Center for Sleep & Respiratory Neurobiology

2nd Annual Research Retreat

June 14, 2005

Levy Conference Center at the
University of Pennsylvania Law School

University of Pennsylvania

Program and Abstracts

PROGRAM

8:30 - 9:00 Poster Mounting

9:00 - 9:05 Introduction
Allan Pack, M.B., Ch.B, Ph.D. - Director, Center for Sleep & Respiratory Neurobiology

Morning Session
***Chairs – Amita Sehgal*, Ph.D. / *Carole Marcus*, M.D.**

9:05 - 9:25 *"Sleep, Experience and Synaptic Plasticity."*
Marcos Frank, Ph.D. - Assistant Professor, Department of Neuroscience

9:30 - 9:50 *"The Effect of Increased Cognitive Workload on Cognitive Impairment During Sleep Deprivation."* Hans Van Dongen, Ph.D. - Research Associate Professor of Sleep and Chronobiology, Department of Psychiatry

9:55 - 10:15 *"Obstructive Sleep Apnea: Are Pregnant Women at Risk?"*
Grace Pien, M.D. – Assistant Professor, Department of Medicine

10:20 - 10:40 *"Obesity, Weight Loss and Obstructive Sleep Apnea."*
Gary Foster, Ph.D. - Associate Professor, Department of Psychiatry
Clinical Director of the Weight and Eating Disorders Program, School of Medicine

10:45 - 11:00 Coffee Break

11:00 - 12:00 **Keynote Lecture:** *"Sleep and Synaptic Homeostasis."*
Giulio Tononi, M.D., Ph.D. Professor, Department of Psychiatry
University of Wisconsin, Madison

12:00 - 1:00 Lunch and Poster Viewing

Special Session: "Dreaming with Adrian Morrison: Lessons & Memories."
Chair – Leszek Kubin, Ph.D.

1:00 - 1:15 Opening Remarks: Alan Kelly, B.V.Sc., M.R.C.V.S., Ph.D.
The Gilbert S. Kahn Dean of Veterinary Medicine

1:15 - 1:45 *"Comparing Sleep Across the Years (Millions of Them!)"*
Joan Hendricks, V.M.D., Ph.D. – Henry and Corinne R. Bower Professor
Department of Clinical Studies-Philadelphia, School of Veterinary Medicine

1:45 - 2:15 *"Alerting and Arousal: Concepts for Understanding State Regulation."*
Larry Sanford, Ph.D. – Professor, Department of Pathology & Anatomy
Eastern Virginia Medical School, Norfolk, VA

2:15 - 2:45 *"REM Sleep: Insights for Psychiatry."*
Richard Ross, M.D., Ph.D. – Professor of Psychiatry, VA Medical Center and
University of Pennsylvania School of Medicine

2:45 - 3:00 Coffee Break

3:00 - 4:30 Poster Session

4:30 - 4:45 Trainee Award Presentation

4:45 - 6:00 Reception to celebrate Adrian Morrison's contributions

FROM THE DIRECTOR:

This is the second annual Research Retreat of the Center for Sleep and Respiratory Neurobiology. The first event was a great success and we look forward to the second. This year's event will be unique. Dr. Adrian Morrison, who started sleep research at the University of Pennsylvania, is becoming an Emeritus Professor. He has, however, recently received another R01 for five years! This seemed, therefore, a good time to celebrate and thank Adrian for his important contributions. Thus, the plan for this Retreat is somewhat different. We have a special symposium at which Adrian's previous trainees will discuss their work, i.e., Dr. Joan Hendricks, Dr. Larry Sanford and Dr. Richard Ross. In addition, we plan to end the Retreat with a reception to honor Adrian. I thank the Organizing Committee: Dr. Narayan Avadhani, Dr. Joan Hendricks and Dr. Leszek Kubin.

Given Adrian's long-term relationship with colleagues in Pisa, Italy, it is fitting that our Keynote Speaker is Dr. Giulio Tononi, who received his training in Pisa. As Adrian has demonstrated, one of the fun aspects of a scientific career is that the world is a small place and one develops collaborators and friends in many countries.

Adrian, I trust that you will accept our thanks for your contributions.

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

Adrian R. Morrison, D.V.M., Ph.D.

As a faculty member at the University of Pennsylvania for almost 40 years, Adrian R. Morrison has been an active teacher and researcher with a remarkably diverse career and record of service to the veterinary profession, the University, and the neuroscience research community.

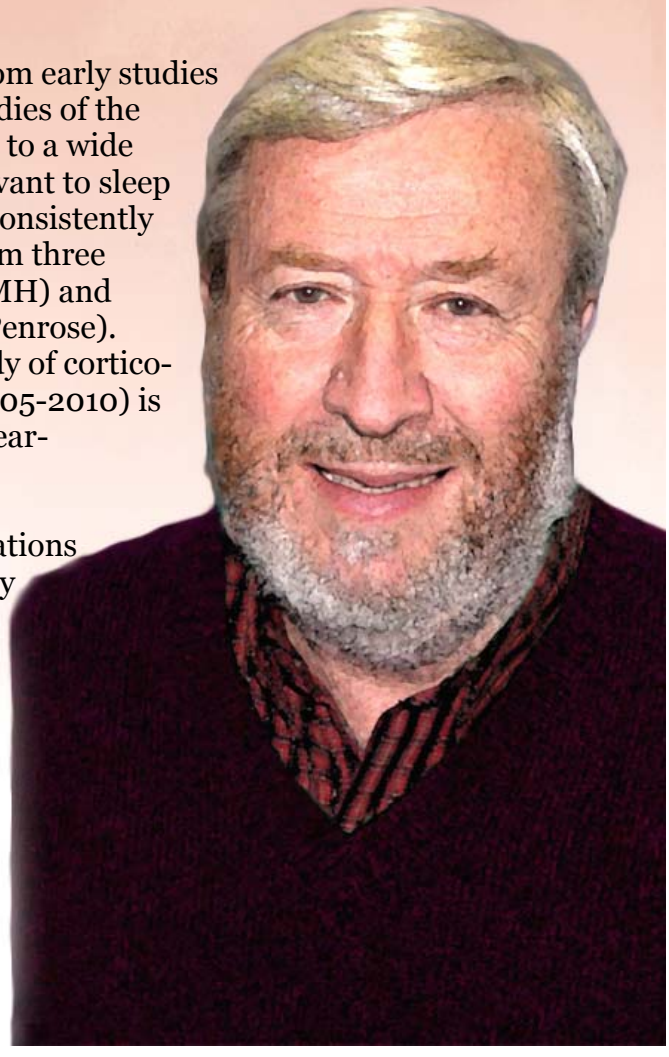
Education

Adrian grew up in rural Lancaster, where he acquired his love of animals. He attended the rigorous Franklin and Marshall College in Lancaster, graduating Phi Beta Kappa in 1957. He then attended Cornell to receive his veterinary training. By 1962, he acquired not only a DVM but also a MS, studying rumen physiology. He was awarded membership in the Phi Zeta veterinary honor society. Subsequently, Adrian received his Penn Ph.D. in 1964, in the Anatomy Department, which was the birthplace of the Institute for Neuroscience, where he worked closely with Bill Chambers, Eliot Stellar and Jim Sprague.

During these scientific formative years, he traveled to Pisa from 1964-65 to study sleep physiology and food with Ottavio Pompeiano. He then returned to Philadelphia in 1965 to complete his postdoctoral training. He joined the Veterinary School's basic science Animal Biology Department in 1966, where he has remained throughout his career. Adrian has continued to obtain varied global research experiences with sabbaticals in Poland and Mexico, as well as frequent return trips to Italy.

Research

Adrian's original research has ranged from early studies of corticothalamic projections, through studies of the vestibular and motor systems during sleep, to a wide array of both clinical and basic studies relevant to sleep pathology. Throughout his career, he has consistently won support against tough competition from three Institutes of the NIH (NINDS, NHLBI, NIMH) and other sources (NATO, Guggenheim, NSF, Penrose). His first NIH grant in 1966 was for the study of cortico-thalamic relationships; his most recent (2005-2010) is concerned with amygdalar modulation of fear-conditioned changes in REM sleep.



In addition to making important observations of the basic physiology and neurophysiology of the sleeping organism, Adrian has worked with colleagues in pulmonary medicine, psychiatry, and veterinary medicine to make contributions to the study of sleep apnea, REM sleep behavior disorder, post-traumatic stress syndrome, narcolepsy, and he made pioneering observations on spontaneous sleep disorders in cats, dogs and horses.

Honors

Adrian has received numerous scientific honors, including a Guggenheim Fellowship in 1984 and the coveted MERIT Award from the NIMH from 1987-1998. Among his many other honors, are several recognizing his courageous service to neuroscience for his work fighting radical advocates of animal rights. One of these, awarded in 1990, is the Rick Simpson Memorial Award, given by the Incurably Ill for Animal Research. Remarkably, his career received recognition by the Sleep Research Society with major awards for two consecutive years, first in 2002 with the Distinguished Scientist Award and again in 2003 as a Significant Early Contributor to Modern Sleep Research. Significant early contributors were recognized for valuable discoveries that were instrumental in advancing sleep science between 1963 and 1972. Finally, Adrian is probably the only Sarkin Yaki (war chief and chief advisor) in the sleep research community. This latter honor is for his service in teaching and supporting education in Butura, Nigeria.

Teaching and Service

Adrian has taught gross anatomy of the dog, cow, and horse to veterinary students throughout his career, as well as numerous neuroscience lectures. He has been invited to give lectures across the country and around the world, including Nigeria, Italy, and Mexico. Adrian has trained six graduate students and ten postdoctoral fellows. Two of his postdoctoral students have received Young Investigator Awards from the Sleep Research Society. His trainees and colleagues have included V.M.D., M.D., as well as Ph.D. students.

Adrian's leadership in the sleep research community at Penn has been manifested in his role in contributing to founding the interdisciplinary Sleep Center, his contribution to the Special Center of Research and Program Project Grants, and the Center for Sleep and Respiratory Neurobiology, and his longstanding membership in both the Institute for Neurological Sciences and the Neuroscience (originally Anatomy) graduate group. Adrian's citizenship within the University includes participation in 30 University committees and dozens of Vet School committees.

Adrian has also been a leader in the national and world sleep research community in numerous roles, including the presidency of both the national Sleep Research Society and the World Federation of Sleep Research Societies.

A remarkable feature of Adrian's service to the wider biomedical research community has been his thoughtful, tireless, and unwavering support for experimental studies of mammals in the face of threats and criminal actions against him by animal rightists. From 1991-1994, he devoted himself officially to this cause as Director of the Office of Animal Research Issues at NIH. He continues to speak and debate on this difficult issue, and has written numerous essays and chapters on the necessity of conducting studies in mammals.

On top of everything else, Adrian and his wife, Ollie, have 5 wonderful, accomplished children, and a spectacularly talented kitty, Buster. Adrian has been active in his community as a scoutmaster and soccer coach, and has received veterinary licenses from Pennsylvania, Ohio and New York.

Keynote Speaker: Giulio Tononi, M.D., Ph.D.

Giulio Tononi received his medical degree and specialized in psychiatry at the University of Pisa, Italy. After serving as a medical officer in the Army, he obtained a Ph.D. in neuroscience as a fellow of the Scuola Normale Superiore, based on his work on sleep regulation. From 1990 to 2000 he has been associated with The Neurosciences Institute, first in New York and then in San Diego. He is currently Professor of Psychiatry at the University of Wisconsin, Madison, where he is studying consciousness and its disorders, as well as the mechanisms and functions of sleep.

In his work on sleep, Dr. Tononi has pioneered the combined use of electrophysiological approaches and molecular biology. His laboratory has discovered striking differences in the expression of certain genes between sleep and waking and identified molecular markers of these behavioral states. Further studies have uncovered the neurophysiological and molecular mechanisms by which the acquisition of new information by the brain is limited to waking states and does not occur during sleep. Recently, his laboratory has demonstrated, based on a variety of behavioral, pharmacological, and molecular criteria, that sleep-like states are present in the fruit fly *Drosophila*. This finding has opened the way to the genetic dissection of sleep using mutant screening and other powerful tools of genetic manipulation available in *Drosophila*. Current work using rat, mouse, and fly models is aimed at understanding the functions of sleep by focusing on the consequences of sleep and sleep deprivation at the cellular and molecular level. Recently, he has formulated a new hypothesis about the function of sleep, according to which sleep serves synaptic homeostasis. This hypothesis has led to several experimental tests, including the recent demonstration that sleep can be induced on a local basis by learning and plasticity. The synaptic homeostasis hypothesis has implications with respect to the neurobiology of mood disorders and the beneficial but transitory effects of sleep deprivation on depression.

In his work on consciousness, Dr. Tononi has addressed the problem of how the activities of functionally specialized areas of the brain can be integrated to give rise to a unified conscious experience. To this end, he has: (1) constructed large-scale computer models based on the anatomy and physiology of the thalamocortical system to study the mechanisms of information integration; (2) developed theoretical approaches aimed at defining and measuring the integration of information within the nervous system; (3) pioneered experimental approaches aimed at characterizing the neural substrate of conscious experience by using neuroimaging and, more recently, transcranial magnetic stimulation. This work has recently led to the formulation of the information integration theory of consciousness. His group is currently investigating some of the predictions of the theory, with particular emphasis on the breakdown of information integration in brain disorders such as schizophrenia.

Dr. Tononi is a frequent lecturer and invited speaker at scientific symposia. He is the author of over 100 scientific publications, co-editor of the volume *Selectionism and the Brain* (with Olaf Sporns), and author of two recent books on the neural basis of consciousness: *A Universe of Consciousness* (with Gerald M. Edelman) and *Galileo and the Photodiode*.

CONTENTS

14. **Kuna ST, Seeger T, Brendel M.**
Intra-Subject Comparison of Polysomnography and a Type 3 Portable Monitor.

15. **Lee JY, Brooks L.**
Positional Differences in Obstructive Sleep Apnea in Children and Adults.

16. **Lu JW, Mann GL, Volgin DV, Ross RJ, Morrison AR, Kubin L.**
Perifornical Hypothalamic Microinjections of Antisense Oligodeoxynucleotides Against β_3 Subunit of $GABA_A$ Receptor Reduce REM Sleep.

17. **Maislin G, Hachadoorian R, Pack F, Staley B, Dinges DF, Pack AI.**
Operating Characteristics of the Multivariable Apnea Prediction Index in Non-Clinic Populations.

18. **Mason TBA, Arens R, Kaplan P, Bintliff BE, Walters AS, Pack AI.**
Sleep in Children with Williams Syndrome.

19. **McGlinchey EL, Banks S, Minkel JD, Dinges DF.**
Effect of Chronic Sleep Restriction on Pre-frontal Cortex Functioning and its Relationship to IQ and Personality.

20. **Meltzer LJ, Mindell JA.**
Sleep and Fatigue in Caregivers of Ventilator-dependent Children.

21. **Meltzer LJ, Mindell JA.**
Validity of 24-Hour Recall Interview for Sleep Patterns.

22. **Minkel JD, Banks S, McGlinchey EL, Dinges DF.**
Relationships among Mood and Neurocognitive Tasks after Five Nights of Partial Sleep Deprivation.

23. **Naidoo N, Cearley C, Zimmerman J, Pack AI.**
A Role for BiP/GRP78 in Recovery Sleep of Drosophila.

24. **Nikonova E, Frank BC, Mackiewicz M, Quackenbush J, Zhang L, Galante RJ, Naidoo J, Pack AI.**
Changes in Components of the Electron Transport Chain in Mouse Cortex with Increases in Wakefulness.

25. **Niyogi S, Price NJ, Rogers NL, Van Dongen HPA, Dinges DF.**
Circulating Norepinephrine Levels in Response to Severe Sleep Deprivation, Caffeine, and Modafinil.

26. **Otto CM, Gaspard RM, Robinson MA, Fox J, Baumgardner JE, Quackenbush J, Pack AI.**
Inflammatory Gene Expression in Macrophages Exposed to Intermittent Hypoxia.

27. **Razavi F, Banks S, Dinges DF.**
Effects of Sleep Restriction and Recovery Sleep on Driving Simulator Test (AusEd) Performance.

28. **Reishtein JL, Maislin G, Dinges DF, Pack AI, Weaver TE, Multisite Group, University of Pennsylvania.** *Intimacy and Sexuality in Obstructive Sleep Apnea: The Effect of Treatment.*

29. **Robinson MA, Baumgardner JE, Fox J, Otto CM.**
Oxygen Substrate Limitation Regulates Nitric Oxide Production by Cytokine-Stimulated Macrophages.

30. **Pawlyk AC, Ross RJ, Jha SK, Brennan FX, Morrison AR.**
The Differential Rapid Eye Movement Sleep Response to Cued and Contextual Fear Conditioning is Suppressed by a Shared Response to Fearful Conditioned Stimuli.

31. **Stakofsky AB, Levin AL, Vitellaro KM, Dinges DF, Van Dongen HPA.**
Effect of Cognitive Workload on Neurobehavioral Deficits during Total Sleep Deprivation.

32. **Tkacs N, Pan Y, Sawhney G, Mann GL, Morrison AR.**
Hypoglycemia Reduces REM Sleep, Increases Arousal, and Activates Locus Coeruleus and Basal Forebrain Cholinergic Neurons in Rats.

33. **Tucker A, Gehrman P.**
Are Insomniacs Less Accurate at Reporting Sleep Times?

34. **Volgin DV, Swan JL, Kubin L.**
Intermittent Hypoxia Alters Hypothalamic Transcription and Increases Pancreatic Insulin-1 Precursor mRNA Expression in Rats.

35. **Yang S, Farias M, Kapfhamer D, Grant G, Abel T, Bucan M.**
Behavioral, Molecular and Biochemical Characterization of Rab3A Mutations that Cause Abnormal Circadian and Sleep Behavior in the Mouse.

36. **Zimmerman JE, Mackiewicz M, Rizzo W, Shockley K, Churchill GA, Pack AI.**
Changes in Gene Expression with Sleep and Wakefulness in Drosophila Brain.

Prevalence and Characteristics of Obstructive Sleep Apnea in Patients Undergoing Gastric Bypass Surgery

Abaluck B, Cartagena S, Hurley S, Pack AI, Schwab RJ, Cantor CR

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

Introduction: Obstructive sleep apnea (OSA) has a high prevalence among obese adults and may lead to perioperative complications if undiagnosed before gastric bypass surgery. We hypothesized that sleep apnea would be common in the gastric bypass population. Therefore, we examined in gastric bypass patients the prevalence of sleep apnea and the relationships of the Multi-variable Apnea Sleep Symptom (MAP) score and the Epworth Sleepiness Scale (ESS) to the apnea hypopnea index (AHI).

Methods: 50 consecutive patients evaluated for gastric bypass from 1/2004 to 4/2004 underwent overnight polysomnography and completed questionnaires (MAP and ESS). Apneas and hypopneas were classified as follows:

Mild apnea: $5 \leq AHI \leq 15$, Moderate apnea: $15 < AHI \leq 30$, Severe apnea: $AHI > 30$, REM-related apnea: present when REM-AHI>twice NREM-AHI and REM-AHI>10

Results: Mean body mass index (BMI) in kg/m^2 was 49.3±8.7 and mean age was 40.6±10.7. Our sample was 78% female and 66% white, 28% African American, and 2% Hispanic. The overall frequency of OSA was 58%; 26% had mild OSA, 10% had moderate OSA, and 22% had severe OSA. The frequency of REM-related OSA was 66%. A linear regression demonstrated the following:

Characteristics	Correlation (r^2)	P Value
BMI and Epworth	.0006	.868
BMI and MAP	.635	<.0001
AHI and Epworth	.00247	.73
AHI and BMI	.0821	.048
AHI and MAP	.346	.0003
AHI and sex	.219	.0006

Conclusion: In our population of gastric bypass patients, the prevalence of sleep apnea was 58%, and the prevalence of REM-related apnea was 66%. ESS did not correlate with AHI, but increased BMI, increased MAP, and male gender correlated with increased AHI. Our data indicate that sleep apnea and particularly REM-related apnea are common in patients undergoing gastric bypass surgery. Therefore, gastric bypass patients should be screened for OSA.

Expanding the Two-Process Model to Describe Cumulative Performance Impairment from Chronic Sleep Loss

Amin DD, Avinash D, Crudele CP, Vacs EF, Dinges DF, Van Dongen HPA

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

Introduction: The homeostatic process S of the two-process model (TPM) saturates rapidly for schedules involving chronic sleep restriction, and cannot predict the waking performance deficits accumulating over days. The equations for process S have fixed asymptotes (U and L), as seen when writing the equations as follows:
$S(t)-U(t)=[S(t-\Delta t)-U(t-\Delta t)]\exp(-\Delta t/Tr)$ during wake;
$S(t)-L(t)=[S(t-\Delta t)-L(t-\Delta t)]\exp(-\Delta t/Td)$ during sleep;
where $U(t)=1$ and $L(t)=0$; Tr and Td are time constants; and Δt is the time step. Based on an idea by Johnson et al. (2004), we expanded the TPM to predict the effects of chronic sleep restriction on psychomotor vigilance task (PVT) performance, by manipulating the asymptotes U and L.

Methods: The asymptotes were modified as a function of prior sleep and wake:
$U(t)=U(t-\Delta t)+Mr\Delta t$ during wake;
$U(t)=U(t-\Delta t)+[1-U(t-\Delta t)][1-\exp(-Md\Delta t)]$ during sleep;
where Mr and Md are rate constants. It was postulated that $L(t)=U(t)-1$. The model parameters were tentatively fixed at previously published values: Tr=18.2h, Td=4.2h, Mr=0.137/h and Md=0.0092/h. Closed-form versions of the equations were derived to assess goodness-of-fit relative to performance in a laboratory sleep restriction experiment involving n=35 subjects (see companion abstract by Avinash et al.). Goodness-of-fit was compared with a null model (i.e., straight line); the original TPM; and our previously hypothesized excess wakefulness model (EWM). To provide an equal basis for comparison, the latter was formulated with fixed critical wake duration ξ=15.84h and computed using scheduled TIB.

Results: Akaike's Information Criterion (AIC) was employed to quantify goodness-of-fit (smaller is better). Expressed relative to the expanded TPM, the AIC was 681.7 for the null model; 322.4 for the original TPM; and –88.2 for the EWM.

Conclusions: The expanded TPM qualitatively captured the pattern of PVT performance changes across days of chronic sleep restriction, and constituted a considerable enhancement of the original TPM. While the EWM provided more accurate predictions of cumulative performance impairment, goodness-of-fit of the expanded TPM will likely increase following optimization of the time and rate constants.

Support:
NASA cooperative agreement NCC 2-1394 with the Institute for Experimental Psychiatry Research Foundation, and NIH grants NR04281, HL70154 and RR00040.

Goodness-of-Fit of an Expansion of the Two-Process Model to Predict Cumulative Performance Impairment Due to Chronic Sleep Restriction

Avinash D, Crudele CP, Amin DD, Vacs EF, Dinges DF, Van Dongen HPA

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

Introduction: In a companion abstract (Amin et al.), we proposed an expansion of the two-process model of sleep regulation to predict cumulative performance impairment due to chronic sleep loss. Here, we compared predictions of the model to psychomotor vigilance task (PVT) performance data from a laboratory experiment involving 14 days of sleep restriction to 4h, 6h or 8h TIB (Van Dongen et al., SLEEP, 2003).

Methods: To determine goodness-of-fit, it was necessary to derive closed-form equations of the model that reflected the experimental design. Defining D as the day number, To as the time of awakening, and Tm as the midpoint of the day (for which predictions were made), these equations were:
For D=0 (baseline): $U(D)=Mr*(Tm-To)+Uo$; $S(D)=U(D)+(So-Uo)*e^{-(Tm-To)/Tr}$.
For D=1..14: $U(D)=Mr*(Tm-To)+1+(Uo-1)*e^{-Md*D*TIB}+\sum_{i=0..D-1}Mr*(24-TIB)*e^{-Md*(D-i)*TIB}$;
$S(D)=U(D)+[(So-Uo)*e^{D*[-(24-TIB)/Tr-TIB/Td]}+e^{-TIB/Td}-1+A]*e^{-(Tm-To)/Tr}$,
where $A=\sum_{k=1..D-1}e^{(D-k)*[-(24-TIB)/Tr-TIB/Td]}*(e^{-TIB/Td}-1)$ if D>1 else A=0.
Initial values: $Uo=1+16*Mr/(e^{8*Md}-1)$; $So=Uo-(1-e^{-8/Td})/(1-e^{-16/Tr-8/Td})$.
Defining Y as observed lapses on the PVT averaged within days and expressed relative to baseline, a mixed-effects regression model was formulated (for D=1..14): $Y=\beta*[S(D)-S(0)]^{\theta}+\varepsilon$. Here θ was a curvature parameter, β a scaling factor with random effect (mean 0, variance ω^2), and ε residual error (mean 0, variance σ^2). Parameters Tr, Td, Mr and Md were tentatively fixed at previously published values: Tr=18.2h, Td=4.2h, Mr=0.137/h and Md=0.0092/h.

Results: With only β, θ, ω^2 and σ^2 as free parameters, the expanded two-process model explained 77% of the variance in the data ($\chi^2=449.2$, $P<0.001$).

Conclusions: This result suggests that the expanded two-process model has potential for the prediction of performance impairment due to chronic sleep loss. Goodness-of-fit will likely increase after optimization of parameters Tr, Td, Mr and Md, for which analyses are ongoing.

Support:
NASA cooperative agreement NCC 2-1394 with the Institute for Experimental Psychiatry Research Foundation, and NIH grants NR04281, HL70154 and RR00040.

Food Cravings: Chronic Sleep Restriction and Mood

Baffy NJ, Banks S, Dinges DF

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

Introduction: There have been few studies that examined the relationship between sleep loss and food cravings. The aim of the present study was to investigate the effects of chronic sleep restriction on food cravings and its association with mood states.

Methods: Preliminary analysis was conducted on 20 healthy subjects (10f, 10m, aged 22-43y, BMI< 30) out of n=45 participating in a sleep restriction protocol with two baseline nights of sleep 10h TIB, followed by 5 nights of sleep restriction to 4h TIB. A validated Food Craving Inventory, with four subscales (sweets, fats, fast-foods and carbohydrates) was administered daily at 2100h during the protocol. The profile of mood states (POMS), with seven subcategories, was given at 2h intervals beginning at 0800h every day. POMS scores were averaged over the day. Food was limited to hospital diets, but food intake was not controlled.

Results: Repeated-measures ANOVA revealed no significant change in total food cravings over the sleep restriction period ($p=0.47$), and no detectable gender difference ($p=0.79$). There was a trend for a relationship between BMI and average food cravings ($p=0.07$). Positive correlations between the tension/anxiety subscale of the POMS and cravings for sweets were found each day during the sleep restriction period ($r=0.27$ to 0.51). Additionally, by the end of the sleep restriction period, subjects who reported greater fatigue on the POMS had less cravings for all food subcategories (fats: $r=-0.46$; sweets: $r=-0.23$; carbs: $r=-0.45$; fast-foods: $r=-0.45$).

Conclusion: It appears that for subjects in a controlled laboratory environment, food cravings are not significantly increased by chronic sleep restriction when food intake is not controlled. However, those subjects who reported being more tense and anxious craved more sweet foods during the sleep restriction period. Furthermore, those subjects who were most fatigued craved less food in general after five days of chronic sleep loss.

Supported by:
A NASA cooperative agreement with the National Space Biomedical Research Institution grant NCC9-58-159, and NIH grants NR004281 and RR00040.

The Effect of Puberty on Upper Airway Dynamic

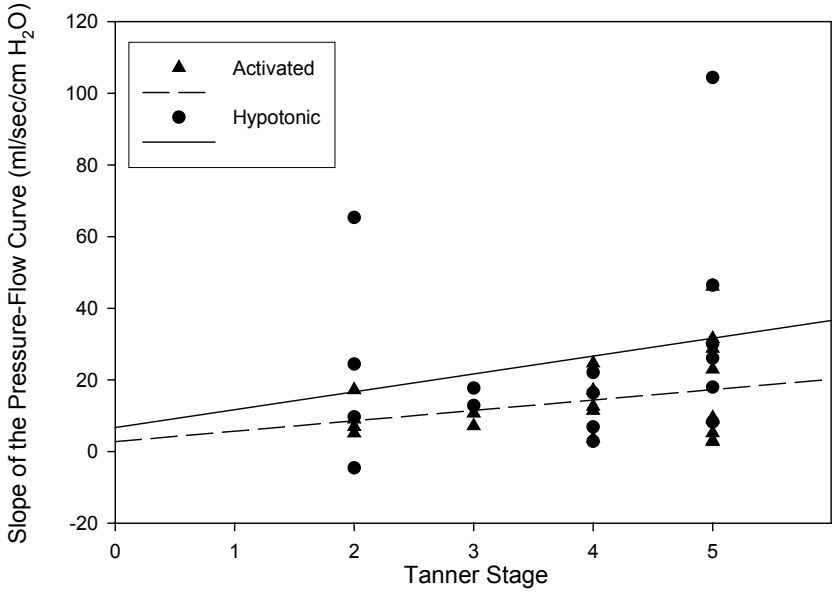
Bandla P, Karamessins L, Samuel J, Pepe M, Marcus CL

The Children's Hospital of Philadelphia, University of Pennsylvania

Introduction: 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. 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.

Fig. 1 Slope vs Tanner Stage



Results: Of the 19 subjects studied thus far, 10 were male and 9 were female. Subjects were of Tanner stages 2-5. Tanner stage showed a trend toward positive correlation with the slope of the PFR with both the activated (r=0.30) (NS) and hypotonic (r=0.23) (NS) techniques (Fig. 1). More subjects are required for significant correlations to be made between gender and the slope of the PFR.

Conclusions: Although limited by the small sample of subjects studied so far, our preliminary results suggest that with increasing Tanner stage, there is an increase in upper airway collapsibility. We speculate that this is probably due to the effect of the sex hormones on the development of the upper airway during the transitional period of puberty. More subjects are needed to determine the relationship between gender specific pubertal changes and upper airway collapsibility.

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

Is the Maintenance of Wakefulness Test Sensitive to Varying Amounts of Recovery Sleep after Chronic Sleep Restriction?

Banks S, Dinges DF

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

Introduction: The Maintenance of Wakefulness Test (MWT) examines an individual's ability to stay awake in an environment of decreased sensory stimulation. Little is known about the sensitivity of the MWT to chronic sleep restriction (CSR) and varying amounts of recovery sleep in normal healthy subjects. The validity of the MWT relies on its ability to distinguish between different amounts of sleep loss.

Methods: Preliminary analyses was conducted on 15 subjects (age=35.4±6.1yr, 8f) out of 35 participating in a laboratory controlled CSR protocol. Subjects underwent 2 nights of baseline sleep (TIB=10h) followed by 5 nights of sleep restriction (TIB=4h) and a recovery night where TIB was given in different doses (2h, 4h, 6h TIB). Subjects were monitored during sleep with polysomnography. Modified single trial (30min) MWTs were conducted between 1430h-1600h on the day after the second baseline night, after the fifth sleep restriction night and after the sleep dose recovery night. Mean sleep latency (MSL) was defined as time to the first appearance of a brief sleep (10sec microsleep).

Results: MWT MSL after 5 nights of CSR was 13.6±10.4min, which differed significantly from baseline (21.1±10.6min; $p=0.01$). The 2h, 4h and 6h TIB doses after 5 nights of CSR to 4h TIB yielded MSL of 5.1±3.4min, 6.6±7.1min and 7.3±3.1min, respectively. The 2h TIB recovery condition resulted in shorter MWT MSL than the final night of 4h TIB ($p=0.007$), but not for the 4h and 6h TIB recovery conditions. All TIB doses (2h, 4h and 6h) yielded MWT MSL significantly below those at baseline ($p<0.001$).

Discussion: Five nights of CSR to 4h TIB significantly increased subjects' sleepiness measured by MWT. Further restriction of sleep to 2h on the recovery night reduced MSL, and the 4h and 6h did not provide improvement. Longer doses of recovery sleep are being studied to determine at what sleep duration MWT normalizes.

Supported by:
NIH NR004281 and RR00040.

Systematic Individual Differences in Delta Wave Expression in NREM Sleep EEG

Baynard MD, Dinges DF, Van Dongen HPA

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

Introduction: Previous investigations in our laboratory revealed systematic individual differences in sleep architecture. The present analyses extend these investigations to delta waves in the NREM sleep EEG.

Methods: As part of a larger study, 5 healthy subjects (age 29.4±5.3; 2 females) were preliminary studied. Each participated in two laboratory experiments with 12h TIB (experiment 1) or 6h TIB (experiment 2) for baseline sleep, 36h controlled total sleep deprivation, and 12h TIB for recovery sleep. Sleep periods were recorded polysomnographically (Vitaport 3; TEMEC), sampled at 128Hz, and scored manually. Artifacts in the EEG (C4–Ax) were removed, and the average amplitude spectrum was computed for every 30s epoch. Subsequently, the mean amplitude of EEG delta waves (0.75–4.75Hz) during NREM sleep was determined for each recording (Vitascore; TEMEC). Analyses were completed for experiment 1 in 2 subjects, experiment 2 in 1 subject, and both experiments in 2 subjects, yielding a total of 14 records. The delta amplitude data were entered into mixed-model ANOVA to assess differences between baseline vs. recovery nights and 6h TIB vs. 12h TIB nights. The intraclass correlation coefficient (ICC) was computed as a measure of systematic individual variability.

Results: Although differences between 6h and 12h TIB nights and between baseline and recovery nights were pronounced, they did not reach significance ($t[4] \leq 1.80$, $P \geq 0.15$). However, there were considerable individual differences (ICC=89.7%; $\chi^2[1]=14.8$, $P<0.001$). Systematic individual differences in mean NREM delta amplitude ranged from 291μV to 3,681μV.

Discussion: There was insufficient statistical power to investigate effects of TIB (6h vs. 12h) and prior sleep deprivation (36h) on NREM delta waves. However, this preliminary investigation yielded evidence of substantial individual differences in the mean amplitude of NREM delta waves (cf. Finelli et al., 2001)—which were systematic across baseline and recovery nights regardless of TIB. Ongoing analyses on a larger sample will confirm if individual differences in NREM delta amplitude constitute a trait.

Support:
NASA grant NAG9-1161 and NIH grants HL70154 and RR00040.

NZO Mouse Is A Mouse Model of Upper Airway Compromise

Brennick MJ, Galante RJ, Pickup S, Schwab RJ, Pack AI

The Center for Sleep and Respiratory Neurobiology, Pulmonary and Critical Care Division, and Department of Radiology, University of Pennsylvania, Philadelphia, PA

Obesity, in particular excess fat in the neck, is a key risk factor for obstructive sleep apnea. We have recently screened several obese mouse models with a high throughput screen based on assessments of activity/inactivity to evaluate sleep and wakefulness in these models. We have found that the New Zealand Obese (NZO) is excessively sleepy based on this screen. (The NZO is the result of a spontaneous mutation of the New Zealand wild type strain (NZW).) The NZO has reduced wakefulness with reduced average length of bouts of wakefulness during the nighttime active period. Moreover, the mouse has a highly unusual phenotype, i.e., it has been observed to sleep in an upright position. These observations suggested to us that this obese mouse, which has obvious increased neck fat, might have upper airway narrowing as occurs in obstructive sleep apnea patients. To address this, we have developed MRI techniques in mouse that allow us to evaluate 3D distribution of fat and reconstruct the upper airway in 3 dimensions. These MRI studies show that the NZO mouse, compared to age- and gender-matched wild type controls (NZW), has: a) marked increase in neck fat; 2) large parapharyngeal fat pads; and 3) decreased size of the upper airway. The NZO mouse is likely to be a model of how excess fat leads to upper airway narrowing that is relevant to obstructive sleep apnea.

Supported by:
NIH grant U01 HL66611.

Can Acupuncture Improve Sleep in Fibromyalgia Patients? *

Chen L, Schumacher HR, Pack AI, Kuna ST.

Rheumatology Division, the University of Pennsylvania, Presbyterian Medical Center and VAMC, the Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA

Purpose:
To study whether acupuncture treatment can improve sleep disturbance in fibromyalgia (FM) patients. Seven published studies used acupuncture to treat FM patients. They all found that acupuncture relieved pain but the sleep improvement was not objectively assessed. In this pilot case study, polysomnograms were used to objectively monitor sleep parameters before and after acupuncture treatment.

Methods:
20 FM patients with complaints of poor sleep were referred by rheumatologists in the Penn Health System. The diagnosis of FM was based on the criteria established by The American College of Rheumatology 1990. Patients were excluded for uncontrolled depression, thyroid diseases, autoimmune diseases and other unstable medical conditions. No medications for pain and sleep were added or changed in the 12 weeks prior to enrollment or during participation in the study. Each patient had 8 weeks individualized acupuncture session once a week based on Traditional Chinese Medicine. All acupuncture treatments for each patient included the acupuncture points for sleep disturbance and pain. Using standard technique, overnight polysomnograms were performed in the sleep laboratory at baseline and following 8 weeks of individualized acupuncture treatment. The following outcomes were measured: Fibromyalgia Impact Questionnaire Score (FIQ), Sleep Efficiency (SE), Sleep Onset Latency (SOL), Arousal Index (AI), Periodic Limb Movements Index (PLM) and Apnea-Hypopnea Index (AHI).

Results:
The 17 females and 3 males ranged from 35-58 years old. Nine patients (45 %) had an AHI > 5, the diagnostic criterion for obstructive sleep apnea. One patient had increased PLM index and was found to have restless leg syndromes. These patients were excluded from the study and referred to sleep medicine specialists for further management.
Of the remaining 10 patients, 7 patents have completed the protocol, 2 were not be able to finish the entire 8 week course of acupuncture treatment due to time conflicts and 1 patient is still undergoing acupuncture treatment. In the seven patients who completed all 8 acupuncture sessions, the FIQ score improved 38.19% (from 57.40 to 35.48); SOL was shortened 65% (from average 69.86 minutes to 24.33 minutes); SE increased 11.09 % from 71.75% to 82.84%; the arousal index decreased 29.31% (from 32.27 events to 22.81 events).

Conclusion:
Acupuncture treatment can help fibromyalgia patients with their sleep disturbance as objectively documented by polysomnographic measures. Acupuncture points for sleep disturbance should be included in acupuncture treatment protocols for FM patients. Randomized controlled studies are needed to confirm and elucidate the efficacy and mechanisms of acupuncture treatment in improving sleep disturbance, pain and quality of life in FM patients.

*This investigation was supported (in part) by the Public Health Services Research Grant M01-RR00040 from the National Institutes of Health and Wawa Fibromyalgia Gift Fund.

Sleep Disruption in Lung Cancer Survivors Compared to Non-cancer Controls

Dean G, Gooneratne N, Nkwuo JE, Rogers AE, Kaiser LR

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

Introduction: Sleep disruption is a common complaint amongst lung cancer survivors, yet little is known about this condition. We examined sleep parameters in long-term elderly lung cancer survivors relative to elderly noncancer controls.

Methods: Lung cancer survivors at least 5 years from diagnosis (N=76) and noncancer controls (N=48) participated. The Pittsburgh Sleep Quality Index (PSQI), demographic and additional questions were completed by telephone survey.

Results: Among the 124 participants, the mean age was 74 (SD=6.6; range=61-89), with 64% female, and 90% Caucasian. Mean lung cancer survival was 7.8 years (SD=1.6; range=5-11); 89% received surgery only. Lung cancer survivors (LCS) spent more hours in bed (8.7 vs. 7.7, $p<.006$), despite spending a similar amount of time asleep (6.5 vs. 6.8, $p=0.24$); they thus had a markedly lower sleep efficiency (77% vs. 89%, $p<.001$). Global PSQI was significantly higher in LCS (5.9 vs. 4.5, $p<.04$), consistent with poorer sleep quality in LCS. Sleep latency was similar (24 min vs. 22 min, $p=.71$). LCS reported using medication to help them sleep more often than NCC ($p<.001$). Dyspnea was significantly worse in LCS (2.6 vs. 1.3, $p<.03$) while pain was significantly worse in NCC (3.3 vs. 2.1, $p<.04$). While the amount of distress from sleep difficulties in LCS was higher than NCC (analog scale, 2.6 vs. 2.4, $p=.62$), it was not significantly different.

Conclusion: These results demonstrate robust differences in sleep parameters between LCS and NCC. While sleep efficiency showed prominent differences between groups, differences of a smaller magnitude were noted in overall perceptions of sleep distress. This suggests habituation towards poor sleep in LCS. Furthermore, the etiology of sleep impairment is different between groups; LCS had more sleep disruption from dyspnea suggesting lingering effects from cancer treatment.

Dorsomedial Pontine Injections of Bicuculline and Carbachol Elicit Similar REM Sleep-Like Effects in Urethane-Anesthetized Rats

Fenik VB, Kubin L

Department of Animal Biology and the Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA

In urethane-anesthetized rats, microinjections of the cholinergic agonist, carbachol, into a discrete region of the dorsomedial pons elicit REM sleep-like episodes that comprise cortical and hippocampal activation, silencing of pontine noradrenergic neurons and a profound suppression of hypoglossal (XII) nerve activity. Similar REM sleep-like effects can be produced by pontine carbachol in chronically instrumented-intact, decerebrate or anesthetized cats. However, carbachol is relatively ineffective in behaving rats; it does not elicit immediate effects, and often increases wakefulness, rather than REM sleep. In contrast, the $GABA_A$ receptor antagonist, bicuculline (BIC), effectively triggers REM sleep-like state in both behaving rats and cats. Our goal was to determine whether BIC and carbachol elicit similar REM sleep-like effects in urethane-anesthetized rats and whether they act at overlapping pontine sites.

In 12 urethane-anesthetized, paralyzed, vagotomized and artificially ventilated rats, we recorded the cortical EEG, hippocampal and XII nerve activity, and injected carbachol (10 nl, 10 mM) and subsequently BIC (10 nl, 0.5 mM or 2 mM) at 26 dorsomedial pontine sites.

Fifty three REM sleep-like responses were obtained. At most sites (18/26), carbachol and BIC could repeatedly elicit REM sleep-like episodes with similar magnitudes of suppression of XII nerve activity (to 18 % ± 3 (SE) and 25 % ± 3 of control, respectively), and patterns of cortical and hippocampal changes. The latencies were shorter for carbachol than BIC (85 s ± 18 vs. 295 s ± 53, n=18, $p<0.001$), with a proportional relationship between the two drugs for injections made at anterio-posterior levels from Bregma -8.3÷-8.7 (Paxinos & Watson, 1986).

Both carbachol and BIC can trigger REM sleep-like effects in urethane-anesthetized rats from at least partially overlapping pontine sites. Both drugs can act repeatedly, thus allowing for acute studies of the underlying cellular behaviors. The results demonstrate a similarity between pontine mechanisms of REM sleep in rats and cats.

Supported by:
HL47600.

[These results were presented at the 19th Annual Meeting of the Associated Professional Sleep Societies, Denver, June 18-23, 2005; *Sleep*, 28 (Suppl.): A9, 2005 (Abstr. 0026).]

The Genetics of Sleep and Starvation Tolerance in Drosophila Melanogaster

Harbison ST, Sehgal A

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

Many hypotheses have been proposed for the purpose of sleep—tissue restoration, thermal and energy regulation, immune system function, and memory consolidation. A wide body of evidence indicates that sleep is influenced by nutritional status, supporting the idea that sleep has a role in tissue restoration and/or energy regulation. We are exploring this hypothesis using P-element insertional mutagenesis in Drosophila melanogaster.

We measured sleep in male and female virgins from 162 homozygous P-element insertion lines previously shown to affect survival time under starvation conditions. These lines were derived from an isogenic Canton-S parental line and differ from the parental line only by the P-element insertion, enabling us to detect mutations affecting sleep without confounding genetic background effects. Lines were tested in blocks, and the mean insertional effect was calculated as a deviation from the contemporaneous parental line mean in each block. We computed the magnitude of mutational variance and estimated 99.9% confidence interval limits using an ANOVA model. Candidate insertions exceeding the 99.9% confidence interval limits were re-tested and verified using a three-way ANOVA that included genotype, experimental block, and sex effects.

Thirty-two P-element insertional mutations had significant effects on sleep. Eight insertions reduced sleep by as much as 4.75 hours as compared to the parental line. The remaining twenty-four lines exhibited more sleep than the control, increasing sleep times as much as 7.61 hours. Twenty of these lines exhibited sex-specific effects, although the same trend (increasing or decreasing) was seen in both sexes for each line.

Thirty-two of the P-element insertions previously shown to affect starvation resistance also had highly significant and sex-specific effects on sleep. Further characterization of these insertions will identify candidate genes involved in both traits and will lead to the elucidation of the molecular basis of the interaction between sleep and nutritional status.

Acknowledgements/funding: The authors would like to thank Trudy Mackay for providing the P-element insertion lines.

Supported by:
CSRN NIH training grant (T32 HL07713) to S. T. H.

Determination of Apnea-hypopnea Index Using Nasal Pressure versus Thermistor Recordings

Jones-Parker M, Staley B, Foster GD, Sanders M, Zammit G, Millman R, Newman A, Freeman J, Warmhold VL, Kuna ST, Sleep AHEAD Subgroup of the Look AHEAD Research Group

University of Pennsylvania, University of Pittsburgh, St. Lukes-Columbia University, Brown University

Introduction: During polysomnography (PSG), nasal pressure and oro-nasal thermistor signals are recorded, either singly or in combination, as surrogate markers of airflow. This study's purpose was to compare indices of sleep-disordered breathing scored using one or both of these airflow signals along with the other recorded signals.

Methods: Overnight home PSGs were performed on 60 adults participating in the Sleep AHEAD study, an ancillary study of Look AHEAD, a multicenter, randomized controlled trial of a weight loss intervention in obese adults with type 2 diabetes. The following signals were recorded (PS2, Compumedics): electroencephalogram (C3A2, C4A1), electrooculograms, pulse oximetry, chest and abdominal movement, nasal pressure, oro-nasal thermistor, and body position. Using standard guidelines, PSGs were separately scored by a registered technologist using: 1) oro-nasal thermistor only, 2) nasal pressure only, and 3) both signals. The technologist was blinded to the unexamined airflow signal on each scoring pass. On a separate set of 32 PSGs, the intraclass correlation coefficient assessing the technologist's intrascorer reliability for apnea-hypoponea index (AHI) was 0.89.

Results: The mean ± SD AHI of 31.8 ± 15.7 events/hr scoring with nasal pressure was greater than the AHI scoring with thermistor (27.6 ± 15.5) or with nasal pressure + thermistor (28.7 ± 15.6) ($p < 0.001$). More hypopneas were scored using the thermistor signal (140 ± 75.7) compared to the nasal pressure (105.7 ± 52.2) or nasal pressure + thermistor (106.7 ± 59.7) signals ($p < 0.05$). The number of apneas scored using nasal pressure (94.1 ± 90.3), thermistor (33.5 ± 56.2), and nasal pressure + thermistor (69.5 ± 77.9) were all significantly different from each other ($p < 0.001$).

Conclusion: When scoring a PSG using a nasal pressure and oro-nasal thermistor signal, either separately or in combination, AHI and number of apneas is greatest when scoring with nasal pressure alone.

Research supported by:
NIH HL 70301, DK57135, DK57002, DK57178, DK56992.

Intra-Subject Comparison of Polysomnography and a Type 3 Portable Monitor

Kuna ST, Seeger T, Brendel M

Center for Health Equity Research and Promotion (CHERP), Philadelphia VAMC;
University of Pennsylvania, Philadelphia, PA

Introduction: Type 3 portable monitors (PM) that record respiratory signals and oxygen saturation, but no signals for sleep staging, are increasingly being used to evaluate patients with suspected sleep apnea. The purpose of this study was to test the performance of a Type 3 PM that records nasal pressure as the surrogate marker for airflow.

Methods: 39 adult males with suspected sleep apnea (mean age 54 ± 9.6 SD yr, mean BMI 35.8 ± 7.0 kg/m^2) performed a home unattended PM recording (Stardust II®, Respironics) followed the next night by an in-lab diagnostic polysomnogram with simultaneous PM recording. The PM recorded the following signals: nasal pressure, rib cage movement, oxygen saturation, heart rate, body position, and snoring. All recordings were scored manually. The apnea-hypopnea index (AHI) on the PM recordings was calculated using recording time.

Results: The AHI was 40.6 ± 35.5 events/hr on in-lab polysomnogram, 36.4 ± 27.7 events/hr on simultaneous PM recording, and 32.1 ± 27.4 events/hr on home recording. Nine of the subjects had an AHI < 15 on the polysomnogram. The correlation coefficient for AHI was 0.92 when comparing polysomnogram with simultaneous PM recording, 0.75 comparing home recording versus polysomnogram, and 0.80 comparing the two PM recordings. Using an AHI ≥ 15 to diagnose OSA on polysomnograms and PM recordings, the PM had a sensitivity of 96.6% and a specificity of 100% on simultaneous in-lab testing, and a sensitivity of 86.7% and a specificity of 77.8% on home testing.

Conclusion: In patients with a high prevalence of sleep apnea, a Type 3 PM monitor using nasal pressure as the airflow signal can both detect and exclude diagnoses of sleep apnea. The greater differences observed between in-lab and home testing were likely due to differences in environment and the known night-to-night variability in AHI.

Research supported by:
CHERP, equipment provided by Respironics, Inc.

Positional Differences in Obstructive Sleep Apnea in Children and Adults

Lee JY, Brooks LJ

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

Rationale: Obstructive sleep apnea (OSA) in adults is generally worse when they sleep in the supine position. However, several studies have suggested that OSA in non-obese children may be worse when they sleep prone. We studied 257 otherwise normal adults and children to determine what factors contributed to positional differences in the severity of OSA.

Methods: 168 children <18 years old and 89 adults with OSA confirmed on polysomnogram were included. Patients, parents, and/or bed partners reported the position the patient's symptoms were worst: supine, prone, lateral, no positional difference, or do not know. The relationships between age, obesity, tonsil size, and severity of OSA with position were assessed by ANOVA and Kruskal-Wallace tests.

Results: The mean (SD) age of the children and adults were 5.17 (4.26) and 48.63 (14.02) years respectively. The adults were more obese than the children (mean body mass index (BMI) 29.85 (7.94) and 22.19 (9.91) respectively, $p<.001$) and had more severe OSA (mean Respiratory Disturbance Index (RDI) 23.54 (27.16) and 15.50 (23.94), $p<.001$). Of the adults who knew whether there was a positional difference in symptoms, 44/74 (59%) reported the supine position was worst, compared to 55/116 (47%) of the children (NS). There was no relationship between worst position and BMI or RDI of adults. However, the children who were worst in the supine position were older ($p=.01$) and more obese ($p<.01$) than those children who were worse in non-supine positions, or reported no positional difference. There was no relationship between worst position and RDI or tonsil size in the children.

Conclusions: Children with OSA who are older and more obese report their symptoms are worse in the supine position, as do adults. This suggests there may be more than one form of OSA, a "pediatric" type that is less obese and has less of a positional component, and an "adult" type associated with obesity and the supine posture.

Perifornical Hypothalamic Microinjections of Antisense Oligodeoxynucleotides Against β_3 Subunit of $GABA_A$ Receptor Reduce REM Sleep

Lu JW, Mann GL, Volgin DV, Ross RJ, Morrison AR, Kubin L

Department of Animal Biology, the Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, and Behavioral Health Service, Veterans Affairs Medical Center, Philadelphia, PA

We previously reported that microperfusion of the perifornical region of the posterior hypothalamus (PF) with the $GABA_A$ receptor antagonist, bicuculline, suppresses REM sleep. We also found that the expression of selected $GABA_A$ receptor subunits varies in PF with both circadian time and sleep need, suggesting that transcriptional changes involving these receptors play a role in the circadian and/or homeostatic regulation of sleep. The $GABA_A$ receptors are pentamers whose properties are determined by their subunit composition. To further investigate the role of transcriptional changes involving PF $GABA_A$ receptors in sleep regulation, we used antisense against the β_3 subunit. This subunit plays an important role in the assembly and intracellular transport of $GABA_A$ receptors; its expression is altered by sleep deprivation, and its mutations in humans are associated with insomnia.

Five adult, male Sprague-Dawley rats were instrumented for chronic recording of the cortical EEG, hippocampal activity and nuchal EMG, and had a microinjection cannula implanted in PF. Following adaptation to the recording conditions, at ~9:00 am, the rats received 0.5 μl microinjections of anti-β_3 subunit, 18-base oligodeoxynucleotide (*oligo*) (2 mM), or scrambled (SC) *oligo* (2 mM), or artificial cerebrospinal fluid (*csf*). The treatments were separated by at least 7 days, with sleep-wake behavior then monitored for 8-9 hrs.

Between 1:00 and 5:00 pm, treatment-related changes in the percentage of slow-wave sleep (SWS) were insignificant; across all subjects and conditions, SWS averaged 48.3% ±1.9 (SE), n=15. In contrast, the percentage of REM sleep was significantly reduced following the β_3 antisense *oligo* injections (10.6% ±1.5) compared to *csf* (18.9% ±1.9; n=5, $p<0.04$). This was mainly due to a decrease in the frequency of REM sleep episodes, from 7.3 hr^{-1} ±0.3 to 5.0 hr^{-1} ±0.8 ($p<0.03$). After SC *oligo*, REM sleep percentage was only slightly reduced, to 15.1% ±1.8.

These data suggest that transcriptional activity in PF that involves the β_3 subunit of the $GABA_A$ receptor contributes to the regulation of REM sleep.

Support:
HL071097.

[These results were presented at the 19th Annual Meeting of the Associated Professional Sleep Societies, Denver, June 18-23, 2005; *Sleep*, 28 (Suppl.): A10, 2005 (Abstr. 0028).]

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

Maislin G, Hachadoorian R, Pack F, Staley B, Dinges DF, Pack AI

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

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

Methods: Study 1 included MAP survey respondents among commercial drivers contained in a randomized list provided by local authorities. The cohort comprised 247 higher (MAP>0.44) and 159 lower risk drivers; with oversampling of higher risk drivers (weighted mean age = 45.4 y; BMI 29.9 kg/m^2). We measured apnea hypopnea index (AHI) in events/hour using in-laboratory polysomnography. The weighted prevalence of apnea (events/hr) was 17.6% (5-<15), 5.8% (15-<30), and 4.7% (>=30). Study 2 includes 301 elders (88 M, 213 F) with subjectively reported excessive daytime sleepiness (mean age = 70.6 y; BMI 29.4 kg/m^2) and with apnea prevalence of 30.2% (5-<15), 23.9%, (15-<30), and 25.9% (>=30). Receiver operating characteristic (ROC) curve analyses were performed for the MAP, BMI, and the apnea symptom-frequency index. The area under the ROC curve (AUC) was used to summarize predictive value. Results were compared to AUC's we reported previously in a sleep disorders clinic population for AHI>==10 (AUC MAP=0.786, BMI=0.734, Symptoms=0.695).

Results: For Study 1, AUC values for AHI>=10 were MAP=0.793, BMI=0.766, Symptoms=0.603. For Study 2, AUC values for AHI>=10 were MAP=0.723, BMI=0.632, Symptoms=0.606. Similar AUC values were found for other cutpoints (e.g., AHI>=5 (AHI>=30), AUC's were 0.781 (0.796) and 0.764 (0.743) for Studies 1 and 2, respectively).

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

Research supported by:
HL60287, HL60756, RR00040, and FHWA contract DTFH61-93-C-00088.

Sleep in Children with Williams Syndrome

Mason TBA, Arens R, Kaplan P, Bintliff BE, Walters AS, Pack AI

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

Introduction: Williams Syndrome (WS) is a human developmental disorder caused by a microdeletion of multiple genes in a defined region of chromosome 7 (7q11.23). Patients with WS have distinctive facies, and may manifest a variety of major phenotypic features, including neurocognitive, cardiovascular, and endocrine abnormalities. We wanted to determine the prevalence and degree of sleep disturbances in children with WS and to explore whether particular sleep features may be characteristic of WS.

Methods: Eligible subjects were males and females ages 2-18 years who met clinical criteria for WS, and who had haplo-insufficiency for the elastin gene as determined by fluorescent *in situ* hybridization (FISH) as a confirmatory test for WS. WS patients were recruited from the CHOP Multispecialty Center for Williams Syndrome. Healthy control subjects without sleep problems were also enrolled. All subjects underwent a test series that included overnight polysomnography as part of an ongoing study.

Results: 27 WS subjects and 14 control children have been studied. WS subjects had decreased sleep efficiency, decreased Stage 2 sleep and increased Stage 3 sleep, as percentages of total sleep time, compared to control children. No statistically significant differences were seen in the arousal/awakening index, apnea index, apnea/hypopnea index, or periodic limb movement index. Two of 27 WS children had a PLMI >5.

Conclusion: Based on the data collected thus far in this study, children with WS have altered sleep architecture with decreased lighter sleep (Stage 2 sleep), and increased deeper sleep (Stage 3 sleep). As these preliminary data may reflect underlying genetic influences on sleep, further study is continuing.

This research was supported by:
NIH grants K23 RR16566 and RR000240.

Effect of Chronic Sleep Restriction on Pre-frontal Cortex Functioning and its Relationship to IQ and Personality

McGlinchey EL, Banks S, Minkel JD, Dinges DF

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

Introduction: Acute total sleep deprivation has been reported to produce decrements in performance mediated by the pre-frontal cortex (PFC). The aim of this study was to investigate PFC tests of planning, verbal fluency and flexibility after chronic partial sleep restriction. The relationship of personality and IQ to these tests was also examined.

Methods: Preliminary analysis was conducted on the first 25 subjects (12m; 13f; aged 22-45y) out of N=45, who participated in a 5 night sleep restriction (4h TIB) protocol following 2 baseline nights (10h TIB). Subjects completed the Millon Index of Personality Styles (MIPS), an IQ task (North American Adult Reading Task; NAART) and half of a verbal fluency task (Controlled Oral Word Association Task; COWAT) at baseline. On the day after the fifth night of 4h TIB subjects completed the second part of the COWAT, Haylings Sentence Completion task (HSC) and the Tower of London test (TOL).

Results: Sleep restriction significantly reduced HSC ($p=0.005$) and TOL performance ($p=0.003$) when compared to normative values (Burgess and Shallice, 1997 and Culbertson and Zillmer, 2001, respectively). In addition, TOL initiation time scores were positively correlated with IQ ($r=0.405$, $p=0.022$) and TOL execution time scores were positively correlated with extraversion ($r=0.435$, $p=0.017$). The HSC task showed no relation to IQ or personality traits. COWAT scores showed no significant changes from baseline ($p=0.846$). However, there was a significant correlation between COWAT scores and IQ both at baseline and after sleep restriction ($r=0.63$, $p=0.003$).

Conclusions: Chronic sleep restriction at 4h per night adversely affected cognitive flexibility (HSC) and planning ability (TOL). However, sleep restriction did not affect all aspects of PFC functioning in the same manner. Moreover, both before and after sleep restriction, subjects with higher IQ had greater verbal functioning. After sleep restriction, subjects with higher IQ had better ability to plan than subjects with lower IQ.

Supported by:
NASA cooperative agreement NCC 9-58-159 with the National Space Biomedical Research Institute, NIH NR04281 and RR00040.

Sleep and Fatigue in Caregivers of Ventilator-dependent Children

Meltzer LJ, Mindell JA

The Children's Hospital of Philadelphia and St. Joseph's University, Philadelphia, PA

Introduction: Caring for a child with a chronic illness significant impacts psychosocial and daytime functioning of the caregiver. However, sleep has been understudied in pediatric caregivers. This study examined sleep patterns and daytime fatigue in caregivers of ventilator dependent children.

Methods: 70 mothers (29 vent caregivers, 41 healthy controls) completed a series of 24-hour sleep pattern recall interviews, the Pittsburgh Sleep Quality Index (PSQI), and the Iowa Fatigue Scale (IFS). Participants were 93% Caucasian, and the mean age was 38.2 years (range 23-49.4).

Results: Significant differences in sleep patterns were found, with caregivers of ventilator dependent children reporting a longer sleep onset latency, $F(1,54) = 5.0$, $p = 0.03$, earlier morning wake time, $F(1,54) = 6.5$, $p = 0.01$, and shorter overall total sleep time, $F(1,54) = 11.0$, $p = 0.002$. In addition, overall sleep quality was poorer for vent caregivers, $F(1,68) = 20.1$, $p < 0.001$. The frequency and reasons for night wakings also significantly differed, with vent caregivers reporting difficulty sleeping at least once a week due to caring for their child's health needs, $X^2(2) = 14.9$, $p = 0.001$, as well as stress related to their child's health, $X^2(2) = 20.3$, $p < 0.001$. There was no difference between the groups in terms of difficulty sleeping due to general stress, $X^2(2) = 1.2$, n.s. Finally, vent caregivers reported more daytime fatigue than caregivers of healthy children, $F(1,68) = 7.5$, $p = 0.008$.

Conclusions: This study demonstrates that caring for a ventilator dependent child results in disrupted sleep patterns and increased daytime fatigue. These sleep disruptions may account for some of the negative daytime functioning frequently reported in the caregiving literature (e.g., elevated depression and anxiety rates). Clinically, these data suggest that additional nighttime support may be needed for caregivers of ventilator dependent children. Future research should focus on the relationship between caregiving demands, sleep patterns, and daytime functioning, as well as include objective measures of sleep patterns.

This study was supported by the National Sleep Foundation's Pickwick Postdoctoral Fellowship in Sleep Medicine.

Validity of 24-Hour Recall Interview for Sleep Patterns

Meltzer LJ, Mindell JA

The Children's Hospital of Philadelphia and St. Joseph's University, Philadelphia, PA

Introduction: Due to the limitations of sleep questionnaires and sleep diaries (e.g., compliance issues, errors in estimation), this study examined the use of a 24-hour recall telephone interview to measure sleep patterns.

Methods: 134 participants were asked to complete a series of 24-hour recall interviews measuring sleep patterns (2 weekday and 2 weekend) over a two week period, the Pittsburgh Sleep Quality Index (PSQI) and a two-week sleep diary. Participants were 91% female and 96% Caucasian (mean age = 38.2 years; range 23-53); 69% were caring for a child with a chronic or serious illness, and 31% had a healthy child.

Results: The 24-hour recall interview was significantly correlated with the PSQI and diary for all sleep variables: bedtime (Recall/PSQI, $r = 0.68$, $p < .001$, Recall/Diary, $r = 0.82$, $p < .001$), wake time (Recall/PSQI, $r = 0.53$, $p < .001$, Recall/Diary, $r = 0.70$, $p < .001$), sleep onset latency (Recall/PSQI, $r = 0.53$, $p < .001$, Recall/Diary, $r = 0.80$, $p < .001$), total sleep time (Recall/PSQI, $r = 0.53$, $p < .001$, Recall/Diary, $r = 0.54$, $p < .001$), and sleep quality (Recall/Diary, $r = 0.75$, $p < .001$). Only 49% of participants completed and returned sleep diaries, while 82% completed all four 24-hour recall interviews. Completion rates for sleep diaries significantly differed based on child's health status (66% of healthy group completed, 41% of affected group completed), X^2 (1) = 7.1, $p = .008$, while no differences were found for recall completion rate.

Conclusions: This study supports the use of a 24-hour recall telephone interview as a subjective measure of sleep patterns. Validity is indicated by the strong correlations with well-established measures and higher completion rate. In addition, based on completion rates, the 24-hour recalls appear to be a more feasible measure of sleep patterns in pediatric populations. Additional research is needed to validate this novel approach, using objective measures of sleep.

Supported by:
the National Sleep Foundation's Pickwick Postdoctoral Fellowship in Sleep Medicine.

Relationships among Mood and Neurocognitive Tasks after Five Nights of Partial Sleep Deprivation

Minkel JD, Banks S, McGlinchey EL, Dinges DF

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

Introduction: Inadequate sleep is associated with negative mood states in healthy populations, but little is known about mechanisms by which sleep deprivation interacts with affect. Individual differences in both performance and subjective mood reports have been found to be stable and trait-like. This study was designed to identify correlates of individual differences in mood changes during sleep deprivation. Based on findings that the prefrontal cortex (PFC) is both implicated in emotion regulation and sensitive to sleep loss, this region was hypothesized to be a neurobiological correlate of mood changes during sleep deprivation. Demographic variables were also investigated as baseline predictors of mood disturbance during sleep deprivation.

Methods: Forty-four healthy adults were restricted to 4 h sleep for 5 consecutive nights in a controlled laboratory setting. Performance was measured across days on 4 tests of PFC function and 1 test of sustained attention. Mood was assessed by self report every 2 h. Age and gender were collected by self-report, and IQ was estimated by a reading test.

Results: Sleep restriction was associated with significant elevation in measures of mood related to energy level and confusion ($p<0.05$), but not to anger, depression, or anxiety. Subjects showed impaired performance on a PFC-mediated task of verbal inhibition ($p<0.05$), but performed normally on PFC-mediated tasks of word fluency, strategic planning, and spatial pattern recognition. None of the PFC-mediated tasks were correlated with individual differences in mood changes. Individual differences in sustained attention however, were significantly correlated with overall mood changes ($r=0.38$, $p<0.05$). Age and gender were not found to significantly predict mood disturbance. Higher IQ scores predicted greater negative mood changes ($r=0.41$, $p<0.05$). Sustained attention and IQ accounted for 24% of the variance in mood disturbance.

Discussion: Mood was only elevated on factors related to energy level and confusion and was significantly correlated with performance on a task of sustained attention, suggesting that subjective mood states gave subjects valid feedback on their level of neurobehavioral impairment. The mood changes observed do not appear to be mediated by differences in PFC function.

A Role for BiP/GRP78 in Recovery Sleep of Drosophila

Naidoo N, Cearley C, Zimmerman J, Pack AI

The Center for Sleep & Respiratory Neurobiology,
University of Pennsylvania, Philadelphia, PA

Introduction: The expression of the heat shock / stress response gene, immunoglobulin binding protein (BiP)/glucose regulated protein 78 (GRP78) has been shown, both in mice and Drosophila, to be increased in the brain following sleep deprivation (SD). We have examined the expression of BiP protein in Drosophila heads following SD and recovery sleep in the clock mutant Cyc^{o} and in Canton-S. In addition, we have examined the effect of alterations in BiP protein levels on sleep recovery in Drosophila.

Methods: The HS Gal4-UAS system was used to generate the BiP over-expressor, (HS-Gal4-WTBiP) and mutant BiP line expressing dominant negative BiP (HS-Gal4-D231S). Flies were maintained at 25°C and 40% humidity in a 12:12 light:dark cycle. Flies were sleep deprived between ZT 16 - ZT 22, using random stimuli mechanically applied throughout the observation period. Time matched controls were also used. For behavioral studies flies were allowed to recover for 2-3 days following SD. Flies were also sacrificed at the end of the deprivation or rest period for protein analysis.

Results: BiP protein expression is increased following SD and returns to normal baseline levels following recovery sleep in CS and Cyc^{o} males. Flies over expressing BiP displayed an increase in recovery sleep over baseline, while flies expressing the dominant negative BiP displayed reduced recovery sleep.

Conclusion: Sleep deprivation elicits the unfolded protein response (UPR) in Drosophila resulting in the increased expression of the molecular chaperone BiP. Recovery sleep restores BiP expression to normal levels. Our data suggest that BiP expression levels and the UPR have an effect on the amount of recovery sleep.

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

Nikonova EV, Frank BC, Mackiewicz M, Quackenbush J, Zhang L, Galante RJ, Naidoo J, Pack AI

The Center for Sleep & Respiratory Neurobiology,
University of Pennsylvania, Philadelphia, PA

Energy homeostasis has been proposed to play a role in sleep/wake regulation. Oxidative phosphorylation (OXPHOS) system located in mitochondria comprises a chain of highly controlled multi-subunit enzyme complexes, whose transcripts originate from both nuclear and mitochondrial genomes. Previously it has been shown that the mRNA for the catalytic subunit of complex IV (cytochrome c oxidase, COX) is up regulated after 3 hrs of sleep deprivation. We used quantitative real-time PCR to assess changes in expression level of multiple genes involved in ATP production. We also examined changes in protein expression for key components of the electron transport chain as well as in COX enzyme activity.

We used C57BL/6J 2-month-old male mice that were sleep-deprived for 3, 6, 9, and 12 hrs after lights on (7AM) by gentle handling. Matching control animals, left undisturbed to sleep, were sacrificed at the same diurnal time. qRT-PCR experiments were conducted on 384-well plates using ABI 7900 technology. Additionally, fresh-tissue mitochondria were used in Western blotting and biochemical assessment of COX enzyme activity.

We demonstrated that after 3 hrs sleep deprivation (3SD) both mitochondrial and nuclear transcripts of key components of complexes in the electron transport chain were up regulated. At the protein level, there were increases after 3 hrs of sleep deprivation in key subunits of complex IV. The key subunit of ATP synthase was increased only after 12SD. Activity of the COX enzyme was significantly higher after 3SD as compared to 3 hrs spontaneous sleep but tended to decline when sleep deprivation was further prolonged.

Our results obtained for mRNA, protein and enzyme activity levels suggest that there is an increase in overall ATP production at least at the beginning of prolonged wakefulness.

Supported by:
NIH grants HL60287 and AG17628.

Circulating Norepinephrine Levels in Response to Severe Sleep Deprivation, Caffeine, and Modafinil

Niyogi S, Price NJ, Rogers NL, Van Dongen HPA, Dinges DF

Division of Sleep & Chronobiology, Department of Psychiatry, and the Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA, USA;
Woolcock Institute of Medical Research, University of Sydney, Sydney, NSW, Australia

Introduction: A series of experiments were performed to determine the effects of caffeine and modafinil on circulating norepinephrine (NE) levels in healthy adults undergoing severe sleep deprivation (SD).

Methods: Four randomized, double-blind, placebo-controlled trials involving 88h SD were completed. N=85 subjects spent 10 days in the laboratory. In two trials, subjects were randomized to either sustained low-dose caffeine (0.3mg/kg/h) or placebo. One trial involved 0h sleep, and the other two 2h naps per 24h (TIB: 0245-0445h; 1445-1645h) across the 88h. In another two trials, subjects were randomized to either modafinil (200mg or 400mg per 24h) or placebo. One trial involved 0h sleep, and the other one 2h nap per 24h (TIB: 0245-0445h) across the 88h. Subjects completed a 30-min computerized neurobehavioral battery every 2h of wakefulness. Plasma samples were taken every 6h via an indwelling venous catheter, and assayed for NE (blind to condition) using RIA. Linear mixed-effects ANOVA was used to evaluate the effects of 0, 1, or 2 naps per 24h; time; day of SD; and drug condition, on NE.

Results: Significant main effects were found for SD day ($p=0.001$), time of day ($p<0.001$), and drug condition ($p=0.044$). An interaction of drug condition by nap by time of day ($p=0.004$) revealed that caffeine during 88h SD without naps produced significantly higher plasma NE, especially during the diurnal period. Naps attenuated this effect in subjects who received caffeine. Modafinil appeared to have no effect on NE.

Conclusion: Sustained low-dose caffeine during SD resulted in a marked increase in plasma NE to levels well beyond those observed for modafinil or placebo. The effect of 2h naps in attenuating the NE increase due to caffeine suggests that homeostatic pressure for sleep contributed to the elevated NE levels; and the confinement of the effects to diurnal periods suggests that the circadian system also influenced NE release.

Support by:
AFOSR grants F49620-95-1-0388, F49620-00-1-0266, NIH grant RR00040, & Cephalon Inc.

Inflammatory Gene Expression in Macrophages Exposed to Intermittent Hypoxia

Otto CM, Gaspard RM, Robinson MA, Fox J, Baumgardner JE, Quackenbush J, Pack AI

University of Pennsylvania, Philadelphia, PA; The Institute for Genomic Research, Rockville, MD

Introduction: Severe obstructive sleep apnea (OSA) is associated with increased risk of cardiovascular disease (i.e. atherosclerosis, stroke, hypertension) independent of obesity. Repetitive nocturnal cycles of intermittent hypoxia (IH) characteristic of severe OSA may represent a mechanism for cardiovascular pathology. We have previously reported microarray results of IH induced-expression of several genes involved in the development and progression of cardiovascular disease. To further expand these studies we compared expression of 23 select genes associated with atherosclerosis, inflammation and the unfolded protein response in macrophages exposed to IH, normoxia and sustained hypoxia.

Methods: RAW 264.7 macrophages were cultured in a specially designed apparatus that allowed for 1) rapid switching between 2 precisely controlled culture oxygen tensions, and 2) collection and freezing of cells without exposure to ambient O_2. Cultures (n=18) were randomly assigned to one of 3 conditions sustained normoxia (40 Torr), sustained hypoxia (8 Torr O_2) or IH (90 sec of 40 Torr and 30 sec of 8 Torr). After 4 hrs, cells were frozen at −70C. RNA purification was followed by quantitative RT-PCR. Serial dilutions of each sample were run with 18S primers and competimers allowing normalization to 18S. All samples were run in duplicate.

Results: Preliminary results demonstrate a significant increase in the expression of genes for the inflammatory mediators, inducible nitric oxide synthase and tumor necrosis factor, in macrophages exposed to IH compared to normoxia.

Conclusions: Cultured macrophages exposed to brief periods of small cyclical changes in PO_2 showed upregulation of inflammatory mediators linked to oxidative stress and associated with cardiovascular disease. These results suggest a possible mechanism linking cycling PO_2 with cardiovascular disease in OSA.

Effects of Sleep Restriction and Recovery Sleep on Driving Simulator Test (AusEd) Performance

Razavi F, Banks S, Dinges DF

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

Introduction: It is known that sleep loss has a detrimental effect on driving performance. The aim of the current study was to measure the sensitivity of driving simulator performance to a night of varying sleep dose following a week of chronic sleep restriction.

Methods: Preliminary analyses were conducted on 10 subjects (age 30 +/- 7.5, 5 females) out of 20 subjects, in a controlled laboratory. The study consisted of 2 nights of baseline sleep (10h TIB) and 5 nights of chronic sleep restriction (4h TIB), followed by randomization to a sleep dose of either 8h TIB or 0h TIB. The 15-minute AusEd was administered every evening between 1830h-2000h and was set to simulate a monotonous rural road at night. Driving performance measures included reaction time (to appearance of trucks on the road ahead), steering deviation from median lane position, and speed deviation beyond the range of 60km/h-80km/h.

Results: On the fifth day of chronic sleep restriction**,** none of the driving simulator parameters differed significantly from baseline (steering deviation, P=0.4; speed deviation, P=0.3; and reaction time, P=0.2) due to subjects continuing to learn the task. After the 0h TIB condition, there was an increase in steering deviation (P= 0.05), and trends toward greater speed deviation (P=0.06) and longer reaction times (P=0.09), compared to the 8h TIB sleep dose condition.

Conclusions: Results from these initial analyses on 10 subjects suggest that following a week of sleep restriction, a night of total sleep deprivation causes a decrease in driving simulator performance relative to an 8h recovery sleep period. Data from additional subjects will resolve the extent to which simulated driving is affected by varying dosages of recovery sleep.

Support:
NASA cooperative agreement NCC 9-58-159 with the National Space Biomedical Research Institute, and NIH grant RR00040.

Intimacy and Sexuality in Obstructive Sleep Apnea: The Effect of Treatment

Reishtein JL, Maislin G, Dinges DF, Pack AI, Weaver TE, Multisite Group (Mahowald M, Kader G, Bloxham T, George C, Greenberg H, Younger J.)

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

Background: Previous reports have indicated that obstructive sleep apnea (OSA) affects interpersonal relationships, and may affect sexuality. However, little is known about alterations in intimacy and sexuality (IS) or improvements following CPAP treatment.

Methods: 156 OSA patients (AHI $\geq$20/hour, age 46.5$\pm$9.5 years, 84% male) were compared with 36 normals (AHI<5, age 42.7$\pm$7.6 years, 64% male). Patients were divided into 3 groups by OSA severity (AHI = 20 to 39; 40 to 59; and AHI = $\geq$ 60). The Intimacy and Sexuality (I and S) subscale of the Functional Outcomes of Sleep Questionnaire was used as the dependent measure. Self-reported sleepiness was measured by the Epworth Sleepiness Scale and objective sleepiness by the Multiple Sleep Latency Test (MSLT). Following initial polysomnography and after 3 mo treatment, subjects again completed the FOSQ, the ESS, and MSLT.

Results: At baseline, OSA patients had greater difficulty when compared to normals on overall IS, and specifically desire, arousal, and orgasm (p <.05). IS correlated significantly with subjective sleepiness (r = -.42, p<.0001). There were no significant differences in IS between severity groups. Participants used CPAP a mean of 4.94 $\pm$ 2.06 hours a night. CPAP mean nightly use for 20-39 group was 4.92 $\pm$ 1.92 hours (range 1.12 - 7.96 hours); 40 to 59 Group was 4.32 $\pm$ 2.24 hours (range, 0 - 7.52 hours); and $\geq$ 60 Group was 5.15 $\pm$ 2.04 hours, with no significant differences among groups. At this time, IS was again significantly related to subjective sleepiness (r = -0.47, p < 0.0001), but not to objective sleepiness. All patient groups improved significantly on overall IS (effect size 0.38-0.93, p<.05), and groups with higher AHI showed larger effect sizes than the groups with lower AHI.

Conclusions: All areas of intimacy and sexuality are adversely affected in OSA. The widespread nature of these impairments points out the importance of probing this critical area during patient assessment. CPAP treatment improves intimacy and sexuality behaviors in people with OSA, with the greatest gains in those with the most severe disease.

Nitric Oxide Production by Activated Hypoxic Macrophages

Robinson MA, Baumgardner JE, Fox J, Otto CM

Biomedical Graduate Studies/Pharmacological Sciences,
University of Pennsylvania, Philadelphia, PA

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

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

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

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

The Differential Rapid Eye Movement Sleep Response to Cued and Contextual Fear Conditioning is Suppressed by A Shared Response to Fearful Conditioned Stimuli

Pawlyk AC, Ross RJ, Jha SK, Brennan FX, Morrison AR

Laboratory for the Study of the Brain in Sleep; Veterinary Medicine, the Center for Sleep and Respiratory Neurobiology University of Pennsylvania and Psychiatry, Philadelphia VA Medical Center, Philadelphia, PA

Introduction: Rapid eye movement sleep (REMS) alterations are commonly observed in studies of the effects of stress and anxiety on sleep. REMS can be divided into two types: sequential (seqREMS), separated from adjacent REMS by less than 3 minutes of intervening wake or non-REMS, and single (sinREMS), separated from adjacent REMS by 3 or more minutes.

Methods: Rats were implanted for recording EEG and EMG. Following recovery, habituation, and baseline polysomnograms, separate groups underwent auditory cued fear conditioning (CFC), consisting of five footshocks either explicitly paired or unpaired with a tone, and contextual fear conditioning (CtxFC), consisting of five footshocks presented in the absence of explicit cues. For both CFC and CtxFC, subgroups were studied in either the presence or absence of fearful conditioned stimuli (CS).

Results: In the absence of CS, REMS recorded on the day following training for the CtxFC group was increased due to an increase in sinREMS (4.7±0.7 to 6.4±0.8 min/hr, $p<0.01$), while REMS was increased in the CFC group due to an increase in time spent in seqREMS (3.8±0.7 to 7.5±1.9 min/hr, $p<0.05$). Time spent in seqREMS for the CtxFC group was unchanged (4.2±0.8 to 4.8±0.6 min/hr, NS), and sinREMS time was not changed for the CFC group (4.9±0.7 to 3.8±0.8 min/hr, NS). In the presence of CS, sinREMS for both groups was significantly decreased (CtxFC: 4.7±0.7 to 2.2±0.4 min/hr, $p<0.05$; CFC: 4.9±0.4 to 3.4±0.5 min/hr, $p<0.05$). Both groups also displayed non-significant decreases in seqREMS (CtxFC: 2.7±0.7 to 1.2±0.4 min/hr, NS; CFC: 3.2±0.5 to 2.1±0.5 min/hr, NS).

Conclusions: We suggest that the different ways in which REMS was upregulated with these two modes of fear conditioning may relate to the different neural mechanisms believed to underlie CFC and CtxFC. Furthermore, we suggest that the similar REMS responses in the presence of conditioned fearful stimuli represent a common fear response.

Supported by:
MH42903, HL07953, and VA Res.

Effect of Cognitive Workload on Neurobehavioral Deficits during Total Sleep Deprivation

Stakofsky AB, Levin AL, Vitellaro KM, Dinges DF, Van Dongen HPA

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

Introduction: The combined effects of cognitive workload and sleep loss have not been systematically studied. This project aimed to compare moderate vs. high levels of cognitive workload, operationalized as a two-fold difference in task duration, during 36h of total sleep deprivation.

Method: 21 healthy subjects (age 28.5±5.5; 11 females) experienced three 36h periods of total sleep deprivation in the laboratory, each separated by two recovery days. Every 2h during the 36h sleep deprivation periods, subjects completed a neurobehavioral test battery. They underwent moderate workload (0.5h test battery) during two of the sleep deprivations and high workload (1.0h test battery) during one deprivation, in random counterbalanced order. For every neurobehavioral variable, outcomes were averaged across the last 24h of sleep deprivation so as to yield a single subject-specific measure of overall impairment for each sleep deprivation. To evaluate the effects of sleep loss on comparable work periods, only responses during the first half of each task in the high workload (i.e., double duration) test bout were analyzed.

Results: The moderate and high workload conditions were compared using a mixed-model ANOVA controlling for the order of conditions. A significant effect of workload was observed on the following tasks: Karolinska Sleepiness Scale, Serial Addition Subtraction Task, Digit Symbol Substitution Task, Word Detection Task, Psychomotor Vigilance Task, VAS for sleepiness, and Effort scale ($F[1,39]>5.90$, $P<0.020$), with increased neurobehavioral impairment during the high workload condition.

Conclusion: These results suggest that higher cognitive workload, operationalized as increased task duration, negatively affects neurobehavioral performance during sleep deprivation, even when controlling for task duration. This effect of cognitive workload has not been recognized in theories of sleep. Moreover, it requires consideration of cognitive workload when developing mathematical models of sleep-wake regulation.

Support:
NIH grants HL70154 and RR00040.

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

Tkacs N, Pan Y, Sawhney G, Mann GL, Morrison AR

School of Nursing, College of Arts and Sciences and School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Research in normal humans shows that nocturnal hypoglycemia increases arousal, decreases sleep efficiency and reduces time in stage 3 and 4 sleep. The purpose of this study was to evaluate hypoglycemia as an arousal stimulus in 8 male Sprague-Dawley rats prepared with EEG and EMG electrodes for recordings of sleep and waking. Recordings were made from 1 PM to 3 PM immediately after subcutaneous saline injection (baseline day) or after insulin 3 U/kg to reduce plasma glucose from 116 mg/dl to 40 mg/dl over the 120 minute recording period. In the second hour after insulin treatment, rats had increased time spent in waking from 23.6% ± 3.5 (SE) to 54.4% ± 4 ($p=.013$). REM sleep was reduced from 22.2% ± 1.4 to 2.0% ± 1.1 ($p<.0001$). SWS showed a modest but not significant decrease from 53.8% ± 4.1 to 42.0% ± 0.7 during hypoglycemia. Additional rats without EEG and EMG surgery were used to assess the effect of afternoon insulin treatment on Fos staining in brain arousal-related neurons. Insulin treatment (n=4) increased Fos-immunoreactivity in neurons counted at 3 levels of the locus coeruleus (LC) to 75 ± 17 while controls (n=2) had an average of 1 Fos-ir cell in LC. Cells double-labeled with Fos and choline acetyltransferase were observed in basal forebrain cholinergic neurons after insulin injection. In the magnocellular preoptic nucleus, these cells totaled 30.4 ± 6.3 in insulin-treated rats (n=7) vs. 5.5 ± 2.8 in controls (n=6). These results provide evidence of hypoglycemic arousal from sleep in a rodent model, and demonstrate that mild hypoglycemia is associated with activation of noradrenergic and cholinergic neuron groups implicated in arousal.

Are Insomniacs Less Accurate at Reporting Sleep Times?

Tucker A, Gehrman P

University of the Sciences/Health Psychology, University of Pennsylvania, Philadelphia, PA

Background: One puzzle of insomnia is that many patients with insomnia overestimate how much sleep they are losing before treatment and overestimate how much sleep they are gaining after treatment. Most of the studies of treatment efficacy do not include a group of people who do not complain of insomnia. Thus, it is not clear if people with primary insomnia, as a group, are less accurate in their perceptions of sleep duration than normal controls.

Objective: To determine whether primary insomniacs are less accurate than normal controls in estimating 1) sleep latency (SOL) and 2) total sleep time (TST). **Literature search:** Review of articles published in OVIDMedline, PsychINFO, and BiblioSleep. **Participants:** 18 studies from 1976 to 2005 that measured both subjective and objective sleep parameters in minutes in a total of 355 normal controls and a total of 533 individuals with primary insomnia.

Results: Insomniacs overestimated sleep latency by an average of 25.97 minutes with a mean effect size of .88 while normal controls overestimated sleep latency by an average of 5.97 minutes with a mean effect size of .41. Insomniacs underestimated TST by an average of 12.99 minutes with an effect size of -.58 while normal controls were fairly accurate, overestimating total sleep time by an average of only 3.05 minutes with a mean effect size of .16. In a series of regressions, neither age nor duration of insomnia was a significant predictor of the difference between subjective and objective measures of SOL or TST in either insomniacs or normal controls (p values from .8 to .4).

Conclusions: Primary insomniacs are less accurate than normal controls in estimating sleep latency and total sleep time although even healthy sleepers tended to overestimate SOL. Discrepancies in estimates of sleep do not appear to be mediated by either age or duration of insomnia.

Intermittent Hypoxia Alters Hypothalamic Transcription and Increases Pancreatic Insulin-1 Precursor mRNA Expression in Rats

Volgin DV, Fenik V, Swan JL, Kubin L

Department of Animal Biology, School of Veterinary Medicine and the Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA

Rationale: The obstructive sleep apnea syndrome (OSAS) commonly coexists with the metabolic syndrome (hypertension, obesity, diabetes). Clinical association studies show that OSAS is an independent risk factor for insulin resistance (reviewed in: Punjabi et al., *Respir Physiol Neurobiol* 136: 167-178, 2003), but the mechanisms of this association are unknown.

Goal: To assess the effect of chronic intermittent hypoxia (IH), a major pathogenic condition of OSAS, on central (hypothalamus) and peripheral (pancreas) transcriptional activity relevant for metabolic regulation.

Methods: Adult Sprague-Dawley rats were subjected to IH for 7 or 35 days (O_2 seen by the animals: 5.5-10% for 70 s followed by 18.9-25% for 80 s, 7:00 am-5:00 pm daily); sham-treated rats were subjected to identical flows of room air. Following the exposure period, the animals were sacrificed and total RNA extracted from the pancreas and tissue micropunches from distinct posterior hypothalamic regions. Selected mRNAs were quantified using RT-PCR. The results are expressed as the number of cDNA copies per ng of total RNA ±SE.

Results: Among the central changes, non-sustained (present only after 7 days) increases occurred for the α_{2A} adrenergic receptor (AR) mRNA in the ventromedial, and for STAT-1 in the dorsomedial, hypothalamus (n=3-4; $p<0.03$ for both). A sustained two-fold increase occurred for the β3 subunit of $GABA_A$ receptor mRNA in the perifornical (PF) hypothalamus (after 7 days: 15,500±9,000 in sham-treated vs. 33,100±1,000 in IH rats; n=3-4; $p<0.01$; after 35 days: 21,100±8,100 vs. 52,300±9,000; n=6; $p<0.03$). In the same region, the α_{2A} AR mRNA level increased after 35 days from 550±95 in sham-treated to 940±121 in IH rats (n=5-6, $p<0.03$). Pancreatic insulin 1 (Ins 1) mRNA expression was also higher in IH than sham-treated rats (after 35 days: 44±13 in sham-treated and 212±59 in IH rats; n=6-8; $p<0.001$).

Conclusions: Chronic IH alters mRNA levels for two inhibitory receptors in the PF hypothalamus. This may lead to increased inhibition of hypothalamic outputs controlling metabolism and/or vigilance. Chronic IH also induces an increase in pancreatic insulin transcription. Since rats subjected to the same IH protocol have a reduced glucose-stimulated insulin release (Fenik et al., *Proc Am Thor Soc*, 2: A232, 2005), IH may activate insulin production but impair its exocytosis.

Supported by:
HL074385.
[These results were presented at the International Conference of the American Thoracic Society, San Diego, CA, May 20-25, 2005; Proc Am Thor Soc, 2: A233, 2005 (Abstract 2699).]

Behavioral, Molecular and Biochemical Characterization of Rab3A Mutations that Cause Abnormal Circadian and Sleep Behavior in the Mouse

Yang S, Farias M, Kapfhamer D, Grant G, Abel T, Bucan M

Department of Genetics, Biology and Penn Center for Bioinformatics,
University of Pennsylvania, Philadelphia, PA;
Ernest Gallo Clinic and Research Center, Emeryville, CA

Rab3A is a neuronal GTP-binding protein that binds synaptic vesicles and regulates synaptic transmission. A mouse mutant, *earlybird*, with a point mutation in the GTP-binding domain of Rab3A (D77G), exhibits anomalies in circadian behavior and homeostatic response to sleep loss (Kapfhamer et al. Nat. Gen. 2002). The D77G substitution in the *earlybird* allele causes reduced GTP and GDP binding, while GTPase activity remains intact. Expression profiling of the cortex and hippocampus of *earlybird* (*Ebd/Ebd*) and null mice (*Rab3A*$^{-/-}$) revealed subtle differences between wild-type and mutant mice, with no common differentially expressed genes. Although mice used for transcriptional profiling were backcrossed for 4 generations to a C57BL/6J background, the most robust changes at the transcriptional level between *Rab3A*$^{-/-}$ and *Rab3A*$^{+/+}$ mice were represented by genes from the 129/Sv-derived chromosomal region surrounding the *Rab3a* gene. Similar to the results of circadian behavior, the *Ebd/Ebd* mice showed a stronger mutant phenotype than the null mice in some behavioral tests; *Ebd/Ebd* have reduced anxiety-like behavior in the elevated zero-maze test, reduced depression-like behavior in the forced swim test, and a deficit in cued fear conditioning, whereas *Rab3A*$^{-/-}$ showed only a deficit in cued fear conditioning.

Acknowledgment: We thank Don Baldwin, John Tobias and Warren Ewens for help with microarray experiments and analysis, Margaret Chou and Jeff Field for advice on GTP/GDT binding experiments and Liz Gurney for help with behavioral analysis, Adam Crystal for providing HEK293 cells, Jessica Ardis for critical reading of the manuscript.

Supported by:
NIH grant (R01 MH604687), and in part, under a grant with the Pennsylvania Department of Health.

Changes In Gene Expression With Sleep and Wakefulness In Drosophila Brain

Zimmerman JE, Mackiewicz M, Rizzo W, Shockley K, Churchill GA, Pack AI.

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

Introduction
Rest in *Drosophila* meets the criteria established for sleep. Many genetic pathways are conserved between flies and mammals. Therefore, the determination of transcriptional regulation in the brain of *Drosophila* could elucidate basic molecular mechanisms of sleep. The aim of this study is to identify wake and sleep dependent gene regulation in the brain of *Drosophila melanogaster*.

Methods
RNA: Total RNA was isolated from pooled brains of 5-day-old adult CS females.

Sleep deprivation**:** Flies were sacrificed at ZT14 for the 0 hour control group and after sleep deprivation for 2, 4 or 6 hours (SD). Time matched controls were also sacrificed (CON).

Mechanical stimulation control**:** Flies were sacrificed at ZT10 for the 0 hour active period control group and after stimulation for 4 hours during a normal active period (MS). Time matched controls were also sacrificed.

Microarray**:** Expression data was determined using the Affymetrix Drosophila Genome Arrays.

Statistics**:** Intensity data was normalized using the robust multi-array average method. Differential expression between experimental groups was determined using the MAANOVA procedure and temporal gene expression was addressed through a linear multiple regression analysis (MR).

Results
252 genes were identified as significant between SD and CON by MAANOVA. MR analysis separated the 252 genes into 9 classes of expression patterns. These 9 classes distinguished 19 genes whose regulation is sleep dependent, 186 dependent on extended wakefulness and 2 circadian genes whose expression is modified by sleep deprivation. 33 of the genes identified by MAANOVA in the sleep deprivation experiment were also differentially expressed in MS and most are part of the immune response of *Drosophila*.

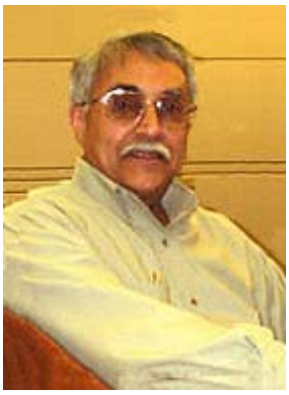
Conclusions
We have identified genes in the Drosophila *brain whose expression is dependent on the sleep state and others induced by stimulation independent of sleep state.*

Welcome to the Second Annual Research Retreat of the Center for Sleep and Respiratory Neurobiology.

We are looking forward to a full day of scientific presentations, discussions, as well as to meeting friends who share a common passion to unravel the mysteries of sleep and understand its meaning for the human condition. As last year, the program includes two symposia, a keynote lecture, and a poster session that will give us a glimpse into the breadth and diversity of sleep research at Penn.

Unique this year are special events to celebrate the contributions to sleep research of Dr. Adrian R. Morrison, a Professor of Anatomy in the School of Veterinary Medicine, who, together with Drs. Dinges and Pack, has laid the foundation for the establishment in 1991 of the Penn's Center for Sleep and Respiratory Neurobiology. On this occasion, the afternoon part of the Retreat is jointly organized by the Center and the Department of Animal Biology of the School of Veterinary Medicine. Also on this occasion, we are extremely pleased to present our keynote speaker, Dr. Giulio Tononi, whose roots go to Pisa, Italy, a place that influenced Dr. Morrison early in his career. Following the keynote lecture, we will hear from three speakers who were introduced to sleep research by Dr. Morrison and then established their own research programs in this field. We will conclude the day with a reception for Dr. Morrison, at which his friends, students and collaborators will have the opportunity to reflect on the man whom we also know as armsleep@vet.upenn.edu.

The Organizing Committee thanks the speakers, poster presenters and, importantly, Maureen Helwig, who spared no effort to put together the program of this special day.



Narayan Avadhani

Joan C. Hendricks

Leszek Kubin

Allan I. Pack

University of Pennsylvania

Sponsored by:
Center for Sleep & Respiratory Neurobiology, School of Medicine and the Department of Animal Biology, School of Veterinary Medicine