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TITLE: **Sleep Homeostasis and Synaptic Plasticity**

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INTRODUCTION:

After a busy day we are sleepy. Yet, how the brain translates this accumulated wake experience into sleep drive and eventually forces us to fall asleep remains a mystery. In this proposal, we aim to identify the neural circuitry that regulates this homeostatic sleep drive by mapping where in the brain sleep need is encoded and where it is translated into sleep drive.

Sleep pressure – the internal drive to sleep – is proposed to be regulated by the interaction of circadian and homeostatic processes. In this two-process model, circadian mechanisms synchronize sleep drive to the day-night cycle while homeostatic sleep pressure responds to wake experience, increasing in parallel with wakefulness and dissipating again during sleep. The homeostatic regulation of sleep remains shrouded in mystery. One of the most exciting recent hypotheses concerning the function of sleep homeostasis is the “synaptic homeostasis” hypothesis. The basic idea is as follows: everyday behavior and learning produce a net increase in synaptic weights in the brain, meaning that the chemical connections between neurons are strengthened. One function of sleep is therefore to downscale or “normalize” all synapses in the brain, while maintaining the relative synaptic strength differences that have accrued through learning.

But how is wake experience translated into sleep drive? Where in the brain does this occur? Is there a discrete sleep drive circuit (a homeostat) that operates in concert with the circadian circuitry or does sleep drive accumulate everywhere in the brain?

To answer these questions we need to study a brain that is highly accessible while still being similar enough to man to be a valuable model organism. The fruit fly *Drosophila melanogaster* is the best candidate, as it comes with a wide variety of genetic tools that allow precise control of gene expression and neuronal activity in discrete parts of the brain. At the same time, neuronal biochemistry is very similar – flies and man respond in a similar manner to wake and sleep promoting drugs.

This proposal aims to tackle these questions by studying where in the fly brain wake experience accumulates and how wake- and sleep promoting brain regions change their activity after sleep deprivation. This will result in a map of the inputs and outputs of the sleep homeostatic circuitry.

ACCOMPLISHMENTS:

Major goals

Task 1A: Determine homeostasis and arousal in null mutants

Task 1B: Attempt rescue of null phenotypes by expressing rescue construct in discrete regions

Task 1C: Verify rescue brain areas by RNAi knockdown (in wildtype) of gene in areas where rescue was successful

Task 2: Quantify wake experience dependent synaptogenesis

Task 3: Test the effect of synaptogenesis on sleep-wake

Keywords: Sleep, Sleep Homeostasis, GRASP, frm1, *Drosophila*

What was accomplished under these goals?

Major Activity 1: to identify circuits where known modulators of sleep homeostasis modulate rebound sleep after sleep deprivation

Specific Objective 1A) Determine homeostasis and arousal in null mutants

We tested sleep homeostasis in *insomniac*, a short sleeping mutant with impaired sleep homeostasis. Wild type flies recover 20-40% of sleep lost during the 24 hours after sleep deprivation. *Insomniac* does not recover any sleep lost.

Specific Objective 1B) Attempt rescue of null phenotypes by expressing rescue construct in discrete regions

To rescue the short sleeping phenotype, we used *inc*⁰⁰²⁸⁵. This is an *insomniac* null mutant with a UAS-*inc* construct. By crossing *inc*⁰⁰²⁸⁵ with a library of GAL4 lines, we can test whether this rescues sleep and/or sleep homeostasis. To test for rescue of the short sleeping phenotype, we crossed *inc*⁰⁰²⁸⁵ to Mushroom Body output neurons (MBONs). The *Drosophila* mushroom body is an important sleep regulating region and consists of sleep promoting and wake promoting subdivisions. When we rescued *inc* in wake promoting regions, this decreased total sleep while rescuing *inc* in sleep promoting regions this increased total sleep.

Specific Objective 1C) Verify rescue brain areas by RNAi knockdown (in wildtype) of gene in areas where rescue was successful

We used RNAi-mediated knockdown on *insomniac* to verify our rescue experiments. Knockdown of *inc* in wake promoting MBONs resulted in increased sleep while *insomniac* knockdown in sleep promoting MBONs resulted in decreased sleep. In 2016, Mark Wu's lab identified a dedicated circuit in the *Drosophila* central brain that encodes sleep homeostasis. This subset of R2 ellipsoid body neurons is capable of generating sleep drive. RNAi-mediated knockdown of *insomniac* in R2 neurons abolished sleep homeostasis without affecting baseline sleep.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

We are currently screening the other modifiers of sleep homeostasis, both pan-neuronally and in the more detailed circuits outlined in aim 1 (dorsal fan shaped body (dFSB), pars intercerebralis (PI), LNV as well as sensory systems such as the antennal lobe and optic lobes).

Major Activity 2: To identify neural circuits where wake experience results in increased synapse formation

Specific Objective 2) Quantify wake experience dependent synaptogenesis

We've used GH146 LexA; LexO sp GFP11; Or92-Gal4 UAS-syb sp GFP 1-10. In this construct, one subunit of GFP is expressed in olfactory projection neurons using the LexA-LexO binary expression system while GFP subunits 1 to 10 are expressed in antennal lobe neurons using Or92-Gal4. These flies were exposed to an odor (10 minutes on, 10 minutes off for four times). If this odor exposure results in synapse formation, we should see GFP expression at the synapse between antennal lobe neurons and projection neurons (compared to unexposed controls of the same genotype).

We've had Inconsistent results with getting synaptic GRASP to work. On average, we only saw GFP staining in 25% of the attempts, but sometimes we also saw GFP expression in flies that were not exposed to odor. We are currently working together with the Gallio lab, who created the synaptic GRASP lines, to resolve these issues and improve our success rate.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, we aim to get synaptic GRASP to work. Instead of testing for connections between a pan-neuronal driver and circuits outlined in Aim 1, we will test for connections between a newly discovered sleep homeostatic circuit, the R2 neurons of the ellipsoid body (Liu, 2016, Cell).

Major Activity 3: To test the hypothesis that altering synapse formation anywhere in the brain alters sleep

Specific Objective 3) Test the effect of synaptogenesis on sleep-wake

We have tested several dFmr1 modifier lines (uas-dFmr1 and EP3517 (overexpression) and three RNAi lines(knock down)) for their ability to change dfmr1 expression and alter sleep architecture. dFmr1 is involved in synaptic pruning and plasticity. We hypothesized that, as published before, dFmr1 overexpression will result in loss of synapses and decreased sleep while dFmr1 knockdown has the opposite effect – increased synapse formation and sleep.

Crossing these lines with a pan-neuronal inducible driver (daughterless geneswitch) did not produce any phenotypes after one or two weeks of induction.

When we crossed the RNAi lines with elav-Gal4, a pan neuronal driver, we found that RNAi knockdown of dFmr1 resulted in *decreased* sleep in two out of three lines. Overexpression seemed lethal. We are currently replicating these results, in combination with PCR to quantify dFmr1 levels.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

We will test fmr1 overexpression and knockdown in circuitry described in Major Activity 1

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS

We encountered several problems in this project:

- 1) The synaptic GRASP technique, which uses split GFP expressed at synapses to quantify synapse formation, is hard to get to work in our lab. One possibility, suggested by the Gallio lab, is that one or more lines were contaminated. We have received new, verified lines and are redoing the experiments
- 2) RNAi-mediated knockdown of *dfmr1* had the opposite result of what has been published – instead of increased sleep we found strongly decreased sleep.

PRODUCTS

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	<i>Matthew Flourakis</i>
Project Role:	<i>Postdoctoral Fellow</i>
Researcher Identifier (e.g. ORCID ID):	?
Nearest person month worked:	1
Contribution to Project:	Initiated GRASP
Funding Support:	-

Name:	<i>Dae Sung Hwangbo</i>
Project Role:	<i>Postdoctoral Fellow</i>
Researcher Identifier (e.g. ORCID ID):	?
Nearest person month worked:	6
Contribution to Project:	<i>Performed genetic sleep experiments</i>
Funding Support:	-

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

W81XWH-16-1-0169

6/1/16 – 11/31/17

0.9 summer, 1.5 academic

Dept. of the Army -- USAMRAA

Role: PI

Total: \$302,910

Contact: Science Officer Sarah Naylor, sarah.a.naylor.ctr@mail.mil

Title: Sleep homeostasis and synaptic plasticity

Project Goal: The goal of this proposal is to study where in the fly brain wake experience accumulates and how wake- and sleep promoting brain regions change their activity after sleep deprivation

Specific Aims:

Aim 1: to identify circuits where known modulators of sleep homeostasis modulate rebound sleep after sleep deprivation

Aim 2: To identify neural circuits where wake experience results in increased synapse formation

Aim 3: To test the hypothesis that altering synapse formation anywhere in the brain alters sleep

W81XWH-16-1-0166

6/1/16 – 11/31/17

0.9 academic, 1.5 summer

Dept. of the Army -- USAMRAA

Role: PI

Total: \$302,910

Contact: Science Officer Sarah Naylor, sarah.a.naylor.ctr@mail.mil

Title: Therapeutic Sleep for Traumatic Brain Injury

Project Goal: This proposal investigates the correlation between TBI-induced sleep disorders and TBI-induced behavioral changes and evaluates whether induced changes in sleep architecture rescue or worsen these behavioral changes.

Specific Aims:

Aim 1: to test the hypothesis that TBI causes either hypersomnia or insomnia in individual flies

Aim 2: to test the hypothesis that correcting impaired sleep patterns can facilitate post-TBI recovery

CBC Catalyst Award

6/1/16 - 5/31/18

0.0 academic, 0.1 summer

Chicago Biomedical Consortium

Role: PI

Total: \$250,000

Contact: Senior Associate Director Kimberly Corn, k-corn@northwestern.edu

Title: Transplanting a prokaryotic oscillator to animals to restore circadian clock function

Project Goal: The goal of this award is to transplant a bacterial clock into animals and engineer this system to drive molecular, physiological and behavioral rhythms.

Specific Aims:

Aim 1. Reconstitute function of the cyanobacterial KaiABC proteins in Drosophila tissue culture cells

Aim 2. Drive transcriptional rhythms in Drosophila cells by fusing Kai proteins to transcriptional activators

Aim 3. Using Kai-meras to drive clock gene and behavioral rhythms in flies

67885MA

10/1/2016 – 10/31/2019 0.45 academic, 0.15 summer

Dept of the Army -- Materiel Command

Role: PI

Total: \$546,186

Contact: Virginia Pasour, U.S. Army Research Office, virginia.b.pasour.civ@mail.mil

Title: Multisensory Integration by Circadian Clocks - Area 3 Mathematics (Biomathematics) and Area 8 Life Sciences (Neurophysiology)

Project Goal: The goal is to understand how circadian clocks integrate sensory information from light and temperature to entrain circadian clocks.

Specific Aims:

Aim 1. From Gene to Neuron: Integrating Transcriptomics, Optical Imaging, and RNA Interference

Aim 2. From Neuron to Circuit: Applying Connectomics and Network Modeling

Aim 3. Determining How Information from Multiple Sensory Modalities Are Integrated to Align the Clock to Environmental Cycles

Note: There is no scientific overlap between these new grants and the current proposal

What other organizations were involved as partners?

Nothing to Report

SPECIAL REPORTING REQUIREMENTS

Not applicable