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13. ABSTRACT (Maximum 200 Words)

The Sleep Consortium at Brandeis consists of 6 PIs, who naturally group into three subsets. Rosbash and Griffith are collaborating on sleep studies in flies (*Drosophila melanogaster*). The two projects are investigating how drugs affect sleep and have identified the $GABA_A$ Receptor gene Rdl as a likely drug target for carbamazepine, a drug which enhances locomotor activity in a dose dependent manner. Preliminary results indicate the inhibitory system potentiates sleep in flies like in mammals, which is the first indication for a role of this system in flies. Birren and Nelson are both interested in specific gene expression in defined subsets of sleep-related neurons as a function of sleep state. These groups will use similar microarray technology, developed and perfected in the Nelson laboratory. Nelson and has perfected RNA isolation, amplification and hybridization from cortical layer 5 pyramidal neurons for microarray analysis of cell-type specific gene expression and has also designed and built an apparatus to implement a sleep deprivation technique in mice. Birren has determined that the brain-derived neurotrophic factor gene is modulated by sleep deprivation in the basal forebrain and has carried out a pilot DNA microarray screen using probes from sleep-deprived and control rat cortex. Turrigiano's and Katz's goal is to understand how plasticity and learning are related to sleep and are focusing on live recording from neurons during task performance and/or development. They have successfully miniaturized electrode arrays for young rats. Katz meanwhile has successfully recorded simultaneous cortical oscillations from amygdala, hippocampus, and cortical layers. In sum good progress is being made on all fronts.

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Introduction

The Sleep Consortium at Brandeis consists of 6 PIs, who naturally group into three subsets. Rosbash and Griffith are collaborating on sleep studies in flies (*Drosophila melanogaster*). These two projects are also interested in drugs and how they affect sleep. Birren and Nelson are both interested in specific gene expression in defined subsets of sleep-related neurons as a function of sleep state. They are currently focusing on different regions of the brain and using different protocols. However, they will both use similar microarray technology – originally worked out in the Nelson laboratory – and will probably end up collaborating extensively. The third group, Turrigiano and Katz, are focusing on live recording during task performance and/or development. The goal is to understand how plasticity and learning are related to sleep. Good progress is being made on all fronts.

Each Aim in the original Statement 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.

Aim 1: To identify the molecular targets of the wakefulness-promoting drug modafinil using forward genetics in *Drosophila* (Griffith)

Introduction

Drosophila exhibit a sleep-like state that shares several characteristics of human sleep (Hendricks et al., 2000; Shaw et al., 2000). Flies therefore potentially provide a useful tool with which to dissect the genetic drive for sleep. Flies entrained in 12:12 light/dark (L/D) cycles have sustained periods of activity and rest; rest occurring primarily during the dark period for female flies (Shaw et al., 2000). The effects of many sleep modulating compounds are thought to be conserved. Our initial intention was to investigate the effects of Modafinil on fly sleep and to use forward genetics to identify its targets. Unfortunately, we have been unable to reproduce the published effects of this drug (Hendricks et al., 2003) and we have reformulated our goals. We will: 1) screen for mutations that disrupt amphetamine-mediated sleep disruption and rebound 2) identify the molecular mechanism of carbamazepine's sleep disrupting effects 3) map the circuitry that controls sleep. These experiments are being carried out in collaboration with the Rosbash lab.

Body

1) Screen for amphetamine-insensitive mutants. We are investigating the effects of stimulatory compounds such as amphetamine to manipulate sleep in flies. The goal of this project is to analyze the 'rebound' effect in sleep-deprived flies in order to determine molecular and tissue specific pathways driving sleep. Amphetamine is fed only during the dark phase to prevent rest, inducing locomotor activity during the normal rest period. During the following light phase, rest is analyzed. Flies fed amphetamine appear to exhibit increased locomotor activity during feeding in a dose-dependent manner. High doses of amphetamine are toxic, and individuals that die exhibit very high locomotor activity immediately before death. We will mutagenize flies with EMS, obtain a baseline activity profile and then screen for lines that have reduced locomotor effects of amphetamine. Secondary screens of these lines will be done to determine if mutations affect sleep/wake cycles, baseline locomotor behavior and rebound sleep.

2) Molecular mechanism of Carbamazepine (CBZ) effects on sleep. In humans, CBZ is used to treat epilepsy, trigeminal neuralgia, bipolar disorder and benzodiazepine withdrawal. The basis of these actions is believed to be due to interaction of the drug with Na^+ channels and $GABA_A$ receptors. In flies, Jose Agosto in the Rosbash lab has discovered that CBZ causes both an increase in locomotor behavior and a decrease in sleep. The effects of CBZ in flies appear to be mediated through the *Rdl* $GABA_A$ receptor since *Rdl* point mutants that are resistant to dieldrin (an insecticide) are also resistant to CBZ. Flies with reduced levels of Rdl protein (*Df/+*) are hypersensitive to the drug. To determine if CBZ interacts directly with the receptor and how it affects GABA responses, we have begun to record from *Xenopus* oocytes expressing wild type

and mutant Rdl. Rdl in this system gives a robust, but quickly desensitizing response to GABA that can be measured electrophysiologically. Application of CBZ appears to selectively stabilize the desensitized state of the receptor. The point mutant that blocks CBZ effects *in vivo* does not show this type of stabilization. These results suggest that the *Rdl* $GABA_A$ receptor is a direct target of CBZ and that this interaction can largely account for the behavioral effects of the drug.

3) Sleep circuits. Circadian and homeostatic circuitries are thought to underlie the sleep-like state in flies. By using cell-specific ablation, it has been shown that the timing of locomotor activity is driven in distinct groups of clock neurons (Stoleru et al., 2004). We therefore analyzed the pattern of sleep-like behavior in flies with genetically ablated circadian neurons in *cry*-Gal4;UAS-*hid* transgenic animals (Stoleru et al., 2004) under L/D and D/D conditions. We observe the same average overall amount of sleep in *cry* ablated animals as controls with respect to each other in both L/D and D/D conditions. In D/D both groups slept twice the average as seen in the L/D condition. These results suggest that while the clock may act to modulate the pattern of sleep, clock neurons are not part of the circuit that generates the sleep state. The robust effects of CBZ on sleep suggest that neurons that express the *Rdl* $GABA_A$ receptor may be critical for either the generation of sleep or the output of the sleep circuit. To gain entrée into this circuit, we will generate antibodies to Rdl and do *in situs* to determine which neurons in the adult brain express this receptor.

Key Research Accomplishments

- amphetamine has biphasic effects on locomotor activity in flies
- circadian clock neurons do not generate sleep
- Carbamazepine enhances locomotor activity by stabilizing the desensitized state of the *rdl* $GABA_A$ receptor

Reportable Outcomes

None

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Hendricks, J. C., Finn, S. M., Panckeri, K. A., Chavkin, J., Williams, J. A., Sehgal, A., and Pack, A. I. (2000). Rest in Drosophila is a sleep-like state. Neuron *25*, 129-138.

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Aim 2: 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.

We are interested in fundamental aspects of sleep in humans, namely, how we sleep and why we sleep? To address these issues, we study the process in the fruit fly Drosophila melanogaster. This choice of organism is dictated by several considerations. It is now almost universally accepted that flies undergo a process similar to mammalian sleep (Hendricks et al., 2000; Shaw et al., 2000). Flies adopt a specific posture and/or resting place during "sleep." The process is regulated by circadian rhythms like in humans, and we have more than two decades of experience studying circadian locomotor activity in Drosophila. These rhythmic cycles include periods of rest now known to encompass Drosophila sleep. There is also evidence for a parallel homeostatic regulatory mechanism (sleep rebound) in flies. Finally, fly sleep is associated with a decrease in sensory responsiveness as well as a decrease in brain activity. Because of these similarities with human sleep, a deeper understanding of fly sleep should illuminate fundamental aspects of the sleep process.

A second interest is drugs that influence sleep and other aspects of brain function. We are particularly interested in pharmaceuticals with no known molecular target or where the target is uncertain or controversial. Drosophila is particularly useful for this kind of search, because a resistant or hypersensitive mutant identifies a potential drug target. The mutant can be chemically induced or a candidate gene approach can be taken. Our studies on sleep and drugs are being conducted in collaboration with the Griffith laboratory

Body

In this first year, we screened a number of human drugs for their effects on fly sleep and circadian rhythms. The most interesting and tractable was carbamazapine (CBZ), a drug used for epilepsy, trigeminal neuralgia and bipolar disorder. There is no well-agreed target, as voltage-gated sodium channels, adenosine receptors and $GABA_A$ receptors have all been proposed to be affected by the drug and the major reason for the therapeutic impact. Many side effects may be due to multiple targets, and there is no in vivo data to support any of these proposed mechanisms. A well-defined mechanism is necessary to make more specific and therefore more effective drugs. Because flies are reported to have all of these channels and receptors, it is likely that the molecule(s) mediating the CBZ therapeutic effect is present in Drosophila.

• **CBZ increases Drosophila locomotor activity markedly and in a dose-dependent manner.** The CBZ effect takes place preferentially during the night or the subjective night – if the assay is being done in constant darkness. (Subjective night is the 12 hr period that would have been night had the entraining 12:12 light-dark cycle not been terminated by placing the flies in constant darkness.) In other words, the active period is less affected, and the inactive-sleep period is strongly inhibited. By assaying specifically sleep rather than just activity, we have been able to document a strong inhibitory effect on sleep as well as a stimulatory effect on activity.

• **Based on the candidate gene approach (and a good guess), we identified the $GABA_A$ Receptor gene Rdl as a likely drug target**. There are two known mutant strains, the most

useful of which contains a point mutant (an A->S substitution in the M2 domain). It was isolated based on resistance to dieldrin. This insecticide is a GABA antagonist, and the mutant strain is also resistant to other GABA antagonists like PTX. Remarkably, this Rdl mutant is almost completely resistant to CBZ. Consistent with the notion that this receptor is a major CBZ target in Drosophila, changing the number and composition of Rdl receptors in vivo (with genetic means) results in alterations in CBZ sensitivity.

This raises a number of specific questions, which we are currently pursuing. First, what is the relationship of the inhibitory-GABA system to sleep in flies? There is nothing currently known about GABA and sleep in flies.

•We have preliminary results indicating that the inhibitory system potentiates sleep in flies like in mammals. This is based on an increase in fly sleep with the GABA agonist Phenobarbital. This then leads to the second question, namely, how does CBZ influence the Rdl receptor? We are currently involved in biophysical studies designed to answer this question, as described in more detail in the Griffith report. Based on the preliminary Phenobarbital phenotype, we favor the idea that CBZ acts as an antagonist, and the biophysics currently suggests that CBZ stabilizes the desensitized form of the receptor (see Griffith report).

Reportable Outcomes

We have nothing concrete at present, although a manuscript is in the initial stages of preparation. I suspect it will be submitted and accepted before the next report in a year.

Conclusion

In addition to the obvious (CBZ stimulates activity, inhibits sleep and acts through the Rdl receptor), there are three unanticipated consequences of these observations. First we think that CBZ will be very useful as a pharmacological agent for sleep-deprivation in flies. It is very difficult to keep flies awake by manual means, even in a "shaking" machine. Second, our detailed observations suggest that longer periods of inactivity may better describe fly sleep than the 1-5 minutes that is generally used n the literature. The arguments here are a bit complicated and will require peer review as well as publication to be fully appreciated, but I think that this conclusion will be well received by the fly-sleep community. Third, I believe that we can learn something fundamental about the anatomical basis of sleep and vigilance in flies. We should be able to discover where CBZ-sensitive Rdl receptor is expressed, i.e., where these inhibitory centers are located. The Griffith lab is examining the circadian system, and we will examine more broadly other areas of the fly nervous system.

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

Introduction

The goal of this project is to determine how gene expression in the basal forebrain is regulated during sleeping and waking. The basal forebrain constitutes an extrathalamic relay from the brainstem to the cortex that has been implicated in the control of sleep. Thus, identification of genes with altered expression in sleep/wake cycles is likely to provide novel information on the regulatory pathways that underlie the control of sleep patterns. In the initial period of this project we have 1) established dissection and culture protocols for mouse and rat basal forebrain, used these cultures to define markers for GABAergic and cholinergic neuron populations, and demonstrated the utility of a cholinergic marker in FACS-based cell isolation. 2) We have carried out sleep deprivation experiments and analyzed expression of a known sleep-regulated gene in the rat cortex and basal forebrain; and 3) we have initiated a pilot DNA microarray screen using cortical probes from sleep deprived and control animals. These experiments have established techniques that will be used for more extensive cell type specific microarray screens. We have demonstrated that our sleep deprivation protocols result in changes in cortical gene expression consistent with previously published studies and have shown, for the first time, sleep-dependent regulation of brain-derived neurotrophic factor in the basal forebrain.

Body

The principle neuron types of the basal forebrain are the cholinergic and GABAergic neurons, both of which project extensively to the cortex and hippocampus. Much attention has been focused on cholinergic basal forebrain projections, which play a major role in the control of arousal, attention, and in the activation of neocortical circuits. The activity of these cortically projecting basal forebrain neurons increases during waking and REM sleep (Detari et al., 1984; Detari and Vanderwolf, 1987; Szymusiak and McGinty, 1986), demonstrating the involvement of this circuitry in sleep regulation. Recently, basal forebrain GABAergic projections have also been implicated in the regulation of sleep cycles with, GABAergic transmission associated with decreased REM sleep (Pollock and Mistlberger, 2003; Sanford et al., 2003). GABAergic transmission within the basal forebrain inhibits acetylcholine release by cholinergic neurons, suggesting that local GABA release is sleep-promoting (Vazquez and Baghdoyan, 2003). The goal of this project is to determine patterns of gene regulation in the basal forebrain that underlie sleep patterns. This issue is complicated by the fact that the different subpopulations of basal forebrain neurons may have different, and potentially opposing, patterns of gene expression during sleeping and waking (Modirrousta et al., 2004). We therefore plan to carry out DNA microarray screens using cell type-specific probes for GABAergic and cholinergic neurons to investigate sleep and waking-dependent changes in gene expression.

Figure 1. Cholinergic (green, VAChT) and GABAergic (red, GAD) neurons in neonatal basal forebrain cultures.

1) Characterization of basal forebrain neuron populations. We have established neuronal cultures

from the basal forebrains of young mice and used them to characterize the different neuronal subpopulations. We have identified two major classes of neurons and defined markers for their identification (Figure 1). GABAergic neurons were labeled with antibodies recognizing the GABA biosynthetic enzyme glutamic acid decarboxylase (GAD). The cholinergic neurons were labeled by staining for the vesicular acetylcholine transporter (VAChT). In these cultures approximately 30% of the neurons expressed a cholinergic phenotype with about 20% staining for GABAergic the marker. Since the basal forebrain is the major site for cholinergic neurons in this region of the brain, these numbers confirm the accuracy of our dissection protocols.

Consistent with previous studies (Sobreviela et al., 1994), we have also demonstrated co-localization of cholinergic markers and the p75 neurotrophin receptor in our basal forebrain cultures (data not shown). We have now successfully used the p75 antibody to isolate p75-positive embryonic neurons, a technique that we plan to apply to isolation of basal forebrain neurons (data not shown).

2) Regulation of BDNF mRNA in sleep deprived animals. We carried out pilot experiments to determine if sleep deprivation regulated expression of a gene previously shown to increase in the brains of sleep-deprived animals. Rats were subjected to gentle manual intervention during their normal sleep period (the light cycle) to disrupt sleep patterns. Following four hours of sleep deprivation, control and sleep deprived animals were sacrificed and RNA was isolated from the cortex and basal forebrain. A second set of animals was exposed to the same treatment during the dark cycle to control for manipulation stress. Since the rats sleep less during the dark cycle, these animals had less sleep deprivation with the same level of intervention. We then analyzed expression of brain-derived neurotrophic factor (BDNF) mRNA using real time PCR. We found that, similar to previous studies (Cirelli and Tononi, 2000), BDNF mRNA increased in the cortex of sleep deprived animals (Fig. 2). Interestingly, when the same intervention treatment was applied to animals during the dark (waking) cycle, the overall increase in BDNF was less, indicating that BDNF levels were being regulated by sleep deprivation rather than the stress associated with the manipulation.

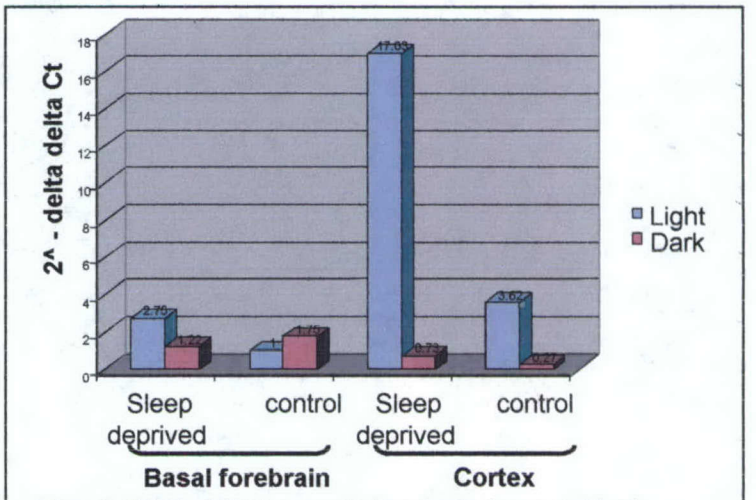

Figure 2. BDNF mRNA increases in sleep deprived cortex and basal forebrain. Real time PCR was used to analyze BDNF mRNA following manual manipuations to stimulate rats during the light cycle (sleep period-blue) or dark cycle (waking period- purple). BDNF mRNA increased during sleep deprivation in both the cortex and basal forebrain.

BDNF mRNA increased in the basal forebrain as well as the cortices of sleep deprived animals (Fig. 2). This increase was seen in animals manipulated during the light (sleep) cycle, but not in animals given the same treatment during the dark (waking) cycle. These data indicate that sleep deprivation and not handling stress underlies basal forebrain regulation of BDNF expression, providing some of the first evidence of sleep-dependent gene regulation in the basal forebrain.

These experiments have validated our ability to assess changes in gene expression in response to altered sleep patterns. In future experiments we will take advantage of a new sleep deprivation protocol designed in Dr. Sacha Nelson's laboratory that has eliminated human intervention during sleep deprivation and reduced stress over longer time periods. This apparatus has been designed for sleep deprivation of mice, permitting us, eventually, to take advantage of available mouse mutants. We will also use specific markers to examine gene expression changes that are specific to the cholinergic and GABAergic subpopulations within the basal forebrain.

3) Microarray analysis of sleep-regulated genes in cortex. In a pilot experiment we probed a Affymetrix Rat 230A Array containing 4699 full-length genes and 10,467 ESTs with probes generated from control and sleep deprived rat cortices. We characterized 259 signals as increased following sleep deprivation and 531 sequences as decreased. Several of genes known to be regulated in during sleep cycles (Cirelli et al., 2004; Nelson et al., 2004), including BDNF and homer, increased during sleep deprivation in our studies. One interesting observation that we will continue to explore is that several myelin-related genes were coordinately down-regulated in sleep deprived animals. This experiment has demonstrated our ability to use microarray screens to effectively monitor changes in gene expression following sleep manipulations.

Key Research Accomplishments

-Established cultures and defined markers for GABAergic and cholinergic neurons in the basal forebrain.
-Developed a sleep deprivation protocol for rats.
-Determined that the brain-derived neurotrophic factor gene is modulated by sleep deprivation in the basal forebrain.
-Carried out a pilot DNA microarray screen using probes from sleep deprived and undeprived rat cortex.
-Identified activity-dependent genes as increased during sleep deprivation and myelin-associated genes as decreased during sleep deprivation.

Reportable Outcomes

-none

Conclusion

Progress during the initial funding period has demonstrated our ability to identify and isolate specific neuronal subpopulations in the basal forebrain, carry out sleep deprivation experiments and analyze gene expression. We have confirmed the efficacy of our protocols by demonstrating appropriate gene-specific regulation during sleep deprivation in cortical neurons. Further, we have demonstrated sleep-dependent regulation of the BDNF gene in the basal forebrain. The basal forebrain is a key regulator of sleep patterns and our ongoing experiments indicate that the identification of gene expression changes in this region will greatly expand our understanding of the molecular regulation of sleep. We will continue this project by carrying microarray screens using probes generated from basal forebrain and from different subpopulations of basal forebrain neurons. Together, these experiments will define cell-specific regulatory processes that control sleep.

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Aim 4: Cell type-specific changes in gene expression across the sleep cycle (Nelson).

Introduction

The primary objective of this project is to identify genes whose expression is altered by Sleep Deprivation (SD). One of the main problems that confounded earlier SD studies was the poor control of physical stress experienced by the animals because of the sleep deprivation techniques (like chronic muscle stress induced by requiring animals to stay on small platforms floating in water in the "flower pot" technique or forced locomotion on a rotating cylinder partially submerged in water)[1, 2]. Further the control animals in these techniques were imperfect as they did not get an equal amount of physical stress.

Body

We have fabricated an apparatus that uses the DOW technique [3] for SD. This technique causes minimal physical stress and equalizes stress for the control and experimental mice. The apparatus consists of a plexi-glass disk that extends in two adjacent plastic cages. The cages are filled with water to a small height below the disk. The disk is mounted on a motor that can be rotated in a random direction using a computer. The control and the experimental mice are implanted with pairs of EEG and EMG electrodes that are used to monitor gross electrical activity of the brain and neck muscle tone, respectively. The recordings are analyzed in real time and the disk is rotated whenever the experimental mouse falls asleep. This causes both the mice to walk in the direction opposite to the rotation of the disk to avoid falling in water. The control mouse can sleep when the experimental mouse is awake. According to our preliminary recordings, the control mouse is deprived of about 30% of its sleep while the experimental mouse is deprived of 75-85% of its sleep. We are still trying to maximize the difference in the sleep lost by the two animals. Food and water are available *ad-libitum*. The animals are maintained on 12 hr:12hr light-dark cycle.

Baseline awake-sleep pattern is recorded for 24 hrs from both the animals after they have recovered from electrode implantation surgery and have acclimatized to the electrode wires and the cage. If the electrode-tissue connections are good, we see a bimodal distribution of EEG and EMG activity from which the awake-sleep thresholds are determined. If the standard deviation of EMG is greater than the threshold value, the animal is considered to be awake. If it is less, then if mean power in the Delta frequency band is higher than the threshold, NREM is scored. If both EMG & Delta power are low, then the mean power in the Theta band is checked. If it is higher than the threshold, REM is scored; otherwise the animal is considered to be awake but quiet. The visually determined state of the mice matches with the state determined by the above algorithm with high probability. The propensity of sleep as scored by above algorithm, increases during light cycle and decreases during dark cycle. Our preliminary recordings also show a small increase in the duration of NREM sleep of the experimental mouse after SD for 24 hours. This is also reflected in an increase in power in the delta frequency band compared to baseline. Thus, other than a few optimizations, we are now ready for using the sleep deprived mice for gene-expression & electrophysiological studies.

The mice used in above studies express a genetically encoded fluorescent protein in a sub-population of neurons. This will allow us to repetitively select a population of neurons for studying the genes whose expression is regulated by SD. We plan to first verify expression in few genes that are known to be regulated by SD using RT-PCR. Following that, we will carry out cDNA hybridization on micro-arrays, which will allow us to study the expression of large

numbers of genes from a very select population of neurons. We have also begun preliminary whole-cell patch clamp recordings from the fluorescently labeled cells in motor cortex slices to study the effects of SD on the electrophysiological properties of neurons in this region. Eventually, we hope that we will be able to correlate the gene expression studies with results from slice-electrophysiology, *in-vivo* recordings, and behavioral studies.

Key Research Accomplishments

- Designed and built apparatus to implement DOW sleep deprivation technique in mice
- Perfected hardware/software for monitoring and scoring sleep/wake EEG and EMG
- Perfected RNA isolation, amplification and hybridization from cortical layer 5 pyramidal neurons for analysis of cell-type specific gene expression

Reportable outcomes

The following were primarily supported by other funding sources, but were also partially supported by the present grant as they represent necessary steps in perfecting the gene expression assays to be used in carrying out the research goals.

- Manuscript: Sugino K., Hempel C.M., Miller M., Hattox, A., Huang, Z.J., and Nelson S.B. (2005). Canonical cortical cell types defined by divergent gene expression. In Preparation.
- Abstract: Sugino K., Hempel C.M., Miller M., Hattox, A., Huang, Z.J., and Nelson S.B. (2004) A Global view of the molecular identity of cortical cell types. Soc. Neurosci. Abst. 611.13.

Conclusion

We have now perfected our apparatus and protocols for reliably producing well controlled sleep deprivation in mice. We have successfully profiled cell-type specific gene expression in different populations of cortical pyramidal neurons and GABAergic interneurons. We are now well prepared to look for sleep deprivation-induced changes in gene expression in these neurons.

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

Introduction

The major goal of this Aim, which in being done in collaboration with Don Katz, is to examine homeostatic cortical plasticity in freely behaving animals using chronically implanted electrode arrays, and determine the role of sleep in this plasticity. Prior to this project, all of our experiments on homeostatic plasticity have been performed using *in vitro* preparations such as brain slices. During the first funding period of this grant, we have developed the necessary methodology to study this phenomenon in the intact animal. Because we need to monitor activity in the upper layers (2/3) of cortex we have had to develop special electrode arrays, as described below, and we now have made significant progress toward this goal.

Body

One of the problems that has limited awake-behaving recordings in the superficial layers (II/III-IV) of the neocortex is the dimpling of brain tissue when electrodes are inserted during surgery. The tissue bends under the pressure due to the size of the bundles of wires (25 μm in diameter) and the electrode array designs that have been used in most experiments of this kind. When the tissue recovers from the dimpling the electrodes are already positioned in deep cortical layer (V-VI) and the superficial ones have been damaged, so that the wires cannot be repositioned closer to the surface. Another issue that we needed to solve is that standard electrode implants require removal of the dura mater above the area of interest. This procedure too damages the superficial portion of the cortex.

Recently Krupa and collaborators designed new electrodes arrays specifically to overcome these problems in adult rats (Krupa et al 2004). The arrays are composed of 16 wires each and limit the dimpling of brain surface because only the wires are lowered in the cortex without the need of support cannulae in the tissue. The wires are spaced about 200 μm in the wire driving bulb, are thinner (only 12 μm) and have high impedance (2-3 MΩ). This allows recording of activity from several non-overlapping neurons. The implant is performed without removing the dura mater. Instead small current pulses are delivered through each individual wire to generate a small hole for the wire to penetrate.

We have imported this technology into the lab and adapted it to our purposes. Our aim is to record single neuron activity in the superficial layers of the monocular portion of the primary visual cortex of juvenile rats (23 days old, p23). 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 (see photo below) and have successfully implanted our first several arrays. The next steps are 1) to use these electrodes to follow cortical activity over time and determine how this activity adapts during visual deprivation, and 2) to determine whether this adaptive, homeostatic plasticity occurs during sleep or wake.

Key Research Accomplishments

1. Miniaturization of electrode arrays for young rats
2. Modification of electrode arrays to record from upper layers of cortex

Reportable Outcomes

None as yet

Conclusion

We successfully adapted a complex chronically indwelling electrode array design for use in visual cortex of developing rat pups. A large number of technical obstacles have been overcome, and several young rats have been implanted; it appears that we have isolated the waveforms of single neurons in the infragranular layers of V1, along with the simultaneous LFP data that will allow us to electrophysiologically determine level and length of sleeping bout. We have also adapted the training rig to enable the long-term (i. e., 24-48 hr) chronic recording and saving of neural data that are required for these experiments, and has outfitted the rig for visual stimulation and video recording of sleep-wake behavior.

All in all, this is remarkable progress on what is a deceptively difficult project. We expect to collect simultaneous single neuron, LFP, and behavioral data within the next few weeks.

References

Krupa DJ, Wiest MC, Shuler MG, Laubach M, Nicolelis MA. Layer-specific somatosensory cortical activation during active tactile discrimination. Science *304* 1989-92 (2004).

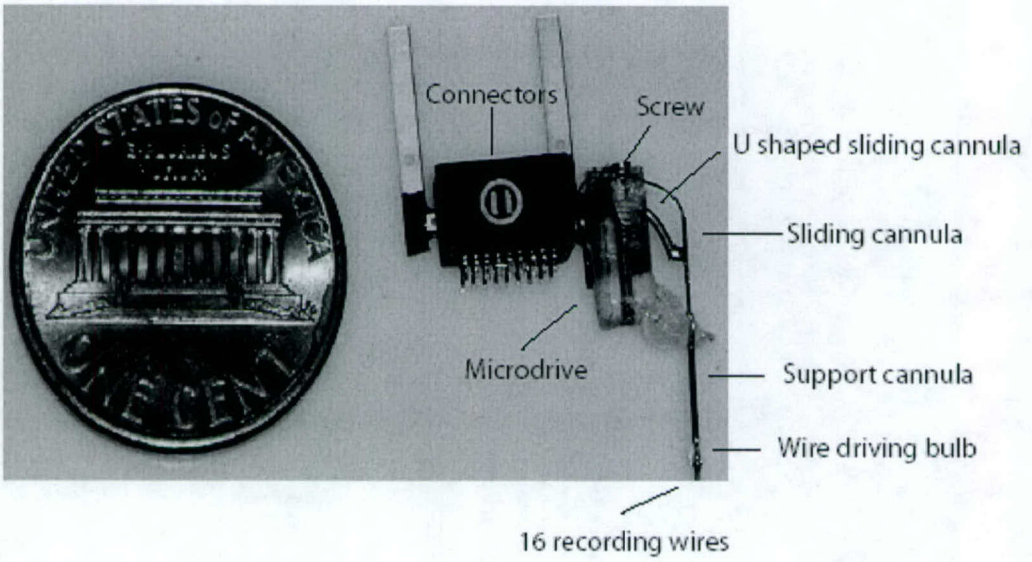

Fig. 1 Cortical electrodes design.

Another problem we needed to overcome was the correct positioning of the implants: rat brain atlases produce very precise coordinates for brain areas, but are based on adult animals. In order to have reliable coordinates for p23 rat monocular visual cortex we performed mapping experiments using extracellular recordings in 5 anesthetized animals and averaged the coordinates obtained. The correct positioning of the testing electrode was determined by neuronal responses from visual stimulation of the contralateral eye only (-5.6 from bregma, 1.8 lateral from midline, about 1mm wide x 1.3 mm long).

Aim 6: To determine whether or not sleep is required for fear conditioning (Katz).

Two Aims of this project are being performed in my lab. The first is my collaboration with Dr. Turrigiano (Aim 5) on sleep-related plasticity of V1 neurons during and following monocular deprivation. Progress on this Aim is described in Dr. Turrigiano's report.

Introduction

This project involves recording multi-site field potential activity during learning of, and sleep following, hippocampal-dependent and hippocampal-independent tasks. We have made progress on setting up the electrophysiology, and on identifying tasks for comparison to fear conditioning.

Body

We will test the importance of sleep-related hippocampal oscillations for consolidation of different types of learning, directly relating task performance to the occurrence and spatial coherence of oscillations during wakedness and sleep. This requires that we: 1) train rats to perform hippocampal-dependent and hippocampal-independent tasks—preferably three tasks, one that is impaired by hippocampal lesion, one that is unaffected by hippocampal lesion, and one that is enhanced by hippocampal lesion; and 2) simultaneously record hippocampal, amygdalar, and cortical oscillations during learning and subsequent sleep episodes, building our ability to inter-relate supposedly learning-relevant rhythms in each region.

Key Accomplishments

- simultaneous amygdalar, hippocampal, and cortical oscillations recorded.
- drowsiness-related oscillations recorded, related to learning and performance.
- hippocampal-antagonistic learning (i. e., learning that is enhanced by hippocampal lesion) identified.
  — importance to project: provides better control/comparison group for hippocampal-dependent learning.

Reportable outcomes

- None

Conclusions

Progress is being made. In the next 12-24 months, it should be possible to run the full experiments in which multi-site recordings are collected during performance of, and sleep episodes following, multiple learning tasks. This should enable us to test whether it is sleep rhythms, hippocampal rhythms, or rhythms in general that contribute most strongly to memory consolidation.

References

NA

Conclusion

There are excellent interactions between the six sleep laboratories at Brandeis. This is obvious within each of the three pairs, where there are intimate interactions between the post-docs and students in the two collaborating laboratories. I suspect, however, that we will see even more and deeper interactions between these three subgroups. It is my hope that some of the *Drosophila* results cross-fertilize with mammalian results and vice versa. Following the circadian rhythm grand synthesis of the last few years, sleep-relevant drugs and gene expression results are also likely to be relevant across species. I also think that some of the live recording results will ultimately interface with the gene expression conclusions. My expectation is that the locations that prove relevant to sleep-relevant effects on plasticity or learning will also be amenable to the gene expression analyses. In these ways, we should observe striking synergies between the different Brandeis projects before the three year project period has elapsed.