

Center for Sleep & Respiratory Neurobiology

3rd Annual Research Retreat

June 13, 2006

*Levy Conference Center at the
University of Pennsylvania Law School*



University of Pennsylvania

Program and Abstracts

- 8:15 – 9:00 Poster Mounting, Registration and Breakfast
- 8:50 – 9:00 Opening Remarks – **Allan Pack, M.D., Ph.D.**
- 9:00 – 9:30 “Centralized PSG Scoring in Multicenter Clinical Trials” **Samuel Kuna, M.D.**
- 9:30 – 10:00 **Caruso HM**, Stakofsky AB, Tucker AM, Dinges DF, and Van Dongen HPA.
“Effect of Cognitive Workload on Delta Power in the NREM EEG of Recovery Sleep Following Acute Sleep Deprivation.”
Mazza E, Fenik P, Zhu Y, Zhan G, Aston-Jones G and Veasey S.
“Identification of Wake Active Neurons Susceptible to Hypoxia-Reoxygenation Injury in Sleep Apnea Model and Mechanisms of Susceptibility.”
Sun Y, Chen J, Kuzel R, Thomas S, and **Kelz MB**. “Norepinephrine Deficient Mice are both Hypersensitive to the Hypnotic Properties of Volatile Anesthetics and Demonstrate Delayed Emergence from Anesthesia.”
- 10:00 – 10:15 Coffee Break
- 10:15 – 10:45 “Interaction between Sleep and the Immune Response in Drosophila.”
Julie Williams, Ph.D.
- 10:45 – 12:00 **Koh K**, Evans JM, Hendricks JC, and Sehgal A.
“A Drosophila Model for Age-Associated Changes in Sleep:Wake Cycles.”
Vecsey CG, Khatib N, and Abel T
“Age-Related Impairment in Long-Term Memory Retention Occludes the Effect of Brief Post-Training Sleep Deprivation in Mice.”
Jha SK, Steinmetz N, Coleman T, and Frank MG
“Increased Firing of Remodeling Neurons in the Visual Cortex during Sleep.”
Joiner W, Crocker A, and Sehgal A.
“Sleep in Drosophila is Regulated by Adult Mushroom Bodies.”
Lu JW, Mann GL, **Fenik VB**, and Kubin L.
“Disinhibition of Perifornical Hypothalamic Neurons Blocks the Ability of Pontine Carbachol to Produce REM Sleep-like State in Anesthetized Rats.”
- 12:15 – 2:00 Poster viewing and Lunch
- 2:00 – 2:30 “Can't Sleep at Night, Can't Function during the Day: Sleep Disorders and Daily Functioning.”
Terri Weaver, Ph.D., RN, FAAN.
- 2:30 – 3:15 **Sawyer AM**, Weaver TE, Canamucio A, Moriarty H, Ferguson J, and Kuna ST.
“A Prospective Cohort Study Examining Race as a Predictor of Adherence to Continuous Positive Airway Pressure Therapy in Obstructive Sleep Apnea.”
Mackiewicz M, Pack AI, and Veasey SC.
“Shift Work-Light Exposure Increases Obesity & Impairs Insulin Tolerance in Mice.”
Mollicone DJ, Van Dongen HPA, and Dinges DF.
“Response Surface Map Analysis of PVT Performance in a Chronic Sleep Restriction Dose Response Experiment With and Without Naps.”
- 3:15 – 3:30 Coffee Break
- 3:30 – 4:00 Additional Poster Time
- 4:00 – 5:00 Keynote Speaker:
“**Entrainment of Human Circadian Rhythms**”
Charles A. Czeisler, M.D., Ph.D.
Frank Baldino, Jr., Ph.D. Professor of Sleep Medicine, Harvard University
- 5:00 – 6:30 Awards, Reception

FROM THE DIRECTOR:



This is the third annual Research Retreat for the Center for Sleep and Respiratory Neurobiology. This has become an important annual event for us. It is a great vehicle to bring together the investigators and trainees studying sleep and its disorders from different Departments and Schools at the University of Pennsylvania. It helps us maintain ourselves as a community of scholars.

This year our keynote speaker is Dr. Charles Czeisler from Harvard, an acknowledged international expert in human sleep/circadian mechanisms. We have continued our trainee poster competition that has proved successful in the past.

This event is one of a series of upcoming events for us. On Monday, June 19, we are having our first cocktail reception from 7:00-9:00 PM in the Paris Ballroom at the Hotel Monaco at the APSS (Sleep) meeting.

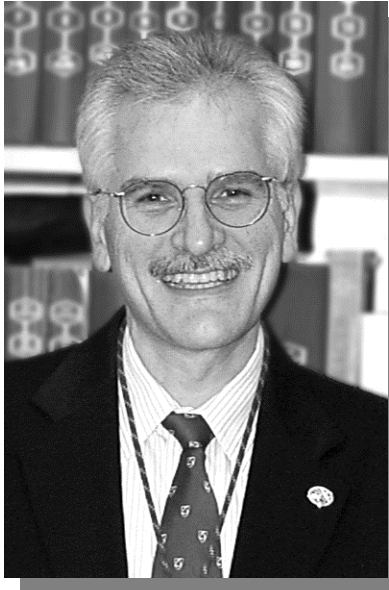
On July 8, at the Pack's, we are having our annual holiday party. This year we are celebrating Iceland Independence Day and our colleagues from Iceland are coming. Icelandair has agreed to fly in two chefs from Iceland and part of the menu will be a Thorablott--old Viking food. We hope that the Icelandic Ambassador to the United States will be attending.

We hope that you will be able to attend both events.

In closing, I would like to thank the Organizing Committee for the Research Retreat, chaired by Dr. Jini Naidoo. The other members of the Committee were: Dr. Marcos Frank, Dr. Susan Harbison, Dr. Nirav Patel, Dr. David Raizen and Dr. Terri Weaver. It is wonderful to see our junior faculty taking ownership of this important event--our annual Research Retreat.

A handwritten signature in black ink, appearing to read "Allan I. Pack". The signature is stylized and fluid, with a long, sweeping flourish extending from the end.

Allan I. Pack, M.B., Ch.B., Ph.D.



Keynote Speaker:

Charles A. Czeisler, Ph.D., M.D.

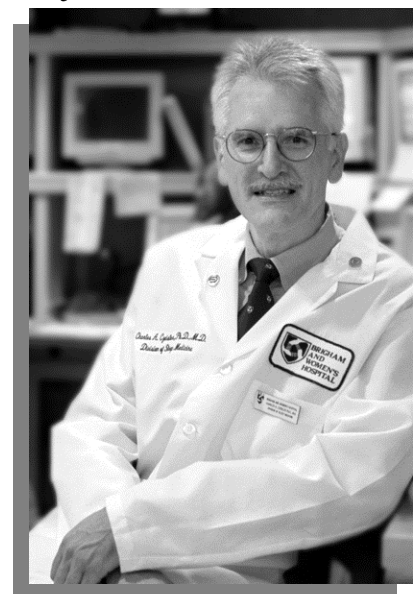
Frank Baldino, Jr., Ph.D. Professor of Sleep Medicine
Harvard University

Charles A. Czeisler, Ph.D., M.D. is Baldino Professor of Sleep Medicine and the Director, Division of Sleep Medicine at Harvard Medical School and Chief, Division of Sleep Medicine, Department of Medicine at the Brigham and Women's Hospital in Boston, Massachusetts.

Dr. Czeisler graduated magna cum laude with a degree in Biochemistry and Molecular Biology from Harvard College. He received his Ph.D. in Neuro-and Bio-behavioral Sciences and his M.D. from Stanford University.

Dr. Czeisler has more than 30 years' experience in the field of basic and applied research on the physiology of the human circadian timing system and its relationship to the sleep-wake cycle. He is Team Leader of the Human Performance Factors, Sleep and Chronobiology Team of NASA's National Space Biomedical Research Institute, which has successfully carried out polysomnographic sleep recording as well as the first double-blind, placebo-controlled trial of a pharmacologic agent ever conducted in space, the results of which were published in the *American Journal of Physiology*. Dr. Czeisler has served on and consulted to a number of national and international advisory committees, including the National Institutes of Health, the Institute of Medicine, the National Academy of Sciences, the Sleep Research Society, the Nuclear Regulatory Commission, the Air Force Office of Scientific Research and the Air Transport Association.

Dr. Czeisler has published over 100 reports in peer-reviewed journals, more than 75 review articles, 5 books/monographs, and numerous research abstracts; he has been a member of the editorial boards of *American Journal of Medicine*, *Journal of Biological Rhythms*, and *Sleep*. He is a recipient of the Robert R.J. Hilker Award in Occupational Medicine (1991), Aschoff's Rule (2001), the E.H. Ahrens, Jr. Lecture Award from the Association for Patient Oriented Research (2002), the William C. Dement Academic Achievement Award from the American Academy of Sleep Medicine (2002), the NIOSH Director's Award for Scientific Leadership in Occupational Safety And Health (2005) and the National Sleep Foundation 2006 Healthy Sleep Community Award (Harvard Work Hours and Health and Safety Group). He is President of the Sleep Research Society, a Fellow of the American Society for Clinical Investigation and of the Association of American Physicians, and is a Diplomate of the American Board of Sleep Medicine.



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Investigating a Dopaminergic Mechanism of Action for Modafinil in Drosophila

Ashmore LJ, Newsom E, Hendricks J, and Pack AI

Center for Sleep and Respiratory Neurobiology, University of Pennsylvania

Introduction: Available evidence suggests that modafinil, a novel wake-promoting drug, is a dopamine transporter (DAT) antagonist which increases dopaminergic (DA) neurotransmission in mammals. *Drosophila melanogaster* also respond to modafinil with decreased rest and disruption of sleep consolidation (Hendricks et al, *Sleep* 26:908, 2003). We tested whether modafinil acts via DAT in *Drosophila* by examining the response of *fumin* flies (*fmn*; DAT null flies who display increased baseline activity levels and decreased sleep) to modafinil. The locomotor activity of wild type and *fmn* flies was monitored, using the Trikinetics DAM system, before and after exposure to modafinil and nisoxetine (a noradrenaline transporter antagonist with a high affinity to *Drosophila* DAT). Changes in total activity and sleep (defined as at least five consecutive minutes of zero activity counts) were compared within flies and between lines. We found that *Fmn* flies are sensitive to both modafinil and nisoxetine, as are wild type flies. The decrease of sleep seen in *fmn* lines on modafinil and nisoxetine was comparable to that of controls. Nisoxetine and modafinil also decreased consolidation of sleep (as measured by number and length of sleep bouts) in both lines, although the timing of these effects differ (modafinil has a main effect on nighttime sleep, whereas nisoxetine's affected activity at all times of day). *Fmn* flies were also resistant to the lethal effects of higher doses of nisoxetine seen in wild type, but more sensitive to those of high modafinil doses. Our preliminary evidence suggests that modafinil does not act via the DAT in *Drosophila*. This is an opposite result to that from DAT-null mice (Wisor et al, *J Neurosci* 21:1787, 2001), who are unresponsive to modafinil, although they also have altered responses to caffeine and hence there may be developmental alterations. Our results question whether modafinil acts through DAT or the dopaminergic system, and indicates that *Drosophila* may be a useful model to investigate modafinil's as yet unknown mechanism of action.

Support:

This research was partly supported by Cephalon, Inc.

The Effect of Puberty on Upper Airway Collapsibility

Bandla P, Mason T, Brooks LJ, Karamessins L, Samuel J, Pepe M, Marcus CL

The Children's Hospital of Philadelphia, University of Pennsylvania,
Philadelphia, PA

Background: Children snore less and have fewer obstructive apneas than adults. In children who develop the obstructive sleep apnea syndrome (OSAS), the disease is found equally among males and females. In contrast, in adults the prevalence of obstructive sleep apnea in males is about twice that of pre-menopausal females. The prevalence of obstructive sleep apnea then increases in females after menopause. This epidemiology suggests that sex hormones play a critical role in upper airway function. During pubertal development, hormone levels increase from minimally detectable to adult levels, in a physiologic fashion making it the ideal natural model to determine the role of sex hormones and gender on upper airway collapsibility.

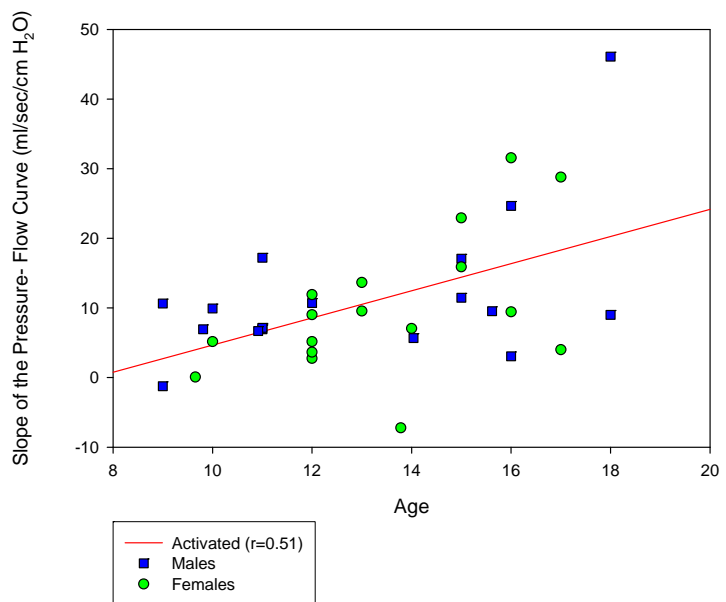
Hypothesis: We hypothesize that upper airway collapsibility is lowest during childhood and increases with age, with a critical transition occurring during puberty.

Methods: Normal, non-obese adolescents of Tanner stages 1 through 5, between the ages of 8-18 were studied. Subjects underwent a baseline screening polysomnogram to ensure normalcy. Clinical Tanner staging was performed. During a second polysomnogram, measurements were made by correlating maximal inspiratory airflow with the level of nasal pressure applied via a mask. The slope of the upstream pressure-flow curve was used to characterize upper airway function. Pressure - flow relationship (PFR) data was obtained using established techniques during both activated and hypotonic upper airway states[1]. The slope of the pressure-flow curve was obtained and correlated with Tanner stage.

Results: 17 male and 17 female subjects have been studied to date. There was a trend toward a positive correlation between Tanner stage and slope of the PFR with both the activated ($r = 0.31$) and hypotonic ($r = 0.11$) techniques (Figure). There was a positive correlation between age and the slope of the activated PFR ($r = 0.52$, $P < 0.01$), but not with the hypotonic PFR ($r = 0.0$).

Conclusions: Although limited by the small number of subjects studied so far, preliminary results suggest there is an increase in upper airway collapsibility with increasing Tanner stage. The correlation with age suggests that part of the increase in collapsibility may be related to airway growth rather than sexual maturity. More subjects are needed to determine the relationship between gender specific pubertal changes and upper airway collapsibility.

Slope vs Age: Activated



1. Marcus, C.L., et al., Developmental changes in response to subatmospheric pressure loading of the upper airway. *J Appl Physiol*, 1999. 87(2): p. 626-33.

Sleep Dose-Response Study of Recovery from Sustained Sleep Restriction

Banks S, Van Dongen H, and Dinges DF

Division of Sleep and Chronobiology, Department of Psychiatry, Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine, Philadelphia, PA

Theoretical models of sleep homeostasis suggest an exponential recuperation of function as recovery sleep duration increases following total sleep deprivation. However, there has been no systematic test of this prediction for recovery following sustained sleep restriction. A dose-response experiment was conducted to determine the nature of recovery following sleep restriction. Sixty-one healthy adults (22-45yr; 36 females) participated in a laboratory-controlled sleep restriction protocol. They underwent 2 nights of baseline sleep (10h TIB), then 5 nights of sleep restriction (4h TIB), followed by randomization to a single recovery night where TIB was either 0h, 2h, 4h, 6h, 8h, 10h or 12h. Sleep was recorded polysomnographically. Subjects completed a 30min neurobehavioral test battery every 2h during wakefulness that included the Psychomotor Vigilance Test (PVT), Karolinska Sleepiness Scale (KSS) and Digit Symbol Substitution Task (DSST). ANCOVA was used to compare across recovery sleep dose conditions controlling for biological variability in the cumulative effects of sleep restriction, baseline performance differences, age and sex. As the recovery sleep TIB was increased in duration following sleep restriction, the 3 outcomes decreased toward normal levels (i.e., fewer PVT lapses, $p < 0.001$; greater number of DSST correct, $p < 0.001$; and lower KSS scores, $p < 0.001$). A linear relationship characterized the sleep-dose recovery functions for PVT lapses and KSS sleepiness, while DSST had both linear and quadratic components. These data suggest recovery of neurobehavioral functions from chronic sleep restriction occurs linearly with increases in TIB up to 12h, at least for measures of alertness/sleepiness (PVT/KSS). The relationship of this function to PSG measures and NREM EEG slow wave activity is being assessed to identify sleep homeostatic mechanisms for recovery from sustained sleep restriction.

Support:

NASA cooperative agreement NCC 9-58-159 with the National Space Biomedical Research Institute, and NIH grants NR 009281 and RR00040

Restless Legs Syndrome and Periodic Limb Movements of Sleep in Pregnant Women

Beothy EA, Pien GW, Nkwuo JE, Pack AI, Schwab RJ, and Staley B

Introduction: Restless legs syndrome has been reported to be common during pregnancy. However, few studies have examined characteristics of RLS in pregnancy or determined whether women with RLS are likely to have periodic limb movements during sleep.

Methods: We recruited 126 pregnant women from the obstetrics practices at our institution. Each woman completed a 4-item questionnaire about the presence and frequency of symptoms of restless legs syndrome and underwent full overnight polysomnography (PSG) during the first trimester. 84 women have also completed questionnaires and PSGs during the third trimester (31-39 weeks). Comparisons were made using unpaired t-tests, chi-squared or Fisher's exact tests and logistic regression as appropriate.

Results: Mean subject age was 27.3 (7.2) years. 76% of subjects were African-American. 40% of subjects were primigravidas. In the first trimester, the prevalence of RLS (symptoms ≥ 2 -4x/month) was 20.6%, increasing to 31.0% by the third trimester with a trend towards statistical significance ($p=0.09$). Mean periodic limb movement index (PLMI) increased significantly from the first to third trimester (1.4/hr v. 3.1/hr, $p=0.03$). The proportion of subjects with PLMI ≥ 15 /hr increased significantly from the first to the third trimesters (PLMI: 4.8% v. 14.3%, $p=0.02$); however, subjects with a PLM Arousal Index (PLMAI) ≥ 15 /hr did not increase significantly (2.4% v. 6.0%, $p=0.12$). We did not observe a significant relationship between the presence of RLS symptoms and either the PLMI or PLMAI. In bivariate analyses (controlled for pregnancy trimester), age, Epworth Sleepiness Score, daytime naps and marital status showed trends towards prediction of RLS symptoms ($p<0.10$). Primiparity, nocturnal sleep time, race, tobacco/alcohol use and use of multivitamins/iron were not predictive of RLS.

Conclusion: The prevalence of restless legs syndrome increased in our subjects with advancing pregnancy. Only a small proportion of women were noted to have periodic limb movements. The presence of PLMS did not correlate with RLS symptoms.

Support:

Supported by grants from the National Institutes of Health (K23-HD-41465 and K24-HL-67848) and American Heart Association (0230190N).

Upper Airway Anatomic Risk Factors for OSA with Digital Morphometrics

Bhat R, Kline N, and Schwab R

Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA

Introduction: MRI and CT scans have shown that pharyngeal structures are larger in apneics compared to controls. Imaging studies are impractical for screening large populations, so we utilized a novel digital morphometric paradigm with a laser ruler to objectively quantify the sizes of pharyngeal structures in apneics and controls. We hypothesized that airway area/width and mandibular length would be smaller and tongue, uvula and lateral wall size would be larger in the apneics than normals.

Methods: Morphometrics with a digital camera and a “virtual laser ruler” were used to quantify structures of the pharynx in 48 controls (40.5+/-15.5 yrs; AHI 1.2+/-1.8; BMI 30.3+/-8.4) and 62 sleep apneics (50.1+/-12.6 yrs; AHI 53.2+/-35.9; BMI 39.3+/-10.3). Sizes of upper airway structures obtained with Image J analysis software, and AHI was determined with polysomnography. Accuracy and reproducibility of the digital morphometrics and laser ruler was validated with multiple phantoms.

Results: The data show that airway area/width were significantly smaller and tongue width, lateral pharyngeal wall width and Mallampati score were significantly larger in apneics compared to controls.

Table 1 - Differences in Pharyngeal Structures

| | Airway width (cm) | Airway Area (cm ²) | Lateral Wall Width (cm) | Mallampati | Mandible Length (cm) | Tongue Width (cm) | Uvula Area (cm ²) |
|----------|-------------------|--------------------------------|-------------------------|------------|----------------------|-------------------|-------------------------------|
| Cases | 0.7+/-0.9 | 0.2+/-0.7 | 2.0+/-0.4 | 3.7+/-0.6 | 9.6+/-9.0 | 5.4+/-0.7 | 0.4+/-0.3 |
| Controls | 1.6+/-0.8 | 1.2+/-1.4 | 1.4+/-0.5 | 2.3+/-1.1 | 9.0+/-0.8 | 4.8+/-0.5 | 0.4+/-0.3 |
| p-value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.02 | <0.0001 | 0.48 |

Conclusion: This study shows that digital morphometrics with a laser ruler can be used to objectively quantify differences in the size of pharyngeal structures in normals and apneics. The data indicate there are significant differences in upper airway measurements between normals and apneics.

Support:

NIH Grant: HL-67948

Effect of Cognitive Workload on Delta Power in the NREM EEG of Recovery Sleep Following Acute Sleep Deprivation

Caruso HM¹, Stakofsky AB¹, Tucker AM², Dinges DF¹, and Van Dongen HPA²

1. Division of Sleep and Chronobiology, Department of Psychiatry, and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine, Philadelphia, PA
2. Sleep and Performance Research Center, Washington State University, Spokane, WA

Introduction: This study investigated the effect of cognitive workload during total sleep deprivation (TSD) on slow-wave activity (SWA) in the NREM sleep EEG.

Methods: 21 healthy subjects (age 28.5 ± 5.5 ; 11 females) spent eleven consecutive days in the laboratory. They underwent three 36h TSD periods. The first TSD was preceded by two baseline nights (12h TIB), and all TSDs were followed by two recovery nights (12h TIB). Every 2h during TSD, subjects completed a moderate (0.5h) workload or high (1.0h) workload neurobehavioral test battery. Moderate cognitive workload testing occurred during two TSDs, and high cognitive workload testing during the third TSD, in randomized, counterbalanced order. Sleep periods were recorded polysomnographically. For the present analysis, 16 subjects each contributed four records: the second baseline night, two recovery nights after moderate workload, and one recovery night following high workload (six records were missing). For every record, SWA (0.75-4.5Hz) in the NREM sleep EEG (Fz, C3, C4, Oz vs. Ax) was assessed. Recovery sleeps following moderate vs. high workload were compared using mixed-effects ANOVA, controlling for baseline, systematic individual differences, order effects, and multiple comparisons.

Results: A significant effect of workload on SWA in the NREM sleep EEG was found for the Oz derivation ($t_{23}=2.76$, $P=0.011$). In recovery sleep following TSD with high workload, compared to moderate workload, SWA increased by $201 \pm 73 (\mu V)^2/Hz$ (mean \pm SE). Significant effects were not found for the other EEG derivations.

Conclusion: Higher cognitive workload during TSD resulted in increased NREM sleep SWA on the occipital EEG derivation during subsequent recovery sleep. Since SWA is considered a marker of sleep homeostasis, this preliminary result indicates that waking cognitive activity (in addition to wake duration) may affect sleep homeostatic regulation. This finding correlates with recent reports suggesting that sleep regulation may be use-dependent. Why the present workload effect was observed only occipitally remains to be investigated.

Support:

NIH grants HL70154 and RR00040

A Comparison of Type 2 Diabetics with and without RLS

Cuellar N, Cantor C, Schutta M, Givens R, Chien D, and Ratcliffe SJ

University of Pennsylvania
Philadelphia, PA

Introduction: Diabetes is the 5th leading cause of death affecting 6.2% of the population, with direct costs of \$91.8 billion dollars and indirect costs of \$40.2 billion dollars. Restless Legs Syndrome is a sleep disorder affecting up to 15% of the population and may compromise diabetic control due to sleep deprivation, fatigue, and depression. Twenty-one per cent of persons with RLS have diabetes, a prevalence over three times that of the general population. There are no reports on the prevalence of RLS in the diabetic population. RLS is significantly associated with self-reported diminished general health and poor mental health, and significantly correlates with age, increasing BMI, and low exercise, all factors that contribute to poor glycemic.

Methods: The study design is a descriptive, comparative study of type 2 diabetics with and without RLS. The sample was recruited from the PENN Rodebaugh Diabetes Center and the PENN Sleep Center as well as RLS Support Groups.

Results: Preliminary findings suggest that 44 of 102 (43%) participants who were screened for the study have RLS; 30 participants completed the surveys with 53% of the diabetics with RLS depressed compared to 43% of non-RLS. Of 7 persons who were receiving insulin for treatment of their Type 2 diabetes, all were in the RLS group. Only 25% of RLS participants who were depressed were being treated for depression compared to 33% of the non-RLS group. Other findings related to fatigue, sleepiness, and quality of life are being analyzed.

Conclusions: Based on our sample, this study indicates that the incidence of RLS in type 2 diabetics may be higher than previously reported. RLS patients with diabetes may have more difficulty managing their diabetes based on the number of RLS patients taking insulin. This could impact the long term outcomes of diabetes i.e. renal disease, blindness, amputation. Rates of depression in persons with RLS are higher than non-RLS diabetics and may affect self-care treatment of diabetes. Since many RLS patients cannot take anti-depressants because of initiating RLS symptoms, this is an important finding related to implications to treat depression. Larger studies need to be performed to confirm these findings. Health care providers should be aware that RLS may impact cost and effectiveness of treatment for diabetes and depression.

Support:

This study was funded by Sigma Theta Tau International Society and the American Association of Diabetic Educators.

A Comparison of Differences in Idiopathic and Secondary RLS in Older Persons

Cuellar N

ABSTRACT

As the population ages, Restless Legs Syndrome (RLS), a sleep disorder common in the elderly, will increasingly become a problem. If presentation of symptoms are different in elders with RLS, issues related to sampling for future studies will be addressed, as well as treatment options for these patients. This pilot study is a descriptive, comparative study to determine differences between idiopathic and secondary RLS on sleep, symptom severity, fatigue, depression and quality of life in elder persons with RLS. The primary aim of this pilot study are to provide preliminary data to determine differences in sleep, symptom severity, fatigue, depression, and quality of life between idiopathic and secondary RLS in older persons with RLS.

The sample for this study is persons with RLS who are over the age of 65 referred from the PENN Sleep Centers, which includes facilities at the Hospital of the University of Pennsylvania, Presbyterian Hospital, Phoenixville Hospital and Doylestown who have met diagnostic criteria for RLS based on International RLS Study Group Criteria. Inclusion Criteria are men and women without dementia, cognitive impairments, or diagnosed sleep disorders other than RLS. Screening of 40 participants from the Sleep Centers will identify persons with primary or secondary RLS. This study was powered to find a difference of 4.0 in the average PSQI score between groups. Assuming 80% power, alpha of 0.5, standard error of 4.5 (from previous studies), using a Student's t-test, we require a sample of 20 subjects per group. The proposed study will provide new data on differences in symptoms between primary and idiopathic diagnosis in persons with RLS and the effects on sleep, symptom severity, fatigue, depression and quality of life. In the event there are no differences between the two groups, evidence will exist for future studies on inclusion and exclusion criteria for sampling. In addition, information will be collected on fatigue, which has not been examined in the RLS population. It is hoped that the preliminary data will lay the foundation for future intervention studies for the older adult with the diagnosis of RLS.

The Majority of Nurses Report Difficulties with Drowsiness Driving Home After Work

Dean GE, Rogers AE, Hwang W, Scott LD, and Dinges DF

Introduction: According to the National Highway Transportation Safety Association (NHTSA), working long hours and/or rotating shifts increases the risk of falling asleep while driving home after work. Since hospital staff nurses often work ≥ 12 consecutive hours, rotate shifts or work nights, they are at high risk for experiencing difficulties remaining alert driving home from work. The goal of this study was to determine how often nurses have difficulty remaining awake driving home and what factors are associated with drowsy driving.

Methods: Two random samples of full time hospital staff nurses from the American Nurses Association (ANA sample = 393 participants) and the American Association of Critical Care Nurses (AACN sample = 502 participants) completed a demographic questionnaire and logbooks with daily information about sleep, alertness on duty, work hours, and errors for 28 days. Summary statistics and parametric tests were used to examine the data.

Results: Two-thirds of the participants reported difficulty remaining awake driving home at least once during the 28-day data-gathering period. On average, nurses struggled to remain alert on their drive home approximately once every four shifts they worked (2920/11,167 shifts). Although 80% of the nurses who worked exclusively night shift reported at least one episode of drowsy driving, over half (58.5%) of the nurses working exclusively day shifts also reported at least one episode of drowsy driving. Drowsy driving was more common when nurses left work between 2400 and 0800 than when nurses left work between 0800 and 1600 or between 1600 and 2400 (46% of the shifts compared to 29% and 18%, respectively). Nurses who reported drowsy driving had significantly longer commutes (32.2 ± 19.5 minutes compared to 25.3 ± 15.9 minutes; $p < 0.001$), and obtained on average 30 minutes less sleep than those who were able to remain alert driving home from work (6.33 ± 3.1 hours versus 6.83 ± 1.64 hours; $p < 0.001$).

Conclusions: Although we expected night shift nurses to report occasional difficulties with drowsy driving, we did not expect that the majority of nurses would report difficulties driving home during the 28-day period. The high number of drowsy driving episodes reported between 0800 and 2400 parallels the findings from a 2003 NHTSA report.

Support:

Financial support for this study was provided by the Agency for Healthcare Research and Quality (R01 HS11963-01) and an American Nurses Foundation Grant (Scott).

Sleep Complaints by Hospital Staff Nurses

Dean GE, Rogers AE, Hwang W, Scott LD, and Dinges DF

Introduction: The 2005 National Sleep Foundation survey found that 54% of respondents reported experiencing at least one symptom of insomnia at least a few nights a week. Hospital staff nurses often work ≥ 12 hour shifts or work nights that may result in increased risk for sleep complaints. This study sought to determine the prevalence and factors associated with sleep complaints in nurses.

Methods: A demographic questionnaire and logbooks with daily information about sleep, alertness on duty, work hours, and errors for 28 days were completed by two samples of full time hospital staff nurses: 393 nurses randomly selected members from the American Nurses Association (ANA) and 502 nurses randomly selected from the American Association of Critical Care Nurses (AACN). Generalized Estimating Equation Logistic regression modeling was used to examine the relationship between sleep complaints and factors that influenced sleep problems.

Results: There were no significant differences in the frequencies of sleep complaints between the samples. Nearly all participants, N=849 (95%), reported at least one symptom suggestive of insomnia. Seventy-nine percent of participants reported trouble falling asleep, 88% had trouble staying asleep and 83% reported waking up too early. While there were no differences in sleep complaints, there were differences in the factors associated with sleep complaints by sample. On work days, caffeine intake (ANA sample) and caring for elderly (AACN) predicted sleep complaints. Female gender (ANA sample) predicted sleep complaints on non-work days. Factors that made sleep complaints less likely on work days included being married (ANA), duration of commute (ANA), years of RN experience (ANA) and type of unit assignment-pediatrics + other (AACN sample). On non-work days, being married (ANA) and number of children (AACN) were factors that made sleep complaints less likely.

Conclusions: Results suggest that the number of sleep complaints of fulltime hospital staff nurses working exceed the number of sleep complaints reported by population-based surveys of adults in the United States. Identified factors that influenced sleep complaints may be used to plan strategies to improve sleep quality in hospital staff nurses.

Support:

Financial support for this study was provided by the Agency for Healthcare Research and Quality (R01 HS11963-01) and an American Nurses Foundation Grant (Scott).

Automating the Polysomnographic Artifact Removal Process

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Background: Valid automated computer sleep staging requires efficient identification and rejection of polysomnography data segments containing artifact. Environmental and biologic sources of artifact (line noise, electrode artifact, eye movements, and electromyographic artifacts) may contaminate scalp electroencephalogram (EEG) recordings, resulting in incorrect automated analysis.

Methods: An automated classification system was developed to identify “extreme artifact”, or segments so severely affected by artifact that no useful information could be recovered. Three features (absolute amplitude, energy, and power spectral density in delta band,) were evaluated using a K-nearest neighbor (*knn*) classifier with a k value equal to 5. The *knn* was trained and tested on data sets manually scored for artifact.

Results: The training set consisted of ~26 minutes each of "clean" and extreme artifact data segment. The quality and usefulness of the extreme artifact module was defined by: Accuracy = ((Sensitivity + Specificity) / 2) x 100. Details of the features and performance of individual features will be discussed. The module was applied to 8 hours of raw EEG data, and results of the automated classifier compared to manual scoring. Sensitivity was 98.7%, specificity 98.7%, and accuracy 93.6%.

Conclusions: This automated classification system for extreme artifact efficiently identified and removed corrupted segments of data with accuracy approaching visual scoring. Optimization of such systems will allow analysis of large polysomnography datasets for which manual artifact rejection would be prohibitively labor-intensive.

Trade-offs in Discretionary Time: Leisure vs. Sleep

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Introduction: Previous studies have shown that pressures of modern society often force people to make a decision between the biological imperative for sleep, and the societal requirement for wakefulness. While much research has been devoted to studying sleep time versus work time trade-offs, little attention has been paid to pleasurable discretionary activities, which are dominated by leisure time and time for sleep. A study was performed to evaluate how leisure time and sleep time co-vary in the American Time Use Survey database (ATUS).

Methods: The 2003 ATUS database is a random national telephone survey of men and women over 15 years of age, conducted by the US Census Bureau for the Bureau of Labor Statistics. ATUS respondents are interviewed about how they spent their time on the previous 24-h period. N=19689 respondents reported some time spent sleeping and in leisure activities. Data are codified into 17 separate categories, which included sleep time and time spent in socializing, relaxing and leisure. The latter was further subcategorized into: socializing and communicating; social events; relaxing and leisure (includes television watching); and arts and entertainment. ANOVA and regression were used to evaluate sleep time relative to leisure time.

Results: Total sleep time varied as a function of total leisure time ($P < 0.001$)—a similar relationship was found between sleep time and certain subcategories of leisure (e.g., television watching). In general, subjects in both short and long sleep categories had more leisure time than those in 7-8h sleep category. Among weekday working adults (N=4363) leisure was higher only for those who slept less than 7h.

Conclusion: It is particularly interesting that the greatest difference in leisure time was explained by the leisure category dominated by activities such as television watching. Further analyses will determine what aspects of leisure are most associated with reduced sleep time.

Support:

NIH NR04281

Intermittent Hypoxia Is Associated with Acute and Sustained Effects on *Drosophila melanogaster* Locomotor Activity

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Introduction: Intermittent Hypoxia (IH) is considered to be an important pathogenic mechanism in Obstructive Sleep Apnea. Mice exposed to long term IH show hypersomnolence, cognitive impairment and areas of apoptosis in discrete hippocampal regions. To our knowledge, no IH model has been developed using *Drosophila melanogaster*. In the present work we set out to study the locomotor response of *Drosophila* to IH as a first step toward characterizing genes that confer susceptibility to IH.

Methods: A timer-controlled solenoid valve system was devised to intermittently deliver compressed room air and 100% Nitrogen into a manifold attached to locomotion monitoring tubes that housed single flies. (Trikinetics, MA) 4-6 day old wildtype Canton-S flies were used. Throughout the assay, flies were maintained at constant temperature in 12 hour light:12 hour dark cycles and the IH cycles were delivered during the latter 6 hours of the dark period for 2 days. Activity was measured for 5 additional days after IH exposure.

Results: Two behavioral phenotypes were observed. The first was an acute cessation of activity, which has previously been described, which was reversible upon cessation of hypoxia. The second consisted of a marked decrease in both daytime and night activity that persisted for 5 days after the IH was given. These flies also had an increase in the number of sleep bouts and a decrease in sleep bout duration, during this period. These effects were only seen when the oxygen nadir of the IH cycles was <1%. A nadir of 2-4% did not result in any significant change in locomotor behavior, perhaps due to the known resistance of *Drosophila* to hypoxic conditions.

Conclusions: IH in *Drosophila* is associated with a biphasic behavioral locomotor response. The second response persists after the IH is administered.

Stress Reactivity in Insomnia

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Introduction: Transient insomnia is a common experience during times of stress, but for most individuals sleep improves once the stressor resolves. It is unclear why some go on to develop chronic insomnia despite resolution of the stressor. One hypothesis is that individuals with chronic insomnia may have a larger response to stress than healthy sleepers. The aim of this pilot study was to examine reactivity to stress in insomnia.

Methods: Five individuals with chronic insomnia (mean age=22) and three healthy sleepers (mean age=23) participated. Subjects were medically and psychiatrically healthy. Each subject spent three nights in the sleep laboratory. On the baseline night subjects completed subjective stress ratings and provided saliva samples for cortisol assay. On the stress night subjects were administered a mild electric shock and told that they would receive additional shocks during the night, although no shocks were actually administered. Given the small sample size collected thus far only descriptive statistics were computed.

Results: On the baseline night, insomniacs reported higher subjective stress, took longer to fall asleep, and had higher cortisol levels compared to controls. Relative to the baseline night, insomniacs had a larger response to stress on all variables whereas controls showed virtually no impact of the stress. For example, insomniacs took twice as long to fall asleep on the stress night compared to the baseline night (199.5 vs. 62.6 minutes) but there was very little change across nights for controls (18.8 vs. 26.3 minutes).

Conclusions: These data provide preliminary support for the hypothesis that individuals with chronic insomnia are more reactive to stress than healthy sleepers. Psychological and pharmacological interventions that specifically target the stress system may hold promise for the treatment of chronic insomnia. Further studies are needed to determine whether elevated stress reactivity is a risk factor for chronic insomnia or if it develops as a result of the disturbance.

iNOS Protein Expression in Macrophages Exposed to Intermittent Hypoxia

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Introduction: Hypoxia influences nitric oxide (NO) production at several levels: gene expression, enzyme activity and substrate availability. Many disease states result in dynamic fluctuations in tissue pO₂ e.g. cyclical recruitment in ARDS and nocturnal desaturations in obstructive sleep apnea. Until recently, *in vitro* studies have been unable to reproduce moderate to high frequency intermittent hypoxia (IH). We hypothesized that IH would induce NOS protein expression and NO production.

Methods: RAW 264.7 macrophages were cultured in a specially designed forced convection culture system. Cultures were randomly assigned to sustained hypoxia (8 Torr O₂), IH (cycles of 40 Torr O₂ for 90 sec and 8 Torr for 30 sec), or sustained normoxia (40 Torr O₂) for 4, 6 or 18 hours. Cell lysates were assayed for inducible nitric oxide synthase (iNOS) and normalized to a constitutive protein (Raf). In the system effluent, collected during the last hour in a limited number of 6 hour experiments, nitrate and nitrite (NOX) were measured by chemiluminescence.

Results: Compared to normoxia, both sustained hypoxia and IH resulted in a significant accumulation of iNOS protein at 6 hours which remained increased at 18 hours. Compared to hypoxia, there was a trend for greater iNOS protein in IH samples at 6 hours. NO production was measured at 0.316, 0.877, 1.877 nM NOX/ng cell lysates/hr in normoxia, hypoxia and IH respectively.

Conclusions: Exposure of murine macrophages to IH and hypoxia leads to increased iNOS. The apparent increase in NO production during IH could be due to an increase in active iNOS or in the availability of oxygen. The dynamic nature of oxygen-regulated NO production has potential importance in several diseases including ARDS and obstructive sleep apnea.

Support:
NIH GM64486

Upper Airway Collapsibility During REM Sleep in Children

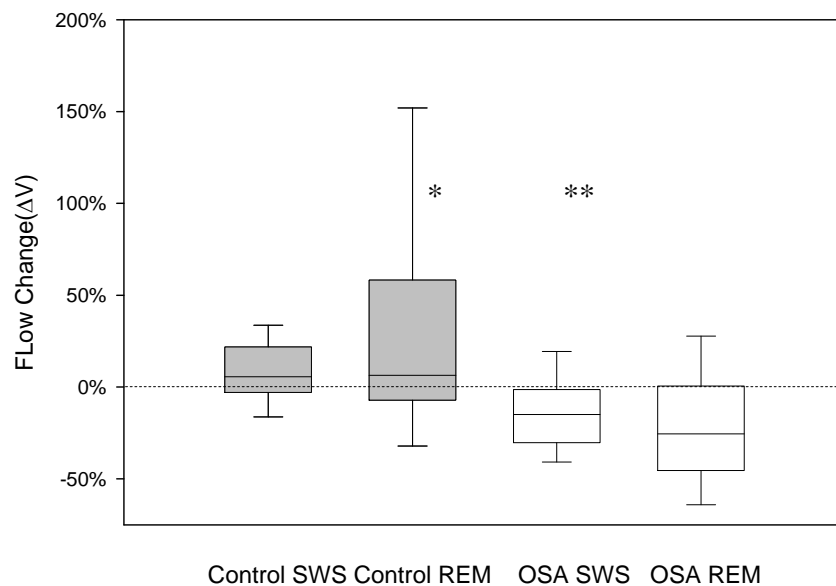
Huang J, Samuel J, Pepe M, Karamessinis L, Mason II TA, Brooks L, and Marcus CL

Rationale: Most of the obstructive sleep apnea (OSA) episodes in children occur during rapid eye movement sleep (REM), a state in which the upper airway muscle tone decreases. We hypothesized that REM-related increases in upper airway (UA) collapsibility would be greater in children with OSA than controls. We used new steady-state techniques to evaluate the upper airway pressure-flow relationship during REM compared to slow wave sleep (SWS).

Methods: 8 children with OSA (age 5 to 11 yrs, BMI 15 to 37 kg/m², AHI 5 to 33/hr) and 15 controls (age 5 to 11 yrs, BMI 16 to 28 kg/m², AHI 0-1.6/hr) were studied. Steady-state pressure-flow relationships were determined by measuring airflow at the holding pressure and at 5 cm H₂O below the holding pressure during SWS and REM. The flow change (ΔV) was defined as $\%(\text{flow at 5 cm H}_2\text{O below holding pressure} - \text{flow at holding pressure})/\text{flow at holding pressure}$.

Results: In SWS, the flow increased in controls (median 6%, range -51 to 46%) but decreased in OSA (median -7%, range -42 to 20%) ($p=0.018$ for OSA vs. controls). In REM, flow increased in controls (median 8%, range -51 to 290%) but decreased in OSA subjects (median -33%, range -65 to 38%) ($p=0.001$ for OSA vs. controls).

Conclusion: We conclude that normal children are able to maintain flow in the face of negative pressure both in REM and SWS, whereas OSA patients can not. However, further subjects are needed to compare the difference in ΔV between REM and SWS, due to the high variability of the response during REM.



Increased Firing of Remodelling Neurons in the Visual Cortex during Sleep

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Occluding vision of one eye (monocular deprivation (MD)) during a critical period of development initiates a rapid remodeling of synaptic weights in favor of the open eye. We have shown previously that ocular dominance plasticity (ODP) is enhanced by sleep and inhibited when neuronal activity in V1 is blocked during sleep. These findings demonstrate that there is an activity-dependent process during sleep that promotes cortical plasticity. To further characterize this mechanism, we measured multi unit activity (MUA) in V1 of freely moving cats before, during and after a period of MD.

Critical period cats were divided into 2 groups (MD and non-MD) and prepared for polysomnographic and micro-wire V1 recordings. In the MD group (n = 4, 34 sites), MUA across sleep-wake was recorded before, during and after a 6 hour right eye (RE) MD. During the MD period cats were kept awake to provide continuous stimulus necessary for synaptic remodelling. The non-MD cats (n = 2, 22 sites) were treated identically except that no MD was performed. Changes in OD at each site were assessed at the end of each period by calculating a LE/RE spike-rate ratio.

We found a significant shift of the neuronal responses towards the open eye only after a period of sleep [LE/RE ratio: (Mean \pm S.E.M.) baseline: 1.02 ± 0.07 ; after MD: 1.06 ± 0.06 ; post-sleep: 1.42 ± 0.16 , $p < 0.01$; paired t-test]. Further, we observed that neurons that were initially RE biased or binocular increased their firing rate during post-MD sleep compared to the baseline period [NREM baseline spikes/sec: 2.14 ± 0.53 , post MD: 3.05 ± 0.64 , $p < 0.01$; REM baseline: 3.05 ± 1.10 post MD: 3.79 ± 1.07 , $p < 0.05$]. However, we did not observe these changes in wake in MD cats, or in sleep/wake from non-MD cats. These results demonstrate first, that sleep is required for the expression of ODP and second, that there are changes in neuronal activity during sleep specific to cortical sites undergoing remodelling.

Support:

NIH RO1 MH 067568 and ASMF 30-CA-05

Sleep in Drosophila is Regulated by Adult Mushroom Bodies

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Sleep could result from global changes in the brain during wakefulness or it could be regulated by specific loci that recruit the rest of the brain into the electrical and metabolic states characteristic of sleep.

Here we address this issue by exploiting the genetic tractability of the fruitfly, *Drosophila melanogaster*, which exhibits the hallmarks of vertebrate sleep. We show that large changes in sleep are achieved by spatial and temporal enhancement of cyclic-AMP-dependent protein kinase (PKA) activity specifically in the adult mushroom bodies of *Drosophila*.

Other manipulations of the mushroom bodies, such as electrical silencing, increasing excitation or ablation, also alter sleep. These results link sleep regulation to an anatomical locus known to be involved in learning and memory.

MRI of NZO and NZW Mice, Identification of a Mouse Strain with a Compromised Upper Airway and Increased Parapharyngeal Fat

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Background: Obesity is the most important factor clinically linked to obstructive sleep apnea. However, it is not merely overall obesity, but rather excess fat in the neck that appears to play the major role in increasing the risk for obstructive sleep apnea. In particular, the volume of the parapharyngeal fat pads, which lie adjacent to the upper airway, is increased in subjects compared to controls {Schwab et al., AJRCCM 173:453-63, 2006}. The New Zealand Obese (NZO) mouse is not only obese but also excessively sleepy.

Hypothesis: We hypothesized that the New Zealand Obese mouse would have large fat deposits in the parapharyngeal region, and a smaller upper airway, thus providing a mouse model that could be used to identify genes leading to fat deposits in the key anatomical regions.

Methodology: To test this hypothesis we used magnetic resonance imaging (MRI) to examine the NZO strain with its genetic background New Zealand Wild Type (NZW), and quantified soft tissue and fat volumes in the pharyngeal, neck, and thoracic and abdominal regions. Paired T-test adjusted for multiple comparisons with a step-down bootstrap method was used. Significance assumed at $P < 0.05$.

Results: In the expiratory-gated images, there was a trend toward smaller airway size in the NZO compared to NZW mice. Volume of tongue and parapharyngeal fat pads, were significantly greater in the NZO compared to NZW mice. In all fat deposits and ratio of fat to tissue in neck, thoracic and abdominal regions were significantly great in the NZO compared to NZW mice.

Summary: The NZO mouse phenotype shows traits that include significant airway narrowing increased pharyngeal and increased fat in head, neck and abdominal regions. Future studies to define the sleep characteristics and genetic background in NZO mouse might be a great benefit towards developing a murine model to understand pathogenesis in obstructive sleep apnea.

A Drosophila Model for Age-Associated Changes in Sleep:Wake Cycles

Koh K, Evans JM, Hendricks JC, and Sehgal A

One of the most consistent behavioral changes that occurs with age in humans is the loss of sleep consolidation. This can be quite disruptive and yet little is known about its underlying basis. To better understand the effects of aging on sleep:wake cycles, we sought to study this problem in *Drosophila melanogaster*, a powerful system for research on aging and behavior. By assaying flies of different ages as well as monitoring individual flies constantly over the course of their lifetime, we found that the strength of sleep:wake cycles decreased, and sleep became more fragmented with age in *Drosophila*. These changes in sleep:wake cycles became faster or slower with manipulations of ambient temperature that decreased or increased lifespan, respectively, demonstrating that they are a function of physiological rather than chronological age. The effect of temperature on lifespan was not mediated by changes in overall activity level or sleep amount. Flies treated with the oxidative stress producing reagent paraquat showed a breakdown of sleep:wake cycles similar to that seen with aging, leading us to propose that the accumulation of oxidative damage with age contributes to the changes in rhythm and sleep. Together these findings establish *Drosophila* as a valuable model for studying age-associated sleep fragmentation, and breakdown of rhythm strength, and indicate that these changes in sleep:wake cycles are an integral part of the physiological aging process.

Molecular Analysis of the Interaction between Sleep and Immune Response in Drosophila

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Although it is generally accepted that there is a connection between sleep and immune response, little is known about the mechanism. To investigate this issue, we studied sleep and the immune response in *Drosophila*. Genome-wide screens have revealed that immune-related genes are up-regulated with sleep deprivation, indicating an interaction between these two physiological systems. cAMP response element binding protein (CREB), a transcription factor known to be involved in long-term memory and circadian rhythm in *Drosophila*, also plays an important role in sleep behavior. cAMP/CREB signaling is known to be inversely related to sleep, such that an increase in CREB signaling increases wakefulness and a decrease in CREB signaling increases sleep. Preliminary data indicate that expression of a dominant negative form of CREB, CREB2b, in *Drosophila* increases the resistance to bacterial infection. Likewise, mutants that increase CREB signaling show decreased resistance. The role of CREB in immune-related signaling was investigated in the *Drosophila* S2 cell culture system. Preliminary results from luciferase reporter assays show an interaction between CREB and NF κ B signaling. Together, these data suggest that cAMP/CREB signaling may mediate the interaction between sleep and immune response.

Support:

Supported in part by a grant from the UMDNJ foundation

Disinhibition of Perifornical Hypothalamic Neurons Blocks the Ability of Pontine Carbachol to Produce REM Sleep-like State in Anesthetized Rats

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Neuronal activity that originates in the perifornical (PF) region of the posterior hypothalamus plays an important role in the maintenance of wakefulness (von Economo, 1930). The recent discoveries that the region contains cells that synthesize orexin, the peptides with wake-promoting properties and actions that prevent narcolepsy/cataplexy, suggested that neuronal activity generated in the PF region can act to suppress rapid eye movement (REM) sleep. We tested this hypothesis in anesthetized rats in which REM sleep-like state can be produced pharmacologically and PF hypothalamic cells, including those that contain orexin, can be activated by the GABA_A receptor antagonist, bicuculline. In 6 urethane-anesthetized, paralyzed and artificially ventilated rats, carbachol injections (10 nl, 10 mM) were made into the dorsomedial pons before and at different times after unilateral microinjections of bicuculline (20 nl, 1 mM) into the PF region. As in our earlier studies, pre-bicuculline pontine carbachol injections readily elicited 2-4 min-long REM sleep-like episodes, comprising an increased power of hippocampal theta-like activity (from 84 ± 11 (SE) to 336 ± 64 mV²/Hz, $p < 0.01$) and increased power of the cortical EEG in the 6-12 Hz range (from 7.6 ± 0.9 to 33 ± 6.0 mV²/Hz, $p < 0.01$). Concurrently, hypoglossal (XII) nerve activity was suppressed (by $74 \pm 5\%$; $p < 0.001$), and the respiratory rate decreased (from 45 ± 2 to 36 ± 2 min⁻¹; $p < 0.01$). Hypothalamic bicuculline injections increased XII nerve activity (maximally by $100 \pm 24\%$; $p < 0.05$), accelerated the central respiratory rhythm (from 45 ± 2 to 48 ± 2 min⁻¹; $p < 0.01$), and shifted rightward the peaks of both hippocampal and cortical power spectra (from 1.0 ± 0.04 to 1.8 ± 0.2 Hz, $p < 0.01$, and from 0.9 ± 0.1 to 1.4 ± 0.1 Hz, $p < 0.01$, respectively). The effects of bicuculline lasted 34 ± 3 min. When carbachol injections were made within 10-50% of the total period of the response to bicuculline, all REM sleep-like effects of carbachol were abolished. They could be elicited again after termination of the effects of hypothalamic bicuculline. These results show that disinhibition of neurons located in the hypothalamic PF region prevents the triggering of REM sleep-like phenomena from the pons, or suppresses their production at multiple levels, including the cortical, hippocampal, motoneuronal, and respiratory rhythm generator.

Support:

Grant: HL-071097

Shift Work-Light Exposure Increases Obesity and Impairs Insulin Tolerance in Mice

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Over 7 million Americans regularly perform nighttime shift work. This subset of adults is at increased risk for obesity and multiple medical illnesses including diabetes. In an effort to begin to determine key factors contributing to the increased propensity towards obesity in shift workers, we have begun to study the effects of shift work ambient light exposure patterns on adult male mice. The aim of this study was to establish whether changes in the diurnal cycle of light and dark, similar to that commonly experience by shift workers, impacts on food intake, body weight and glucose metabolism. We hypothesized that changes in the diurnal pattern of sleep and wakefulness in mice leads to symptoms comparable to metabolic syndrome in humans.

Male C57/B6/J mice were randomized to a shift work-like light-dark schedule for three months during or typical light exposures 12:12 light:dark schedule (n=10/condition) across which the locomotor activity, food intake and changes in the body weight were continuously monitored. Mice were provided with both high fat/high calorie and standard rodent chow food pellets. We have assessed weight gain across the 3 months exposure, food preference, caloric intake, body composition, glucose level and glucose response to insulin, and compared them to the control mice.

We have demonstrated that total sleep time, number and duration of sleep bouts, did not differ between “shift” and control mice; however, there were significant differences in the temporal distribution of sleep and wakefulness across a 24 hrs day. Disruption of the light-dark cycle in shift mice lead to a 15% (p=0.03 by ANOVA) increase in body weight; indeed a significant difference was observed after only after 7 days of shift work-like light-dark cycle. There were no differences in the amount of food intake or in the pellet preference between shift and control mice. DEXA scan indicated a higher content of whole body and abdomen fat in shift mice (~20% increase in the whole body and abdomen; p=0.005 and p=0.01, respectively). Shift mice exhibit higher normal and fasting glucose level and the insulin tolerance test indicated an impaired response (55% reduction in the AUC response to insulin challenge, p=0.01).

Our data suggest that changes in the light-dark cycle similar to that experienced by shift workers lead to symptoms of metabolic syndrome. These changes are likely due to a decrease in the metabolic rate. Additional experiments on the energy expenditure, circadian variation in metabolic hormones, and cholesterol/triglyceride blood chemistry of shift mice are in progress.

Support:

NHLBI grant HL60287

An Animal Model of the Sleep Disturbances in PTSD: Micro-Architecture Changes in REM Sleep

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Introduction: We have recently reported (Jha et al., 2005) that in rats reexposure to tones previously paired with shocks results in a subsequent alteration in REM sleep (REM). In that study, the tones were presented 24 hr after tone-shock pairings. Post traumatic stress disorder (PTSD), however, can be evident for decades after the trauma. Therefore, one purpose of the current study was to attempt extending the findings temporally by presenting the tones again two weeks post-conditioning.

Methods: Five male Sprague-Dawley rats were surgically implanted with electrodes for the measurement of EEG and EMG signals. Subjects were habituated to the recording apparatus and hookup over a number of days, and a baseline sleep recording was taken. Rats received five pairings of a 5-sec tone, which co-terminated with a 1-sec, 1 mA footshock, every 30 sec. Subjects were placed into a neutral chamber both 24 hr and 2 wks after conditioning. Due to headcap and other problems, only three animals were tested at two weeks. On each occasion, five tones were presented, and sleep was then recorded for 4 hr. Time spent freezing was also noted in all conditions.

Results: The most pronounced changes were observed at two weeks. Although the amount and percent of REM did not change, REM architecture changed dramatically. The number of sequential REM episodes (seqREM)(those occurring at < 3-min intervals) and their total duration were decreased. Conversely, the number and duration of single REM episodes (sinREM) (appearing > 3 min apart) increased at two weeks. The “switch” from seqREM to sinREM was so dramatic that a significant negative correlation was observed between the two ($r = -0.98$). Further, the amount of time that the animal spent freezing upon being reexposed to the tones negatively correlated with seqREM and positively correlated with sinREM.

Conclusions: Thus an increase in a behavioral index of anxiety (freezing) predicted a decrease in seqREM and an increase in sinREM. This suggests that conditioned anxiety may cause REM changes, specifically a difficulty in initiating REM. These data also support clinical observations that total REM amount is often not altered in PTSD patients, but REM microarchitecture is very different.

Support:

RO1-MH072897

Identification of Wake Active Neurons Susceptible to Hypoxia-Reoxygenation Injury in Sleep Apnea Model and Mechanisms of Susceptibility

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Obstructive sleep apnea affects tens of millions of individuals worldwide, and refractory impaired wakefulness in these individuals is a major health concern. Using a murine model of oxygenation patterns in sleep apnea we have previously identified NAD(P)H oxidase-dependent carbonylation injury in selective wake-active groups with lasting wake impairments. We hypothesize that the hypoxia/reoxygenation events activate NAD(P)H oxidase in select wake active neurons through angiotensin _{1A} receptor (AGTR_{1A}) activation in wake neurons. We hypothesize that AGTR+ noradrenergic locus coeruleus (LC) and dopaminergic ventral periaqueductal gray (vPAG) neurons are susceptible to hypoxia-reoxygenation injury and that NAD(P)H oxidase mediates this injury. Eight to 10 week-old male AGTR_{1A}^{-/-} mice and AGTR wild type mice were exposed to either chronic intermittent hypoxia (intermittent reductions in ambient FIO₂ from 21% to 10% for 5 seconds every 90 seconds) or sham intermittent hypoxia (ambient FIO₂ fluctuations from 21% to 19%) for 6 months (n=6-10). Animals were then deeply anesthetized, transcordially perfused with 4% paraformaldehyde and their brains sectioned at 20 μm. Sections containing LC and vPAG were immunohistochemically stained for tyrosine hydroxylase (TH), activated caspase-3, cleaved (p85) poly ADP-ribose polymerase (PARP) and NAD(P)H oxidase subunits, p67^{phox} and rac1. Some sections stained for TH were counterstained with Neutral Red. The results of the study distinguish noradrenergic locus coeruleus and dopaminergic ventral periaqueductal gray neurons as the wake-active groups most susceptible to injury and neural loss in this model. In addition, NAD(P)H oxidase is found in these neuronal groups and is activated by chronic intermittent hypoxia. Transgenic absence of functional AGTR_{1A} receptors prevented injury to catecholaminergic neurons. In conclusion, these data show that activation of NAD(P)H oxidase by AGTR_{1A} in catecholaminergic neurons contributes to the increased susceptibility of these neurons to intermittent hypoxic injury and suggests that NAD(P)H oxidase or AGTR_{1A} blockade may prevent further injury and possibly improve function in key wake-promoting neural groups. Moreover, we propose some similarity in the mechanisms of sleep apnea oxidative injury to the cardiovascular, hepatic and endocrine systems.

Effect of Introversion-Extroversion on Mood during Chronic Sleep Restriction

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Introduction: It has been reported that introverts report more anger and hostility. The aim of this study was to investigate the effects of introversion-extroversion on mood during 5 nights of sleep restriction.

Methods: Analyses were conducted on 33 healthy adults (17m; 16f; aged 22-45y), who participated in a 5-night sleep restriction protocol (4h TIB) following 2 baseline nights (10h TIB). The Millon Index of Personality Styles (MIPS) was used to identify extroverts (n=25) and introverts (n=8). Throughout the study, subjects completed the POMS and the psychomotor vigilance test (PVT) every 2 hours during wakefulness. POMS total mood disturbance (TMD) scores were used as the primary outcomes, but analyses were also run on POMS subscales.

Results: Sleep restriction significantly increased PVT lapses in both introverts and extroverts (both $p < 0.001$), with no differences between them. On the first 3 days of the study (baseline days 1 and 2, and sleep restriction day 1), introverted subjects reported significantly greater total mood disturbance compared to extroverts ($p = 0.006$). A repeated measures general linear model with baseline day 1 differences in TMD as a covariate, revealed that TMD increased steadily across days of sleep restriction ($p = 0.006$), but there was no interaction with introversion-extroversion ($p = 0.798$). Thus, by the final day of sleep restriction, initial mood differences between introverts and extroverts were no longer significant ($p = 0.404$). Analyses of POMS subscales generally fit the results for TMD, except for anger/hostility ratings, which remained higher for introverts throughout the study ($p = 0.017$).

Conclusions: These data suggest that sleep loss affects mood states and PVT performance similarly in introverts and extroverts, despite the former showing higher mood disturbance when not sleep deprived.

Support:

NASA cooperative agreement NCC 9-58-159 with the National Space Biomedical Research Institute, NIH NRO4281 and RRO0040

A Night of Sleep Deprivation Decreases Positive Mood in Healthy Subjects

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Abstract: This study tested the effects of sleep deprivation on ratings of positive affect (PA) and negative affect (NA). Subjects who experienced one night of sleep deprivation (n=8) experienced decreases in PA ($p < 0.05$) without increases in NA ($p = 0.69$). The control group (n=5) reported no significant changes in either PA or NA.

SUMMARY

Introduction: Sleep deprivation impairs a wide range of cognitive functions (Durmer & Dinges, 2005), but less is known about how it affects mood. Studies using the Profile of Mood States (POMS) suggest that sleep deprivation changes mood, but only on factor scores that overlap considerably with sleepiness (Penetar et al, 1993). This study was conducted to evaluate changes in mood using the Positive and Negative Affectivity Schedule (PANAS), which has a better balance of positive and negative items and less overlap with sleepiness than the POMS.

Method: Data were taken from a laboratory study of sleep deprivation. Healthy adult subjects (N=13) volunteered for a 48 hour study where they were randomized to receive either 9 hours or 0 hours of sleep opportunity on the first night of the study. On the second night, all subjects received 10 hours of sleep opportunity to insure adequate recovery. The PANAS was administered in the afternoon (between 1:00pm and 4:00pm) on both days of the study and change scores were calculated. Effect sizes were calculated by comparing the change scores for the control group (n=5) to the change scores of the sleep deprived group (n=8).

Results: Sleep-deprived subjects experienced a significant decrease in Positive Affect (PA; $p = 0.02$) but no significant increase in Negative Affect (NA; $p = 0.69$). Subjects in the control condition did not indicate significant changes in either PA ($p = 0.59$) or NA ($p = 0.14$). Effect sizes indicated that sleep deprivation produced a large decrease in PA ($d = 1.07$). Furthermore, the effect was not limited to a single item. Although the change in ALERTNESS had the largest effect size ($d = 1.78$), change scores on four of the other positive items (INTERESTED, ATTENTIVE, DETERMINED, and INSPIRED) were also large ($d > 0.70$). The effect of sleep deprivation on 3 other positive items (EXCITED, ENTHUSIASTIC, and PROUD) was moderate ($d > 0.30$). Two of the positive mood items (STRONG and ACTIVE) increased relative to the control group. Although the change in NA overall was not significant, sleep deprivation had a large effect on some of the negative items. UPSET, SCARED, and IRRITATED had large effect sizes for increases ($d > 0.70$) and the item NERVOUS ($d = -0.88$) had a large effect size for a decrease.

Conclusion: Although the study is limited by small sample size, these data suggest that sleep deprivation has a substantial effect on mood beyond that mediated by reduced alertness. The finding that 8 out of 10 positive items decreased while only 4 out of 10 of the negative items increased suggests that sleep deprivation produces a state of relatively neutral mood rather than a state of intense negativity. The POMS is not likely to pick up these changes because it is heavily biased toward negative states. The PANAS as well as the POMS should therefore be used in future studies of sleep deprivation. It is also important to note that sleep deprivation was associated with increased feelings of strength and decreased nervousness, suggesting that the effect of sleep pressure on mood may not be exclusively deleterious.

Support:
NR04281

Response Surface Map Analysis of PVT Performance in a Chronic Sleep Restriction Dose Response Experiment With and Without Naps

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Introduction: This study investigated the effect of a range of restricted nocturnal sleep schedules with and without diurnal naps on psychomotor vigilance test (PVT) performance. The objective was to determine whether split sleep schedules with reduced sleep time could serve to increase total wake time while preventing cumulative reductions in alertness.

Methods: N=93 healthy adults (aged 21–49y; 39 females) participated in a 10-night sleep restriction protocol where they were assigned to one of 18 sleep regimens. These conditions involved restricted nocturnal anchor sleep (4.2h, 5.2h, 6.2h or 8.2h TIB) and a diurnal nap (0.4h, 0.8h, 1.2h, 1.6h, 2.0h or 2.4h TIB) or no nap. Neurobehavioral performance was tested at 2h intervals during scheduled wakefulness. PVT performance lapses (sleep inertia bouts excluded) were averaged by day for each subject. Response surface maps were constructed to examine cumulative impairment across days as a function of anchor sleep and nap sleep durations.

Results: The rate of degradation in PVT performance across the 10 restriction days was found to be accurately described by a linear function of daily total TIB (i.e., anchor + nap), with greater total TIB per 24h resulting in fewer PVT lapses ($\chi^2[1]=4.9$, $p=0.03$). Differentiating between anchor and nap sleep durations did not result in significantly improved goodness-of-fit ($\chi^2[1]=0.3$, $p=0.58$); nor did differentiating between each of the 18 different conditions separately ($\chi^2[16]=18.3$, $p=0.31$).

Conclusions: Across a range of chronic nocturnal sleep restriction conditions (4.2h to 8.2h TIB) with and without diurnal nap (0h to 2.4h TIB), neurobehavioral performance as measured by PVT lapses was primarily a function of total TIB per 24h—regardless of how sleep was divided among nocturnal anchor sleep and diurnal nap sleep periods. This suggests that split sleep schedules offer no clear advantage over monophasic sleep patterns with regard to preventing cumulative impairment from chronic sleep restriction.

Support:

NASA cooperative agreement NCC 9-58-159 with the National Space Biomedical Research Institute

Changes in Components of the Electron Transport Chain in Mouse Cortex with Increases in Wakefulness

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Introduction: Homeostatic sleep drive resulted from accumulation of extra cellular adenosine in sleep-regulating areas of mammalian brain is largely described in literature. Numerous attempts to describe mechanisms facilitating adenosine accumulation resulted in the following: (1) both mitochondrial and nuclear energy-related genes are up-regulated after 3 but not 8 hrs of sleep deprivation in cortex; (2) uncoupling protein 2 gene (*Ucp2*) is up-regulated after 8 hrs of sleep deprivation in cortex; (3) activity of cytochrome c oxidase (COX) is up-regulated after 3 hrs of sleep deprivation in multiple brain regions. Thus we hypothesized that after long-term (12 hrs) sleep deprivation, there is overall decline in ATP production and intracellular ATP/AMP ratio. We believe that could provide the means by which accumulation of adenosine, a byproduct of ATP degradation, is facilitated in extended wakefulness.

Methods: We used 2-month old C57B6 male mice kept at 7AM: 7PM lights on/off cycle. Sleep deprivation was conducted by gentle handling. Animals were monitored by electronic beam splitting (AccuScan). The six (6) test groups (n= 6-8 animals each) were as the following: (1) sleep-deprived for 3 hrs after lights on (3SD), (2) sleep-deprived for 12 hrs after lights on (12SD), (3) 3 hrs spontaneous sleep control (3C), (4) 12 hrs after lights on control (7PM, lights off), (5) 3 hrs after lights off, or spontaneous wake control (3SW), and (6) 7AM (lights on). Animals should have spent 75% asleep/awake (>40 sec paradigm) for the preceding 3 hrs (3SS and 3SW groups) in order to be used as controls. We used freshly isolated mitochondria in our studies. We measured (1) protein levels of mitochondrial COXI, nuclear COXIV, ATPB subunits of oxidative phosphorylation (OXPHOS) as well as uncoupling protein UCP2 using Westerns; (2) activity of cytochrome c oxidase; and (3) phosphorylation of AMP-dependent protein kinase (AMPK) by Westerns as an indicator of the decline in ATP/AMP ratio.

Results: We found that (1) electron transport chain proteins are up-regulated after 3 but not 12 hrs of sleep deprivation in mouse cortex, except for the catalytic COXI subunit of cytochrome c oxidase, which was increased after both 3 and 12 hrs of sleep deprivation; (2) activity of cytochrome c oxidase enzyme did not change throughout the course of 12-hr sleep deprivation; (3) uncoupling protein UCP2 is up-regulated only after 12 hrs of sleep deprivation in mouse cortex; (4) there is an increased phosphorylation of alpha-AMPK subunit after 12 hrs of sleep deprivation.

Conclusions: Changes in ATP production machinery on protein level imply the increased energy demands likely aimed to compensate for the increased neuronal load in sleep deprivation. Activity of crucial COX enzyme – marker of neuronal activity - once elevated after 3 hrs of sleep deprivation, does not change throughout the course of 12-hr sleep deprivation in mouse cortex, nor does the protein level of its catalytic COXI subunit. That confirms that short-term energy supply is mainly executed on mitochondria level by mitochondrial sources. With longer duration of sleep deprivation, there is increased uncoupling of oxidative phosphorylation via nuclear UCP2 ultimately leading to decreased ATP production. The increase in phosphorylated alpha-AMPK subunit implies that intracellular ATP/AMP ratio drops as wakefulness is prolonged. Thus, changes in components of the electron transport chain on protein level with long-term sleep deprivation suggest overall decline in ATP production in mouse cortex. This can be a common mechanism among sleep/wake brain areas potentially leading to accumulation of adenosine.

Support:

Grant HL-60287

Regional and Temporal Distribution of Neutrophils in a Model of Acute Lung Injury

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Introduction: Acute lung injury (ALI) is recognized as a heterogeneous condition that progresses over time. Dependent regions of the lung are prone to collapse and/or alveolar flooding; mid-regions are prone to tidal recruitment and intermittent hypoxia; and non-dependent regions of the lung are prone to overdistension and stretch. We hypothesized that neutrophil accumulation would vary with spatial position and with time in a surfactant depletion model of acute lung injury.

Methods: Following saline lavage in NZW rabbits, we adjusted mechanical ventilation to provide tidal recruitment in approximately 25% of the lung as determined by a previously described fast intra-arterial fluorescence quenching oxygen probe. Lungs were harvested at prior to or at 0, 1.5, 3, or 6 hours after lavage; then formalin fixed at a constant distending pressure of 30 cm H₂O; sectioned into 8-9 slices from nondependent to dependent regions; and cut into histologic sections. After staining with H&E, investigators blinded to the origin of the section obtained 10 digital images from each section. Semi-automated image analysis and manual cell counting was performed for each image. The influence of time and location on the number of alveolar neutrophils was tested by repeated measures 2 way ANOVA.

Results: Both time and location were significantly related to alveolar neutrophil accumulation. The dependent regions had significantly higher neutrophil counts than the nondependent regions. Alveolar neutrophil counts increased over time.

Conclusions: In this surfactant depletion model of ALI, neutrophil accumulation showed substantial spatial and temporal heterogeneity. Dependent regions of the lung exposed to cyclical recruitment had greater neutrophil accumulation than nondependent regions exposed to excessive stretch.

Support:

DFG Ma2398/3-1, NIH GM64486

Prediction of Improvement with CPAP Treatment for OSA

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Introduction: Continuous positive airway pressure (CPAP) treatment leads to improvement in sleepiness and function in obstructive sleep apnea (OSA) patients. This secondary analysis was undertaken to determine if the amount of improvement could be predicted.

Methods: 176 OSA patients from 7 sites in US and Canada were tested at baseline and 3 months post-CPAP on several outcomes, including sleepiness (Epworth Sleepiness Scale [ESS] & Multiple Sleep Latency Test [MSLT]), and function (Functional Outcomes of Sleep Questionnaire (FOSQ)). Fixed regression models for change from baseline included age, BMI, apnea hypopnea index (AHI), CPAP use, site, and baseline value. Continuous variables were mean centered. Site specific intercepts were estimated to account for site differences.

Results: Baseline mean values were: age 46.7 yr, AHI 63.9, BMI 38.0, MSLT 6.8 min, ESS 15, & FOSQ total 14.7. Mean CPAP use was 4.7 hrs/night. Three months treatment with CPAP led to significant improvements in MSLT, ESS and FOSQ total ($p < .001$). The full regression model predicted 50% of the change in ESS, 37% of the change in MSLT, and 55% of the change in FOSQ total (all $p < .0001$). The strongest predictor in each equation was the baseline value ($p < .0001$); CPAP use was a significant predictor for ESS ($p < .0001$) and FOSQ total ($p = .02$). Baseline AHI was a weak predictor of change for all, and age was a weak predictor for MSLT and ESS. Site specific intercepts were significant for all outcomes ($p < .01$).

Conclusions: Baseline measurements of key symptoms such as sleepiness and function can predict who might have the greatest response to treatment. These predictions could also be used in pretreatment counseling to increase adherence to CPAP treatment, and enhance post treatment outcome. Further research is needed to develop a profile of those who would optimally respond to treatment.

Support:

NHLBI, Respironics, Inc., Nellcor Puritan Bennett Inc., DeVilbiss Health Care Inc., & Healthdyne Technologies, Inc.

Excessive Daytime Sleepiness Impairs Self-care in Persons with Heart Failure

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Background: Excessive daytime sleepiness (EDS) has been proposed as a cause of poor self-care, but little data exist to support this relationship.

Methods: We conducted a cross-sectional descriptive pilot study of 63 stable patients with chronic heart failure. EDS was measured with the Epworth Sleepiness Scale (ESS). Self-care was defined as treatment adherence and decision-making about symptoms and measured with the Medical Outcomes Study adherence scale and the Self-Care of Heart Failure Index (SCHFI) and its three subscales: maintenance, management, confidence. Higher scores indicate better self-care. Data were collected in home or office settings.

Results: The sample was mostly male (55.6%), white (52.4%), high school educated (48.4%), *M* age 58.4±13.8 yrs. Most (55.5%) had a moderate or high number of comorbid illnesses, many were severely functionally compromised (50.8% NYHA class III/IV). Mean ESS score was 8.9 ± 5, but 33% scored ≥11 and were categorized as EDS. Adherence scores were lower in those with EDS: (22.8±31.3 vs. 29.5±35.9). Self-care scores were lower in those with EDS than those without (SCHFI: 207.9±37.8 vs. 213.6±35.6; Figure).

Conclusions: Preliminary data suggest that EDS may be one cause of poor self-care in persons with heart failure. Further research is needed to confirm these results and to explore mechanisms by which EDS impairs self-care.

Nitric Oxide Consumption by Cytokine-Stimulated Macrophages?

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Introduction: Using forced convection cell culture, we previously reported rapid reversible inhibition of nitric oxide (NO) production by cytokine-stimulated macrophages due to acute hypoxia (seconds). The apparent inducible nitric oxide synthase K_m for oxygen was 28 Torr, much higher than 5 Torr, the reported K_m in cell lysates. We suspect the increased K_m is due to multiple mechanisms, one of which could be increased NO consumption during hypoxia.

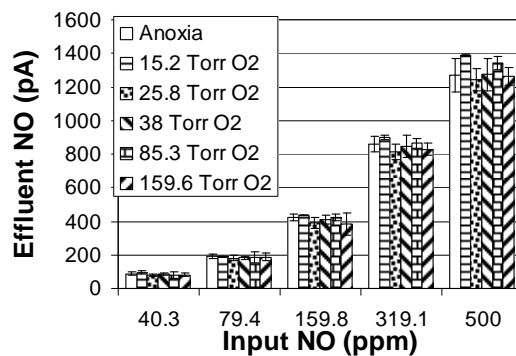
Methods: Using an NO electrode, effluent NO from the forced convection system was directly and continuously measured. Experiments were performed with and without cells at oxygen tensions between 8 and 160 Torr ($n \geq 2$). Maximum effluent NO was defined as the NO concentration measured without oxygen or cells in the system. Cellular consumption of exogenous NO was measured after completely inhibiting endogenous NO production with 1400W.

Results: The effluent NO concentration was independent of oxygen tension and the presence of cytokine-stimulated macrophages.

Conclusions: NO consumption did not affect the apparent K_m in our model. The lack of consumption by cytokine-stimulated RAW 264.7 cells is consistent with reports that these stimulated cells do not produce significant amounts of superoxide, the predominant intracellular scavenger of NO. We are currently investigating other mechanisms to explain the high apparent K_m detected in our model.

Support:

NIH GM64486



Effect of Morning Versus Afternoon Arithmetic Testing in Lower, Middle, and Upper School Students and Adults

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Introduction: Teens have a circadian tendency for later bed and wake times. Because of this delayed sleep phase tendency, teens may be especially sleepy in the morning. Several high schools have delayed school start times. The primary purpose of this project is to see how time of testing may affect test performance in lower, middle, and upper school students and adults.

Methods: 4th, 8th, and 11th graders and adult teachers from a Philadelphia school completed a timed, two minute, simple arithmetic test during first period (AM testing) and in the mid-afternoon (PM testing). AM and PM tests were matched for problem type and level of difficulty. Participants also completed questionnaires on prior sleep time and subjective alertness scale (0: sleepy- 10: very alert).

Results: 16 fourth graders (average age 9.4 years), 23 eighth graders (13.7 years), 20 eleventh graders (16.7 years), and 10 adult teachers (36.7 years) completed AM and PM questionnaires and testing (Table 1). All students and adults appeared partially sleep deprived (for their ages) prior to testing. All groups rated higher afternoon alertness compared to AM alertness, though this difference was least for the 4th graders and significant only for 8th graders (p=0.0487).

| Table 1 | Age (years) | N (M/F) | Prior Sleep Time (Hours) | AM Alertness | PM Alertness |
|--------------------------|-------------|--------------|--------------------------|--------------|--------------|
| 4 th Graders | 9.4 | 16 (10M/6F) | 9.2 | 6.4 | 6.8 |
| 8 th Graders | 13.7 | 23 (14M/9F) | 8.0 | 5.7 | 6.7 |
| 11 th Graders | 16.7 | 20 (11M/9 F) | 7.4 | 4.7 | 5.5 |
| Adults | 36.7 | 10 (7M/3F) | 6.9 | 5.5 | 6.5 |

All groups had more correct answers during afternoon testing compared to morning testing (Table 2). Only eighth graders appeared to have more incorrect answers on morning compared to afternoon testing (p=0.0242).

| Table 2 | #AM Correct | #PM Correct | Compare: AM vs PM Correct | AM: #Incorrect | PM: #Incorrect | Compare: AM vs PM Incorrect |
|--------------------------|-------------|-------------|---------------------------|----------------|----------------|-----------------------------|
| 4 th Graders | 12.6 | 14.3 | 1.7 | 0.6 | 0.4 | 0.1 |
| 8 th Graders | 9.5 | 10.8 | 1.3 | 1.7 | 1.0 | 0.7 |
| 11 th Graders | 16.3 | 19.1 | 2.7 | 1.0 | 1.1 | -0.3 |
| Adults | 10.9 | 12.8 | 1.9 | 1.5 | 1.5 | 0.0 |

Discussion: All student and adult groups appeared sleep deprived. All rated themselves as more alert in the afternoon. All groups had more correct answers for afternoon testing but only 8th graders had more incorrect answers during morning testing. If correct answers show the degree of alertness and incorrect answers show the degree of inattentiveness, then 8th graders appear at greatest risk for decreased alertness and increased inattentiveness in the morning. This may have implications on learning and testing during morning hours during Middle School.

A Prospective Cohort Study Examining Race as a Predictor of Adherence to Continuous Positive Airway Pressure Therapy in Obstructive Sleep Apnea

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Introduction: Adherence to continuous positive airway pressure therapy (CPAP) remains a challenge in the management of obstructive sleep apnea (OSA) patients. There have been no consistent predictors of adherence identified in this population. In adherence studies conducted in other medical conditions, racial differences in adherence were documented. There have been few studies examining the contribution of race to CPAP adherence, with one retrospective study finding a trend for differences. The purpose of this study is to prospectively investigate the relative contribution of race (non-Hispanic Caucasian vs. non-Hispanic African American) to CPAP adherence.

Methods: Ninety-eight participants were consecutively enrolled prior to diagnostic polysomnogram (PSG) with 61 meeting the inclusion criteria post-PSG (AHI \geq 15), 34 (55%) non-Hispanic Caucasian and 27 (44%) non-Hispanic African American. Prior to titration and one week after treatment, participants completed a demographic form, Epworth Sleepiness Scale (ESS), Functional Outcomes of Sleep Questionnaire, Profile of Mood States, and a self-efficacy measure for sleep apnea. CPAP adherence, mask-on time, was measured at one-week and at 30-days post treatment initiation.

Results: One-week CPAP adherence data were available for 49 subjects (29 Caucasians; 20 African Americans); 30-day data were available for 41 subjects (25 Caucasian; 16 African American). A multiple regression model of mean nightly duration of use at one week that included AHI, ESS, and race was statistically reliable ($p = .046$). Only race was an independent predictor of adherence with African Americans applying CPAP 2.072 hours (95% CI, 0.573, 3.571) less than Caucasians ($p=0.0080$). Similar results were also found for the predictive model of 30-day adherence with mean nightly use of African Americans being 2.234 hours (95% CI, 0.696, 3.772) less than Caucasians ($p=0.0057$).

Conclusions: Race-based differences in objectively measured CPAP adherence do exist between African Americans and Caucasians. The findings support the urgent need to gain an understanding of culturally rooted aspects of adherence behaviors, specifically elucidating African American patient perceptions of both diagnosis and treatment as contextual contributors to CPAP adherence.

Support:

VA Stars & Stripes Healthcare Network Competitive Pilot Project Fund

The Role of AMP-Activated Protein Kinase in Sleep-Wake State

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The physiological functions of sleep remain elusive. One hypothesis is that sleep serves to replenish energy stores in the brain that are depleted during wakefulness. The AMP-activated protein kinase (AMPK) is phosphorylated and activated when energy consumed exceeds energy produced and hence, AMP increases. Once activated, AMPK preserves ATP by decreasing energy consuming processes and increasing energy-producing processes. Therefore, if energy stores in the brain are depleted during wakefulness due to increased metabolic activity and repleted during sleep, we would predict that phosphorylation of AMPK in the brain would increase with sleep deprivation. We found that following 12 hours of sleep deprivation, phosphorylation of AMPK increases by 35% in the whole brain ($p < 0.05$). In order to begin to assess if this phosphorylation of AMPK is universal throughout the brain or region specific, we measured AMPK phosphorylation in the cortex. With 3 hours of sleep deprivation, phosphorylation of AMPK increases by 30% in the cortex ($p < 0.05$). These data are consistent with the hypothesis that energy stores are depleted during progressive wakefulness. Our current studies aim to address the following three points: a) to establish the time course of AMPK phosphorylation following sleep deprivation b) to demonstrate that the effects of sleep deprivation on AMPK phosphorylation are due to a loss of sleep per se and not stress c) to assess if AMPK phosphorylation is increased in the wake-promoting cholinergic basal forebrain following sleep deprivation. Interestingly, numerous studies have suggested that neurons of the cholinergic basal forebrain play a critical role in modulating sleep-wake state in an energy state-dependent manner. A future question we wish to address is does manipulation of AMPK in the basal forebrain affect sleep-wake state?

Support:
NIH

Effects of Modest Sleep Restriction on the Inflammatory Marker C-reactive Protein

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Introduction: Sleep complaints and short sleep duration are associated with increased cardiovascular morbidity. Findings from previous sleep deprivation studies suggest that sleep loss itself may contribute to inflammation and cardiovascular risk. One inflammatory marker of particular interest to cardiovascular risk is high-sensitivity C-reactive protein (hsCRP), which is the major marker of the acute phase response in humans. Our laboratory has recently demonstrated that severe sleep debt, incurred by either 88 hours of total sleep deprivation or 10 days of partial sleep deprivation (4 hours sleep per night), significantly increased plasma CRP levels in healthy adults. However, it remains unclear if milder bouts of sleep deprivation, which commonly occur in everyday life, have similar effects on CRP levels.

Methods: Thirty-seven participants (16 men and 21 women; mean age, 30.9+7.0 years) were enrolled in the current study. All participants were medically and psychologically healthy. Subjects underwent two nights of baseline sleep and then were randomized to either a partial sleep deprivation or control group. In the sleep deprivation condition, participants underwent five nights of partial sleep restriction (four hours time in bed per night), followed by one night of randomly assigned recovery sleep and a final night of 10 hours of sleep. The recovery night conditions were categorized as either ~high recovery" (6-12 hours time in bed for sleep) or ~low recovery" (0-4 hours time in bed). In the control condition, participants spent 10 hours in bed each night. The additional night of ~recovery" sleep allowed for comparison of possible dose-dependent effects of sleep deprivation. Blood samples were collected prior to sleep restriction (Time 1), following the fifth night of 4-hour sleep restriction (Time 2), and following the final 10-hour night of sleep (Time 3).

Results: When comparing the control and partial sleep deprivation groups, there were no significant differences in the log-transformed CRP values at any time point (all p 's > 0.3). However, there were significant differences in CRP levels between the Time 1 and Time 3 blood draws within the low recovery partial sleep deprivation group ($t=-2.22$, $p=0.048$). Significant changes in log-transformed CRP levels were also found in the pooled partial sleep deprivation groups between the Time 2 and Time 3 blood draws ($t=-2.34$, $p=0.027$) and the Time 1 and Time 3 blood draws ($t=-2.61$, $p=.015$). No comparisons within the control condition or the ~high recovery" partial sleep deprivation groups reached statistical significance.

Discussion: Although a five-day period of partial sleep deprivation was not sufficient to produce a significant increase in CRP levels in healthy adults, an additional night of sleep restriction (as occurred in the ~low recovery group") resulted in significant increases in CRP levels after a subsequent recovery night of 10h time in bed. These findings suggest that even modest sleep restriction may potentiate inflammatory processes. Future research will address whether there are differential vulnerabilities to this sleep-restriction induced inflammatory response, and search for possible psychosocial, neurobehavioral, and biological predictors of those most vulnerable to it.

Norepinephrine Deficient Mice are both Hypersensitive to the Hypnotic Properties of Volatile Anesthetics and Demonstrate Delayed Emergence from Anesthesia

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Introduction: Despite 160 years of widespread use, mechanisms of anesthetic-induced unconsciousness remain unknown. We speculate that volatile anesthetics specifically inhibit wake-active arousal centers to mediate hypnosis, defined as a lack of perceptive awareness to a non-noxious stimulus. The aim of the present study was to investigate the role of the noradrenergic wake-active arousal centers in mediating induction as well as emergence from anesthesia.

Methods: Dopamine Beta Hydroxylase Deficient and hemizygous littermate control mice of both genders, aged 8-14 weeks were used in this study. Mice were maintained on a mixed genetic background of * *. Behavioral assays to evaluate anesthetic sensitivity were 1) the loss of righting reflex, a commonly used surrogate marker of anesthetic-induced hypnosis, and 2) EEG/EMG analysis of anesthetic depth. In addition, to determine whether deficiency of central or peripheral dopamine stores could modulate anesthetic sensitivity, mice were given a subcutaneous injection of L-DOPS and benserazide, a peripheral decarboxylase inhibitor that permits selective restoration of NE in the CNS but not in the periphery.

Results: The ED₅₀ hypnosis dose for three inhaled volatile anesthetics (halothane, isoflurane, and sevoflurane) was significantly reduced by 10-30% in DBH null mice. Moreover, DBH null mice took significantly more time to regain the righting reflex, with more than a two-fold delay in emergence following halothane, isoflurane, and sevoflurane anesthesia. Administration of L-DOPS and benserazide restored normal isoflurane sensitivity in DBH null mice. Preliminary analysis of EEG signals acquired from DBH null and hemizygous control littermates (n=4 per group), has demonstrated that DBH mice show global slowing of EEG as well as burst suppression at low inspired concentrations of isoflurane while control mice do not.

Discussion: It is well known that acute exposure to psychostimulants such as cocaine or amphetamine cause resistance to induction of general anesthesia. Here we demonstrate that congenital deficiency of central but not peripheral norepinephrine (and epinephrine) causes hypersensitivity to anesthetic-induced hypnosis as well as delayed emergence from anesthesia. Results in DBH null mice support a scheme in which induction of general anesthesia is partially dependent upon inhibition of wake-active adrenergic centers. Conversely, emergence from anesthesia is also partially dependent upon re-activation of wake-active adrenergic centers.

Support:

Foundation of Anesthesia Education and Research grant, Department of Anesthesiology and Critical Care startup funding, and NIH grant MH073168

Age-Related Impairment in Long-Term Memory Retention Occludes the Effect of Brief Post-Training Sleep Deprivation in Mice

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Brief sleep deprivation (SD) produces impairments in memory for contextual fear conditioning, a hippocampus-dependent form of memory, when mice are tested 1 day after training. In the current study we asked if these deficits persist when long-term memory retention is tested, and if aged mice have altered susceptibility to the effects of sleep deprivation on memory retention. 80 young adult (2-4 months) and 46 aged (22-24 months) male C57BL/6 NIA mice received single-shock contextual fear conditioning and half of the mice were then deprived of sleep by gentle handling for 5 hours post-training (SD), whereas the remaining mice were left undisturbed in their home cages (NSD). A 5-minute retention test was performed at either 1 or 30 days after training. Mice also received retention tests in an altered context at either 2 or 31 days after training to determine the specificity of memory for the trained context. Memory for the context was quantified as percent freezing, measured every 5 seconds. As expected, sleep deprivation in young mice significantly reduced freezing in the trained context 1 day after training (44.2% +/- 2.6% and 32.9% +/- 2.3% for NSD and SD mice, respectively, $p < 0.05$), and these deficits persisted for at least 30 days (51.4% +/- 4.4% and 39.1 +/- 3.2% for NSD and SD mice respectively, $p < 0.05$). These findings demonstrate that post-training sleep deprivation prevents the consolidation of stable memories, and does not simply affect the ability to recall memories 1 day after training. Sleep deprivation in aged mice produced a similar impairment in contextual memory at 1 day after training (51.2% +/- 4.6% and 27.9% +/- 3.7% for NSD and SD mice respectively, $p < 0.05$), but no deficits were observed when aged mice were tested 30 days after training (38.8% +/- 5.8% and 31.3% +/- 3.2% for NSD and SD mice respectively, $p > 0.05$). This difference between young and old mice appeared to stem from age-related deficits in long-term memory, and suggests that these deficits occlude the effect of sleep deprivation. Further research will be necessary to determine the molecular targets of age and sleep deprivation that mediate these deficits in hippocampus-dependent memory.

The mRNA Level of a Putative Transcriptional Regulator of GABA_A Receptor β_1 Subunit Is Reduced by Stimulation of Hypothalamic GABA_A Receptors In Vitro

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Sleep is associated with an increased release of GABA in the posterior hypothalamus (Nitz & Siegel, 1996). We found that the rat β_1 subunit ($r\beta_1$) of GABA_A receptor (GABA_AR) mRNA level is increased in the perifornical region of hypothalamic slices *in vitro* following superfusion with a GABA_AR antagonist, gabazine, and that the same mRNA is also increased *in vivo* following short-term sleep deprivation (Volgin & Kubin, APSS2003; 2005). GABA suppresses human β_1 subunit ($h\beta_1$) promoter activity and β_1 subunit mRNA expression *in vitro*, with a 72-bp construct containing tripled initiator element (3xInr) being sufficient to mediate this effect (Russek *et al.*, 2000). Our computational analysis revealed that both the sequence for $h\beta_1$ 3xInr and a region predicted to contain $r\beta_1$ promoter have a binding site for the nuclear DEAF-1 related transcriptional regulator (NUDR). This prompted us to investigate whether hypothalamic NUDR mRNA levels in rat hypothalamic slices *in vitro* are sensitive to stimulation of GABA_ARs. Posterior hypothalamic slices from adult rats were superfused for 1.5 h with artificial cerebrospinal fluid (ACSF), or ACSF containing a GABA reuptake blocker (NO-711; 20 μ M), or ACSF containing NO-711 with the GABA_AR antagonist, gabazine (20 μ M). mRNA levels of NUDR, c-fos, and a housekeeping gene, tubulin, were then quantified in 700 μ m tissue punches from the perifornical region using reverse transcription followed by real-time polymerase chain reaction. The data are expressed as cDNA copy numbers per 1 ng of total RNA in the sample \pm SE. The NUDR mRNA levels were 990 \pm 140 (n=8) following incubation with ACSF; 540 \pm 140 (n=6) in NO-711 (p<0.05 vs. ACSF), and 930 \pm 85 (n=6) in NO-711 with gabazine (p<0.04 vs. NO-711 only). In contrast, c-fos and tubulin mRNA levels were not altered. These data show that stimulation of posterior hypothalamic GABA_ARs decreases the mRNA level of NUDR, a factor that may stimulate transcription of $r\beta_1$. This may lead to transcriptional downregulation of $r\beta_1$ that occurs during stimulation of GABA_ARs, as well as during sleep.

Support:

HL-071097 and ASMF # 26-CA-04

Hypothalamic Levels of mRNA for Melanin-Concentrating Hormone (MCH) and Inhibitory GABA_A and Adrenergic Receptors are Increased Three Days After Exposure to Recurrent Hypoglycemia

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The perifornical region of the posterior hypothalamus (PeF) plays important roles in arousal, autonomic control and metabolic regulation. Hypoglycemia potently induces expression of Fos, an immediate-early gene product and component of the AP-1 transcription factor, in PeF neurons; this may have a lasting impact on various homeostatic processes that are integrated in this brain region. Data from juvenile patients treated with insulin for type 1 diabetes mellitus suggest that recurrent hypoglycemic insults impair the ability to respond to hypoglycemia as an arousing stimulus (“hypoglycemia unawareness”). Here, we used a juvenile rat model to test whether recurrent hypoglycemic episodes alter the levels of selected hypothalamic genes important for the regulation of sleep and metabolism. We exposed young rats (25-30 days old) to 3 days of afternoon subcutaneous insulin injection (n=5) or 3 days of saline injection (n=5). Three days later, the rats were euthanized at a constant circadian time (1 PM-2 PM), brains were rapidly collected and transverse sections obtained from the posterior hypothalamus. Tissue punches were taken bilaterally from the PeF region, RNA was isolated and reverse transcribed. Quantitative PCRs were carried out (Roche LightCycler) using primers for two hypothalamic neuropeptides, prepro-orexin and melanin concentrating hormone (MCH), as well as for selected subunits of GABA_A receptors and for adrenergic receptors because these receptors have established roles in the hypothalamic regulation of both sleep and metabolism.

Gene expression levels (mean \pm SEM) were measured as the target cDNA copy number/1 ng total RNA. Insulin-treated rats had significantly higher mRNA levels for MCH, GABA_A receptor α 5 and β 1 subunits, and adrenergic α _{2A} receptors, whereas prepro-orexin mRNA was not different between groups. Since GABA_A and adrenergic α _{2A} receptors mediate inhibitory effects, these data suggest that recurrent hypoglycemia may cause a prolonged inhibition of PeF neurons. This may be one mechanism by which recurrent juvenile hypoglycemia can lead to lasting suppression of hypoglycemic arousal and autonomic counterregulatory responses.

| mRNA for | Insulin group (n=5) | Saline group (n=5) | Significance (p) |
|-----------------------------------|--------------------------------|-------------------------------|-----------------------------|
| MCH | 1,020,000 \pm 120,000 | 410,000 \pm 89,000 | 0.0036 |
| GABA _A α 5 | 10,200 \pm 1100 | 4,600 \pm 670 | 0.0028 |
| GABA _A β 1 | 9,300 \pm 1,200 | 5,200 \pm 570 | 0.0135 |
| Adrenergic α _{2A} | 1,400 \pm 110 | 880 \pm 100 | 0.0086 |

Identifying Novel Sleep Mutants in Drosophila melanogaster

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Sleep is a fundamental and evolutionarily conserved behavior, whose role in human health and disease is becoming increasingly recognized. Despite its importance, its function remains mysterious and its molecular mechanisms poorly understood. The relevance of a molecular dissection of sleep is underscored by the finding that the molecular characterization of a related process in fruit flies, the circadian clock, led to the identification of the genetic basis of a human sleep disorder (familial advanced sleep phase syndrome). The recent discovery that the fruit fly *Drosophila melanogaster* sleeps allows for the use of powerful forward genetic techniques to identify the molecular pathways involved in sleep initiation, maintenance, and homeostasis. The overall goal of this work is to use *Drosophila* as a system to identify novel molecules that function to regulate sleep. Using a collection of roughly 3,000 chemically mutagenized lines, we have conducted a screen for flies that show a significant reduction in daily sleep time. From this screen, we have identified 7 mutants that are “short-sleepers.” We will present initial phenotypic characterization and genetic mapping data for several of these mutants.

Gender Differences in Daily Functioning in Patients with Obstructive Sleep Apnea

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Rationale: Gender-related differences in the presentation of Obstructive Sleep Apnea (OSA) have recently gained recognition as an important clinical problem. Although differences have been examined in upper airway anatomy and function, polysomnographic findings, hormone physiology, and some clinical features such as sleep symptoms and comorbidities, there has been little focus on differences in daily functioning.

Methods: To examine pre-treatment gender differences in several aspects of daily functioning, a secondary analysis of 176 OSA patients (152 men and 24 women) who participated in a multicenter study of CPAP treatment was completed. Functional status, sleepiness, affect, alertness, and neuropsychological functioning were compared between male and female groups. Functional status was measured with the Functional Outcomes of Sleep Questionnaire (FOSQ), subjective daytime sleepiness with the Epworth Sleepiness Scale (ESS), and mood with the Profile of Mood States Total Mood Disturbance (TMD) subscale.

Results: The potential confounding factors, age, BMI, and respiratory disturbance index (RDI), did not differ between men and women. The Total score of the FOSQ were significantly lower in women.

Conclusions: Within a pre-treatment sample, the womens' ability to conduct multiple daily behaviors was more adversely affected than men despite similar age, BMI, and apnea severity. This may reflect the tendency of women to self-disclose the impact of illness more than men. However, further studies are needed to evaluate the underlying mechanism of this observation.

Support:

NIH HL53991, HL60287; Respironics, Inc., Nellcor Puritan Bennett Inc., DeVilbiss HealthCare, Inc.

Anxiety as a Strong Predictor of Health-related Quality of Life in Patients with Obstructive Sleep Apnea

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Introduction: Obstructive sleep apnea (OSA) has been recognized as a great public health hazard and health-related quality of life (HRQOL) impairment in OSA is an increasingly important consideration. Identification of clinical factors related to HRQOL can assist in targeting supportive interventions to improve HRQOL in this enormous population around the world. This paper reports a study exploring the biological and physiological factors and common symptoms of OSA which are predictive of HRQOL in a Chinese population.

Methods: A total of 108 patients (87 men and 21 women) with newly diagnosed OSA were assessed for HRQOL using the Calgary Sleep Apnea Quality of Life Index, for daytime sleepiness using the Epworth sleepiness scale. Zung self-rating depression scale and Zung self-rating anxiety scale were used to assess the symptoms of depression and anxiety. The severity of OSA was measured by Polysomnographic (PSG) variables including the apnea-hypopnea index and the mean and minimum oxygen saturation. The associations between OSA severity, sleepiness, depression, anxiety, and HRQOL were examined by Pearson linear correlation. Hierarchical multiple regression analyses were performed to determine predictors to the total score and each dimension of HRQOL.

Results: Forty-six patients (42.6%) self-rated for a depressed mood and 21 patients (19.4%) were anxious. Fifty-nine patients (54.6%) were hypersomnolent. Significant correlations were observed between depression and sleepiness ($r=.210$, $p<.05$), and between anxiety and sleepiness ($r=.220$, $p<.05$). Hierarchical multiple regression analysis identified anxiety, sleepiness, together with age and gender predicted 45.2% of the variance in the total score of HRQOL ($R^2=.452$, $p<.001$). Anxiety was the strongest significant predictor of the total score and every domain of HRQOL.

Conclusion: The findings suggest that psychological disturbances such as anxiety have a strong impact on HRQOL in patients with OSA. A comprehensive evaluation and intervention for psychological disturbances are highly important for improving HRQOL in this population.

This year marks our third annual full day Research Retreat of the Center for Sleep and Respiratory Neurobiology (CSRN). This retreat has come to serve an increasingly important role for the Penn Sleep Community. From its inception, the philosophy of the CSRN has been to be an interdisciplinary research community that is united by its common interests in sleep and sleep disorders. That this philosophy has been successful is evident by the tremendous growth and scope of sleep research done on campus and by the fact that we continue to be the first or second in the country in NIH funding for sleep research. We have grown in both numbers of researchers as well as in geographic distribution. Research labs and centers stretch across campus as far West as 38th street at the School of Veterinary medicine, as far East as 31st street at the new Translational Research Labs, as far North as our new sleep clinic at 3624 Market Street and as far South as the VA. But this growth has also led to the challenge of gathering together to share our research with one another and exchange ideas. This research retreat therefore provides this important function.

This year our format is a bit different from past years with a greater focus on showcasing our young researchers. Students and Post-docs will give most of the talks this year and we have allotted a full two hours for the poster presentation. We encourage everyone to visit the posters. As the organizing committee, we trust that this day will be enjoyable and scientifically enriching for all.



Dr. Jini Naidoo



Dr. David Raizen



Dr. Terri Weaver



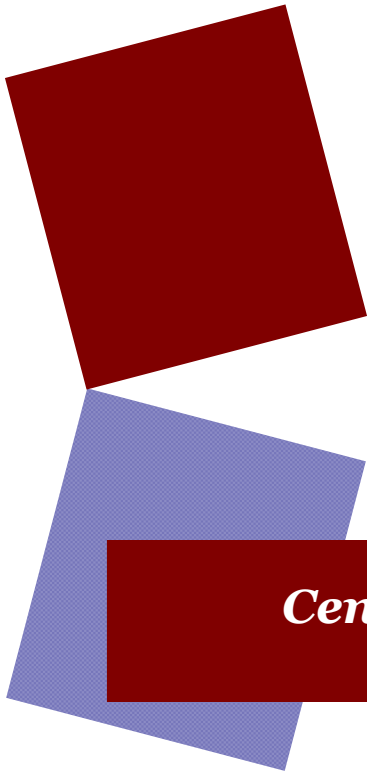
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