Center for Sleep & Respiratory Neurobiology

4th Annual Research Retreat

June 22, 2007

Levy Conference Center at the
University of Pennsylvania Law School

University of Pennsylvania

Program and Abstracts

Center for Sleep and Respiratory Neurobiology
Research Retreat Program

8:00-8:50 **Poster Mounting and Breakfast**

8:50-9:00 **Opening Remarks - Allan Pack, M.D., Ph.D.**

9:00-10:15 **Sleep Regulation**

Deepa Avinash
"Slow Wave Activity Dynamics during Consecutive Weeks of Sleep Restriction to 4 Hours per Day"

Miroslaw Mackiewicz
"An In-Silico Analysis of Genes in the QTL for Sleep Homeostasis in Mice"

Nirinjini Naidoo
"Loss of Functional Homer Leads to Sleep Fragmentation in Drosophila"

Mark Wu
"Identification and Characterization of Sleepless, a Novel Gene Critical for Sleep in Drosophila"

Michel Halassa
"Astrocytes Regulate Sleep Homeostasis"

10:15-10:30 **Coffee Break**

10:30-12:00 **Sleep Disorders: Clinical Observations to Basic Mechanisms**

Norma G. Cuellar
"Discovering the Impact of RLS in Type 2 Diabetes"

Subhajit Chakravorty
"The Relationship Between Sleep Disturbances and Alcohol Craving"

Jingtao Huang
"Respiratory Sensation during Sleep in Children with the Obstructive Sleep Apnea Syndrome"

Sigrid Veasey
"Activation of 153GADD/CHOP in Upper Airway Motoneurons in a Murine Model of Sleep Apnea"

Richard Ross
"Long-term Effects of Cued Fear Conditioning on REM Sleep Microachitecture and Phasic Activity in Rats"

Irma Rukhadze
"Fos Expression in Pontine Noradrenergic Neurons Negatively Correlates with the Duration of Carbachol-Induced REM Sleep-like State in Urethane-anesthetized Rats"

12:00-2:00 **Lunch and Poster Viewing**

2:00-3:00 **Sleep Restriction and Sleep Purpose: Observations and Consequences**

Christina J. Calamaro
"The Relationship Between Short Sleep Duration and Obesity in Adolescents"

Siobhan Banks
"Response to Sleep Restriction Depends Upon Pre-Existing Sleep Debt"

Namni Goel
"Phenotyping Neurobehavioral and Cognitive Responses to Partial Sleep Deprivation"

Sara J. Aton
"Cortical Plasticity"

3:00-3:15 **Coffee Break**

3:15-4:00 **Sleep, Drugs, and the Real World**

Les A. Gellis
"Sleep Hygiene Practices of Good and Poor Sleepers in a Nationwide Internet-Based Sample"

Melisa Moore
"The Relationships Between Sleep Time, Sleepiness, and Psychological Functioning in Adolescents"

Julie Seibt
"Hypnotic Effects on V1 Plasticity"

4:00-4:30 **Poster Viewing**

4:30-5:30 **Keynote Lecture**

H. Craig Heller
"The Deep, Deep Sleep of Hibernators: Amazing Regulatory and Neural Plasticity"

5:30-6:30 **Reception and Awards Presentation**

Table of Contents

From the Director

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

This is the fourth annual research retreat of the Center for Sleep and Respiratory Neurobiology. This annual event plays an increasingly important role in the calendar of the academic activities of the Center. As the event has evolved, it has increasingly been focused on the research of our junior faculty and trainees. We see this increasingly as their day. This year we received 63 abstracts, which is the largest number to date. They are from many different areas of science. This speaks to the robust nature of our research programs and the collegiality that we have fostered.

We have another excellent keynote speaker—Dr. Craig Heller—and we look forward to his lecture.

I am very grateful to the organizing committee, chaired by Dr. David Raizen. The members of the committee—Sara Aton, Siobhan Banks, Jingtao Huang and Norma Cuellar—are to be congratulated for their efforts. I am also grateful to our able administrative staff—Kim Battillo, Jen Montoya and Daniel Barrett—for their help in putting on this event.

A Note from the Organizing Committee:

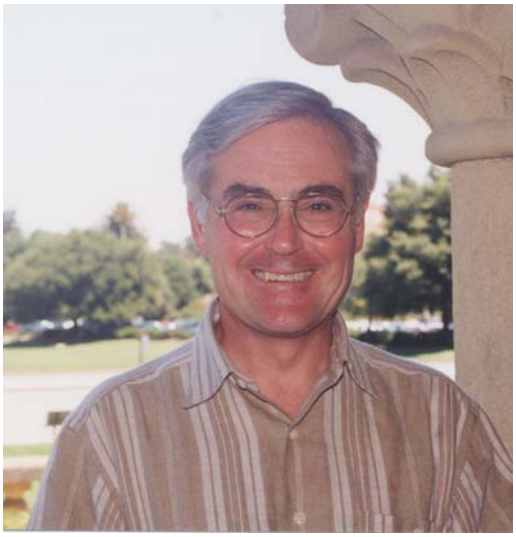
The Organizing Committee welcomes you to the fourth annual full day Research Retreat of the Center for Sleep and Respiratory Neurobiology (CSRN). We are happy and proud to note that abstract submissions to the retreat this year numbered more than sixty, a record number. This retreat brings together the large and multidisciplinary cohort of sleep scientists and clinicians at the University of Pennsylvania. Such a gathering facilitates sharing data and ideas between diverse sleep disciplines - an essential fuel to spark ideas and new collaborations. In addition, we view this retreat as an opportunity for some of the younger members of our community to get additional exposure in the form of oral and poster presentation. In designing the schedule for the day, we chose to increase the number of oral presentations by young investigators. But even with this increased number of short talks, we could include only a small fraction of the abstracts as oral presentations. Over forty abstracts will be presented in a poster format only. We therefore left ample time for poster viewing after lunch. We encourage everyone to stay for the full day, and in particular, to make every effort to see the posters. As the Organizing Committee, we trust that this day will be enjoyable and scientifically enriching for all.

The Committee:

David Raizen	Instructor of Medicine, Neurology
Sara Aton	Postdoctoral Fellow, Neuroscience
Norma Cuellar	Assistant Professor, School of Nursing
Siobhan Banks	Assistant Professor, Unit for Experimental Psychiatry
Jingtao Huang	Postdoctoral Fellow, Children's Hospital of Philadelphia

Keynote Speaker

H. Craig Heller, Ph.D.
Department of Biological Sciences
Stanford University



Dr. Heller is the Lorry I. Lokey/ Businesswire Professor of Biological Sciences at Stanford University. He received his B.S. degree from Ursinus College and a Ph.D. from Yale University. He was a postdoctoral fellow at Scripps Institution of Oceanography before assuming a faculty position at Stanford University.

Dr. Heller's research has been in the areas of: CNS regulation of body temperature, mammalian hibernation, and sleep and circadian neurobiology, and human physical performance. Some of the contributions he and his colleagues have made in the area of sleep include: demonstrations of homologies between hibernation and sleep, characterizations of interactions between sleep and thermoregulation, the early development of REM and NREM sleep in rats, the role of adenosine in control of NREM sleep delta power, and hypotheses on functions of REM and NREM sleep.

Current work in his laboratory includes neural plasticity in sleep and hibernation, the role of clock genes in sleep, and various aspects of circadian rhythms in a unique mammalian model system that can be made arrhythmic with a change in photoperiod. In addition, his laboratory is involved in studies of human thermal physiology and its effects on performance. The lab has developed a technology for rapidly changing the heat content of the human body and has used that to achieve large improvements in physical conditioning and endurance for work in the heat. Medical applications are also being developed including its effect on sleep in non-neutral thermal environments.

Dr. Heller is the author or coauthor of about 200 scientific papers as well as a widely used undergraduate textbook of biology.

SLEEP REGULATION

Slow Wave Activity Dynamics During Consecutive Weeks of Sleep Restriction to 4 Hours per Day

Avinash D[1], Banks S[1], Van Dongen HPA[2], Dinges DF[1]

[1]Division of Sleep & Chronobiology, Department of Psychiatry, Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine [2]Sleep and Performance Research Center, Washington State University, Spokane

Introduction: While sleep restriction (SR) to 4h per night results in cumulative waking neurobehavioral deficits, inconsistent results have been reported for the SR response of NREM EEG slow wave activity (SWA)—the putative marker of sleep homeostasis.

Methods: As part of a larger study on N=80 subjects, N=27 healthy adults (29±7y, 13 females) underwent 2 nights of baseline sleep (B; 10h TIB), 5 nights of sleep restriction (SR1-5; 4h TIB), one variable night of randomized TIB (C1; 0,2,4,6,8,10,12h), 5 more nights of sleep restriction (SR6-10; 4h TIB), and 2 recovery nights (R; 10h TIB). EEG was recorded at baseline, restriction nights 1 and 5 (SR1a, SR5a), variable night (C1), restriction nights 6 and 10 (SR6, SR10), and recovery night (R1) and analyzed for NREM SWA (0.5-4.5Hz, sampled at 120Hz) for the C3-Ax derivation. After artifact removal, FFT analysis was performed in 5s bins, and the average delta power for every 30s epoch was computed.

Results: N=16 subjects from the 6h, 8h, 10h and 12h C1 TIB sleep doses were pooled and SWA was analyzed in the first 5 days of SR. Compared to baseline, SWA was reduced on SR1 and SR5 by 36% ($p<0.001$) and 31% ($p<0.001$) respectively; there was no significant difference between SR1 and SR5. For subjects who received 6h-12h TIB on C1, SWA increased by 36% ($p=0.001$) on C1 relative to SR5. SWA decreased from C1 to SR6 by 28% ($p=0.01$) and from SR6 to SR10 it decreased further by 16% ($p=0.05$). On R1 (10h TIB), SWA increased by 46% ($p<0.001$) over SR10 and by 12% ($p=0.05$) over baseline.

Conclusion: These results provide preliminary evidence that SWA undergoes homeostatic changes with SR. SWA is reduced when sleep is restricted to 4h TIB, and increased when TIB is subsequently increased to 6h-12h. These SWA effects were found on both the first and second week of sleep restriction.

Support: This research was supported by NASA cooperative agreement NCC 9-58-159 with the National Space Biomedical Research Institute and NIH RR00040.

An In-Silico Analysis of Genes in the QTL for Sleep Homeostasis in Mice

Mackiewicz M[1], Paigen B [2], Naidoo N[1], Pack AI[1]

[1]Division of Sleep Medicine/Department of Medicine and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania; [2]The Jackson Laboratory, Bar Harbor, ME

Introduction: EEG delta power reflects sleep intensity and is correlative with the homeostatic response to sleep loss. The time course of accumulation of delta power varies among inbred strains. Segregation of the rebound of delta power in BxD RI strains has identified a region on chromosome 13 (*Dps1*) that contains genes that modify rate of accumulation of delta power (Franken et al., 2001). We performed a comprehensive analysis of genes in this region to identify potential candidate genes that may underlie the accumulation of delta power after sleep deprivation.

Methods: Using a set of bioinformatic tools and the relevant databases we performed (1) annotation of genes in the *Dps1* region; (2) haplotype analysis of *Dps1* region in B6, D2 and BxD RI strains; (3) analyses of SNP in exons, introns and regulatory regions; (4) included data from microarrays about genes changing expression between sleep and sleep deprivation. The top candidate genes to explain *Dps1* QTL were selected based on the SNP and gene expression data.

Results and Conclusions: Through the in silico analysis, the list of 239 genes in the *Dps1* region was narrowed to 4 top candidates. We identified the *Homer1* gene as a potential candidate to explain *Dps1* QTL. *Homer1* is a scaffolding protein involved in glutamate signaling. There are SNP differences between inbred mice in the promoter of the *Homer1* gene. These changes likely impact the binding of transcription factors and may lead to differential increase in *Homer1* in mouse strains with sleep deprivation.

Loss of Functional Homer Leads to Sleep Fragmentation in Drosophila

Ferber M, Pack AI, Naidoo N

Center for Respiratory Neurobiology, University of Pennsylvania School of Medicine

Introduction: Disruption of sleep architecture occurs in key human diseases such as narcolepsy, in which there is behavioral state instability, and in sleep-maintenance insomnia where there is instability only of the sleep phase. With the exception of the orexin/hypocretin system not much is known about the molecules that may contribute to behavioral state stability. Behavioral studies with the Homer loss of function mutant suggest that Homer may be one such molecule. Homer is a group of synaptic scaffolding proteins that are known to interact with both the group I metabotropic glutamate receptors and IP3 calcium receptors and is thought to mediate signaling between them.

Methods: Sleep-wake behavior was determined in young (5-7 day old) male and female Homer ^{R102} transgenic flies (n=200) maintained in 12: 12 light: dark conditions. Canton-S (CS) flies were used as background controls (n=200). Using the 5 minutes of inactivity definition of sleep we determined the following parameters: % time active and sleeping, number of sleep and wake bouts, mean length of sleep and wake bout, maximum length of the sleep and wake bout, number of activity counts per bout.

Results: While we observe no change in the % time sleeping and the % time active between Homer mutant flies and the background CS flies, Homer mutant flies do display a greater number of both sleep and wake bouts than the control background strain and also exhibit shorter sleep and wake bouts. There are significant differences in wake bout number ($p<0.01$), wake bout length ($p = 0.02$), sleep bout length ($p<0.01$) and number of sleep bouts ($p<0.01$) in both male and female Homer ^{R102} mutant flies compared with CS flies.

Conclusion: The scaffolding protein Homer appears to be required to maintain both sleep and wake state stability in Drosophila.

Identification and Characterization of Sleepless, a Novel Gene Critical for Sleep in Drosophila Melanogaster

Joiner W*, Koh K*, Wu M*, Yue Z, Sehgal A
*these authors contributed equally to this work and are listed alphabetically

Departments of Neurology and Neuroscience, Division of Sleep Medicine; Howard Hughes Medical Institute University of Pennsylvania School of Medicine

Introduction: Sleep is a fundamental and evolutionarily conserved behavior. Despite its importance, the function of sleep remains unknown, and the molecular mechanisms underlying sleep are poorly understood. It has been demonstrated that the fruit fly *Drosophila melanogaster* sleeps, and we are exploiting the genetic power of this model system to identify novel genes required for sleep.

Methods and Results: We have been carrying out large-scale forward genetic screens for sleep mutants in *Drosophila melanogaster*. To date, we have screened over 6,000 mutant lines and found several lines with severe reductions in daily sleep. One of these mutants, which we have named *sleepless*, has the most extreme short sleeping phenotype. Compared to controls that sleep ~12 hrs/day, *sleepless* mutants sleep ~1 hr/day. *sleepless* mutants bear a transposon insertion in the coding region of a novel gene. Deficiencies that remove the gene fail to complement the short sleeping phenotype, and precise excision of the transposon rescues the mutant phenotype, demonstrating that disruption of the *sleepless* gene is responsible for the short-sleeping phenotype. The *sleepless* gene contains sequence for a putative signal peptide, is enriched in the brain, and is RNA-edited, suggesting that SLEEPLESS could be a novel brain-specific signaling molecule critical for sleep. Further characterization of *sleepless* is ongoing, including cellular localization of *sleepless* mRNA and protein, studying the effects of overexpression of SLEEPLESS protein, and determination of whether SLEEPLESS protein levels covary with sleep need.

Conclusion: *sleepless* encodes a novel protein critical for sleep in *Drosophila melanogaster*.

Conflict of Interest: The authors have no conflicts of interest to disclose.

Trainee Award Eligible

Astrocytes Regulate Sleep Homeostasis

Halassa MM, Fellin T, Munoz JR, Haydon PG, Frank MG

Department of Neuroscience, University of Pennsylvania School of Medicine

Introduction: Research over the last few decades has implicated adenosine as an important molecule for mediating sleep homeostasis. However, the source and mechanism of action of adenosine is still largely unknown. We have shown that a significant amount of extracellular adenosine is derived from astrocytes (Pascual et al, 2005). Astrocytes release chemical transmitters (a process known as gliotransmission) to influence neuronal activity. One such gliotransmitter is ATP, which is rapidly degraded into adenosine to suppress synapses.

Methods: We have developed a transgenic mouse in which gliotransmission is attenuated by the overexpression of a dominant negative SNARE domain (dnSNARE) selectively and conditionally in astrocytes in regions including the cortex and basal forebrain. This manipulation relieves adenosine-dependent synaptic suppression. Therefore, we used the dnSNARE mouse to ask whether gliotransmission is necessary for this behavior.

Results: dnSNARE mice (N=5) and wildtype littermate controls (N=4) (8-10 weeks of age) were implanted with EEG and EMG electrodes and acclimated to a 12:12 light-dark (LD) cycle; lights on at 6 A.M. Baseline recordings were acquired for 48 hrs before mice were sleep-deprived for 6 hrs (starting at 6 A.M.) and allowed to recover. We found the average vigilance states across 24 hrs to be similar between the two groups. However, following sleep deprivation, dnSNARE mice showed a significantly less robust increase in nonREM delta power compared to controls and a faster return to baseline.

As dnSNARE gene expression is regulated by doxycycline (Dox), we fed both groups a Dox diet for two weeks to turn of transgene expression and repeated these studies. We found a complete reversal of the dnSNARE phenotype with this manipulation: transgenic mice had the same nonREM delta power as controls following sleep deprivation.

Conclusion: These studies indicate an important role for astrocyte-dependent gliotransmission in sleep homeostasis.

The cGMP-Dependent Protein Kinase Gene Foraging Promotes Sleep in Drosophila

Zimmerman JE, Pack AI, Raizen DM

Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine

Introduction: To test the possibility that the cGMP dependent protein kinase (PKG), which we have recently shown promotes sleep-like behaviour in *C. elegans* (see D. Raizen poster), regulates sleep in other animals, we compared sleep in Drosophila strains that differed in the activity of a PKG protein encoded by the *foraging* (*for*) gene, the PKG Drosophila protein most similar to worm PKG gene *egl-4*.

Methods: To measure sleep, we employed a novel video digital subtraction principle we call the Subtraction Analysis of Video Images (SAVI). The SAVI approach offers increased accuracy for the estimation of sleep parameters over the traditional beam splitting method (see J. Zimmerman poster). Adult female flies 7 days old were imaged every 5 seconds using Retiga digital video cameras for 24 hours, 12 hours white light and 12 hours IR light. SAVI was used to estimate total sleep and mean sleep bout lengths.

Two Drosophila strains, for^{R} and for^{S2}, were used for this study. The for^{R} strain was isolated from the wild and has high levels of PKG activity (Sokolowski, 1980). The for^{S2} strain was derived from the for^{R} strain by mutagenesis followed by extensive backcrossing to yield an isogenic low PKG activity strain (Pereira and Sokolowski, 1993).

Results: We found that the for^{s2} Drosophila strain, which has low PKG activity, slept less (672.9 ±186.8 minutes / 24 hr) than the for^{R} strain from which it was derived (807.7 ± 177.5 minutes/24 hr), p=0.006, and has higher PKG activity. Mean wake bouts duration is increased in for^{s2} (24.1 ± 7.3 minutes per bout) in comparison to for^{R} flies (16.8 ± 6.2 minutes per bout), p=0.0005, whereas sleep bout duration is unchanged, p=0.8.

Conclusion: Therefore, *foraging*/PKG promotes the initiation but not the maintenance of sleep in *Drosophila* and, as in *C. elegans*, higher PKG activity is associated with more sleep in Drosophila. This conserved genetic regulation of quiescence in nematodes and arthropods supports the idea that sleep-like behavior is evolutionarily ancient. In addition, this finding establishes PKG as a heretofore-unrecognized regulator of sleep-like states.

Role of Octopamine in Drosophila Sleep

Crocker A, Sehgal A

Center for Sleep and Respiratory Neurobiology, University of Pennsylvania Medical School

Recent efforts by different labs have opened up the field of *Drosophila* genetics for detailed analysis of sleep. Similar to mammals, *Drosophila* have an increased arousal threshold during sleep, show stereotypic rest positions, and show rebound sleep following a period of deprivation. In addition, *Drosophila* offers distinct advantages over mammals, including short generation time, genetic tractability and spatial and temporal control over transgenic expression. By taking advantage of these benefits of *Drosophila* we have begin to work out signaling pathways important to sleep. We have early preliminary evidence that octopamine (the *Drosophila* homolog of nor-epinephrine) is acting as a wake promoting signal in *Drosophila*. Preliminary data shows that decreases in octopamine levels lead to increased sleep and when octopamine levels are raised there is a corresponding decrease in sleep.

Support: A.C. was supported by NIH HL07953

REM Expression Increases Over A 5-Day Period of Sleep Restriction

Kalra GK[1], Banks S[1], Van Dongen HPA[2], Dinges DF[1]

[1]Division of Sleep and Chronobiology, Department of Psychiatry, and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine; [2]Sleep and Performance Research Center, Washington State University, Spokane

Introduction: It is controversial whether sleep restriction can result in REM sleep homeostasis. We used a chronic sleep restriction paradigm to examine this issue.

Methods: N=58 healthy subjects (22-44y) participated in an 11-day controlled laboratory protocol that included 2 nights of baseline sleep (TIB=10h) followed by 5 nights of sleep restriction (TIB=4h) at 04:00-08:00 hours. PSG data from the first and fifth sleep restriction nights (SR1 and SR5) were analyzed for latency to NREM and REM sleep, and minutes of stages 2, SWS and REM sleep.

Results: Paired t-tests revealed no significant difference between SR1 and SR5 in the following sleep latency measures: latency to sleep onset, latency from stage 1 to 2, latency from wake to SWS, latency from stage 1 to SWS, and latency from stage 2 to SWS. In contrast, REM onset latency was shortened on SR5 relative to SR1 (mean difference = 8.62min, $t = 2.28$, $p = 0.026$). The amount of REM sleep also increased significantly (mean difference = 7.88min, $t = 3.48$, $p = 0.001$), as did the amount of stage 2 time (mean difference = 9.06min, $t = 3.04$, $p = 0.004$).

Conclusion: The reduction in REM latency in the absence of a change in NREM latency, as well as an increase in REM time on the fifth night of sleep restriction relative to first night of sleep restriction, suggests the possibility of elevated homeostatic pressure for REM sleep engendered by sleep restriction. It is unlikely that REM latency shortened because of circadian phase advancement since circadian delay (not advance), accompanies late night sleep restriction. These data suggest that REM sleep homeostasis may play a role in the neurobehavioral effects of late night sleep restriction.

Support: NIH NR004281 and RR00040

Trainee Award Eligible

Aging Attenuates the Unfolded Protein Response to Sleep Deprivation

Naidoo N, Master M, Pack AI

Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine

Introduction: Earlier studies from our group indicated that acute sleep deprivation in mice leads to up regulation of BiP and induction of the unfolded protein response (UPR). We have now extended the study to examine the effect of acute sleep deprivation on the BiP response and UPR in aged mice.

Methods: Aged (24 months old) C57BL/6 mice were sleep deprived for 3, 6, 9 and 12 hours (n=8/timepoint) starting at lights on (7AM) and were sacrificed at the end of the deprivation period. Mice that had been left undisturbed and sacrificed at the same time points were used as diurnal controls (n=8/timepoint). Expression of BiP, the key cellular marker of the UPR, was determined by Western blots. We also determined the phosphorylation status of PERK, a sensor of the UPR and regulator of protein translation. Expression of ubiquitin and the pro-apoptotic protein CHOP (C/EBP homologous protein) were also determined.

Results: Unlike in young sleep deprived animals, BiP protein levels remained unchanged in the cerebral cortex of sleep deprived aged animals compared to control animals sacrificed at the same diurnal time. In addition, the basal expression of BiP was reduced in aged animals compared with young animals ($p<0.01$). We also observed no phosphorylation of PERK in the old sleep deprived mice suggesting that protein translation was not attenuated. Both control and sleep deprived mice displayed an increase in the level of ubiquitination compared to young mice ($p= 0.01$). CHOP levels were also increased in both control and sleep deprived old mice.

Conclusion: The lack of BiP up regulation and the absence of PERK phosphorylation suggest that the UPR is attenuated or not induced in mouse cerebral cortex following sleep deprivation. Increased ubiquitination in the old animals suggests that more proteins are misfolded and are being targeted for degradation while the increased expression of CHOP suggests that cell death/apoptotic pathways are being activated.

Defective Photoresponse on Sleep in Melanopsin Deficient Mice

Tsai J, Bourgin P, Heller C

Stanford University

Melanopsin is a photopigment located exclusively in retinal ganglion cells involved in irradiance level detection. Melanopsin is required for light resetting of the biological clock through dense innervations to the suprachiasmatic nucleus, the circadian pacemaker (Panda et al., 2002; Ruby et al., 2002). Melanopsin cells likewise influence the pupillary light reflex via retinal projections to the pretectal area. The ventrolateral preoptic nucleus (VLPO), a key structure for sleep induction, is also a target of melanopsinergic fibers (Gooley et al., 2003). Sleep positive neurons in the VLPO have firing rates 2-3 times faster during sleep than wake (Sherin et al., 1996). Light influences sleep in two ways: entraining the circadian control of sleep and exerting a masking effect on the expression of sleep. The neural substrate of the light masking effect on sleep remains unknown. We hypothesize that melanopsin-containing retinal ganglion cells convey light information to the VLPO, thereby effecting sleep and waking independently of the circadian drive. If our hypothesis is correct, there should be no masking effect in melanopsin knockout mice (Opn4 -/-).

To test our hypothesis, we recorded sleep-wakefulness cycles in Opn4 -/- (n=8) and wildtype Opn4 +/+ (n=8) mice. Animals were implanted with EEG/EMG electrodes. Vigilance states were recorded under the following conditions: (i) 72 hours baseline; (ii) 1-hour light pulse administered during the dark period; (iii) and 1-hour dark pulse given during the light period. Our results confirm the lack of photoresponse on sleep in melanopsin-deficient mice.

SLEEP DISORDERS: CLINICAL OBSERVATIONS TO BASIC MECHANISMS

Cine-MRI Shows Airway Inspiratory and Expiratory Compromise in New Zealand Obese Mice

Brennick MJ, Pack AI, Kim E, Pickup S, Schwab RJ

Introduction: Obesity is the most important factor clinically linked to obstructive sleep apnea (OSA). We have previously reported that New Zealand Obese (NZO) mice have significantly increased parapharyngeal fat and tongue volume compared to New Zealand Wild type (NZW) mice. The effect of these anatomic factors on airway cross-sectional area (CSA) during the respiratory cycle is relevant to OSA, yet not well understood. We hypothesized that upper airway cross sectional area in NZO vs. age-matched NZW mice is reduced during both inspiratory and expiratory phases.

Methods: In anesthetized spontaneously breathing NZO (N=5) and NZW (N=5) mice, magnetic resonance imaging, triggered by a chest wall pressure sensor, was used to obtain a time series (6 images @ 0.075 sec spacing) of 1 mm thick axial images encompassing the pharynx. CSA in the combined naso- and oropharynx at inspiratory and expiratory times, at matched caudal and mid-pharyngeal loci were compared between NZO and NZW strains (t-test; significance *, at $p<0.05$).

Results:

Pharyngeal CSA (mean±SD, mm)	NZW	NZO	NZW vs. NZO
Caudal			
Inspiratory	2.64±1.68	1.01±0.44	p<0.04*
Expiratory	3.40±1.91	0.59±0.21	p<0.01*
Insp-Exp	-0.76±1.00	0.42±0.07	p<0.02*
Mid-Pharyngeal			
Inspiratory	0.42±0.07	0.39±0.08	p<0.29
Expiratory	2.33±2.22	0.41±0.07	p<0.06
Insp-Exp	-1.91±2.22	-0.02±0.09	p<0.06

Caudal location is near distal edge of soft palate.

Conclusions: In the caudal pharynx, airway CSA is reduced in NZO vs. NZW mice, during inspiration and expiration. Reduced CSA in NZO mice may be due to increased parapharyngeal fat and tongue volumes compared to NZW. These data suggest that obesity may compromise the pharyngeal airway through an anatomic mechanism.

Support: NIH grants HL67948, HL072067, EB01780.

Conflict of interest: None.

Sleep-wake Patterns of Genioglossal EMG at the Base and Tip of the Tongue in the Rat

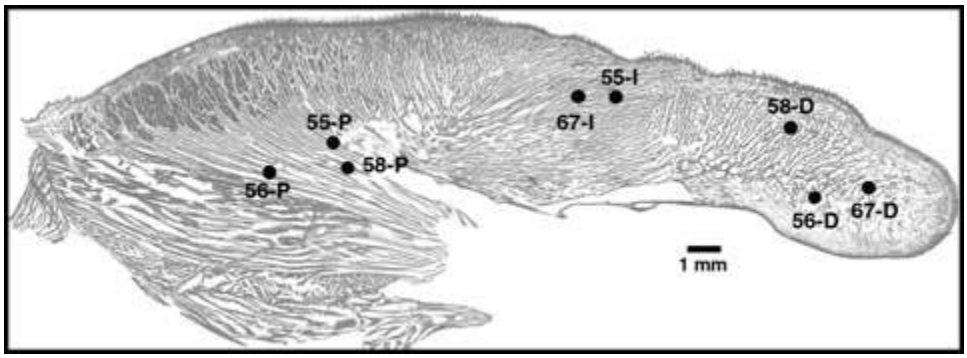
Lu JW, Kubin L

Department of Animal Biology, University of Pennsylvania School of Medicine

Introduction: In rats, the sleep-wake pattern of lingual EMG is different from that of neck postural muscles: whereas nuchal EMG progressively declines from high and variable during wakefulness to atonia with occasional twitches during REM sleep, lingual muscles become nearly atonic during slow-wave sleep (SWS) and then progressively increase their phasic activity during REM sleep (Lu *et al.*, Respir. Physiol. Neurobiol, 2005). Our goal was to test whether sleep-wake-related changes in lingual EMG are different in the basal and distal compartments of the tongue muscle.

Methods: Rats were instrumented and adapted for recording of lingual EMG from two locations within the tongue, nuchal EMG, cortical EEG and hippocampal activity. In some rats, diaphragmatic EMG also was recorded to determine the relationship between phasic bursts in lingual EMG and the phase of the respiratory cycle. Data from two middle hours of baseline recording conducted with each rat between noon and 4 pm were analyzed. The records were scored and root mean squares of EMG activities determined in successive 10 s intervals.

Results: Three recording sites within the tongue were localized, at autopsy, near the base of the tongue, 3 near the tip of the tongue, and 2 at intermediate locations (see figure). When normalized by the mean level of activity during wakefulness, the mean lingual EMG near the base of the tongue was 8.7%±4.0(SE) during SWS and 27%±5 during REM sleep. For the distal recording sites, the mean levels were 8.9%±2.2 and 27%±3, respectively (not different from the base of the tongue). Rhythmic activities intermittently occurred in all lingual locations in wakefulness and REM sleep, but the rhythms were not respiratory.



Conclusion: In contrast to upper airway motor tone in obstructive sleep apnea subjects, in normal rats, lingual EMG in both basal and distal compartments of the tongue reaches a nadir during SWS, rather than REM sleep, and does not exhibit significant respiratory modulation.

Support: HL071097. We thank Ms. Jennifer L. Branconi for image processing.

Conflict of interest: None.

Racial Differences in Upper Airway Dynamics During Sleep

Tapia IE, Huang J, Bandla P, Karamessinis LR, Marcus CL

Sleep Center, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine

Introduction: Obstructive sleep apnea is more prevalent in African Americans (AA) than Caucasians (C). Consequently, we hypothesized that normal AA children, even if asymptomatic, had an inherently more collapsible upper airway during sleep than normal C children.

Methods: We compared the upper airway pressure-flow response to subatmospheric pressure between normal, non-obese, non-snoring, age-matched AA and C children. During sleep, maximal inspiratory airflow was correlated with the level of nasal pressure applied via a mask. Depending on the pattern of pressure applied either the hypotonic or the neuromotor activated upper airway could be assessed. The slope of the upstream pressure-flow relationship (SPF), representing upper airway conductance, was used to characterize UA function.

Results: 25 AA and 25 C (mean age 13 $\pm$ 3 [SD] yr) were studied. The median activated SPF for AA was 9.5 ml/s/cm H_2O *vs.* 9.0 ml/s/cm H_2O for C (NS). The median hypotonic SPF for AA was 18.3 *vs.* 9.0 ml/s/cm H_2O for C (NS).

Conclusion: There is no difference in upper airway dynamics during sleep between AA and C. The increased prevalence of OSAS in AA cannot be explained by a more collapsible upper airway.

Speculation: The known increased prevalence of OSAS in AA is probably not due to changes in ventilatory drive or craniofacial structure. A proposed cause for this difference is exposure to environmental factors.

Conflict of interest: None.

Trainee Award Eligible

Effect of Obesity on the Size of Upper Airway Soft Tissues in Sleep Apneics and Normals

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Introduction: Obesity is associated with sleep apnea. Studies have shown a strong correlation between the size of the upper airway soft tissues and the presence of sleep apnea; however, the relationship between these structures and obesity is unclear. We hypothesized that increased obesity will have an effect on upper airway soft tissue structures, and will specifically lead to larger tongue width and greater modified Mallampati score.

Methods: A validated morphometric device with a digital camera and laser ruler was used to examine the pharynx of 75 apneics and 61 normals categorized into 4 groups based on BMI. Upper airway measurements were obtained with Image J analysis software. AHI was determined with polysomnography.

Results: Among apneics, tongue width was comparable between all 4 BMI groups, while modified Mallampati score increased with BMI ($p<0.05$ comparing BMI >45 to BMI 35-44.9). Among normals, tongue width in the BMI <25 group was significantly smaller than in all other groups ($p< 0.05$). Modified Mallampati scores were significantly greater ($p<0.05$) in the heavier BMI groups compared to the lighter BMI groups.

Conclusions: Data from normals demonstrate that increased obesity results in larger tongue width and greater Mallampati scores. In apneics Mallampati scores also increased with BMI category. The data suggest that obesity affects the size of upper airway structures.

Upper Airway Soft Tissue Structure in Different BMI Categories
(Apneics - top 4 rows; Normals - bottom 4 rows)

BMI range (kg/m)	Sample Size	Tongue Width (cm)	Modified Mallampati Score	AHI
>45	28	5.4±0.6	3.9±0.3	47.4±38.3
35-44.9	23	5.4±0.7	3.6±0.7	52.7±36.3
25-34.9	23	5.4±0.7	3.6±0.7	57.9±30.1
<25	1	5.6±0	4±0	44±0
>45	10	5.3±1.1	3.6±0.7	4.5±3.3
35-44.9	18	5.2±0.6	3.1±1.0	3.2±3.8
25-34.9	15	5.1±0.5	2.4±1.2	1.4±1.8
<25	18	4.5±0.4	1.9±1.2	1.0±1.8

Support: NIH grant HL67948.

Trainee Award Eligible

Obstructive Sleep Apnea in Patients Undergoing Gastric Bypass Surgery – A Tertiary Center Experience

Sareli AE[1], Williams NN[2], Korus G[2], Raper SE[2], Hurley S[1], Lang R[1], Cartagena S[1], Pack AI[1], Schwab RJ[1], Cantor CR[1]

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Introduction: The patient population that is evaluated for gastric bypass surgery is characterized by very high body mass index (BMI). As a result; obstructive sleep apnea (OSA) is highly prevalent. If undiagnosed before gastric bypass surgery, untreated OSA may lead to perioperative complications. We examine the prevalence and characteristics of OSA in the patient population that is considered for gastric bypass surgery.

Methods: 354 consecutive subjects, evaluated for gastric bypass surgery from 11/1/2005 to 1/31/2007 underwent overnight polysomnography and completed questionnaires (Multi-variable Apnea Sleep Symptom (MAP) score and the Epworth Sleepiness Scale (ESS)). Apneas and hypopneas were classified as follows: Mild apnea: $5 \leq AHI \leq 15$, Moderate apnea: $15 < AHI \leq 30$ and Severe apnea: $AHI > 30$.

Results: Mean BMI (kg/m^2) was 49.96±10.72 (range 29.85 to 101.8) and mean age was 43.87±10.88 (range 19 to 67). Our sample was 81% female and 19% male. 77% of subjects provided information regarding race. The distribution of race was 48% white, 51% African American, and 1% other (Asian and Hawaiian-Pacific Islander). The overall frequency of OSA was 77%. 30% of subjects had mild OSA, 20% had moderate OSA, and 27% had severe OSA.

Conclusions: In this population of subjects evaluated for gastric bypass surgery, the prevalence of sleep apnea was 77%. The prevalence of severe obstructive sleep apnea was 27%. Our data indicate that sleep apnea is common in patients that are evaluated for gastric bypass surgery. Our population sample represents all-comers who are evaluated for gastric bypass surgery (as opposed to patients evaluated for gastric bypass surgery who are considered "high risk" based on historical factors and clinical examination).

Future analysis of our database will aim to develop a predictive tool for OSA in this population group based on age, sex and menstrual status (i.e. pre/ post or perimenopausal) BMI, neck circumference, MAP, ESS and existing medical conditions. In addition, we will investigate whether or not correlations exist between subject BMI, neck circumference, medical history, ESS and MAP scores and the following parameters: distribution of sleep stages, oxyhemoglobin saturation nadirs, AHI and REM AHI.

Trainee Award Eligible

Predictors of Continuous Positive Airway Pressure Use during the First Week of Treatment

Ye L, Maislin G, Pack AI, Hurley S, Dinges DF, McCloskey S, Weaver TE

University of Pennsylvania

Introduction: CPAP adherence remains the foremost concern in the management of OSA. About half of OSA patients use their CPAP nightly and the pattern of adherence is established during the first week. The purpose of this study was to identify predictors of CPAP use during the first treatment week.

Methods: Patients with AHI ≥ 15 and prescribed CPAP were recruited. Adherence was measured using a microprocessor monitor within the machine. The ResMed Autoset-Clinical System set to manual CPAP pressure was used to measure residual events, mask leak, and airflow limitation. Self-efficacy was measured with the Self Efficacy Measure for Sleep Apnea and CPAP side effects were assessed using daily diaries.

Results: Mean CPAP use during the first week from 90 patients (54% male) was 3.45±2.67 h/night. Regression models examined the contribution of demographic characteristics, disease severity, self-efficacy, and treatment efficacy. Being less intimate was the only side effect significantly correlated with CPAP adherence (r = -.30). A three covariate model including data from 56 patients accounted for 25.3% (p = 0.002) of the variance in mean CPAP use. Reduced CPAP use was simultaneously associated with less intimacy (p = 0048), being African American (p = 0011), and higher residual AHI (p = 0044).

Conclusions: These data suggest the need to assess the impact of CPAP treatment on intimacy and troubleshoot aspects of the treatment that interfere with sexual relations. Assessing the presence of residual AHI may be important in promoting CPAP adherence. Why race affects CPAP use needs further exploration.

Conflict of Interest: T. Weaver: Research equipment from Respironics, Inc. and Protech; funding from Respironics Sleep and Respiratory Foundation, FOSQ License Agreements with Sanofi-Aventis Pharmaceutical, Sleep Solutions, Merck & Co, Inc., Jazz Pharmaceutical; consultant for Jazz Pharmaceutical and Sanofi-Aventis.

Adult Perceptions of the Diagnosis and Treatment of Obstructive Sleep Apnea: A Mixed Methods Study Examining the Influence of Obstructive Sleep Apnea Patients' Experiences in Context on Continuous Positive Airway Pressure Adherence Outcomes

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Introduction: Recent studies have identified differences in adherence to continuous positive airway pressure therapy (CPAP) between African American and Caucasian adult obstructive sleep apnea (OSA) patients, possibly contributed to by specific health beliefs, diagnosis and treatment perceptions, and self-efficacy. This study elucidated and examined beliefs and perceptions about the OSA diagnosis and treatment with CPAP and self-efficacy relative to CPAP adherence outcomes among adults with OSA.

Methods: The study employed a mixed methods concurrent nested design. The predominant method was qualitative (semi-structured, individual interviews) with an embedded quantitative data collection procedure (quantitative measurement of CPAP adherence). Nineteen participants were consecutively enrolled prior to diagnostic polysomnogram (PSG) with 16 meeting the inclusion criteria post-PSG (AHI $\geq$ 15), six (38%) non-Hispanic Caucasians, nine (56%) non-Hispanic African Americans, and one (6%) other. Within one week of diagnostic PSG and within 5-30 days post-CPAP treatment, participants completed semi-structured interviews. The first interview focused on individual perceptions of the diagnosis, the perceived health effects of the diagnosis, pre-treatment perceptions of CPAP, and the social and cultural precedents that contributed to health-seeking and healthcare decision-making behaviors relative to the individual's sleep problems. The second interview focused on the perceived effects of treatment with CPAP, supportive mechanisms or barriers to incorporating CPAP into daily life, and how beliefs and perceptions about the diagnosis, the associated risks of the diagnosis, and the treatment experience may impact on decisions and commitments to use CPAP. CPAP adherence, mask-on time, was measured after the first week of CPAP treatment. Adherence data were used during the second interview to promote discussion of barriers/facilitators to CPAP use. Adherence data were also used in the analysis phase to identify and describe adherers and nonadherers in terms of qualitative themes that emerged from content and narrative analysis of the interview data.

Results: Post-diagnosis and post-treatment perceptions and beliefs were defined within cases and across cases for the sample through analysis of the transcribed interview text data. Cases were defined as adherers or nonadherers, using the mean (SD) and median nightly CPAP use for the first week of treatment. Adherence was defined with the application of an *a priori*-determined mean nightly CPAP use cut-point. Across-case beliefs and perceptions, both post-diagnosis and post-treatment, were then separated into adherent and nonadherent cases to examine consistencies and differences in beliefs and perceptions about OSA and CPAP that contribute to CPAP adherence outcomes and offer exploratory suggestion as to why differences in CPAP adherence exist among ethnically-diverse OSA patients.

Conclusions: Elucidating adult OSA patient perceptions of both diagnosis and treatment as contextual contributors to CPAP adherence provides new knowledge with regard to socio-cultural influences on differences in the use of CPAP treatment among ethnically-diverse OSA patients. These findings provide investigative insight for developing and testing culturally congruent adherence measures and interventions to enhance health and functional outcomes for people living with OSA.

Support: Research support by NINR F31 NR009315 (Sawyer).

Trainee Award Eligible

The Relationship Between Sleep Disturbances and Alcohol Craving

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Purpose: Prior literature has established a link between alcohol dependence and sleep disorders. The sleep disorders seen include insomnia, decreased sleep duration and polysomnographic changes, which include decreased total sleep time as well as abnormalities of NREM and REM sleep. These sleep problems are especially common during the early and sub-acute phases of detoxification and may be associated with relapse. Similarly, the concepts of alcohol craving and reward have been determined to be associated with relapse and are considered targets for medication development. The purpose of this study was to characterize the association between these two constructs.

Design: The sample included 307 alcohol-dependent subjects who presented for participation in an adaptive treatment study of naltrexone. Assessment measures used for this study included standardized measures for sleep problems, alcohol consumption and alcohol craving (prior to treatment with naltrexone). Alcohol consumption was measured by the Time Line Follow Back Measure (TLFB) and alcohol craving was measured by the Penn Alcohol Craving Scale (PACS). Sleep problems were assessed using the Medical Outcomes Study Sleep Scale (MOS Sleep Scale).

Results: As expected, alcohol craving was associated with the percent days of heavy drinking in the subjects ($p = 0.018$). After controlling for prestudy drinking the cravings were associated with sleep abnormalities. Cravings at baseline were associated with somnolence ($p = 0.024$) and sleep problems ($p < 0.001$). We also note that Caucasian subjects ($n = 202$) had higher intensity of cravings than the African American subjects ($n = 94$; $p = 0.002$).

Conclusion: As previously noted, sleep problems are common among treatment-seeking, alcohol-dependent adults. Results from this study suggest that sleep problems are highly associated with craving states. Based on these results, sleep problems should be considered in the interpretation of clinical trials of anti-craving medications or the phenomenology of craving.

Trainee Award Eligible

Fibromyalgia Patients without Sleep Apnea?

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Purpose: Sleep disturbance is a common complaint of fibromyalgia (FM) patients. Increased spontaneous arousal has been well documented in FM but there is controversy regarding any association with sleep apnea. There have been reports that there is increased incidence of sleep apnea in FM patients. We have reviewed 135 FM patients' medical records in rheumatology clinics at the University of Pennsylvania and confirmed an increased incidence of sleep apnea in this group of patients. The purpose of this study is to compare age, sex, BMI and sleep study indexes of FM patients with sleep apnea (FMSA) versus FM patients without sleep apnea (FMNSA).

Methods: 135 FM patients' medical records in rheumatology clinics were reviewed. These patients were all diagnosed with FM by rheumatologists at the University of Pennsylvania using ACR criteria. Their sex, age, BMI, co-existing medical conditions, and sleep study records were recorded. Overnight polysomnograms were performed in the sleep laboratory. Using standard techniques, a computer data acquisition and analysis system recorded the following signals: electroencephalogram (C3A2, O2A1); bilateral electrooculograms; submental and bilateral tibialis anterior electromyograms; impedance plethysmography of the rib cage and abdomen, airflow at the nose and mouth (nares pressure), body position, pulse oximetry, and tracheal breath sounds.

Results: Patients' ages ranged from 20-84 years old. Of the 40 patients with sleep studies, 25 had an apnea-hypopnea index >5 and were found to fulfill diagnostic criteria for obstructive sleep apnea. The documented percentages of sleep apnea in these 135 fibromyalgia patients were at least 19.4% for female and 32% for male. Both were higher than reported for sleep apnea in the general population (9% for female and 24% for male). The average age of FMSA was 47.08±9.57 and similar with FMNSA which was 46.07±12.54. Interestingly, there were 24% male in FMSA compared only 13% male in FMNSA. There was no statistical difference (p=0.32) in average BMI in the FMSA group (30.33±6.89) versus FMNSA group (27.93±7.47). There was significant difference (p=0.004) in the arousal index between these two groups, 24.41±16.10 in FMSA group and 12.69±8.13 in the FMNSA group. Otherwise there were no differences in total sleep time, sleep efficiency, and sleep onset latency, percentages of REM and NREM in FMSA and FMNSA

Conclusion: Sleep apnea occurs more often than generally recognized in patients with FM. Males are more numerous in the fibromyalgia patients with sleep apnea than without sleep apnea. The arousal index is significantly increased in FM patients with sleep apnea. It potentially can cause worsened FM symptoms. Treating the sleep apnea might improve FM symptoms.

Trainee Award Eligible

Symptoms of Restless Legs Syndrome in Older Adults: Outcomes on Sleep Quality, Sleepiness, Fatigue, Depression, and Quality of Life

Cuellar NG, Strumpf NE, Ratcliffe SJ, Cantor CR

Objective: To compare differences in sleep quality, sleepiness, fatigue, depression, and quality of life by severity of symptoms in Restless Legs Syndrome (RLS) in older adults.

Design: Descriptive, comparative study, cross-sectional design.

Setting: Penn Sleep Center at the University of Pennsylvania and RLS Support Groups in Philadelphia

Participants: 39 adults, 65 years of age and older, diagnosed with RLS with symptoms at least 3 nights per week. Participants were stratified by symptom severity based on scores from the RLS Symptom Severity Scale. Exclusion criteria: dementia, cognitive impairments, and sleep disorders other than RLS.

Measurements: Sleep quality, measured by the Pittsburgh Sleep Quality Index (PSQI), was the primary outcome. Secondary outcomes were sleepiness, fatigue, depression, and quality of life measured by the Epworth Sleepiness Scale (ESS), Fatigue Severity Scale (FSS), Center for Epidemiological Studies – Depression (CES-D), and RLS-Quality of Life Instrument (RLS-QLI), respectively.

Results: Significant differences were found in subjective sleep quality (P=0.007) and sleep duration (P=0.042), as well as in the PSQI global score (P=0.007). RLS-QLI sleep quality (b=-0.12, 95% CI=-0.18 to -0.06, P<0.001) and sleepiness (b=0.35, 95% CI=0.09 - 0.61, P=0.010) were significantly related to the PSQI global score. Subjects with severe symptoms were 5 times more likely to use medication to treat RLS (OR=5.3, 95% CI=1.2 - 22.2).

Conclusion: The severity of RLS symptoms in older adults affects not only sleep quality but many aspects of quality of life including social functioning, daily functioning, and emotional well-being.

Support: We gratefully acknowledge support for this study from the Hartford Center of Geriatric Nursing Excellence and the Frank Morgan Jones Fund at the University of Pennsylvania, School of Nursing, Philadelphia, PA. Supported in part by NIH Grant M01-RR00040.

Discovering the Impact of RLS in Type 2 Diabetes

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University of Pennsylvania, School of Nursing, Philadelphia Pennsylvania

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

Purpose: The purpose of this study was to identify the prevalence of RLS in type 2 diabetes and compare participants with and without RLS on glycemic control, sleep, sleepiness, fatigue, and depression.

Research Question: Is there a difference in type 2 diabetics with and without RLS on glycemic control, sleep, sleepiness, fatigue, and depression?

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

Results: Of 102 participants who were screened, 44 (43%) had RLS; 39 participants completed the surveys (mean age 60.8 years old) with 18 Caucasians, 3 Asians, 15 African Americans, and 3 mixed. Average weight of the group was 177 lbs. (range of 110-249) with a BMI of 33.13 (range of 19.64 – 51.39) with only 7 in a normal weight. For type 2 diabetics, significant relationship with ethnicity and RLS status was found ($p=0.26$) with Caucasians 5 times more likely to have RLS (OR=5.0, 95% CI=1.28-1961). Only 5 participants had primary RLS. While not found to be statistically significant, 44% of the participants with RLS used insulin in conjunction with oral agents compared with 29% of non-RLS participants. Type 2 diabetics with RLS reported significant difference in quality of sleep ($p=0.001$), sleep latency ($p=0.04$), sleep efficiency ($p=0.035$), sleep medications ($p=0.000$), and daytime dysfunction ($p=0.005$). Subjects with RLS had almost double the average global PSQI score when compared to those without RLS, 12.8 vs. 6.7, respectively. In the total group, higher HgbA1c were positively correlated with sleepiness while global PSQI scores were significantly positively correlated with fatigue ($r=0.58$, $p=0.002$) and depression ($r=0.74$ $p<0.001$). As well, fatigue and sleepiness were positively correlated ($r=0.36$, $p=0.04$).

Conclusions: Based on our sample, this study indicates that the incidence of RLS in type 2 diabetics may be higher than previously reported. RLS significantly affects sleep which may impact diabetes management. Larger studies need to be performed to confirm these findings. Health care providers should be aware that RLS may impact cost and effectiveness of treatment for diabetes.

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

Spontaneous Breathing in Children with Congenital Central Hypoventilation Syndrome in REM vs. NonREM Sleep

Marcus CL[1], Schwartz MS[1], Bandla P[1], Samuel JM[1], Pepe ME[1], Panitch HB[1], Bradford RM[1], Colrain IM[2], Mosse YP[1], Huang J[1]

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Introduction: Congenital central hypoventilation syndrome (CCHS) is a rare condition characterized by generally adequate ventilation during wakefulness but alveolar hypoventilation during sleep, to the point where patients need mechanical ventilation. The early literature suggested that hypoventilation in children with CCHS was less severe during rapid eye movement (REM) than nonREM sleep. This conclusion was drawn from a few case reports and has not been tested rigorously. We hypothesized that there would be no difference in terms of hypoventilation between REM and nonREM sleep in children with CCHS.

Methods: Nine subjects with CCHS, aged 4 months to 20 years, were studied during sleep while being mechanically ventilated on their home settings. Spontaneous ventilation during REM and nonREM sleep was evaluated briefly by disconnecting the ventilator under controlled circumstances. Once the end-tidal PCO_2 rose to more than 60 mm Hg or SaO_2 dropped to less than 85%, patients were placed back on their ventilators.

Results: Arousal occurred in 56% of REM trials vs. 32% of nonREM trials (NS). Central apnea occurred in 25% of REM vs. 68% of nonREM trials (p=0.026). The average minute ventilation (V_E) during the challenge was 30±26% (mean ± SD) of baseline in REM vs. 12±17% in nonREM (p=0.036).

Conclusions:

1. Children with CCHS have hypoventilation and central apnea when breathing spontaneously during sleep. However, patients who are usually adequately ventilated during sleep frequently arouse in response to gas exchange abnormalities.
2. The hypoventilation in CCHS is more severe during nonREM than REM sleep.

Support: This study was supported by NIH grants M01-00240, U54-RR023567, R01-HL58585 and a research grant from Respironics.

Respiratory Sensation during Sleep in Children with the Obstructive Sleep Apnea Syndrome

Huang J[1], Marcus CL[1], Melendres MCS[2], Karamessinis LR[1], Pepe ME[1], Samuel JM[1], Abi-Raad RF[2], Trescher WH[2], Colrain IM[3]

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Introduction: Children with obstructive sleep apnea syndrome (OSAS) have blunted respiratory sensation which may contribute to the pathogenesis of the disease. Respiratory sensation can be tested by measuring respiratory-related evoked potentials (RREPs). RREPs are obtained by occluding the airway briefly during inspiration and measuring the resultant cortical EEG. Adults with OSAS have been shown to have a significantly smaller N550 component than controls in nonREM sleep. RREPs in children are dominated by an earlier negative component, the N350, which is also measurable in REM sleep. We hypothesize that children with OSAS have smaller N350 responses than normal children.

Methods: Nine children with OSAS and 10 controls slept wearing a mask connected to a nonrebreathing valve. Flow was measured using a pneumotachograph connected to a face mask and a differential pressure transducer. Pressure within the mask was measured by a pressure transducer. Multiple 400 ms inspiratory occlusions were performed during stage 2, slow wave (SWS) and REM sleep. EEG activity was averaged and RREPs were determined at Fz, Cz and Pz. N350 amplitude was analyzed with a site x sleep state x diagnosis ANOVA.

Results: Three OSAS patients had no obvious RREP waveforms and thus had a zero voltage input into the analysis. OSAS patients had significantly smaller N350 amplitudes than controls ($p<0.05$). The site (largest at Fz, $p<0.001$), and sleep state (largest in stage 2, $p<0.01$) factors were also significant. T-tests for SWS and stage 2 Fz data, excluding the subjects with aberrant responses, showed patients' response to be significantly smaller in both sleep states (SWS, $p<0.01$; stage 2, $p<0.05$).

Discussion: Children with OSAS have impaired neural processing of respiratory load information during sleep. We speculate that this may be a factor in the pathophysiology of childhood OSAS. Alternatively, it may result from chronic hypoxemia/hypercapnia or sleep disruption.

Support: This study was supported by NIH grants M01-00240, U54-RR023567, R01-HL58585 and a research grant from Respironics.

Trainee Award Eligible

Afferent Respiratory Processing in Children with the Congenital Central Hypoventilation Syndrome

Huang J[1], Colrain IM[2], Bandla P[1], Schwartz MS[1], Pepe ME[1], Samuel JM[1], Panitch HB[1], Bradford RM[1], Mosse YP[1], Marcus CL[1]

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Introduction: Congenital central hypoventilation syndrome (CCHS) is characterized by generally adequate ventilation during wakefulness but alveolar hypoventilation during sleep, to the point where patients need mechanical ventilation. Respiratory mechanoreception has not been studied in CCHS during sleep. Respiratory sensation can be tested by measuring respiratory-related evoked potentials (RREPs); RREPs are thought to be mediated in part by mechanoreceptors. RREPs are obtained by occluding the airway briefly during inspiration and measuring the resultant cortical EEG. In children sleep RREPs are dominated by an N350 component. We therefore hypothesized that children with CCHS have abnormal respiratory mechanoreception during sleep as reflected by a smaller N350 component.

Methods: Eight subjects with CCHS and 12 controls slept with a nonrebreathing valve connected to either a facemask or a tracheostomy tube. Flow was measured using a pneumotachograph and a differential pressure transducer. Pressure from the mask or tracheostomy was measured by a pressure transducer. Multiple, 400 ms inspiratory occlusions were performed during stage 2, slow wave (SWS) and REM sleep. EEG activity was averaged and RREPs were determined for each sleep stage at Fz, Cz and Pz. N350 amplitude was analyzed with a site x sleep state x diagnosis ANOVA.

Results: RREPs were produced by all patients and controls. Patients with CCHS had significantly smaller N350 amplitudes than controls ($p < 0.05$). The site (largest at Fz, $p < 0.001$), and sleep state (largest in stage 2, $p < 0.001$) factors were also significant. None of the interaction terms was significant.

Conclusion: While subjects with CCHS have a significantly smaller N350 than controls, they nevertheless demonstrate a robust RREP response during sleep. Previous studies raised the speculation that patients with CCHS have functional chemoreceptors but have abnormal central integration of chemoreceptor input. This study supports the presence of intact mechanoreception, but leaves open the possibility of abnormal central integration of respiratory afferents.

Support: This study was supported by NIH grants M01-00240, U54-RR023567, R01-HL58585 and a research grant from Respironics.

Trainee Award Eligible

Sleep Disturbances Among Cancer Survivors

Marie E, Gooneratne N, Palmer S, Keddem S, Barg F

Introduction: Sleep-related complaints are common among cancer survivors, yet there has been little research focused on the origins of these difficulties (Malone M et al., J R Soc Med 1994; Savard J et al., J Clin Oncol 2001). Davidson et al. looked at the self-reported sleep-disturbances of 982 cancer patients and reported an increased incidence of fatigue, sleepiness, repetitive leg movements, and sleeping more than usual among those with lung cancer compared to those with other forms of cancer (Davidson et al., Social Science & Medicine, 2002). At the same time, 36.8% of lung cancer patients reported insomnia, making the prevalence of insomnia in lung cancer second only to its prevalence in breast cancer patients, 37.8% of whom reported insomnia. While these finding are provocative, conclusions we can draw from it are limited, as there was great variability in the time the information was collected relative to the date of diagnosis and the percentage of those in each cancer group who had received treatment in the last six months.

Methods: Surveys were mailed to individuals listed in a Pennsylvania state registry of cancer survivors. The mailing was limited to those whose initial diagnosis was within the last three to four years. A total of 614 people responded, with a mean age of 60 years. The survey asked about various types of problems encountered by cancer patients both during and after treatment and to what extent their perceived needs were met. Included were questions concerning sleep disturbances since diagnosis, including whether "sleeping too little or too restlessly" and "sleeping too much" had not been a problem, been a "small problem," "moderate problem," or "significant problem." The survey also included extensive questioning about demographics, medical history, and the physical, financial, and emotional burdens experienced by participants since their cancer diagnosis.

Planned Analysis: Over 30% of the respondents reported sleep disturbances since diagnosis. Analysis will test the hypothesis that lung cancer survivors more frequently experience sleep disturbances than survivors of cancer overall, three to four years after initial diagnosis. We will also look for possible relationships between frequency of sleep disturbances and medical history, current health issues, cancer treatment type, and burdens related to illness. We hypothesize that cancer treatments vary in their association with sleep troubles and that advanced age, comorbid illness burden, symptom distress, and unmet emotional needs are associated with sleep troubles in cancer survivors. We will run a multivariate analysis of risk factors for poor sleep to determine which of these factors are most relevant.

Conclusion: Assessment of the risk factors associated with sleep disturbances in cancer survivors both across and within cancer types will encourage greater awareness among physicians and earlier intervention for those most at risk. Further investigation into the origins of sleep disturbances in high-risk groups allows for the examination of cause-effect relationships between illness and sleep disturbances. Besides the known cognitive, emotional, and physical benefits of improved sleep, there is increasing evidence that sleep may play a role in regulating several hormones that can impact cancer cell activity, suggesting that improved sleep may be especially important for the long-term health cancer survivors (Sephton, S., Brain, Behavior and Immunity, 2003).

Sleep Behaviors and Sleep Quality in Children with Autistic Spectrum Disorders

Souders MC

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Background: Children with Autistic Spectrum Disorders (ASD) are at increased risk for sleep disturbances. Core deficits of ASD and their underlying neurophysiology may predispose children to intrinsic and extrinsic stressors that threaten sleep. Poor sleep in children can alter learning, attention and performance and adversely affect sleep quality in parents. About 75% of parents report a sleep disturbance with their child with ASD.

Objectives: Describe the sleep behaviors and sleep quality in a cohort of children with ASD, ages 4-10, (24 Autism, 24 PDD-NOS and 24 Asperger Disorder) as compared to 72 typical controls.

Methods: Cases were randomly recruited from the Regional Autism Center (RAC) at The Children's Hospital of Philadelphia. RAC cares for over 2000 children with ASD, approximately 50% of the school age children with ASD in the state of Pennsylvania. By selecting a random sample, the prevalence of sleep disturbances can be estimated. Case diagnosis was confirmed with the DSM-IV-TR, ADOS-G or ASDS. Controls were screened with the SCQ. Sleep behaviors and quality are described utilizing a questionnaire, CSHQ, sleep diaries and 10 nights of actigraphy. The actigraph was placed into a small pocket, secured in place with Velcro, on the non-dominant shoulder area of a snug fitting T-shirt. Actigraphic raw data were translated into sleep measures with the Actigraphic Scoring Analysis (ASA) program for an IBM-compatible PC, using the Sadeh algorithm (Sadeh et al., 2000).

Results: To date, data have been collected on 16 cases (mean age of 6.8) and 22 controls (mean age of 7.3). The ASD group includes 2 children with Asperger, 7 children with PDD-NOS and 7 children with Autism, 4 females and 12 males, 31.5% minority. The control group has 10 females and 12 males and a 25% minority representation. 60% of children with ASD are taking a medication to aid sleep. No controls are taking sleep aids. Preliminary analyses show that ASD cases have longer sleep latency ($p<0.006$) and shorter total sleep time ($p<0.028$) as compared to typically developing controls. There is also a trend toward decreased sleep efficiency in ASD children compared to controls ($p<0.0564$). These actigraphy data suggest that children with ASD have significant sleep disruption compared to control subjects when assessed by objective measures. Moreover, there is phenotypic variability among ASD children with regard to these key measures. Also, parents of children with ASD report greater sleep anxiety, parasomnias and daytime sleepiness on the CSHQ compared to parents of control subjects.

Conclusion: Strong descriptive epidemiological data on a well described ASD group compared to controls utilizing standardized measures of sleep behaviors and quality will provide a foundation for future studies of etiology and intervention.

Trainee Award Eligible

Support is Essential for Heart Failure Patients with Excessive Daytime Sleepiness

Riegel B, Dickson V, Goldberg L

Introduction: Social support is widely regarded as an important contributor to success in managing chronic illness. Yet, clearly some patients need support more than others. We hypothesized that patients with heart failure (HF) who were sleepy would need more support for self-care than HF patients who were not sleepy.

Methods: A cross-sectional study was conducted with 117 out-patients with HF recruited from the HF clinic of a large urban university-affiliated medical center. Social support was measured with a single item asking, "How would you rate the quality of the support you receive from others?" and evaluated on a 4-point scale from poor to very good. Self-care was measured with the management scale of the Self-Care of HF Index (SCHFI), which reflects decision-making required to respond to HF symptoms. Scores are transformed to a 100-point scale. Sleepiness was measured using the Epworth Sleepiness Scale (ESS), a measure of the propensity to doze in various situations. Scores on the ESS were categorized as sleepy (>10) or not sleepy (≤10). Linear regression analysis was used to test the contribution of support to self-care management in sleepy and not sleepy HF patients.

Results: 94 of 117 (80%) of the patients were symptomatic and able to judge their ability to manage their HF symptoms. Self-care management scores were poor (68.98 ± 18 out of 100) and support ratings were high (3.5 ± 0.7 out of 4) overall. Only 30% of the sample was sleepy, but in these patients, support was a significant predictor of self-care management (R^2 = 38.5, p=0.002). When the relationship was retested in those who were not sleepy, no relationship was evident (R^2=0.01, p=0.39).

Conclusion: Support is particularly important for HF patients who are sleepy. This patient population has numerous risk factors for sleepiness (e.g., sleep-disordered breathing, medication regimens that fragment sleep, nocturia), so screening for excessive daytime sleepiness is essential. In those who are sleepy, assuring that support is available may be one way of increasing patient self-care and improving clinical outcomes.

Conflict of interest: None.

New Synthesis of Cervical Trkb Protein is Necessary for A2a Receptor-Induced Phrenic Motor Plasticity

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Adenosine 2a receptor agonists can mimic neurotrophins by transactivating Trk receptors *in vitro*. Here we show that A2a receptor activation *in vivo* transactivates TrkB receptors near phrenic motoneurons in rats, which in turn elicits long-term increases in phrenic motor output. Adult male Sprague Dawley rats were anesthetized, vagotomized, neuromuscularly paralyzed, and mechanically ventilated. A phrenic nerve was isolated, cut distally, and the proximal stump placed on bipolar recording electrodes. Phrenic motor activity was recorded continuously throughout the experiment. An intrathecal catheter was placed between the first and second cervical vertebrae with the tip of the catheter overlying the C4 spinal segment (which contains the majority of phrenic motoneurons in rats). A stimulating electrode was positioned in the ventrolateral funiculus at the C2 spinal segment to evoke short-latency (< 0.7 ms) phrenic compound action potentials before and after intrathecal drug administration. After recording baseline spontaneous phrenic motor output and evoked potentials, an A2a receptor agonist, CGS 21680 (2 $\mu g.kg^{-1}$), was injected intrathecally over C4. We found that spinal A2a receptor activation strengthened synaptic pathways to phrenic motoneurons (evoked potential facilitation: 23 ± 5%; of baseline, $p<0.05$) and induces facilitation of spontaneous phrenic motor output (90 ± 11%; of baseline at 2 hr post-injection; $p<0.05$).

To investigate possible TrkB-dependent mechanisms contributing to A2a-induced phrenic motor facilitation, we harvested the C4 spinal segments at the end of each experiment. A2a receptor activation increased expression of an immature TrkB isoform (80 kD) in the ventral cervical spinal cord (western analysis: 31 ± 9%, above control; $p<0.05$). Immunohistochemistry localized the increase in TrkB protein to within C4 motoneurons. To determine the role of TrkB synthesis in A2a-induced phrenic motor facilitation, siRNAs targeting TrkB mRNA (50 nM) were injected intrathecally 2 hrs prior to CGS 21680. TrkB RNAi abolished A2a-induced phrenic motor facilitation and prevented new synthesis of 80 kD TrkB. Intrathecal CGS 21680 also increased phosphorylation of the immature ($TrkB_{80}$: 23 ± 5% above controls, $p<0.05$), but not mature ($TrkB_{150}$), TrkB isoform. Activation of TrkB protein occurred without changes in expression of the TrkB ligand, BDNF (measured using ELISA). TrkB RNAi prevented A2a-induced phosphorylation of TrkB protein. Furthermore, pretreatment with the Trk inhibitor, K252a (100 $\mu g.kg^{-1}$, intrathecally), blocked A2a-induced phrenic motor facilitation. Thus, new synthesis and phosphorylation of immature (presumably intracellular) TrkB protein is necessary for A2a-induced phrenic motor plasticity. These experiments represent the first description of Trk receptor transactivation *in vivo*. A2a receptor agonists may provide a novel approach to treating patients with motor impairments, such as occur with spinal cord injuries and ALS.

Support: NIH grant HL69064.

Towards Understanding the Relationship Between Obstructive Sleep Apnea, Insulin Resistance and Visceral Fat

Ahmed MM, Teff K, Gooneratne N, Tadesse M, Schwab RJ, Pack AI

Introduction: The goal of our proposed study is to further characterize the relationship between obstructive sleep apnea (OSA) and insulin resistance (IR), accounting for the potential confounding effects of visceral adipose tissue (VAT) volume. We hypothesize that given our current understanding of the disease process, OSA will be associated with IR, independent of VAT. IR has a well-described role in the pathogenesis of type 2 diabetes mellitus and cardiovascular disease. The consequences of OSA are far-reaching with significant morbidity and mortality. Prior authors have demonstrated an association between OSA and IR, independent of obesity. Visceral adipose tissue (VAT) is the most metabolically active adipose tissue and plays a central role in the development of IR. Conventional anthropometric measures assess VAT poorly. Moreover, measures of VAT are also associated with OSA severity. Prior studies examining the relationship between OSA and IR have not accounted for VAT in their observations.

Study Design, Methods and Planned Analysis: We have proposed a cross-sectional study to test our hypothesis that OSA will be related to IR independent of VAT. The Iceland Sleep Apnea Cohort (ISAC) study is a large longitudinal study taking place in Iceland to identify key genetic polymorphisms associated with OSA. We propose measuring apnea severity, VAT volume and IR at study enrollment in this cohort. IR will be measured using the well-validated homeostatic model of assessment (HOMA-IR) and will be treated as a continuous outcome variable. All PSG's will be read by a certified sleep research technician at the Penn Sleep Reading Core Lab. VAT will be measured using MRI following a standard, validated protocol to measure fat content. Univariate analyses will allow us to determine crude rates of association between outcome and exposure variables. Stratified analysis will allow us to control for the effects of VAT. Finally, we will develop an explanatory multivariate model using linear regression methods. Our a priori analysis of power demonstrates that given the fixed sample size of 250 patients in the parent study, power (1-beta) = 0.99.

Strengths, Limitations and Future Directions: Our cross-sectional design is an appropriate preliminary step in examining the relationship between OSA and IR. This will be a relatively large study with adequate power and uses definitive measures of VAT and a well-validated measure of IR. However, our design allows only for demonstration of association, not causality. In addition, the time sequence of exposure and outcome cannot be known. However, it does set the stage for planning future assessments in the cohort after initiation of CPAP. Furthermore this work will contribute towards more detailed characterization of OSA patients hopefully allowing us to identify those at greatest risk of mortality and potentially intervening earlier.

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OSA Symptoms Are More Common Among African American than Caucasian Women

Beothy EA, Sammel MD, Freeman EW, Lin H, Pien GW

Introduction: Recent studies suggest that African Americans are at greater risk for obstructive sleep apnea (OSA) than Caucasians. In addition, postmenopausal women have an increased risk of OSA compared to premenopausal women. We examined the relationship between race, menopausal status and OSA symptoms in a large group of African American women using the Multivariable Apnea Prediction (MAP) Index, which asks about OSA symptoms and is helpful in determining the likelihood of having OSA.

Methods: The Penn Ovarian Aging Study (POAS) is a longitudinal cohort study of African American and Caucasian women going through the menopausal transition. We administered the MAP questionnaire to 269 POAS participants 8 years after study inception. Linear regression analyses were performed to examine relationships between race, menopausal status, BMI and change in BMI (baseline to time of questionnaire administration) and the MAP apnea subscale score.

Results: Mean subject age was 48 (SD 3.5) years. 49.4% of subjects were African American. Women were classified as premenopausal, early transition, late transition or postmenopausal as defined by bleeding patterns; using these criteria (PENN-5), 37.5% of women were premenopausal, 43.0% in the menopausal transition and 19.5% were postmenopausal. In unadjusted analyses, the mean apnea score among African American women was nearly double that of Caucasian women (0.79 (SE 0.08) v. 0.39 (0.08), p=0.0009). Menopausal status was not a significant predictor of OSA symptoms. Race remained a significant predictor of OSA symptoms (p=0.04) after adjustment for current BMI, change in BMI over time, and menopausal status.

Conclusions: Middle-aged African American women are more likely to experience symptoms of OSA than their Caucasian counterparts. Although menopausal status did not predict OSA symptoms, OSA symptoms in our cohort of menopausal women increased with higher BMI and larger increases in BMI over time. Studies to document whether OSA is more common among African-American than Caucasian women should be performed to further investigate these findings.

Support: Supported by grants from the National Institutes of Health (K23 HD41465, R01 AG12745).

Development of Icelandic Sleep Apnea Cohort (ISAC)

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Introduction: Iceland is a small island in the middle of the Atlantic. It has several advantages for biomedical research: a) the population is relatively small (of the order of 300,000), but large enough to study common disorders such as obstructive sleep apnea; b) it has one health care delivery system with excellent medical records; c) about ¾ of the population live in the capital (Reykjavik) or immediate surrounds; d) the relationship of every living Icelander can be determined from the genealogy database; e) the population is extremely supportive of biomedical research and recruitment rates are high.

Methods: To take advantage of this, we have established a collaboration with the University of Iceland, the Sleep Center at Penn and deCODE Genetics (CEO: Dr. K. Stefansson). Over the last 2.5 years the group at the University of Iceland Hospitals has recruited 2 main groups of subjects: a) all patients in Iceland currently on CPAP (retrospective sample) and b) all patients newly diagnosed with OSA. In the latter group we obtain much more phenotyping data—abdominal and upper airway MRI, serum for biomarkers, fasting insulin and glucose. We call the former the Icelandic Sleep Apnea Cohort (ISAC) and the latter JACOB (son of ISAC) (Junior Apnea Cohort with OBservations). For both cohorts all subjects have an overnight sleep study and all of these are being scored at Penn.

Results: DNA has also been obtained on all subjects in both groups. A genetic linkage study is currently in process. A future genome-wide association study is in the planning stage.

Discussion: This is a rich resource for future patient-oriented research.

Support: NIH grant HL072067.

Sleep, Breathing, and Neurobehavior in COPD: Pilot Study

Reishtein JL, Kuna ST, Weaver TE

Introduction: Deficits in neurobehavior have been shown in COPD, possibly associated with the poor sleep and oxygen desaturations experienced by patients. There has been limited exploration of these associations. We present preliminary data describing the association between pulmonary parameters and sleep quality, and the impact of sleep quality on neurobehavior.

Methods: Subjects with COPD (FEV_1 < 60% predicted for gender, age, and height) were recruited from a pulmonology clinic during routine visits. For one week, subjects wore an ambulatory pulse oximeter (one day) and accelerometer, and completed a sleep diary. All subjects completed the Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality Index (PSQI), a 4 word memory recall test, a 10 minute psychomotor vigilance task (PVT), a computerized digit-symbol substitution test (DSST), and a finger tapping test.

Results: Nine subjects (mean age 65.0 $\pm$ 7.29 years, FEV_1 45.50 $\pm$ 13.06% predicted, and FEV_1/FVC 51.13 $\pm$ 15.83) have been studied. Sleep quality is below normal; with PSQI global scores ranging from 5 to 11 (mean 7.78 $\pm$ 2.11). PSQI correlated moderately with number of minutes during sleep SO_2 < 88% (r = 0.45), but minimally with baseline SO_2 or the SO_2 nadir. Additionally, PSQI had a moderate correlation with FEV_1% predicted (r = -0.44), but small correlation with FEV_1/FVC (r = -0.27). Sleep quality also demonstrated small to moderate correlations with neurobehavioral measures, including short term memory (r = -0.36), PVT (reaction time r = 0.69, trend p = 0.08; lapses r = 0.59), finger tapping (r = -0.46), and executive function (DSST r = -0.24).

Conclusion: Although we present a small sample size as data collection is ongoing, these preliminary data suggests that pulmonary parameters are related to sleep quality and neurobehavioral performance. The nature of this relationship will be delineated in the modeling of these variables using a larger sample.

Support: Hartford Center for Geriatric Nursing Excellence Frank Morgan Jones Fund grant for pilot study, University of Pennsylvania; T32-HL07953.

Microinjections of Clonidine into the Noradrenergic A7 Cell Region Reduce Hypoglossal (XII) Nerve Activity in Urethane-Anesthetized Rats

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Introduction: The withdrawal of noradrenergic excitation from XII motoneurons by local microinjections of an α1 adrenergic antagonists into the XII nucleus region reduces XII nerve activity and nearly eliminates any further decrement of that activity during experimentally induced REM sleep-like state (Fenik *et al.*, 2005). Since pontine A7 neurons send major noradrenergic projections to the XII nucleus (Rukhadze & Kubin, 2007), we investigated whether selective inhibition of A7 neuronal activity results in a reduced XII nerve activity.

Methods: In 13 urethane-anesthetized, paralyzed, vagotomized and artificially ventilated rats, we recorded XII nerve activity, arterial blood pressure, hippocampal activity and cortical EEG. Clonidine, an α2-adrenergic agonist that inhibits noradrenergic cells, was microinjected (0.75 mM, 20-40 nl) aiming at the A7 group on one side, or sequentially both sides. Brainstem sections were immunostained for tyrosine hydroxylase (TH) and, in some rats, also for c-fos as marker of cell activity.

Results: Ten of the 21 clonidine injections made to date were placed within less than 200 μm from the A7 group. These injections caused a 29% ± 3 (SE) ($p<0.01$) decrease of XII nerve activity and only a small reduction of the respiratory rate (from 40 min^{-1} ± 1 to 37 min^{-1} ± 1, $p<0.05$). The remaining 11 injections were placed farther away from the A7 group and were ineffective. In 3 rats with unilateral clonidine injections placed close to the A7 group, a lower percentage of TH-positive A7 neurons expressed c-fos on the injected side (24% ± 9) than on the opposite side (41% ± 4), indicating that clonidine was effective in reducing the level of activity in norepinephrinergic neurons located near the injection site.

Discussion: We conclude that A7 neurons are an important source of noradrenergic excitation that significantly contributes to the maintenance of spontaneous XII nerve activity. A silencing of A7 cells that is likely to occur during sleep (see abstract by Rukhadze *et al.* at this Retreat) can, therefore, contribute to the depression of upper airway motor tone.

Support: HL-47600.

Conflict of interest: None.

Loss of Hypoglycemic Arousal after Recurrent Hypoglycemia in Juvenile Rats

Tkacs N, Pan Y, Morrison AR

Introduction: Nocturnal hypoglycemia that fails to result in arousal from sleep is common in children, adolescents, and adults with type 1 diabetes mellitus. Recurrent nocturnal hypoglycemia contributes to and sustains hypoglycemia-associated autonomic failure and hypoglycemia unawareness.

Methods: We tested the ability of three episodes of insulin-induced hypoglycemia in young rats to alter arousal responses to hypoglycemia tested three weeks later. Prepubertal rats (ages 25-28 days) were given three days of insulin injections to induce hypoglycemia averaging 34 mg/dl, lasting at least 60 minutes (Juv Hypo, n=5). Control rats were given saline injections (Juv Control, n=8). Rats were recovered for three weeks, during which they were surgically prepared for sleep recordings (implantation of EEG and EMG electrodes) and for blood sampling (jugular venous catheter). At age 7 weeks, after habituation to the recording conditions, sleep was recorded from 1 PM to 3 PM on a baseline day (saline 1 ml/kg injected subcutaneously at 1 PM). The following day, sleep was recorded from 1 PM to 3 PM after a 1 PM injection of insulin (3 U/kg sc).

Results: During sleep recording, the plasma glucose response to insulin was not different between groups, averaging 53 ± 2 mg/dl in Juv Control rats, and 54 ± 4 mg/dl in Juv Hypo rats. Juv Control rats demonstrated a robust hypoglycemic arousal response. Percent time awake on the baseline day was 18.8 ± 1.9, while the percent time awake on the insulin day was 40.7 ± 2.4, a 233% increase in time awake during hypoglycemia. Juv Hypo rats had no arousal response to insulin injection, with 21.1 ± 1.4% time awake on the baseline day, and 21.0 ± 2.1 % time awake during hypoglycemia. There was a significant effect of day (saline vs. insulin) as well as group (Juv Control vs. Juv Hypo) on the percent time spent in Wake (effect of day: $F=24.3$, $p < 0.001$; day*group interaction: $F=24.6$, $p < 0.001$). There was a significant interaction of group and day on non-rapid eye movement (REM) sleep (day*group interaction: $F=9.4$, $p=0.011$), but not on REM sleep.

Discussion: Our data show that significant loss of hypoglycemic arousal can be demonstrated weeks after recurrent juvenile hypoglycemia in rats. We expect that this model will be very suitable for translational interventional studies of treatments for this serious complication of insulin treatment for diabetes mellitus.

Support: Juvenile Diabetes Research Foundation Innovative Award to N. Tkacs

Conflict of Interest: The authors declare no conflict of interest.

Predilection for Amyloid Precursor Protein Accumulation in GABAergic REM Sleep Neurons with Early Dysfunction in a Murine Model of Alzheimer's Disease

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Introduction: Alzheimer's disease results in significant sleep disturbances. Sleep/wake disturbances in Alzheimer's disease include impaired or blunted circadian rhythms, reduced or absent stage III/IV NREM sleep, behavioral state instability for sleep and wake states, and impaired REM sleep. We have previously shown that reductions in REM sleep occur at 6 months of age in the most widely used model of Alzheimer's disease, the Tg2576 mouse. Although memory impairments also occur at about the same age, there are several advantages to using REM sleep to study the onset of neuronal dysfunction. In light of the well-defined GABAergic and cholinergic neuronal circuitry underlying REM sleep and a gradual loss of REM sleep, deterioration in REM sleep should provide a valuable tool with which to understand the mechanisms underlying early injury in Alzheimer disease. We hypothesized that impairments in REM sleep rebound after sleep deprivation might occur prior to reductions in baseline REM sleep.

Methods: Tg2576 mice and wild type littermates at 6-7 wks of age were implanted for EEG/EMG recordings of baseline, 6 hr sleep loss and recovery sleep. REM sleep rebound was not present in the majority of Tg2576 mice (12±4 mins/hr vs. 17±6 mins/hr). Mice were perfused with paraformaldehyde, and brains were cryopreserved and sectioned for double label immunohistochemistry (GAD67 and human amyloid precursor protein). Silver labeling of amyloid precursor protein was used to enhance visualization.

Results: In the brains of mice with impaired REM sleep rebound, amyloid precursor protein was localized specifically in the GABAergic neurons in the ventrolateral periaqueductal gray and sub locus coeruleus. Immunoelectron microscopy revealed swollen endoplasmic reticulum with amyloid precursor protein in GABAergic neurons suggesting early endoplasmic reticulum injury in GABAergic REM on neurons in this model.

Discussion: Thus, there is early involvement in the brainstem in this model of Alzheimer's disease, and this injury appears to primarily involve the GABAergic REM sleep on neurons. Identification of a neuron, an affected organelle and early behavioral abnormality should facilitate elucidation of early injury in Alzheimer's disease.

Conflict of Interest: There are no conflicts of interest.

Activation of 153GADD/CHOP in Upper Airway Motoneurons in a Murine Model of Sleep Apnea

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Introduction: Obstructive sleep apnea is a common syndrome with significant neurological and cardiovascular sequelae. The disease typically progresses over time. Mechanisms of progression of the disease are unknown. In a rodent model of sleep apnea oxygenation patterns, we have identified impaired whole nerve responses to serotonergic and glutamatergic excitation in the hypoglossal nucleus. We hypothesize that endoplasmic retitculum stress is present at baseline in upper airway motoneurons, and that with exposure to the oxygenation patterns of sleep apnea, this stress is augmented and injurious.

Methods: To test this, we exposed C57BL/6J adult male mice to long-term (8 wks) intermittent hypoxia, and then procured tissue for immunoblotting, immunofluorescence and electron microscopy to describe the effects of long-term intermittent hypoxia (LTIH) on ER stress in the motoneurons.

Results: GRP78(BiP) and p-PERK were evident in motoneurons at baseline and did not increase following exposure to LTIH. In contrast, 153GADD/CHOP and caspase 12 immunofluorescence increased by over 50% in the facial, dorsal motor nucleus of vagus and hypoglossal nuclei ($p<0.05$, $p<0.001$, $p<0.01$, respectively). Electron micrographs of choline acetyltransferase peroxidase-labeled motoneuron sections with gold-labeled 153GADD/CHOP showed that LTIH resulted in nuclear translocation of 153GADD/CHOP and marked upregulation in both dendrites and soma. Mitochondrial swelling and increased autophagy were also observed. In light of the important role of SOD1 aggregates in neurodegenerative motoneuron disease, we examined with western SOD1 aggregation, finding marked misfolding and dimerization and polymerization of SOD1.

Discussion: We hypothesize that oxygenation patterns observed in the millions of individuals with obstructive sleep apnea result in substantial damage to motoneurons, including the upper airway motoneurons and this may contribute to disease progression. In addition, we hypothesize an important disease interaction between severe obstructive sleep apnea and motoneuron diseases, including amyotropic lateral sclerosis.

Support: This work was supported in part by NIH HL080492.

Frequency of Intermittent Hypoxia Influences iNOS Protein Expression in Macrophages

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Introduction: Many disease states result in dynamic fluctuations in tissue pO_2, e.g., high frequency cyclical recruitment in ARDS and moderate frequency nocturnal desaturations in obstructive sleep apnea. Until recently, *in vitro* studies have been unable to reproduce moderate to high frequency intermittent hypoxia (IH). We have previously demonstrated that 6 hours of moderate frequency (30 cycles per hour) IH (between 40 and 8 Torr O_2) is a potent stimulus for the induction of inducible nitric oxide synthase (iNOS) gene, iNOS protein and the metabolites of NO synthesis, nitrite and nitrate. We hypothesized that 6 hours of high frequency (10 cycles per minute) IH (between 40 and 8 Torr O_2) would induce iNOS protein expression and NO production.

Methods: RAW 264.7 macrophages were cultured in a specially designed forced convection culture system. Cultures were randomly assigned to sustained hypoxia (8 Torr O_2; n=6), IH (cycles of 40Torr O_2 for 6 sec and 8 for 6 sec; n=6), or sustained normoxia (40 Torr O_2; n=6) for 6 hours. Western blot of cell lysates were probed for inducible nitric oxide synthase (iNOS). The signal was normalized to a constitutive protein (Raf) and compared by one-way ANOVA.

Results: Culture of macrophages for 6 hours at 8 Torr induced significantly more iNOS than cells exposed to high frequency IH or normoxia ($p<0.001$). Moderate frequency (30 cycles/hour) IH resulted in significantly more iNOS than high frequency IH with 10 exposures to 8 Torr per minute.

Conclusions: Although the cumulative time of hypoxic exposure was twice as long (180 min) in high frequency IH as in moderate frequency IH (i.e., 90 min from 6 hours of IH with 30 seconds at 8 Torr, every 2 minutes), high frequency IH failed to induce iNOS. The design of the system provided identical peak and nadir at all frequencies. These results suggest that frequency of intermittent hypoxia is more important than the cumulative duration of hypoxic exposure.

Support: NIH GM64486.

Fos Expression in Pontine Noradrenergic Neurons Negatively Correlates with the Duration of Carbachol-Induced REM Sleep-Like State in Urethane-Anesthetized Rats

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Introduction: Noradrenergic (NE) neurons of the locus coeruleus (LC) and A5 group are silenced during both rapid eye movements sleep (REMS) and REMS-like state elicited in urethane-anesthetized rats by pontine microinjections of carbachol (Aston-Jones & Bloom, 1981; Fenik *et al.*, 2002). Withdrawal of noradrenergic excitation is a major factor contributing to REMS-related depression of activity in hypoglossal (XII) motoneurons that innervate the genioglossus, an important upper airway dilator (Fenik *et al.*, 2005). NE projections to XII motoneurons originate in multiple pontomedullary NE cell groups (Rukhadze & Kubin, 2007), including A7 that has yet unknown pattern of activity across the sleep-wake cycle. Our goal was to determine whether c-Fos immunohistochemistry can be used to assess the behavior of pontomedullary NE cells during experimentally induced REMS-like state and then use this approach to study the behavior of A7 neurons. We used a model of REMS in which, in anesthetized rats, dorsomedial pontine microinjections of carbachol can repeatedly elicit multiple REMS-like episodes (Kubin, 2001).

Methods: In 17 urethane-anesthetized, paralyzed, vagotomized and artificially ventilated rats, we recorded the cortical EEG, hippocampal and XII nerve activity, and arterial blood pressure. Rats received different numbers of pontine carbachol (10 mM, 10 nl) or saline (10 nl) injections that over a 3 h period prior to sacrifice produced REMS-like episodes of a total duration that ranged from 0 to 71 min. Brainstem sections were immunohistochemically processed for Fos and tyrosine hydroxylase (TH), markers for cell activity and catecholaminergic neurons, respectively. Fos-positive cells in A1, A1/C1, C1, A2, caudal A5, rostral A5, A7 and sub-coeruleus (SubC) regions were counted relative to all TH-positive cells in these groups. The percentage of those expressing Fos was then correlated with the total duration of REMS-like state using a linear regression.

Results: The percentage of Fos-positive TH cells was negatively correlated with the total duration of REMS-like state for the A7 ($R=-0.72$, $P<0.001$) and rostral A5 ($R=-0.70$, $P<0.01$) groups bilaterally and for SubC region ($R=-0.53$, $P<0.05$) contralaterally to the carbachol injection site. In A1, A1/C1, C1, A2 and caudal A5 neurons, a similar trend occurred but was not statistically significant. There was no correlation with the total dose of carbachol injected for any of the NE groups, whereas Fos expression in pontine reticular formation neurons located adjacent to the injection site and directly affected by the drug was positively correlated with the dose of carbachol ($R=0.72$, $P<0.01$).

Discussion: The negative correlation between Fos expression in A5 and SubC neurons and the duration of REMS-like state is consistent with silencing of these cells during REMS, as previously determined by electrophysiological recordings. This validates the use of Fos as a marker of reduced NE cell activity. Our result with A7 cells shows that, similar to A5 and LC neurons, they also reduce their activity during REMS-like state. Their silencing may, therefore, contribute to upper airway hypotonia during REMS.

Support: HL-47600.

Conflict of interest: None.

Trainee Award Eligible

Long-Term Effects of Cued Fear Conditioning On REM Sleep Microarchitecture and Phasic Activity in Rats

Madan V, Brennan FX, Ross RJ, Horbal AA, Dunn GA, Mann GL, Morrison AR

Introduction: Re-exposure to a fear-conditioned cue (CS) 24 hr post-conditioning (short-term effect, Day 1) alters REM sleep (REMS) architecture. To extend our findings, we investigated the disturbances in REMS microarchitecture and REMS phasic activity (myoclonic twitches) 14 days post-conditioning (long-term effect). In addition, we examined the effects of re-exposure to the CS on freezing, a common behavioral index of fear.

Methods: Male Sprague-Dawley rats (n=6) were prepared for polysomnographic recording and habituated to a neutral chamber. One day after a 4-hr baseline sleep recording (BL), they were presented, at 30-sec intervals, with five 5-sec tones, each co-terminating with a 1-sec, 1 mA footshock. Rats were returned to the neutral chamber on Day 1 and again on Day 14, and were presented with five tones on each occasion. Behavior was videotaped for offline scoring of freezing, and sleep was recorded for four hr. The number of myoclonic twitches during REMS was counted.

Results: Significant alterations in REMS microarchitecture were observed on Day 14. There were significant decreases in the amount of time spent in sequential REMS (REMS episodes at $\geq$ 3-min interval) (BL: 13.4$\pm$0.7 min; Day 14: 5.2$\pm$2.8; $p<0.05$) and the number of sequential REMS episodes (BL: 9.2$\pm$1.3; Day 14: 3.5$\pm$2.0; $p<0.05$). There were significant increases in the total amount of time spent in single REMS (REMS episodes at > 3-min interval) (BL: 16.2$\pm$1.5; Day 14: 27.0$\pm$2.8; $p<0.001$) and the number of single REMS episodes (BL: 7.0$\pm$0.4; Day 14: 10.7$\pm$1.2; $p<0.05$). Also, there were significant increases in freezing ($p<0.001$) and myoclonic twitches ($p<0.05$) on Day 14. Changes in REMS microarchitecture and twitches correlated significantly with freezing behavior.

Conclusion: Fear-induced alterations in REMS microarchitecture and phasic activity could depend on the incubation of fear memories over 14 days.

Support: Supported by R01-MH072897.

Conflict of interest: None.

SLEEP RESTRICTION & SLEEP PURPOSE: OBSERVATION & CONSEQUENCES

Macromolecule Biosynthesis - A Key Function of Sleep

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Introduction: Genomic approaches have been successfully applied to studies of gene expression in the central nervous system during sleep and wakefulness; however, the temporal dimension has not been thoroughly studied. We therefore sought to identify dynamic changes in gene expression associated with different durations of sleep and extended wakefulness. We proposed that determining genes which change expression during sleep can point to possible function(s) of sleep.

Methods: Male C57BL/6 mice were subjected to 3, 6, 9 and 12 hrs of total sleep deprivation (n=5/time point). Sleeping mice were left undisturbed, and were sacrificed at the same diurnal time points as sleep deprived mice. Transcript levels in the cerebral cortex and hypothalamus were assayed by microarrays using the GeneChip Mouse Genome 430 2.0 array.

Results: There were significant differences in gene expression between behavioral states; we identified 3988 genes in the cerebral cortex and 823 genes in the hypothalamus with altered expression patterns between behavioral states; 2090 genes in the cerebral cortex and 409 genes in the hypothalamus were defined as changing during sleep. The largest classes of over-represented genes increasing expression with sleep were those involved in biosynthesis and transport. There was up-regulation of genes encoding enzymes involved in cholesterol synthesis and proteins of lipid transport, genes involved in synthesis of proteins, heme, maintenance of vesicle pools, as well as antioxidant enzymes and genes encoding proteins of energy-regulating pathways.

Conclusions: We postulate that during sleep there is rebuilding of multiple key cellular components in preparation for subsequent wakefulness.

The Relationship between Short Sleep Duration and Obesity in Adolescents

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Introduction: Obesity continues to be a major health problem because of increasing prevalence and relation to medical consequences such as increased risk for diabetes mellitus, hypertension, heart disease, and cancer (Healthy People 2010). Obesity also has important social and economic consequences, accounting for almost $100 billion in obesity-related costs in the United States (U.S.). The identification of potentially modifiable risk factors, such as sleep duration, for increased body weight could lead to preventative strategies. Short sleep duration produces hormonal changes that have been associated with an increased risk of obesity. There has been limited study of the relationship between sleep duration and obesity in young people. Therefore, the purpose of this study is to determine the association between short sleep duration in adolescents and weight gain leading to obesity. Further association of short sleep duration and obesity will be evaluated in light of ethnicity and low socioeconomic status, as several studies have shown that low socioeconomic status (SES) and minority groups have a higher prevalence of obesity.

Methods: Specific aims achieved through analysis of data from the National Longitudinal Study of Adolescent Health (Add Health), a survey of 90,000 youths, grades 7 to 12, that provides national representation of the U.S. middle and high school populations in three waves, spanning from 1994~2002. Add Health explores the causes of health-related behaviors of adolescents and their outcomes in young adulthood. Add Health also seeks to examine how social contexts (families, SES, ethnicity, friends, peers, schools, neighborhoods, and communities) influence adolescents' health behaviors. The study population (n=13,000) will be obtained from Wave I survey (1994~1995) and Wave II follow-up survey (1996~1998).

Statistical Analysis: The primary outcome for this study is BMI>95th percentile. The primary analysis included descriptive statistics, bivariate, and multivariable analyses to further explore the relationships between the independent variables of interest and BMI.

Results: Thirty-six percent of adolescents slept 8 h each night in Wave I; 35% slept 8 h in Wave II. Adolescents who slept <6 h per night were almost twice as likely to have increased BMI compared to those who slept 6 to 8 hours per night, specifically in males (p<0.001). Longitudinal analyses suggest the effect of shortened sleep duration in Wave I significantly predicted BMI in Wave II (p<0.01). Analyses of income demonstrated that children with <6 h sleep, and in a lower income household, had increased BMI in Wave II (p<0.0001). Hispanics, Asians and non-Hispanic blacks had significantly less sleep that non-Hispanic white children (p<0.0001, p<0.01, and p=0.0002, respectively). Longitudinal analyses suggest an interaction effect of race (non-Hispanic black and Hispanic) and shortened sleep duration in Wave I as significantly predictive of BMI in Wave II (p<0.0001 and p=0.001, respectively).

Discussion: By understanding shortened sleep duration and association with weight gain leading to increased BMI, future studies can then focus on tools for health care providers to identify and then intervene with behavior related to shortened sleep. Improvement in sleep, therefore, can lead to a decrease in comorbidities such as obesity.

Conflict of Interest: There is no conflict of interest for the authors.

Effects of Inadequate Recovery from Sleep Restriction on the Inflammatory Marker C-Reactive Protein

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Introduction: Short sleep durations are associated with increased cardiovascular morbidity. One inflammatory marker associated with cardiovascular risk is high-sensitivity C-reactive protein (CRP), which has also been found to increase significantly in response to severe sleep debt. However, it remains unclear if milder sleep deprivation has similar effects on CRP.

Methods: 91 healthy adults (42 men and 49 women; 30.1±6.7 years) were randomized to either a sleep deprivation or control group following two nights of baseline sleep. In the deprivation condition, participants underwent 5 nights of partial sleep restriction (4h time in bed [TIB]), followed by two nights of randomly assigned recovery sleep. The sleep from both recovery nights were pooled and categorized as either "high recovery" (12-20h total TIB) or "low recovery" (0-10h total TIB). Control participants spent 10h TIB/night. Blood samples were collected prior to sleep restriction (BD1), following the fifth night of 4h TIB (BD2), and following the second night of randomized recovery sleep (BD3).

Results: Preliminary analyses showed no statistical effect of the 5-night partial sleep deprivation on CRP levels (comparison of BD1 and BD2); however, additional sleep deprivation was associated with increased CRP levels at BD3. Data analyses on log-transformed CRP values revealed a significant increase in CRP levels between BD1 and BD3 ($t=-2.22$, $p=0.026$) in the "high recovery" group and a significant increase in CRP between BD2 and BD3 ($t=-2.19$, $p=.029$) in the "low recovery" group. No comparisons within the control condition reached statistical significance.

Conclusions: Although a five-day period of partial sleep deprivation was not sufficient to produce a significant increase in CRP levels in healthy adults, an additional night of sleep restriction (as occurred in the "high" and "low" recovery groups) resulted in elevated CRP levels. These findings suggest that even modest sleep restriction may potentiate inflammatory processes.

Support: NIH/NINR R01 NR04281.

The Role of NMDA Receptors in Sleep-Dependent Cortical Plasticity

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Introduction: We have previously shown that sleep enhances a canonical form of *in vivo* synaptic remodeling in the visual cortex (V1), triggered by monocular deprivation (MD) during a critical developmental window (known as ocular dominance plasticity [ODP]). The effects of sleep on ODP are mediated by unknown, activity-dependent mechanisms.

Methods: We investigated the role of NMDA receptors (NMDArs) in this process by infusing the NMDAr antagonist APV into V1 during a 6-hour sleep period following brief MD. ODP was assessed by intrinsic signal imaging and single-unit recording of responses in V1 to stimuli presented to the deprived and non-deprived eyes (DE and NDE, respectively).

Results: A large shift in neuronal responses toward the open eye occurred when V1 was infused with vehicle during post-MD sleep, but this shift was abolished in V1 infused with APV. Further analyses revealed that V1 changes underlying ODP in vehicle-infused cats involved both depression of DE responses and potentiation of NDE responses. MD alone (without subsequent sleep) induced depression of DE responses, but failed to potentiate NDE responses. APV treatment during post-MD sleep appeared to selectively block the sleep-dependent potentiation of NDE responses, and also blocked maintenance of DE depression during sleep.

Conclusion: These findings demonstrate that sleep promotes synaptic remodeling by strengthening synapses via NMDAr-dependent cellular mechanisms.

Support: This work supported by NIH R01 MH067568 and a National Sleep Foundation Pickwick Postdoctoral Fellowship.

Conflict of Interest: The authors have no conflicts of interest to disclose.

Trainee Award Eligible

Changes in Hippocampal Gene Expression After Sleep Deprivation in Mice

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Introduction: The present study was conducted to determine the effects of sleep deprivation on gene expression within the hippocampus in order to better understand the molecular basis of hippocampal-dependent memory deficits related to sleep deprivation.

Methods: Two groups of C57BL/6J mice (n=9-10 per group) were either sleep deprived by gentle handling for 5 hr or allowed to remain undisturbed in their home cages. Transcriptional changes were then examined using RNA extracted from hippocampi using the Affymetrix mouse genome 430v2 microarray. RNA from hippocampal tissue obtained from separate groups of sleep deprived or non-sleep deprived mice (n=5 per group) was used for qPCR validation of microarray results. Protein products of validated genes are currently being examined in hippocampal-dependent models of learning and memory.

Results: A preliminary analysis of microarray data revealed that 5 hrs of sleep deprivation significantly altered hippocampal expression of 75 genes (D-score $\geq$ 6.0). Thirty-one genes showed increased expression after sleep deprivation, whereas 44 genes had decreased expression. Transcripts modulated by sleep deprivation coded for a variety of functional classes of proteins. Highlighted here are four genes that are upregulated by sleep deprivation and that have been validated by qPCR ($p<0.03$): Elk1 (synaptic plasticity), Tsc22d3 (stress/glucocorticoid induced transcriptional regulation), Prkab2 (AMP kinase, energy homeostasis), and Mmp9 (extracellular matrix/adhesion). Further validation experiments are currently being conducted. Tsc22d3 was chosen for further study due to its involvement in stress/glucocorticoid-related transcriptional regulation and for its potential role in the effects of emotional stress on hippocampus-dependent learning and memory processes.

Discussion: The present study was designed to identify hippocampally-expressed genes that are modulated by periods of sleep deprivation. Previous work in this laboratory has demonstrated that the certain forms of hippocampus-dependent memory (e.g., contextual fear memory) are facilitated by post-acquisition sleep (Graves, LA 2003). Indeed, contextual fear learning was further shown to decrease wakefulness and increase NREM sleep (Hellman, K 2007). Thus, the identification of genes impacted by sleep deprivation may also give insight into the molecular mechanisms by which sleep deprivation results in memory deficits. One particularly interesting phenomenon is the effect of stress on memory formation. Further work is underway investigating the potential role of Tsc22d3 and other genes in contextual fear learning and its interactions with sleep.

Brief Sleep Deprivation Produces Deficits in cAMP/PKA-dependent Forms of Synaptic Plasticity in the Mouse Hippocampus that May Be Rescued by Phosphodiesterase Inhibition

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Introduction: Brief sleep deprivation produces profound deficits in hippocampus-dependent memory, but the cellular and molecular pathways affected by sleep deprivation remain unclear. To address this question, we studied the effects of sleep deprivation on several forms of hippocampal long-term potentiation (LTP) with different sets of molecular requirements.

Methods: LTP studies were performed in hippocampal slices taken from young adult C57BL/6J mice that were sleep deprived by gentle handling for 5 hours or left undisturbed in their home cages. CA1 field excitatory post-synaptic potentials were evoked by Schaffer collateral stimulation.

Results: We found that 4-train, theta-burst, and forskolin-induced LTP, all of which produce stable forms of LTP whose maintenance is dependent on cAMP/PKA signaling, transcription, and translation, were greatly impaired in sleep-deprived mice. In contrast, basal synaptic properties and 1-train LTP, which is not dependent on cAMP/PKA, transcription, or translation, were unaffected. Interestingly, LTP induced with a combination of forskolin and IBMX, a broad-spectrum phosphodiesterase inhibitor, was unaffected by sleep deprivation, and preliminary data suggest that deficits in 4-train LTP can be rescued application of the PDE4-specific inhibitor rolipram.

Discussion: Together, these findings demonstrate that brief sleep deprivation has selective effects on cellular and molecular mechanisms underlying the maintenance of long-lasting forms of LTP, and may act to disrupt cAMP/PKA signaling by altering phosphodiesterase activity in the hippocampus.

Sleep Deprivation and Stress Have Additive Effects on Negative Mood States

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Introduction: The following study was designed to investigate the subjective effects of stress on sleep deprived and control subjects.

Methods: This experiment was conducted in a hospital-based sleep deprivation laboratory. Healthy adult volunteers (N=29, female = 15) were randomly assigned to a night of no sleep (n=14 deprivation condition) or a 9-hours sleep opportunity (n=15 control condition). Subjects were tested under low and high stressor conditions once on day 1, before the experimental manipulation and again on day 2, after the experimental manipulation. The low-stressor condition included relatively easy cognitive tasks and positive feedback on performance. The high-stressor condition included more difficult cognitive tasks and negative feedback on performance. After each bout of testing, subjects completed the Profile of Mood States (POMS). All subjects received 10 hours of sleep opportunity on the second night of the study.

Results: The high-stressor bouts were associated with greater reports of Total Mood Disturbance (TMD) on the POMS than the low-stressor bouts. This was true on day 1 ($p<0.01$, $d=0.68$) before the sleep manipulation and on day 2 for both the sleep deprived ($p=0.02$, $d=0.68$) and control subjects ($p<0.01$, $d=0.68$). The sleep deprived subjects reported greater levels of TMD than control subjects after both low stress ($p<0.01$, $d=1.06$) and high stress ($p<0.05$, $d=0.87$). The interaction of stress level and experimental condition was not significant ($p=0.53$).

Conclusion: These data suggest that sleep deprivation and stress each have negative effects on mood which are additive rather than interactive.

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

Conflict of Interest: The authors have no conflicts of interest to disclose.

Digit Symbol Substitution Task Performance in a Chronic Sleep Restriction Experiment with and without Naps

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Introduction: This study investigated the effect of a range of restricted nocturnal sleep schedules with and without diurnal naps on Digit Symbol Substitution Task (DSST) performance, a measure of cognitive throughput well established to be sensitive to sleep loss. The objective was to determine whether split sleep schedules with reduced TST could increase total wake time without reductions in performance.

Methods: N=90 healthy adults (21–49y; 38 females) participated in a 10-night sleep restriction protocol where they were randomized to 1 of 18 sleep schedules that involved restricted nocturnal anchor sleep (4.2h, 5.2h, 6.2h or 8.2h TIB) and a diurnal nap (0.4h, 0.8h, 1.2h, 1.6h, 2.0h or 2.4h TIB) or no nap. Neurobehavioral performance was tested at 2h intervals during scheduled wakefulness. Total DSST correct responses (sleep inertia bouts excluded) were averaged within each subject on each day. Response surface maps (RSMs) with increasing degrees of freedom were fitted to examine the rate of degradation of DSST performance across the 10 sleep-restriction days for each of the 18 conditions.

Results: A linear RSM of daily total TIB (i.e., anchor + nap) was found to explain 67% of the variance in DSST performance across days, with greater total TIB per 24h resulting in more correct answers ($\chi^2[1]=5.6$, $p=0.045$). An RSM differentiating between each anchor and nap sleep duration resulted in significantly improved goodness-of-fit ($\chi^2[8]=20.5$, $p=0.009$), but the additional model complexity (8 more parameters) only explained an additional 2% of variance.

Conclusions: During chronic nocturnal sleep restriction with and without diurnal naps, DSST performance was primarily a function of total TIB per 24h. Differentiating between anchor and nap sleep duration provided marginal improvement in explained variance, suggesting a more complex relationship possibly including learning effects.

Support: This work is supported by the National Space Biomedical Research Institute through NASA NCC 9-58 and the Institute for Experimental Psychiatry Research Foundation.

Trainee Award Eligible

Executive Functioning Following Five Nights of Sleep Restriction

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Introduction: Total sleep deprivation has been reported to produce decrements in neuropsychological performance mediated by the prefrontal cortex (PFC), but it is unknown whether partial sleep restriction (PSR) has such effects. The aim of this study was to investigate PFC-mediated tests of planning, verbal fluency and flexibility following chronic partial sleep restriction.

Methods: N=133 healthy subjects (29.9 ± 6.8y, 68 female) participated in a controlled laboratory protocol. N=120 underwent 2 nights of baseline sleep (10h TIB) followed by 5 nights of sleep restriction (4h TIB). At baseline, subjects completed the PFC-related tasks: North American Adult Reading Task (NAART) and 1 letter-set (C,F,L) of the Controlled Oral Word Association Task (COWAT). Following the fifth night of 4h TIB, subjects completed the remaining half of the COWAT (letters P,R,W), the Tower of London (TOL) and the Hayling and Brixton tests (HBT). N=13 served as 10h TIB per night control subjects.

Results: Mann-Whitney U tests were conducted to test for differences between the control and sleep-restricted groups. A significant difference was found between the control and the sleep-restricted groups in the Hayling scaled errors score of response inhibition ($p=0.028$). No other differences were significant between the control group and experimental group on any of the other tasks (4 other HBT outcomes; 3 NAART outcomes; 7 TOL outcomes; 2 COWAT outcomes).

Conclusions: Previous studies have elucidated a known anatomical basis for increased involvement of Broca's Areas 9, 10 and 45 during Hayling response inhibition as compared to response initiation. These results suggest that 5 nights of restriction of sleep to 4h TIB has a specific effect on these areas. It remains unclear as to why this effect has not been shown in any of the other PFC tests examined.

Support: National Space Biomedical Research Institute through NASA NCC-9-58; NIH NR04281 and RR00040.

Neurobehavioral and Cognitive Differences During Total Versus Partial Sleep Deprivation

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Introduction: Because individuals show varying neurobehavioral and cognitive responses to sleep loss, we examined the same individuals in repeated studies to systematically assess the differential impact of partial sleep deprivation (PSD) versus total sleep deprivation (TSD).

Methods: N=19 healthy adults (30.5y ± 6.9y; 11 females) completed 2 separate laboratory protocols: a PSD study (2 nights of baseline sleep [TIB=10h] followed by 5 nights of sleep restriction [TIB=4h]), and a TSD study (one night of baseline sleep [TIB=8h] followed by 40h without sleep). In both studies, the 10-min Psychomotor Vigilance Test (PVT), Digit Symbol Substitution Task (DSST), and Karolinska Sleepiness Scale (KSS) were administered every 2h while awake. Paired t-tests examined differences in measures between studies.

Results: Baseline values for the 3 outcomes did not differ significantly between studies. TSD produced significantly greater changes in all 3 outcomes than 5 nights of 4h PSD (PVT lapses were more frequent with TSD [$p=0.02$]; DSST correct responses were fewer with TSD [$p<0.001$]; and KSS ratings were higher with TSD [$p<0.001$]). Despite TSD producing greater neurobehavioral impairment than PSD, changes in PVT reaction times induced by TSD and PSD were highly correlated ($r=0.95$, $p<0.001$).

Conclusion: Forty hours of total wakefulness is more debilitating than 5 nights of 4h sleep (20 hours of total wakefulness/day) on neurobehavioral, cognitive and self-rated measures in the same subjects undergoing both protocols.

Support: Department of Homeland Security's Transportation Security Laboratory Human Factors Program (FAA #04-G-010); National Space Biomedical Research Institute through NASA NCC 9-58; NIH NR004281; NIH RR00040.

Phenotyping Neurobehavioral and Cognitive Responses to Partial Sleep Deprivation

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Introduction: Differential vulnerability to sleep loss assayed by cognitive and behavioral responses has been demonstrated in subjects undergoing total sleep deprivation, but has not been quantified in partial sleep restriction protocols. This study defined such heterogeneity by phenotyping responses to chronic partial sleep loss.

Methods: 120 healthy adults (M=29.9y ± 6.8y; 60 females) completed 2 baseline sleep nights (TIB=10h) followed by 5 sleep restriction nights (TIB=4h). 13 subjects were controls (M=29.0y) completing 7 nights of 10h TIB. The 10-min Psychomotor Vigilance Test (PVT), Digit Symbol Substitution Task (DSST), Karolinska Sleepiness Scale (KSS) and "Fresh-Tired" visual analog scale (VAS) were administered every 2h on all days. PVT responses (baseline to sleep restriction day 5) identified 3 groups: Type 1 responders (n=24; 1+ SD below the mean); Type 3 responders (n=22; 1+ SD above the mean); and Type 2 responders (n=74; within 1 SD of the mean). ANOVA compared differences in DSST, KSS, and VAS outcomes to sleep restriction among PVT response types.

Results: Baseline DSST, VAS and KSS measures did not differ among the PVT response groups. Compared with Type 1 PVT responders, Type 3 responders had fewer correct DSST responses (p=0.007), and higher KSS (p=0.02) and VAS fatigue scores (p=0.003) during sleep restriction. By contrast, Type 1 PVT responders and control subjects showed no differences between baseline and sleep restriction on DSST, KSS, and VAS measures.

Conclusion: Type 3 responders categorized for PVT vulnerability to sleep restriction showed consistent vulnerability in cognitive (DSST) performance, subjective sleepiness and fatigue. By contrast, Type 1 responders were similar to control subjects, showing neither cognitive nor subjective responses to partial sleep loss. Thus, stable inter-individual differences may exist in neurobehavioral vulnerability to partial sleep loss.

Support: National Space Biomedical Research Institute through NASA NCC 9-58; NIH NR004281; NIH RR00040.

Response to Sleep Restriction Depends Upon Pre-Existing Sleep Debt

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Introduction: Cumulative effects of chronic sleep restriction on behavioral alertness have been carefully documented, but little is known about the role of pre-existing sleep debt on the subsequent response to sleep restriction. This issue was addressed by determining whether a single night of sleep restriction to 4h TIB following partial recovery from a sleep debt resulted in the same degree of neurobehavioral deficit as that found after a single night of sleep restriction to 4h TIB following a period without sleep debt.

Methods: N=13 subjects (M=29.4y; 5 females) participated in a laboratory-controlled protocol, undergoing 2 nights of baseline sleep (B1-B2; 10h TIB) followed by 5 nights of sleep restriction (SR1-SR5; 4h TIB), then a recovery night (R1; 8h-12h TIB) followed by another night of sleep restriction (SR6; 4h TIB). A 10 min Psychomotor Vigilance Test (PVT) was completed every 2 h (08:00h to 20:00h) as part of a neurobehavioral test battery. PVT lapses were averaged within days for each subject. Change scores were calculated between B2 and SR1 (acute sleep restriction after no sleep debt), and between R1 and SR6 (acute sleep restriction after sleep debt). Change scores between the two conditions were compared using a Wilcoxon signed ranks test.

Results: PVT lapses increased by an average of 2.96 per test bout after SR1 following baseline sleep. Lapses increased an average of 5.73 per test bout after SR6 following a single recovery sleep preceded by 5 nights of prior sleep restriction. The differences between the lapse increase was significant ($p=0.033$).

Discussion: The change in PVT performance upon acute sleep restriction to 4h TIB after incomplete recovery from prior sleep debt was nearly twice the change in PVT performance upon acute sleep restriction after a period without sleep debt. Thus, when recovery from sleep debt is incomplete, neurobehavioral vulnerability to further sleep restriction appears to be disproportionately increased.

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

Change in Psychomotor Vigilance Test Lapses Predicts Change in Digit-Span Memory Performance During Sleep Restriction

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Introduction: It is controversial whether lapses of attention measured by the Psychomotor Vigilance Test (PVT) relate to other cognitive deficits due to sleep deprivation. To address this issue, we compared changes induced by sleep restriction in PVT lapse rates to changes in Digit Span (DS) working memory capacity performance from the Wechsler Adult Intelligence Scale, to determine if those people who had greater lapse rates also had greater memory capacity deficits across days of sleep restriction.

Methods: N=38 healthy adults (M=30yr; 19 males) underwent 2 baseline nights (10h TIB), followed by 5 sleep restriction nights (4h TIB/night) in a controlled laboratory setting. Subjects completed a 35-min. test battery that included a computerized visual DS task and a 10-min. PVT. The battery was completed every 2h from 08:00 to 21:00. Mean performance on each day for each subject, and change scores between baseline day 2 and sleep restriction day 5 were calculated for PVT lapses and DS total scores.

Results: Sleep restriction resulted in progressive increases in PVT lapses ($p<0.001$; linear trend $p<0.001$). DS total score decreased slightly but not reliably across sleep restriction days ($p=0.156$). While baseline performance on the PVT and DS tasks was uncorrelated, change in lapses and memory capacity from baseline to sleep-restriction day 5 was correlated ($rho=-0.53$, $p<0.01$), indicating that the more subjects lapsed on the PVT in response to sleep restriction, the more they declined in DS performance.

Conclusions: Subjects who were more severely affected by sleep restriction as manifested in their greater increases in lapses during vigilant attention performance, also had greater declines in working memory capacity over 5 nights. This suggests these neurobehavioral effects are not entirely orthogonal, but instead share some common biological basis via homeostatic sleep drive.

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SLEEP, DRUGS, & THE REAL WORLD

Synthetic Work Performance Following Five Nights of Sleep Restriction

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Introduction: Laboratory performance tests that simulate aspects of work processes have been referred to as synthetic work tasks. The aim of this study was to investigate the effect of sleep loss and learning on a classic synthetic work (SYNWORK) performance battery.

Methods: N=41 healthy subjects (28.9 +/- 6.8y, 21 female) participated in a controlled laboratory protocol involving 2 nights of baseline sleep (10h TIB), followed by 5 nights of sleep restriction (4h TIB), followed by recovery sleep. N=4 subjects had 10h TIB each night as a control. SYNWORK performance testing was conducted twice each day (10:30h-12:00h and 18:30h-20:00h) for 15 minutes each session, and consisted of 4 tasks performed simultaneously: an arithmetic task, a Sternberg (working memory) task, an auditory vigilance task, and a visual tracking task. A score for each task was recorded, as well as the total score for each session. Mixed model ANCOVA controlling for baseline day one was conducted to examine the effects of sleep restriction.

Results: There were significant improvements across sleep restriction days in the arithmetic ($p=0.001$) and Sternberg ($p<0.001$) tasks, and the total SYNWORK score ($p=0.045$), but the effects of sleep restriction were evident only on arithmetic performance ($p=0.010$) and total SYNWORK score ($p=0.01$).

Discussion: The only SYNWORK task that was differentially affected by sleep restriction (relative to the control condition) was arithmetic performance, which was also the only subject-paced (as opposed to work-paced) task in SYNWORK. Thus it appears that sleep restriction resulted in subjects being slower on this task, and since this task can contribute more points to the SYNWORK total score than the other tasks, total score was also differentially affected by sleep restriction. These findings replicate a long-standing observation in sleep deprivation research—namely that cognitive and psychomotor slowing are the dominant effects of sleep loss on subject-paced tasks.

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Sleep Quality in Greater Philadelphia: An Investigation of its Social Stratification and its Relation to the Social-Health Gradient

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Background: In its evolution, the Sleep Medicine field has focused on sleep disorders. Sparse literature is available on the cultural context of sleep in relation to social class, urban settings, and the way people get socialized around sleep. The public health ramifications of poor sleep are enormous and importantly, under-recognized. Current national health initiatives such as 'Healthy People 2010' lack mention of healthy sleep practice in their objectives. It is critical that we begin to understand, in an inter-disciplinary manner, how the people are sleeping and why. We must link societal value of sleep with targeted intervention to enhance behavioral change. Addressing healthy sleep practice as part of healthy living is imperative given the understanding we have of sleep and its functions.

Methods: This is a policy-driven project aimed at raising awareness and attention of the public, healthcare workers, local and regional organizations and policy-makers. Advocating the importance of study and its findings to these various agencies of 'health promotion' is a first important step. To assess sleep quality amongst different socioeconomic classes (SES), a Leikert scale-based sleep question was added to the Philadelphia Health Management biannual survey: this is a cross-sectional sample of approximately 10,000 adults acquired via random telephone digit dialing. Census weighting will be used to adjust for non-responders and to reflect the target population from which sampling was undertaken. Initial analysis included reporting the distribution of sleep amongst different sociodemographic categories in addition to income, education, and self-reported health outcomes. We plan to develop a multi-variable regression model addressing sleep quality as the dependent variable and the associations or confounding with various socioeconomic variables. Thirdly, the mediating effect of sleep will be examined by assessing the extent to which coefficients for the effect of ethnicity and SES variables on health are attenuated by the inclusion of sleep as a covariate. Finally, the novel use of Geographical Informational Systems (GIS) will allow the examination of sleep quality cartographically, providing important insight to the neighborhood level differences in sleep quality.

Results (using census weighting)**:** 9894 subjects answered the sleep question. Greater than 50% of males (n=4515) and females (n=5379) reported sleep quality score of ≥ 4 (1 –restless, 5-restful). However, there is a significant difference in sleep quality between genders (female reporting less restful sleep and more restless sleep than men, $p<0.0001$), by age group with 75+ age group reported the highest percentage of restful sleep (43%) compared with 24% in the 18-39 age group ($p<0.0001$), and level of education attained ($p<0.001$). Significant racial differences in sleep quality exist: a higher proportion of African American (12%) and Latino (14%) subjects reported restless sleep compared with Caucasians (8%) and Asians (3%) ($p<0.0001$). Marriage is associated with the lowest frequency of reported restless sleep (7%) compared to non-married groups (12-14%, $p<0001$). The frequency of restless sleep decreases in a linear fashion with increase in income category ($p<0.0001$). A significantly higher proportion of restful sleepers report good/excellent health than other sleep categories ($p<0.0001$).

Conclusions: Preliminary analysis has demonstrated that there are significant demographic, race and socioeconomic-based differences in sleep quality**.** There are also differences in self-reported health based on sleep quality. The findings support the need to understand the social differences in sleep quality in order to apply targeted culture-specific intervention. The use of GIS will allow us to examine the neighborhood effect upon sleep quality as opposed to the effect of individual attributes and/or behaviors.

Trainee Award Eligible

Effects of Fatigue from Night Work and Sleep Loss on Simulated Threat Detection Performance

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Introduction: Fatigue from night work and sleep loss can increase the risk of human error during critical visual search tasks in the real world. This study is the first to investigate the effects of night work and sleep loss on threat detection performance.

Methods: We developed a simulated luggage search task (SLST), consisting of more than 5,800 unique simulated X-ray images of luggage, organized into 30 stimulus sets of 200 bags each. 25% of the bags contained either a gun or a knife. N=24 healthy volunteer subjects performed a 200-bag search every 2 hours during a 34-hour period of prolonged wakefulness, starting at 8 am. Based on hits and false alarms, A'—the standard measure of signal detection theory— was calculated, with high values of A' indicating good threat detection performance. Night work (9 pm to 7 am, 13h-23h awake) and sleep loss (9 am to 5 pm, 25h-34h awake) performance were compared to nonsleep-deprived daytime performance (1h-11h awake).

Results: During night work, hit rate (HR) decreased on average by 1.7% ($p=0.151$), while false alarm rate (FAR) increased by 2.5% ($p<0.001$), leading to a significant decrease in detection accuracy A' of 2.8% ($p<0.001$). Trial duration decreased significantly by 55 s ($p<0.001$). During sleep loss, HR decreased on average by 3.5% ($p=0.008$), while FAR increased on average by 0.9% ($p=0.318$), leading to a significant decrease accuracy of 2.3% ($p=0.001$). There were also prominent time on task effects on HR and FAR, not affecting detection accuracy.

Conclusions: Fatigue from night work and sleep loss adversely affects performance on a task that simulates threat detection demands. Thus, fatigue may pose a risk for errors in tasks involving detection of threats, unless countermeasures for fatigue are deployed.

Support: This investigation was sponsored by the Department of Homeland Security's Transportation Security Laboratory Human Factors Program (FAA #04-G-010), and by NIH grant M01-RR00040.

Conflict of Interest: David F. Dinges received grants from Cephalon, honoraria from Cephalon and Jazz Pharmaceuticals and consulted for Cephalon, Merck, Novartis, Pfizer, GSK, Mars Masterfoods, and Procter & Gamble.

American Time Use Survey: Sleep Time and Its Association with Waking Activities

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Introduction: Both short and long self-reported sleep time have been found to be associated with increased risks of morbidity and mortality. However, the reasons for short and long sleep times in the population are unknown, although it is frequently assumed to be biological more so than behavioral. To gain some insight into the contributions various behavioral (lifestyle) factors may make to sleep duration, we investigated sleep time and its association with waking activities using the American Time Use Survey (ATUS) database, a population sample of US citizens.

Methods: Data were pooled from the 2003, 2004 and 2005 ATUS databases, accounting for cross-sectional data on N=47,731 respondents above the age of 14.

Results: Adjusted multiple linear regression models showed that, by a wide margin, the largest reciprocal relationship to sleep was found for work time, with travel time a distant second. Sleep time was minimal while work time was maximal in the age group 45-54yr. Only short sleepers spent more time for socializing, relaxing and leisure, while both short and long sleepers watched more TV than the average sleeper. The extent to which sleep time was exchanged for waking activities was shown to strongly depend on age and gender.

Conclusion: Work time, travel time, and time for socializing, relaxing and leisure are the primary activities exchanged for sleep time among Americans. These activities may be confounding the frequently observed association between short and long sleep on one hand and morbidity and mortality on the other hand, and should be controlled for in future studies.

Support: NIH NR04281

Disclosure/conflict of interest: David F. Dinges received grants from Cephalon, honoraria from Cephalon and Jazz Pharmaceuticals and consulted for Cephalon, Merck, Novartis, Pfizer, GSK, Mars Masterfoods, and Procter & Gamble.

Sleep Hygiene Practices of Good and Poor Sleepers in a Nationwide Internet-Based Sample

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Introduction: Experimental investigations show that many sleep hygiene variables cause sleeping difficulties, yet little is known about the frequency and impact of these behaviors in the community. This investigation explored the frequency of sleep hygiene behaviors in the United States, and assessed whether these practices are associated with poor sleep.

Methods: Data were collected through a national internet-based investigation with the assistance of StudyResponse, a non-profit research group based at Syracuse University. Panelists associated with StudyResponse were sent email messages regarding an ongoing study about "sleeping patterns and sleep-related behaviors", and interested persons were directed to the online study materials. Email messages were sent to 4945 residents of the United States, and 701 individuals completed the questionnaires. After excluding for nightshift employment and symptoms of sleeping disorders other than insomnia, 359 participants remained. An investigator-designed questionnaire, derived from The International Classification of Sleep Disorder's criteria for 'Inadequate Sleep Hygiene', was used to assess sleep hygiene characteristics. Participants were classified as good or poor sleepers based on scores from the Pittsburgh Sleep Quality Index.

Results: The final sample included 237 (66%) good sleepers and 122 (34%) poor sleepers. Only one out of 19 sleep hygiene variables were performed greater than three times per week. Sleep hygiene variables with the highest frequency totals were as follows. Participants watched television in bed 2.54 (SD = 2.89) days per week, worried, planned, or thought about important matters in bed 2.80 (SD 2.41) days per week, and drank caffeine between 5 and 10 hours before bedtime 3.18 (SD = 2.91) days per week. MANOVA revealed that poor sleepers were more likely to have poor sleep $= 0.975, \Lambda$scheduling behaviors (Wilks' $F(3, 351) = 2.96$, $p < 0.05$.), engage in activating or $= 0.924, \Lambda$arousing activities near bedtime (Wilks' $F(4, 352) = 7.22$, $p < 0.001$), engage in bed activities $= .918, \Lambda$other than sleep (Wilks' $F(4, 349) = 7.81$, $p < 0.001$), and sleep in uncomfortable $= 0.957, \Lambda$environmental conditions (Wilks' $F(4, 354) = 4.0$, $p < 0.01$). Specifically, poor sleepers were significantly more likely to engage in an inconsistent sleep schedule, worry, plan, or think about important matters in bed during the day and at bedtime, sleep in a noisy environment, and sleep in an uncomfortable temperature. Caffeine usage, alcohol usage, and napping frequency were unrelated to poor sleep.

Conclusion: Sleep hygiene practices in the United States are generally good, however, there is a clear correlation between sleep hygiene practices and poor sleep. Treatment and education programs should focus on the possible negative influence of inconsistent sleep schedules, excessive cognitive activity in the bed, and uncomfortable sleep environments.

Conflict of Interest: None.

The Relationship Between Sleep Time, Sleepiness, and Psychological Functioning in Adolescents

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Introduction: Normative biological, psychological, and social changes during adolescence contribute to insufficient sleep time, irregular sleep schedules, and sleepiness. Such changes in sleep may lead to psychosocial consequences such as depressed mood and behavior problems. The aim of the current study was to examine the association between sleep time, sleepiness, and psychological functioning (e.g., symptoms of anxiety, depression, externalizing behaviors, and perceived health) in adolescents.

Methods: This cross-sectional sample, comprised of 247 adolescents (48.5% female, 54.3% ethnic minority, mean age of 13.7 years), was recruited from an ongoing community-based cohort study of sleep and health. Data were collected via 5-7 day actigraphy, the Epworth Sleepiness Scale (ESS), physical exam, and parent, teacher, and adolescent questionnaires. It was hypothesized that less mean total sleep time, more variability in sleep time, and more sleepiness would be associated with higher scores on measures of anxiety, depression, externalizing behaviors, and lower scores on a measure of perceived health.

Results: The mean ESS score was 7.9 (+/- 4.47), and scores ranged from 0-23. Higher ESS scores were associated with higher scores on measures of anxiety ($p<0.001$) and depression ($p<0.01$), and lower scores on a measure of perceived health ($p<0.001$) when controlling for previously identified covariates (e.g., age, ethnicity, gender, Tanner stage, socioeconomic status, body mass index, prematurity, ADHD, vacation status, and mean total sleep time). Other relationships between sleep variables (e.g., sleep time and variability in sleep time) and psychological variables were not found. Additionally, less total sleep time and higher night-to-night variability were associated with higher ESS scores.

Conclusions: Future studies should include objective measurement of sleepiness and behavioral alertness to clarify whether the relationship between sleepiness and psychological functioning is due to sleepiness per se or to a general negative bias. In addition, clinicians should consider sleepiness when conducting psychological assessments.

Effects of Benzodiazepine and Non-Benzodiazepine Hypnotics on Sleep Architecture and Sleep-Dependant Plasticity

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Introduction: Currently approved treatments for insomnia include drugs that bind to different sites on the GABA receptor. While these compounds improve sleep initiation and maintenance to varying degrees, they are accompanied by a number of side-effects including alterations in sleep architecture and impairments in cognitive functioning. For example, benzodiazepines impair the acquisition and recall of newly formed memories in awake subjects (i.e., anterograde amnesia). In contrast, very little is known about how hypnotics influence the consolidation of learned information; a process that may occur during sleep.

Methods: We examined the effects of benzodiazepine (Triazolam) and non-benzodiazepine (Zolpidem and Ramelteon) hypnotics in recently described model of sleep-dependent synaptic plasticity. Monocular deprivation in developing cats causes a rewiring of visual cortex in favor of the open eye. This is enhanced by sleep and inhibited by sleep deprivation (Frank *et al.*, 2001. To test the effects of hypnotics on this process, cats had one eye closed followed by ad lib sleep in combination with systemic hypnotic administration. Polysomnographic data were collected to quantify changes in sleep architecture, and cortical plasticity was assessed with micro-electrode recording and intrinsic signal optical imaging.

Results: We found that only Zolpidem appeared to impair sleep-dependent plasticity. Zolpidem and Triazolam suppressed REM sleep to varying degrees, while Ramelteon had no effect on sleep architecture. REM suppression, however, was unrelated to the loss of plasticity as Triazolam-treated cats (which showed the greatest REM loss) showed normal sleep-dependent plasticity.

Discussion: These findings show that certain hypnotics may interfere with processes underlying brain remodeling that occur during sleep.

Trainee Award Eligible

Modafinil Does Not Promote Wakefulness by Inhibiting Dopamine Reuptake in Drosophila

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Introduction: Modafinil is a novel wake-promoting agent widely prescribed to treat excessive daytime sleepiness, but its mechanism of action is still unknown. Evidence from mammals suggests that it enhances dopamine neurotransmission by inhibiting the dopamine transporter (DAT). Modafinil has been shown to colocalize with dopaminergic terminals *in vivo*, to bind DAT *in vitro*, and DAT knockout mice do not respond to modafinil. However, its effects on mammals differ greatly from most other DAT antagonists (such as cocaine, Ritalin, and amphetamines), and there is also evidence of involvement of the noradrenergic system. *Drosophila*, which lack noradrenaline as a dominant neurotransmitter system, present a convenient system for testing the hypothesis that modafinil promotes wake predominantly by inhibiting DAT. *Drosophila* also respond to modafinil --their daily activity rhythms are altered and they sleep less-- and they have only one DAT gene.

Methods: We compared the responses of wild type flies and two lines of flies mutant for DAT (*fumin*; *fmn*) to modafinil, as well as to methamphetamine.

Results: Wild type flies sleep less under the influence of both drugs, and are hyperactive on methamphetamine. Unexpectedly, *fmn* flies are hypersensitive to the wake-promoting effects of modafinil. However, as we expected, the *fmn* mutation abolishes the hyperactivity and decreased sleep effects of methamphetamine.

Discussion: Our data strongly suggest that modafinil has a novel mechanism of action that does not involve blocking dopamine reuptake via the DAT. We are considering testing whether dopamine neurotransmission, thought to be a key regulator of arousal in Drosophila, is even necessary for the wake-promoting effects of modafinil.

Trainee Award Eligible

The Ontogeny of Hypnotic Response: A Preliminary Study on the Effects of Zolpidem in the Developing Ferret (Mustela Putorius Furo)

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Introduction: In a recent US survey, 25% of primary care pediatricians reported prescribing hypnotics to child patients presenting with behavioral sleep problems (Owens et al., 2003). Hypnotics are increasingly used to treat insomnia in children and adolescents (Mindel et al., 2006). However, little basic research exists to explain the mostly unknown side effects and long term consequences of these hypnotics. Evidence suggests that the developing brain will respond differently to hypnotics than the adult brain. To begin to address this issue, we examined the effects of a commonly prescribed non-benzodiazepine (BDZ) hypnotic, Zolpidem (Ambien), on sleeping patterns in the developing ferret.

Methods: Sleep-wake electroencephalogram (EEG) and electromyogram (EMG) baseline data were collected in six ferrets (ages P35-36) following injection of DMSO. After twenty-four hours, either 2 mg/kg or 20 mg/kg Zolpidem in DMSO were injected and a further twenty-four hours later, a third counterbalanced injection of 20 mg/kg or 2 mg/kg Zolpidem in DMSO was given. One of these ferrets underwent the same procedure at age P49 to assess for any age-dependent changes.

Results: We find that in P35-36 ferrets, Zolpidem suppresses rapid-eye-movement (REM) sleep and increases non-REM (NREM) sleep, although the effects did not appear to be dose-dependent. But, there was no effect of Zolpidem on state duration or state amounts. An unusual finding was that immediately following injection, Zolpidem induced an abnormal EEG state (high amplitude, high frequency EEG activity with little EMG activity) the duration of which was dose dependent. Suppression of both NREM and REM sleep in the older animal indicates that Zolpidem may have different effects in older animals. Additionally, while the abnormal EEG state did appear in the older ferret, its duration was much shorter than in younger ferrets.

Conclusion: Taken together, these results suggest that there is an age-dependent response to Zolpidem.

Conflict of Interest: The authors disclose that they have no conflict of interest.

METHODOLOGY

Maintenance of Wakefulness Test: Reliability and Predictors in Normal, Healthy Subjects

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Introduction: The Maintenance of Wakefulness Test (MWT) examines an individual's ability to stay awake in an environment of decreased sensory stimulation. In an effort to get novel data on its psychometric properties in a healthy non-sleepy population, we evaluated the relationship of MWT sleep latency to other variables, and assessed its test-retest reliability.

Methods: N=9 healthy adults (23-37y; 6 females), underwent 11 consecutive laboratory nights of 10h TIB (10:00pm to 08:00am), and completed the Composite Scale of Morningness and Eveningness (CSME), Eysenck Personality Inventory (EPI), Beck Depression Inventory (BDI), the Karolinska Sleepiness Scale (KSS) and Psychomotor Vigilance Test (PVT). Modified single trials (30 min) of MWT were conducted between 14:30h-16:00h on days 2 and 7; sleep latency was defined as time to first appearance of sleep (10 sec). Nocturnal PSGs were recorded before daytime MWTs. Change scores were calculated between day 2 and 7 for MWT sleep latency, PVT, KSS and total sleep time (TST).

Results: The test-retest reliability of the MWT was r=0.65 (p=0.05). MWT scores increased from day 2 (M=15.4 min) to day 7 (M=22.2 min) by an average of 6.8 minutes (p=0.047), despite TST decreasing across nights (M=34 min, p=0.053). Forward stepwise multiple regression on the MWT difference score between day 2 and day 7, with age, gender, CSME, BDI, EPI, KSS, PVT and TST as predictors revealed that only CSME predicted MWT change across days (adj R square=0.40, p=0.04). Evening types had larger improvement in MWT latency from day 2 to 7.

Conclusions: MWT latency increased and was relatively reproducible among healthy adult subjects receiving 10h TIB. Evening types appeared to benefit more by 10h TIB.

Support: NIH NR 004281 and RR00040.

Development of a Shorten Version of the FOSQ

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Introduction: Efficacy of CPAP treatment does not rely solely on documentation of elimination of apneas and hypopneas but also on evaluation of clinical outcomes. The Functional Outcomes of Sleep Questionnaire (FOSQ), a reliable and valid instrument, is widely used in research and clinical practice to measure activities sensitive to sleep disruption. The purpose of this study was to develop a shorter version for clinical application that maintains the validity of the original instrument.

Methods: Data from a multisite study of OSA patients (Sample 1) (n =155, age 46.3 $\pm$ 9.18 years; BMI = 37.7 $\pm$ 8.49 kg/m^2; AHI = 63 $\pm$ 31) was utilized in the development of the short version. Of the 30 original items, 10 questions representing each subscale (General Productivity, Social Outcome, Activity Level, Vigilance, Intimate Relationships and Sexual Activity) were selected if they met the criteria of having a normal distribution of responses, had the largest pre- to post-treatment effect size within subscale. The instrument was then prospectively tested on a second, independent sample of OSA patients (Sample 2)(n =25 age 48.10$\pm$ 10.10 years ; BMI= 33,98 $\pm$ 6.18 kg/m^2, AHI= 51 $\pm$25).

Results: Psychometric evaluation of the FOSQ-10 was performed using Sample 2. Internal consistency of the FOSQ-10 was Cronbach's alpha = 0.866. Pre-tx correlations of the FOSQ-10 with the original FOSQ were: r = 0.83 - 0.96 for subscales and r = 0.96 for total scale; after 3-months tx: subscale range r = 0.90 - 0.95 and total scale r = .97, all p-values= <0.0001. Paired t-tests comparing the total scores of the FOSQ-10 with the original FOSQ showed a statistical difference at baseline (mean =00.63, $p < 0.0001$) but not after 3 mo tx. Significant changes in the Total score following treatment were found for both instruments, however, the change was slightly larger for the short version (FOSQ-10 effect size = 1.43 vs. original FOSQ effect size =1.36, p-values <0.0001).

Conclusions: The FOSQ-10 is a psychometrically strong instrument that is rapidly completed and easily scored. It shows promise as a robust instrument for the clinical evaluation of CPAP efficacy.

Conflict of Interest: T. Weaver: Research equipment from Respironics, Inc. and Protech; funding from Respironics Sleep and Respiratory Foundation, FOSQ License Agreements with Sanofi-Aventis Pharmaceutical, Sleep Solutions, Merck & Co, Inc., Jazz Pharmaceutical; consultant for Jazz Pharmaceutical and Sanofi-Aventis.

AMP-Activated Protein Kinase Phosphorylation is Dependent on Tissue Preparation

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Introduction: AMP-activated protein kinase (AMPK) is activated when energy consumption exceeds energy production and the ATP/AMP ratio decreases. AMPK acts to shift cellular metabolism from energy-consuming anabolic processes to energy-producing catabolic processes. AMPK decreases cholesterol, fatty acid and protein synthesis and therefore preserves cellular ATP. AMPK exists as a heterotrimer consisting of an α, β and γ subunit. AMPK is activated when the catalytic α subunit is phosphorylated on Thr172 by upstream kinases. Therefore, phosphorylation of the α subunit is frequently used as a measure of AMPK activation. However, measurement of AMPK phosphorylation *in vivo* can be technically challenging since AMPK phosphorylation is altered by numerous kinases and phosphatases. To determine the most accurate method of measuring AMPK phosphorylation in the mouse brain, we compared three different methods of sacrifice and dissection.

Methods: One group was sacrificed by cervical dislocation and the brain dissected on ice, one group was sacrificed by cervical dislocation and the brain immediately frozen in liquid nitrogen and dissected frozen and one group was sacrificed by a 3.5 kW microwave (which rapidly heats the brain and inactivates all the enzymes) and dissected on ice. Furthermore, since AMPK phosphorylation is labile, we assessed the stability of the phosphorylation by comparing samples that were freeze/thawed (as per normal laboratory practice) and subsequently denatured and boiled to samples that were immediately denatured and boiled.

Results: We found that the freeze/thawing greatly increased phosphorylation of AMPK in both the ice dissected and frozen brains ($p<0.001$ for each) but there was no change in the microwaved brains with freeze thawing. We also found that in the absence of freeze/thawing, the phosphorylation states were similar for all three groups. Total α subunit of AMPK (including both phosphorylated and non-phosphorylated forms) was similar in all groups. Therefore, freeze/thawing of samples increases phosphorylation of AMPK and introduces variability that may mask biological changes. The freeze/thawing likely increases AMPK phosphorylation by allowing the upstream kinases of AMPK to phosphorylate it. Immediate denaturing and boiling appears to render these kinases inactive and prevents their activation of AMPK. This freeze/thaw effect is not evident in the microwaved brains most likely because microwaving renders the kinases and phosphatases that act on AMPK inoperative thus preserving the phosphorylation state of AMPK.

Conflict of Interest: There are no conflicts of interest.

Three-State Predictive Models using Digital Video Analysis in Mice

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Introduction: Sleep can be identified in mice using video based quiescence criterion (Pack, Phys Genomics '06). Our goal is to determine whether we can detect sleep substates using video. We are aiming to distinguish between WAKE, NREM, and REM.

Methods: We recorded 24-hours of data from seven C57BL/6 male mice. EEG and EMG signals were used to score sleep stages and video frames were simultaneously acquired at a rate of 10/sec. Digital video analysis methods were used to extract the following parameters: speed, aspect ratio, and the area of the mouse. These variables were used to build predictive models for minimizing the classification error of the three states.

Results: We performed 10-fold cross-validation procedure whereby 90% of one mouse data is randomly selected for training, and 10% reserved for validation. This in mouse approach achieved an overall accuracy of 90-92% for each of the 7 mice. Models derived by training on one mouse and then tested on another achieve an error rate on the three-class problem of 13-14%. Surprisingly, despite the fact that our classification algorithm is designed to minimize the overall misclassification rate of the three states, we were 58% accurate at the identification of REM sleep, demonstrating that there is strong REM signal in the video-based parameters.

Discussion: Inspection of video-based measurements revealed that during REM sleep, the area of the mouse increases relative to its area during surrounding NREM sleep epochs. We postulate that this increase in mouse area is explained by REM-related muscle atonia.

A Digital Video Analysis Method to Study Sleep in Drosophila Melanogaster

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Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine

Introduction: To address limitations in the estimation of sleep of *Drosophila melanogaster* using infrared beam crossing technology we have developed a digital video analysis technology to identify behavioural quiescence.

Methods: Wild type Canton-S (CS), and *white* (w^{1118ex}) flies were collected upon eclosion and kept overnight on standard cornmeal agar media, then singly transferred into tubes 6 cm long containing 5% sucrose/1% agar. Flies were maintained under 12 hours light: 12 hours infrared (IR) (wavelengths 950 nm and >) at 25˚C and constant humidity for six days before behaviour was recorded.

For aged females, w^{1118ex} flies were kept in groups of 8-10 animals initially on standard media under 12 hr light:12 hr dark at 25˚C and constant humidity for 38 days with transfer to new vials every 3 to 4 days. The aged flies were then transferred singly to individual monitor tubes as above.

For direct comparison to the Drosophila Activity Monitoring System (DAMS) (Tri Kinetics, Waltham MA), the monitor tubes were placed in the system as normal and eight adult flies were simultaneously recorded every 5 seconds using a Retiga (QImaging, Surrey BC) digital video camera for a complete 12 hour light: 12 hour IR cycle.

Results: An infrared beam break system in wide use, the DAMS system, has several limitations for studying sleep. During the lights on period, flies are making significant movements that do not break the infra red beam that are counted as extended daytime sleep using DAMS. This is seen in the total day time sleep determination for video versus DAMS: 7 day old w^{1118ex} females 245.5 ± 84.7 vs. 414.0 ± 67.3 minutes ($p = 0.0033$); 7 day old w^{1118ex} males 302.3 ± 92.2 vs. 485.6 ± 35.4 minutes ($p = 0.0013$); 45 day old w^{1118ex} females 71.4 ± 80.6 vs. 216.7 ± 140.2 minutes ($p < 0.0001$); 7 day old CS males 324.5 ± 22.3 vs. 407.7 ± 34.1 minutes ($p = 0.0008$). The missed movements are also seen in the day time mean sleep bout length determination for video versus DAMS: 7 day old w^{1118ex} females 19.2 ± 12.7 vs. 35.0 ± 12.5 minutes ($p = 0.0398$); 7 day old w^{1118ex} males 17.7 ± 5.2 vs. 67.1 ± 50.3 minutes ($p = 0.0393$); 45 day old w^{1118ex} females 6.9 ± 4.1 vs. 13.2 ± 9.8 minutes ($p = 0.0158$). Night time total sleep was found to be significantly less for young w^{1118ex} and CS males (video 614.5 ± 14.8 minutes vs. DAMS 629.6 ± 13.0 minutes [$p = 0.0045$] and video 324.5 ± 22.3 minutes vs. DAMS 407.7 ± 34.1 minutes [$p = 0.0078$], respectively) and for old w^{1118ex} females (video 431.8 ± 156.8 minutes vs. DAMS 594.3 ± 87.7 minutes [$p= 0.0016$]).

Conclusion: We use a new method of video analysis to measure quiescence in *Drosophila melanogaster* to show the error rate associated with the use of DAMS to identify sleep and that this error rate is dependent upon animal gender, age and genotype. The accuracy for the identification of sleep bout duration using our new video method which we are calling Subtraction Analysis of Video Images For Sleep research (SAVIS) is superior to DAMS and can possibly lead to fundamentally different biological conclusions.

Index

K

L

M

N

O

P

R

S

T

V

W

Y

Z


SINE MORIBUS