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14. ABSTRACT This Phase II proposal (G. Tononi-PI) resulted from the combination of two independent projects in Phase I: "Rapid discovery of continuous performance and power-nap compounds through large-scale mutagenesis in Drosophila" (G. Tononi-PI, C. Cirelli-CoPI) and "Avian models of sustained wakefulness" (R. Benca-PI, N. Rattenborg-CoPI). The goal of both projects was to find ways to postpone temporarily the need for sleep, and do so in a way that is completely safe. In Phase I, the strategy had been to study animal models of sleeplessness, including models created through mutagenesis (flies) or naturally-occurring models (birds), in order to identify druggable targets that can be exploited to produce prolonged					
15. SUBJECT TERMS drosophila short sleeping phenotype Kv potassium channel migration					
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a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER 608-263-6063

Report Title

Rapid Discovery of Continuous-Performance Compounds and Povernap Compounds Through Large-Scale Mutagenesis in Drosophila

ABSTRACT

This Phase II proposal (G. Tononi-PI) resulted from the combination of two independent projects in Phase I: “Rapid discovery of continuous performance and power-nap compounds through large-scale mutagenesis in Drosophila” (G. Tononi-PI, C. Cirelli-CoPI) and “Avian models of sustained wakefulness” (R. Benca-PI, N. Rattenborg-CoPI). The goal of both projects was to find ways to postpone temporarily the need for sleep, and do so in a way that is completely safe. In Phase I, the strategy had been to study animal models of sleeplessness, including models created through mutagenesis (flies) or naturally-occurring models (birds), in order to identify druggable targets that can be exploited to produce prolonged wakefulness in the absence of performance decrements or health risks. The overall goal for Phase II was, for each model, to determine the specific mechanisms and genes responsible for sleep reduction and then to develop drugs that could produce temporary sleeplessness. This effort has resulted in the identification of 2 potentially promising pharmacological tools to reduce/postpone the need for sleep, i.e. compounds acting on voltage-dependent (Shaker-like) potassium channels and nicotinic agents. Results are described in detail in 6 peer-reviewed publications.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Rattenborg NC, Mandt BH, Obermeyer WH, Winsauer PJ, Huber R, Wikelski M, Benca RM (2004) Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). Plos Biology 2: 924-936.

Cirelli C, Bushey D, Hill S, Huber R, Kreber R, Ganetzky B, Tononi G (2005) Reduced sleep in Drosophila Shaker mutants. Nature, 434: 1087-1092.

Cirelli C, Lavaute TM, Tononi G (2005) Sleep and wakefulness modulate gene expression in Drosophila. J of Neurochemistry 94:1411-1419.

Bushey D, Huber R, Tononi G, Cirelli C (2007) Drosophila Hyperkinetic mutants have reduced sleep and impaired memory. Journal of Neuroscience, 27: 5384-5393.

Douglas CL, Vyazovskiy VV, Southard TL, Chiu S-Y, Messing A, Tononi G, Cirelli C (2007). Sleep in Kcna2 knockout mice. BMC Biology, 5(1):42.

Jones S, Pfister-Genskow M, Benca RM, Cirelli C (2008). Molecular correlates of sleep and waking in the brain of the white-crowned sparrow. J of Neurochemistry 105: 46-62.

Jones S, Pfister-Genskow M, Cirelli C, Benca RM (2008) Changes in brain gene expression during migration in the white-crowned sparrow. Brain Research Bulletin, in press.

Number of Papers published in peer-reviewed journals: 7.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

0

(d) Manuscripts

Number of Manuscripts: 0.00

Number of Inventions:

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Fankhauser, Jeffrey	0.33
Jones, Stephany	0.36
Landsness, Eric	0.62
FTE Equivalent:	1.31
Total Number:	3

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Balduzzi, David	0.54
Bushey, Daniel	0.16
Douglas, Christopher	0.64
Gilestro, Giorgio	1.00
Hanlon, Erin	0.64
Huber, Reto	0.50
Vyazovskiy, Vladyslav	0.64
FTE Equivalent:	4.12
Total Number:	7

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Benca, Ruth	0.29	No
Cirelli, Chiara	0.60	No
Tononi, Giulio	0.19	No
FTE Equivalent:	1.08	
Total Number:	3	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
various - estimate 0.30/student	0.30
FTE Equivalent:	0.30
Total Number:	1

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PhDs

NAME

Total Number:

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	
Baker, Tracie	0.46	No
Hannan, Ciaran	0.60	No
Jaja, Tamika	0.50	No
Luebke, Anne	1.00	No
Obermeyer, William	0.27	No
Pfister-Genskow, Martha	0.60	No
FTE Equivalent:	3.43	
Total Number:	6	

Sub Contractors (DD882)

Inventions (DD882)

**From 24/7 flies and birds to 24/7 humans:
The University of Wisconsin Continuous Performance Project**

Ruth Benca, Chiara Cirelli, Giulio Tononi

STATEMENT OF THE PROBLEM STUDIED

This Phase II proposal resulted from the combination of two independent projects in Phase I: “Rapid discovery of continuous performance and power-nap compounds through large-scale mutagenesis in *Drosophila*” (G. Tononi-PI, C. Cirelli-CoPI) and “Avian models of sustained wakefulness” (R. Benca-PI, N. Rattenborg-CoPI). The goal of both of these projects was to find ways to postpone temporarily the need for sleep, and do so in a way that is completely safe. The strategy for both projects has been to study animal models of sleeplessness, including models created through mutagenesis (flies) or naturally-occurring models (birds), in order to identify druggable targets that can be exploited to produce prolonged wakefulness in the absence of performance decrements or health risks. During Phase I, we demonstrated in both projects that models exist or can be produced that fully meet the goals of the CAP initiative. By using large-scale mutagenesis in flies, we have discovered some lines that need minimal amounts of sleep and yet perform impeccably. Moreover, for several of these lines we have identified the responsible genes, opening the way to targeted drug development. By studying migratory birds, we have shown that, during migration, birds can dramatically restrict their need for sleep for several days and yet carry out their tasks flawlessly. We have also found specific transcripts that are upregulated in their brains during migration, which will also lead to the identification of potential drug targets.

The overall goals for Phase II was to characterize the mechanisms for these models and determine how to switch them on safely in humans. Based on discussions with DARPA at the June 2003 Downselection Meeting, we selected to combine the two projects in Phase II for two reasons: (1) The overall approach for each model, which was to determine the specific mechanisms and genes responsible for sleep reduction and then to develop drugs that can produce temporary sleeplessness, was generally the same; and (2) It was likely that responsible genes were similar in the various models, which could not only suggest the most likely targets to pursue, but also reduce duplication of efforts.

The Phase II proposal was therefore structured along 3 subprojects and 3 tasks:

SUBPROJECTS

Subproject 1: Fly models and molecular biology; Cirelli, project coordinator

Subproject 2: Bird models and neurophysiology; Benca, project coordinator

Subproject 3: Translation into mammalian models; Tononi, project coordinator

TASKS

Task 1a: Conclusion of target identification for continuous performance compounds in flies and birds

- A. Sleep deprivation resistant flies
 - Continue to screen EMS-mutagenized lines
 - Characterize candidate lines behaviorally
 - Characterize candidate lines genetically and molecularly
- B. Short sleeper flies
 - Continue to screen EMS-mutagenized lines
 - Characterize candidate lines behaviorally
 - Characterize candidate lines genetically and molecularly
- C. Unihemispheric sleep in pigeons
 - Continue brain collection from bihemispheric and unihemispheric sleep deprived pigeons
 - Screen for candidate genes using differential display
 - Compare cellular, physiological, cognitive effects of unihemispheric vs. bihemispheric sleep deprivation
 - Characterize candidate genes
- D. Migratory sleeplessness in sparrows
 - Continue brain collection for baseline, preparatory, migratory, and recovery phases
 - Screen for candidate genes using differential display
 - Compare cellular, physiological, cognitive effects of migratory sleeplessness vs sleep deprivation
 - Characterize candidate genes

Task 1b: Down-selection of identified fly/bird targets for drug discovery

- Microarray gene expression profiling of mutant fly lines to identify downstream targets
- qPCR analysis of candidate migratory genes in other migratory species
- Proteomics on selected tissues
- Bioinformatic down-selection based on comparative analysis of state-dependent gene expression in other species (UW database) and appropriate mammalian homologues
- Construction of constitutive and/or conditional transgenic fly lines
- Construction of constitutive and/or conditional knock-down fly lines (RNAi)

- Construction of constitutive and/or conditional transgenic mouse lines
- Construction of constitutive and/or conditional knock-out mouse lines
- Antisense strategies to demonstrate biological and behavioral effects of candidate genes in birds/rats
- Antibody strategies to demonstrate biological and behavioral effects of candidate genes in birds/rats

Task 2: In vitro screening of continuous-performance compounds against targets selected in Task 1b

- Complete screening of compound libraries against potassium channel targets, selectivity profiling
- Physiochem, ADMET, and wide profiling testing for potassium channel compounds
- Assay development for other candidate targets, High throughput screening, Initial medicinal chemistry
- Preclinical studies (Xention or tbd)
- IND-enabling studies (Xention or tbd)

Task 3: In vivo testing of continuous-performance candidate compounds identified in Task 2

- Scale up apparatus for testing drug effects on sleep/waking and performance in flies/birds/rats
- Test candidate compounds for effects on sleep/waking and performance in flies/birds/rats (if needed, monkeys)

In conclusion, the overall goal was to provide DARPA, by the end of Phase II, with a radically new pharmacological tool to safely reduce or postpone the need for sleep and has no negative effects on physical and cognitive performance. Such a tool would profoundly impact military operations by minimizing the high cost of sleep deprivation and its consequences and by extending the envelope of what is feasible, irrespective of the job requirement, branch of service, or theater of operation. It can also be expected that such a tool would find extensive applications in other areas of society, from the transportation industry to medical services. Most importantly, such a tool would achieve the goal of the CAP program: make sure that our soldiers and special forces do not lose their lives because they lost their sleep.

SUMMARY OF THE MOST IMPORTANT RESULTS

The findings related to this project have resulted in 7 publications (see Bibliography), where the results are described in detail. The reprints are attached as Appendixes. Below is a brief summary of the general accomplishments. The overall goal has been met by the identification of 2 potentially

promising pharmacological tools to reduce/postpone the need for sleep, i.e. compounds acting on voltage-dependent (Shaker-like) potassium channels and nicotinic agents.

Task 1

- More than 15,000 EMS/P lines were screened, spanning the 3 major Drosophila chromosomes
- Fifteen sleep deprivation resistant and/or short sleeper mutant lines characterized behaviorally
- Four sleep deprivation resistant and/or short sleeper mutant lines characterized genetically and molecularly; results for 2 lines already published (Shaker lines: (Cirelli et al., 2005b); Hyperkinetic lines: (Bushey et al., 2007); results for the other two lines close to publication (PKA, Fragile X Mental Retardation Protein))
- Completed screening for candidate genes using differential display and cDNA arrays during migration and in unihemispheric sleep; results have already been published: (Jones et al., 2008a) (Jones et al., 2008b)
- Compared cellular, physiological, cognitive effects of migratory sleeplessness vs sleep deprivation; this analysis has resulted in two publications (Rattenborg et al., 2004) (Jones et al., 2008b)
- Completed microarray gene expression profiling of mutant fly lines to identify downstream targets (Cirelli et al., 2005a): Shaker lines were used for this analysis
- Complete down-selection of identified fly/bird targets for drug discovery to 2 druggable targets: voltage-dependent potassium channels and nicotinic agonists identified as potential candidates

Task 2

- Complete screening of compound libraries against potassium channel targets and nicotinic agonists, selectivity profiling
- Physiochem, ADMET, and wide profiling testing for potassium channel compounds and nicotinic agonists

Task 3

- Scaled up apparatus for testing drug effects on sleep/waking – vigilance in rats
- Tested candidate compounds for effects on sleep/waking and performance in rats: results for potassium channel compounds have been published (Douglas et al., 2007) or presented in abstract form at scientific meetings (Douglas et al., 2006; Faraguna et al., 2006); results for nicotinic agents have been presented in abstract form at scientific meetings (Faraguna et al., 2007)

Bibliography

Bushey D, Huber R, Tononi G, Cirelli C (2007) Drosophila Hyperkinetic mutants have reduced sleep and impaired memory. J Neurosci 27:5384-5393.

Cirelli C, LaVaute TM, Tononi G (2005a) Sleep and wakefulness modulate gene expression in Drosophila. J Neurochem 94:1411-1419.

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- Douglas CL, Vyazovskiy V, Southard T, Faraguna U, Cirelli C, Tononi G (2006) Voltage-dependent potassium channel Kv1.2: effects on sleep and EEG power spectrum of intracortical injections of an anti-Kv1.2 antibody. . *Sleep* S29:110.
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- Faraguna U, Vyazovskiy VV, Douglas CL, Tononi G, Cirelli C (2007) Effects of intracortical microinjections of nicotinic agonists on sleep regulation. *Sleep Suppl.*:80.
- Jones S, Pfister-Genskow M, Benca RM, Cirelli C (2008a) Molecular correlates of sleep and wakefulness in the brain of the white-crowned sparrow. *J Neurochem* 105:46-62.
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