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14. ABSTRACT Work on sleep at Brandeis focuses on Drosophila melanogaster as well as the more traditional rodent models. The Drosophila works aims to exploit the genetic advantages of this organism yet still learn about aspects of sleep relevant to humans. The major finding has been that the human therapeutic Carbamazepine is a potent sleep-deprivation agent in flies. Current data indicate that its effects are mediated through the Rdl GABAA receptor, which has implications for the role of this drug in humans. One of the rodent laboratories is focused on the regulation of sleep and waking in the basal forebrain. The goal is to identify gene expression changes in its cholinergic neuronal subset, and specific neuron purification has been accomplished. Another rodent laboratory is studying the effects of sleep deprivation on the intrinsic electrophysiology and gene expression properties of neocortical neurons. Interesting changes in firing properties of layer 5 pyramidal neurons have been observed, and gene expression assays from these cells are underway. The final two projects involve the role of sleep in homeostatic plasticity and fear conditioning. These are being done both in vivo, in freely behaving animals, and "ex vivo, in cortical slices after sleep deprivation or training.					
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Table of Contents

Cover	01
SF 298	02
Table of Contents	03
General Introduction	04
Aims from the original Statement of Work, funded from January 15, 2004-January 14, 2008	
Aim 1 (Griffith) and Aim 2 (Rosbash):	05-07
Aim 3 (Birren):	08-10
Aim 4 (Nelson):	11-13
Aim 5 (Turrigiano):	14
Aims from the supplementary Statement of Work covered by contract amendment P00002, funded from January 15, 2005-January 14, 2008	
Mass Spectrophotometry Aim (Agar):	15-16
Bioinformatics Aim (Hong):	17-18
General Conclusions	19
Appendices.....	NA

General Introduction

There were eight PIs at Brandeis with a common interest in sleep. During the past year, Dr. Katz's research interests have lead away from sleep leaving seven labs dedicated to sleep investigation. Although the investigators run autonomous research programs with individual reports, every research program has interactions with one or more other of the other laboratories. This is a characteristic of Brandeis, i.e., close and productive inter-laboratory collaborations. Indeed, it is anticipated that all publications on sleep from Brandeis will reflect collaborations between two or more laboratories as we are a highly interactive research community.

Finally, I emphasize that the sleep community at Brandeis is relatively young. Most of us have no long-term track record in this field and have become interested in sleep only in the past few years. So we all began this work de novo. As a consequence, the group is only now entering a mature phase. As such, much of the work initiated over the past almost two years should reach fruition (i.e., publication) during the next year or two. Moreover, we are only now getting up to full speed, and many of these projects are continuing into the next phase. Please see the individual reports for more detail.

Each Aim in the original and supplemental Statements of Work is being pursued by an individual laboratory at Brandeis University. As such the introduction, body, key research accomplishments, reportable outcomes, conclusions, and references for each aim are reported as separate sections by the PI of the laboratory who is pursuing the aim. There are also several inter-lab collaborations, which should be obvious from the individual lab aims. They are presented in the chronological order they were funded. Aims 1-5 from the original Statement of Work, funded 01/15/04 to 01/14/08 and in the third year of research, are followed by the Mass Spec and Bioinformatics Aims proposed in the supplementary Statement of Work, funded 01/15/05 to 01/14/08 and in the second year of research. Aims 1 and 2 are described together, as the Griffith and Rosbash labs are working together on these *Drosophila*-sleep projects.

Aims 1 and 2: To identify the molecular targets of the wakefulness-promoting drug modafinil using forward genetics in *Drosophila* (Griffith). To determine the role played by *Drosophila* clock neurons in regulating sleep and to identify other groups of neurons in the *Drosophila* brain involved in regulating sleep and wakefulness (Rosbash)

Introduction

Drosophila exhibits a sleep-like state, previously referred to as “rest,” that shares multiple characteristics with human sleep (Hendricks et al., 2000; Shaw et al., 2000). Flies maintained in standard 12:12 light:dark (L:D) cycles have sustained periods of sleep, which occur predominantly during the dark period for female flies (Shaw et al., 2000). The effects of many sleep modulating compounds are thought to be conserved between flies and humans (Hendricks et al., 2000; Shaw et al., 2000), but there is little current understanding of the circuitry or channels that drive sleep in *Drosophila*. Consistent with its contribution to the understanding of many biological phenomena, the study of sleep will undoubtedly provide insight into human sleep and its regulation. Flies therefore provide a useful model system not only to dissect the genetic and biochemical underpinnings of sleep drive but also as a platform to screen for novel sleep and vigilance drugs.

Body

1) Role of clock neurons in sleep architecture.

In humans and flies, sleep occurs in a circadian pattern, with relatively more sleep during the night than during the day. Little is known about the circuitry underlying generation or timing of sleep in flies. To determine if the cells that make up the core of the circadian clock were important for generation of sleep, as opposed to just regulation of its timing, we expressed the cell death gene *hid* under control of *cry-GAL4*. This GAL4 driver expresses in almost all of the cells of the clock, and elimination of these cells with HID renders flies completely arrhythmic. Examination of sleep in these flies indicates that while the timing of sleep is disrupted, the total amount of sleep remains the same. These results suggest that the circadian clock regulates the timing of sleep, but is not critical for its generation in either L:D or D:D conditions.

To further investigate the mechanisms that underlie modulation of sleep by the clock we expressed transgenes encoding the *Shaw* potassium channel or *Shaw* RNAi in subsets of the clock circuit. Expression of *Shaw*, which is a leak channel, would be expected to block firing while expression of *Shaw* RNAi will enhance firing in cells whose resting potential is regulated by endogenous *Shaw* (Hodge et al., 2005). Animals expressing *tim-GAL4;UAS-Shaw* or *tim-GAL4;UAS-Shaw/pdf-GAL80* fall asleep earlier in both the day and night. These data suggest that sleep latency is controlled by the

dorsal group of clock neurons, including LN_{ds} and DN_s. Flies expressing *pdf-GAL4;UAS-ShawRNAi* have significantly less sleep in both the day and the night. These results are consistent with the activity of LN_{ds} normally acting to suppress sleep.

2) GABAergic circuits that control sleep.

In humans, both onset and maintenance of sleep is regulated by GABAergic transmission, and many of the important therapies for sleep disorders modulate GABA receptors. The Rosbash lab has defined a role for the GABA_A receptor RDL in fly sleep. We have begun to define the circuits in which this receptor acts. Expression of the *Shaw* potassium channel under control of *GAD2B-GAL4* significantly reduces both daytime and nighttime sleep. This suggests that inhibition of GABAergic transmission, by decreasing excitability of these neurons, is enough to disrupt total sleep.

3) Role of the GABA_A receptor subunit RDL in sleep.

We are currently in the final stages of preparing a manuscript for publication on the role of GABA_A receptors in regulating sleep onset and sleep maintenance in *Drosophila*. This paper describes strong evidence that these two ubiquitous features of sleep can be assayed in flies and are affected by GABA_A receptors like in mammals. However, the two systems have major difference in regulation. This conclusion stems from experiments examining fly strains with GABA_A receptor subunit (*Rdl*) mutants, with altered kinetics of desensitization, as well as strains with altered *Rdl* gene dose. The analysis of sleep onset and sleep maintenance in these strains shows that sleep onset is affected more strongly by the mutants affecting RDL desensitization kinetics, whereas sleep maintenance is more sensitive to gene dosage manipulations. We interpret these results to indicate that there are major differences in the circuits that regulate sleep onset and sleep maintenance. These differentially exploit the biophysical properties of the RDL receptors to provide behavioral complexity. Given the pivotal role of the GABA_A receptor complex in the pharmacological treatment of several neurological disorders as well as sleep, we propose that targeting specific aspects of GABA_A receptor function such as its desensitization kinetics is a potential route for the design of drugs to target differentially specific aspects of sleep such as only sleep onset.

4) Effect of carbamazepine on fly sleep.

Based on a connection between human circadian rhythms and manic-depressive (bipolar) disorder, we began an investigation of whether therapeutic drugs for this disease, especially those with no known or well-agreed upon target, might have an effect in flies. The purpose was to use *Drosophila* genetics and identify the missing, behaviorally relevant, drug-target. We were most keen on lithium, but this drug has only a modest and difficult to track phenotypic effect. In contrast, carbamazepine (CBZ), a drug used for bipolar disorder and even more frequently for epilepsy, dramatically reduces sleep and increases locomotor activity. This is surprising, since its effect on humans suggests that it should have the opposite effect on sleep, namely, act as a hypnotic. To address this paradox, we assayed the physiological effects of the drug on *Drosophila* RDL and human GABA_A channels in the *Xenopus* oocyte system. The results were interesting and consistent with an opposite effect on flies and human GABA_A receptors. CBZ exerts its effect on the fly RDL channel by enhancing channel desensitization. The *Rdl*^{A302S} mutant channel, which desensitizes poorly, is resistant to CBZ. Importantly, flies carrying this mutation are partially resistant to the behavioral effects of CBZ, specifically to the potent effects of CBZ on sleep onset. This supports the idea that desensitization of RDL is critical for onset. Although we

intend to publish the physiology and even the behavior (several labs are now using CBZ to sleep-deprive flies, as it is completely dependable and much more convenient than shaking), we have been slowed by the fact that the sleep maintenance effects of CBZ are almost certainly mediated by actions on other targets. Straightening out this phenomenon has caused the delay in writing up this story.

Key Research Accomplishments

- Defining a role for the of GABA_A receptor in sleep latency and sleep maintenance in *Drosophila*
- Defining an effect of the drug carbamazepine on fly sleep.
- Defining a role for the circadian neurons in sleep
- Defining an effect of GABAergic cells in sleep

Reportable Outcomes

The first three key research accomplishments will result in manuscripts shortly.

Conclusion

We have begun to combine genetics and pharmacology to address sleep in *Drosophila*. The results indicate that GABA_A receptors and clock neurons are important in ways that resemble their contributions to mammalian sleep. We have also defined a role for carbamazepine, which might help address its unknown mechanism of action as a human therapeutic. Finally, we are at the beginning stages of examining GABAergic cells, which have not been very well studied in the fly system. Given the important role of the receptors, we anticipate that these inhibitory cells will be important and we intend to define their relationship to the clock neuronal system.

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Aim 3: Regulation of gene expression during sleeping and waking (Birren).

Introduction

Basal forebrain cholinergic and GABAergic neurons contribute to a complex regulatory circuit that controls mammalian sleep patterns. Our goal is to understand how gene expression changes in the different components of this circuit regulate sleep-wake cycles. Basal forebrain cholinergic neurons project to the cortex and hippocampus where they modulate cortical circuits associated with arousal and attention (Berger-Sweeney et al., 2001). Cholinergic neurons are active during waking and REM sleep (Detari et al., 1984; Detari and Vanderwolf, 1987; Szymusiak and McGinty, 1986), contributing to cortical activity associated with the waking state. The GABAergic neurons form local connections within the basal forebrain, as well as co-projecting to cortical and hippocampal targets. GABAergic neurons have increased activity during non-REM sleep (Pollock and Mistlberger, 2003; Sanford et al., 2003), regulating the activity of the cholinergic neurons in the circuit, and contributing to the regulation of sleep-wake cycles (Vazquez and Baghdoyan, 2003). While the importance of this system in sleep regulation is well established, little is known about the transcription patterns that underlie the sleep-wake transitions. A basic premise of our work is that mass analysis of gene expression in multiple cell types subserving opposing functions during sleeping and waking cannot provide an in-depth picture of sleep regulatory pathways. We are therefore investigating patterns of gene expression during sleep deprivation in subpopulations of neurons in the basal forebrain. These experiments will permit us to begin to construct a gene-based regulatory circuit for the control of sleep-wake cycles.

Body

In previous periods we confirmed overall changes in gene expression in rat basal forebrain tissue following sleep deprivation. We also established approaches for the identification and isolation of cholinergic neurons within the basal forebrain to provide a purified population for microarray screens. We defined basal forebrain regions containing choline acetyltransferase-expressing neurons and identified the p75 low affinity neurotrophin receptor as a selective marker for cholinergic neurons within the medial septal nucleus and the horizontal and vertical limb of the diagonal band of Broca. We used the p75 cell surface marker and fluorescence-activated cell sorting to purify basal forebrain cholinergic neurons from adult mice. We have continued this project over the past year by investigating experimental approaches for sleep deprivation in mice, isolating p75-expressing medial septal cholinergic neurons from control and sleep-deprived mice, and carrying out DNA microarray screens on these different populations. In contrast to the modest changes in gene expression changes seen in analysis of mixed neuron types in sleep-deprived animals, our preliminary analysis shows that sleep deprivation results in large changes in expression in a number of genes, including genes known to play a regulatory role in neural circuits.

1) Establishment of a mouse sleep deprivation protocol. A long term-goal of this project is to investigate sleep patterns in mouse models deficient for sleep-regulatory genes identified in our microarray screens. For this reason, and to take advantage of available mouse Affymetrix microarrays, we tested several different approaches for sleep deprivation in mice. Our goal was to minimize interventional and handling stress while obtaining significant sleep deprivation in an experimentally amenable system. After experimenting with a rotating platform and a direct handling approach, we have chosen a rotating wheel system for our sleep deprivation studies. In this system a mouse is positioned in a motorized rotating wheel that moves very slowly. The mouse has to periodically move to avoid falling as the wheel rotates over the course of a six hour period. In control experiments mice are placed in the apparatus for the same period of time, but the motorized wheel is intermittently shut off during the six hour experiment. Thus, control animals share the same environment, but are less sleep deprived. In

both conditions there is a minimum of handling stress, and food and water are constantly available. While the slow movement of the wheel minimizes the total amount of exercise, it is also possible to carry out an exercise control in which mice are placed in the wheel without any movement for the same period. We have now subjected mice to this sleep deprivation and the control protocol. Following sleep deprivation, mice were sacrificed and p75-expressing basal forebrain cholinergic neurons were isolated using fluorescence-activated cell sorting.

2) *Microarray analysis of sleep deprived and control basal forebrain cholinergic neurons.* Mouse Affymetrix microarrays (mouse 430 set, 39,000 transcripts) were screened with probes derived from basal forebrain (medial septal nucleus) cholinergic neurons from sleep deprived and control mice. We found 374 transcripts that showed a greater than 4-fold change in expression following sleep deprivation, suggesting that there are large changes in gene expression within individual subpopulations of basal forebrain neurons. Within this group of 374 transcripts, 271 transcripts showed an increase following sleep deprivation and 103 showed a decrease. Regulated transcripts represented several different gene classes with changes in genes associated with neuronal differentiation, neurotransmitter regulation, neurotrophin signaling pathways, and synaptic proteins. Intriguingly, neurotransmitter receptor genes for both cholinergic and GABAergic systems were regulated, suggesting potential regulatory interactions between these systems.

We are now in the process of repeating our microarray screens with RNA derived from sleep deprived and control animals. We will also include additional control conditions to determine the contribution of stress and exercise responses to transcriptional changes. We will verify the results of our microarray screens using real-time PCR to examine expression levels of candidate genes in cholinergic neuron RNA isolated from sleep-deprived and control animals. The identification of candidate sleep regulatory genes from among the verified group will be guided by studies of proteins involved in the regulation of neural circuits. We will examine the neuronal subtype-specific expression patterns of candidate genes during sleep and waking using immunostaining and/or *in situ* hybridization of intact tissue. In future experiments we will develop neuron isolation methods for additional neuronal subtypes within the basal forebrain to permit cell type-specific microarray analysis. These studies will permit us to define the interacting patterns of gene expression that underlie mammalian sleep regulation.

Key Research Accomplishments

- Establishment of a mouse sleep deprivation model that controls for handling stress.
- Isolation of a purified population of adult basal cholinergic neurons from sleep deprived and control mice.
- Demonstration of cholinergic-specific gene expression changes in sleep deprived mice.

Reportable Outcomes

-none

Conclusion

The cholinergic and GABAergic neurons of basal forebrain provide form a key neural circuit for the regulation of sleep patterns. The experiments in this study are defining the specific role of the cholinergic neurons in the regulation of sleeping and waking. This information is the first step in defining a dynamic pattern of gene expression across a neural circuit that regulates sleep-wake cycles and will shed light on the genes that regulate transitions between these states.

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Aim 4: Effects of sleep deprivation on neuronal physiology and gene expression (Nelson).

Introduction

In the last report we presented preliminary whole cell recording data from layer 5 pyramidal neurons of the primary motor cortex from control and sleep deprived mice.

Specifically, we observed that neurons from sleep deprived animals fired at lower frequencies upon injection of depolarizing current pulses as compared to yoked control mice. This was consistent with the observation of a nearly 26 % increase in membrane conductance in case of sleep deprived mice compared to the yoked control.

We then set out to investigate the underlying ion channels that could be contributing to the increase in conductance upon sleep deprivation. Hyper-polarization activated mixed cation conductance, I_h , and inwardly rectifying potassium conductance K_{IR} were isolated but were not found to be significantly different between the control and sleep deprived mice. Further experiments showed no significant differences in intrinsic excitability or membrane conductance between sleep deprived and yoked control mice after 24 hour sleep deprivation. However, both these groups had significantly increased membrane conductance (normalized to capacitance) compared to cage control animals (cage control: 91 ± 6 S/F, yoked control: 106 ± 4 S/F, sleep-deprived: 103 ± 3 S/F). Our interpretation of these results is that the variable increase in membrane conductance in our sleep deprivation experiments may be caused by stress due to restricted mobility (McDermott, LaHoste et al. 2003) and/or to surgical implantation of recording electrodes for monitoring EEG and EMG. It is possible that the sleep-deprived mice were more stressed than the control mice in our preliminary experiments in which we observed an increase in membrane conductance and a higher current threshold for action potential firing.

Unfortunately, gene expression analysis was also relatively unrevealing. In a pair of experiments comparing expression in pyramidal neurons from the cortices of deprived and yoked controls, 30 transcripts were differentially expressed by 1.5 to 2.5 fold, but no obvious pattern of affected genes was apparent, and false positives could not be rigorously excluded. We feel confident that we have excluded the possibility of huge changes in gene expression in these neurons following 24h sleep deprivation, but the confound of stress may have made more modest changes difficult to detect.

We have decided in the coming period to focus our efforts on a neural cell type known to be involved in the regulation of sleep and alertness, the noradrenergic neurons of the locus coeruleus (LC). This brain stem nucleus projects widely throughout the brain, and is implicated in the modulation of sleep/wake states and vigilance. LC neuronal activity is strongly regulated across the sleep/wake cycle. We obtained mice that express GFP under the control of the tyrosine hydroxylase promoter. This allows us to unambiguously identify the catecholaminergic neurons in the LC in brain slice physiology experiments and to isolate them for gene expression experiments. Our initial experiments have focused on characterizing the baseline electrophysiology of these neurons and the complement of ion channels that they express. Although intrinsic electrophysiological properties of these neurons have previously been studied, little is known about the specific ion channels that mediate those properties. These channels may represent important targets for regulating alertness.

LC neurons have depolarized resting membrane potentials (between -40 to -50 mV) and fire spontaneous action potentials in the slice at frequencies $\sim 0.2 - 5$ Hz. We carried out cell-type specific mRNA profiling and found that LC neurons express a very different set of channels from those we have previously identified in cortical and hippocampal pyramidal neurons and interneurons. Specifically, we have found that LC neurons lack expression of all but one splice variant of the KCNQ subunits that

mediate the M-current. Physiologically they lack an M-current defined with a pharmacological blocker (XE-991) and this may contribute to their depolarized resting membrane potential. These neurons are also unusual in that they lack the HCN1 subunit that contributes in most other neurons studied to the hyperpolarization-activated cation current. Physiologically LC neurons lack a postsynaptic I_h , although this current can also be expressed in axon terminals where it presynaptically regulates transmitter release. If this is the case in LC neurons, it is due to expression of the HCN2 and HCN3 subunits. We have also identified a calcium channel regulatory subunit that is selectively expressed in LC neurons. We hypothesize that this could be important in regulating the substantial resting calcium current that these neurons are known to possess, that may contribute to their spontaneous activity. In order to test this we are in the process of obtaining knock-out animals produced by Verrity Letts at Jackson labs. Finally, we have also examined the ligand gated receptors expressed by these neurons. We have identified the serotonin receptor likely to mediate the known interaction between the serotonergic Raphe nucleus and the LC (Htr1d). We have also confirmed the prior observation that LC neurons lack the $\alpha 1$ subunit of the GABA-A receptor and have found that this is correlated with GABA-A mediated IPSCs that have slow kinetics, as expected from other systems. A paper describing these correlated gene expression and physiological results is in preparation.

We have also begun a collaboration with Jeff Agar to examine protein level expression in LC neurons. These neurons express a rich complement of peptides at the prepro RNA level. We have facilitated Jeffs proteomic experiments on LC neurons in brain slices and will help him correlate RNA and protein expression of peptides in these neurons.

After completing our baseline characterization of these neurons we will look for circadian changes in gene expression and electrophysiology in these neurons and will study the effects of sleep deprivation. For the sleep deprivation studies we will switch to a briefer (6 h) and less invasive method of achieving sleep deprivation (gentle handling, addition of novel objects to cage). Assuming proteomic approaches are successful, these will be used to complement analysis at the RNA level.

Key Research Accomplishments

- Found that previously observed physiological consequences of deprivation on firing properties of layer 5 pyramidal neurons in mouse motor cortex were most likely due to stress of deprivation.
- Carried out RNA isolation, amplification and hybridization experiments from cortical layer 5 pyramidal neurons, and found that deprivation-induced changes in gene expression in this cell type are modest.
- Studied the baseline physiological properties of Locus Coeruleus (LC) neurons known to be important in regulating sleep and vigilance.
- Used cell-type specific expression profiling to identify multiple channels specifically present and absent from LC neurons that contribute to their unique physiological properties.

Reportable outcomes

- A paper describing our physiological and gene expression results on LC neurons is in preparation

Conclusion

Changes in the physiology and gene expression of a neocortical pyramidal neuron subtype are subtle and eclipsed by the effects of stress. Basic physiological properties of locus coeruleus neurons and the specific ion channels that mediate them have been identified. These studies have identified

multiple potential targets for manipulations of alertness in mammals. Further work will address circadian and sleep related changes in LC physiology and gene expression.

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Aim 5: Role of Sleep in Homeostatic Plasticity (Turrigiano).

Introduction

The major goals of this Aim are to examine the role of sleep in homeostatic cortical plasticity. We have taken two approaches: one, to examine this in freely behaving animals using chronically implanted electrode arrays, and an “ex vivo” approach in which animals are first sleep deprived and then brain slices are made to examine changes in cortical microcircuitry. The in vivo approach has proved more difficult than anticipated and so in future we are shifting our focus to the ex vivo approach.

In vivo approach:

We have had numerous technical difficulties with this approach, largely because we are trying to record from superficial cortical layers of young animals. We had to reduce the size and weight of the arrays to implant them on small animals without affecting their ability to move freely. We now have a working electrode design, but have run into significant problems getting stable recordings from implanted animals, because for the age animals we require the brain is still growing. While we have had some success the yield (number of neurons we can stably record from) has been low. In order to move to older animals, we have had to first show that homeostatic plasticity is present in older (adult size) animals. We have now verified that homeostatic plasticity is present in upper layers of cortex in older animals, so the next step is to try implanting our modified electrodes into older animals with slower growth. We are continuing with this approach, but at a reduced level of effort. Don Katz continues to serve in an advisory role.

Ex vivo approach:

To supplement the *in vivo* approach to studying the modulation of cortical plasticity by sleep, we are also taking an ex-vivo approach that has been very successful in my lab. In collaboration with Sacha Nelson’s lab we have shown that sleep deprivation depresses cortical function as assayed ex vivo in mouse motor cortex. My postdoc Kiran Nataraj has shown that a similar depression of cortical activity occurs in visual cortex; this suggests that sleep deprivation produces a generalized depression of cortical activity. This could have a profound impact on cortical synaptic plasticity, which depends on activity propagation through cortical networks. The next steps will be to determine the mechanism of this depression, and to ask whether synaptic plasticity is impaired. In addition we are asking whether synaptic scaling induced by visual deprivation is prevented by sleep deprivation.

Key Research Accomplishments

1. Miniaturization and modification of electrode arrays for young rats in upper cortical layers.
2. Demonstration of generalized depression of cortical activity induced by sleep deprivation in rodents.
3. Demonstration that synaptic scaling operates in adult animals.

Reportable Outcomes

We expect to have a paper out on the sleep deprivation effects on cortical activity shortly.

Conclusions

Due to difficulties with in vivo recordings we are shifting our focus to an ex vivo approach to examining the interactions between sleep and homeostatic plasticity.

Mass Spectrophotometry Aim: To examine qualitative and quantitative protein expression changes during sleep and wake (Agar)

Introduction

Dr. Jeffrey Agar was hired at Brandeis in June 2005 as an Assistant Professor of Chemistry. His research focuses on mass spectrometry, a technique that will augment research on the mechanisms of sleep and complement microarray gene expression and bioinformatics research.

Body

Since his hire one and a half years ago, priorities have been to remodel laboratory space, choose the proper mass spectrometry equipment, and then procure, install, and make functional this equipment. These goals have now been met and the laboratory is now fully operational. The Agar laboratory has also invented a technique for tissue preparation for mass spectrometry imaging, and has submitted an article to "Nature Methods" on this topic, acknowledging the support of the US DOD. A post doctoral fellow, Satish Murari, was hired and is spearheading collaboration between the Agar laboratory, the Rosbash laboratory, and the Nelson laboratory to study sleep. In these studies, transgenic mice that contain specific neurons that are labeled with fluorescent proteins, are used. The fluorescently labeled cells are used as spatial markers, to allow the researchers to know exactly which location they are measuring within the brain. These studies take advantage of MALDI imaging capabilities, that allow protein imaging with 100 micrometer resolution, approaching the size of large cells and certainly making possible the observation of important changes within small brain regions, and expertise in mouse genetics and anatomy. This approach is particularly well-suited for assaying changes in neuropeptide composition in defined brain nuclei, which accompany sleep-deprivation for example. For future experiments, regions of the brain known to be integral to the sleep process, such as the SCN, will be dissected from awake and asleep mice, and changes to peptides and proteins that result from sleep will be characterized. The ultimate goal of these studies is to search for major changes in protein composition in brain regions important for sleep regulation as a function of sleep and wake or as a function of sleep deprivation.

In general terms, the mass spectrometry facility now has the capacity to perform the following experiments with our new equipment: 1) MALDI-mass spectral imaging; 2) Two-dimensional protein and peptide separation on-line with MS; 3) In-gel digests to identify proteins from gels; 4) Whole-protein post-translational modification studies; 5) Protein conformational changes by hydrogen/deuterium exchange and on-line digest; 6) Protein binding partners; 7) Quantitation with or without labeling (ICAT, ITRAQ, etc); 8) MALDI and electrospray FTMS for the identification of small molecule structure; 9) Gas phase reactions within the FTMS. We have placed an emphasis on data analysis, and are using the same project management database that HUPO (human proteomics organization) uses to store the entire human proteome.

Key Research Accomplishments:

Two manuscripts have been submitted by the Agar group, one has been accepted, and one is in review. One manuscript involves proteomics methods development for the study of protein post-translational modification, and the second involves the development of MALDI mass spectrometry imaging methods for proteomics at high resolution.

Reportable Outcomes:

The Agar laboratory has also invented a technique for tissue preparation for mass spectrometry imaging, and has submitted an article to "Nature Methods" on this topic, acknowledging the support of the US

DOD. This will enable high resolution proteomics, to complement microarray data. A provisional patent has been filed on techniques for MALDI imaging of tissues.

Conclusions:

The mass spectrometry laboratory has a number of unique capabilities and is among the most advanced laboratories in the world. It is anticipated that this advanced technology will be an integral component of research on the mechanisms of sleep at Brandeis.

Bioinformatics Aim: To assist sleep labs at Brandeis University with analysis of microarray data (Hong)

INTRODUCTION

Dr. Hong is specialized in bioinformatics and computational biology and was hired by Brandeis University in August 2005. During the last year, he continued building up his computational systems biology group and recruited three more Ph.D. students. His group is now conducting research on high-content image analysis and biological network modeling.

BODY (Research)

(a) High-Content Image Analysis

High-content screening (HCS) is a powerful high-throughput technology for generating cellular images that are rich in phenotypic information and has been widely used in drug discovery. Recently, HCS has been combined with RNA interference (RNAi), a revolutionary approach for silencing gene expression and generating functional data, to become an essential image-based high-throughput method for studying genes and biological networks through RNAi-induced cellular phenotype analyses. Never before in history could cellular images be generated at terabyte scales in matter of days. However, the advancement of experimental technologies usually creates great challenges for data analysis. We have developed a prototype called imCellPhen (interactive mining of cellular phenotypes) for interactively analyzing HCS images. Our initial focuses are neuron images generated in genome-wide studies using *Drosophila* disease model. A NIH proposal has been prepared based on the above preliminary results.

(b) Biological Network Modeling

Cellular activities, organ developments, and animal behaviors are governed by complex biological networks, which contain dynamically interacting components coupled via inter- and intra-cellular signaling and transcriptional regulations. We have developed a methodology for elucidating the structure of biological networks by integrating heterogeneous biological data such as phenotype and gene expression data. We chose *C. elegans* vulval induction, which is a paradigmatic example of animal organogenesis with extensive experimental data, as our first application. The model generated by our methodology is capable of simulating vulval induction under 37 different genetic conditions. Our model also contains several hypothetical relationships between network components, and hence can serve as guidance for designing future experiments.

REPORTABLE OUTCOMES:

- NIH Funding Application. Proposal title: Intelligent Interfaces for Interactively Analyzing High-Throughput Cellular Images. This is the second submission. The first submission received a promising score and was encouraged to submit a revision.
- Ph.D. student recruited since last report: Xiaoyun Sun, Chen Lin, and Yuanyuan Yue, Computer Science, Brandeis University.
- Invited speaker: Symposium on Image Analysis at Harvard Medical School. April 4, 2006.
- Delegate: Frontiers in Live Cell Imaging Meeting, NIH, Natcher Conference Center, Bethesda, MD USA, April 19-21, 2006.
- Invited speaker: 2006 Workshop on Multiscale Biological Imaging, Data Mining & Informatics, Santa Barbara, CA, USA, Sept 7-8, 2006.

CONCLUSION:

The Hong group is building up the strength and has demonstrated its potential by developing a few bioinformatics tool for systems biology research. The group will continue its research on the systematic studies of biological systems by integrating heterogeneous biological data including biological sequences, microarray data, proteomics data, and images of cells treated by RNAi and various chemical compounds.

Conclusion

The sleep consortium at Brandeis has entered a mature phase and much of the work initiated over the past three years is reaching fruition (i.e., publication) and anticipate multiple publications in the coming 12 months. The addition of Drs. Agar and Hong over the past few years has greatly aided the sleep efforts at Brandeis by bringing to the group advanced mass spectrometry and bioinformatics tools, respectively.