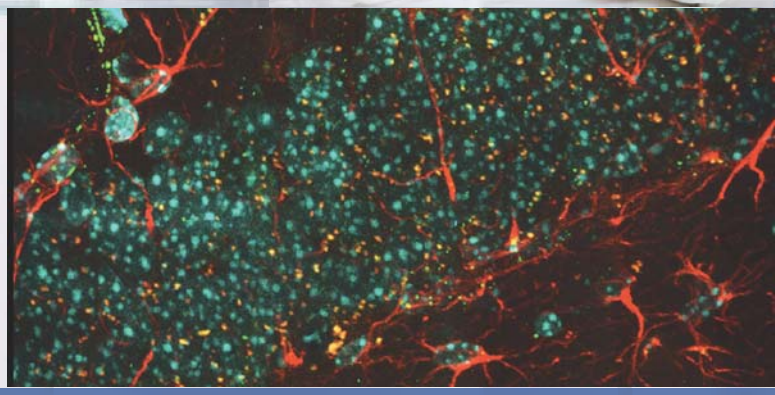
7th annual

CSRN Research Retreat

Friday, May 21, 2010

Bodek Lounge, Houston Hall



Penn Medicine

Center for Sleep and Respiratory Neurobiology

2010 CSRN Research Retreat Schedule

8:30 – 9:00 AM **Poster Mounting, Continental Breakfast**

9:00 – 9:15 AM **Opening Remarks:**

Allan I. Pack MBChB, PhD – Director, Center for Sleep and Respiratory Neurobiology

Eliot Friedman, MD – 2010 CSRN Research Retreat Committee Chair

9:15 – 10:15 AM ***Sleep Problems Across The Lifespan***

Jingtao Huang, PhD - Upper airway collapsibility and genioglossus activity in children with sickle cell disease

Michael Grandner, PhD - Sleep complaints and fatigue decline across the lifespan: getting older does not necessarily mean poor subjective sleep and daytime fatigue

Ashdin Tavaria, MD - Self treatment of insomnia by the elderly

Bilgay Iczi-Balserak, PhD - SDB and daytime napping are associated with higher glucose levels in pregnant women

10:15 – 10:45 AM **Break & Poster Session**

10:45 – 12:00 PM ***Neural Subtrates of Sleep and Circadian Rhythms***

Justin DiAngelo, PhD - The control of lipid storage by the circadian clock and age in *Drosophila* melanogaster

Jason Moore, BS - Neurons in the ventrolateral preoptic nucleus play a key role in anesthetic-induced hypnosis

Jason Wester, BS - The impact of local circuit dynamics in layers 4 and 2/3 of rodent somatosensory cortex on response properties to thalamic input in vitro

Michelle Dumoulin, PhD - Exploring the role of REM sleep in ocular dominance plasticity consolidation

Namni Goel, PhD - The *COMT* Val158Met polymorphism predicts interindividual differences in sleep homeostatic responses to chronic partial sleep deprivation

12:00 – 1:30 PM **Lunch & Poster Session**

1:30 – 2:45 PM ***Updates on Sleep Disordered Breathing***

Kristan Singletary, PhD – ER stress and age-related dysfunction of wake-active neurons

Diane Lim, MD - Irreversible ER stress in the hippocampus parallels lasting memory impairment in sleep apnea model

Francois-Louis Comyn, DDS, MS - Effects of mandibular divergence and tongue volume on obstructive sleep apnea syndrome severity

Amy Sawyer, PhD - Does a neurobehavioral and mood dose response to CPAP exist in obstructive sleep apnea patients treated with CPAP?

Clinical Case Presentation

Lori Panossian, MD - The case of the man with abnormal movements in sleep

2:45 – 3:00 PM **Break**

3:00 – 4:00 PM **Keynote Speaker**
Dr. Juliane Winkelmann – Max Planck Institute for Psychiatry
“Genetics of Restless Leg Syndrome”

4:00 – 5:00 PM **Reception and Awards Presentation**

A Message from the Organizing Committee

Pictured: Dr. Eliot Friedman, Dr. Michael Grandner and Amanda Crocker

Dear Colleagues and Friends,

We thank you for your participation in the 7th annual Center for Sleep and Respiratory Neurobiology Research Retreat. We are honored to have as our keynote speaker, Dr. Juliane Winkelmann who is a world renowned investigator into the genetics of restless leg syndrome and periodic limb movement disorder.

This year's retreat will allow participants to experience the high caliber current research being conducted by members and collaborators of the Center for Sleep and Respiratory Neurobiology. We will be showcasing work from a wide range of fields that span from basic science research in invertebrate as well as mammalian murine models to clinical science research in humans that go from translational and genetic work to epidemiological population studies in children, adults and the elderly.

Our presentations today come from a wide variety of schools and Departments from within the University, including the School of Medicine, the Children's Hospital of Philadelphia, the School of Nursing and the Veterinary School. This wide representation reflects the richness and diversity of sleep and circadian rhythm research available at the University of Pennsylvania. Our hope is that retreat participants take in the wide breadth and scope of research available from within our institution thereby fostering a spirit of synergy that enhances the scientific quality of our work and makes the Center for Sleep and Respiratory Neurobiology a national reference for sleep research.
Finally, the committee members would like to thank the tireless work of Ms. Margaret Higgins and Ms. Jennifer Montoya in organizing this retreat.

The Committee:

Dr. Eliot Friedman is an Instructor in Medicine in the Division of Sleep Medicine. He works in the Sehgal laboratory and has collaborated with Dr. Max Kelz in the development of a *Drosophila* model to study the genes and neuronal circuits responsible for arousal from hypnotic effects of volatile anesthetics and how they relate to normal awakening from natural sleep. He also studies the effects of acute intermittent hypoxia on sleep.

Dr. Michael Grandner is a postdoctoral fellow at the Center for Sleep and Respiratory Neurobiology. His research interests include health outcomes associated with habitual sleep practices and involves projects exploring the relationships between short sleep duration and health and population-level sleep disturbances.

Amanda Crocker is a final year PhD student in the Sehgal lab. She is currently preparing to defend her thesis on "Sleep in *Drosophila"*.

Dr. Joseph Daley is a postdoctoral fellow in the Division of Sleep Medicine. He received his MD/PhD degrees from Emory University, and completed his neurology residency at Yale. Dr. Daley plans to focus his research on the basic neurobiological mechanisms of sleep.

Dr. Margaret Souders is a postdoctoral fellow in the School of Nursing. Her research focuses on sleep in children with autism.

From the Director, Allan I. Pack, MBChB, PhD

This is our seventh annual research retreat. We hope that today will inspire everyone to get away from their usual work day and participate and celebrate the amazing work going on in the Center for Sleep. Once again this year, we are delighted to see the exciting research being conducted by our trainees. And again, our established faculty are well represented as well, where some of the research presented here has appeared in prestigious journals.

Our keynote speaker, Dr. Julianne Winklemann comes to us from the Max Planck Institute of Psychiatry and will speak about her recent exciting research in the field of genetics of restless legs syndrome.

We received almost 60 abstracts, with 14 being presented as talks. Our committee, chaired by Eliot Friedman, and including Amanda Crocker, Joseph Daley, Michael Grandner and Margaret Souders, did a wonderful job in choosing some of the most interesting science going on in the Center to be presented to you today.

CSRN Research Retreat Keynote Speaker: Dr. Juliane Winkelmann

Professor Juliane Winkelmann was born in Munich, Germany, and received her medical education at the Semmelwies University in Budapest, Hungary, as well as the Ludwig Maximillians University in Munich. She completed her Neurology training at the Hammersmith Hospital in London as well as the Klinikum Grosshadern, the Ludwig-Maximilians University and the Max Planck Institute for Psychiatry where she most recently served as Medical Director. She also received advanced training in Neurogenetics at the Institute of Human Genetics at the Hemholtz Zentrum of Munich. In 2003 she became the head of the Genetics of Restless Leg Syndrome (RLS) Research Group at the Institute of Human Genetics of the Hemholtz Zentrum in Munich and in 2004 became the Head of the Neurological Genetics group as well as head of the outpatient clinic for RLS at the Max Planck Institute of Psychiatry. Since 2008 she has worked at the Munich Technical University where she holds the post of specialist in Neurology and senior physician of the RLS outpatient clinic. She has been a prolific investigator in the field of RLS and has established successful research collaborations across Germany, Austria and Canada that have led to the identification of the first genetic risk factors for RLS.

Table of Contents

The Effects of Sustained Sleep Restriction on Driving Simulator (AusEd) Performance

Arroyo S[1], Banks S[2], Dinges DF[1]

[1]Division of Sleep and Chronobiology, Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA, USA
[2]Center for Sleep Research, University of South Australia, Australia

Introduction: Little work has been done on the effects of chronic sleep restriction on simulated driving performance. This study examined driving simulator performance after a period of 5 nights of sleep restriction to 4h time in bed (TIB).

Methods: N = 86 healthy adults (30.3 ± 6.8y; 46 females) participated in a controlled laboratory protocol. N = 77 underwent 2 baseline nights (10h TIB/night, 10pm-8am), followed by 5 sleep restriction nights (4h TIB/night, 4am-8am). N = 9 were randomized to a control group that received 10h TIB per night. The 20-minute driving simulator (AusEd) was administered every morning between 1030h-1215h and was set to simulate a monotonous rural road at night. Driving performance measures included steering deviation (StD) from median lane position (measured in centimeters) and speed deviation (SpD) from 70km/h. Driving performance measures were averaged among subjects. Paired and independent t-tests were used to examine the differences between days and groups.

Results: There was no significant change from baseline day 2 (B2) to sleep restriction day 5 (SR5) in the sleep-restricted subjects StD (p=0.19) or SpD (p=0.96), while the control group improved their StD (p=0.02) performance but not SpD (p=0.09). The sleep restricted group differed at SR5 from the controls in StD (p=0.005) but not SpD (p=0.67).

Conclusions: Sleep restriction interfered with subjects' ability to improve steering variability performance on a 20-minute version of the AusEd driving task, when compared to well rested subjects who improved performance with each da y of the study. These results are consistent with the increased variability in sustained attention tasks (e.g., PVT) produced by sleep restriction, and they support concerns that sleep restriction may contribute to driving risks.

Supported by: NIH NR004281 and CTRC UL1RR024134

Network Mechanisms for Sleep-Dependent Consolidation of Plasticity in the Visual Cortex

Sara J. Aton, Julie Seibt, Michelle Dumoulin, Tammi Coleman, Marcos G. Frank

Department of Neuroscience, University of Pennsylvania

Introduction: Several findings suggest that sleep has beneficial effects for processes dependent upon synaptic plasticity, such as memory consolidation. Recent studies have shown that cortical areas engaged in a learning task are “reactivated” during subsequent sleep, with coordinated replay of activity patterns seen during prior waking experience, and specific, localized changes in EEG activity. However, it is unclear what function this reactivation may subserve, and what cellular and network-level mechanisms underlie sleep facilitation of synaptic plasticity.

Methods: Altered visual experience during a critical developmental window profoundly influences the development of responses in the primary visual cortex (V1), in a canonical form of *in vivo* synaptic remodeling known as ocular dominance plasticity (ODP). If one eye is deprived of sight (monocular deprivation; MD), most cortical neurons gradually lose responsiveness to deprived eye (DE) stimuli and become more sensitive to non-deprived eye (NDE) stimuli. Our lab has previously shown that following 6 h of waking MD experience, ODP can be consolidated by as little as 6 h of *ad lib* sleep, and involves potentiation of NDE responses. Sleep-dependent consolidation of ODP involves cellular changes that mimic long-term potentiation (LTP) of glutamatergic synapses *in vitro* (*i.e.*, NMDA receptor-dependent kinase activation, and phosphorylation of AMPA receptor subunit GluR1, during the first 1-2 h of sleep), and is associated with enhanced multiunit activity across the visual cortex. On a site-by-site basis, local enhancements of V1 multiunit activity during early post-MD sleep are positively correlated with overall ODP consolidation measures. It is possible that enhanced multiunit activity reflects increased firing rate and or synchrony among individual V1 neurons, or changes in spike timing between neurons, all of which could facilitate activation of LTP-like intracellular pathways and functional potentiation of NDE responses. To clarify the network-level mechanisms involved in sleep-dependent consolidation of ODP, we recorded populations of individual V1 neurons in freely-moving, freely-sleeping animals over a baseline period of *ad lib* sleep and waking, 6 h of waking MD (or waking visual experience without MD), and 6 h subsequent sleep, using custom-made stereotrode arrays. At intervals (following baseline, following MD (or control experience), and following subsequent sleep), visual responses and were assessed in these neurons during presentation of stimuli to the left (NDE) and right (DE) eyes, in order to track the consolidation of ODP.

Results: Our preliminary findings suggest that ODP consolidation during post-MD sleep occurs in regular-spiking (putative glutamatergic) V1 neurons, but does not occur in fast-spiking (putative GABAergic) neurons. Regular-spiking neurons also show enhanced firing rates during the first 2 h of post-MD sleep (relative to baseline), while firing rates are unchanged in fast-spiking neurons. Moreover, synchrony of firing between pairs of regular-spiking and fast-spiking neurons is reduced during post-MD sleep.

Conclusions: These data suggest a change in the balance of excitation and inhibition across the cortex during consolidation of ODP. In addition to these changes, our preliminary recordings suggest that patterns of neuronal activation across V1 during waking MD are reiterated during subsequent sleep. Altogether, our data suggest that consolidation of ODP during post-MD sleep is associated with: 1) changes in the balance of activity between putative glutamatergic and GABAergic neuronal populations, and 2) "replay" of network activity patterns associated with prior visual experience.

Age-Related Deterioration of Recovery Sleep in *Drosophila melanogaster*

Marishka K. Brown

Center for Sleep and Respiratory Neurobiology, University of Pennsylvania

Sleep is a universal process that has been demonstrated in organisms ranging from invertebrates to mammals. In *Drosophila melanogaster*, short periods of 'rest' have similar characteristics as mammalian sleep, which among other facets; consist of circadian and homeostatic regulation. The homeostatic component governs the aspect involving recovery sleep, i.e. the longer animals are in a state of wakefulness, either normal or forced, the more profound the ensuing sleep period will be. Alterations in sleep quality have been demonstrated during the aging process. These age-related changes produce a marked deterioration in an individual's ability to initiate and sustain sleep, which consequently leads to excessive daytime sleepiness. Evidence suggests that sleep homeostasis diminishes with advancing age, a process that modifies the efficiency of nighttime sleep and increases the tendency toward daytime sleep. Reports have demonstrated that flies that have been sleep deprived experience an intense period of recovery sleep that results in more consolidated daytime sleep. In this study, we used wild-type Canton-S flies to assess recovery sleep in aging populations of *D. melanogaster*. Using 9 to 13 day old flies as our young controls, we sleep deprived aged flies (between 5 - 9 weeks) for 6hr during lights off (rest period) and observed recovery sleep. We found that at 5 weeks, there was no significant difference between these animals and the young controls. However, at 7 weeks, the animals seem to require more sleep than the young controls to recover and experience a slight delay in returning to an active state. The older animals, which consist of the 8 and 9 week populations, require more extensive periods of recovery sleep. This dysfunctional recovery sleep process could be a consequence of the fragmented sleep seen in aged flies. Since there are clear demarcations seen between the adult and aged populations, we next want to determine if the decomposition of recovery sleep can be restored by pharmacological intervention.

GABA Transaminase Affects Sleep in Drosophila and Is Increased in *Sleepless* Mutants

Wen-Feng Chen*[1], Mallory Sowick[1], Wenyu Luo[2], and Amita Sehgal[1,2]

[1]Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, Pennsylvania;
[2]Department of Neuroscience, University of Pennsylvania, Philadelphia, Pennsylvania

Sleep is an essential daily physiological state that is accompanied by dramatic changes in neuronal excitability and is regulated by numerous neurotransmitters. In *Drosophila melanogaster*, loss of the *sleepless* (*Sss)* gene product leads to a >80% reduction in sleep amount. *Sleepless* encodes a glycosylphosphatidyl inositol-anchored membrane protein that interacts with the potassium ion-channel SHAKER. We used 2D-Differential in Gel Electrophoresis (2D-DIGE) to identify proteins that are altered in the brains of *sss* mutant flies and found that levels of GABA transaminase (GABAT) are enhanced in the mutants. GABAT catalyzes the turnover of GABA, which is a sleep-promoting neurotransmitter in both flies and mammals. Consistent with this role of GABA, *Drosophila* mutant for GABAT show increases in daily sleep amount, mainly in nighttime sleep. The mutants also have consolidated sleep indicated by longer sleep episodes and reduced number of sleep episodes. In addition, the sleep arousal threshold is increased in the mutants. However, locomoter activity of these flies is rhythmic and shows no difference in circadian period length when the flies are tested in freerunning (constant darkness) conditions. These results indicate that the mutation in GABAT largely affects sleep homeostasis rather than circadian clocks. Experiments are under way to determine if GABAT contributes to the sleep phenotype in *sss* mutants.

Upper Airway Anatomic Structures Between Apneic Men and Women, Matched on age, BMI and AHI

L Chi[1], M Thorne-Fitzgerald[1], Francois-Louis Comyn[1], T Gislason[2], ES Arnardottir[2], B Benediktsdottir[2], H Einarsdóttir[2], S Juliusson[2], A Pack[1], RJ Schwab[1]

[1]Center for Sleep and Respiratory Neurobiology, University of Pennsylvania School of Medicine, Philadelphia PA; [2]Landspitali University Hospital and Medical Faculty, University of Iceland, Reykjavik, Iceland

Rationale: We currently have limited knowledge on the differences in the pathogenesis of OSA in men and women. We hypothesize that upper airway soft tissue, craniofacial structure and volume of soft tissue enclosed inside oral cavity would be larger in apneic men than apneic women after matching for BMI, AHI and age, but the relationship between upper airway soft tissue and craniofacial structures would be similar in apneic men and women.

Methods: We used 3D MRI analysis techniques to study the upper airway soft tissue and craniofacial structure in untreated apneic subjects as part of the Iceland Sleep Apnea Cohort (ISAC) Study. There were 73 men and 73 women, matched for BMI (within 1 kg/m^2), AHI (within 5 per hour) and age (within 5 years).

Results:

Measurements	Difference* Paired T-Test		t	p
	Mean	SD	df = 72	
Parapharyngeal fat pad, mm^3	2498	4227	4.14	0.0001
Retroplatal lateral pharyngeal wall, mm^3	3773	4640	5.69	<0.0001
Retroglossal lateral pharyngeal wall, mm^3	3152	4156	5.31	<0.0001
Total soft tissue, mm^3	43571	28081	10.86	<0.0001
Soft palate, mm^3	2349	2899	5.67	<0.0001
Genioglossus, mm^3	24081	17004	9.91	<0.0001
Tongue fat, mm^3	2049	2815	5.04	<0.0001
Total airway length, mm	9.92	9.51	7.30	<0.0001
Mandibular depth, cm	0.34	0.86	2.51	0.0164
Mandibular body length, cm	0.83	1.03	5.09	<0.0001
Mandibular width, cm	0.48	0.49	4.72	0.0001
Hyoid to sella distance, cm	1.43	1.16	7.87	<0.0001
Hyoid to C3 distance, cm	0.61	0.54	7.17	<0.0001
Intramandibular volume, mm^3	50183	39694	8.39	<0.0001
Ratio of total soft tissue volume to Intramandibular volume	-0.16	1.99	-0.67	0.50

Definition of abbreviations: SD = standard deviation. df = degree of freedom. C3 = the third cervical vertebra. Difference* = Men - Women

Conclusions: The data support our a priori hypotheses that the upper airway soft tissue volume, craniofacial structures and volume of soft tissue enclosed inside oral cavity are larger in apneic men than apneic women. However, the relationship of soft tissue volume to intramandibular volume was similar in men and women. For the same severity of OSA, after matching important confounding factors, the anatomic structures compensate for each other in men and women.

Craniofacial Structures and Upper Airway Soft Tissue Differences Between Caucasian and African American Females with Obstructive Sleep Apnea

FL Comyn, RJ Schwab, SJ Ratcliffe, EA Beothy, BE Staley and GW Pien

Introduction: Upper airway structure in patients with sleep apnea is thought to be dependent on ethnicity. Based on the literature we hypothesized that in sleep apneic Caucasian women craniofacial structures would be smaller and upper airway soft tissues would be larger in African American women.

Methods: We performed upper airway imaging (1.5 Tesla MR scanner) and overnight sleep studies in 60 untreated apneic women (AHI > 15 events/hr). We used a 3D reconstruction analysis (Amira 4.1.2) to study soft tissue and craniofacial structures. Statistical analysis was performed using unpaired t-tests (significance: $p < 0.05$).

Results: The study included 25 apneic Caucasian women (mean age 53.8 years (SD 8.2), mean AHI 25.9 (SD ± 15.7) and mean BMI 28.9 ± 7.5)) and 35 apneic African American women (mean age 53.7 years ± 3.9), mean AHI 27.6 ± 13.3) and mean BMI 32.1 ± 8.2)). There was no statistical difference in age and AHI between the Caucasian and African American women but African American women had higher BMI ($p = 0.04$). Mean values of each of the soft tissues (soft palate, tongue and lateral walls) and the maxillo-facial variables (saddle angle, mandible and hyoid bone) analyzed were both significantly smaller ($p < 0.05$) in the Caucasian patients compared to the African American subjects; however the mandible was significantly narrower in the African American subjects (see table). The volume of the parapharyngeal fat pads was not different between the two groups.

	CAUCASIAN FEMALE (n = 25)		**AFRICAN AMERICAN FEMALE (n = 35)**		
	Mean	**SD**	**Mean**	**SD**	**P value**
ANB (^{0})	4	2.6	5.6	2.9	**0.04**
Saddle Angle (^{0})	126.1	5.8	129.3	5.3	**0.04**
Mandibular Depth (cm)	5.7	0.5	6.1	0.7	**0.007**
Total mandibular Length (cm)	13.1	0.8	13.5	0.5	**0.03**
Mandibular Divergence (^{0})	70	4.6	61	5.2	**< 0.0001**
Hyoid to C3 (cm)	3.1	0.4	3.3	0.3	**0.03**
Soft Palate (cm³)	8.8	1.4	10.1	2.7	**0.04**
Genioglossus (cm³)	72	12.8	82.2	15.4	**0.008**
Total Tongue (cm³)	93.5	15.2	106.4	18.5	**0.005**
Retropalatal Lateral Walls (cm³)	8.4	2.9	11	3.4	**0.003**
Retroglosal Lateral Walls (cm³)	8	2.6	9.9	3.3	**0.001**
Total Lateral Walls (cm³)	16.9	3.9	21	5.1	**0.001**
Parapharyngeal Fat Pads (cm³)	5.7	1.9	5.6	1.3	**0.08**

Conclusions: Based on similar AHI between the two groups our findings indicate that in Caucasian apneic women craniofacial structures and upper airway soft tissues are smaller compared to African American middle-aged apneic women.

This abstract is funded by: NIH R01HL085695.

Effects of Mandibular Divergence and Tongue Volume on Obstructive Sleep Apnea Syndrome Severity

FL Comyn, M Thorne-Fitzgerald, T Gislason, AI Pack, ES Arnardottir,B Benediktsdottir, S Juliusson, H Einarsdottir and RJ Schwab

Introduction: Craniofacial structures and tongue volume play a major role in the pathogenesis of sleep disorder breathing. We hypothesized that a combination of a decrease in mandibular divergence (angle formed by the two branches of the mandibular corpus) and an increase in tongue volume would increase the severity of OSA more than for each factor independently.

Methods: Untreated apneic patients had upper airway MRIs (1.5 Tesla scanner) and overnight sleep studies as part of the Iceland Sleep Apnea Cohort (ISAC). We used 3D reconstruction techniques (Amira 4.1.2) to analyze their craniofacial and soft tissue structures. Statistical analysis was performed using multivariable ANOVA tests to examine the effect of mandibular divergence and tongue volume on AHI and then pairwise comparisons between groups were performed. Our population was stratified into 3 groups based on the mean ratio of the mandibular divergence over tongue volume; group 1 included subjects who had 1 SD above the mean, group 2 included subjects into the mean and group 3 included subjects who had 1 SD below the mean (see Figure1).

Results: The study included 440 Caucasian male subjects (mean age 53.1 ± 10.5 (SD), mean BMI 32.2 ± 4.7 and mean AHI 43.2 ± 19.4).We showed that in this population a decrease in the mandibular divergence or an increase in the tongue volume alone is associated with an increase of AHI (see Figure 2). Group 1 which had the narrowest mandible (mean 67.2^0 ± 5.2) and the largest tongue volume (mean 127 cm³ ± 13.8), had a significantly ($p < 0.02$) higher AHI (mean 47 events/h ± 20.1) compared to the group 2 (mean AHI 43.6 events/h ± 19.5; mean mandibular divergence: 72.3^0 ± 4.5; tongue volume: 107.7 cm³ ± 14.5) and the group 3 (mean AHI 37.9 events/h ± 17.2; mean mandibular divergence: 75.5^0 ± 4.3; tongue volume: 88.1 cm³ ± 15.2) (see Figure2). Pairwise group comparisons showed that this significant difference was only between group 1 and 2 ($p = 0.02$) and group 1 and 3 ($p = 0.006$). The association between other craniofacial structures (cranial base, mandibular length, maxilla, hyoid bone) and tongue volume did not show as large an effect on AHI severity.

Conclusions: Our results support our initial hypothesis that both a decrease in mandibular divergence and an increase in tongue volume significantly increase the severity of OSA. These data indicate that both craniofacial and soft tissue structures are important in mediating the severity of OSA.

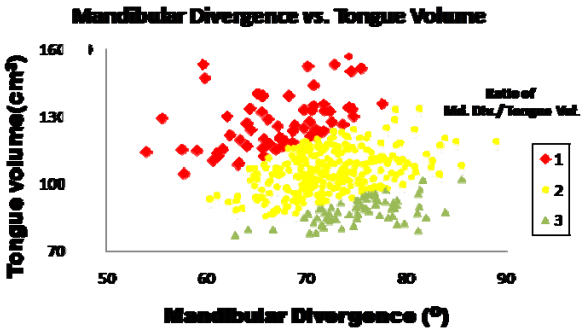


Figure 1

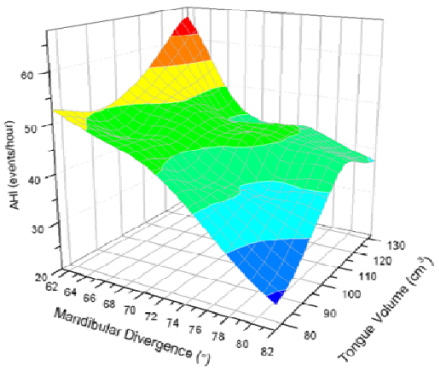


Figure 2

This abstract is funded by: R01 HL072067

Trainee Award Eligible

Overlapping Neural Circuitry Underlying Sleep and Metabolism in *Drosophila*

A Crocker[1], J DiAngelo, R Erion, A Watson, A Sehgal [1,2]

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

Introduction: *Drosophila* provides an ideal model for understanding both the network and cellular roles of neurotransmitters in sleep and metabolism. Recently we showed that octopamine, the norepinephrine homolog in *Drosophila*, is important for maintaining arousal on the fly. We found that this signal is mediated through the insulin producing neurons known as the Dilp2 neurons found in the pars intercerebralis brain region. This led us to ask whether octopamine was playing a role in the metabolic function of the fly.

Results and Methods: In this study we looked at Dilp2 (Drosophila Insulin Like Peptide 2) protein levels, pAKT levels (downstream signal of Dilp2), and triglyceride levels. We found that increased octopamine signaling through either expression of an excitatory Trp A1 channel or a bacterial sodium channel in octopamine producing neurons led to increases in Dilp2 staining, pAKT levels and triglycerides. Conversely flies that lack the OAMB receptor (octopamine receptor found on Dilp2 neurons and mediating the arousal effect) show decreased Dilp2 staining, pAKT levels, and triglycerides. Interestingly we also find that octopamine can mediate metabolic signals independent of Dilp2 when it is enriched non-neuronally.

Conclusion: This research provides strong support for the overlap of neuronal function of octopamine in both sleep and metabolism. It also points out the complexity of addressing whether sleep is necessary for normal metabolic function or is normal metabolic function required for normal sleep. It is also possible they are completely independent while overlapping in circuitry.

Reduced GABAergic Synaptic Transmission During Lethargus, a *C. elegans* Sleep-Like State

Nooreen S. Dabbish and David M. Raizen

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

Introduction: Lethargus is a behaviorally-quiescent period during *C. elegans* development characterized by the sleep-like properties of reversibility, increased arousal threshold, and homeostasis [Raizen et al 2008]. Interested in studying the mechanisms of synaptic alterations during sleep, we used pharmacological and optogenetic approaches to study synaptic transmission at the neuromuscular junction (NMJ) during lethargus.

Methods: Muscle tone is controlled by a balance of excitatory acetylcholine (Ach) and inhibitory GABA. We used the acetylcholinesterase inhibitor aldicarb to assay synaptic transmission at the NMJ. Aldicarb causes the accumulation of Ach in the synaptic cleft, leading to a hypercontractive paralysis. Increased Ach transmission or decreased GABA transmission accelerates this paralysis. We assayed worms on 1 mM aldicarb, prodding them three times every fifteen minutes to check for paralysis. Optogenetic experiments were performed using strains expressing Channelrhodopsin in either GABAergic or Cholinergic neurons. L4 larvae cultivated on agar containing all Trans Retinal were transferred singly to an unseeded NGM plate and imaged on a fluorescent microscope. $GABA_A$ agonist experiments used 10mg/ml sodium oxybate for 15 min and 0.5 mM muscimol for 30 min. On GABA agonists, worms display a “rubberband phenotype” in which they contract and relax without displacement. The severity of the rubberband phenotype in response to anterior touch was assayed using a 0-5 scale.

Results: Worms in lethargus demonstrate hyper-sensitivity to aldicarb. During adult satiety, another quiescent behavioral state, worms were also aldicarb-hypersensitive, demonstrating another similarity between lethargus quiescence and adult quiescence. The aldicarb hypersensitive *unc-25* mutants, which lack GABA, demonstrate a wild-type time-course to paralysis during L4 lethargus. This suggests that the mechanism of hypersensitivity involves reduced GABAergic neurotransmission. In support of this mechanism, we found a reduced behavioral response (body elongation) to optogenetic stimulation of GABAergic motor neurons during lethargus. We observed that worms in lethargus are resistant to both muscimol and sodium oxybate, two GABA agonists, suggesting that a post-synaptic mechanism is responsible for the decreased GABAergic signaling.

Conclusion: Our observations demonstrate a surprising decrease in GABAergic signaling during lethargus. The resistance to $GABA_A$ agonists observed during lethargus supports a post-synaptic mechanism. Several explanations may account for this phenomenon. There could be high GABAergic signaling during early lethargus, which leads to a homeostatic down-regulation of the GABA receptor [Bruce Bamber, unpublished result]. Alternatively, the reduced synaptic transmission may reflect a developmental process. There is extensive remodeling of the GABAergic motor neurons during the first larval stage, and post-synaptic silencing during L1 lethargus may function in this process. Reduced GABAergic signaling during subsequent lethargus periods may be a reiteration of the L1 stage. We are excited to further characterize the mechanism of decreased GABAergic synaptic transmission during lethargus and to explore these and other possible explanations.

Abrupt Loss of Consciousness in a Commercial Trucker: A case report

Joseph T. Daley, MD, PhD

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

A 58 year old man employed as long distance truck driver with PMH of hypertension, Type 2 diabetes, coronary artery disease s/p angioplasty, and obesity was referred for a sleep evaluation following an episode while on the job.

He describes awakening from sleep at the end of his typical 10-hour mandatory rest, and pulling out of his parking space. Shortly thereafter, without any aura or prodrome, he lost consciousness. The next thing he recalls is his truck facing directly into the side of another parked truck in the rest stop following a collision. There was no post event confusion. He denied any symptoms of hypoglycemia before or after the event.

The patient lost his employment, and was referred for a sleep and cardiac work-up by his primary doctor. His cardiac evaluation to date (EKG, echocardiogram) did not reveal any abnormalities.

He reports a highly irregular sleep schedule due to his job. He also had experienced infrequent unintended sleep episodes when inactive, but never while driving. He endorsed loud snoring every night for many years, with infrequent witnessed apneas. His Epworth sleepiness scale was 15/24.

Review of symptoms was positive for 30 pound weight gain in last 2 years.

Social history revealed current 1.5ppd smoker, a rare, single alcoholic beverage (<1/week), and caffeine intake of 2-3 20oz mugs of coffee daily and 2L diet Mountain Dew daily.

His pertinent physical exam findings were:
Height: 6'1"; Weight 295 lbs; BMI: 39
E/N/M/T: Neck circumference 20"
Lateral and anteroposterior narrowing of the oropharynx
Swollen erythematous uvula
Modified Mallampati Class III oral airway

An ambulatory polysomnogram was arranged within 2 weeks, which demonstrated an apnea-hypopnea index (AHI) of 68 (all sleep was prone). His oxyhemoglobin saturation nadir was 66%, and 53% of TST was spent with oxyhemoglobin saturation <90%.

Following this study, the patient underwent a home based auto-titrating positive airway pressure, which identified a setting of 11 cm of water as an effective pressure.

At his first follow up visit, compliance data showed usage on 21/23 days, for an average of 5:18 min on days used. His average residual AHI was 3.5.

Discussion: Drowsy driving is an important and often underestimated public health issue. Because of their long, monotonous hours on the road, shift work, insufficient sleep and other factors, commercial drivers are at an increased risk. In addition, given their demographics, sleep apnea likely plays an important role for many individuals. Guidelines for screening, treatment, and re-certification have been developed, but have yet to be adopted formally by regulatory agencies. The fact that they frequently transport hazardous cargo, or many passengers in the case of bus drivers, indicates a higher need for scrutiny than in non-commercial drivers and emphasizes the need for awareness of this issue among transportation screeners, primary care physicians, and truck drivers themselves.

The Control of Lipid Storage By the Circadian Clock and Age in *Drosophila melanogaster*

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The circadian clock drives a number of processes in a rhythmic fashion including locomotor activity and sleep-wake cycles. Additionally, a number of metabolic processes such as feeding and insulin secretion and sensitivity are recognized to be under circadian control, specifically by peripheral clocks. Recently, we identified a feeding rhythm that is regulated by a peripheral clock in the fat body of *Drosophila*. To determine which physiological processes in addition to feeding are controlled by the *Drosophila* fat body clock, microarray analysis was performed on RNA isolated from the adult fat body. This analysis revealed a number of cycling genes including those involved in metabolism, detoxification and reproduction. Since lipid metabolic genes were over-represented in the fat body cycling genes, we hypothesized that the circadian clock controls lipid levels. While fat body triglycerides do not cycle and are unchanged in animals that lack clocks in all tissues, flies with an ablated fat body clock have decreased lipid levels and those without an intact central clock have increased triglycerides. Interestingly, fat body triglycerides are also elevated in flies expressing the Alzheimer's disease-causing amyloid protein Aβ both pan-neuronally and specifically in the clock neurons. Together, these data indicate that lipid storage is controlled by the interaction between the fly's central and fat body clocks and suggest a potential role of the circadian system in the development of metabolic disorders associated with age.

The Effects of Cognitive Workload During Sleep Restriction on Polysomnographic Measures

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Introduction: Sleep physiology is believed to reflect homeostatic sleep drive and circadian dynamics, but little systematic research has been conducted on whether the intensity of waking activity influences sleep. We investigated the effects of high cognitive workload (HW) versus low cognitive workload (LW) on polysomnographic (PSG) measures following both sleep restriction (SR) and no sleep restriction (NSR).

Methods: N=25 healthy adults (31.5±8.9y;10f) completed 3 baseline (8h TIB/night) nights, followed by 5 SR (4h TIB/night) or NSR (8h TIB/night) nights in a laboratory setting. Subjects were randomized to 1 of 4 experimental conditions: HW+SR (N=6); HW+NSR (N=3); LW+SR (N=8); LW+NSR (N=8). The HW vs. LW conditions differed in cognitive testing intensity and duration. Standard PSG sleep measures were collected on the 3rd night of baseline (B3) and the 5th night of SR (SR5) or NSR (NSR5). Sleep records were visually scored using standard scoring criteria by an experimentally-blind trained scorer. One-way and mixed-model (night×condition) ANOVAs compared differences across all 4 conditions; paired t-tests examined changes from B3 to SR5/NSR5 for each group.

Results: There were no significant differences in B3 PSG measures across the 4 conditions. The HW+NSR and LW+NSR groups showed significant increases in Stage 3 %TST ($p \leq .04$) from B3 to NSR5, with significantly larger increases in the HW group ($p=0.002$). By contrast, the HW+SR and LW+SR groups showed significant—but not differential—increases in SE and SWS%TST, and decreases in TST, SOL, WASO, Stage 1 and 2%TST, and REM duration from B3 to SR5 (all $p<0.05$).

Conclusion: The preliminary results from this experiment suggest that cognitive workload may influence non-REM sleep physiology, but more data will be required to confirm this finding. Sleep restriction had the expected robust effects on sleep physiology, and cognitive workload did not appear to affect the homeostatic sleep response to partial sleep restriction.

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Exploring the Role of REM Sleep in Ocular Dominance Plasticity Consolidation

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Introduction: Sleep is important for the consolidation of ocular dominance plasticity (ODP) in the cat primary visual cortex (V1) [1]. Activation of the Ca^{2+}-calmodulin dependent kinase (CaMKII) and extracellular signal-regulated kinase (ERK) cascades, as well as phosphorylation of the AMPA receptor subunit GluR1, occurs during sleep in remodeling V1 neurons and may underlie ODP consolidation [2]. However, the respective contributions of NREM and REM sleep to these processes are unknown. NREM and REM are characterized by distinct neuromodulatory tone and cortical activity patterns, raising the possibility that these states may play distinct roles in consolidation. In this study, we investigated the role of REM sleep in the consolidation of cortical plasticity.

Methods: Cats at the peak of the critical period of visual development (postnatal day 28-34) were used, and underwent polysomnography recording throughout the experiment. After a baseline sleep period, cats underwent monocular deprivation (MD) for 6 hours during waking to induce cortical remodeling. MD was followed either by 1 hour of normal sleep, 1 hour of REM sleep deprivation (RSD) or 1 hour of NREM fragmentation (NF). NF served as a control for the nonspecific effects (increased wake time, suppression of delta power, and decreased sleep continuity) of RSD. In the first set of experiments, immediately following these manipulations, V1 tissue was collected for Western blot analysis to quantify levels of total and phosphorylated GluR1, ERK1/2, and CaMKIIα/β. In the second set of experiments, optical imaging of intrinsic cortical signals and single unit recording were performed immediately following the sleep/wake manipulations to measure the magnitude of the ocular dominance shift in V1.

Results: RSD attenuated the shift in ocular dominance that occurs during uninterrupted sleep, indicating that REM sleep plays a role in cortical consolidation. Of the proteins examined by Western blot analysis, ERK1/2 phosphorylation levels showed the greatest change, with lower ERK1/2 phosphorylation in the visual cortex of RSD animals than sleeping or NREM-fragmented animals. This suggests that one mechanism underlying REM-dependent consolidation of ODP is the activation of the ERK cascade.

Conclusion: These results suggest that activation of the ERK cascade during REM sleep, possibly due to the unique neuromodulatory tone and cortical activity patterns that occur during REM, may consolidate ODP. However, previous studies using this model have implicated NREM, but not REM, sleep in cortical consolidation [1, 2]. One potential reason for this discrepancy is that the current study examined only the first hour of post-MD sleep, whereas the previous studies measured ODP after 6 hours of sleep. This raises the possibility that ODP consolidation relies on both early REM and late NREM during post-MD sleep. Together, these studies suggest that REM and NREM sleep play separate but complementary roles in ODP consolidation.

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Novel Automated Assay of Anesthetic Sensitivity in *Drosophila*

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Introduction: Our group has previously developed a high throughput behavioral assay to study anesthetic sensitivity in the *Drosophila* model system by measuring cessation of locomotor activity during the circadian mediated evening peak of locomotor activity. However, this assay has two constraints: experiments are a) limited to active circadian times and b) dependent on spontaneous locomotor activity. In order to free ourselves from these constraints, we developed a new assay to measure the response of flies to an automated shaking stimulus.

Methods: In order to test the effects of continued shaking on the normal circadian locomotor activity pattern, 4- to 7-day-old female flies from three wild-type strains (*Iso*31, *RC1*, and white eyed *RC1* or *WRR*) were randomly assigned to conditions of a) non-shaking or b) about fourteen hours of shaking at a rate of once per minute starting at ZT10.2 and ending at ZT0. One five-minute break after two hours of shaking was introduced to measure return to baseline movement in the absence of shaking. Movement was measured as beam-breaks in *Drosophila* activity monitors (DAMS, Trikinetics, MA) recorded in 10-second bins for up to 24 hours, while the 10-second bin in which shaking occurred was removed during analysis in order to eliminate beam breaks directly caused by flies being flung by the shaking. We then generated induction and emergence curves to Isoflurane during the circadian peak of activity under the following conditions: a) non-shaking with Isoflurane exposure, b) non-shaking without anesthetic, c) shaking with Isoflurane exposure, and d) shaking without anesthetic. We defined our anesthetic endpoint as cessation of movement for the 5 minutes following the administration of a given dosage of Isoflurane.

Results: All three strains maintained high levels of movement through the 14-hour period of shaking (which included periods of normal locomotor quiescence), while the movement of the non-shaken flies followed the expected circadian pattern. After all shaking ended, shaken flies showed less movement than non-shaken flies for about seven hours, which we interpret as a rebound effect. There were no significant changes in EC_{50} for induction in shaken flies with respect to non shaken flies. However, emergence occurred at a statistically higher dose of anesthetic for two genotypes (*Iso31* and *RC1*) and a trend in the same direction was observed for the third strain (WRR).

Conclusion: Shaking can induce a high level of locomotor activity in *Drosophila* for extended periods of time including normally quiescent circadian times. We did not detect an effect of shaking on induction from Isoflurane but did discover a facilitating effect on emergence. We hypothesize that shaking stimulates endogenous arousal circuits that are important for emergence and not induction, which is consistent with previous work from our group showing that induction and emergence are regulated by different neural circuits. However, based on our current experiments, we are confident that high levels of movement may be obtained in flies at quiesecent locomotor times and also that anesthesia can abolish locomotor activity due to shaking. Our new assay therefore enables a range of high-throughput automated behavioral assays of anesthetic sensitivity to operate free of the constraints of endogenous circadian influences and spontaneous locomotor behaviors.

Newborn with Hypercapnic Respiratory Failure: A Case of Congenital Central Hypoventilation Syndrome (CCHS)

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Introduction: CCHS is a rare condition that is characterized by abnormal autonomic control of breathing resulting in alveolar hypoventilation that is more marked during slow-wave sleep (NREM). There is no subjective or objective drive to breathe during sleep despite progressive hypercapnia and hypoxia. The severity of the disease may range from hypoventilation during sleep with adequate ventilation during wakefulness to severe hypoventilation during both sleep and wakefulness. Affected individuals require some form of assisted ventilation. I described the case of a new born with hypercapnic respiratory failure requiring intubation.

Case: this is a healthy 32 years old woman, with no pregnancy complications who presented to the Children Hospital of Philadelphia for a routine C-section on 6/16/2009. The newborn was noted to be hypotonic with poor color and weak cry. He received deep suctioning for clear fluid and blow-by O2 for 2 minutes, with improvement in color. Apgar were 8, 8 and9. The baby was transferred to the well being nursery and placed on 0.5 L via nasal canula (NC). Patient had desaturations to 80's with attempts to wean to room air (RA) and was admitted to NICU. On admission he was afebril with a heart rate of 145, blood pressure 67/42, respiratory rate 36 and oxyhemoglobin saturation 100% on 25%FiO2. He was extubated the same day to NC and weaned to RA. A pneumogram showed central apnea and periodic breathing. On 6/18 he was noted to have desaturations and apneic episodes requiring tactile stimulation. An arterial blood gas showed evidence of severe respiratory acidosis with a pH of 7.09, CO2 of 88, O2 of 64 and was reintubated. Head ultrasound and EEG were normal. An echocardiogram showed PDA with left to right shunt. A lumbar punction was normal. A sleep study showed central apneas with hypoventilation. The PCR for PHOX2B was positive.

Discussion: CCHS is an autosomal dominant disease with incomplete penetrance caused by a defect in the PHOX2B homeobox gen. There is a deficiency of automatic control of breathing mainly during quiet sleep (NREM) when breathing is controlled almost entirely by the automatic system. Therefore, ventilation is more severely affected during quiet sleep. Affected infants may immediately fail to breathe on delivery and require assisted ventilation from the time of birth. As their oxygen saturation fails and their CO2 levels rise, affected infants demonstrate no increase in RR or effort and usually do not arouse or appear distressed. Patients usually present in the first few months of life after episodes of severe apnea, or a respiratory arrest. Other patients presents with symptoms of cor pulmonale secondary unrecognized hypercapnia and hypoxia. Some infants present with frank apnea or respiratory arrest upon falling asleep. CCHs patients also manifest symptoms of autonomic dysfunction, difficulty feeding or breath holding spells. This disease represents a primary physiologic abnormality of integration of chemoreceptor input to central ventilator controllers, rather than abnormalities in the chemoreceptor themselves. Most children have no pathological lesion in the brainstem, on autopsy and MRI. However, they have decrease neuronal signal in areas including cerebellar vermis when challenged with hypercapnia and hypoxia on functional MRI when compared with controls. During infancy and early childhood, they require mechanical ventilation when sleeping. Within a month to a year, many infants will be able to maintain adequate ventilation while awake and ventilatory responses to hypercapnia or hypoxia are not regained in CCHS at any age. A relationship between the PHOX2B genotype and the need for continuous ventilatory dependence has been demonstrated.

SIRT1: Metabolic Regulation of Wakefulness

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Sleepiness and impaired alertness occur in numerous metabolic disturbances, aging, and neurodegenerative processes, where sleepiness is generally believed to be a direct consequence of activating sleep-promoting neurons. *We seek to begin to test a complimentary explanation: metabolic dyshomeostasis directly modulates wake neuron function.* Recently we found that sleep apnea oxygenation exposures and aging impose wake impairments and substantive injury on populations of wake neurons. Could there be a metabolically sensitive pathway necessary for both function and protection of wake neurons? Sirtuin 1 (SIRT1) is one of seven mammalian orthologs of the longevity-associated yeast silent information regulator 2 (Sir2). Of the seven mammalian orthologs, SIRT1 is closest to Sir 2 and is both a nuclear and cytoplasmic sirtuin orchestrating numerous, largely protective, transcriptional and translational processes. SIRT1 is a nicotinamide adenine dinucleotide $(NAD)^+$ dependent deacetylase for select histone and nonhistone proteins. As such, SIRT1 activity is highly dependent upon local metabolic and redox status. Mild oxidative challenges activate SIRT1; more severe oxidative stressors suppress its activity. SIRT1 activation of peroxisome proliferator activated receptor γ (PPARγ) coactivator 1 (PGC1)α triggers an armament of mitochondrial biogenesis, optimization of mitochondrial function and anti-oxidant defense. At the same time SIRT1 deacetylates other key players in energy homeostasis FOXO1, FOXO3 and STAT3, and inactivates proinflammatory mediator NFκ-β. SIRT1 regulates circadian function in peripheral cells at multiple levels. To begin to understand whether SIRT1 contributes to wakefulness, we first studied sleep/wake activity in mice with transgenic knockout or knock down of SIRT1. SIRT1-/- and -/+ mice showed marked impairments in wakefulness (25% reductions in wake time for both lights on and lights off. Diurnal rhythm was reduced. Wake bout lengths were unchanged and REM sleep was increased, suggesting that the wake impairments were in part a consequence of monominergic wake neuron dysfuction. To distinguish between a developmental role for SIRT1 in wakefulness vs. an adult acquired wake impairment, we created conditional brain SIRT1 knock down mice using SIRT1 lox P mice with brainstem injections of AAV 2.9 CMV cre-recombinase. After three weeks the vector was effective in knocking down >50% of th eSIRT1 imunoreactivity in bran regions of wake active neurons. SIRT1 floxed mice relative to wild type mice injected with the same virus showed 20±4% reductions in total wake time, and in lights on and lights off periods. There were no shortened sleep/wake bouts or REM sleep entries to suggest orexinergic injury. Reduced wake time after a novel social encounter and the total reductions in wake time are most consistent with injury to the catecholaminergic neurons.

This work demonstrates a clear role for SIRT1 in the regulation of wakefulness and suggests a critical role for SIRT1 in the function of catecholaminergic wake neurons. It is anticipated that aging and other conditions involving metabolic dyshomeostasis will result in similar wake impairments.

The *COMT* Val158Met Polymorphism Predicts Interindividual Differences in Sleep Homeostatic Responses to Chronic Partial Sleep Deprivation

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Introduction: The *COMT* Val158Met polymorphism modulates cortical dopaminergic catabolism and has been reported to predict prefrontal cortex functioning and cognitive performance in healthy subjects. This polymorphism has also been associated with interindividual differences in brain alpha oscillations during total sleep deprivation and with modulating the effects of modafinil on waking functions, but not on sleep-deprivation-induced changes in recovery sleep. We determined whether the *COMT* Val158Met polymorphism related to sleep homeostatic responses and cumulative neurobehavioral deficits during chronic partial sleep deprivation (PSD).

Methods: 20 *MetMet*, 64 *ValMet*, and 45 *ValVal* healthy adults (29.9±6.9y; 63 females) completed 2 baseline (10h TIB/night) nights, followed by 5 consecutive PSD (4h TIB/night) nights in a controlled laboratory experiment assessing physiological sleep responses and neurobehavioral measures (cognitive performance and executive function tests, subjective sleepiness and fatigue, MWT). Comparisons were made across the 3 genotypes. In our sample, *MetMet* genotypic and *M* allelic frequencies were significantly higher in Caucasians than African Americans; reported results were significant after statistically controlling for ethnicity.

Results: *MetMet* subjects had significantly larger declines in SWE during chronic PSD. *ValVal* subjects had significantly shorter REM latency at baseline and more stage 1 sleep across baseline and chronic PSD. The genotypes did not differ significantly at baseline in demographic characteristics, habitual sleep, circadian phase, cognitive performance, or physiological or subjective sleepiness. All genotypes demonstrated comparable cumulative decreases in cognitive performance (PVT, Digit Span), and increases in subjective and physiological sleepiness (KSS, MWT) to PSD, with increasing daily inter-subject variability. The genotypes did not differ on executive function tasks (Hayling, COWAT).

Conclusion: The *COMT* Val158Met polymorphism related to individual differences in sleep homeostatic, but not neurobehavioral, responses to chronic PSD. Thus, the *COMT* Val158Met polymorphism may be a novel genetic marker for predicting such differential sleep responses resulting from sleep deprivation.

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*DQB1*0602* Allele Predicts Interindividual Differences in Physiological Sleep Structure, Sleepiness and Fatigue During Baseline and Chronic Partial Sleep Deprivation

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Introduction: The human leukocyte antigen *DQB1*0602* allele is closely associated with narcolepsy, a neurological disorder characterized by excessive daytime sleepiness, fragmented sleep, and shortened REM latency. We evaluated whether *DQB1*0602* was a novel marker of inter-individual differences by determining its relationship to sleep homeostatic, sleepiness and cognitive responses to baseline and chronic partial sleep deprivation (PSD) conditions.

Methods: 37 *DQB1*0602* positive and 92 *DQB1*0602* negative healthy adults (29.9±6.9y;63f) completed 2 baseline (10h TIB/night) nights, followed by 5 consecutive PSD (4h TIB/night) nights in a controlled laboratory experiment assessing physiological sleep responses and neurobehavioral measures (cognitive performance and executive function tests, subjective sleepiness and fatigue, MWT). Comparisons were made between *DQB1*0602* groups. *DQB1*0602* allelic frequencies did not differ significantly between Caucasians and African Americans.

Results: During baseline, although *DQB1*0602* positive subjects were significantly sleepier and more fatigued by self report (KSS, VAS, POMS), they showed greater sleep fragmentation, and decreased sleep homeostatic pressure and differentially sharper declines during the night (measured physiologically by NREM EEG slow-wave energy [SWE]). During PSD, despite SWE elevation comparable to *DQB1*0602* negative subjects, *DQB1*0602* positive subjects were sleepier and showed more fragmented sleep. Moreover, they showed differentially greater reductions in REM latency and smaller reductions in stage 2 sleep, along with differentially greater increases in fatigue. Both groups demonstrated comparable cumulative decreases in cognitive performance (PVT, Digit Span) and increases in physiological sleepiness (MWT) to PSD, and did not differ on executive function tasks (Hayling, COWAT).

Conclusion: *DQB1*0602* positivity in a healthy population may represent a continuum of some sleep-wake features of narcolepsy. *DQB1*0602* was associated with inter-individual differences in sleep homeostasis, physiological sleep, sleepiness and fatigue—but not in cognitive measures—during baseline and chronic PSD. Thus, *DQB1*0602* may represent a genetic biomarker for predicting such individual differences in both basal and sleep loss conditions.

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Sleep Complaints and Fatigue Decline Across the Lifespan: Getting Older Does Not Necessarily Mean Poor Subjective Sleep and Daytime Fatigue

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Introduction: It is generally believed that sleep deteriorates with age. This concept is supported by a variety of studies that show increased prevalence of insomnia in older cohorts. To further explore the age by insomnia interaction, the occurrence of sleep initiation and maintenance problems and daytime fatigue complaints were evaluated in an existing, large scale database known as the Behavioral Risk Factor Surveillance System (BRFSS).

Methods: Sleep Initiation and Maintenance Problems (SIMP) and Daytime Fatigue Complaints (DFC) were explored in 159,856 adult (age=18-99+yrs) participants of the BRFSS. SIMP was measured with, “Over the last 2 weeks, how many days have you had difficulty falling asleep or staying asleep or sleeping too much?” DFC was measured with, “Over the last 2 weeks, how many days have you felt tired or had little energy?” Responses were dichotomized, ≥6 days indicating a “complaint.” Outcome variables were age, general health, and depression. All analyses adjusted for race/ethnicity, income, education and healthcare access.

Results: Across all age groups, women reported more SIMP and DFC. Further, decreasing general health and mild and severe depression were associated with SIMP and DFC. In contrast, both SIMP and DFC steadily declined across the lifespan, with fewest endorsements in the oldest respondents (80+yrs). For SIMP, ORs (reference=80+) declined from 18-54yrs, rose slightly then declined again after 59yrs in men. The pattern was similar for women, except a more marked rise was noted, from 40-59yrs. The pattern was similar for DFC.

Conclusions: Despite the confirmatory findings that sex, health, and depression predict SIMP and DFC, age was not found to be associated with the reported frequency of sleep continuity disturbance or daytime fatigue complaints. These results suggest that the age by insomnia interaction is mediated by factors other than those associated with physiologic aging.

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Sleep-Related Attitudes, Beliefs and Practices in Black and White Adults

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Introduction: There have been very few explorations of how social and cultural factors influence sleep, especially with respect to culturally-determined sleep-related beliefs and practices.

Methods: Forty-one Black/African-American (BAA) and 32 White/European-American(WEA) completed a questionnaire on beliefs/attitudes/practices regarding sleep. Groups were compared using Mann-Whitney U.

Results: Groups were equivalent for age (M=65(WEA);72(BAA)), gender (92%F WEA; 88%F BAA), and income. Groups differed for education, with more college-educated WEA (72%WEA; 17%BAA). For complaints, BAA reported more snoring (p=.002). There were no differences in obtained TST or perceived sleep need, but the difference ([need]-[obtained]) was significant(p=.004). Only 3.1% of WEA reported needing more sleep than they received, while 29.2% of BAA reported needing more. BAA were more likely to read/watch TV(p=.0003), drink alcohol(p=.004), or start their day(p=.0007) if they could not sleep. In bed, BAA were more likely to read/watch TV(p=.000007), eat/drink(p=.0003), worry/think(p=.0002), and argue/be angry(p=.006). Fewer BAA report being motivated to make time for sleep (p=.0005). Trends for sleepiness being due to laziness and bad habits (p=.04) or boredom(p=.02) were found in BAA. BAA were more likely to believe that lying in bed with eyes shut was as good as sleeping (p=.004). WEA were more likely to believe that sleep is related to health (p=.005) and ability to enjoy daytime(p=.004). BAA were less likely to “Strongly Agree” that dozing while driving a vehicle is serious (p=.00005). When asked if sleep is related to health problems such as obesity, blood pressure, diabetes, and depression, Most WEA(71.8%) and BAA(85.4%) were “Unsure” or disagreed(p=.52). No differences for exposure to sleep-related information were found (p=.41), and both groups reported that they did not discuss sleep problems with their doctor (p=.81) and their doctor did not discuss sleep with them (p=.29).

Conclusion: Important cultural differences in knowledge and perception were found. These findings have important public health implications in terms of developing effective sleep education interventions that include consideration of cultural factors.

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Sleep Disturbance in Children and Adolescents with ADHD: Unique Effects of Stimulant Medication and ADHD Subtype

Jocelyn R. Helwig, Lisa J. Meltzer, Jodi A. Mindell, George J. DuPaul, Thomas J. Power

ADHD is among the most common childhood psychiatric disorders, estimated to affect approximately 3% to 5% of elementary school-aged children, with 11% to 37% reporting sleep disturbance (American Psychiatric Association, 2000; Owens, Spirito, McGuinn, & Nobile, 2000). Disturbed sleep can result in daytime sleepiness and behavioral difficulties that affect cognitive functions in children, such as attention and memory, as well as exacerbate symptoms of ADHD (Fallone, Owens, & Deane, 1998; Owens, 2005). The primary aim of this study was to determine the prevalence of ICD-9 sleep disorders as diagnosed by pediatric primary care providers in children and adolescents with ADHD across medication status and ADHD subtype.

Electronic medical records were reviewed for 5916 patients (6-18 years) diagnosed with ADHD and seen for a well-child visit in 2007. Information was collected on ICD-9 sleep diagnoses, demographic variables (age, sex, race), ICD-9 ADHD subtype (ADHD with and without hyperactivity), and stimulant medications commonly used to treat ADHD.

Across all ages, 7.3% of patients had an ICD-9 diagnosis for a sleep disorder. The most common diagnoses were nocturnal enuresis (3.2%), sleep disorder NOS (2.5%), and sleep disordered breathing (1.5%). Predictors of sleep disorders included age, ADHD subtype, and race. Specifically, children ages 6-12 years were more likely to have a sleep diagnosis than adolescents ages 13-18; Children with ADHD without hyperactivity were more likely than children with ADHD with hyperactivity to have a sleep diagnosis; And Black children were more likely than White children to have a sleep diagnosis.

The 7.3% of patients given a ICD-9 sleep diagnosis is lower than prevalence rates reported in other epidemiological studies, suggesting that primary care providers may be under-diagnosing sleep disorders in this population of children and adolescents. These results suggest the need for additional education and support in the diagnosis of sleep disorders in children and adolescents with ADHD.

Upper Airway Collapsibility and Genioglossus Activity in Children with Sickle Cell Disease

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Both anatomical and neuromuscular factors contribute to the pathogenesis of obstructive sleep apnea (OSA) in children. Adenotonsillar hypertrophy is thought to be a major contributor to OSAS in patients with sickle cell disease (SCD). However, the effect of neuromuscular factors has not been investigated. We hypothesize that SCD patients with OSAS have a blunted neuromuscular response to subatmospheric pressure loads during sleep, making them more likely to develop upper airway collapse.

Subjects with SCD with and without OSA underwent pressure-flow measurements during sleep using intraoral surface electrodes to measure genioglossal EMG (EMGgg). Two techniques were applied to decrease the nasal pressure to subatmospheric levels, resulting in an activated and relatively hypotonic upper airway. The area under the curve of the inspiratory EMGgg moving time average was analyzed. EMGgg activity was presented as a percentage of baseline. Changes in EMGgg in response to decrements in nasal pressure were presented as the slope of the EMGgg vs. nasal pressure (slope of EMGgg-P_N).

18 SCD-controls and 4 SCD-OSA were studied. For SCD-controls, median (range); 95% confidence intervals of the slopes of EMGgg- P_N were -11.6 (-27.9 to -1.9); -18.5 to -8.1 %*cm H_2O^{-1} using the activated technique versus -1.9 (-11.3 to 1.5); -6.1 to -1.4 %*cm H_2O^{-1} using the hypotonic technique (p=0.03). For SCD-OSA, slopes of the EMGgg using the two techniques were -3.3 (-2.6 to 3.5); -9.6 to 4.5 versus -3.8 (-4.0 to 3.3); -7.8 to 3.6 %*cm H_2O^{-1}, respectively (p=NS). Activated PN-EMGgg was greater in SCD-controls than SCD-OSA (p = 0.01), but hypotonic PN-EMGgg was similar (p=0.47).

SCD-OSA have decreased genioglossal muscle activity in response to subatmospheric pressure loads compared to SCD controls. We speculate that neurologic impairment, possibly related to vaso-occlusive episodes, may affect upper airway activation during sleep, contributing to OSAS in this population.

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SDB and Daytime Napping are Associated with Higher Glucose Levels in Pregnant Women

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Introduction: During pregnancy, the majority of women experience sleep disturbances including sleep-disordered breathing (SDB) and day-napping. SDB may be associated with gestational diabetes (GDM), and longer day-napping in older adults was found to be associated with a higher risk of diabetes. However the health consequences of both SDB and day-napping during pregnancy are poorly understood.

Methods: A group of 104 pregnant women who underwent a full polysomnography in the first trimester and a glucose challenge test (GCT) at any point during pregnancy, and completed Multivariable Apnea Prediction (MAP) questionnaire, Pittsburgh Sleep Quality Index (PSQI) and a demographic questionnaire were studied. Bivariate and multivariable linear regression analyses were performed to determine the clinical characteristics that were significantly associated with higher GCT results. Using GCT values as the dependent variable, sleep and demographic variables having a P<0.2 in a bivariate analysis were reevaluated in full linear regression models as independent variables.

Results: In bivariate linear regression analysis, variables with a P<0.2 were first-trimester BMI (kg/m2) (β=0.51, p=0.12), first-trimester neck circumference (β =1.89, p=0.02), parity (β=8.14, p=0.09), first-trimester AHI≥15 (β=49.91, p=0.004), habitual snoring (β=19.27, p=0.01), second-trimester nap duration (β=0.07, p=0.02), steady night-shift work (β=-13.09, p=0.12), self-reported nocturnal sleep duration (β=-0.04, p=0.10), PSQI-sleep duration (β=4.25, p=0.08), PSQI-sleep efficiency (β=2.74, p=0.14) and global PSQI score (β=0.88, p=0.18). In the final multivariable model, first-trimester AHI≥15 (β=38.88, p=0.02) and second-trimester nap duration (β=0.06, p=0.03) were significantly associated with increasing GCT values after adjustment for BMI, parity, steady night-shift work and nocturnal sleep duration.

Conclusion: SDB and longer daytime nap duration are associated with higher GCT values. However, we did not find such an association between nocturnal sleep duration and higher GCT levels. SDB and longer daytime naps which disturb the sleep-wake pattern may increase sympathetic activation. These events may impair glucose tolerance. Since even minor degrees of increased glucose intolerance are associated with adverse perinatal outcomes, further research is needed to determine whether pregnant women with SDB and longer day-napping are at risk for GDM and other adverse perinatal outcomes.

Young Children's Sleep Practices in Pediatric Primary Care

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Introduction: Sleep problems are highly prevalent in young children; however little research has examined the documentation of early childhood sleep practices in the pediatric setting. The purpose of this study was to examine documented sleep practices during well-child visits in a large primary care network.

Methods**:** A chart review was conducted of well-child visit for all children less than 5 years of age seen across 32 pediatric practices affiliated with the Children's Hospital of Philadelphia in 2007. The sample included 22,427 infants (0-1 years), 30,208 toddlers (1-3 years) and 21,661 preschoolers (4-5 years); 51% were male, 54% Caucasian, and 27% African American.

Results: Seven percent of infants had documentation of a specific sleep practice. The most commonly documented sleep hygiene practices for all infants were feeding to sleep (2.7%) and same bed co-sleeping (1.8%). For infants 3-month olds and older, night wakings (5.3%) were the most common. In toddlers, problematic night wakings (5.9%) and bedtime resistance (0.5%) were the most common behaviorally based sleep problems, while 2.5% of toddlers were noted to co-sleep (same bed). Preschoolers were more likely to have documented "sleeping through the night" (20%) than night wakings (3.3%) or bedtime resistance (0.2%). Two percent of preschool-aged children were noted to same bed co-sleep with a caregiver, while 3% were noted to sleep in their own bed.

The Effects of Cognitive Workload During Sleep Restriction on the 10-minute PVT

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Introduction: Although sleep loss degrades cognitive functions, there are little theoretical or experimental data addressing whether waking cognitive activity potentiates the effects of sleep loss. We investigated the effects of high cognitive workload (HW) versus low cognitive workload (LW) on the well-validated 10 minute Psychomotor Vigilance Task (PVT) during both sleep restriction (SR) and no sleep restriction (NSR).

Methods: N=29 healthy adults (33.7±9.3y; 13 females) completed 3 baseline (8h TIB/night) nights, followed by 5 SR (4h TIB/night) or NSR (8h TIB/night) nights in a laboratory setting. Subjects were randomized to 1 of 4 experimental conditions: HW+SR (N=11); HW+NSR (N=7); LW+SR (N=7); LW+NSR (N=4). The HW vs. LW conditions differed in cognitive testing intensity and duration. The PVT was administered 6 times each protocol day during diurnal waking hours. Baseline values were derived from the third baseline day (B3). Daily values for PVT lapses (>500ms RT) were calculated by averaging scores from all test bouts that day. A mixed-model ANOVA (day:within-subjects factor; sleep and workload:between-group factors) was conducted; paired t-tests examined differences from B3 to SR5/NSR5 for each group.

Results: PVT lapses for the 4 groups did not differ at B3 ($p=0.273$). There was a significant main effect of day on PVT lapses ($p=0.019$) and a significant interaction with sleep (SR vs. NSR) for lapses ($p=0.017$), whereby the SR groups showed significantly more lapses than the NSR groups ($p=0.02$). By contrast, the interaction with workload was not significant for lapses ($p=0.799$). The HW+SR group showed the most dramatic increases in PVT lapses ($p=0.018$) from B3 to SR5.

Conclusion: The preliminary results from this experiment suggest that sleep restriction had the expected effects on PVT lapse rates. However, higher cognitive workload under sleep-restricted conditions may further potentiate deficits in PVT performance.

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Trainee Award Eligible

Validation of Single-Item Sleep Measures from Epidemiological Studies

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Introduction: Many epidemiological studies demonstrate negative health outcomes associated with self-reported short sleep duration, insomnia, and poor sleep quality. However, these studies typically use single-item measures of the sleep parameter of interest. Relatively little is known about whether these questions reflect responses to validated measures of sleep and daytime fatigue.

Methods: The current study examined the criterion validity of 7 questions, taken from 3 epidemiological surveys: the Behavioral Risk Factor Survaillance System (Sleep Complaints, Daytime Fatigue), Philadelphia Health Management Corporation (Sleep Duration, Sleep Quality, Sleep Debt), and the Cancer Prevention Study II (Sleep Duration, Insomnia). Our approach included the following: (1) correlations of single-item measures among each other, (2) correlations of single-item measures to previously-validated scales (Pittsburgh Sleep Quality Index (PSQI) and Fatigue Severity Scale (FSS)), and (3) a receiver-operator curve analysis of days of Insomnia complaint and clinical threshold values on standardized instruments.

Results: Many of the 7 items correlated moderately with each other. Neither Sleep Duration item correlated with Insomnia or Sleep Quality, which did not correlate highly with each other. The two (differently-worded) Sleep Duration questions were not collinear. Single-item measures had moderate correlations with PSQI and only sleep debt was significantly correlated with FSS. Questions that asked about insomnia symptoms (Sleep Complaints, Insomnia) tended to have moderate correlations (r=0.35-0.38) with the PSQI while the item for Sleep Quality and those for Sleep Duration had borderline large correlations (r=0.50-0.52). A threshold of 4 nights of insomnia per month had the optimal test characteristics as a threshold for potentially identifying poor sleep quality with associated daytime fatigue as measured using clinical cutoffs on the PSQI and FSS.

Conclusions: These results indicate that the single item measures commonly used to assess several sleep parameters are related to well-validated multi-component measures of similar constructs, but are not necessarily interchangeable. The finding that sleep characteristics (duration and quality) correlated better with the PSQI than sleep complaints or insomnia may be that “insomnia” is perceived as a disease state, and thus was less likely to be endorsed than more general questions. This is in line with other research that has demonstrated that sleep quality and insomnia are separate constructs. We also noted that of the various measures of sleep, sleep debt had the strongest link to daytime fatigue, suggesting that sleep debt may be more associated with daytime fatigue than insomnia or sleep quality.

Upper Airway Soft Tissue Differences in Apneic Adolescent Children Compared to BMI-Matched Controls

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Introduction: Subjects with the obstructive sleep apnea syndrome (OSAS) have been shown to have upper airway narrowing. In young children this upper airway narrowing is due to adenotonsillar hypertrophy, whereas in adults it is primarily due to increased upper airway soft tissues. However, the upper airway anatomic risk factors in the adolescent population are largely unknown. We hypothesized that the upper airway soft tissue structures would be larger in the obese adolescents with OSAS compared to age and body mass index (BMI) matched controls.
Methods: Obese adolescents with OSAS aged 12-16 years were compared to age and BMI-matched obese controls and age-matched thin controls. Subjects underwent upper airway magnetic resonance imaging (MRI) with a 3T Siemens Vision System. Amira 4.1.2 software suite was used in the reconstruction and volumetric analysis of the upper airway images. Data were compared in the three groups using one way ANOVA and then post hoc pairwise comparisons between groups were performed.
Results: 10 obese apneics (mean BMI 37.9 ± 9.2 [SD] kg/m2, BMI Z-score 2.40 ± 0.31, AHI 14.0 ± 12.5/hr) were compared to thin controls (14 subjects, BMI 20.7 ± 2.9 kg/m2, BMI Z-score 0.19 ± 1.25, AHI 0.21 ± 0.37/hr) and obese controls (16 subjects, BMI 37.0 ± 6.7 kg/m2, BMI Z-score 2.32 ± 0.39, AHI 0.42 ± 0.42/hr). Significant difference between the three groups were found in the volumes of the genioglossus, total tongue, retroglossal lateral walls with and without tonsils, parapharyngeal fat pads, adenoids, and total soft tissue ($p < 0.05$). Post hoc pairwise comparisons showed that these significant group differences were primarily related to variance between the thin control and obese OSAS groups in the volumes of the genioglossus, total tongue, parapharyngeal fat pads, adenoids, and total soft tissue but not between the obese controls and obese apneics. However, the retroglossal lateral walls with tonsils ($p = 0.008$) and without tonsils ($p = 0.021$) were shown to be significantly larger in the obese OSAS group compared to the obese control group.

Table 1. Upper Airway Soft Tissue Volumes

Structure	Thin Controls		Obese Controls		Obese OSAS		*p*-value Between Groups
	Mean (mm3)	Std. Dev.	Mean (mm3)	Std. Dev.	Mean (mm3)	Std. Dev.	
Soft Palate	7331.6	1818.3	9184.6	2739.2	7866.3	791.3	0.107
Genioglossus	60540.0	13495.2	72545.3	16065.2	77783.4	13724.6	0.017*
Total Tongue	85518.4	21128.9	100063.2	19647.4	108120.0	18148.7	0.024*
Retropalatal (RP) Lateral Walls	11447.7	2401.7	13859.5	3983.7	13051.9	4708.3	0.217
Retroglossal (RG) Lateral Walls	7197.5	3208.3	8134.3	3671.5	12416.3	2801.9	0.001*
Parapharyngeal Fat Pads	3724.5	1392.2	5435.4	2054.4	6464.5	2250.4	0.004*
Tonsils	7006.1	2576.9	6564.1	2552.8	6696.1	2844.1	0.898
Adenoids	2448.9	1449.4	3604.5	1980.7	4697.6	1190.5	0.007*
Total Soft Tissue	125647.6	24284.6	147466.4	25964.4	160748.0	25896.8	0.006*
RP Lateral Walls Minus Tonsils	4441.6	2084.3	7295.3	3110.0	6355.7	4633.1	0.067
RG Lateral Walls Minus Tonsils	191.4	3170.9	1570.2	4059.6	5720.2	4136.6	0.002*

Conclusion: Adolescents with OSAS have increased upper airway retroglossal lateral walls compared to BMI-matched controls. This suggests that the upper airway structure in adolescents with OSAS is similar to that of adults with OSAS, and that adenotonsillar hypertrophy does not play a major etiologic role in this age group.
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Association of Aleep Apnea with Allergic Disease and Related Symptoms

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Introduction: There is increasing evidence of a bidirectional association between sleep-disordered breathing (SDB) and allergic diseases. However, evidence documenting the association of various allergic diseases and related symptoms with SDB in adults is limited. Therefore, the aims of this study were to investigate; 1) whether SDB was related to a history of and/or current allergic diseases and 2) if SDB was associated with allergic symptoms.

Methods: We included 4,577 adults (aged ≥20 years) from the National Health and Nutrition Examination Survey database 2005-2006. The frequency of physician-diagnosed asthma, hay fever, eczema, and having allergies, and associated symptoms over the past 12 months were assessed by questionnaire. SDB included habitual snoring (HS: ≥5 nights per week) and having been diagnosed with sleep apnea (SA).

Results: Prevalences of HS and SA were 41.1% and 6.1% for men and 25.4% and 3.0% for women, respectively. After adjusting for possible covariates, SA was associated with approximately 2.0-fold increase in the odds of hay fever and eczema in men and 3.0- to 4.2-fold higher risks for asthma and allergy in women. HS had no independent relationship with any allergic diseases in either gender. SA was associated with episodes of asthma and hay fever and general symptoms related to allergic responses during the past 12 months in men only; HS was relate to sneezing/runny/blocked nose.

Conclusion: This study demonstrates an association between SA and allergic diseases, both having inflammatory mechanisms. However, HS, the milder end of SDB spectrum, has no association. There also appears to be a gender bias where symptom manifestation occurs in men with SA, but not in women. The gender difference as well as potential mechanistic links between SDB and allergic diseases needs further exploration.

Comparisons of CPAP Adherence and Its Effects on Functional Outcomes in Sleepy and Non-Sleepy Patients

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Background: Some recent papers suggest that continuous positive airway pressure (CPAP) treatment might not be used by patients with sleep apnea who do not manifest the primary symptoms, excessive daytime sleepiness (EDS). However, it is also unclear, given the limited evidence, whether there are differences in outcomes between those who are and are not excessively sleepy. The aims of this study were to investigate 1) if there was a difference in CPAP adherence between sleepy and non-sleepy patients with obstructive sleep apnea and 2) if the functional outcomes of CPAP adherence were the same in these two groups.

Methods: One hundred and seventy-six patients diagnosed with moderate to severe obstructive sleep apnea (apnea-hypopnea index (AHI) ≥15) were included in this study. Participants were classified as sleepy if their Epworth Sleepiness Scale (ESS) score was >10 and non-sleepy if their score was below this cut-off score. We defined good CPAP adherence as ≥ 4 h mean hours of CPAP use. To evaluate functional outcomes, patients underwent psychomotor vigilance task, Digit Symbol Substitution Test, and Probed Memory Recall Test at baseline and after three months of CPAP treatment. Other measures included the Functional Outcomes of Sleep Questionnaire (FOSQ) and Pittsburgh Sleep Quality Index.

Results: There were 134 sleepy and 42 non-sleepy patients. There was no difference in mean hours of CPAP use in sleepy and non-sleepy groups, after adjustment for baseline BMI and AHI. The difference in FOSQ between baseline and after 3 mo treatment was significantly ($p=0.0045$) higher in sleepy group (least squares means ± standard error: 3.8±0.3) than in non-sleepy group (1.5±0.6), even in patients with good adherence to CPAP treatment. However, none of other outcomes were different in sleepy and non-sleepy patients.

Conclusion: We showed that CPAP adherence and its effects on functional outcomes were not different in sleep apneics with and without symptoms of daytime sleepiness, except for FOSQ. This suggests that non-sleepy patients as well as sleepy patients should be under appropriate treatment for sleep apnea.

Identification of *dyschronic*, a Gene Required for Circadian Output in *Drosophila*

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Many aspects of our behavior and physiology are under circadian control. Our daily sleep:wake cycle is one prominent example. The molecular mechanisms underlying circadian oscillations of the core clock components and resetting of the clock by light have been investigated in detail. In contrast, how the clock output controls rhythmic behavior is not well understood. Through a forward-genetic screen for *Drosophila* circadian mutants, we have identified a novel clock gene, *dyschronic (dysc)*. Homozygous *dysc* mutants display arrhythmic locomotor activity in constant darkness, while heterozygous mutants exhibit wild-type circadian rhythmicity. Notably, the core clock protein PERIOD in central clock cells undergoes circadian cycling in *dysc* mutants, suggesting that the gene is involved in circadian output. *dysc* mutants bear a transposon insertion in an intron of an uncharacterized gene. Deficiencies that remove the *dysc* gene do not complement the arrhythmic phenotype, and precise excision of the transposon reverts the mutant phenotype. These data demonstrate that disruption of the *dysc* gene is responsible for the arrhythmic phenotype. The *dysc* gene contains multiple protein-protein interaction domains, suggesting that the gene product forms a scaffold for a protein complex. Ongoing experiments examine the molecular and genetic interactions between *dysc* and other circadian output pathway genes.

Behavioral and Molecular Characterization of Novel *Clock* Alleles

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The neurobiology, genetics and molecular basis of circadian rhythms is best understood in *Drosophila melanogaster*. These daily rhythms are brought about by a molecular feedback loop in which a master transcriptional activator, CLOCK (CLK), acts with its partner, CYC, to regulate the expression of canonical clock genes. However, most of our understanding of CLK function and regulation comes from one dominant negative (Clk^{Jrk}) and one semi-dominant allele (Clk^{ar}) in *Drosophila*. Here, we report a PBac insertion and a point mutation in *Clk* which correspond to a hypomorph, and an antimorph respectively. In addition, we isolated a putative null allele carrying a gross rearrangement of the *Clk* locus. The homozygous putative null mutants as well as the antimorphic allele of *Clk* are behaviorally arrhythmic and display almost undetectable levels of *per* transcript. However, in the heterozygous condition, the mutations exhibit period lengthening similar to that seen in Clk^{Jrk} heterozygotes. On the other hand, flies homozygous for the hypomorphic allele of *Clk* exhibit a ~ 25.5 hr period (~30% of these flies are arrhythmic) and dampened *per* and CLK cycling. Our preliminary experiments suggest that the long period phenotype of *Clk* heterozygotes can be partially rescued by pan-neuronal expression of PERIOD (PER), suggesting that PER is the major determinant of circadian period length in the Drosophila clock. *Clk* mutants also show deficits in their response to light. Like the original Clk^{Jrk} allele, the newly isolated *Clk* antimorph shows increased nocturnal activity in the presence of light:dark cycles. Furthermore, the hypomorphic mutants are highly sensitive to very dim intensity light, as shown by a larger phase shifts in response to brief pulses presented during the early and late night. CRY levels are high in all *Clk* mutants examined, and may contribute to these aberrant light responses.

Non-Inferiority of Functional Outcome in Ambulatory Management of Obstructive Sleep Apnea: Results of the VSATT Clinical Trial

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Rationale: Home portable monitor testing is increasingly being used to diagnose patients with obstructive sleep apnea (OSA) and initiate them on CPAP. Only a few studies have evaluated the functional outcome of this ambulatory management of OSA.

Methods: We conducted a 2 site randomized parallel groups study of the functional improvement on CPAP treatment in veterans with OSA randomized to either standard in-laboratory polysomnographic testing (in-lab group) or home unattended testing (home group). Home testing consisted of an overnight recording with a type 3 portable monitor (Embla) followed by at least 3 nights using an autoCPAP apparatus (Respironics). The primary outcome measure was the change in total score on the Functional Outcomes of Sleep Questionnaire (FOSQ) following 3 months of CPAP treatment with an a priori non-inferiority delta of -1 points. Non-inferiority was tested by determining whether the lower bound of a 95% one-sided confidence interval for the group difference in ANCOVA adjusted mean changes exceeded -1.0 pts. Preliminary analysis was performed on an evaluable cohort defined as CPAP initiated and having complete baseline and 3-month data. Final analyses will be modified intent-to-treat.

Results: Of the 296 randomized patients (95% males), 223 were initiated on CPAP and 185 completed the 3-month follow-up (N=86 in-lab group, N=96 home group). The mean Multivariable Prediction Index at baseline, a measure of OSA severity, was 0.78 in the in-lab group and 0.76 in the home group ($p = 0.25$). There was no difference between the two groups in mean FOSQ total score at baseline ($p = 0.55$). The mean improvement at 3 months was 1.93 (SD=2.47) in the in-lab group and 1.81 (SD=2.83) in the home group (p value = 0.75). The adjusted group difference (at-home minus in-lab) in mean change from baseline to month 3 in FOSQ total score, controlling for baseline FOSQ and investigative site, was -0.04 (SE=0.34) with $p=0.90$. The lower bound of the 95% non-inferiority CI was -0.60. Since $-0.6 > -1.0$ the null hypothesis that at-home testing is clinically inferior was rejected ($p<0.05$) based on the evaluable cohort.

Conclusion: This randomized clinical trial demonstrated non-inferiority of functional outcome following 3 months of CPAP treatment in patients with OSA randomized to either standard in-laboratory polysomnographic testing versus unattended home testing with portable monitors.

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An Insulin-Like Signaling Pathway Mediates the Homeostatic Response to Deprivation of Lethargus Quiescence, A Sleep-Like State In *C. Elegans*

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Introduction: Lethargus is a developmentally regulated period of quiescent behavior that has physiological and genetic similarities to sleep, including a homeostatic response to deprivation (Raizen et al, Nature 2008). Little is known about the genetic and anatomical basis of the homeostatic process in any organisms. We hypothesized that metabolic signaling by the insulin-like signaling pathway would be affected by sleep deprivation in *C. elegans*. We studied DAF-16/FOXO, a transcription factor that is activated by nuclear translocation under conditions of low insulin receptor signaling,

Methods: Worms in lethargus were deprived of quiescence by swimming for 30 minutes. A GFP::DAF-16 fusion protein was used to monitor the subcellular location of DAF-16. The extent of nuclear GFP was assessed by ranking fluorescent images after blinding to the experimental conditions. The homeostatic response to deprivation of lethargus was assessed by measuring the latency to response of two different aversive stimuli, each of which is detected by the worm by different mechanisms. First, we used the previously studied 1-octanol avoidance assay. Second, we developed a novel assay, avoidance of blue light (450-490nm). Worms show an accelerated return to prolonged sleep-like response latencies to both of these aversive stimuli following deprivation of lethargus quiescence.

Results: Deprivation of quiescence during lethargus results in nuclear translocation of DAF-16 in at least two tissues indicating a reduction of insulin signaling. We found functional evidence that deprivation of lethargus quiescence causes reduced insulin-like signaling by showing that after deprivation, there is an increased propensity to form dauers, a developmental state that is promoted by reduced insulin signaling.
daf-16 mutants show an impaired homeostatic response to deprivation of quiescence during lethargus. We tested four independent alleles to confirm this effect. Introduction of *daf-16* in several tissues showed rescue of this defect in the homeostatic response, suggesting a non-cell autonomous mechanism for *daf-16* role in this response. In contrast to *daf-16* mutants, mutants in *daf-2*, which encodes an insulin-like growth factor receptor and in which *daf-16* is constitutively active, show an exaggerated homeostatic response to deprivation of lethargus quiescence.

Conclusion: These data connect the regulation of sleep-like states with regulation of metabolism, We propose that insulin-like growth factor signaling may play a similar role in sleep regulation in more complex organisms, including humans. The results of these studies provide a potential mechanistic explanation for the observed effects on insulin signaling following sleep deprivation in humans (Spiegel et al, Lancet 1999).

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Upper Airway Dynamics in Response to Negative Expiratory Pressure (NEP) During Wakefulness in Children with Sleep-Disordered Breathing (SDB)

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Introduction: Upper airway collapsibility is a major factor in the pathophysiology of SDB. Studies in adults have shown that flow-limitation in response to negative expiratory pressure (NEP) during wakefulness may be a potential method to asses upper airway collapsibility in SDB. We hypothesized that NEP could distinguish between normal children and children with SDB even during wakefulness.

Methods: Three groups were recruited: Controls (AHI< 1.5/h; no snoring); snorers (AHI <1.5/h; habitual snoring) and obstructive sleep apnea syndrome (OSAS, AHI ≥ 1.5/h). During wakefulness, NEP of -5cm H2O was applied during the first second of expiration. Upper airway muscle activity was measured using intra-oral electrodes. The ratio of the area under the expiratory flow-volume curve during NEP compared to tidal breathing (RatioNEP) was calculated for 0.25, 0.5, 0.75 and 1 second time points. Similarly, integrated EMG moving average area under the curve during NEP as a ratio of baseline, was measured (RatioEMG).Groups were compared using ANOVA.

Results: 12 controls (14 ± 2 [mean + SD] yr; AHI 0.1 ± 0.1/hr), 9 snorers (11 ± 3yr, AHI 0.7 ± 0.3/hr) and 11 OSAS (13 ± 3yr., AHI 14.6 ± 14.7) were studied. Snorers were younger than the other two groups ($p=0.022$). There were significant differences in the RatioNEP between controls and snorers ($p<0.01$), and controls and OSAS ($p<0.05$), at all time points. However, there were no significant differences between snorers and OSAS. For RatioEMG,no significant differences were found between groups.

Conclusion: RatioNEP distinguishes between normal children and children with SDB, be it snoring or OSAS, indicating that these children have a more collapsible upper airway even during wakefulness. However, it does not differentiate between snorers and OSAS. Upper airway EMG activity was similar between groups.

Periodic Legs Movements During Sleep in Children: Frequency Distributions Across Sleep Stages and Total Sleep Time (TST)

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Introduction: In adults, periodic leg movements during sleep (PLMS) occur primarily in stages N1 and N2. However, sleep stage-specific occurrence of PLMS has not been evaluated in children. We hypothesized that PLMS in children would be greater in N1 and N2, despite the relatively high percentage of N3. Further, to better understand PLMS in children, we examined the distribution of PLMS within intervals of TST to assess circadian variation and differences between subjects with PLMI ≥5 /h versus <5/h.

Methods: We examined polysomnograms of children 1.5 - 18 years old with a periodic limb movement index (PLMI) ≥2/h. Studies were scored using standard criteria. TST was divided into thirds: T1, T2 and T3. The PLMI was determined in each sleep stage (N1- N2, N3 and REM) and in each third of TST for each subject. Generalized Estimating Equations (GEE) models were used to examine the effects of sleep stage and sleep period on the PLMIs.

Results: 67 subjects met study criteria, with a mean age of 8±4.3 years. Of these, 44% had a PLMI ≥ 5.The total PLM index was significantly greater in N1 and N2 vs N3 ($p<0.0001$), and in N1 and N2 vs REM ($p <0.0001$). No differences were seen for N3 vs REM sleep. Both the PLMI without arousals and the PLMI with arousals were elevated in N1 and N2 compared to N3 and REM. There were no differences across TST, except for the PLMI ≥ 5 group, which had a significantly higher total PLMI in T2 vs. T3 ($p=0.026$).

Conclusion: In this novel study of children, PLMI's were greater in N1 and N2, compared with N3 and REM stages (similar to adults), while PLMI's seldom differed across TST. These findings support a sleep-stage association with PLMs in childhood, without any clear relationship to intervals of TST.

Irreversible ER Stress in the Hippocampus Parallels Lasting Memory Impairment in Sleep Apnea Model

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Rationale: Obstructive Sleep Apnea is associated with cognitive dysfunction that is only partially reversible with Continuous Positive Airway Pressure (CPAP) treatment. Rodent models of sleep apnea oxygenation show hippocampal dependent memory deficits. A persistent injury has yet to be identified to explain residual impairments.

Objective: To determine whether sleep apnea oxygenation results in a persistent unfolded protein response (UPR) in the hippocampus, paralleling memory impairment.

Methods: After hypoxia/reoxygenation or sham-treatment for 8 weeks, adult mice were recovered in normoxia for 2 wks before undergoing spatial memory testing with fear conditioning and recovered in normoxia for 10 weeks before undergoing the Barnes maze. Perfused hippocampi were examined for endoplasmic reticulum stress indices.

Measurements and main results: Mice exposed to hypoxia/reoxygenation displayed persistent memory impairments in the Barnes, $p<0.01$, while no lasting impairment was observed in contextual fear conditioning. Hypoxia/reoxygenation increased phosphorylated pancreatic kinase R-like ER kinase (p-PERK) and CCAAT/enhancer-binding protein-homologous protein (CHOP) in the CA1 hippocampus during exposures. Despite a ten-week recovery in normoxia CHOP protein and mRNA remained elevated, along with cleaved activated transcription factor-6 (ATF-6) and BiP/GRP78 mRNA.

Conclusion: Herein, we report that sleep apnea oxygenation exposures result in lasting UPR in the CA1 hippocampus. This is likely to contribute to sleep apnea residual memory impairments.

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Can the Onset of Dependency in Activities of Daily Living (ADLs) be Delayed in Cognitively Impaired Older Adults with Short Total Sleep Time?

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Nocturnal sleep in cognitively impaired nursing home residents is frequently short. Short sleep time in healthy adults impairs waking cognitive and motor function and accordingly may hasten onset of ADL dependency in cognitively impaired older adults. Social activity and exercise (walking/high-intensity resistance training) improves older adults' cognitive and motor functioning. However, it is unknown whether social activity and/or exercise will improve cognitively impaired nursing home residents' ability to complete ADLs. Therefore, this study examined the effect of 7-weeks of social activity, exercise, and combined social activity/exercise compared to usual care on ability to complete ADLs in cognitively impaired nursing home residents.

A secondary data analysis was conducted on a subset of thirty-two participants (mean age 80.28 ± 9.35 years; 16 women; mean TST = 4.8 hours, SD ± 2) from "Effect of Activities and Exercise on Sleep in Dementia" (R01 NR7771). Participants were randomized to social activity (n=8), exercise (n=9), social activity/exercise (n=6), usual care (n=8). The Nursing Home Physical Performance Test (performance test) and actigraphy measured outcomes at baseline and post-intervention.

ANCOVA revealed no statistically significant change in ability to complete ADLs. However, compared to baseline, the mean performance test score in the exercise group increased by .89 (±4.0) and by 0.50 (±3.1) in the social activity/exercise group, whereas, the usual care group decreased by 0.25 (±2.1). Mean total sleep time increased 24 minutes and 20 minutes in the exercise and social activity/exercise groups, respectively, while the usual care group mean decreased by 6 minutes.

Since a ≥0.5 point change in the performance test indicates a clinically meaningful change, our findings indicate that exercise alone and exercise combined with social activity may improve ADL function and sleep. Further research is needed to investigate the relationships among exercise, function, and sleep in older adults with cognitive impairment.

The Effects of Cognitive Workload During Sleep Restriction on Subjective Sleepiness and Fatigue

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Introduction: Although sleep loss increases ratings of sleepiness and fatigue, whether waking cognitive activity potentiates the effects of sleep loss on these measures remains unknown. We investigated the effects of high cognitive workload (HW) versus low cognitive workload (LW) on the Karolinska Sleepiness Scale (KSS) and a visual analog scale of fatigue (VAS) anchored by "fresh as a daisy" and "tired to death", during both sleep restriction (SR) and no sleep restriction (NSR).

Methods: N=30 healthy adults (33.0±9.3y;13 females) completed 3 baseline (8h TIB/night) nights, followed by 5 SR (4h TIB/night) or NSR (8h TIB/night) nights in a laboratory setting. Subjects were randomized to 1 of 4 experimental conditions: HW+SR (N=11); HW+NSR (N=7); LW+SR (N=8); LW+NSR (N=4). The HW vs. LW conditions differed in cognitive testing intensity and duration. The KSS and VAS were administered 6 times each protocol day during diurnal waking hours. Baseline values were derived from the 3rd baseline day (B3). Daily values for KSS and VAS were calculated by averaging scores from all test bouts that day. A mixed-model ANOVA (day:within-subjects factor; sleep and workload:between-group factors) was conducted; paired t-tests examined differences from B3 to SR5/NSR5 for each group.

Results: KSS and VAS scores for the 4 groups did not differ at B3 ($p=0.750$ and $p=0.899$, respectively). There was a significant main effect of day on KSS and VAS scores ($p's<0.0001$), and a significant interaction with sleep for both variables ($p's<0.0001$). However, the interaction with workload was not significant (KSS:$p=0.234$; VAS:$p=0.071$). Specific examination of the HW+SR group showed large, significant increases in KSS and VAS scores ($p's<0.0001$) from B3 to SR5.

Conclusion: The preliminary results from this experiment suggest that sleep restriction had the expected robust effects on subjective sleepiness and fatigue. Higher cognitive workload under sleep-restricted conditions may potentiate perceptions of sleepiness and fatigue.

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Increased Tongue Muscle Fat in Obese vs. Lean Zucker Rats by *In-vivo* Magnetic Resonance Spectroscopy

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Introduction: Obesity is thought to be the most important risk factor for obstructive sleep apnea yet we do not understand how obesity may predispose to obstructive sleep apnea (OSA). We have previously shown that obese Zucker rats (OB) compared to lean Zuckers (LZ) have more narrow pharyngeal airways. Understanding the linkage between increased tongue muscle fat, increased tongue volume and reduced airway size may help in understanding the pathogenesis of OSA in obese humans. We hypothesized that OB compared to LZ rats have increased tongue muscle fat, measurable with magnetic resonance spectroscopy (MRS).

Methods: 4 OB and 4 LZ rats, age-matched 19-21 weeks, with OB = 689 ± 36g > LZ = 428 ± 7.0g, $p < 0.001$. Rats were anesthetized with isoflurane (2% in O_2) and body temperature was maintained at 37°C. We examined 3 voxels ($3mm^3$) in each rat using a 2 step MRS protocol with water suppression and followed by no water suppression. A Litz volume coil was shimmed to 1/2 line width of 20 Hz or better with respiratory-gated MR sequence that employed (512 averages, with TR = 2000ms and TE = 14ms). Gain was constant (40) to allow post acquisition analysis and comparisons. Voxels were placed at the base of the tongue, in the tongue mid-section (1/2 distance: anterior-to-posterior) and in the body of the masseter muscle. Fat to water ratio was calculated with MacNuts® software that used curve fitting to determine the area under water or lipid peak from the acquired spectra.

Results: The table shows the Fat to Water ratio obtained from MRS spectra for lean and obese Zucker rats. (Partial results included; missing data denoted as N/A)

Animal ID	Tongue Base	Tongue Mid-Section	Masseter Mus.
LZ44	N/A	3.24	N/A
LZ41	2.36	8.99	N/A
LZ43	1.12	3.31	0.07
LZ42	1.98	3.76	0.12
Average ± STD	1.82 ± 0.64	4.83 ± 2.78	0.01 ± 0.03
OB41	6.91	N/A	N/A
OB44	9.54	N/A	N/A
OB42	5.70	10.75	1.31
OB43	11.03	7.82	1.59
Average ± STD	*8.30 ± 2.43	9.28 ± 2.07	1.45 ± 0.19

Fat/water ratio for OB rats at the tongue base was significantly greater than LZ rats (paired t-test, $p < 0.05$, marked *)

Conclusion: Preliminary data suggest that there is a greater volume of fat in the base of the tongue in obese rats compared to lean rats. Work is on going to further quantify the fat/water ratios in additional animals. Biochemistry analysis of fat content in the dissected tongue tissues in each animal will be used to corroborate the MRS analysis. Additional data is being obtained to measure the volume of the tongues using MRI volumetric analyses.

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Systematic Strategies to Find OSA in Volunteer Commercial Drivers

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Background: Screening for obstructive sleep apnea (OSA) in commercial drivers is imperative because OSA is common in this group, causes daytime sleepiness and may lead to vehicular crashes.

Purpose: We evaluated whether data obtained during a simulated pre-employment examination can identify latent cases of OSA among commercial drivers.

Participants: We solicited Commercial Driver's License (CDL) holders in Philadelphia who were 18-65 years of age using internet advertisements. We excluded drivers who were using positive airway pressure (PAP) or supplemental oxygen, or had hypoxia due to cardiopulmonary illness.

Methods: For each subject, we simulated a federal medical-certification examination, and we recorded demographics, body mass index (BMI), neck circumference (NC), apnea symptoms (Sx) and Epworth Sleepiness Scale score (ESS). Using BMI, age, gender and symptoms, we computed the multivariable apnea prediction (MAP) for each driver (range 0=no risk to 1=maximal risk). We did full sleep studies in the subjects' homes. From these studies, we determined: 1) the 4% desaturation index *(*ODI4); 2) portable Apnea-Hypopnea Index (Portable AHI) using oximetry, nasal pressure and respiratory effort monitoring; and 3) AHI (PSG-AHI) using EEG, oximetry, nasal pressure, and respiratory effort; we defined hypopneas using a 50% drop in flow with 4% desaturation. We computed correlation coefficients and area-under-curve (AUC) values using receiver operating characteristic (ROC) curves. We considered any apnea (AHI ≥ 5/h) and severe apnea (AHI ≥30/h).

Results: We enrolled 55/59 (93%) males, of whom 58% were Caucasian and 41% were African-American. The mean ± SD age was 44.1 ± 8.5. A total of 10 (17%) had ESS > 10. A total of 53/57 (93%) had at least mild apnea with AHI ≥ 5/h, 29 (51%) had AHI ≥ 15/h, and 16 (28%) had AHI ≥ 30/h. Subjects tended to be obese, with large neck circumference, and widely ranging levels of sleepiness (Table 1). For MAP, the Spearman Rank and Pearson correlation coefficients were 0.53 and 0.52, respectively, indicating excellent discriminatory power. For oximetry, these values were 0.77 and 0.92, respectively, and for portable sleep studies, 0.84 and 0.94. AUC values indicated that MAP, overnight home oximetry and portable sleep studies have high discriminatory power for finding any apnea or severe apnea.

Table 1 Subject characteristics

	Mean ± SD	Range
BMI (kg/m^2)	31.9 ± 6.2	21.6−51.0
NC (cm)	42.5 ± 4.1	34.2.−54.5
ESS score	7.5 ± 5.3	0.0−22.0
MAP index	0.6 ± 0.2	0.1−0.9
ODI4	12.9 ± 18.9	0.0−79.0
Portable AHI4	12.7 ± 19.5	0.0−79.5
PSG-AHI	26.7 ± 23.8	1.6−88.9

Table 2 Spearman Rank and Pearson correlation coefficients

	Age	NC	BMI	Sx	ESS	MAP	ODI4	Portable AHI4	PSG AHI
Spearman	0.22	0.29	0.34	0.38	0.29	0.53	0.78	0.84	1
Pearson	0.23	0.30	0.34	0.41	0.45	0.52	0.92	0.94	1
AUC any	0.601	0.601	0.717	0.548	0.582	0.695	0.811	0.887	1
AUC severe	0.670	0.679	0.715	0.756	0.730	0.820	0.910	0.960	1
N	57	57	57	54	57	54	57	57	57

Conclusion: In this research setting, there was a high prevalence of self-reported sleepiness. OSA was also highly prevalent, and could be readily identified by MAP, home oximetry and portable sleep studies in the home. However, future analyses must avoid reliance on symptoms, since subjective data may be inaccurate in an occupational setting.

Neurons in the Ventrolateral Preoptic Nucleus Play a Key Role in Anesthetic-Induced Hypnosis

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Despite 160 years of clinical use, the neural mechanisms through which general anesthetics act remain unknown. One possibility is that anesthetics exert their hypnotic effects by acting on the endogenous arousal neural circuitry, including the wake-promoting orexinergic neurons of the hypothalamus and the sleep-promoting GABAergic and galaninergic neurons of the ventrolateral preoptic nucleus (VLPO). We have previously demonstrated that orexinergic neurons play an essential role during emergence from general anesthesia but not during anesthetic induction (Kelz et al., 2008). Here, we present evidence that the VLPO plays a similarly critical role in the induction of anesthetic hypnosis. *Characterization of the effect of anesthetics on VLPO neurons.* We used c-Fos immunohistochemistry to analyze the activity of VLPO neurons in brain slices of mice sacrificed after two hours of anesthetic exposure. Whereas anesthetic exposure produced a decrease in the number of c-Fos-positive nuclei in most brain areas, this is not true for the VLPO: exposure to the volatile anesthetics isoflurane or halothane produced a rapid, dose-dependent increase in the number of c-Fos-positive nuclei in the VLPO, implying that hypnotic doses of volatile anesthetics increase firing rates of VLPO neurons. Furthermore, this effect is also observed *ex vivo* in partially-deafferented hypothalamic slices, indicating that the activation of VLPO neurons may be at least partially due to a direct effect of the anesthetics. *Functional significance of the VLPO in anesthetic-induced hypnosis.* To determine whether activation of the VLPO is necessary for anesthetic-induced hypnosis, galanin-saporin was used to produce targeted lesions of VLPO neurons. While this study is still ongoing, the effects of the lesion appear to be very time-dependent, suggesting that increasing sleep debt following the lesion may be opposing the anesthetic-resistance effects of the lesion. In order to avoid the sleep-disturbance confounds inherent in a chronic VLPO lesion, we have also done preliminary studies looking at the effects of acute activation and deactivation of VLPO neurons on anesthetic sensitivity. Mice that are maintained at hypnotic levels of isoflurane anesthesia for one hour regain consciousness in 4.8 ± 0.6 minutes after discontinuation of the anesthetic. However, mice that received a targeted injection of the synaptic activity blocker tetrodotoxin regained consciousness within one minute following the injection while the anesthetic was still being administered, and mice that received a targeted injection of the long-lasting glutamate agonist ibotenic acid took an average of 328.2 ± 36.0 minutes following discontinuation of the anesthetic to regain consciousness. In summary, the identification of a population of anesthetic-active neurons in VLPO suggests that the endogenous arousal neural circuitry may play a critical role in the generation and dissipation of anesthetic-induced unconsciousness. We are currently in the process of determining whether the population of VLPO neurons active during general anesthesia is the same as the sleep-active population.

The Effects of Cognitive Workload During Sleep Restriction on Executive Function Measures

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Introduction: Although sleep loss degrades cognitive functions, little theoretical or experimental attention has been paid to determining whether waking cognitive activity potentiates the effects of sleep loss. We investigated the effects of high cognitive workload (HW) versus low cognitive workload (LW) on two classic executive function tasks following both sleep restriction (SR) and no sleep restriction (NSR).

Methods: N=33 healthy adults (33.9±9.1y; 15 females) completed 3 baseline nights (8h TIB), followed by 5 nights of SR (4h TIB) or 5 nights of NSR (8h TIB) in a controlled laboratory setting. Subjects were randomized to 1 of 4 experimental conditions: HW+SR [N=11]; HW+NSR [N=6]; LW+SR [N=8]; LW+NSR [N=8]. The HW vs. LW conditions differed in the intensity and duration of cognitive workload. The Controlled Oral Word Association Test (COWAT; work production speed) was administered on baseline night 2 (B2) and the 5th night of SR (SR5) or NSR (NSR5); the Hayling task (initiation speed and response suppression) was administered on SR5/NSR5. One-way and mixed-model (night×condition) ANOVAs compared differences across all 4 experimental conditions; post-hoc Bonferroni-adjusted probabilities were used for significant group differences.

Results: Hayling performance was significantly different across the 4 conditions ($p=0.025$); the SR+HW group had significantly higher scores than the SR+LW group ($p=0.017$), but the NSR+HW and NSR+LW groups did not differ. By contrast, the COWAT did not significantly differ across the 4 conditions at B2 ($p=0.777$) and SR5/NSR5 ($p=0.591$), though all groups showed a significant improvement from B2 to SR5/NSR5 due to learning ($p<0.001$).

Conclusion: Workload did not affect executive functioning under fully rested conditions. Our preliminary results suggest that sleep restriction promoted better response suppression of word generation, but this result is not likely to sustain once the sample size is increased.

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The Effects of Cognitive Workload and Sleep Restriction on the Maintenance of Wakefulness Test

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Introduction: Chronic partial sleep deprivation decreases the ability to resist sleep as measured by the MWT. It has been assumed that the nature of cognitive activity during wakefulness does not affect sleep latency. We tested this assumption by investigating the effects of high cognitive workload (HW) versus low cognitive workload (LW) on MWT following both sleep restriction (SR) and no sleep restriction (NSR).

Methods: N=33 healthy subjects (33.6±9.1y;15f) completed 3 baseline (8h TIB/night) nights, followed by 5 SR (4h TIB/night) or NSR (8h TIB/night) nights in a laboratory setting. Subjects were randomized to 1 of 4 experimental conditions: HW+SR (N=10); HW+NSR (N=7); LW+SR (N=8); LW+NSR (N=8). The HW vs. LW conditions differed in cognitive testing intensity and duration. Modified 30-minute single-trial MWTs were conducted between 1445h-1600h after the 3rd night of baseline (B3) and after the 1st, 4th and 5th nights of SR (SR5) or NSR (NSR5). Sleep latency was defined as time to the first microsleep (10-sec EEG theta) or 30 minutes if no sleep occurred. A mixed-model ANOVA (day:within-subjects factor; sleep and workload:between-group factors) was conducted; paired t-tests examined differences from B3 to SR5/NSR5 for each group.

Results: MWT latencies for the 4 groups did not differ at B3 ($p=0.882$). The main effect of day on MWT ($p<0.0001$) and an interaction with sleep ($p=0.001$) were significant but the interaction with workload was not ($p=0.821$). Specific examination of the HW+SR group showed a significant decrease in MWT sleep latency ($p<0.001$) from B3 to SR5.

Discussion: The MWT, as expected, was sensitive to sleep loss, while the impact of high cognitive workload on the ability to resist sleep was less pronounced. These preliminary results suggest that MWT sleep latency may be influenced by cognitive workload, but a larger sample size is needed to elucidate the exact relationship between these factors.

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ER Stress and Age-Related Dysfunction of Wake-Active Neurons

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Introduction: Fragmentation of wakefulness and sleep are expected outcomes of advanced aging. We hypothesize that metabolically active wake neurons are susceptible to age related changes in the unfolded protein response and that dysfunction with age might contribute to the observed wake impairments. In this series of experiments we sought to more fully characterize age-related changes in wakefulness and then, in relevant wake neuronal populations, explore functionality and endoplasmic reticulum homeostasis.

Methods: Young (2 month old) and aged (12, 24 month old) mice underwent electroencephalographic/electromyographic recordings to measure wakefulness bout length distribution and average sleep latency in the active period. Brains were then procured for functional assays of wake neurons (c-fos response) during wake and for ER stress markers.

Results: Aged mice display impaired wakefulness with fragmentation and significantly more sleep to wake transitions with 209$\pm$37 bouts compared to 105$\pm$47 in the young mice during the lights off period (n=9/age group, p=0.0001). In addition wake bout durations are significantly shorter in the aged mice compared to the young mice (2.2+0.5 vs 6.2+3.7 min, p=0.006). Young mice show c-fos activation in approximately 60% of dopaminergic (VPAG) and noradrenergic (LC) neurons and 80% c-fos activation in orexin neurons. 12 month old mice show marked reduction in c-fos activation in VPAG and LC (<20%), while orexinergic neurons have 50% activation. At 24 months c-fos activation remains low in the catecholaminergic neurons and additional reductions are observed in the orexin neurons (30% activation). Coninciding with the reduced c-fos staining we find increased immunoreactivity of the ER stress marker P-PERK, and the pro-apoptotic marker, CHOP. Intervention studies using the drug salubrinal in 12 month old mice are underway to determine a mechanistic link between these observations. Preliminary data indicates that salubrinal which prevents disinhibition of protein translation and leads to CHOP induction fragments sleep and wake.

Conclusions: Aged mice display wake fragmentation with shorter wake bout durations and many more sleep to wake transitions. This may be due to impaired function of the wake active neurons though the ER stress response.

Intermittent Hypoxia Triggers Release of Macrophage Mediators That Decrease Cardiac Myocyte Survival

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Introduction: Obstructive sleep apnea (OSA) leads to nocturnal intermittent hypoxia (IH). OSA is associated with oxidative stress and a pro-inflammatory state. OSA has been recognized as a risk factor for cardiac morbidity; although the mechanism is not known. IH triggers a series of cellular responses as a result of hypoxia and repeated reoxygenation. Mediators released by cells exposed to IH can initiate signaling in normal cells. We tested the effect of soluble factors released from intermittently hypoxic macrophages on the survival and expression of inflammatory mediators in cardiac myocytes cultured under standard conditions.

Methods: A murine macrophage cell line (RAW264.7) was cultured in a forced convection cell culture system. Cells were randomly assigned to either normoxia (40 Torr O_2) or intermittent hypoxia at a frequency consistent with OSA (cycles of 90 seconds of 40 Torr O_2 followed by 30 seconds of 8 Torr O_2) in DMEM/5%FBS equilibrated with 5% CO_2. Conditioned media was collected for 10 min following 6 and 18 hours of culture (flow rate 0.256ml/min). Neonatal rat cardiac myocytes were grown to confluence on fibronectin coated wells in DMEM/5%FBS. After growth to confluence, the myocyte culture media was replaced with conditioned media that had been collected from the macrophages. For controls, the media was replaced with fresh DMEM/5%FBS. The myocytes were then cultured for 6 or 24 hours in a standard incubator. Apoptosis was evaluated with anti-caspase 3 staining. Expression of inflammatory mediators was evaluated by western blot for inducible nitric oxide synthase (iNOS), macrophage migration inhibitory factor (MIF) and tumor necrosis factor (TNF).

Results: Exposure to conditioned media from IH macrophages increased expression of activated caspase-3 in adherent cardiac myocytes at 6 hours. Effluent from macrophages exposed to OSA-frequency IH but not normoxia induced significant expression of MIF, iNOS and TNF in normoxic cardiac myocytes.

Conclusions: Macrophages exposed to IH release soluble factors that alter cardiac myocyte protein expression and survival. IH may be a mechanism contributing to cardiac morbidity in OSA.

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The Case of the Man with Abnormal Movements in Sleep

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A 60-year-old man presents with a history of abnormal movements in sleep that have been occurring for decades. He has 30-second long episodes once or twice a week consisting of head turning to the left, eyes “glazing over,” drooling and groaning. Afterwards he will be aware but confused, and unable to talk for approximately 1.5 minutes. These episodes never occur while he is awake. The patient also has obstructive sleep apnea but is not adherent to CPAP therapy. He underwent a polysomnogram, which demonstrated frequent epileptiform discharges predominantly in the frontal regions with spike-and-slow-wave morphology, occurring during non-REM sleep. These discharges lasted for up to 15 seconds and were frequently associated with respiratory hypopneas. The clinical history and the EEG together point to a diagnosis of Nocturnal Frontal Lobe Epilepsy (NFLE). This condition is often inherited in an autosomal dominant manner and is associated with mutations in neuronal nicotinic acetylcholine receptors. Although the mutation results in net inhibition of cortical pyramidal neurons, seizures are thought to occur when the waves of inhibition are followed by rebound synchronized neuronal excitation. A previous publication reported a patient with NFLE whose seizures all occurred immediately following K-complexes; a third of these seizures and K-complexes were associated with sound-induced microarousals from sleep (El Helou et al, Clin Neurophys, 2008). I hypothesize that my patient’s hypopneas result in microarousals that then trigger his epileptiform discharges, analagous to the sound trigger in the published case report. Studies have demonstrated that untreated obstructive sleep apnea worsens seizure control in various types of epilepsy (Chihorek et al, Neurology, 2007). There is also evidence that frequent nocturnal seizures can adversely affect upper airway control in sleep and worsen obstructive sleep apnea severity (Foldvary-Schaefer et al, Epilepsia, 2008). CPAP therapy for this patient’s obstructive sleep apnea may reduce microarousals and thus improve seizure frequency.

Response to Hypercapnia During Sleep in Obese Adolescents With and Without the Obstructive Sleep Apnea Syndrome (OSAS)

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Introduction: The prevalence of obesity and resultant OSAS is increasing in adolescents. However, many obese adolescents do not develop OSAS, despite having a presumably narrower upper airway. The reasons for this are unclear but may be related to upper airway neuromotor control. We hypothesized that ventilatory and genioglossal EMG (EMGgg) responses during sleep are decreased in obese adolescents with OSAS compared to BMI-matched controls.

Methods: Subjects underwent polysomnography using surface electrodes to measure EMGgg. CO_2 was introduced, sufficient to raise the transcutaneous PCO_2 by 3 mmHg. Ventilatory parameters were calculated for each breath before and during the hypercapnic challenge. The area under the curve of the filtered rectified inspiratory EMGgg moving time average was analyzed and compared to the baseline before the challenge.

Results: 8 subjects with OSAS (age 15±1 [mean ± SD] yr, BMI z-score 2.5±0.5, AHI 21±21/hr) and 5 controls (15±2 yr, BMI z score 2.0±0.4, AHI 0±0/hr) were studied. During CO_2 challenges, controls increased minute ventilation ($p=0.002$), primarily by increasing flow ($p=0.014$) and tidal volume ($p=0.007$). Controls had a significant increase in EMGgg ($p=0.027$). In contrast, OSAS also had a change in minute ventilation ($p=0.008$), but this was due more to changes in the duty cycle ($p<0.001$) than flow ($p=0.139$) or tidal volume ($p=0.031$). OSAS had no significant change in EMGgg.

Conclusions: Obese adolescents without OSAS have ventilatory and upper airway neuromotor responses to hypercapnic challenges during sleep. In contrast, adolescents with OSAS do not mount an upper airway response to CO_2, but instead compensate for the hypercapnic load by changing respiratory timing. We speculate that obese adolescents without OSAS maintain protective upper airway reflexes during sleep, whereas those who go on to develop OSA do not.

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The Case of the Girl with Groaning During Sleep

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A 21 year old girl with a seizure disorder treated with a vagal nerve stimulator (VNS) was referred for a polysomnogram (PSG) to evaluate whether her involuntary sleep groaning may be related to underlying obstructive sleep apnea. She has a one year history of groaning and moaning on expiration during sleep, not associated with any snoring or apneic events. On her PSG, intermittent EMG activation for 30 second intervals; groaning that appeared to correlate with VNS activation and periods of decreased airflow were seen. She had an apnea-hypopnea index (AHI) of 0.4 and there was an increase of groaning in REM sleep. There have been reports of VNS-induced respiratory events during sleep. (Malow BA et al, Neurology 2000). The stimulus frequency of her VNS was lowered, but the frequency of seizures increased and the groaning persisted.

The clinical history and polysomnogram findings point to a diagnosis of catathrenia. Catathrenia is defined as a parasomnia in the International Classification of Sleep Disorders Diagnostic and Coding Manual (ICSD-2), but there is debate about its classification and its response to CPAP. The hallmark of catathrenia is inspiration followed by protracted expiration during which a prolonged or fragmented sound is produced, and this recurs especially during REM sleep. The quality of groaning in catathrenia is monotone, and often presents with a morose or sexual connotation, causing a social problem for some patients. Patients are generally unaware, but parents and bed-partners can be troubled.

A previous publication reported 7 patients that presented over 5 years with a chief complaint groaning during sleep (Guilleminault, C et al, *Sleep.* 2008) I hypothesize that in catathrenia patients with evidence of upper airway obstruction, treating the obstruction positive airway pressure or upper airway surgery can decrease the prevalence of catathrenia. The patients were all 20-34 years old, female, with a BMI < 25 and used nasal CPAP for 1 month which eliminated the expiratory sound, confirmed by bed partner report. Due to anatomic evidence of a small upper airway and presence of flow limitation on polysomnogram, surgical treatment was recommended. All of the patients had resolution of groaning following an adenotonsillectomy with or without use of an oral appliance. There was another case report of a 62-year-old woman with OSA and catathrenia that responded to CPAP. (Iriarte et al, *Neurology*, 2006). CPAP therapy may provide resolution of this patient's catathrenia.

Exercise Reduces Apnea-Hypopnea Index in Cognitively Impaired Older Adults

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Introduction: In this study, we evaluated the effect of exercise (high intensity physical resistance strength training and walking) on the apnea-hypopnea index (AHI) in institutionalized older adults.

Methods: As a part of a randomized controlled trial, 97 institutionalized older adults (81.82 y; 52 F; Mini-Mental State Examination [MMSE]score=20.44, SD=6.97) received 7 weeks of strength training to the arm and hip extensors 3 days a week, and 2 days a week they walked with a research assistant for up to 60 minutes. Of the 35 possible intervention days the mean attendance was 29 days (SD=7.2) or 81% attendance. Muscle strength improved 107.69% for the arm extensors and 92.84% for the leg extensors. The control group (n = 47; 82.34 y; 30F; MMSE score=20.34) did not receive any intervention and participated in the usual activities of nursing homes and assisted living centers. Two nights of polysomnography were averaged at baseline and at post-intervention to obtain the AHI.

Results: ANCOVA adjusted for baseline level showed a significant decrease in AHI for the exercise group relative to the control ($F_{1,141}$ = 4.30; p=.04). Adjusted means showed a decrease in AHI from 20.22 (SE=1.385) to 16.72 (SE=.964). Correlation of change in AHI with change in muscle strength was non-significant (arm extensors, r=.15; hip extensors, r=.18).

Conclusion: Strength training and walking improved the AHI in older adults. A potential mechanism is the strengthening of pharyngeal and glossal muscles during training.

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Sleep Apnea in a Child with Achondroplasia

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Case: A 3 year, 5 month old male with achondroplasia presented to the Sleep Clinic at CHOP with a diagnosis of mild obstructive sleep apnea (OSA) on routine polysomnography (PSG). The otolaryngologist had recommended adenotonsillectomy (T&A), but the patient's father, an infectious disease physician, hesitated to remove the tonsils due to concerns of impaired immune function. This visit was a second opinion.

History of present illness included mild snoring not heard outside the bedroom, on about 50% of nights. Also present were apneic periods, noted when his mother held him, not accompanied by respiratory effort or grunting. There were no parasomnias or other sleep disorders.

Past medical history was significant for emergency Cesarean section for maternal bradycardia during induction of labor. Infant apgars were high, and no resuscitation or oxygen was required postnatally. Achondroplasia was diagnosed at birth. His height and weight have been steady at the 50^{th} percentile for age based on achondroplasia growth charts. Speech was delayed secondary to mild hearing loss, and muscle tone was slightly decreased but within normal limits for a child with achondroplasia. He experienced frequent congestion. There was no history of asthma. Past surgery included only bilateral myringotomy (PE) tubes at 2 years of age, which had since fallen out. He is scheduled for PE tube replacement in 2 months. A PSG two months prior to this consult showed central apneas 1.9/hr, obstructive hypopneas 2.2/hr, no obstructive apneas, nadir oxygen saturation 89%, and ETCO2 46.3. Other findings were within normal limits.

On physical exam, he was alert and interactive. His weight was 17.8 kg, height 79.2 cm, BMI 20.2, pulse 119, BP 106/64 and oxygen saturation by pulse oximetry 97% on room air. Lung sound were clear, with mild inter- and subcostal retractions and no digital clubbing. Visualization of the oropharynx was difficult due to noncooperation, but tonsillar tissue was seen. Mid-face hypoplasia was present with a normal jaw. His spine was straight.

Background: Achondroplasia is an inherited, autosomal dominant disorder, the most common form of dwarfism. Clinical features include short stature, long narrow trunk with protuberant abdomen, shortening of proximal limbs and hands, large head with frontal bossing, mid-face hypoplasia, lumbar lordosis, and hypotonia. Central nervous system defects include compression at the cervicomedullary junction and hydrocephalus. Sleep-disordered breathing may be present in 85% of children with achondroplasia, including hypoxemia, central apnea, obstructive sleep apnea, and abnormal electromyographic activity of accessory muscles. Sudden, unexpected death in infants with achondroplasia is not uncommon and is attributed to cervicomedullary compression leading to central respiratory control abnormalities and ultimately, apnea.

Recommendations: Adenoids and tonsils continue to grow until about 12 years of age and can significantly obstruct the upper airway. This child is at risk of increasing upper airway obstruction due to continued adenotonsillar growth during childhood. Midface hypoplasia and decreased muscle tone with increased work of breathing in achondroplasia also place him at increased risk of upper airway obstruction and oxygen desaturations. Despite the mild degree of OSA, given his young age and risk factors, T&A was recommended.

Do Cognitive Perceptions Influence CPAP Use?

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Rationale**:** Nonadherence to CPAP increases health risks associated with OSA. Early identification of those at risk for nonadherence is critical to reducing untoward outcomes in OSA.

Objectives**:** To examine if disease-specific cognitive perceptions influence adherence to CPAP treatment.

Measures**:** Objective CPAP adherence was objectively measured following the first week and first month of treatment. Subjects completed the Self-Efficacy Measure for Sleep Apnea questionnaire that assesses perception of risk, outcome expectancies, and perception of treatment benefit (self-efficacy), at baseline, post-CPAP education, and post-one week CPAP treatment.
Methods: A prospective cohort study included 62, middle-aged (57.2 yrs [CI 95% 54.6-59.9]) subjects (34 [54%] Caucasians; 28 [45%] African Americans) with severe OSA (AHI 44.7 events/hr [CI 95% 38.4-51.0]). Following full-night diagnostic and CPAP titration polysomnograms, CPAP was initiated.

Results**:** Average daily CPAP adherence was 4.01hrs (CI 95% 3.67-4.66) at one week and 3.43hrs (CI 95% 2.74-4.12) at one month. No domains measured at baseline predicted 1-wk CPAP adherence in the reduced or full linear regression models. Self-efficacy measured at baseline, post-education, and after one-week of CPAP use predicted CPAP adherence at 1 mo (p=0.009, p=0.021, p=0.008, respectively). Moreover, self-efficacy assessed post-education prior to initiation of CPAP treatment also predicted 1-wk CPAP adherence (p=0.004). Outcome expectancies measured at 1 wk predicted adherence at 1-wk (p=0.001) and 1 mo (p=0.008).

Conclusions: Cognitive perceptions influence adherence to CPAP use, but only within the context of knowledge of CPAP treatment and experience with the treatment.

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Does a Neurobehavioral and Mood Dose Response to CPAP Exist in Obstructive Sleep Apnea Patients Treated with CPAP?

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Introduction: Obstructive sleep apnea (OSA) patients' functional and sleepiness outcomes are associated with duration of CPAP use or dose. Neurobehavioral and mood outcomes relative to CPAP dose are not well-defined. The study objective was to examine the relationship between achieving "normal" neurobehavioral and mood outcomes as a function of CPAP dose.

Methods: A multi-site, international prospective observational study included severe OSA patients (n=149) with baseline testing before treatment and after three months of CPAP. Subjects with abnormal baseline values were included in the dose response analysis using probit analysis. Neurobehavioral measures included Psychomotor Vigilance Task (PVT; Sustained attention), Digit Symbol Substitution Test (DSST; Cognitive processing), Probed Memory Recall Test (memory); Mood measures included Profile of Mood States (POMS). Objective CPAP use was overtly monitored. A validated cut point value of 265msec for PVT was used to estimate response probabilities as a function of mean CPAP use; selected cut point values for the DSST, Probed Memory Recall test, and POMS were used such that 25% of the sample was defined as having "normal" response at baseline.

Results: Subjects were middle-aged (46.8 ± 8.8 yrs) severe OSA patients (AHI 64.3 ± 29.1 events/hr). Responders, defined by the normal response thresholds for individual study outcomes, had overall longer CPAP use than nonresponders, except within the subsamples for DSST and Probed Memory Recall ($p<0.05$). Among POMS subsample, 42.9% (42/98) achieved the criterion for response with a significant association between likelihood of response and mean CPAP use ($p=0.017$). Improvement in POMS appeared to occur with 2 hrs CPAP use without further improvement with additional CPAP use. The PVT subsample included 26.9% (14/52) responders with significant association between likelihood of response and mean CPAP use ($p=0.021$). PVT improvement occurred with 6 or more hrs of CPAP use. No dose response was observed for DSST or Probed Memory Recall ($p=0.593$; $p=0.821$, respectively).

Conclusion: Sustained attention neurobehavioral outcomes and mood are associated with CPAP dose in CPAP-treated severe OSA. Future studies examining these relationships across OSA disease severity categories are needed to more robustly describe CPAP dose response for neurobehavior and mood outcomes.

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Age-Related Dysfunction of Wake-Active Neurons

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Introduction: Sleep/wake quality changes as humans age. Many individuals experience increased nighttime awakenings and have difficulty staying awake during the day. This is likely attributed to age related neuronal dysfunction. Expression of BiP/GRP78, an ER molecular chaperone in wake-active neurons, decreases over age yielding accumulation of misfolded proteins and an increase in apoptotic factors. In young animals, but not aged animals BiP is upregulated in response to an acute stressor such as sleep deprivation. Catecholaminergic, but not orexinergic wake-active neurons also express NADPH oxidase (NOX) which interferes with BiP function, leading to neuronal dysfunction.

We predicted transgenic mice with reduced BiP (+/-) would have wakefulness impairments correlated to less neuronal activity. Additionally, we predicted wake-active neurons expressing NOX would show less activity during prolonged wakefulness in 24 month aged mice versus 3 and 12 month aged mice and that these neurons show dysfunction at an earlier age than wake-active neurons not expressing NOX.

Methods: In BiP (+/-) and wild-type mice, EEG recordings and/or beam breaks monitored activity. Animals were sleep deprived for 6 hrs during the lights-on period. We used immunohistochemistry to compare neurons double-labeled for TH or OXA and c-fos between groups of mice.

Results: 24 month wild-type animals show less c-fos activity in wake-active neurons (22.6%) when compared to 12 and 3 months of age (45% and 44% respectively $p<0.05$). A reduction in wakefulness coincided. Wake-active neurons expressing NOX show a reduction in c-fos activity in comparison to orexinergic neurons at 3 months but not 12 or 24 months ($p<0.05$). In BiP (+/-) mice wakefulness impairments were also seen; neuronal activity will be compared.

Conclusion: Age-related dysregulation diminishes maintenance of wakefulness.

Self Treatment of Insomnia by the Elderly

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Introduction: Sleep disorders are common in the elderly, with up to 57% of older adults complaining of difficulties with their sleep[1]. These sleep disorders can be associated with an increased risk of driving accidents [2-4], cardiovascular mortality [5], falls[6], depression[7] and cognitive deficits [8]. Despite the significant prevalence of sleep disorders, many older adults do not seek evaluation or treatment of their sleep problems and instead engage in a number of self-treatment activities[9, 10]. Potential self-treatments include pharmacological aids (sedatives), alternative therapeutics (melatonin), and non-pharmacological activities (exercise or reading). The purpose of this study was to explore the different types of self-treatment strategies that were being used by older adults for their insomnia. The understanding of common self-treatment strategies amongst older adults with insomnia is an important aspect of providing effective care for their sleep disturbances.

Study Methods: Our study consisted of adults over the age of 65 who were recruited from a database of elderly subjects in the greater Philadelphia area. Subjects were drawn from the Penn Partners in Healthy Living Program, an elder outreach program affiliated with the University of Pennsylvania Health System.

Results: A total of 242 study questionnaires were completed. There was an average of 4.8 treatments used per study participant (SD 2.9, range 0-13). There was a statistically significant correlation between the number of treatments attempted and the PSQI sleep quality score ($r=0.37$, $p<0.0001$) suggesting that study participants who had worse sleep quality had attempted more sleep treatments. Prescription sleeping pills were felt to be the most effective treatment option (2.5), and ear plugs (1.0) were felt to be the least effective. Comparison of z-score normalized values revealed that only prescription medications had a perceived efficacy that was significantly larger than the pooled average efficacy of the other methods to the Bonferroni adjusted p-value criteria of 0.0024.

Discussion: This study examined a wide array of treatment options used by elders for the management of their insomnia. The high usage rates of several treatments highlights the importance of inquiring about the broad range of treatment choices made by older adults as they seek to address their sleep complaints.

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Serotonin 2A Receptor mRNA Levels, But Not mRNAs for Other Excitatory Receptors that Mediate Wake-Related Activation of Hypoglossal (XII) Motoneurons, Are Higher in the XII Nucleus at Wake Onset Than At Sleep Onset

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Introduction: Motor activity of the tongue is high during wakefulness in association with various voluntary behaviors, whereas during sleep tongue muscles are relatively quiescent. Serotonin (5-HT), norepinephrine and orexins (OX) are some of the major mediators of wake-related activation of XII motoneurons that innervate the tongue. We investigated whether mRNA levels for the receptors that mediate activating effects of these modulators vary in the XII nucleus in association with the rest-activity cycle.

Methods: Adult Sprague-Dawley rats were decapitated under deep isoflurane anesthesia at 8-9 AM (rest/sleep onset; n=8-10 animals) or at 6-7 PM (near active period onset; n=7-9 animals), medullary slices were obtained, 500 µm tissue micropunches were extracted from the motor XII nucleus and the region of the sensory external cuneate nucleus, RNA was extracted, reverse-transcribed and subjected to quantitative PCR. 5-HT_{2A}, 5-HT_{2C}, $\forall_{1A}$- and $\forall_{1B}$-adrenergic, and OX-2 receptor mRNAs were quantified relative to the number of tubulin copies in each sample.

Results: In the XII nucleus, 5-HT_{2A} receptor mRNA levels were higher in the samples collected at active period onset than in those collected at rest onset (15±4 (SE) vs. 3±1.7 per 1000 copies of tubulin cDNA; $p<0.01$), whereas the other mRNAs did not exhibit significant differences. In the external cuneate nucleus, mRNA levels for 5-HT_{2A}, 5-HT_{2C}, and $\forall_{1B}$-adrenergic receptors were 3-6 times lower than in the XII nucleus, and no mRNA exhibited significant circadian variation, although $\forall_{1B}$-adrenergic receptor tended to be twice higher at active period onset ($p=0.056$).

Conclusion: If these mRNA changes lead to proportional changes in functional membrane receptors, our data suggest that excitatory actions of 5-HT on XII motoneurons are reinforced as a result of wake-related increase in availability of 5-HT_{2A} receptors. In obstructive sleep apnea patients in whom sleep-related upper airway hypotonia facilitates airway obstructions, low levels of 5-HT_{2A} receptors during rest period may contribute to sleep-disordered breathing. (Supported by grant HL-047600.)

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The Impact of Local Circuit Dynamics in Layers 4 and 2/3 of Rodent Somatosensory Cortex on Response Properties to Thalamic Input In Vitro

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The state of cortical circuits and how they respond to inputs is affected by different types of anesthesia and transitions from waking to sleep. Under anesthetics that produce slow oscillations similar to those observed during natural sleep, the rodent primary somatosensory "barrel" cortex responds to whisker input with a large network event that lasts hundreds of milliseconds and recruits multiple cortical columns. However, under anesthetics that do not produce slow oscillations but instead a low amplitude desynchronized background synaptic activity (more closely resembling the waking state), responses to whisker input are much shorter in duration and primarily engage a single cortical column. Altering the state of the cortical network may provide insights into how the circuits of the primary input layer 4 and its immediate target layer 2/3 respond to and process afferent input.

Slices of rodent barrel cortex can be prepared that preserve thalamocortical connections and offer a powerful tool for studying cortical responses to afferent input. Such slices can be made to produce slow oscillations when maintained in a medium that contains extracellular ionic concentrations similar to those found in situ. Furthermore, slices can be easily exposed to drugs that alter the network state, such as neuromodulators. Here we use thalamocortical connected slices of rat barrel cortex to investigate how excitation travels from layer 4 to layer 2/3 and then across columns during slow oscillations and in the presence of acetylcholine, a prominent neuromodulator of the cortex during waking. Using a combination of intracellular recordings and voltage-sensitive dye imaging, we show that when slices are generating the slow oscillation, afferent input results in a large network response that begins in a single column and then spreads to multiple adjacent columns. This wave of network activity engages all layers, and interestingly appears to travel from layer 2/3 to layer 4 in the adjacent columns. After bath application of acetylcholine, the slow oscillation is disrupted and responses to an input are significantly shorter in duration and remain confined to the primary input column and one or two neighboring columns.
Thus neuromodulation by acetylcholine changes the state of the cortical circuitry such that any one input cannot dominate the response. This would allow for integration of multiple inputs, as is necessary during waking.

Effects of Visceral Neck Fat on AHI and BMI in the Icelandic Sleep Apnea Cohort (ISAC)

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Introduction: Obesity is the primary risk factor for OSA. Increased BMI is strongly associated with AHI. The specific relationship between visceral neck fat and OSA severity has not been fully examined. We hypothesized that increased visceral neck fat would be associated with increased AHI, BMI, and upper airway fat.

Methods: 135 male subjects (mean age 53.57±10.0, mean BMI 31.54±4.2, mean AHI 40.30±20.6) had upper airway MRIs as part of the ISAC study. Visceral and subcutaneous fat between C3 and C7 was measured using upper airway sagittal images (Figure 1). We examined the relationship for males only between BMI and AHI and both C3-C7 total visceral fat and C3-C7 average visceral fat adjusted for C3-C7 length by stratifying BMI (BMI≤30, 30<BMI<35, BMI≥35 kg/m^2) and AHI (AHI≤30, 30<AHI<50, AHI≥ 50 events/hour). C3-C7 visceral fat and adjusted C3-C7 visceral fat were compared between males and 54 females (mean age 58.5±8.9, mean BMI 33.0±4.7, mean AHI 38.8±17.1). Relationships between neck fat, tongue volume, tongue fat, and parapharyngeal fat pads were examined. Pearson's correlations and ANOVA were used.

Results: Both total and adjusted visceral fat were significantly associated with increased AHI and BMI. There are significant differences in the BMI and AHI categories for visceral C3-C7 fat and for adjusted C3-C7 visceral fat. Analysis confirmed that subcutaneous fat is not associated with AHI.

	BMI ≤ 30 n = 50	**30 < BMI < 35 n = 55**	**BMI ≥ 35 n = 30**	
	Mean (SD)			**p**
Visceral C3-C7 Fat (SD)	168.24 (39.28)	207.11 (49.18)	230.04 (33.25)	<0.001
Visceral C3-C7 Fat adjusted (SD)	23.47 (5.23)	29.17 (6.81)	32.23 (5.17)	<0.001
	AHI ≤ 30 n = 49	**30 < AHI < 50 n = 48**	**AHI ≥ 50 n = 38**	
	Mean (SD)			**p**
Visceral C3-C7 Fat (SD)	196.56 (40.70)	186.04 (52.70)	214.29 (49.50)	0.03
Visceral C3-C7 Fat adjusted (SD)	27.53 (5.63)	26.1 (7.43)	30.08 (6.96)	0.02

Pairwise contrasts between BMI categories were all significant (p<0.04). However, pairwise contrasts for AHI were only significant between the 30<AHI<50 and AHI≥50 categories (p=0.01 for both C3-C7 and adjusted C3-C7 visceral fat). Men showed significantly more total C3-C7 visceral fat than women (p=0.035). However, there was no significant difference between genders regarding adjusted C3-C7 visceral fat. The data show no significant correlations between visceral fat and tongue fat or parapharyngeal fat pads. However, there is a significant correlation between total visceral fat and total tongue volume (r=0.26, p<0.001).

Conclusions: Increases in C3-C7 visceral fat are associated with both increased AHI and BMI. Additionally, the correlation of total C3-C7 visceral fat with tongue volume suggests that tongue volume is secondary to tongue fat. This indicates that C3-C7 visceral fat has an important effect on the severity of OSA.

Circadian and Homeostatic Regulation of SIRT1 in Wake-Active Neurons

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In peripheral tissues, SIRT1 serves as a critical metabolic rheostat for circadian rhythms and can directly modify the circadian period. SIRT1 deacetylation of BMAL and PER, represses CLOCK:BMAL transcription, and delays PER2 degradation. Additionally SIRT1 regulates CLOCK function. In turn, CLOCK:BMAL regulates the circadian rhythm of SIRT1 activity through transcriptional regulation of NAMPT, the rate limiting NAD^+ synthesizing enzyme of the salvage pathway. This bidirectional SIRT1-circadian relationship has been shown in hepatocytes and fibroblasts, but we believe that a similar relationship in WAN would be beneficial by directly fine-tuning the circadian wake response for metabolic derangements. A first and necessary step is to determine whether SIRT1 and the major enzymes regulating its activity (NAD synthesizing enzymes, NAMPT and NMNAT1-3) demonstrate circadian rhythmicity. We have begun this exploration with micropunches of brain testing two circadian time points (zeitgeber 6 and 12) and at zeitgeber 12 we tested homeostasis by comparing responses in sleep allowed mice to responses in mice kept awake for 6 hours. To measure mRNA, we designed Taqman primer probes sets, each with confirmed superb sensitivity ($<10^3$ copies, $r^2 > 0.95$) RNA was purified in tissue samples, from which quantitative RT-PCR was run.

Results: Three brain regions were compared: cortex, lateral hypothalamus (orexinergic neuron nucleus) and dorsal pons (locus coeruleus nucleus). As expected Per2 increased from ZT6 to ZT12 in all three regions. SIRT1 and NAMPT (rate-limiting NAD^+ synthesizing enzyme) both increased with Per2 ($p<0.01$). More pronounced differences were observed with sleep loss. Here all enzymes increased dramatically, and NMNAT1 (nuclear SIRT1 NAD^+ synthesis enzyme and SIRT1 chaperone also increased in all three regions.

Conclusions: There are circadian and homeostatic influences for SIRT1 regulation in the brain. SIRT1 and its NAD^+ synthesizing enzymes show increased transcription in response to circadian time and history of sleep allowance. The elevated SIRT1 and enzymes is likely to enhance wakefulness at lights out and with short term sleep loss by increasing SIRT1 activity. We anticipate a very different response with longer sleep loss, where we expect SIRT1 and the NAD^+ synthesizing enzymes to be reduced, suggesting loss of this protective mechanism. Future studies are needed to measure and localize protein changes for SIRT1, NMNAT and NAMPT.

SIRT1: A Neuroprotectant for Catecholaminergic Neurons

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There is a particular fragility of wake-active neurons (WAN) in aging and in neurodegenerative processes. These neural groups are essential for alertness, optimal mood and cognitive performance; yet, heightened susceptibility of WAN is poorly understood. Injury to WAN is beginning to be recognized as an important source of morbidity in several neurodegenerative processes. Degeneration of the locus coeruleus noradrenergic neurons is observed in Alzheimer's disease and Parkinson's disease. In Parkinson's disease neuronal loss in the locus coeruleus can be as pronounced as loss of dopaminergic neurons, and loss of locus coeruleus neurons in Parkinson's correlates with cognitive impairment and hypersomnolence. Post-mortem brains from Parkinson's subjects have shown a loss of orexinergic neurons, with low CSF and prefrontal cortex orexin levels. Histaminergic and cholinergic neurons and their projections can be reduced in early Alzheimer's and Parkinson's. In Huntington's disease while CSF levels of orexin are within normal limits, the number of orexinergic neurons and orexin levels within the prefrontal cortex are reduced, and inclusion bodies are evident in orexin neurons in the hypothalamus. Serotoninergic WAN injury is apparent in Parkinson's disease, and in the neocortex in Alzheimer's disease and in frontotemporal dementia. In Alzheimer's disease, the dorsal raphe serotoninergic neurons can show tau fibrillary changes prior to forebrain pathology.

We recently identified oxidative injury to WAN and impaired wakefulness in a murine model of sleep apnea, and in a separate study we identified age-related endoplasmic reticulum stress injury in select WAN with impaired wakefulness. Seeking a common modulator of wake neuron function and health, we have begun to explore whether a key silence information regulator 2 homolog, SIRT1, could both regulate wake neuron activity and offer protection from physiological perturbances. As an NAD^+ dependent deacetylase, its activity is closely linked to metabolic status.

To examine a role for SIRT1 in the health and function of wake-active neurons we have explored two complimentary models (1) transgenic homozygotes and heterozygotes for congenital SIRT1 loss and wild type littermates and (2) conditional SIRT floxed knock down mice using an AAV CMV cre-recombinase vector injected into the brainstem and forebrain.

Results: We have begun with a focus on locus coeruleus and orexinergic neurons. Both SIRT1-/- and -/+ mice revealed small somata, thickened truncated, vacuolated axons and dendrites. There are reduced orexinergic boutons present in the locus coeruleus. Tyrosine hydroxylase and orexin-A transcription in each wake nucleus is markedly reduced. In target brain regions of the locus coeruleus, including the dentate gyrus of the hippocampus, we find reduces noradrenergic projections but also reduced glial cell line-derived neurotrophic factor (GDNF) despite normal levels of GDNF mRNA in surrounding tissue. In the conditional SIRT1 knock down mice, findings were primarily limited to catecholaminergic neurons.

Conclusions: Catecholaminergic neurons in the adult animal rely critically on SIRT1 for function and integrity. Longer-term loss of SIRT1 may also impact negatively on the health of other wake-active neurons. It is anticipated that metabolic dyshomeostasis with aging predisposes individuals to heightened vulnerability of wake-active neurons, and this, in turn, likely contributes to cognitive impairment as well as sleep/wake disturbances in aging.

Author Index