10th Annual CENTER FOR SLEEP AND CIRCADIAN NEUROBIOLOGY

CSCN

research retreat

Thursday, May 30, 2013

8:00 a.m. – 5:00 p.m.

Levy Conference Center,
Penn Law School

Perelman School of Medicine
University of Pennsylvania

www.med.upenn.edu/sleepctr

2013 CSCN Research Retreat Program
Thursday, May 30, 2013
Levy Conference Center, Penn Law School

8:00 – 8:30 a.m. Poster Mounting
Continental Breakfast will be served.

8:30 – 8:40 a.m. Opening remarks
Allan I. Pack, Center Director

8:45 – 9:30 a.m. **Session 1: Sleep & Neurodegeneration**

Jason Gerstner - "Amyloid-beta Induced Sleep Fragmentation is Rescued by Fatty-acid Binding Proteins in *Drosophila*"

Marishka Brown - "Aging Induced ER Stress Alters Sleep and Sleep Homeostasis"

Lama Chahine - "Questionnaire-based Diagnosis of REM Sleep Behavior Disorder in Parkinson's Disease"

9:35 – 10:35 a.m. **Session II: Obstructive Sleep Apnea, Obesity & Metabolism**

Julio Chirinos - "A Randomized Trial of Continuous Positive Airway Pressure Therapy, Weight Loss, or Both for Obese Adults with Obstructive Sleep Apnea"

Victoria Pak - "Obstructive Sleep Apnea, Obesity and Cellular Adhesion Molecules: Impact of 2 Years of CPAP Treatment"

Beverly Shin - "Respiratory-Related Dynamic Upper Airway Changes in Obese Apneics after Weight Loss"

Zhou Fang – "Enhanced Resting-State Striatal Connectivity Correlates with Increases in Daily Caloric Intake after
Sleep Deprivation"

10:35 a.m. Break

10:45 – 11:45 a.m. **Session III: Neuroanatomy & Functional Connections**

Robbert Havekes - "Sleep Deprivation Impairs Memory by Altering Cofilin and Actin Signaling"

Hilary McCarren - "Stimulation of the α2a Adrenergic Receptor in the Ventrolateral Preoptic Area Promotes Arousal"

Anand Venkataraman - "Bmal1^{fbrainKO} Mice Exhibit Deficits in Learning and Memory Processes"

Matthew Nelson - "The Molecular Logic of *C. elegans* Sleep: A Single Sleep-Promoting Neuron Inhibits a Wake-Promoting Pair of Neurons via Neuropeptide Signaling"

11:45 a.m. – 1:45 p.m. **Lunch and Poster Session**

1:45 – 2:30 p.m. **Session IV: Circadian/Homeostatic Sleep**

Brian Altman – "Oncogenic c- and N-Myc Disrupt Circadian Rhythm"

Mathias Basner - "A Randomized Trial of a 3-hour Protected Nap Period in Medical Interns"

David Garbe - "Cooperative Interaction between Phosphorylation Sites on PERIOD Maintains Circadian Period in *Drosophila*"

2:30 p.m. Break/Judges Meeting

2:45 p.m. Introduction of Inaugural Adrian R. Morrison Keynote Address – Dr. Allan Pack

3:00 – 4:00 p.m. **Adrian R. Morrison Keynote Address**
Joseph Takahashi, PhD, Loyd B. Sands Distinguished Chair in Neuroscience, University of Texas Southwestern
"Molecular Architecture of the Circadian Clock in Mammals"

4:00 p.m. Reception and Presentation of Awards

Message from the Director
Allan I. Pack, MB, ChB, PhD

We are pleased to be celebrating our 10th Annual CSCN Research Retreat. Again this year, faculty and trainees come together to present an impressive array of state-of-the-art basic science, clinical and translational studies in sleep and circadian research. This year we have moved back to the Levy Conference Center in the Law School.

Our thanks once again go to this year's Research Retreat Committee, co-chaired by Lori Panossian and Bernie Sunwoo, for choosing an interesting program to be presented to you today.

This year the keynote address will be the first Adrian R. Morrison Address. Named for emeritus professor of veterinary medicine Adrian Morrison, it is our way of honoring Dr. Morrison's legacy.

Dr. Morrison's laboratory did seminal work on the mechanisms of REM sleep. He showed that REM without atonia could occur if certain regions in the pons were lesioned. He produced an animal that "lived out their dreams" during REM sleep. This observation in animals preceded the description of REM behavior disorder in humans by many years. Dr. Morrison's laboratory also identified the mechanisms of generation of ponto-geniculo-occipital waves in REM sleep and that homeostatic control of temperature was lost in REM sleep. He is a past president of the Sleep Research Society and a past president of the World Federation of Sleep Research Societies. In addition to his outstanding work in establishing the field of sleep research, Dr. Morrison was also on the forefront of establishing policy on the ethics and proper use of animals in the advancement of medical knowledge. He was president of the National Animal Interest Alliance and served on the boards of Americans for Medical Progress and the Pennsylvania Society for Biomedical Research. In 1991 he received the Scientific Freedom and Responsibility Award of the American Society for the Advancement of Science in honor of his dedication to the responsible use of animals in research and his courageous stand in the face of personal risk against attempts to curtail animal research essential to public health.

Today, we welcome Dr. Joseph Takahashi, who will be giving the first Adrian R. Morrison Address. Dr. Takahashi has pioneered the use of forward genetics and positional cloning in the mouse as a tool for discovery of genes underlying complex behaviors. He discovered the first mammalian clock gene that led to a description of a conserved circadian clock mechanism in animals.

Dear Friends and Colleagues,

We would like to welcome you to the 10th Annual Center for Sleep and Circadian Neurobiology Research Retreat! The talks and posters presented here today represent the far-reaching breadth and multidisciplinary focus of the sleep community at the University of Pennsylvania. We would like to thank all of the trainees and faculty who submitted abstracts this year. Their work demonstrates the productivity, innovation and diversity of sleep/wake and circadian research being conducted within the School of Medicine and beyond, including the Children's Hospital of Philadelphia, the School of Nursing, the Philadelphia VA Hospital and the School of Veterinary Medicine. We are also honored to host our keynote speaker, Joseph Takahashi, Ph.D. Chair of the Department of Neuroscience and an Investigator of the Howard Hughes Medical Institute at University of Texas Southwestern Medical Center. Dr. Takahashi's pioneering discovery of the mouse and human clock genes, using forward genetics and positional cloning techniques, led to his ground-breaking description of the mammalian circadian clock mechanism. He remains a leader in the field and his current work continues to explore the molecular and genetic basis of circadian rhythms.

We hope that the wide range of research topics, from basic science to clinical and epidemiologic studies, will stimulate increasing collaboration, introduce researchers to innovative techniques and new areas of inquiry, and inspire novel approaches towards the study of sleep and consequences of sleep loss.

Sincerely,

The 2013 Retreat Committee:

Lori A. Panossian, MD, MS, is co-chair of this year's committee. She is a Clinical Associate in the Division of Sleep Medicine and a Postdoctoral Research Fellow in the Center for Sleep and Circadian Neurobiology. She is currently working under the mentorship of Dr. Sigrid Veasey, studying the neurobiology of wake-active neurons. Her work focuses on mechanisms of neuronal susceptibility to sleep fragmentation and the role of disrupted autophagy in wake neuron injury.

Bernie Sunwoo, BSc, MBBS, is an Assistant Professor of Clinical Medicine with dual appointments in the Division of Sleep and the Division of Pulmonary, Allergy and Critical Care. Her clinical interests are in sleep related breathing disorders and chronic hypoventilation.

Robbert Havekes PhD, is currently a Research Associate in the Department of Biology, under the supervision of Dr. Ted Abel. Dr. Havekes' research investigates the cellular and molecular mechanisms by which sleep loss leads to cognitive impairments with a focus on the mammalian hippocampus.

Miranda Lim, MD, PhD, is a Clinical Associate in the Division of Sleep Medicine and Department of Neurology, and the Measey Senior Research Fellow in the Department of Medicine. Her post-doctoral research focuses on the neural mechanisms underlying sleep disturbances in mild traumatic brain injury in mouse models, under the dual mentorship of Dr. Allan Pack in the Center for Sleep and Circadian Neurobiology and Dr. Akiva Cohen in the Division of Pediatric Neurology at CHOP.

Hengyi Rao, PhD, is currently a Research Assistant Professor of Neurology. Dr. Rao's primary research interests involve the use of multimodal functional brain imaging to study brain function before and after sleep deprivation, as well as neural mechanisms underlying inter-individual differences in cognition and behavior.

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Oncogenic c- and N-Myc Disrupt Circadian Rhythm

Brian J. Altman[1]; Annie Hsieh[1]; Arvin Gouw[1]; Anand Venkataraman[2]; Bo Li[1]; David I. Bellovin[4]; Dean W. Felsher[4]; M. Celeste Simon[1]; John B. Hogenesch[2]; and Chi V. Dang[1,3]

[1]Abramson Family Cancer Research Institute, [2]Department of Pharmacology, Institute for Translational Medicine and Therapeutics, [3]Division of Hematology-Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania; [4]Division of Medical Oncology, Departments of Medicine and Pathology, Stanford School of Medicine

Circadian rhythms are regulated by feedback loops comprising a network of factors that regulate Clock-associated genes. Chronotherapy seeks to take advantage of altered circadian rhythms in some cancers to better time administration of treatments to increase efficacy and reduce toxicity. Taking advantage of cancers that have substantially different circadian rhythms, or are 'out of phase', with normal tissues, could open a wide therapeutic window to make them vulnerable to chemotherapy or targeted drugs at different times than normal tissue. However, there is currently no basis to identify which cancers have disrupted circadian rhythms and would be amenable to chronotherapy. c- and N-Myc are oncogenic transcription factors translocated or amplified in many cancers. While the role of Myc in circadian rhythm is currently unknown, it may affect circadian rhythm by binding to the same E-box promoter regions used by the central regulators of circadian rhythm, Clock/Bmal1. Thus, we hypothesized that Myc may disrupt circadian rhythm through inappropriate engagement of E-box promoters.

Here we show in neuroblastoma, osteosarcoma, and hepatocellular carcinoma cells that overexpressed Myc specifically upregulated the negative circadian regulator Rev-erbα, which in turn decreased expression of Bmal1. Importantly, Myc-expressing cells showed dramatically disrupted circadian oscillations, which could be partially rescued by inhibiting expression of Rev-erbα. Together, these data suggest that Myc-driven cancers have altered circadian oscillation due to upregulation of Rev-erbα, and that cancers driven by Myc may thus be good candidates for chronotherapy.

We thank the following funding sources: NIH R01CA051497, R01CA57341, LLS 636311

Trainee Award Eligible

Sleep Synchronizes Transcription in The Lung

Ron C. Anafi[1 * §], Renata Pellegrino[1,2,3 *], Keith R. Shockley[4], Micah Romer[1], Sergio Tufik[3], Allan I. Pack[1]

[1] Division of Sleep Medicine and Center for Sleep and Circadian Neurobiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA; [3] Universidade Federal de São Paulo _ UNIFESP; [4]Biostatistics Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC

Introduction: Recent work has demonstrated that small changes in temperature, such as those expected from the normal physiology of sleep, are powerful time cues for peripheral circadian oscillators. As a result, we hypothesized, that sleep could synchronize these peripheral oscillators with behavioral rhythms. By extension, such changes are expected to induce a measureable change in the uniformity of peripheral transcription among sleeping and sleep-deprived animals.

Methods: Using microarrays we compared gene expression in lung tissue from sleeping and sleep-deprived mice euthanized at the same diurnal times. Eight or nine animals were sacrificed at 4 different times from both groups. Bartlett's test of homoscedasticity (uniform variance) was applied to the expression data from the five groups of experimental animals (baseline and sleeping for 3, 6, 9, and 12 hours) not subjected to artificial deprivation (FDR cutoff <1%). We then applied the non-parametric, but less powerful, Brown-Forsythe test (p-value <.01) to ensure that transcripts were not identified as a result of a few spurious outliers or departures form normality.

Results: A total of 210 probes and 189 unique genes met the combined criteria for sleep/circadian related changes in inter-animal variability. These transcripts all showed consistent and systematic changes in inter-animal variability over the course of uninterrupted sleep. As a group, they showed increasing uniformity as the uninterrupted sleep period progressed. In contrast, among sleep deprived animals the same transcripts showed the opposite temporal pattern and demonstrated increased variability with extended deprivation. These transcripts were highly enriched for gene ontology annotations describing oxidation and reduction.

Conclusion: Our data suggest a unique role of sleep in synchronizing transcription in peripheral tissues. These results suggest that the pathophysiologic consequences of sleep deprivation may be intimately connected with circadian disruption.

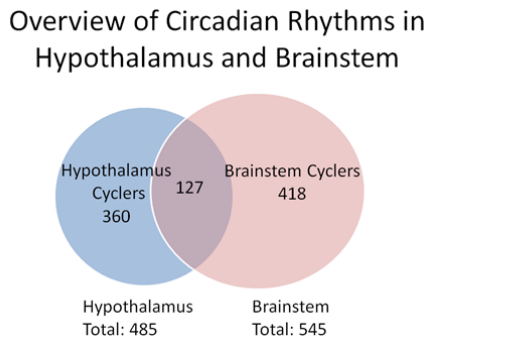
Support: This research was supported in part by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences. This work was also supported National Institutes of Health Program Project Grant AG-17628 to AP, a Physician Scientist Training Award from the American Sleep Medicine Foundation to RA, and support from a K12 (HL090021).

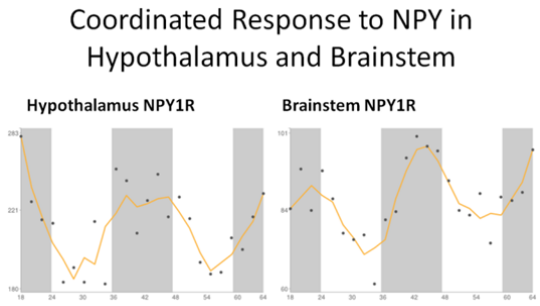
Circadian Integration of Metabolism and Sleep Homeostasis in the Hypothalamus and Brainstem

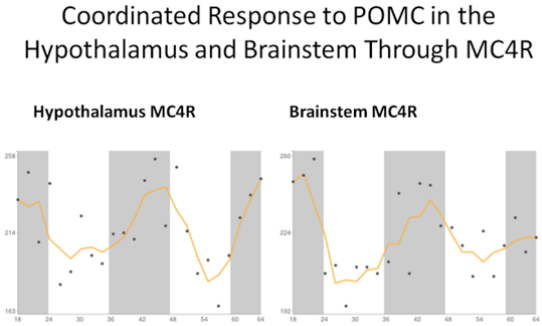
Heather I. Ballance, Ray Zhang, Nick Lahens, Michael E. Hughes, John B. Hogenesch

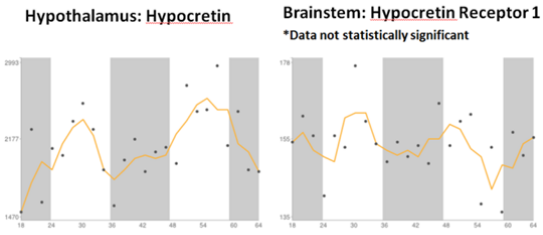
The hypothalamus and brainstem contain key populations of neurons that modulate appetite, feeding activity, and rest cycles. Several neurotransmitters and their corresponding receptors include NPY, which stimulates feeding, and POMC, which decreases feeding, are expressed in the hypothalamus and brainstem. To investigate how the circadian clock may regulate expression of RNA in these two metabolic centers, hypothalamus and brainstem tissues were harvested every two hours over the course of 48 hours. RNA from this collection was submitted for microarray analysis using the Affymetrix Mouse 1.0 ST platform. The resulting data were analyzed for circadian cycling and statistical significance using JTK-CYCLE. Interestingly, RNA transcripts for receptors that respond to NPY and POMC not only show circadian expression, but peak at similar circadian time points in the hypothalamus and brainstem. The data furthermore show circadian expression of transcripts that influence wake and sleep cycles, including hypocretin and acetylcholine, along with glutamate and GABA, which control the balance of excitation and inhibition in the hypothalamus and brainstem.

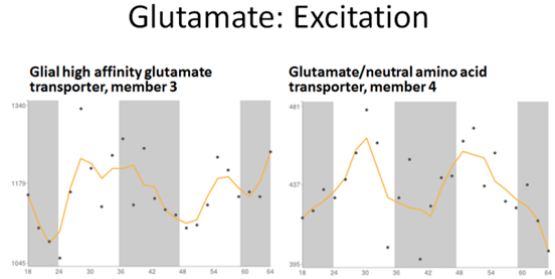
Data source: http://bioinf.itmat.upenn.edu/circa-dev/query

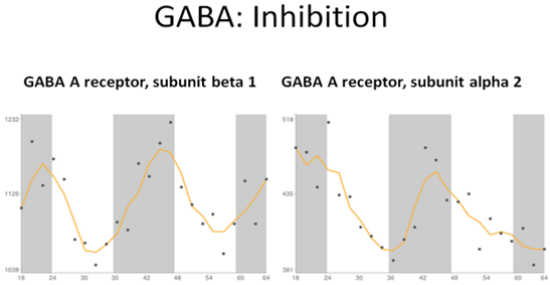












Trainee Award Eligible

Are Those with More Physically Demanding Jobs More Likely to Exhibit Short/Long Sleep Duration?

Holly Barilla MS, Charles Corbitt, Michael L. Perlis, and Michael A. Grandner PhD

Introduction: Short and long sleep are important health indicators, and it is important to understand the social and environmental context of short and long sleep in order to better characterize these individuals in the general population settings. One hypothesis is that those who get short or long sleep may be due to job related stress.

Methods: Data from the 2009 Behavioral Risk Factor Surveillance System was used (N=17,329 adults provided complete data). Sleep duration was assessed with, "On average, how many hours of sleep do you get in a 24-hour period?" and was subsequently categorized as short (≤6hours) or long (≥9hours) sleep. Job activity was classified as low ("mostly sitting or standing"), moderate ("mostly walking"), or high ("mostly manual labor"). Covariates included age, sex, race/ethnicity, income, education, overall physical and mental health, and minutes/day of moderate and vigorous activity. Multinomial logistic regression analyses evaluated whether job activity (vs low) was associated with likelihood of short or long sleep (vs normal).

Results: Compared to those in low activity jobs, those in moderate activity jobs were more likely to be short sleepers (RRR=1.26,p=0.001) and long sleepers(RRR=1.70,p<0.001), and those in high activity jobs were more likely to be short sleepers(RRR=1.62,p<0.001). After adjustment for covariates, those working moderate activity jobs were more likely to be short sleepers (RRR=1.23,p=0.005) and long sleepers(RRR=1.49,p=0.009), and those working high activity jobs were more likely to be short sleepers(RRR=1.45,p<0.001).

Conclusions: Individuals working in more high activity jobs are more likely to exhibit high-risk sleep durations, irrespective of other demographic, socioeconomic, and health factors that may be associated with those jobs.

Supported by R21ES022931, K23HL110216, UL1RR024134, R01AT003332, and R01MH077900. We wish to thank the Centers for Disease Control and Prevention for collecting these data and making them available, and the BRFSS participants.

Trainee Award Eligible

Insomnia With and Without Depression and Their Association with Daytime Dysfunction and Occupational Performance

Holly Barilla, MS[1], Michael A. Grandner PhD[2], Michael L. Perlis, PhD[1] , and Philip R. Gehrman PhD[1]

[1]*Behavioral Sleep Medicine Program, Department of Psychiatry, University of Pennsylvania*
[2]*Center for Sleep and Circadian Neurobiology, Division of Sleep Medicine, University of Pennsylvania*

Introduction: Many studies have documented that insomnia is associated with the self-report of daytime dysfunction and poor work performance. Most studies have shown this association using survey methods with non-standard determinations for the classification of insomnia. The present study explores daytime and occupational performance differences (as compared to good sleepers), in patients with primary insomnia, primary insomnia comorbid with major depression, and primary insomnia with remitted depression.

Methods: Participants (Good Sleepers [GSs]; Primary Insomnia [PIs]; Insomnia + Major Depression [MDDs]; Insomnia + Remitted Major Depression [MDDRs]) completed a series of questionnaires including the Insomnia Severity Index (ISI), Daytime Functioning Scale (DFS, Part1= Problems Functioning, Part2= Impact of Sleep), Quick Inventory of Depressive Symptoms (QIDS), and the Occupational Impact of Sleep Questionnaire (OISQ). Linear regression analyses evaluated ISI, QIDS, DFS1, DFS2, and OISQ as outcome, and group as predictor (vs. good sleepers), adjusted for age and sex.

Results: Compared to GSs (n=20), PIs (n=21) reported higher ISI (12.51,$p<0.0001$), QIDS (B=6.18,$p<0.0001$), and DFS2 (B=11.41,p=0.001). MDDs (n=16) reported higher ISI (B=11.75,$p<0.0001$), QIDS (B=13.11,$p<0.0001$), DFS1 (B=6.35,p=0.028), and DFS2 (B=7.54,p=0.045). MDDRs (n=21) reported higher ISI (B=11.04,$p<0.0001$), QIDS (B=10.29,$p<0.0001$), DFS1 (B=5.52,p=0.039), and DFS2 (B=6.95,p=0.048). While group differences were not apparent for the OISQ, when the insomnia groups were combined, a significant difference was observed (B=-18.20,p=0.029).

Conclusion: Individuals with Primary insomnia exhibited depression and were likely to ascribe their problems with daytime function to their insomnia (though the problems functioning were not different than those of good sleepers). Both the Depression groups, exhibited sleep continuity disturbance, depression, problems with daytime function, and were more likely to ascribe their problems with daytime function to their insomnia. The lack of findings with the occupational measure is likely due to smaller effects and/or the reduced statistic power when classifying patients with insomnia in terms of their subgroup status.

*This work was supported by **R01MH077900.***

Trainee Award Eligible

Sociodemographic and Behavioral Determinants of Short Sleep

M. Basner,[1] A. Spaeth, [1] D.F. Dinges[1]

[1]*University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA*

Introduction: Although habitual short sleep is associated with reduced alertness and cognitive performance, disease precursors, negative health outcomes, and mortality, 35%-40% of the adult US population report sleeping less than the recommended 7-8 hours on weekday nights. There is a need to identify characteristics and behaviors that predispose individuals to short sleep.

Methods: Analyses are based on a representative sample of Americans 15 years and older participating in the 2003 to 2011 American Time Use Survey (N=124,517).

Results: In multiple adjusted models, age (45-54 years), female gender, black ethnicity, high family income, being separated or widowed, having 3 or more household children, and working multiple jobs were all associated with a higher prevalence of short sleep duration ($\leq$ 6 hours; all $P<0.05$). Time spent working for pay, traveling, and grooming were the primary behavioral determinants of short sleep duration for both genders and virtually all age ranges after weighing each activity by the number of respondents engaging in it and by the average duration of the activity in those who engage in it. With every hour that work or educational activity started earlier in the morning, sleep time decreased by approximately 20 minutes.

Conclusions: Behavioral interventions to increase sleep for individuals who sleep less than 6 hours per night should focus on populations most at risk and concentrate on making the start times for work and educational activities later and more flexible when possible, and on reducing morning and evening commute times. Raising awareness that obtaining sufficient sleep may be as important as exercise and diet may be an additional way to promote public health.

Acknowledgments: Supported by NIH NR004281 and by the National Space Biomedical Research Institute through NASA NCC 9-58. The American Time Use Survey was sponsored by the Bureau of Labor Statistics and conducted by the U.S. Census Bureau. The Eating and Health module was sponsored by the U.S. Department of Agriculture‘s Economic Research Service and the National Institutes of Health‘s National Cancer Institute.

Craniofacial and Intraoral Anatomical Differences between Obese White and Black Americans as Measured With Digital Morphometrics

C.B. Bearn[1], F.L. Comyn, R.J. Schwab[1]
[1]*University of Pennsylvania School of Medicine, Philadelphia, PA*

Introduction: It is well established that there is a relationship between obstructive sleep apnea (OSA) and upper airway anatomy. It has also been reported that there is an increase in apnea severity among black Americans after adjusting for obesity and sex. However, there are very few studies that directly examine anatomical differences between white and black Americans. Based on previous studies, we hypothesized that lower facial height and intraoral soft tissue structures would be larger in black subjects in apneics and controls.

Methods: Subjects were recruited from the University of Pennsylvania Sleep Center. Fifty white and 50 black subjects were retrospectively matched on AHI (within 3.23±3.22 events/hr), BMI (within 2.28±1.73 kg/m^2), age (within 4.26±3.62 years) and gender. There was no difference in AHI, BMI, age, or weight between matched subjects ($p>0.1$). Subjects with an AHI≤10 events/hr were defined as controls and subjects with AHI≥15 events/hr were defined as apneic. Anatomical measures were taken from 3 craniofacial photographs (frontal; profile; and neck extended) and 3 intraoral photographs (mouth open with the tongue in the mouth; tongue extended; and tongue depressed with phonation). A laser ruler projected a known measure into each photograph and was used to convert pixel measures to centimeters. Differences in upper airway anatomy were evaluated using paired t-tests. **Results:** In this population of obese subjects, black apneics had a significantly larger lower facial height, smaller mandibular divergence, nasiolabial angle, and facial divergence compared to white apneics (Table 1). There was not a significant difference in the length of the mandible or corpus (Table 1). Black apneics also had significantly larger lateral mouth width, mouth opening area, and tongue width (Table 2). The directionality of other soft tissue intraoral structures was non-significantly larger in black apneics (Table 2). Black controls had a significantly larger lower facial height compared to white controls. Black controls also had a smaller mean neck width than white controls (Table 1).

Conclusion: Black apneics exhibited larger tongue and uvula measures and a smaller mandibular divergence (risk factors for sleep apnea). White apneics demonstrated a larger nasiolabial angle, smaller lower facial height, and smaller intraoral opening. Although differences between black and white controls were not significant, the direction of the differences was analogous to the apneic sample. These differences indicate that the upper airway anatomical risk factors of OSA differ significantly between white and black patients.

Table 1 Differences in Craniofacial Anatomy Between White and Black Americans Within Controls and Apneics; (n) mean ± SD

Photo	Measure	Units	Controls			Apneics		
			White	Black	[1]p	White	Black	[1]p
Front Photo	Lower Facial Width	cm	(10) 11 5 ± 0 6	(10) 11 4 ± 1 1	0 69	(27) 12 3 ± 0 9	(27) 12 2 ± 0 8	0 65
	Upper Facial Width	cm	(10) 12 3 ± 0 5	(10) 12 2 ± 1 0	0 90	(25) 12 5 ± 0 7	(25) 12 6 ± 0 7	0 69
	Neck Width	cm	(5) 9 47 ± 0 58	(5) 8 93 ± 0 76	**0.027**	(12) 10 4 ± 0 9	(12) 10 6 ± 0 9	0 50
Profile Photo	Mandibular Divergence	degrees	(11) 110 9 ± 10 2	(11) 106 7 ± 6 5	0 29	(31) 118 3 ± 11 3	(31) 110 9 ± 6 2	**0.0034**
	Mandibular Length	cm	(10) 6 91 ± 0 56	(10) 7 23 ± 0 87	0 22	(28) 7 19 ± 0 82	(28) 7 34 ± 1 08	0 56
	Mandibular Angle	degrees	(12) 108 4 ± 7 7	(12) 104 9 ± 5 9	0 20	(30) 112 0 ± 10 4	(30) 106 9 ± 8 4	0 057
	Upper Facial Height	cm	(9) 4 47 ± 0 54	(9) 4 62 ± 0 20	0 47	(29) 4 63 ± 0 45	(29) 4 61 ± 0 55	0 89
	Lower Facial Height	cm	(10) 5 48 ± 0 68	(10) 5 97 ± 0 53	**0.037**	(28) 5 42 ± 0 60	(28) 6 04 ± 0 61	**0.0004**
	Nasiolabial Angle	degrees	(12) 122 1 ± 6 3	(12) 114 7 ± 25 2	0 35	(32) 118 9 ± 9 5	(32) 111 7 ± 11 8	**0.022**
	Facial Divergence	degrees	(8) 162 9 ± 5 2	(8) 158 0 ± 4 9	0 14	(26) 166 1 ± 6 7	(26) 158 0 ± 5 1	**0.0001**
	Submental Area	cm^2	(7) 16 1 ± 2 9	(7) 14 8 ± 3 5	0 40	(15) 21 3 ± 6 7	(15) 18 0 ± 3 0	0 088
Neck Extended Photo	Mandibular Width	cm	(6) 9 37 ± 0 66	(6) 9 19 ± 0 64	0 64	(20) 9 69 ± 0 66	(20) 9 63 ± 1 52	0 87

[1] p-value from paired t-tests

Table 2 Differences in Intraoral Anatomy Between White and Black Americans Within Controls and Apneics; (n) mean ± SD

Photo	Measure	Unit	Controls			Apneics		
			White	Black	[1]p	White	Black	[1]p
Mouth Open, Tongue in the Mouth	Lateral Mouth Width	cm	(9) 5 40 ± 0 82	(9) 5 96 ± 0 86	0 19	(24) 5 78 ± 0 75	(24) 6 49 ± 0 99	**0.0085**
	Tongue Width	cm	(2) 5 32 ± 0 29	(2) 5 25 ± 0 20	ISS	(10) 5 27 ± 0 65	(10) 5 46 ± 0 54	0 42
	Mouth opening Area	cm^2	(11) 20 6 ± 4 9	(11) 22 8 ± 8 0	0 41	(28) 21 9 ± 5 4	(28) 26 2 ± 6 9	**0.024**
Tongue Extended	Tongue Width	cm	(9) 4 94 ± 0 65	(9) 5 27 ± 0 48	0 23	(24) 5 09 ± 0 51	(24) 5 48 ± 0 66	**0.020**
	Tongue Length	cm	(10) 4 89 ± 0 77	(10) 5 81 ± 1 36	0 079	(19) 5 05 ± 1 06	(19) 5 79 ± 1 41	0 15
	Tongue Area	cm^2	(10) 20 2 ± 4 5	(10) 24 8 ± 6 9	0 12	(20) 21 1 ± 5 5	(20) 26 0 ± 7 5	0 054
Tongue Depressed, with phonation	Uvula Width at the Airway	cm	(4) 0 903 ± 0 150	(4) 0 676 ± 0 085	ISS	(14) 0 795 ± 0 150	(14) 0 890 ± 0 181	0 12
	Uvula Width at the Soft Palate	cm	(3) 0 905 ± 0 038	(3) 1 339 ± 0 135	ISS	(7) 1 06 ± 0 26	(7) 1 20 ± 0 16	0 078

[1] p-value from paired t-tests; ISS = Insufficient Sample Size for statistical analysis

Aging Induced ER Stress Alters Sleep and Sleep Homeostasis

Marishka K. Brown, May T. Chan, John E. Zimmerman, Nicholas E. Jackson and Nirinjini Naidoo

Center for Sleep and Circadian Neurobiology, Division of Sleep Medicine, University of Pennsylvania, Philadelphia, PA

Introduction: Alterations in the quality, quantity and architecture of baseline and recovery sleep have been shown to occur during aging. *Drosophila melanogaster* shares several characteristics with mammalian sleep, including circadian and homeostatic regulation. Sleep deprivation induces endoplasmic reticular (ER) stress and upregulates a protective signaling pathway termed the unfolded protein response (UPR), which reduces the aggregation and accumulation of misfolded/unfolded proteins and shields the cell from injury. A key mechanism of the UPR involves increasing the levels of endogenous chaperones that bind to misfolded proteins. The effectiveness of this adaptive response, as well as other UPR elements including chaperone levels, is diminished by age. We have previously shown that increasing endogenous chaperone levels enhances recovery sleep in *D. melanogaster*. These results suggested that an exogenous chaperone could alleviate age-related sleep dysfunctions.

Methods: We compared sleep parameters in young and aged flies at baseline and after 6 h of sleep deprivation. Animals were either control or treated with 5mM PBA for 48 h. Molecular/biochemical studies were carried out to determine UPR markers induction over aging and with and without sleep deprivation.

Results: Acute administration of the chemical chaperone sodium 4-phenylbutyrate (PBA) reduced ER stress and ameliorated age-associated sleep changes in *Drosophila.* PBA consolidated both baseline and recovery sleep in aging flies. The behavioral modifications of PBA were linked to its suppression of the UPR. PBA decreased splicing of XBP1 and upregulation of p-eIF2α, both markers of the UPR, in flies that were subjected to sleep deprivation. Directly activating ER stress through protein misfolding in young flies fragmented baseline sleep and altered recovery sleep.

Conclusions: Age-related alterations in baseline and recovery sleep were ameliorated by the chemical chaperone PBA. Inducing ER stress directly correlates to changes in sleep.

Trainee Award Eligible

The Association between Liquid Consumption and Sleep Pattern in School Children

Siyuan Cao and Jianghong Liu

School of Nursing, University of Pennsylvania

Objectives: Nutritional factors have been thought to play a role in sleep behavior. However, few studies have investigated the consumption of liquids on sleep patterns. The aim of this study is to assess the frequency of liquid consumption on sleep patterns and the differential effects of the type of liquids on this pattern.

Method: A sample of 1978 4-6 graders (mean age 11 years old) from four elementary schools in Jintan City, Jiangsu Province of China participated in a sleep and health survey. Children were asked to complete a self-administered questionnaire including demographics, sleep behavior, and nutritional intake. Parents completed the Child Sleep Habit Questionnaire (CSHQ). Liquid consumption was self-reported by children at the same time as the self-report of their sleep habits. Parents were asked: "Generally speaking, how often do your child drink the following items? Water, tea, soft drinks," with the possible responses "never," "rarely," "sometimes," and "frequently." Descriptive statistics, correlations, and and independent sample t-tests analyses were performed to examine the differential effects of liquid consumption frequency and sleeping habits.

Results: For the consumption of water, responses gave 0.35% "never," 1.4% "rarely," 8.3% "sometimes," and 89.9% "frequently." Due to the small response sizes of the "never," "rarely," and "sometimes" groups, these groups were combined to serve as low liquid consumption (while the "frequently" group served as the high liquid consumption group. The same grouping was used for other liquids in the study. There was significant correlation between water consumption and 8 CSHQ subscales: bedtime resistance ($r=-0.125$, $p<0.001$), sleep onset delay ($r=0.094$ $p<0.01$), sleep anxiety ($r=-0.157$, $p<0.001$), night wakings ($r=-0.174$, $p<0.001$), parasomnias ($r=-0.173$, $p<0.001$), sleep disordered breathing ($r=-0.199$, $p<0.001$), and daytime sleepiness ($r=-0.116$, $p<0.01$).

Conclusions: Our study suggested that children who consume more water may have better quality sleep and less sleep problems. Future research needs to include objective sleep measures to overcome the limitation of current study with only subjective measures.

Trainee Award Eligible

Questionnaire-based Diagnosis of REM Sleep Behavior Disorder in Parkinson's Disease

Chahine LM, Daley J, Horn S, Colcher A, Hurtig H, Cantor C, Dahodwala N

Objective: To validate a combination of patient and bed-partner questionnaires for diagnosis of rapid eye movement (REM) sleep behavior disorder (RBD) in Parkinson's Disease (PD).

Background: RBD is present in up to 40% of PD patients and may portend more severe PD manifestations. It can also pose increased risk of injury, but is treatable. Definitive diagnosis of RBD requires demonstration of REM sleep without atonia on polysomnogram, but this is costly, time-intensive, and not practical for large-scale studies.

Design/Methods: We prospectively validated the patient-administered REM sleep behavior disorder questionnaire (RBSQ) and bed-partner-administered question 1 of the Mayo questionnaire in a convenience sample of 75 PD patients. A diagnosis of RBD was made based on International Classification of Sleep Disorders-II criteria: polysomnographic evidence of REM sleep without atonia and a clinical history of dream enactment behavior, determined through interview with the patient and his/her bed-partner.

Results: 75 PD patients (51 male, 68 Hoehn and Yahr stage I and II) participated. 48 had a clinical history of RBD. A cut-off on the RBSQ of 7 classified 78% of those with RBD correctly. Among those who achieved REM sleep (n=65), sensitivity was 74.2% (95% CI 55.1-87.5) for the RBSQ alone but was 100% [95% CI 86.3-100] when a combination of both questionnaires was compared to the gold standard of polysomnogram-confirmed RBD. Specificity was highest at 82.4% [95% CI 64.8-92.6] for the RBSQ used alone.

Conclusions: A combination of patient and bed-partner questionnaires is a useful tool to detect RBD in PD. Questionnaire-based diagnosis is more practical than diagnostic criteria that require polysomnographic confirmation. Combined use of patient and bed-partner questionnaire to diagnose RBD in the PD population would be an ideal screen. It is hoped that accurate questionnaire-based diagnosis will facilitate large-scale cohort studies to better understand the implications of RBD in PD.

Trainee Award Eligible

GABA Transasminase is Required for the Loss of Sleep in *Drosophila sleepless* Mutants

Wen-Feng Chen and Amita Sehgal

Sleep is an essential process and yet mechanisms underlying it are not well understood. Loss of the *Drosophila sleepless* (*sss*) gene increases neuronal excitability and diminishes daily sleep, providing an excellent model for exploring the underpinnings of sleep regulation. Using a proteomic approach we found that in *sss* fly brains protein levels of CG7433, a γ-aminobutyric acid transaminase (GABAT) that acts in mitochondria, are increased post-transcriptionally and GABA levels are reduced. Loss of *GABAT* increases daily sleep and improves sleep consolidation. Importantly, disruption of the *GABAT* gene completely suppresses the sleep phenotype of *sss* mutants, demonstrating that increased GABAT in *sss* is relevant for this phenotype.

Trainee Award Eligible

A Randomized Trial of Continuous Positive Airway Pressure Therapy, Weight Loss, or Both for Obese Adults with Obstructive Sleep Apnea

Julio A Chirinos, MD, PhD[1,2]; Indira Gurubhagavatula, MD[1,2]; Karen Teff, PhD[1]; Daniel Rader, MD[1]; Thomas Wadden, PhD[1]; Raymond Townsend, MD[1]; Gary Foster, PhD[3]; Greg Maislin, MS, MA[1]; Hassam Saif, MD[1]; Preston Broderick, MSc[1]; Jesse Chittams, MS[4]; Alexandra L. Hanlon, PhD[4]; Allan Pack, MD, PhD.[1]

[1]University of Pennsylvania School of Medicine/Hospital of the University of Pennsylvania; [2]Philadelphia VA Medical Center; [3]Temple University School of Medicine; [4]University of Pennsylvania School of Nursing

Background: Obesity and obstructive sleep apnea (OSA) tend to co-exist and are associated with inflammation, insulin resistance, dyslipidemia and hypertension. The causal effect of OSA vs. obesity on these cardiovascular risk factors is unclear and cannot be confidently discerned in cross-sectional studies. We designed this mechanistic randomized trial to assess assess the incremental effect of combination therapy (weight loss and CPAP) over each individual therapy in obese patients with OSA is unknown.

Methods: We randomly assigned 181 subjects with obesity (body mass index>30 kg/m^2), moderate-to-severe OSA (Apnea-Hypopnea Index $\geq$15), and a C-reactive protein (CRP) level >1.0 mg/L to undergo one of 3 interventions for 24 weeks: (1) Continuous positive airway pressure (CPAP); (2) Weight Loss; (3) Both CPAP therapy and weight loss. We assessed the effect of these interventions on C-reactive protein levels, insulin sensitivity (assessed with a frequently-sampled intravenous glucose tolerance test), fasting lipid parameters and blood pressure in both intent-to-treat analyses and pre-specified analyses including only compliant subjects.

Results: A total of 136 subjects completed the study. Weight loss, but not CPAP therapy, resulted in a sustained reduction in CRP, insulin resistance, LDL-cholesterol, LDL-particle concentration, apoprotein B and a sustained increase in LDL-particle size at 24-weeks (all P<0.05). For these endpoints, changes in the combination arm were significantly greater than those observed in the CPAP-only group (all P<0.05), without significant differences between the combination arm and the weight-loss only arm. In contrast, both weight loss and CPAP therapy resulted in significant sustained reductions in systolic and mean arterial pressure (P<0.0001). For these endpoints, changes in the combination therapy arm were significantly greater than both monotherapy arms. In pre-specified per-protocol analyses including only adherent subjects, combination therapy was associated with a much larger effect on systolic BP (14.1 mmHg-reduction) than that the effect associated with either CPAP monotherapy (3.0 mmHg-reduction; P vs. combination therapy arm <0.0001) or weight loss monotherapy (6.8 mmHg-reduction; P vs. combination therapy arm = 0.02).

Conclusions: Among obese patients with moderate-to-severe OSA, combination therapy with CPAP and weight loss is superior to either therapy alone to improve the cardiovascular risk profile. Our results suggest an important causative role for OSA on high blood pressure, whereas obesity is the predominant causative factor related to inflammation, dyslipidemia and insulin resistance.

Obstructive Sleep Apnea in Infants with Cleft Palate and Tongue-based Airway Obstruction

Cielo C[1], Taylor JA[2], Vossough A[3], Bradford RA[1], Radcliffe J[4], Sullivan A[5], and Marcus CL[1]

Children's Hospital of Philadelphia, [1]Sleep Center, [2]Division of Plastic and Reconstructive Surgery, [3]Division of Radiology, [4]Division of Child Development, [5]Research Institute

Introduction: Isolated cleft palate (ICP) and craniofacial disorders resulting in tongue-based airway obstruction (TBAO) are risk factors for obstructive sleep apnea syndrome (OSAS). The contribution of craniofacial anatomy to OSAS and the effect of OSAS on development in this age are unknown. We hypothesize that there is an increased prevalence of OSAS in both populations, that craniofacial parameters are inversely related to severity of OSAS, and that OSAS contributes to neurodevelopmental impairment in this population.

Methods: Consecutive infants with unrepaired ICP or TBAO were prospectively recruited. Subjects underwent polysomnography (PSG), neurodevelopmental evaluation (Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III), and lateral neck radiograph for cephalometrics.

Results: 13 subjects aged 11.3±9.4 weeks (mean±SD) were evaluated, including 7 (53%) with TBAO and 7 (53%) boys. Both the ICP and TBAO groups had substantial OSAS, but the TBAO group had more abnormalities seen on PSG (Table 1). There was correlation between AHI and four cephalometric measurements, some of which were measures of midface hypoplasia and others markers of retrognathia (Figure 1). Eight subjects completed Bayley-III testing (this testing could not be completed in the sickest patients). The Bayley-III cognitive composite scores were low (83.6±13) compared with published normal values of 100±15 (Figure 2). With this small sample size, there was not a significant correlation between Bayley-III cognitive composite scores and AHI.

Conclusion

Infants with ICP and TBAO have an AHI that is substantially higher than normal values for this age. There is a positive correlation between AHI and degree of retrognathia and midface hypoplasia. There is substantial cognitive delay in this infant population. We speculate that cognitive delays may be in part related to the presence of obstructive sleep apnea. More research is needed to determine whether obstructive sleep apnea is persistent and if there are long term developmental consequences in this population.

Enhanced Resting-State Striatal Connectivity Correlates with Increases in Daily Caloric Intake after Sleep Deprivation

Fang Z, Spaeth AM, Zhu S, Taylor A, Goel N, Basner M, Detre JA, Dinges DF, Rao H

Center for Functional Neuroimaging, Department of Neurology, and Division of Sleep and Chronobiology, Department of Psychiatry, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

Introduction: Sleep loss is a recognized risk factor for overeating and obesity, however the underlying neural mechanisms remain unclear. Previous imaging studies have indicated that sleep loss may enhance neural responses to food stimuli in the dopaminergic striatal pathways. In this study, we examined the effects of one night of total sleep deprivation (TSD) on resting-state striatal functional connectivity (FC) and its relationship to changes in caloric intake.

Methods: A total of 21 healthy adults (11 females, age 22-50 yrs, BMI 19.7-28.9) were scanned at rest on three occasions between 7-9 am using a standard EPI sequence on a Siemens 3T MR scanner: a baseline (BS) scan after 9h normal sleep, a SD scan after 24h TSD, and a third scan after two nights (20h) recovery sleep (RS). FC analyses using caudate as the seed region were conducted using SPM8 and the REST toolbox. Food and beverage intake was weighed before and after subjects ate a meal. Participants' food consumption was ad libitum and recorded. Changes in daily caloric intake were calculated and correlated with FC alteration following SD.

Results: Participants' caloric intake per hour was significantly higher following SD compared to BS ($p<0.001$). FC analyses showed no differences between BS and RS. However, SD significantly enhanced connectivity to caudate in the thalamus, dorsolateral prefrontal cortex, superior parietal cortex, precuneus, and putamen, and reduced connectivity in the sensorimotor regions (all $p<0.001$). Caudate-putamen connectivity changes correlated with increases in caloric intake from BS to SD ($r=0.62$, $p=0.003$).

Conclusion: Our results show that SD significantly altered resting-state striatal connectivity which predicted increases in daily caloric intake after sleep loss, supporting the role of the dopaminergic system in overeating. These findings suggest that disrupted dopamine pathway connectivity may be one mechanism by which SD increases food intake.

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Trainee Award Eligible

Cooperative Interaction between Phosphorylation Sites on PERIOD Maintains Circadian Period in *Drosophila*

David S. Garbe[1], Yanshan Fang[†], Xiangzhong Zheng[1], Mallory Sowcik[1], Rana Anjum[3,‡], Steven P. Gygi[3], and Amita Sehgal[1,2,§]

[1]Department of Neuroscience, [2]Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Translational Research Center, Philadelphia, PA; [3]Department of Cell Biology, Harvard Medical School, Boston, MA

Introduction: Circadian rhythms in Drosophila rely on cyclic regulation of the period (per) and timeless (tim) clock genes. The molecular cycle requires rhythmic phosphorylation of PER and TIM proteins, which is mediated by several kinases and phosphatases such as Protein Phosphatase-2A (PP2A) and Protein Phosphatase-1 (PP1).

Methods: Here we used mass spectrometry to identify 35 "phospho-occupied" serine/threonine residues within PER, 24 of which are specifically regulated by PP1/PP2A. We found that cell culture assays were not good predictors of protein function in flies and so we generated per transgenes carrying phosphorylation site mutations and tested for rescue of the per^{01} arrhythmic phenotype.

Results: Surprisingly, most transgenes restore wild type rhythms despite carrying mutations in several phosphorylation sites. One particular transgene, in which T610 and S613 are mutated to alanine, restores daily rhythmicity, but dramatically lengthens the period to ~30hrs. Interestingly, the single S613A mutation extends period length by 2-3 hours, while the single T610A mutation has a minimal effect, suggesting these phospho-residues can substitute for each other to a large extent. Conservation of S613 from flies to humans suggests that it has a critical role in determining circadian period. Biochemical data indicate defects in overall phosphorylation and altered timely degradation of PER carrying the double or single S613A mutation(s). Immunohistochemical analysis revealed that PER-T610A/S613A exhibits an extended period of protein expression during the middle of the day. Our results also suggest that PER undergoes nuclear-to-cytoplasmic redistribution in some clock cells prior to its daily decline.

Conclusions: Together these data identify specific phosphorylation events that are critical for PER stability and circadian period, demonstrate that cooperativity between phosphorylation sites maintains PER function, and reveal novel features of PER regulation.

Trainee Award Eligible

Propofol Anesthesia in *Drosophila*

Benjamin Gardner, Qing Cheng Meng[1], Max Kelz[1], Julie A. Williams

Center for Sleep and Circadian Neurobiology, [1]Department of Anesthesiology and Critical Care, University of Pennsylvania Perelman School of Medicine

Introduction: Sleep and anesthetic-induced hypnosis share many neurophysiologic features, such as the breakdown of effective cortical communication accompanying loss of consciousness, indicating an overlap in the neuronal circuitry underlying both states. Unlike the volatile anesthetics, the intravenous hypnotic propofol putatively satisfies the homeostatic drive for sleep. Pre-existing sleep debts are discharged identically under propofol anesthesia as during natural rebound sleep, while new sleep debts do not accrue under propofol anesthesia. Moreover, sleep plays an important role in the maintenance of a robust immune system, and the acute increase in sleep prompted by infection has been proposed to enhance immune function. Using the Drosophila model, we investigated a ternary link between propofol anesthesia, sleep and the immune response by first determining if propofol treatment abolishes behavioral responsiveness in flies as it is known to do in humans. We next induced an immune response by infection with Gram-negative bacteria after propofol sedation to test the hypothesis that propofol would either act as an immunosuppressant or confer an immunological benefit by exogenously enforcing a rest period prior to infection. Finally, we evaluated the notion that propofol sedation may substitute for natural sleep using a mechanical sleep deprivation paradigm.

Methods & Results: Daily sleep was measured in Canton-S wild-type flies incubated at 25 ˚C in a 12:12 h light:dark cycle. Female flies were loaded into the Drosophila Activity Monitoring system (DAM system; Trikinetics Inc.) to measure their locomotor activity and sleep. Propofol was administered by transferring flies to activity tubes with sucrose food containing either 1 mM propofol or equivalent concentrations of vehicle. We found that flies are sedated by propofol in a dose-dependent manner. Moreover, subjecting flies fed propofol to vibratory stimuli revealed that anesthetized subjects have an increased arousal threshold compared to naturally sleeping flies, permitting us to distinguish between natural sleep and propofol-induced hypnosis.

To evaluate whether propofol sedation satisfies the homeostatic need for sleep, we subjected flies to six-hour SD in the latter half of the night, followed by propofol exposure in the morning hours. Recovery sleep in the group fed propofol after SD was indistinguishable from a non-deprived group that received propofol at the same time. Results will be presented examining the absorption and clearance of ingested propofol using High-Performance Liquid Chromatography (HPLC), allowing behavior to be correlated to fly tissue propofol concentrations at different time points. Finally, we investigated whether treatment with propofol would impair immune function by infecting flies with Gram-negative bacteria after nighttime propofol sedation. The survival of flies fed propofol was not found to be significantly different from un-anesthetized subjects. In flies, gram-negative and gram-positive bacteria activate different aspects of the innate immune response, and hence ongoing studies are focused on infection of anesthetized flies using gram-positive bacteria an effort to discriminate between propofol-mediated immunomodulation that may be restricted to a specific pathway comprising *Drosophila* innate immunity.

Conclusion: In summary, we found that treatment with propofol sedates flies in a dose-dependent manner. To better understand whether differences in sleep exhibited by the group fed propofol after deprivation arises from increased sleep pressure or residual anesthetic, the pharmacokinetic features of propofol ingestion will be characterized using HPLC. Together, these findings establish *Drosophila* as a suitable genetic model to evaluate mechanisms of propofol anesthesia.

Trainee Award Eligible

Amyloid-beta Induced Sleep Fragmentation is Rescued by Fatty-acid Binding Proteins in *Drosophila*

Jason R. Gerstner, May T. Chan, Olivia Lenz, Cory Pfeiffenberger, Allan I. Pack

Sleep amount and quality are known to decline with age. This effect is even more pronounced in Alzheimer's disease (AD), and is a major contributing factor for institutionalization. Amyloid-beta (Aβ) aggregation increases during AD, and is associated with disruption of sleep. Sleep/wake disturbances may also accelerate the neurodegenerative process. Therefore, identifying changes in sleep prior to clinical onset may serve as a prodromal marker to facilitate interventions that delay AD progression. Molecular mechanisms which contribute to disturbed sleep in AD are not known and therefore present a challenge for development of therapeutic strategies. Fatty-acid binding proteins (Fabp) are small chaperones that shuttle long-chain fatty-acids such as docosahexaenoic acid, a lipid known to reduce Aβ plaque burden and restore cognitive deficits in AD mouse models. Fabp expression cycles based on time-of-day, has been implicated sleep and memory processes, and is reduced at synapses following aging. Transgenic flies which express Aβ are syndromal to human AD, and have progressive cognitive deficits and neurodegeneration. Here, we were interested in characterizing the effects of Fabp expression on sleep in a Drosophila AD model. Flies carrying a transgene that induces the expression of human Aβ42 peptide under the control of a neuronal promoter were examined for changes in sleep using a video monitoring assay. Aβ42-flies sleep was compared with control flies with or without the presence of another transgene that overexpresses the Drosophila Fabp gene. We observed Aβ flies have significantly reduced sleep in both daytime and night-time at ages which precede memory loss and neurodegeneration. The reduction in sleep observed in Aβ flies is rescued with flies that carry a Drosophila Fabp transgene. These data suggest that sleep can serve as a prodromal marker in an AD animal model, and that Fabp may be a novel therapeutic target for the treatment of AD symptoms.

Trainee Award Eligible

Neurobehavioral and Physiological Effects of High Cognitive Workload and Chronic Sleep Restriction

Goel N, Braun ME, Dinges DF

Division of Sleep and Chronobiology, Department of Psychiatry, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Introduction: Although sleep loss degrades cognitive functions, little attention has been devoted to determining whether waking cognitive activity potentiates the effects of sleep loss, even though time on task is often considered an important fatigue factor in addition to time awake. This experiment evaluates the effects of variation in cognitive workload and variation in sleep restriction on behavioral attention, fatigue, sleepiness, physiological alertness and executive functioning.

Methods: N=63 healthy adults (33.2 ± 8.7y; 29 females) completed a 10-day controlled laboratory experiment with randomization to one of four conditions (moderate cognitive workload [MW] + sleep restriction [SR]; high cognitive workload [HW] + SR; MW + no sleep restriction [NSR]; HW + NSR). SR entailed 5 nights at 4h TIB; NSR entailed 5 nights at 8h TIB. Subjects had 3 workload test sessions/day of either 120 min (HW) or 60 min (MW).

Results: High workload, regardless of the presence of sleep loss, significantly increased subjective fatigue and sleepiness (VAS, KSS; p's<0.05), but did not significantly affect behavioral attention (PVT), physiological alertness (MWT) or executive functioning (COWAT and Hayling tests; all p's >0.05). Sleep restriction produced significant cumulative increases in PVT lapses, fatigue, and sleepiness and decreases in PVT response speed and MWT sleep onset latencies (all p's<0.05). There were no significant interactions between workload and sleep restriction for any of the outcome measures.

Conclusion: The results of this study provide the first experimental evidence that the amount of cognitive workload produces negative effects on subjective (e.g., fatigue and sleepiness) aspects of neurobehavioral performance independent of sleep loss. Cognitive workload is an important factor worthy of consideration in a broad range of laboratory and applied settings in which demanding workloads are common.

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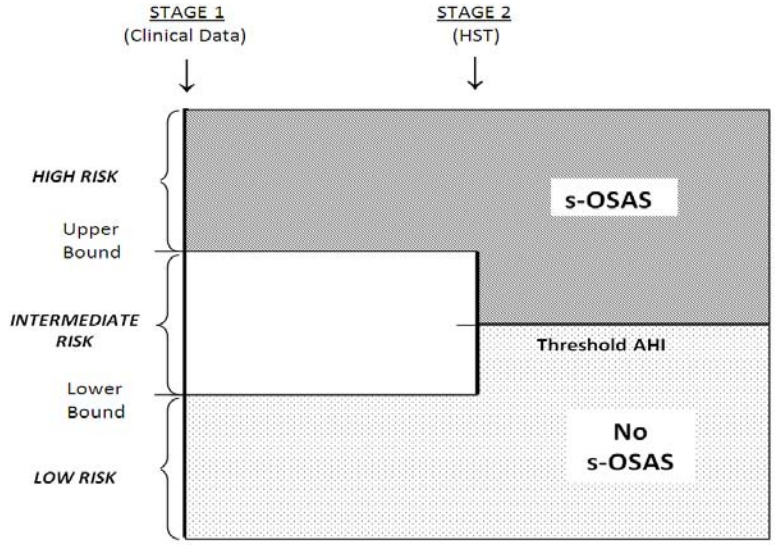
Screening for Severe Obstructive Sleep Apnea Syndrome in Hypertensive Outpatients

Indira Gurubhagavatula MD, MPH;[1, 2, 3] Barry G. Fields MD; [2,3] Christian R Morales MD;[2] Sharon Hurley;[2] Grace W Pien MD, MSCE;[2,3] Lindsay C. Wick;[2] Bethany A. Staley;[2] Raymond R Townsend MD;[4] Greg Maislin MS, MA[2]

Pulmonary and Critical Care and Sleep Section, Philadelphia VA Medical Center, Philadelphia, Pennsylvania,[1] Center for Sleep and Circadian Neurobiology,[2] Division of Sleep Medicine,[3] Renal Electrolyte and Hypertension Division,[4] Department of Medicine, University of Pennsylvania Medical Center, Philadelphia, PA

We attempted to validate a two-stage strategy to screen for severe obstructive sleep apnea syndrome (s-OSAS) among hypertensive outpatients, with polysomnography (PSG) as the gold standard. Using a prospective design, we recruited outpatients with hypertension from medical outpatient clinics. Interventions included: 1) assessment of clinical data; 2) home sleep testing (HST); and 3) 12-channnel, in-laboratory PSG. We developed models using clinical or HST data alone (single-stage models) or clinical data in tandem with HST (two-stage models; Figure) to predict s-OSAS. For each model, we computed area-under-receiver-operating-characteristic curves (AUC), sensitivity, specificity, negative likelihood ratio, and negative post-test probability (NPTP). Models were then rank-ordered based upon AUC values and NPTP. HST used alone had limited accuracy (AUC=0.727, NPTP = 2.9%). However, models that used clinical data in tandem with HST were more accurate in identifying s-OSAS, with lower NPTP: 1) facial morphometrics (AUC=0.816, NPTP=0.6%); 2) neck circumference (AUC=0.803, NPTP=1.7%); and 3) Multivariable Apnea Prediction Score (AUC = 0.799, NPTP =1.5%) where sensitivity, specificity and NPTP were evaluated at optimal thresholds. Therefore, HST combined with clinical data can be useful in identifying s-OSAS in hypertensive outpatients, without incurring greater cost and patient burden associated with in-laboratory PSG. These models were less useful in identifying OSAS of any severity.

Figure. Study Design (Two-Stage Models)



Abbreviations: HST = home sleep test; AHI = apnea-hypopnea index; s-OSAS = severe OSA associated with sleepiness

Trainee Award Eligible

Sleep Deprivation Impairs Memory by Altering Cofilin and Actin Signaling

Robbert Havekes[1], Jonathan P. Day[2], Vibeke M. Bruinenberg[1,3#], Jennifer H.K. Choi[1], Shane G. Poplawski[1], Kyle S. Krainock[1,4], Arnd Baumann[5], Peter Meerlo[3], Sara J. Aton[6], Miles D. Houslay[7], George S. Baillie[2], Ted Abel[1].

[1]Department of Biology, University of Pennsylvania, Philadelphia, PA; United States; [2]Institute of Cardiovascular and Medical Science, Wolfson and Davidson Buildings, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland, United Kingdom; [3]Center for Behavior and Neurosciences, University of Groningen, Groningen, The Netherlands; [4]Department of Psychology, Swarthmore College, Swarthmore, PA;, United States.[5]Forschungszentrum Juelich, Institute of Complex Systems, Zelluläre Biophysik , Juelich, Germany; [6]LSA Molecular, Cellular, and Developmental Biology, Kraus Building, University of Michigan, Ann Arbor, MI; [7]Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, London, United Kingdom.

Sleep deprivation is a significant public health issue affecting millions of people. Insufficient sleep attenuates brain function, particularly targeting the hippocampus, and contributing to cognitive disorders and psychiatric diseases. However, the molecular mechanisms by which sleep deprivation impairs memory storage are not defined. We show that sleep deprivation alters hippocampal actin dynamics through increased activity of the filamentous actin-severing enzyme cofilin. Viral expression of inactive mutant cofilin selectively in hippocampal excitatory neurons makes memory consolidation resistant to sleep loss. The sleep deprivation-induced increase in cofilin activity and associated memory deficits are prevented by increasing cAMP levels or suppressing PDE4A5 function using novel pharmacogenetic and viral approaches. Transient sleep loss alters molecular signaling cascades within hippocampal neurons, leading to changes in actin dynamics and impairments in memory storage.

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Factors Associated with Excessive Daytime Sleepiness

Jingying He, Ke Shen, Chen Zhu, Jenny Wang, Xianchen Liu, Jianghong Liu

Objective: To examine the prevalence of excessive daytime sleepiness (EDS) and the factors (child, family, emotional problems) correlates EDS in Chinese children

Methods: A community sample of 3914 4-6 graders from 4 elementary schools in Jintan city participated in a sleep and health survey. Of the sample, 52.2% were boys and mean age was 10.99 (SD=0.99). Children were asked to complete a self-administered questionnaire including demographics, sleep behavior, and emotional problems and parents (n=2396) completed the Child Sleep Habit Questionnaire (CSHQ). Seven items were used to indicate daytime sleepiness, namely, feeling very sleepy during the day, falling asleep very easily, taking a long time to become alert, having difficulty getting out of bed in the morning, needing adult or siblings to wake up, waking up in negative mood, feeling physically tired during the day, watching TV or riding in car.

Results: The prevalence rates of parent- and self-reported EDS range from 41.0%-74.1% versus 59.7%-77.7% for "rarely", 4.3%-29.1% versus 15.1%-21.1% for "sometimes", and 6.1%-31.1% versus 7.2%-23.3% for "usually". EDS was more prevalent in boys than in girls. Multiple factors are associated with EDS. Specifically, both parent- and self-reported EDS were associated with sleep duration at weekdays (self-report: β=-0.216, p=0.015; parent-report: β=-0.173, p=0.013), short sleep duration (self-report: β=0.214, p=0.006; parent-report: β=0.201, p=0.001) and sleep anxiety (self-report: β= 0.232, p=0.008; parent-report: β=0.272, p=0.000). Parent-reported EDS was also associated with parent-reported night waking (β=0.327, p=0.001), parasomnias (β=0.196, p=0.001) and sleep disorder breathing (β=0.294, p=0.006). In addition, self-reported EDS was associated with self-reported internalizing problem (β=0.091, p<0.001).

Conclusion: Excessive daytime sleepiness (EDS) was very prevalent in Chinese adolescents as reported by both parents and adolescents themselves. Excessive daytime sleepiness was significantly associated with sleep problems/difficulties and internalizing problems in Chinese adolescents. Future study may include objective measure of sleep behavior in order to accurately assess the relationship between EDS and adolescents' behavioral outcomes.

A Screening Algorithm for Obstructive Sleep Apnea in Pregnancy

Bilgay Izci-Balserak MA, PhD[1,2,3], Indira Gurubhagavatula, MD, MPH[1,2,4], Brendan T. Keenan, MS[1,2], Grace W. Pien MD, MSc[1,2]

[1]Center for Sleep and Circadian Neurobiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Division of Sleep Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [3]School of Nursing , University of Pennsylvania, Philadelphia, PA; [4]Pulmonary and Critical Care and Sleep Section, Philadelphia VA Medical Center, Philadelphia, Pennsylvania

Introduction: Obstructive sleep apnea (OSA) is associated with maternal and fetal complications. Its recognition may have important implications during pregnancy. Upper airway narrowing is a main determinant of OSA. Its evaluation with other existing objective and subjective tools could be useful for identifying OSA. We aimed to develop a screening tool combining subjective and objective measures to identify OSA in the pregnant population.

Methods: Data were collected from a completed cohort study of OSA including 108 women in the first trimester and 87 in the third. Participants underwent full polysomnography, completed Index of Sleep Apnea (ISA) of Multivariable Apnea Prediction index, Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index, and had OSAHS Score (sum of Mallampati score, body mass index categories and tonsil size). Bivariate regression analyses were performed. Each bivariate logistic regression variable with a suggestive ($p<0.2$) associated with apnea risk was forwarded into a multivariate analysis to determine the best performing tool/s. The accuracy of the models was assessed using receiver operating characteristics curves for AHI≥5 in both trimesters.

Results: Mean age was 27.4±7.0. Twelve women in the first and 21 in the third trimester had AHI≥5. In the first trimester, the model combining age, BMI and NSaO2 was the best predictor of OSA risk. The AUC of this model was 0.838 (95% CI, 0.708 – 0.968). In the third trimester, the model which included age and BMI was the best performing model to determine OSA risk (AUC 0.812; 95% CI, 0.709 – 0.916). Further analysis demonstrated the first trimester age, BMI and ISA score are the best determinants of OSA risk in the third trimester (AUC 0.781; 95% CI, 0.654-0.908).

Conclusion: The existing screening tools assessing sleep problems, SDB symptoms alone and the upper airway physical exam are poor predictors of OSA risk in pregnant women. BMI, age, $NsaO_2$ and ISA are the most valuable determinants of OSA risk in pregnant population.

Self-Reported and Scheduled Sleep in Spaceflight

Jones CW,[1] Basner M,[1] Mollicone DJ,[2] Dinges DF[1]

[1]Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, [2]Pulsar Informatics Inc., Philadelphia PA

Introduction: Fatigue induced by chronic partial sleep deprivation, circadian misalignment (e.g., slam shifts), and work overload (e.g., EVAs) is one of the more prominent risks to astronaut cognitive performance during long-duration spaceflight. Despite the necessity of sleep, maximizing sleep in spaceflight is difficult due to high work demand and environmental factors on the International Space Station (ISS).

Methods: Scheduled sleep data were acquired from the flight plan timelines used by the crew onboard ISS. Self-report sleep, sleep quality and workload ratings were collected as part of the Reaction Self Test (3-minute PVT-B) currently deployed on the ISS by our group. N=18 astronauts (50±4y; 4f) reported in-flight sleep data on a total of 657 non slam-shift sleep periods. Baseline data was also collected an N=199 sleep periods before flight.

Results: Baseline data on an N=199 sleep periods revealed an average reported sleep duration of 6.7h (SD±1.17h). During flight, astronauts reported an average sleep duration of 6.38h (±1.48h), approximately 2.12h less than the average scheduled sleep period of 8.5h. Only 7.8% of reported sleep durations were equal to or longer than scheduled sleep periods. Astronaut's ratings of sleep quality were generally high (87.7% of ratings ≥5 on a 10 point scale, avg. 6.55±1.73). Additionally, astronaut's ratings of workload were generally high (80.0% of ratings ≥5 on a 10 point scale, avg. 5.79±2.20).

Conclusion: Astronauts on the ISS are scheduled to a nightly average of 8.5 hours time in bed. However, astronauts frequently report sleeping only 6.38 hours each night on ISS. Although sleep quality on ISS is generally rated by astronauts as good, the use of hypnotics to ensure sleep during scheduled sleep periods is not uncommon. Chronic sleep deprivation will have performance implications on critical tasks and there is a need to protect and promote astronaut sleep periods while living and working on the ISS.

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Trainee Award Eligible

Role of a Steroid Hormone Nuclear Receptor E75 in *Drosophila* Circadian Clock

Shailesh Kumar[1], Dechun Chen[2], Christopher Jang[2], Amita Sehgal[1,2]

[1]Howard Hughes Medical Institute, [2]Department of Neuroscience, University of Pennsylvania Perelman School of Medicine, Philadelphia PA

A modular misexpression system was used to carry out systematic gain-of-function genetic screens to identify novel genes involved in regulating circadian rhythms; we found several genes whose overexpression led to arrhythmic behavior. The system is based on inducible expression of genes tagged by insertion of a P-element vector carrying a GAL4-regulated promoter oriented to transcribe flanking genomic sequences. Out of the several genes identified in this screen, we particularly focused on a nuclear receptor protein E75 (or Eip75; Ecdysone Induced Protein 75). The E75 gene expression is known to be induced by ecdysone treatment in the pre-adult as well as adult stages. The steroid hormone 20-hydroxyecdysone and its EcR/USP receptor are vital during insect development for coordinating molting and metamorphosis. However, the function for this hormone signaling system is largely unknown in adult stage. Recent studies indicate that the hormone and receptor are present and active in adults and those mutations decreasing hormone or receptor levels affect diverse processes such as reproduction, sleep-wake behavior, stress resistance, and lifespan. In our study we found that E75 directly acts as *Clk* repressor in the central pacemaker cells as well as in the peripheral clocks. The overexpression as well as the knockdown of *E75* gene in the all clock neurons leads to arrhythmic or weak circadian behavior. The overexpression and knockdown of *E75* in all clock cells also abolishes the molecular cycling of PER in the central pacemaker neurons as well as in the peripheral clock cells under light dark conditions. Interestingly, PER has been proposed to act as a de-repressor of *Clk* gene expression, however the underlying molecular mechanisms are unknown (Glossop et al 1999). We have uncovered the molecular mechanism by which that the PER protein mediates the de-repression of *Clk* gene. Based on cell culture assays we found that PER directly interacts with E75 to remove it from the *Clk* promoter and relieves the *Clk* gene repression. In addition, we also found that E75 appear to mediate stress response (nutritional and temperature) to the circadian clock; flies lacking E75 in the central clock cells become completely arrhythmic under stressful conditions whereas those with optimal amount of E75 do not.

Trainee Award Eligible

Polysomnographic Validation of Actigraphy in Healthy Adults

Langguth JJ, Jones CW, Ecker AJ, Basner M, Dinges DF

Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Introduction: Actigraphy is a widely used technique to infer sleep-wake behavior, but published validation studies using behavioral observation and polysomnography (PSG) in healthy subjects in controlled environments are rare. The objective of this study was to validate Philips Respironics Actiwatch AW64 with PSG via epoch-by-epoch agreement of concurrent behavioral states.

Methods: N=22 healthy adults (mean age 35.1 ± 9.0 y [SD]) participated in a protocol that included 115 laboratory days with scheduled 8h time in bed (TIB) periods. Subjects continuously wore the actigraph on the wrist of the non-dominant arm. The actigraph analysis software Actiware 5.59.0015 automatically assigns 3 discrete behavioral states (i.e., active wake, resting wake, sleep). For this analysis, resting wake and active wake were combined in the wake category. Obvious misclassifications in the automatic scoring of the actigraphy software were corrected by human scoring independent of validation criteria (this was < 1.5% of all data). Objective documentation (i.e., validation criteria) of wakefulness was accomplished by continuous behavioral monitoring of subjects, while sleep was verified by PSG during scheduled daily sleep periods.

Results: In a double-blind epoch-by-epoch analysis (N=331,200 thirty-second epochs), the Actiwatch algorithm correctly identified 97.0% of sleep epochs (sensitivity) and 96.2% of wake epochs (specificity). The overall accuracy of the algorithm for a 24-h period was 96.4%, with an almost perfect chance-corrected agreement (κ=0.914). Validation analyses confined to only 8-h TIB periods for sleep correctly identified 97.0% of sleep epochs (sensitivity), 46.4% of wake epochs (specificity), and had 89.9% accuracy with a moderate agreement (κ=0.509). Actiwatch sleep time overestimated PSG sleep time for the 24-h period by 26.4 min (95% CI 18.0-34.8 min; P < 0.0001).

Conclusion: These results confirm the utility of the Actiwatch for the investigation of rest-activity cycles in healthy adults.

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Trainee Award Eligible

Dietary Branched Chain Amino Acids Ameliorate Sleep Disturbances after Mild Traumatic Brain Injury: Orexinergic and Glutamatergic Mechanisms

Miranda M. Lim[1,2,3], Jaclynn Elkind[4], Guoxiang Xiong[4], Ray Galante[3], Jingxu Zhu[3], Lin Zhang[3], Jie Lian[3], Akiva S. Cohen[4], Allan I. Pack[1,3]

[1]Division of Sleep Medicine, Dept of Medicine, University of Pennsylvania; [2]Department of Neurology, University of Pennsylvania; [3]Center for Sleep and Circadian Neurobiology, University of Pennsylvania; [4]Division of Neurology, Dept of Pediatrics, Children's Hospital of Philadelphia

Introduction: Chronic sleep disturbances after traumatic brain injury (TBI) have been reported in up to 72% of patients. The neural mechanisms underlying sleep disturbances after TBI are unknown. Branched chain amino acids (BCAA -- precursors to 50% of de novo glutamate synthesis in brain) are decreased in hippocampus after mild TBI. We hypothesize that sleep disturbances after TBI result from imbalances in cellular metabolism which affect excitatory glutamate inputs onto sleep-wake circuits, including the orexin pathway in the hypothalamus.

Methods: We used a widely accepted mouse model of mild TBI, fluid percussion injury (FPI), which recapitulates many features of human mild TBI including neuronal cell loss, gliosis, ionic perturbation, and memory deficits. Brain-injured mice underwent EEG/EMG implantation for sleep staging and power spectral analysis. A separate cohort of mice was given dietary BCAA supplementation under the same experimental conditions.

Results: Brain-injured mice show significant sleep disturbances for at least 4 weeks post-injury, including an inability to sustain wakefulness and increased sleep fragmentation compared to sham surgery controls. BCAA dietary supplementation ameliorated sleep disturbances by significantly increasing wakefulness and consolidating sleep. Double-labeled orexin and Fos (a marker of neural activation)- positive cells in the lateral hypothalamus were significantly decreased after brain-injury, suggesting impaired activation of orexin neurons during the awake state. BCAA dietary supplementation significantly increased the number of double-labeled orexin and Fos-positive cells, suggesting rescued activation of orexin neurons during the awake state.

Conclusion: Our model offers a unique opportunity to study the neural mechanisms underlying sleep disturbances after mild TBI, and suggests that chronic dysfunction of orexin neurons via glutamatergic inputs occurs after mild brain injury. BCAA dietary intervention is a promising therapy for sleep disturbances after TBI.

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Trainee Award Eligible

Is Childhood Lead Exposure Associated with Sleep Problems in Early Adolescence?

Jianghong Liu, Jenny Wang, Xianchen Liu

School of Nursing, University of Pennsylvania

Introduction: Lead exposure has been linked to mental and behavior developmental problems in children. However, little is known about the impact of lead exposure on children's sleep. This study examined the association between blood lead levels (BLL) and sleep problems as reported by both parents and children themselves in a sample of Chinese children.

Method: 1,656 preschool children participated in a cohort study of lead and health in Jiangsu, China. BLL was measured when children were aged 3-5 years and sleep was assessed when children were at grade 4-6. The Chinese version of the Child Sleep Habits Questionnaire (CSHQ) was used to assess parent-reported child's sleep and an adolescent sleep questionnaire (AHQ) was used to assess self-reported sleep. A total of 665 children with complete data on BLL and sleep were included for the current study.

Results: Mean age of the sample at sleep assessment was 11.05 (SD=0.88), 51% were males. Mean BLL at ages 3-5 was 6.26μg/dl (SD=2.54). There were significant correlations between BLL and 3 CSHQ subscales: Sleep onset delay ($r=.113$, $p<.01$), Sleep duration ($r=.139$, $p<.001$), and Night waking ($r=.089$, $p<.05$). After adjusting for child age, sex, school and parental education, sleep onset delay was still significantly associated with BLL. Difficulty initiating sleep (15.2% vs 10.0%, $p>.05$), difficulty maintaining sleep (23.9% vs 18.6%, $p>.05$), early morning awaking (19.6% vs 12.1%, $p>.05$), excessive daytime sleepiness (26.1% vs 9.0%, $p<.001$), and use of sleep pills (6.5% vs 1.8%, $p=.03$) were more prevalent in children BLL ≥ 10.0μg/dl than in those children BLL<10.0μg/dl.

Conclusion: Our findings suggest that elevated BLL at ages 3-5 is associated with increased risk for sleep problems in early adolescence. Further research is warranted to examine the long-term impact of childhood lead exposure on sleep and the mechanism between lead and sleep problems.

Rapid eye movement sleep (REMS) is increased during the lights-on period in young rats subjected to moderate alcohol exposure during early postnatal period

Graziella L. Mann and Denys V. Volgin

Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Introduction: Prenatal alcohol exposure (AE) can have a detrimental effect on the development of sleep-regulatory mechanisms. We previously reported that a rat model of fetal alcohol spectrum disorders exhibits upregulation of hypothalamic $GABA_A$ receptors and elevated response to sedative effect of $GABA_A$ receptor agonist, gaboxadol, during the lights-on (rest) period when tested on postnatal days (PD) 29-52. Using the same rat model, we now investigated whether a moderate early postnatal alcohol exposure (AE) changes sleep/wake behavior in young adult rats.

Methods: Alcohol (3.0 g/kg/day) was administered to male rats via intragastric intubations on PD4-9, a period equivalent to human brain development during the third trimester of pregnancy (AE group; N=3). Control pups were sham-intubated (S group; N=3). On PD31-33, rats were instrumented for radiotelemetric recording of sleep/wake behavior. On PD44-47, a 72 h-long recording session started at ~9AM. Sleep scoring was performed using Somnologica software. Wake, slow-wave sleep (SWS), and REMS were distinguished in 10 s epochs and quantified over 48 h-period starting on the second recording day. For each rat, data averaged from two successive lights-on (7AM-7PM) and lights-off (7PM-7AM) periods were subjected to statistical analysis.

Results: During the lights-on phase, REMS was significantly elevated in AE rats (17.1%±0.9 (SE) vs. 13.8%±0.4 in S rats; $p=0.03$), mainly due to an increased number of REMS episodes (69.3±0.4 vs. 51.0±3.0 in S; $p=0.02$). During the lights-off (active) phase, REMS amounts did not differ significantly between the groups, but AE group tended to have reduced REMS (4.2%±0.7 vs. 6.1%±0.4 in S; $p=0.08$). Differences in SWS and wake were not significant during either phase.

Conclusion: Pubertal male rats subjected to moderate early postnatal AE have increased amounts of REMS during the lights-off (rest) circadian phase. This may be related to increased levels of $GABA_A$ receptors in the hypothalamic perifornical region that we previously described in this model.

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Stimulation of the α2a Adrenergic Receptor in the Ventrolateral Preoptic Area Promotes Arousal

Hilary McCarren[1, 2, 5], Michael R. Chalifoux[1], Bo Han[1], Matthew A. Fleisher[1], Sheryl G. Beck[2, 3], Abraham J. Wyner[4], Max Kelz[1, 2, 3, 5]

[1]Anesthesia and Critical Care, University of Pennsylvania, Philadelphia, PA; [2]Pharmacology, University of Pennsylvania, Philadelphia, PA; [3]Neuroscience, University of Pennsylvania, Philadelphia, PA; [4]Statistics, University of Pennsylvania, Philadelphia, PA; [5]Center for Sleep and Circadian Neurobiolgy, University of Pennsylvania, Philadelphia, PA

Introduction: Anesthetic induced hypnosis arises in part from specific actions of anesthetic drugs upon the endogenous circuits regulating sleep and wakefulness. We have previously demonstrated that isoflurane directly depolarizes sleep-promoting ventrolateral preoptic (VLPO) neurons. Neighboring non-sleep active VLPO neurons are not depolarized by general anesthetics. However, the behavioral significance of these effects has been called into question. We hypothesized that acute pharmacologic modulation of adrenergic signaling in VLPO would counteract anesthetic-induced hypnosis both ex vivo and in vivo.

Methods: Whole-cell current clamp recordings were conducted on 200µm VLPO-containing slices obtained from C57B6/J mice. Cells were categorized as putative sleep-promoting based upon a hyperpolarizing response to norepinephrine (NE). To determine the mechanism of adrenergic-induced hyperpolarization, the highly specific alpha2A agonist, dexmedetomidine was bath applied. In 7/7 NE hyperpolarized VLPO neurons, 100nM dexmedetomidine also elicited a hyperpolarization (-43±2.7mV to -50.0±2.3mV, p=0.0014). Multiplex RT-PCR performed on cytoplasmic aspirates from single neurons confirmed the presence of alpha2A, 2B, and 2C adrenoceptors in the dexmedetomidine-hyperpolarized neurons. Conversely, in 3/3 NE depolarized VLPO neurons dexmedetomidine did not significantly alter resting membrane potential.

Results: Having demonstrated that dexmedetomidine hyperpolarizes putative sleep-promoting neurons, we explored the effects of adrenergic ligands in vivo. Indwelling bilateral cannulae were used to deliver 25nl of adrenergic drugs into VLPO of 0.8% isoflurane-anesthetized mice or into mice with bilateral cannulae implanted 500um more caudally. Arousal state behavioral scores ranging from 0 (no movement) to 4 (full return of righting reflex) were assigned for the 10-minute period prior to drug and the 5-minutes following drug.

Conclusions: Dexmedetomidine infusion significantly increased arousal, while saline, α1 agonist phenylephrine, and NE did not ($p<0.05$). Dexmedetomidine failed to rouse animals exposed to a deeper state of isoflurane anesthesia. These results suggest that stimulation of α2A adrenergic receptors in VLPO promotes arousal.

T32 HL007713-18, R01 GM088156, Penn Department of Anesthesiology & Critical Care.

Trainee Award Eligible

Small Molecule Screen in Adult *Drosophila* Identifies VMAT as a Regulator of Sleep

Aleksandra Nall[1], Amita Sehgal[1,2]

[1] Cell and Molecular Biology Program, University of Pennsylvania School of Medicine; [2] Department of Neuroscience, Howard Hughes Medical Institute, University of Pennsylvania School of Medicine

Sleep is an important physiological state, but its function and regulation remain elusive. In *Drosophila melanogaster*, a useful model organism for studying sleep, forward genetic screens have identified important sleep-modulating genes and pathways; however, the results of such screens may be limited by developmental abnormalities or lethality associated with mutation of certain genes. To circumvent these limitations, we used a small molecule screen to identify sleep-modulating genes and pathways. We administered each of 1280 pharmacologically active small molecules to adult flies and monitored their sleep. We found that administration of reserpine, a small molecule inhibitor of the vesicular monoamine transporter (VMAT) that repackages monoamines into presynaptic vesicles, resulted in an increase in sleep. Supporting the idea that VMAT is the sleep-relevant target of reserpine, we found that VMAT null mutants have an increased sleep phenotype, as well as an increased arousal threshold and resistance to the effects of reserpine. However, although the VMAT mutants are consistently resistant to reserpine, other aspects of their sleep phenotype are dependent on genetic background. These findings indicate that small molecule screens can be used effectively to identify sleep-modulating genes whose phenotypes may be suppressed in traditional genetic screens. Mutations affecting single monoamine pathways did not affect reserpine sensitivity, suggesting that effects of VMAT/reserpine on sleep are mediated by multiple monamines. Overall, we identify VMAT as an important regulator of sleep in Drosophila and demonstrate that small molecule screens provide an effective approach to identify genes and pathways that impact adult *Drosophila* behavior.

The Molecular Logic of *C. elegans* Sleep: A Single Sleep-promoting Neuron Inhibits a Wake-promoting Pair of Neurons via neuropeptide signaling

Nelson M.D. and Raizen D.M.

Sleep is ubiquitous in the animal kingdom and many molecular mechanisms of sleep regulation are conserved (Crocker and Sehgal, 2010). The simplicity of the nervous system of the roundworm *Caenorhabditis elegans*, combined with our complete understanding of the synaptic connectivity, provides a unique opportunity to dissect the circuitry of sleep regulation at a single cell resolution. Epidermal growth factor (EGF) signaling promotes sleep in mammals and fruit flies (Kramer et al, 2001; Foltenyi et al, 2007) but the mechanism has been opaque. In *C. elegans,* activation of the single peptidergic interneuron ALA by the epidermal growth factor LIN-3 induces sleep (Van Buskirk and Sternberg, 2007), but the neurotransmitter released by ALA to induce quiescence has been unknown. We report that the ALA neurotransmitters are FLP-13 neuropeptides. The gene *flp-13* is expressed in ALA; over-expression of *flp-13* induces sleep during normally active periods; *flp-13* mutants are defective in EGF-induced sleep; and this defect is rescued by restoring *flp-13* expression specifically in the ALA neuron. We have identified a FLP-13 receptor encoded for by the gene *frpr-4*. In collaboration with Drs. Janssen and Schoofs (U. Leuven), we have shown that FLP-13 neuropeptides potently activate the G-protein coupled receptor FRPR-4 in a heterologous cell culture system. Over expression of *frpr-4* induces sleep, which requires the function of its ligand FLP-13, thereby demonstrating *in vivo* functional interactions between the identified ligand and receptor. *frpr-4* is expressed in a pair of highly connected interneurons, the RIAs, which we have previously shown to secrete the somnogenic neuropeptide NLP-22. NLP-22 is similar to the mammalian, circadian-regulated, anorexigenic hormone neuromedin S (NMS) (Mori et al, 2005; Ida et al, 2005). *nlp-22* mRNA shows cyclical expression in synchrony with sleep behavior and is downstream of a clock regulated by LIN-42/PERIOD. Somnogenic effects of NLP-22 require inhibition of a cAMP-dependent protein kinase (PKA) mediated pathway. Surprisingly, acute optogenetic activation of the RIA neurons is wake-promoting and not sleep-promoting, indicating that in addition to NLP-22, RIA releases a wake-promoting neurotransmitter, and that this wake-promoting effect dominates in this acute activation paradigm. Thus, we have defined a flip-flop mechanism of how a sleep-promoting neuron (ALA) inhibits a wake-promoting neuron (RIA). Moreover, we demonstrate that a wake-promoting neuron can express a sleep-promoting neurotransmitter, providing a new mechanism for sleep homeostasis at the single cell level. Given the conserved molecular nature of sleep regulation, we propose that similar logic operates in other animals, including humans.

Trainee Award Eligible

Sleep Disturbance Partially Mediates the Relationship between Intimate Partner Violence and Physical/Mental Health

Linden Oliver MA, Andrea Segal BA, Michael L. Perlis, PhD, and Michael A. Grandner, PhD

Introduction: Intimate partner violence (IPV) is a worldwide health concern and an important risk factor for poor mental/physical health. Little is known about whether IPV leads to sleep disturbance. If so, it is possible that sleep problems may mediate this relationship, and future sleep interventions could mitigate the negative effects of IPV.

Methods: Data from the 2006 Behavioral Risk Factor Surveillance System (BRFSS) was used (N=34,975). IPV was assessed as any history of being (1)threatened by (THREAT), (2)physically hurt by (HURT), or (3)forced to have sex with (SEX) an intimate partner, and, further, as (4)being forced to have sex (SEXyr) or (5)being physically injured (HURTyr) by an intimate partner within the past year. These survey items were coded yes/no. Sleep disturbance was assessed as difficulty falling asleep, staying asleep, or sleeping too much at least 6 of the last 14 days. Logistic regression analyses, adjusted for age, sex, race, income, education, and physical/mental health, assessed whether IPV predicted sleep disturbance. Sobel-Goodman tests assessed whether relationships between IPV and physical/mental health were partially mediated by sleep disturbance.

Results: All IPV variables were associated with sleep disturbance. THREAT was associated with sleep disturbance (OR=2.798,$p<0.0001$), as was HURT (OR=2.683,$p<0.0001$), SEX (OR=3.237,$p<0.0001$), SEXyr (OR=7.741,$p<0.0001$), and HURTyr (OR=7.497,$p<0.0001$). In mediation analyses, all IPV variables were associated with mental health ($p<0.0001$), and all were associated with physical health ($p<0.007$) except SEXyr. Sleep disturbance partially mediated all relationships (Sobel $p<0.0005$ for all tests). Mediation was around 30%, ranging from 18% (HURTyr and mental health) to 41% (HURT and physical health).

Conclusions: IPV was strongly associated with current sleep disturbance above the effect of demographics and overall mental/physical health, even if the IPV happened in the past. Further, sleep disturbance partially mediates the relationship between IPV and mental/physical health. Sleep interventions may potentially mitigate negative effects of IPV.

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Trainee Award Eligible

Obstructive Sleep Apnea, Obesity and Cellular Adhesion Molecules: Impact of 2 Years of CPAP Treatment

Victoria M. Pak, PhD*[1,2], Brendan T. Keenan, MS*[1], Nicholas Jackson, MPH[1], Michael A. Grandner, PhD[1], Greg Maislin, MS, MA[1], Richard J. Schwab, MD[1,3], Erna Sif Arnardottir, MS[4,5], Bryndis Benediktsdottir, MD[4,5], Thorarinn Gislason, MD, PhD**[4,5], Allan I. Pack, MBChB, PhD**[1,3]

*co-lead authors; **co-senior authors

[1]Center for Sleep and Circadian Neurobiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [2]Biobehavioral Research Center, School of Nursing, University of Pennsylvania, Philadelphia, PA; [3]Division of Sleep Medicine/Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [4]University of Iceland, Reykjavik, Iceland; [5]Landspitali Hospital, Reykjavik, Iceland

Introduction: Elevated levels of intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) may contribute to cardiovascular disease and are associated with Obstructive Sleep Apnea (OSA) and obesity. The interaction between CPAP and obesity in determining levels of these molecules is unknown.

Objectives: We investigated associations between adhesion molecule changes and CPAP usage after 2 years of treatment, and examined whether these associations differed based on obesity.

Methods: 309 OSA patients referred for CPAP from the Icelandic Sleep Apnea Cohort were studied. The mean (SD) BMI was 32.4(5.1), they had severe OSA [AHI=45.0(20.2)] and 79% were male. Subjects were stratified by BMI at baseline (<30, 30-35, ≥35). Fasting blood was drawn to assess adhesion molecules (measured via ELISA) in untreated subjects and again 2 years after CPAP initiation.

Measurements and Main Results: There were 177 full (≥4 hours/night), 44 partial (<4 hours/night), and 88 non CPAP users. We observed significant change in ICAM-1 ($p<0.001$) and VCAM-1 ($p=0.012$) change between the 3 CPAP usage groups. For ICAM-1, the strongest association was among the most obese subjects ($p<0.001$). In each case, we observed significant differences between full and non-users; non-users had significant increases in ICAM-1 and VCAM-1 levels compared to no change in full users.

Conclusion: In a moderate to severe OSA population, adequate CPAP usage protects against increases in cellular adhesion molecules observed in non-users over a two year period. For ICAM-1, this association is dependent on obesity, with a strong association in subjects with a BMI≥35.

Trainee Award Eligible

Wake Neuron Injury and Impaired Hypercapneic Arousal in Chronic Sleep Fragmentation

Lori Panossian, MD*, Yanpeng Li, MD*, Jing Zhang, MD, Yan Zhu, MD, Guanxia Zhan, MD, Yu-Ting Chou, MD, Polina Fenik, MS, Seema Bhatnagar, PhD, Sigrid Veasey, MD
*Authors contributed equally to study

Study Objectives: Delayed hypercapneic arousals from obstructive events occur in some patients with obstructive sleep apnea, where the longer latency to arousal promotes more severe oxyhemoglobin desaturations. We hypothesized that long-term sleep fragmentation (SF) results in injury to hypercapneic-responsive wake-active neurons and impairs the hypercapneic arousal response.

Design: Adult male mice were implanted for sleep state recordings and then randomly assigned to 4 weeks of either orbital platform SF (SF4wk, 30 events/hr) or control (Ct4wk) conditions prior to behavioral and histological evaluation.

Measurements and Results: SF was successfully achieved across the 4 wk study, as evidenced by an increased arousal index, $p<0.01$ and shortened sleep bouts, $p<0.05$, while total sleep/wake times were unaffected by rotor SF. A multiple sleep latency test performed at the onset of the dark period showed a reduced latency to sleep in SF4wk mice ($p<0.05$). The hypercapneic arousal latency was increased, Ct4wk, 64±5sec to SF4wk, 154±6sec, $p<0.001$, and remained elevated after 2wk recovery (101±4sec, $p<0.001$). C-fos activation in noradrenergic, orexinergic, histaminergic and cholinergic neurons was reduced in response to hypercapnia ($p<0.05$-0.001). LC and orexinergic projections into the cingulate cortex were analyzed and were reduced in SF4wk, $p<0.01$. Corticosterone levels were unchanged across conditions.

Conclusions: Rotor SF is effective for extended periods. SF4wk imparts injury to wake neurons and impairs function, including lasting impaired arousal responses to hypercapnia, an effect that may contribute to progression of apnea and poor ventilatory arousals in sleep apnea.

Trainee Award Eligible

Cul3 and the BTB Adaptor Insomniac are Key Regulators of Sleep Homeostasis and a Dopamine Arousal Pathway in Drosophila

Cory Pfeiffenberger[1], Allan Pack[1], Ravi Allada[2]

[1]University of Pennsylvania, Center for Sleep and Circadian Neurobiology; [2]Northwestern University, Center for Sleep and Circadian Biology

Introduction: Sleep is homeostatically regulated; such that sleep drive reflects the duration of prior wakefulness. Yet despite the discovery of genes important for sleep, a coherent molecular model for sleep homeostasis has yet to emerge.

Methods: We performed a reverse genetics screen in Drosophila using standard genetics techniques. Sleep was monitored with the DAM system from Trikinetics. Sleep deprivation experiments were performed using a mechanical sleep deprivation device that resulted in ≥ 90% sleep deprivation. Dopamine levels were modulated pharmacologically by adding 3-iodo-tyrosine or DOPA to the food, and measured by HPLC. CoIP and western blots were performed using standard techniques. Immunostaining was performed using standard techniques.

Results: We have identified an insertion in the BTB domain protein CG32810/inc that exhibits one of the strongest sleep phenotypes thus far observed, a ~10h sleep reduction. Importantly, this is coupled to a reduced homeostatic response to sleep deprivation, consistent with a disrupted sleep homeostat. Knockdown of the INC-interacting protein, the E3 ubiquitin ligase Cul3, results in reduced sleep duration, consolidation, and homeostasis, suggesting an important role for protein turnover in mediating INC effects. Interestingly, inc and Cul3 expression in post-mitotic neurons during development contributes to their adult sleep functions. Similar to flies with increased dopaminergic signaling, loss of inc and Cul3 result in hyper-arousability to a mechanical stimulus in adult flies. Furthermore, the inc sleep duration phenotype can be rescued by pharmacological inhibition of tyrosine hydroxylase, the rate-limiting enzyme for dopamine biosynthesis.

Conclusions: Taken together, these results establish inc and Cul3 as important new players in setting the sleep homeostat and a dopaminergic arousal pathway in Drosophila.

Reduced Resting-State Connectivity between PCC and Hippocampus Correlates with Memory Performance Changes after Sleep Deprivation

Rao H, Fang Z, Zhu S, Hu S, Basner M, Goel N, Detre JA, Dinges DF

Center for Functional Neuroimaging, Department of Neurology and Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Introduction: Sleep plays an important role in learning and memory processing while sleep deprivation (SD) loss impairs behavioral and neural capacity to encode new memories, particularly in the hippocampal complex. Recent neuroimaging studies have demonstrated the attenuation effects of SD on hippocampus activity during memory encoding tasks, while the effects of SD on hippocampal function during resting states remain unclear. In this study, we examined the effects of 1 night of total SD (TSD) as well as 2 nights of recovery sleep (RS) on resting-state functional connectivity (FC) and its relationship to episodic memory.

Methods: We scanned 29 healthy adults (14 females, 22-50y) while at rest (0700-0900) on a Siemens 3T scanner using a standard EPI sequence. The baseline (BS) scan was after 9h normal sleep. The second scan was after 24h TSD. A third scan followed 20h RS. FC analyses, using the hippocampus and posterior cingulate cortex (PCC) as the seed regions, were conducted via SPM8 and REST toolbox. Subjects completed a behavioral hippocampus-dependent scene encoding and recognition task each afternoon. Changes in memory performance (hit rate, false alarm, accuracy, response bias) were calculated and correlated with FC alteration following TSD.

Results: FC analyses showed that TSD significantly reduced functional connectivity between the PCC and the hippocampus compared with BS and RS ($p<0.001$), although no connectivity differences were found between BS and RS. TSD significantly reduced participants' response accuracy ($p=0.003$) and conservative bias ($p=0.02$), and increased false alarms ($p<0.001$) during the memory task. Decreases in PCC-hippocampus connectivity correlated with changes in hit rate ($r=0.51$, $p=0.005$) and response bias ($r=-0.52$, $p=0.004$) following TSD.

Conclusions: This study demonstrated that SD significantly reduced PCC-hippocampal connectivity. The observed correlation between PCC-hippocampal connectivity decreases and poorer memory performance suggests that disrupted connectivity changes in memory circuits may be one mechanism by which sleep loss affects memory processing.

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Individualized Neurocognitive Assessment Toolkit for Spaceflight Fatigue (NeuroCATS)

J. S. Schneiderman[1], R. C. Gur[2], D. F. Dinges[1], D. J. Mollicone[3], C. G. Mott[3], T. McCann[3], Z. T. Roberts[1], J. Hansen[2], A.P. Savitt[2] and M. Basner[1]

[1]Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, [2]Brain Behavior Laboratory, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, and [3]Pulsar Informatics Inc., Philadelphia, PA

Introduction: Undisturbed sleep of sufficient length is of paramount importance in all settings where successful completion of essential tasks is critical. This is especially true for space flight where the detection of cognitive deficits induced by fatigue and other space flight stressors has been identified as a top priority by NASA.

Methods: The goal of this project is to develop NeuroCATS as a rapid, accurate, reliable and valid procedure for measuring the effects of fatigue, circadian misalignment and work-overload as well as the cognitive impact from other spaceflight conditions. NeuroCATS consists of 10 brief neurocognitive tests covering a wide range of cognitive domains with known cerebral representation established via fMRI administered using adaptive computerized testing: motor praxis, visual object learning, fractal 2-back, abstract matching, line orientation, emotion recognition, matrix reasoning, digit symbol substitution, balloon analog risk, and psychomotor vigilance. We are completing the evaluation of the sensitivity of the 10 NeuroCATS tests to acute total and chronic partial sleep restriction in healthy adults participating in laboratory protocols at the University of Pennsylvania.

Results: Preliminary data show that battery administration time averaged between 15-25 minutes. Depending on the cognitive test and the selected outcome variable, the 10 tests differed in their sensitivity to fatigue induced by sleep loss.

Conclusions: After validation for sensitivity to fatigue from sleep loss is finalized in the laboratory, astronaut norms will be generated, and feasibility for space flight will be assessed in astronauts on the International Space Station.

This study was supported by the National Space Biomedical Research Institute through NASA NCC 9-58, NIH R01NR004281, NIH UL1TR000003 and Office of Naval Research N00014-11-1-0361.

Trainee Award Eligible

Stability of Trait-Like Vulnerability to Chronic Sleep Restriction over Long Time Intervals

Selame L, Dinges DF, Goel N

Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Introduction: Re-exposure to sleep loss within a few weeks reveals trait-like differential neurobehavioral vulnerability. We sought to determine whether such trait-like response deficits are maintained over longer time intervals (months to years) between chronic sleep restriction (SR) exposures.

Methods: 14 healthy adults (22–50y; 7 females) completed 2-3 baseline (8h–12h TIB) nights followed by 5 consecutive SR nights (4h TIB) in two separate laboratory experiments. The duration between the two experimental exposures to SR ranged from 2.5 to 71.5 months (median = 27 months). Neurobehavioral testing included the 10-min Psychomotor Vigilance Test (PVT), Digit Span (DS), Digit Symbol Substitution Test (DSST), and Karolinska Sleepiness Scale (KSS), every 2h during wakefulness. The intraclass correlation coefficient (ICC) for each measure was computed as the ratio of between-subjects variance to the sum of the between- and within-subjects variances using data from 0800h to 2000h after the fifth night of SR.

Results: Subjects who displayed vulnerability to SR on their first exposure also displayed vulnerability to SR on their second exposure, as evident by high ICCs: PVT lapses + false starts, ICC = 0.939; PVT response speed, ICC = 0.972; DSST correct, ICC = 0.732; DS correct, ICC = 0.604; and KSS, ICC = 0.779.

Conclusion: Neurobehavioral vulnerability to SR experiences, separated by a median of ~2 years, showed trait-like stability in both performance and subjective measures, as evident in the stability of substantial inter-individual variance (60%–97% across measures). These are the first data to confirm the stability of phenotypic neurobehavioral responses to SR over a longer period of time. The results are relevant for predicting neurobehavioral responses in individuals who are exposed to SR, chronically or intermittently, across months and years.

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Trainee Award Eligible

Stability of Trait-Like Vulnerability to Total Sleep Deprivation over Long Time Intervals

Selame L, Dinges DF, Goel N

Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Introduction: Re-exposure to acute total sleep deprivation (TSD) following a couple of weeks reveals trait-like differential neurobehavioral vulnerability. We sought to determine whether such trait-like response deficits are maintained over longer time intervals (months to years) between acute sleep loss exposures.

Methods: 15 healthy adults (22–49y; 9 females) completed 1-2 baseline (9h–12h TIB) nights followed by 36h of acute TSD in two separate laboratory experiments. The duration between the two experimental exposures to TSD ranged from 3.5 to 70 months (median = 8 months). Neurobehavioral testing included the 10-min Psychomotor Vigilance Test (PVT), Digit Span (DS), Digit Symbol Substitution Test (DSST), and Karolinska Sleepiness Scale (KSS), every 2h during wakefulness. The intraclass correlation coefficient (ICC) for each measure was computed as the ratio of between-subjects variance to the sum of the between- and within-subjects variances using data after 2200h/0000h of TSD.

Results: Subjects who displayed vulnerability to TSD on their first exposure also displayed vulnerability to TSD on their second exposure, as evident by high ICCs: PVT lapses + false starts, ICC = 0.679; PVT response speed, ICC = 0.861; DSST correct, ICC = 0.864; DS correct, ICC = 0.966; and KSS, ICC = 0.898.

Conclusion: Neurobehavioral vulnerability to TSD experiences, separated by a median of 8 months, showed trait-like stability in both performance and subjective measures, as evident in the stability of substantial inter-individual variance (68%–97% across measures). These are the first data to confirm the stability of phenotypic neurobehavioral responses to TSD over a longer period of time. The results are relevant for predicting neurobehavioral responses in individuals who are exposed to acute TSD, chronically or intermittently, across months and years.

Work funded by the Department of the Navy, Office of Naval Research (Award No. N00014-11-1-0361); CTRC UL1TR000003; NIH R01 HL102119; AFOSR FA9550-05-1-0293.

Trainee Award Eligible

A Randomized Trial of a 3-hour Protected Nap Period in Medical Interns

Shea JA,[1,2,3] Dinges DF,[4] Small DS,[3,5] Basner M,[4] Zhu J,[2,3] Norton L,[2,3] Novak C,[2,3] Bellini LM,[2] Dine CJ,[2] Volpp KG[1,2,3,6]

[1]Center for Health Equity Research & Promotion, Philadelphia Veterans Affairs Medical Center, [2]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, [3]Leonard Davis Institute Center for Health Incentives and Behavioral Economics, [4]Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, [5]Department of Statistics, the Wharton School, University of Pennsylvania, [6]Department of Health Care Management, the Wharton School, University of Pennsylvania

Introduction Previous work has demonstrated the effectiveness of protected sleep periods of 5 hours for internal medicine interns in increasing amount slept and alertness after extended shifts. Here we evaluate intern and patient outcomes associated with protected nap periods of 3 hours that are personnel neutral.

Methods Randomized controlled trial in 61 medical interns at the Philadelphia VA Medical Center (PVAMC) Medical Service and Oncology Unit of the Hospital of the University of Pennsylvania (HUP). Four-week blocks were randomly assigned to either a standard intern schedule (extended duty overnight shifts of up to 30 hours), or sequential protected sleep periods with cell phone sign out between 00:00-03:00 (early shift, intern 1) and 03:00-06:00 (late shift, intern 2). Study participants wore wrist actiwatches, completed sleep diaries, and performed a 3-minute version of the Psychomotor Vigilance Tests (PVT-B).

Results On 97.4% of intern on call nights, cell phones were signed out as designed. Interns at HUP had significantly longer sleep durations during protected nap periods compared to controls (HUP early shift: 2.40 vs. 1.55 hours, $p<0.0001$; HUP late shift: 2.44 vs. 1.55 hours, $p<0.0001$). At PVAMC sleep duration was longer only for the late shift group (PVAMC late: 2.40 vs. 1.90 hours, $p<0.0001$). PVT response speed was significantly faster in the intervention group after on-call nights at the PVAMC but not at HUP. There were no differences in patient outcomes between standard schedule months vs. intervention months.

Conclusions A protected nap period of 3 hours resulted in more sleep during call and reductions in periods of prolonged wakefulness. The current design on sequential naps was personnel neutral and appears feasible to integrate into scheduling designs that have trainees working extended (24+4) shifts.

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Redeye (Rye), a Nicotinic Acetylcholine Receptor, is Required to Maintain Sleep Length and Sleep Homeostasis in Fruit Fly

Mi Shi, Zhifeng Yue, and Amita Sehgal

Howard Hughes Medical Institute and Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Acetylcholine (Ach) is a well-known neurotransmitter that promotes wakefulness in animals. Here, we have identified a short-sleep mutant named as *redeye (rye)* through a genetic screen in fruit fly followed by whole-genome deep sequencing. Rye encodes a nicotinic acetylcholine receptor (nAchR), but unexpectedly reduces fly sleep length in its mutant form. Reducing RYE expression through RNA interference or silencing RYE neuron function decreases sleep duration, which indicates that RYE is required to maintain a normal sleep length. While *rye* mRNA level remains constant during a regular 24h light-dark (LD) cycle, RYE protein level cycles. Such posttranscriptional regulation of RYE correlates with the sleep/wake cycle in fruit fly. Furthermore, sleep deprivation at late night when RYE is normally low in a regular LD cycle increases RYE expression dramatically, indicating that RYE reflects sleep homeostasis and probably functions as a sleep promoting factor. Like mammal, there are several nAchR paralogs in the fly genome. While acetylcholine promotes arousal in general, we propose that Ach promotes sleep as well via activating specific RYE expressing neurons. Therefore, mutation of *rye* leads to sleep disruption as well as decreasing RYE dosage through RNAi and RYE neuron silencing. In mammal, brain acetylcholine level is high during wakefulness and is also high in REM stage during sleep. The identification of a novel nAchR essential for sleep maintenance in fruit fly may contribute to revelation of nAch function during REM stage in mammal.

Trainee Award Eligible

Respiratory-Related Dynamic Upper Airway Changes in Obese Apneics After Weight Loss

B.H. Shin, D.L. Morelli, C.B. Bearn, R.J. Schwab
Center for Sleep & Circadian Neurobiology, University of Pennsylvania, Philadelphia, PA

Rationale: It has been shown that the percentage change in airway caliber during awake respiration is significantly greater in apneics as compared to normals due to increased airway collapsibility. We hypothesized that weight loss would decrease upper airway collapsibility in obese apneics as demonstrated by larger minimum and smaller maximum airway areas, as well as smaller differences between maximum and minimum airway areas (a measure of airway compliance).

Methods: Dynamic MRI and polysomnography data were collected on 25 obese (BMI≥30 kg/m3) apneics (AHI≥15 events/hour) before and after participation in a six month weight-loss program. For the dynamic imaging, three sets of 100 axial slices were taken at the mid-soft palate, mid-tongue, and mid-epiglottis. Maximum and minimum slice areas were determined by averaging the largest and smallest five slice areas at each position. After completing the program, 10 subjects lost ≥5% weight, and 15 were weight stable. Comparisons between pre- and post-weight loss were made using paired t-tests.

Results: Subjects who lost ≥5% weight had larger minimum airway areas at the mid-tongue and mid-soft palate, as well as smaller maximum airway areas at the mid-tongue and mid-epiglottis (Table 1). Post weight loss subjects also showed a decreased difference between maximum and minimum airway area at the mid-tongue and mid-epiglottis. Weight stable subjects showed little change in minimum airway areas at the mid-soft palate and mid-tongue and little change in maximum airway areas at all locations. In contrast to those who lost weight, weight stable subjects showed little change in maximum-minimum airway area differential at the mid-tongue and an increase in maximum-minimum area change at the mid-epiglottis. Moreover, after completing the weight loss program, subjects who lost ≥5% weight exhibited smaller differences in minimum-maximum airway areas at all positions as compared with weight stable subjects (mid-soft palate P=0.374, mid-tongue P = 0.140, mid-epiglottis P= 0.675). In all subjects, no changes were significant, possibly because of the small sample size.

Conclusions: These data suggest that a weight loss of ≥5% causes decreased airway collapsibility during wakefulness in obese apneics as shown by a smaller range of dynamic change in the upper airway post-weight loss. Improvements in AHI post-weight loss may be due in part to this decrease in airway compliance.

Table 1: Differences in Airway Areas in 5% Weight Loss and Weight Stable Obese Apneics

	Apneics Pre 5% Weight Loss (n=10)	Apneics Post 5% Weight Loss (n=10)	P-value	Weight Stable Apneic Pre Weight Loss Program (n=15)	Weight Stable Apneic Post Weight Loss Program (n=15)	P-value
Variable	Average ± SD	Average ± SD		Average ± SD	Average ± SD	
Mid Soft Palate Max Airway Area (mm^2)	126.9 ± 53.5	134.8 ± 55 5	0.6522	141.1 ± 73.9	138.6 ± 94.0	0.8962
Mid Soft Palate Minimum Airway Area (mm^2)	67.6 ± 41.2	74 3 ± 56.5	0.7163	41.2 ± 27.5	57.3 ± 50.7	0.2237
Mid Soft Palate Δ Max-Min Airway Area(mm^2)	59.3 ± 37.8	60 5 ± 35.4	0.9211	99.9 ± 76.5	81.3 ± 71.9	0.1711
Mid-Tongue Maximum Airway Area (mm^2)	233.8 ± 200.6	196.6 ± 108.7	0.3399	194.7 ± 98.8	190.7 ± 96.9	0.8704
Mid-Tongue Minimum Airway Area (mm^2)	108.6 ± 62.9	119.6 ± 70 3	0.5782	72.0 ± 62.0	68.5 ± 50.7	0.8537
Mid-Tongue Δ Max-Min Airway Area (mm^2)	125.2 ± 171.6	77 1 ± 59.5	0.2472	122.7 ± 68.7	122.2 ± 88.6	0.9777
Mid-Epiglottis Maximum Airway Area (mm^2)	285.0 ± 238.7	218.4 ± 131.8	0.2782	257.3 ± 109.9	232.2 ± 97.3	0.4179
Mid-Epiglottis Minimum Airway Area (mm^2)	123.8 ± 53.3	108 1 ± 72.7	0.4552	140.9 ± 98.7	105.9 ± 65.3	0.2056
Mid-Epiglottis Δ Max-Min Airway Area (mm^2)	161.2 ± 218.3	110 3 ± 107.7	0.3301	116.4 ± 61.0	126.3 ± 57.9	0.6262

Trainee Award Eligible

Effects of Weight Gain on Tongue Fat in Obese and Lean Zucker Rats by *In-vivo* Magnetic Resonance Spectroscopy

Shinde SA[1], Brennick MJ[1], Bearn, CB[1] Delikatny EJ[2], Schwab RJ[3]

[1]Department of Medicine; [2] Department of Radiology; [3]Sleep Section and Pulmonary and Critical Care Sections, Department of Medicine; Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Introduction: Obstructive sleep apnea (OSA) is a common disorder with a prevalence that is linked to an epidemic in obesity. The focus of this study was to further examine the relationship between obesity and OSA. Studies have shown that obese Zucker rats (OBZ) to have significantly more tongue fat than lean Zucker rats (LNZ). The objective of this study was to examine tongue fat longitudinally in obese and lean Zucker rats that gain weight. We hypothesized that weight gain in OBZ will cause a larger increase in tongue fat than in LNZ rats.

Methods: 6 age-matched OBZ and 6 LNZ rats were studied at two time points: 6-8 weeks and 12-14 weeks (Table1). Each animal was anesthetized with isoflurane (2% in O_2). We examined 2 voxels (mm^3) in each rat using a 2-step magnetic resonance spectroscopy (MRS) protocol: water suppression, followed by no water suppression. In a 4.7 Tesla magnet, MRS of the tongue voxel was acquired using a respiratory-gated MRS sequence with 512 averages; repetition time (TR) = 2000ms; echo time (TE) = 14ms. One voxel was in the posterior tongue and a second voxel was in the masseter muscle. The fat to water ratio was the area of lipid to unsuppressed (normalized) water peak, from the best-fit spectral curves by MacNuts® (AcornNMR.com)**.**

Results: There was a significant increase in weight and fat/water ratio in the tongue and masseter in both the LNZ and OBZ groups. The change in fat/water ratio was much larger in the tongue than in the masseter for both obese and lean rats. Although there was a significant increase in the fat/water ratio in both the lean and obese groups, the change was larger in the obese group (Table 1).

Table1 Changes in Fat/Water Ratio over time in LNZ and OBZ Rats

	LNZ (n=6)				OBZ (n=6)			
	Time pt. 1	Time pt. 2	Change	p-value	Time pt. 1	Time pt. 2	Change	p-value
Age (wks)	7.67 ± 0.52	11.67 ± 0.52	4.00 ± 0.89	<.0001	7.33 ± 0.52	11.83 ± 0.41	4.50 ± 0.84	<.0001
Weight (g)	189 ± 49	312 ± 38	123 ± 45	0.0008	252 ± 54	619 ± 13	367 ± 64	<.0001
Fat/water ratio of tongue	1.14 ± 0.38	2.76 ± 0.31	1.62 ± 0.63	<.0001	1.58 ± 0.44	3.88 ± 0.27	2.30 ± 0.48	<.0001
Fat/water ratio of Masseter	0.235 ± 0.085	0.562 ± 0.286	0.327 ± 0.276	0.0371	0.428 ± 0.118	1.195 ± 0.282	0.767 ± 0.312	0.0006

Conclusion: Our data shows that weight gain in OBZ rats is associated with a larger increase in tongue fat compared LNZ. This disproportionate deposition of fat in the tongue may play an important role in the narrowing of the airway in OBZ rats.

Fat Infiltration in the Tongue of Obese vs. Lean Zucker Rats

Shinde SA[1], Brennick MJ[1], Delikatny EJ[2], Schwab RJ[3],
[1]*Department of Medicine; Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*

Introduction: The causal relationship between obesity and sleep apnea is unclear. But obese mice show increased tongue fat compared to lean control mice. We examined tongue size and fat content in obese Zucker rats (OBZ) compared to lean Zucker (LNZ) rats using non-invasive methods that could be validated with post-mortem biochemical and histological analyses. We hypothesized that fat infiltration in the tongue would be significantly greater in the OBZ rats in comparison to the age matched LNZ rats.

Methods: Tongue fat was evaluated through 4 different techniques: Dixon imaging, spectroscopy, colorimetry, and histology. Upper airway Spin-echo MRI included Dixon fat discriminated images that were analyzed for fat volume. Magnetic resonance spectroscopy (MRS) of the tongue and masseter muscle was conducted. Voxels were placed in the posterior and mid tongue sections, and in the masseter muscle. Fat-to-water ratios were calculated with MacNuts® software. Overall fat-to-water ratio for the tongue was calculated by averaging the ratios of the posterior and mid tongue sections. Lipid extraction and enzyme colorimetry (StanbioTX) was used to obtain triglyceride content of postmortem tissue extracts from the tongue and masseter muscles. Tissue histology was performed and used to qualitatively evaluate fat distribution.

Results: OBZ rats weighed significantly more than the LNZ rats (p=0.001, Table 1). OBZ rats also had a significant increase in the fat/water ratio in the tongue (p=0.002 Table 1). Although there was also an increase in the fat/water ratio of the masseter (a typical upper airway skeletal muscle) between OBZ and LNZ rats, the magnitude of this difference was much less than that of the tongue. The biochemical analysis showed significantly greater tongue fat in OBZ compared to LNZ (p<0.0006), but no significant difference in the masseter muscle fat between OBZ and LNZ (Table 1). Results from Dixon analysis showed that fat content was greater in OBZ than LNZ in both posterior and anterior sections of the tongue (Table 1). Histology shows, there was visibly more intracellular and extracellular fat in the obese rat (Fig 1).

Conclusion: Multiple imaging modalities show that there is significantly more fat in the tongue of OBZ rats compared to LNZ. However, there were no significant increases in masseter muscle fat. We speculate that the greater increase in tongue fat with obesity may contribute to metabolic and mechanical impairment of normal tongue function having a role in previously reported airway narrowing in obese Zucker rats.

Table 1 Differences between LNZ and OBZ mice; mean ± SD (n)

Measure		LNZ	OBZ	p-value	Statistical Test
Spectroscopy Sample	Weight (g)	438 ± 35 (13)	639 ± 47 (11)	0.001	GLM
	Age (weeks)	15.3 ± 1.5 (13)	15 3 ± 1.3 (11)	0 95	GLM
	Fat/water Ratio Tongue	1.9 ± 1.1 (13)	5.5 ± 2.9 (11)	0.002	GLM
	Fat/water Ratio Masseter	0.35 ± 0.42 (4)	1.15 ± 0.61 (6)	0.038	Students t-test
Biochemistry Sample	Weight (g)	450 ± 43 (8)	637 ± 31 (10)	0.001	GLM
	Age (weeks)	14.5 ± 0.93 (8)	14.9 ± 0.90 (10)	0.46	GLM
	Tongue TriGly (mg/g)	0.82 ± 0.26 (8)	3.57 ± 1.7 (10)	0.0006	GLM
	Masseter TriGly (mg/g)	0.28 ± 0.08 (8)	0.28 ± 0.11 (10)	0 94	GLM
Dixon Sample	Posterior Tongue Volume (mm^3)	7.7 ± 3.2 (7)	15 ± 5.8 (7)	<0.01	GLM
	Anterior Tongue Volume (mm^3)	26.1 ± 2.8 (7)	40 ± 10.9 (7)	<0.01	GLM

GLM = Generalized Linear Model

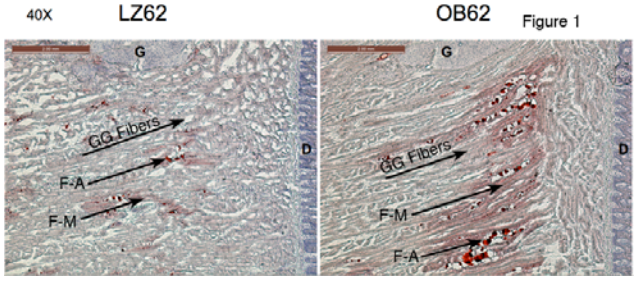

Figure 1: Tongue tissue samples from similar mid-sagittal locations in lean (LZ62) and obese (OB62) Zucker rats with Oil Red O stain at 40X (upper left bar = 200 µm). There is much more intracellular and extracellular fat in the obese rat. Arrows show: Fat in adipose cell (F-A); fat in muscle cell (F-M); the direction of the genioglossus muscle fibers (GG Fibers) to dorsal (D) or mucosal surface. (G) = glands near posterior base of tongue.

Trainee Award Eligible

Sleep Fragmentation Induces the Unfolded Protein Response in *Drosophila melanogaster*

Laura Shook[1,2], Marishka Brown[2], Nirinjini Naidoo[2]

Biological Basis of Behavior Program,[1,2] Center for Sleep and Circadian Neurobiology[2]

Introduction: The endoplasmic reticulum (ER) is the site of secretory and integral protein folding and post-translational modification. Perturbation of ER homeostasis leads to protein misfolding, accumulation and aggregation. The ER responds to this perturbation/stress by activating a 'quality control mechanism' called the Unfolded Protein Response (UPR). The molecular chaperone BiP is the master regulator of the UPR and targets misfolded proteins for degradation or re-folding. Earlier work from our laboratory demonstrated that acute sleep deprivation leads to upregulation of the UPR. In this study, we hypothesize that sleep fragmentation will perturb ER homeostasis, and will subsequently lead to induction of the UPR and an upregulation of BiP. We also hypothesize that the reverse association may hold true: that UPR activation will induce sleep fragmentation.

Methods: Sleep fragmentation was carried out mechanically and pharmacologically. Flies were subjected to a random 1 second pulse within any given 15 minute interval. Relative BiP expression was measured by western blots in both the *Drosophila* sleep fragmented flies and the control flies. Tunicamycin was used to induce ER stress. This drug inhibits N-linked glycosylation which leads to protein misfolding. Flies were placed into locomotor tubes containing a sucrose/agar media and either vehicle, 6μM or 12μM of tunicamycin and allowed to acclimate for 24 hrs. Sleep-wake behavior was then recorded for 2 days.

Results: Tunicamycin treatment fragmented daytime and nighttime sleep. This was demonstrated by an increase in nighttime sleep bout number in males (48.5% at 6 μM, $P<0.05$) and females (59.4% at 12μM, $P<0.01$). Total daytime sleep in males (38.2% at 6μM and 81% at 12μM, $P<0.001$ and $P<0.0001$ respectively) and nighttime sleep bout duration in females (59.4% at 12μM, $P<0.05$) were both significantly decreased. BiP expression following sleep fragmentation is currently being assessed.

Conclusion: Our results indicate that protein misfolding and ER stress induces sleep fragmentation. It is expected that sleep fragmentation will lead to increased BiP levels.

Effects of Two Five-Day Bouts of Chronic Sleep Restriction on Caloric Intake in Healthy Adults

Spaeth AM[1], Goel N[2], Dinges DF[2]

[1]*Department of Psychology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, USA*
[2]*Division of Sleep and Chronobiology, Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA*

Introduction: We have previously shown that caloric intake increases when bedtime is delayed until 0400h. The current study examined the effects of two separate bouts of chronic sleep restriction (SR) on caloric intake under ad libitum feeding conditions to determine if the effects of SR on caloric intake cumulatively increase with repeated exposure to sleep loss.

Methods: N=19 healthy subjects (35.7 ± 9.16y; 10 females) participated in a laboratory-controlled protocol. Subjects underwent 2 baseline nights (10h TIB/night; 2200h-0800h), 10 SR nights (4h TIB/night; 0400h-0800h), and 4 recovery nights (12h TIB/night; 2200h-1000h). The 10 SR nights were split into two bouts of 5 nights each and were separated by either 1 or 3 nights of recovery sleep. Food and drink consumption was ad libitum: subjects had 3 meals/day, access to snacks and an optional late night meal during SR nights. Caloric intake was recorded daily and analyzed using The Food Processor SQL program. Repeated measures ANOVAs were used for analyses.

Results: Consistent with previous findings, subjects consumed more calories when bedtime was delayed until 0400h compared to days when bedtime was at 2200h (*p*'s<0.05). Daily intake during the second five-day bout of SR was significantly greater than intake during days following baseline and recovery sleep (*p*'s<0.05) but was not significantly different from intake during the first five-day bout of SR (SR1-5=3166.5 calories/day, SR 6-10=3028.6 calories/day; *p*=0.07). Intake during days following recovery sleep was significantly reduced compared to baseline intake (*p*<0.05). The proportion of calories derived from each macronutrient was similar across all protocol days.

Conclusion: During both five-day bouts of SR, subjects consumed more calories during days when bedtime was delayed until 0400h. The increase in caloric intake was similar during both bouts of SR; therefore, the effect of sleep restriction on energy balance does not appear to cumulatively increase with additional days of restriction.

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Distinct clustering of locations and activity patterns among ventrolateral medullary cells recorded during the atonia of REM sleep elicited by pontine carbachol in urethane-anesthetized rats

Georg M. STETTNER, Yanlin LEI, Kate BENINCASA HERR and Leszek KUBIN
Department of Animal Biology, University of Pennsylvania, Philadelphia, PA

Rapid eye movement sleep (REMS) is generated in the brainstem by a distributed network of neurochemically distinct neurons of which some are activated (REMS-on cells) while others are suppressed or silenced (REMS-off cells) during REMS episodes. Various concepts regarding the role of medullary REMS-on and REMS-off cells have been advanced based on juxtaposition of electrophysiological data with information about cellular neurochemistry and connectivity. However, REMS behaviors of cells located in the caudal ventrolateral medullary reticular formation region (VLM) have not been unexplored. This region is of significant interest because it contains noradrenergic A1 and adrenergic C1 neurons which play an important role in cardiovascular regulation and may also contribute to state-dependent changes in activity of cranial motoneurons that innervate upper airway muscles. Our objective was to examine REMS-related changes in activity of cells located in the VLM and relate this information to neurochemical identity and other electrophysiological and functional features of these cells.

Since the efficiency of sampling of cell activities from the caudal medulla in behaving animals is limited, we used an acute animal model of REMS based on a urethane-anesthetized rat. In this model, small injections of a cholinergic agonist, carbachol, into the dorsomedial pons are used to repeatedly trigger REMS-like episodes that comprise cortical and hippocampal activation with a concomitant suppression of activity in respiratory-modulated hypoglossal (XII) motoneurons. The model well represents tonic features of REMS. As such, it is especially suitable for investigation of the cellular mechanisms underlying the triggering and maintenance of REMS episodes and the associate tonic changes in activity of central neurons.

In 18 urethane-anesthetized, paralyzed and artificially ventilated Sprague-Dawley rats, we recorded cortical EEG, hippocampal activity, XII nerve activity and single cell activity from VLM during REMS-like episodes elicited by pontine carbachol (10 nl, 10mM). Recording sites were marked with Pontamine blue dye, the animals were perfused, the medulla was sectioned and brain sections containing the marked sites were immunohistochemically processed for dopamine-β-hydroxylase (DBH), a marker for noradrenergic and adrenergic neurons. Cell recordings at sites located closer than 50 μm from one or more DBH-positive cells were operationally regarded as obtained from adrenergic or noradrenergic neurons.

We studied activity of 52 cells. They were classified into four groups: silenced cells (n=26; baseline firing rate of 7.1±0.8(SE) Hz); suppressed cells (n=10; firing rates were reduced from 15.3±2.2 to 7.3±1.1 Hz); activated cells (n=11; baseline firing rate of 4.0±1.9 Hz increased to 10.2±0.7 Hz); and cells whose activity was unchanged during REMS-like episodes (n=5). All but two activated cells were found near one or more DBH-positive neurons and all but two were located in the VLM region containing the adrenergic C1 group. The activated cells had longer half-widths of action potentials than the silenced or suppressed cells (0.23±0.01 ms vs. 0.19±0.01 ms; p=0.024) and longer half-widths of afterpotentials (0.48±0.03 ms vs. 0.40±0.02 ms; p=0.033). Among the 36 cells that were silenced or suppressed, 29 were localized within the lateral reticular nucleus (LRt), and only 7 were found near a DBH-positive cell. Our data indicate that adrenergic C1 cells are activated, rather than suppressed, during REMS. Interestingly, we also established that LRt cells are suppressed or silenced, which indicates that spinal afferent input transmitted to the cerebellum and reticular formation through the LRt is diminished during REMS. This may distort the spatial representation of body position during this state of sleep.

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Prospective Study of Polysomnographic Variables Predictive of Peri-operative Complications After Adenotonsillectomy in Children with Obstructive Sleep Apnea

Thongyam A[1], Marcus CL[1], Lockman JL[3], Cornaglia MA[1], Caroff A[2] , Gallagher PR[4], Traylor JT[1], Rizzi MD[2], Elden L[2]

[1]Sleep Center, [2]Otolaryngology Division, Department of Surgery, [3]Anesthesiology and Critical Care Medicine Department, [4]Clinical and Translational Research Center, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA

Introduction: Retrospective studies have attempted to predict peri-operative risk following adenotonsillectomy in children with obstructive sleep apnea (OSA). However, there have been few large prospective studies. We hypothesized that certain demographic and polysomnographic (PSG) variables predicted both respiratory and general peri-operative complications in the postoperative period after adenotonsillectomy in children with OSA.

Methods: Consecutive children <18 years of age who underwent adenotonsillectomy for OSA at Children's Hospital of Philadelphia within 12 months of PSG were followed prospectively, and intra-operative and post-operative complications were recorded. Finally, caregivers were called 2 weeks after discharge to ascertain late complications. Complications were classified as non-respiratory (e.g., inadequate oral intake, hemorrhage and arrhythmias) and respiratory (e.g., pulmonary edema or need for supplemental oxygen, airway, CPAP, intubation). Data were analyzed using two-sample t-tests, Mann-Whitney tests, and point-biserial correlations.

Results: 198 subjects included 56 (28.3%) <3 years of age, 48 (24.2%) obese, 26 (13.1%) preterm and 38 (19.2%) with serious comorbidities. In this high risk population, 35.9% had respiratory complications and 38.4% non-respiratory complications. There was a significant correlation between certain PSG parameters and respiratory complications as follows: log arousal index ($p=0.029$), SpO_2 nadir ($p=0.003$), % total sleep time with $SpO_2<90\%$ ($p=0.002$), % total sleep time with end-tidal CO_2 >50 mmHg ($p=0.027$); with a trend for log apnea hypopnea index ($p=0.058$). There were no significant correlations between PSG parameters and non-respiratory complications. There was a significant association between obesity and non-respiratory ($p=0.011$) but not respiratory ($p=0.12$) complications. Age, history of prematurity and comorbidities were not associated with increased complications.

Conclusion: PSG predicted respiratory peri-operative complications but not non-respiratory complications in children with OSA. Obese children are at increased risk for non-respiratory but not respiratory complications. PSG is useful in predicting which children are at high risk for adenotonsillectomy and may therefore benefit from post-operative admission.

NIH HL58585, REDCap

Trainee Award Eligible

The Regulation of a Sleep Subprogram in an Invertebrate Genetic Model Organism

Trojanowski N, Fang-Yen C, Raizen D

Introduction and Background: During sleep, locomotion and feeding cease and there is an increased threshold for arousal response to sensory stimuli. Identifying the neural circuits regulating each of these subprograms of sleep is necessary for understanding the function of conserved sleep/wake genes. We focus on understanding feeding quiescence in the nematode genetic model system *Caenorhabditis elegans*, the only organism for which the entire synaptic connectivity of the nervous system has been mapped. The *C. elegans* nervous system, which contains only 302 neurons, regulates many complex behaviors similar to those seen in mammals, including learning, memory, and sleep. *C. elegans* sleep occurs during a larval transition period called lethargus and demonstrates fundamental behavioral and molecular genetic similarities to mammalian sleep. The small size and established connectivity of the *C. elegans* nervous system is a great asset for identifying the neural circuits that mediate sleep subprograms, as it allows identification of the specific roles of individual neurons.

C. elegans feed on bacteria via contraction/relaxation motions of its pharynx, a tubular neuromuscular pump. The pharyngeal nervous system consists of 20 neurons of 14 types, and its only connection to the somatic nervous system is through a pair of gap junctions. Pharyngeal pumping occurs continuously throughout the animal's life except during lethargus. The mechanism by which pharyngeal pumping is inhibited during *C. elegans* sleep is unknown. I have focused initially on understanding how pumping is regulated by pharyngeal neurons with the future goal of using this information to understand how feeding quiescence is regulated during sleep.

Methods: I have developed a novel approach that allows dynamic manipulation of neural circuits *in vivo* at single neuron resolution in intact, behaving animals. I use targeted optogenetic stimulation, achieved by arbitrarily shaping a laser beam with a digital micromirror device, and behavioral observation via machine vision, to quantify changes in feeding after dynamic manipulation of the activity of single neurons.

Results and Conclusions: I have shown that two pairs of bilaterally symmetric cholinergic motor neuron types, called MC and M2, directly stimulate pumping. I have further shown that the paired cholinergic interneurons I1 are excitatory to both MC and M2. These data are consistent with prior ultrastructural reconstructions of the pharyngeal nervous system: The MC and M2 neurons form gap junctions on each other and chemical synapses on the muscle, and the I1 interneuron forms chemical synapses on MC and M2. Previous work indicates that the MC neurons stimulate pumping via nicotinic neurotransmission. Using genetic mutants, I found that the MC (and M2) neurons also excite pumping via a cholinergic non-nicotinic mechanism. We are currently indentifying other cholinergic receptors involved in feeding and exploring the function of the connection between the pharyngeal and somatic nervous systems with the ultimate goal of understanding the roles of conserved sleep/wake genes in suppressing feeding during sleep.

Trainee Award Eligible

Bmal1^{fbrainKO} Mice Exhibit Deficits in Learning and Memory Processes

Anand Venkataraman, Robbert Havekes, Ted Abel and John Hogenesch

The time-of-day effect on cognitive performance has been investigated by various behavioral tools and is supported by anecdotal evidence. However, the molecular details of the interaction between the circadian and cognitive systems remain underexplored. Recent studies suggest an endogenous slave-oscillator in the hippocampus, the primary seat of all learning and memory processes. Furthermore, in mammals *Arntl* (*Bmal1*) is the only indispensable transcription factor in the core transcriptional and translational feedback loop (TTFL) of the circadian-clock. Global deletion of the Bmal1 locus (Bmal1$^{-/-}$) leads to arrhythmic locomotor activity but also many other behavioral and physiological deficits, complicating the relationship between the clock and these phenotypes. We hypothesized that conditional tissue-specific deletion of Bmal1 in the hippocampus will function as a proxy for disabling the 'hippocampal-clock' leading to learning and memory deficits. To test this, we ablated the Bmal1 loci in post-natal mouse hippocampi using the CamKII-Cre driver line (referred hereafter as Bmal1^{fbrainKO}). The circadian locomotor activity rhythms of Bmal1^{fbrainKO} are comparable to wild-type mice. However, the behavioral performance of Bmal1^{fbrainKO} in hippocampal-dependent tasks like classical-fear conditioning and spatial-object recognition was significantly impaired compared to littermate controls. We also observed differences in the hippocampal molecular-network of Bmal1^{fbrainKO} and wild-type mice that provides some insight into the molecular correlates of this performance.

Assessing Chinese Children's Sleep Behavior using Youth Self-report Sleep Questionnaire (YSRSQ)

Amy Yang, Siyuan Cao, Xianchen Liu, and Jianghong Liu

School of Nursing, University of Pennsylvania

Introduction: Sleep problems are important issues for the pediatric populations. While Children's Sleep Habits Questionnaire is used for parents report, however, no youth self-report sleep questionnaire is currently available. The main purpose of the study is to evaluate the agreement among self report (YSRSQ) and parent-report (CSHQ) on sleep questionnaires

Method: 3081 10-12 years old pairs of Chinese children and parents completed Youth self-report sleep questionnaire (YSRSQ) and Chinese version of Children's Sleep Habits Questionnaire (CSHQ) respectively to assess the children's sleep habits. YSRSQ contains 41 items newly developed by a Chinese research team. Spearman's correlations were used to assess the agreement between CSHQ and YSRSQ on parasomnias, sleep disordered breathing problems, and day time sleepiness problems.

Results: Prevalence of Self-report sleep problems ranges from 3.9% for "child wets bed at night" to 49.6% for "child is restless and moves a lot during sleep". All correlations between the two reports were positive and significant ($p<0.001$ for all), ranging from 0.126 to 0.268, indicated a significant but low agreement between child and parent reports. Although some Kappa values were low, all categorical variables showed acceptable reliability when examined for percent agreement between test and retest (range 43%–92% agreement)

Conclusions: Although the agreement between the two reports was low to moderate, we believe both CSHQ and YSRSQ questionnaire can provide useful information on evaluating children's sleep patterns. Factor analysis is needed to better understanding the number of syndromes in YSRSQ.

Trainee Award Eligible

Attenuation of the Unfolded Protein Response by Modafinil in *Drosophila melanogaster*

Alice Yu[1,2] and Nirinjini Naidoo[2]

[1] *Vagelos Scholars Program in Molecular Life Sciences,* [2]*Center for Sleep and Circadian Neurobiology*

Introduction: 2-[(Diphenylmethyl) sulfinyl]acetamide (modafinil), an FDA approved for the treatment of narcolepsy, has been reported to sustain wake in individuals without the negative side effects common to stimulant and amphetamine-use such as, dizziness, insomnia, and increased rates of addiction. Modafinil's mechanism of action, however, remains to be characterized. We have previously shown that sleep deprivation/prolonged wake in *Drosophila melanogaster* induces the unfolded protein response (UPR). Preliminary studies indicate that prolonged wakefulness as a result of modafinil treatment does not lead to an increase in BiP expression. We have also found that chemical chaperone treatment also attenuates the UPR. Based on modafinil's structural similarity to this chemical chaperone, 4-phenyl butyric acid (4-PBA), we hypothesized that modafinil attenuates the UPR. We also hope to gain insight into the mechanisms governing natural wake and attention.

Methods: *Drosophila melanogaster* were treated with modafinil (0.7mg/mL) for 3 days, and behavior was monitored by video. *Drosophila* tissue was then probed for markers of activated UPR. Westerns were used to measure BiP levels and RT-PCR was used to assay XBP1 splicing. *Drosophila Schneider 2* cells were pre-treated with either modafinil vehicle (negative control), 4-PBA (positive control), or modafinil, before activation of the UPR by the reducing agent dithiothreitol (DTT). Cellular extracts were then probed for spliced XBP-1 mRNA, which is a marker of activated UPR.

Results*: Drosophila* treated with modafinil slept significantly less (40% decrease after 2 days). There was no increase in BiP expression levels as assayed by western blots and DNA gels showed no spliced XBP-1 mRNA following modafinil treatment. In S2 cells, increasing concentrations of DTT led to a dose dependent activation of the UPR, shown by increasing XBP-1. After 4-PBA treatment, a higher concentration of DTT was required for full UPR activation A similar effect is expected in modafinil treated S2 cells.

Conclusion: Modafinil induced wakefulness does not activate the UPR. Modafinil may be acting as a chemical chaperone to prevent induction of the UPR.