian rhythm in melatonin across the 26h ($p<0.001$). In the 8.2h condition there were no significant differences in melatonin profiles between assessment days. In the 4.2h sleep-restriction conditions, with dark onset delayed by 2h, there was a significant delay in melatonin onset (approximately 2h; $p\leq0.005$). Sleep restriction was able to alter the circadian system, as evidenced by changes in the timing of melatonin secretion. This effect may be due to changes in light-dark exposure and/or the reduced sleep period.

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Changes in Upper Airway Size during Tidal Breathing in Children with OSA

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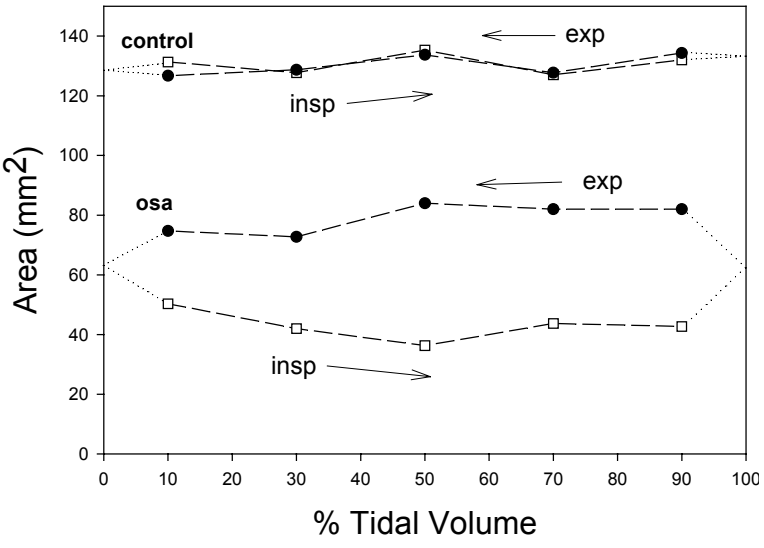
The upper airway was previously found to be more collapsible in children with obstructive sleep apnea (OSA). We performed volume-gated fast MRI to evaluate airway motion at mid-tonsillar region during tidal breathing (TV) in 3 OSA and 3-matched controls, age 4.2±2.0y and 4.4±2.6y, respectively.

Methods: Studies were performed under sedation. We used the gating system of the Siemens Sonata, 1.5T. Volume triggering during TV was performed automatically at 10, 30, 50, 70, and 90% of inspiration and expiration using thoraco-abdominal motion sensed by bellows as the respiratory signal. Axial images were obtained using a 2D truefisp sequence with parameters: TR/TE/alpha = 4.6/2.3/50deg; FOV=200x150; MA=256x128; Half Fourier acquisition, AT=300 ms sec/slice. Airway cross-sectional area was measured at mid-tonsillar region for each increment of the respiratory cycle using 3DVIEWNIX.

Results: Mean cross-sectional area vs. % of TV breath volume during the inspiratory (open squares) and expiratory cycle (closed circles) of the 3 pairs of subjects are shown in the figure. Our findings demonstrate: 1. Changes during tidal breathing are smaller in the control group. 2. The mean cross-sectional area during each phase of TV is smaller in OSA subjects compared to controls. 3. Area change is noted in the OSA group during TV. Area is relatively constant throughout the inspiratory phase and dilates during expiration.

Discussion: Absence of airway narrowing during inspiration could suggest a neural activation mechanism during inspiration. Increased airway cross sectional area during expiration demonstrates effective compliance of this region and is probably secondary to increased intraluminal driving pressure during expiration.

Supported by: HL-62408



Hypoglycemia Reduces REM Sleep, Increases Arousal, and Activates Locus Coeruleus and Basal Forebrain Cholinergic Neurons in Rats

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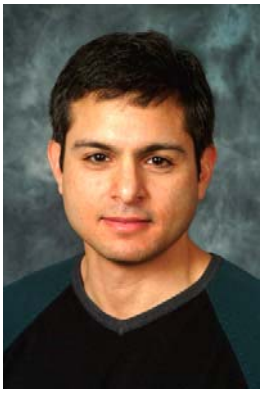
Research studies in normal humans show that nocturnal hypoglycemia is an arousing stimulus, decreasing sleep efficiency and reducing time in stage 3 and 4 sleep. The purpose of this study was to evaluate hypoglycemia as an arousal stimulus in 8 male Sprague-Dawley rats prepared with EEG and EMG electrodes for recordings of sleep and waking. Recordings were made from 1 PM to 3 PM, immediately after subcutaneous saline injection (baseline day) or after insulin 3 U/kg to reduce plasma glucose from 116 mg/dl to 40 mg/dl over the 120 minute recording period. Insulin treatment increased time spent in waking from 29% ± 2 (SE) to 50% ± 4 ($p=.003$). REM sleep was reduced from 18% ± 2 to 6% ± 1 ($p=.0001$). SWS showed a modest but not significant change from 53% ± 3 to 45% ± 4 during hypoglycemia. Additional rats without EEG and EMG surgery were used to assess the effect of afternoon insulin treatment on Fos staining in brain arousal-related neurons. Insulin treatment (n=4) increased Fos-immunoreactivity in neurons counted at 3 levels of the locus coeruleus (LC) to 75 ± 17 while controls (n=2) had an average of 1 Fos-ir cell in LC. Cells double-labeled with Fos and choline acetyltransferase were observed in basal forebrain cholinergic neurons after insulin injection. In the magnocellular preoptic nucleus, these cells totaled 37 ± 10 in insulin-treated rats vs 2 in controls. These results provide evidence of hypoglycemic arousal from sleep in a rodent model, and demonstrate that mild hypoglycemia is associated with activation of noradrenergic and cholinergic neuron groups implicated in arousal.

In the mid-1980s, a trio of pioneering scientists interested in sleep research – David Dinges, Adrian Morrison and Allan Pack – embarked upon a collaboration which led, in 1991, to the creation of the Center for Sleep and Respiratory Neurobiology at the University of Pennsylvania. Since its inception, the CSRN has evolved into a vibrant multidisciplinary program that draws its membership from schools across the university and now includes over 100 members. The research interests of Center members span a range from circadian biology to sleep-disordered breathing and sleep deprivation, and pursue excellence in both basic and clinical research. In addition, the CSRN plays a major role in clinical program delivery and the education of undergraduates, medical students, resident physicians and fellows, nursing students, and Ph.D. students and post-doctoral fellows.

As the sleep research community at the University of Pennsylvania continues to grow, the timing is right for the first CSRN Research Retreat. It will be a great opportunity to interact with colleagues both old and new, and to learn more about the exciting projects taking place in the various research groups in the center. With two symposia, a data blitz session, poster presentations and Dr. Masashi Yanagisawa as the keynote speaker, we are confident that the retreat will be scientifically fruitful. As the organizing committee, we hope that the CSRN Research Retreat will become an annual stimulus for promising new ideas and long-lasting collaborations.

Grace Pien

Julie Williams



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