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Modeling Sleep-related Activities from Experimental Observations - Initial computational frameworks for understanding sleep function(s)

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ABSTRACT

Sleep disorders affect ~70 million Americans, an estimate that is likely an underestimate, based on recent data. Though sleep is considered an essential behavior, the biological function of sleep is not known, which has limited the development of safe and effective therapies. We hypothesize that sleep serves some sort of restorative function that affects the entire organism. We propose to model activities associated with sleep using a "bottom-up" approach that utilizes computational modeling to link sleep-related protein activities. In this paper, we show how data from our investigation of proteins correlate to sleep-wake in the cortex of rats. We believe that understanding the underpinnings of sleep at all levels of the body's organizational hierarchy holds great promise for the future of neuroergonomics research and practice.
Keywords: Sleep-wake behavior, biological timing, cellular underpinnings of sleep, interactions across brain regions, organ-organ interactions, protein expression, sleep deprivation, neuroergonomics, health maintenance.

INTRODUCTION

A comprehensive and ever-growing literature on the evolutionary conservation of sleep and sleep patterns [Zeppelin 2000] and the debilitating effects of sleep deprivation [Bonnet 2000] in all animals studied to date [Cirelli, Huber, Gopalakrishnan, Southard & Tononi 2005] indicates that sleep is an essential behavior, similar to eating, drinking, and mating. How and why the brain orchestrates shifts in vigilance states are fundamental questions in sleep research.

That a balance between waking and sleep is important to maintain health and productivity in humans is supported by statistics associated with sleep disturbances. Sleep disorders affect approximately 70 million Americans, with associated costs estimated at billions of dollars per year. Similar to other physical anomalies, sleep disorders generally result from extremes in behavior- i.e., too much or too little sleep. While it is recognized that these extremes in behavior are determined by a combination of genetics and environment, sleep deprivation is the more prevalent of these extremes in our 24 h, 21st century society and individuals that consistently do not get enough sleep, exhibit cumulative decrements in cognitive and psychomotor performance [Owens 2001]. For example, cognitive performance was decreased by 30-40% in military personnel deprived of sleep for one night, with further declines in performance (60-70%) after a second night of sleep deprivation [Westcott 2005]. In addition, as people age, alterations in sleep (the inability to fall asleep, and/or stay asleep) are also correlated with cognitive and/or psychomotor performance [Ohayon, Carskadon, Guilleminault & Vitiello 2004]. Moreover, recent findings in adolescent and young adults indicate there are positive correlations between inadequate sleep and the development of obesity, cardiovascular disease and type-2 diabetes [Knutson & Van Cauter 2008; Mullington, Haack, Toth, Serrador & Meier-Ewert 2009]. Thus chronic sleep deprivation may negatively impact the function of several different organs in the body. Hence, from the point of view of neuroergonomics research, understanding the underpinnings of sleep at all levels of the body's organizational hierarchy holds great promise for improving and sustaining physical and mental capabilities and performance. As discussed below, the biological function (s) of sleep (i.e., what is restored during sleep) is not known, though an overall restorative function for sleep is widely accepted. To appreciate sleep function however, requires an understanding of the cellular correlates and mechanisms that underlie sleep, an area of sleep research that remains poorly characterized. The following sections provide an overview of the complexities that underlie sleep and our efforts to identify biological function (s) using systems biology approaches.

CHARACTERISTICS OF SLEEP

The evolution of sleep research began with classical "top-down" experimental approaches that revealed a complex hierarchy underlies this relatively simple behavior. Sleep-wake behavior is orchestrated by a number of brain regions interacting with one another via the differential release of a variety of neurotransmitters and peptides. In addition, the lack of sensitive, high-throughput biotechnologies to characterize the cellular correlates of sleep in these regions did not become available until relatively recently. As a result, the specific biological function(s) of sleep remain unclear. Proposed functions include the maintenance of body temperature [McGinty & Szymusiak 1990; Wehr 1992], energy homeostasis [Benington & Heller 1995; Adam 1980; Walker & Berger 1980], immune function [Majde & Krueger 2005; Opp 2005], synaptic plasticity [Tononi & Cirelli 2006] and memory consolidation/reconsolidation [Born, Rasch & Gais 2006; Stickgold &

Walker 2005; Stickgold & Walker 2007]. One clinical consequence of these gaps is that drugs designed to treat sleep disorders may have a number of undesirable side effects, including addiction. Considering the number of people with sleep disorders and the potential that insufficient sleep can lead to other pathologies, there is an urgent need to develop safer and more effective drugs to treat sleep disorders.

The identification of sleep-wake states is based on a combination of observational and electrophysiological recordings, parameters that are similar in all vertebrates studied. Characteristics include a specific sleeping site, typical body posture, physical quiescence, an elevated arousal threshold, rapid state reversibility, and regulatory capacity, i.e., compensation for sleep after sleep loss. Waking is characterized by irregular, low voltage fast waves and high muscle activity. Non-rapid eye movement sleep or slow wave sleep (SWS) is characterized by high voltage slow waves and decreased muscle activity. In humans, this state is divided into four stages, with Stages 1 and 2 considered "light" sleep, and Stages 3 and 4 regarded as "deep" sleep or SWS. Rapid eye movement (REM) sleep, the "other" sleep state, is characterized by low voltage fast waves in the EEG, and virtual atonia of the neck muscles, as well as rapid eye movements. Together, SWS and REM states provide the basis of sleep architecture. Though sleep architecture and sleep stage classifications differ between rats and humans, the process of falling asleep is similar and can be characterized by a progressive decrease in wakefulness that is followed by SWS. Under normal conditions, SWS precedes REM sleep.

Sleep-wake behavior is regulated by a combination of circadian and homeostatic factors [Borbely 1982; Borbely & Achermann 1999]. The circadian control of sleep-wakefulness is associated with the regulation of sleep timing and emanates from the master pacemaker in the suprachiasmatic nucleus (SCN) [Edgar 1995]. Homeostatic factor(s) are associated with the regulation of sleep drive, sometimes expressed as sleep need. The homeostatic influence on sleep is suggested by the observations that longer periods of wakefulness result in an increased need for sleep, cumulative bouts of SWS and REM sleep are necessary to dissipate the sleep drive [Levine, Roehrs, Stepanski, Zorick & Roth 1987; Stepanski, Lamphere, Roehrs, Zorick & Roth 1987], and sleep-wake transitions persist even after lesion of the suprachiasmatic nucleus, the master circadian clock [Tobler, Borbely & Groos 1983]. The nature of the homeostatic drive is unknown; however, an increase in an endogenous factor(s) that accumulates during wakefulness and dissipates during sleep has been postulated to regulate homeostatic drive. At the electrophysiological level, this buildup is reflected by a gradual increase in slow wave activity (SWA; 0.1–4 Hz) during waking that dissipates with sleep. In rats, an increase in SWA accompanies sleep deprivation, reaching an asymptote after ~12 h of continuous waking [Tobler & Borbely 1986]. SWA declines exponentially during the recovery sleep that follows SD. As a result of these considerations, sleep research has primarily used total and selective SD paradigms as tools to investigate the homeostatic component of sleep and to distinguish SWS and REM effects on sleep. However, recent demonstrations that the targeted disruption of core circadian clock genes affects sleep duration, sleep structure and EEG delta power and core circadian gene expression in the cortex appears dependent on prior sleep-wake history

[Franken & Dijk 2009] underscore the complexities involved in studies designed to distinguish circadian from homeostatic effects at the cellular level. Our data examining Per-2 levels, a circadian transcription factor, following SD/RS are consistent with these findings [Greco, unpublished]. Thus while homeostatic and circadian processes have different origins, at the cellular level, they may interact directly with one another to control behavior.

INITIAL STEPS

To address the question of sleep function(s), a "bottom-up" analysis was undertaken to identify putative cellular correlates of sleep within the brain. Using high through-put mRNA and protein technologies, changes in the expression of proteins [Vazquez, Hall & Greco 2009; Vazquez, Hall, Witkowska & Greco 2008; Basheer, Brown, Ramesh, Begum & McCarley 2005] and/or mRNAs [Cirelli, Gutierrez & Tononi 2004] associated with energy metabolism, re-dox state, and synaptic plasticity were shown to underlie sleep-wake bouts that occur during the latter portion of the lights-on period. In addition, comparison of protein profiles across spontaneous sleep in young and old rats indicate that processes like synaptic plasticity that are controlled by phosphorylation [Ramakers 2002] may be compromised in old animals from damage to intracellular organelles and macromolecules (i.e., DNA, proteins, and lipids) caused by the accumulation of reactive oxygen and nitrogen species, ROS and RNS, respectively [Balaban, Nemoto, Finkel 2005; Calabrese, Giuffrida Stella, Calvani, Butterfield 2006; Joseph, Shukitt-Hale, Casadesus, Fisher 2005]. ROS/RNS species may also affect other post translational modifications (i.e., acetylation) involved in the regulation of other systems integral to the maintenance of circadian effects on biological timing [Borrelli, Nestler, Allis, Sassone-Corsi 2008]. SD also generates ROS and RNS species; thus mitigation of the responses described herein may also provide insights into aging.

Our experimental approach has several unique features. In particular, the specific mix of sleep-wake behavior is controlled with high precision at the time of sacrifice [Vazquez, Hall, Witkowska, Greco 2008]. Once sleep- and/or wake-related proteins and their related activities are determined in the brain, putative interactions between brain regions and with other organs across these states can be mapped and compared using systems biology approaches. With this strategy in mind, we have embarked upon some initial protein analysis studies and we are starting to use the results to develop a predictive computational systems biology model of the dynamics of sleep.

As sleep deprivation studies are traditionally used to differentiate sleep timing (circadian) from sleep need (homeostatic regulation), we believe that using this paradigm will provide insight into mechanisms associated with both time of day and sleep-wake components of biological timing.

INTEGRATED BIOLOGICAL SYSTEM MODELING

Our approach to developing a predictive model of the biological systems underlying the responses to sleep deprivation combines statistical analysis, logical representation of subsystems, processes (also known as executable or symbolic systems biology) and interactions, model abstraction and hybrid systems methods that accomodates different levels of detail, types of measurments, and missing information. Subsystem models can be combined using module calculi that are part of the logical framework and linking rules. This approach will be applied to integrating models of multiple subsystems and pathways, with behavioral, realtime, and homeostatic measurements. We are not aware of any existing work that attempt to model such diverse subsystems or such a the range of data types.

Traditionally, biological models have been represented using simplified drawings (cartoons) capturing relations between key components (A activates/regulates B) and tables relating model components and experimental observations. Such models are invaluable to build initial insights and give overall structure, and can be used to guide our initial model development. However these models don't scale, it is difficult to understand the consequences when two models are combined, and they are not directly suited to computational processing.

Computational systems biology is an approach to overcome limitations of informal models, and to bring the power of computation to understanding the results of biological data. Such models have generally been based either on systems of differential equations or statistical analysis of high throughput data. The differential equations approach can be used to answer questions about dynamic (kinetic) aspects of a system, such as change of concentration or expression of one or more molecules over time, under different conditions. One problem this approach faces is the lack of experimental data for rate parameters, thus techniques for inferring or fitting parameters must be used. Simple curve fitting is often used to measure responses with circadian components. Recent work has shown that adding biochemical components to the equations can improve model fidelity. The statistical approach can be used to infer correlations between changes in different components and is frequently applied to understand transcriptomic data. The resulting interaction graph can be used, for example, to identify highly connected components, to determine subgraphs that correspond to biological function or processes. Such models give useful high level insights, but are not adequate to explain underlying mechanisms or to predict effects of change.

SYSTEM-WIDE MODELING APPROACH

Integration of proteomics and gene expression data from non-human animal studies into a computational model of baseline behaviors is a rational and innovative means to examine key links between sleep deprivation , the resulting behavioral responses (adaptive or dysfunctional), and other effects of inadequate sleep. We envision that these models may eventually be used to support the neuroergonomics community, in the study of human sleep characteristics and effects. In addition, they may offer a

means to assay the efficacy of existing sleep-wake drugs, to aid the discovery /development of novel drugs to modulate sleep-wakefulness, and to facilitate studies designed to establish safe and effective drug treatment regimens *in silico*.

To examine putative functional interactions between proteins identified by mass spectrometry and sleep-wake behavior within the frontal cortex, we have used Pathway Logic, a symbolic computational modeling system and signaling knowledge base, to look for common functional path(s). One resulting "hit" linked proteins associated with cellular transport/cytoskeletal support and signal transduction to synaptic plasticity, a property of neurons which underlies higher executive behaviors like memory, cognition and learning [Guzman-Marin, Ying, Suntsova, Methippara, Bashir, Szymusiak, Gomez-Pinilla, McGinty 2006; Stickgold, Walker 2007]. We are currently testing the signal transduction pathway identified by Pathway Logic. Our plan is to develop a symbolic systems biology model to support the interpretation of the data resulting from sleep studies to identify biomarkers and mechanisms underlying adaptive and maladaptive responses to sleep deprivation and to predict the effects of modulating target markers on the response.

The symbolic computational system model provides a framework for integrating the diverse types of measurements. As data is incorporated into the model, we will be able to look for underlying mechanisms that correlate the different observations, across data types, time and sleep conditions. The intent is to identify significant correlations and dependencies between model features, and by using the underlying computational system model to identify processes/pathways affected by components with significant changes. These analyses will be used to explain and validate potential markers that are linked to adaptive and maladaptive responses to sleep disorders.

Our underlying hypothesis is that the restorative function(s) fulfilled during sleep affect the entire body. This lends itself to using a broad systems approach to sleep exploration, which draws us to examine the signaling processes between the brain and the rest of the body – specifically across the blood-brain barrier. We therefore plan to look for markers of sleep deprivation in blood, as well as unique protein expression in liver, which may be signals controlling the metabolic processes that correlate with effective sleep states. By adopting this system-wide approach, we anticipate that a connected whole-body model will emerge, which is supported by the data and adds confidence to the theory. For example, perhaps the blood is carrying sleep-related signals from the brain, that control what the liver is doing.

The modeling effort involves the construction of a baseline system level model, which can then be used to predict the effects of modulation and to interpret experimental results. The proposed multi-level baseline model will incorporate and integrate models from several subsystems, and will include both non-human and human sub-models (based on data availability). It will incorporate biological similarities (functional, structural) to assist in the creation of a broad human model of the intracellular underpinnings of adaptive/maladaptive responses to sleep disorders. Figure 1 shows the architecture of the system model.

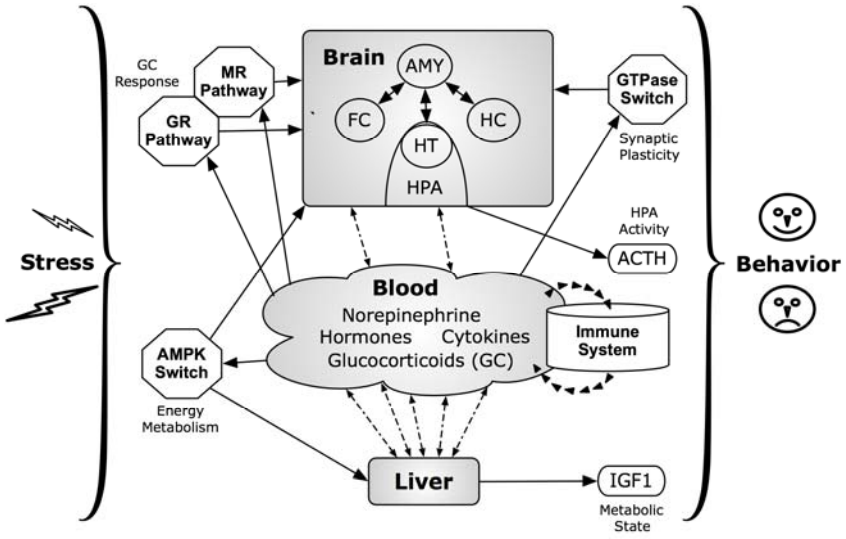

FIGURE 1. System level model of response to stress (sleep deprivation), reflecting interaction with its environment via stress inputs (on left, bold represents high stress, light represents low stress) and behavioral response on the right (managing stress, smile; negative stress effects, frown).

The figure shows three main system model components - brain, blood, and liver - along with signals flowing between components that serve as modulators of behavior. Brain submodels correspond to key regions: Frontal Cortex (FC), Amygdala (AMY), Hippocampus (HC), and Hypothalmus (HT). HPA (Hypothalamic-Pituitary-Adrenal axis) modulates cognition by propagating signals from the HT. ACTH (Adrenocorticotropic hormone) is an indicator of HTA activity. Blood serves as a transport system for signaling molecules such as norephinaphrin, hormones, glucocorticoids (GC), and cytokines, including a providing a connection with the immune system. Liver cell behavior is modulated by signals from the blood system and from the AMP activated protein kinase (AMPK) switch. Insulin growth factor, IGF1, is a key indicator of the metabolic state. Octagons represent pathways through which received signals (arrows into octagons) are transduced into signals modulating behaviors of target components (arrows out of octagons). Thus, the GR and MR pathways are glucocorticoid-like receptor pathways that control energy metabolism depending on glucocorticoid level. The GTPase switch integrates signals from molecules including ephrins, and neurotrophins to modulate features such as synaptic plasticity.

Information for the baseline system model will be curated from existing knowledge about interactions, behaviors, and phenotypes available in the literature and databases. As noted earlier, we expect model construction to initially focus on the major subsystems - brain, liver, and blood. Beyond the high-level systems view, information about specific processes, switches and pathways involving selected proteins and metabolites will be curated and linked into the system level model.

We plan to use existing systems biology knowledge bases (KBs), including Pathway Logic (pl.csl.sri.com), PANTHER (pantherpathway.org), and BioCyc (biocyc.org), as a starting point and collect additional information as needed from external pathway databases, such as KEGG and the NCI Nature Protein Interaction databases, and published literature. The collected information supporting the computational model will be stored using a formal KB representation with a well-defined schema/ontology. A system-level signaling KB helps to organize data to enable extraction of specific models and search for relevant patterns and pathways. Model elements will be extracted from the resulting KB by query. The KB will also be a source of facts for initial model validation and consistency tests.

Computational model elements will be represented using a combination of logical constraints and a rule-based formalism integrated using an algebraic signature. They will be combined using logical module operations and analyzed using formal reasoning tools such as model checking, constraint solving, and model abstractions. A neural model will be developed from data obtained from regions of the brain linked to the modulation and control of adaptive/maladaptive cognitive response(s) to stress. Sleep-related inputs and outputs for each region will be modeled and will include regional interconnections.

CONCLUSION

Our long-term vision for this work is to develop a comprehensive, system-level computational modeling and analysis framework for exploring sleep-related processes and responses in the brain, liver, and other organs. For example, a neural model of the effects of sleep across brain regions will help characterize the modulation and control of the cognitive impacts of SD/RS. A model that maps sleep-related processes in the liver will help describe the relevant effects of energy metabolism and other expression markers. A third model, using the levels of signaling molecules in blood, provides a means to represent the blood as a transport system for organ-organ intercommunications. The result will be a high-level, holistic system view of the dynamics of sleep throughout the body.

This type of computational model can be used to understand interactions between subsystems, as well as to predict possible consequences of perturbing the system. Genetic variation in primary protein sequence represents one example of such a perturbance, where the altered genes may produce non-functional proteins, proteins that are always active, or do something else that deviates from the norm. Similarly, altered neural interconnections can occur, hence sending signals to unexpected places. We anticipate that our computational modeling framework will prove to be a valuable tool for neuroergonomic analysis of these important issues.

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