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What Makes a Good Homeostat? Influencing circadian and sleep-wake regulation for prevention and intervention in mood and anxiety disorders

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Abstract

All living organisms depend on homeostasis, the complex set of interacting metabolic chemical reactions for maintaining life and well-being. This is no less true for psychiatric well-being than for physical well-being. Indeed, a focus on homeostasis forces us to see how inextricably linked mental and physical well-being are. This paper focuses on these linkages. In particular, it addresses the ways in which understanding of disturbed homeostasis may aid in creating classes of patients with mood and anxiety disorders based on such phenotypes. At the cellular level, we may be able to compensate for the inability to study living brain tissue through the study of homeostatic mechanisms in fibroblasts, pluripotent human cells, and mitochondria and determine how homeostasis is disturbed at the level of these peripheral tissues through exogenous stress. We also emphasize the remarkable opportunities for enhancing knowledge in this area that are offered by advances in technology. The study of human behavior, especially when combined with our

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greatly improved capacity to study unique but isolated populations, offers particularly clear windows into the relationships among genetic, environmental, and behavioral contributions to homeostasis.

Keywords

homeostasis; circadian rhythms; psychiatry; genetics; mood disorders; anxiety disorder

Introduction

The second International Scientific Group of Circadian Rhythm Experts (INSPIRE) conference was held April 24-26, 2014 in Viareggio, Italy. It was attended by 27 senior basic and clinical scientists from around the world each of whom was accompanied by a junior colleague. Several of many of whom formed the authorship group for this report, the conference covered the spectrum of research on homeostasis as it relates to sleep-wake regulation and to mood and anxiety disorders. The conference provides a unique opportunity for considering how these various parameters interact to produce health or illness. This report summarizes the conference presentations and seeks to capture the very interesting and lively interchange among the varied group of scientists attending.

Circadian Coherence in Disease and Health

Overview

The opening plenary address was given by Michael Hastings (MRC-Laboratory of Molecular Biology, Cambridge) who presented an up-to-date account of the importance of circadian coherence in health and disease. In particular, Hastings considered the question of whether “cerebral circadian coherence was the secret to good sleep” by examining the consequences of circadian incoherence both within the suprachiasmatic nucleus (SCN), the site of the circadian clock, and between the SCN and other brain regions implicated in regulating the sleep/wake cycle. His introductory statements considered Borbely's two-component model (Borbely & Achermann, 1999), which suggests both the quality and timing of sleep and wakefulness result from the finely tuned interaction between homeostatic and circadian components. The homeostatic process keeps track of the time spent awake whereas the circadian process sets internal time of day. Sleep occurs when the homeostatic drive outweighs the circadian signal for wakefulness. During sleep the homeostatic drive dissipates and ultimately wakefulness is restored by resurgent circadian activation. A key, unaddressed question is whether circadian regulation of sleep is solely a property of the SCN, or a property of a distributed network of local brain clocks. Following a brief overview of the SCN as the circadian pacemaker and its role in synchronizing the cellular clocks in both the brain and periphery, Hastings went on to consider the impact of circadian incoherence on health in both animal models and humans. If multiple circadian systems within the brain and periphery are forced out of alignment with the SCN by genetic, environmental, or emotional insults, does this compromise health and in particular, the sleep/wake architecture? Hastings noted that novel techniques to either delete or alter the periodicity of specific cell types and/or regions, now provide an opportunity to tease apart

the specific roles of the circadian and homeostatic processes in controlling sleep and wakefulness.

SCN and peripheral clocks

Circadian rhythms have a period of approximately 24h and are generated by an internal pacemaker located in the hypothalamic SCN, which drives and synchronizes our physiology and behavior to the 24 hour environment. At the molecular level, the circadian clock consists of auto-regulatory transcriptional/translational feedback loops where Period (Per) and Cryptochrome (Cry) genes are driven by E-box-dependent transcriptional activation mediated by Clock and Bmal1 heterodimers. The translated proteins translocate back into the nucleus to repress their own transcription. The time constants for the production, post-translational modification and degradation of the protein products mean that this whole process takes approximately 24h to complete, hence circadian (circa= approximately, dian= one day). In addition to the core clock mechanism, these genes also synchronize E-box mediated clock-controlled output genes. This mechanism enables the SCN to direct time-dependent activation of genes to control local physiology and metabolism throughout the body. Hastings outlined the current technology used to examine the SCN pacemaker, and in particular the circadian activity of E-boxes using a novel transgenic mouse where the Cry1 promoter drives firefly luciferase activity, revealing the exquisite spatial and temporal complexity of the molecular oscillations across the several thousand neurons of the SCN circuit (Maywood et al., 2013). In addition to the Cry genes, the Per genes are also necessary for the expression of circadian rest/activity behavior and SCN pacemaker activity since null mutations or ENU-induced mutations (Bae et al., 2001; Maywood et al., 2014; Anand et al., 2012); can disrupt or alter the periodicity of these overt rhythms. However, the SCN is not the only circadian clock, as circadian oscillations of Per-driven bioluminescence in NIH 3T3 fibroblasts persist for several days at the individual cell level. Importantly, however, these cell-autonomous rhythms are not synchronized, as there are no synaptic interactions or other temporal communications between the cells (Welsh et al., 2004). A defining feature of the SCN therefore, is the ability for inter-cellular cross-talk to maintain the temporal and spatial synchrony of the circuit. So powerful is this synaptic and paracrine communication that the SCN can maintain coherent circadian oscillations for many months when isolated in vitro (Maywood et al., 2011; Brancaccio et al., 2013).

Circadian coherence and health

The SCN is, therefore, a self-sustaining circadian clock, and is able to keep the rest of the brain and peripheral tissues in synchrony via its behavioral, hormonal and nervous efferent cues. To be adaptive, this circadian network needs to be synchronized to solar time, so that its period is exactly 24 hours and internal day and night are correctly phased to the light dark cycle. This is dependent on the second defining property of the SCN - it is the only component of the network to receive, directly and indirectly, photic information from the retina. So what is the physiological importance of this circadian network, and more specifically what is the particular role of peripheral circadian clocks? In one example, a recent proteomic study by Hastings' group showed that the expression of hepatic enzymes important in carbohydrate metabolism underwent major changes in abundance as a function of circadian time (Reddy et al., 2006). This enables the hepatic clock to anticipate and

thereby accommodate daily metabolic challenges linked to the cycle of feeding (during wakefulness) and fasting (during sleep). Another example from the Weitz laboratory demonstrated directly the importance of circadian coherence in metabolic health by ingesting a Cre recombinase genetic mouse model crossed with a mouse where the *Bmal1* gene has been floxed (Lamia et al., 2008). This produced a mouse with liver-specific deletion of an essential clock component i.e. a “clockless liver”. In normal mice, the liver clock controls the expression of the glucose transporter that facilitates release of glucose into the circulation. This is a rhythmic event, peaking during the hours of fasting, thereby buffering circulating glucose levels. In the liver mutant animals, expression of the transporter remains permanently at basal levels, and these animals exhibit progressive hypoglycemia during the fasting phase. Thus, the loss of the local clock deprives the animal of the ability to accommodate to and anticipate the rhythmic daily metabolic challenges associated with the sleep/ wake cycle. Hastings used the analogy of a modern day factory, where components for the assembly line arrive as and when they are needed, to describe the importance of these findings; for the efficient control of physiological processes like glucose metabolism the necessary genes and their products need to be available “just in time”. If they are present too soon or too late this will lead respectively to inefficiency or compromise. Therefore local clocks can deliver requirements for internal physiology to meet the rhythmic needs imposed by daily behavior. Is the concept of local clocks, in particular brain clocks relevant to human physiology? A study by Li et al applied time-of-death analysis to gene expression data from post-mortem human brain and revealed cyclic gene expression in the dorsolateral prefrontal cortex, which included known circadian genes including *Per1-3* and *Bmal1* (Li et al., 2013). Furthermore their phasing was consistent with data derived from diurnal animal models. Thus local clock gene networks are active in the human brain.

These networks of local circadian clocks are widely distributed across the brain centers that are involved in the regulation of sleep and wakefulness and cognition (Hastings et al., 2008). Sleep and wakefulness are global states across the neuraxis. For the entire network to operate effectively, the local circadian clocks need to be synchronized to each other and to external solar time, and this is achieved by efferent synchronizing signals from the SCN. These local clocks control the timing of tissue-specific transcriptomes so that different sets of genes are activated and/or inactivated. The particular constellations of genetic activation ensure that in the alternating brain states of wakefulness and sleep, the physiological and metabolic demands are appropriately timed to allow the brain to anticipate the circadian transitions between sleep and wakefulness. Therefore, misalignment of the sleep and wakefulness cycles with circadian phase, or genetic perturbation of the clock mechanism in the SCN and/or locally could compromise sleep and wakefulness, the efficient regulation of energy metabolism and/or consolidation of memory and the regulation of mood (See Figure 1; adapted from Kyriacou & Hastings, 2010).

Novel technologies to study consequences of circadian incoherence

Experimental tests of the hypothesis presented by Hastings require refined approaches to manipulate specific genes in both a temporally and spatially specific manner. The recent advances in genetic and pharmacogenetic tools for such purposes have enhanced the ability of scientists to look at such circuit-level interactions, and this is ideally suited to unravelling

the circadian and homeostatic components of the sleep/wake cycle. In particular, the Cre-lox system where Cre recombinase, a site-specific enzyme which catalyzes recombination between 2 loxP sites within DNA, under the control of promoter regions of interest can result in deletion, inversion or translocation of the targeted allele in selected cell types and tissues. By using this technology Hastings looked at the consequences for sleep when there was circadian incoherence between the SCN and other brain regions. Targeting specific cell populations in the SCN to delete a gain-of-function mutant allele, in this case the casein kinase 1 epsilon (CSK1 ϵ), resulted in a chimeric mouse where the SCN had a period of 24h and so a circadian rest-activity cycle of 24 hours, even though local clocks in brain and periphery retained intrinsic periods of 20h (unpublished observations Smyllie, Maywood, Hastings). EEG recordings of such mice revealed a marked contribution of local clocks to the structure of sleep (Maywood et al. unpublished). A second example, in which the local clock of the histaminergic cells in the tuberomammillary body (TMN), was genetically targeted, showed how sleep structure and sleep-dependent memory are also dependent upon brain circadian function outside the SCN (unpublished observations, Wisden et al). Taken together the results underlined the complex, non-unitary nature of the circadian component of sleep regulation and its interaction with homeostatic mechanisms. Returning to the factory analogy to end his talk, Hastings suggested that these local clocks deliver genes encoding enzymes, ion channels etc. 'just in time', where and when in the brain they are needed to sustain the anticipated global states of sleep and wakefulness, and their dependent neural functions. Extending the analogy reveals very clearly the potential disturbance to sleep and sleep-dependent cognition and mood that would arise from circadian misalignment.

Reformulation of Psychiatric Diagnosis Based on Disturbed Homeostasis

Mood and anxiety homeostasis and the daily rhythms of life

Mental health and substance use disorders account for 60% of the years lost to disability in 15-34 year olds. The majority of these mental health disorders are mood and anxiety disorders. Professor Ian Hickie (University of Sydney) proposed that the reformulation of these disorders based on disturbed circadian processes might improve prevention, early intervention, mental and physical outcomes and ultimately reduce disability in this group.

The working hypothesis is that circadian dysfunction underpins some (but not all) mood disorders, particularly mania, atypical depression and persistent fatigue states. It is this dysfunction that may result in age-dependent phenotypes, observable from infancy (e.g. difficulties in establishing regular sleeping patterns) to late adolescence and early adult life. The potential causes of circadian dysregulation were suggested to be in part genetic. For example, morningness versus eveningness (i.e. peak alertness and a preference for activity in the morning versus the evening) is a heritable trait (Klei et al., 2005; Vink et al., 2001), an unstable clock that switches very easily or the capacity for the clock to switch. Genetic effects would interact with age-specific environmental events. If correct, such a model would have potential for reverse translation into relevant animal models.

The focus of the presentation was on the recruitment of young people (aged 16-19) presenting with mood disturbances. A three pathway model was proposed to describe these

states at their onset, when symptom clusters often do not meet the thresholds needed for conventional diagnoses (Hickie et al., 2013): a primarily anxiety pathway, a circadian pathway and a developmental pathway. Around 25-30% of clinical cases are that of circadian depression. i.e., they exhibit a disruption of the 24-hour sleep-wake and circadian systems as the fundamental biology. The experience of circadian depression is understood as a pathophysiological experience rather than purely psychological; one that might be described as prolonged jetlag, low sense of vitality, loss of interest and difficulty concentrating and sleeping.

Hickie highlighted the utility of actigraphy to measure repeated activity cycles across the day (as opposed to sleep), particularly for use within large population studies. Motoric activation, as a measure of circadian dysfunction, (captured by actigraphy) is both highly reliable across species and better characterizes the experience of mania than changes of mood, within adolescent samples. It is the variability of motoric activation across a two week period which allows insight into the robustness of an individual's clock. Data were presented in relation to mood; depressed mood was associated with less activity throughout the day in depressed participants and increased sleep time was associated with increased activity later in the day for Bipolar I patients, but not controls.

Study of the heritable components of bipolar disorder provides a way to identify reliable components of any cross-sectional model. Thus, the concept that mania and depression are both part of the same underlying diathesis is challenged by family studies (Hickie, 2014; Merikangas et al., 2014). Instead, there is a high heritability for the activity component of mania. The largest heritable part of depression is the atypical form. Removing the atypical and activity parts of major depression leaves a very low heritability. This suggests that a conceptualization of bipolar disorder that puts mania and depression together along a single dimension might be misleading. Instead, Hickie presented an alternative model (see Figure 2; Hickie, 2014) that places bidirectional motor or psychic activation as central, with depression and psychosis constructs along separate unipolar axes.

In support of the activation hypothesis, data were presented from the National Comorbidity Survey indicating that those with fatigue and mood or anxiety disorders are the most disabled (Lamers & Merikangas, 2013). The prevalence of hypomanic symptoms is relatively high; 18% of respondents endorsed all five hypomanic symptoms, yet there is very little genetic contribution to this. Conversely, a twin study of monozygotic and dizygotic twin pairs revealed that there is a strong genetic influence on adolescent sleep-wake patterns, specifically sleep initiation and maintenance (Sletten et al., 2013).

Clinical data were presented to further support the concept of circadian-based mood disorders. With regard to sleep wake patterns, 62% of depressed bipolar disorder patients exhibited a delayed sleep phase, compared to 30% of patients with unipolar depression and 10% of controls (Robillard et al., 2013a). A later sleep schedule at baseline predicted worse mania at follow up. Those with bipolar disorder exhibited more unstable circadian cycles, with decreased circadian rhythmicity and later cycles. Bipolar samples also evidenced later dim light melatonin onset and overall reduced melatonin secretion, i.e. less area under the curve (Robillard et al., 2013b). Current disability and mania symptoms were related to

delayed melatonin onset. These data converge to indicate a circadian disruption in bipolar patients.

Looking at sleep across disorders, there is evidence of later sleep onset in depression, anxiety, and bipolar groups (but not psychosis) compared to controls. There is also evidence of later sleep offset in depression, anxiety, bipolar disorder, and psychosis compared to controls. All four clinical groups spend more time in bed compared to controls and there is evidence of a longer total sleep time in those with bipolar disorder and psychosis, but not depressed or anxious patients (Robillard et al., 2014). In terms of chronotype, all four groups show a shift towards eveningness on a morningness–eveningness questionnaire, but this effect is more pronounced in the affective disorders group than in psychosis.

Hickie closed his presentation by describing current work in the realm of circadian therapeutics. He views this as a potentially promising area for improving outcomes in those with circadian-based mood disorders.

Autism spectrum disorders and circadian functions: Identification of biological pathways

Marion Leboyer (Henri Mondor Hospital, Paris) discussed the biochemical abnormalities underlying severe sleep dysfunctions and circadian disturbances observed in autism spectrum disorders (ASDs). Researchers are searching for biological pathways involved in ASD in order to identify biomarkers to better understand the mechanisms, and hopefully to find treatments for these disorders.

ASDs are complex, heterogeneous and multifactorial, characterized by impaired social communication and stereotyped behaviors. A variety of other psychiatric symptoms are frequently observed in ASD, including circadian disturbances and sleep disorders (40-80%; Silvertsen, 2012). They persist from infancy to adulthood and affect the health of the whole family. The etiology of ASD is considered to be mostly genetic (Constantino, 2010), but the heritability is complex (Huguet et al., 2013), involving genes associated with syndromic forms of ASD (Moss & Howlin, 2009) (Fragile X syndrome, Rett syndrome, tuberous sclerosis), genes associated with high risk of ASD (*SHANK3*, *Neuroligins*), and susceptibility genes, particularly genes involved in melatonin synthesis such as *ASMT* (Pagan, 2011).

Melatonin is synthesized from serotonin via two steps: the first one involves the enzyme AANAT, which is particularly important in determining the phase of the melatonin rhythm, and produces the N-acetylserotonin (NAS); the second involves ASMT, which controls the amplitude of melatonin production. Biochemical abnormalities in this pathway have been widely reported. Among them, elevated whole blood serotonin is the most replicated finding (Gabriele et al., 2014). A deficit in melatonin has also been described in several studies based on plasma or urine of individuals with ASD (Rossignol, 2011). The mechanisms of these impairments and the clinical correlates, however remain largely unknown.

Whole blood serotonin, platelet NAS and plasma melatonin were assessed in 278 patients with ASD, their 506 first-degree relatives and 416 sex- and age-matched controls, recruited by the PARIS (Paris Autism Research International Sib-pair) study (Pagan, in press). This

study confirmed the previously reported hyperserotonemia in ASD (40% of patients) as well as the deficit in melatonin (51%). In addition, this study revealed an increase in NAS (47%). To a lesser extent, these biochemical impairments were also observed in the first-degree relatives of patients. A score combining impairments of serotonin, NAS and melatonin distinguished between patients and controls with a high sensitivity and specificity. AANAT and ASMT were then explored in platelets and in post-mortem tissues, in both the intestinal tract and the pineal gland in a small sample of subjects. Reduction of the two enzyme activities was confirmed in these tissues and a correlation of ASMT activity with melatonin level was found.

What are the genetics of melatonin deficit in ASD? Leboyer presented a meta-analysis for AANAT and ASMT genetic variants. There was only a minor contribution of ASMT variants to melatonin level, even though AANAT and ASMT variants have been associated with ASD and bipolar disorders. And what are the clinical correlates of the biochemical alterations? While no correlations were found using clinical data such as diagnosis or ADI scores, only insomnia revealed a significant association with melatonin deficit in patients. Clinical sleep markers were then assessed in a subgroup of high functioning adults with ASD, using actigraphy, the Pittsburgh Sleep Quality Index (PSQI) and other questionnaires, and showed poor subjective sleep quality and longer sleep onset latency in patients.

Considering the evidence for sleep and circadian disturbances as well as biochemical impairments of the serotonin-NAS-melatonin pathway in ASD, what are the consequences for therapeutic approaches? Based on the results presented, melatonin has been identified as a therapeutic target for sleep disturbances associated with ASD. These findings may open the door for personalized medicine in this population.

Genetics of mood and sleep disorders

Overview

Joseph Takahashi (UT Southwestern Medical Center) introduced the session by quoting Donald Rumsfeld, former United States Secretary of Defense, who once famously delineated problem-solving into “known knowns,” “known unknowns,” and “unknown unknowns.” Takahashi stated that this session would be about using genetics to solve the problem of the “unknown unknowns.” In other words, the goal of this session was to illustrate how genetics is really the only way to dissect complex systems without any previous understanding of the underlying mechanisms or processes of disease. The specific diseases under discussion in this session included major depression, bipolar disorder and narcolepsy.

Genetic analysis of major depression in 12,000 Chinese women

Jonathan Flint (Oxford University) presented a genetic study of major depression (MD) in a Chinese population. He began by outlining the difficulties of dissecting out the genetic contributions to depression. Typically in disorders like schizophrenia, there are an increasing number of associated genes detected with an increase in the sample size. In depression, however, approximately 30,000 cases and 30,000 controls would be needed to observe at least one genetic genome-wide association with confidence. Therefore, the chance of finding

a single hit is small. Because of the varying disease prevalence, depression is more like weight than height in terms of prevalence relative to sample size. So, how can a study be designed to take advantage of this? One way to reduce heterogeneity is by only looking at severely depressed patients. In the study China, Oxford and VCU Experimental Research on Genetic Epidemiology (CONVERGE) was described throughout the rest of the talk, 6000 cases and 6000 controls were compared. Patients were women age 40 and over who had experienced multiple episodes of major depression (MD). Risk factors such as personality, childhood sexual abuse, stressful life events, poor parenting, and low social support were assessed to account for heterogeneity. Furthermore, the co-morbidity of other disorders such as anxiety, dysthymia, and melancholia were determined to assess the specificity of MD.

China was chosen as the site of the study because of the large population with a good health system that could provide access to large numbers of participants. Sixty hospitals across 30 cities in eastern China participated in four years of data collection. The entire genome of the 12,000 participants was sequenced at BGI (Beijing Genomics Institute), although because of cost considerations, each genome only had approximately 1.7X coverage. While it is possible to look at sequence variants in these data, with this amount of coverage there are likely many gaps or holes. In addition to the nuclear genome, however, the mitochondrial genome of all of these participants was also sequenced to ~70x coverage in these samples. DNA from saliva was used for all of the sequencing. Interestingly, the total amount of mitochondrial DNA (mtDNA) was increased in cases compared to controls. To test whether drugs that patients with MD often take were the cause of this change in mtDNA, mouse saliva was sampled after treatment with anti-depressant drugs and the mtDNA actually went down, demonstrating that drug use was unlikely the cause of this change. To bolster the human data, the mitochondrial finding was replicated in a separate, smaller cohort using data from blood.

Next the relationship between changes in mtDNA and MD was discussed. Patients with MD have more stressful life events. For example, there is an increased incidence of childhood sexual abuse (odds ratio of ~10) in cases of MD compared to controls. In addition, there is a significant correlation between both stressful life events and childhood sex abuse with increased mtDNA. To test directly this correlation between stress and mtDNA, experiments were carried out in mice. If mice are stressed, there is a concomitant increase in mtDNA in saliva. However, among additional organs tested only the liver demonstrates this relationship, not the brain, ovaries, or muscle. Stressed mitochondria are functionally impaired as indicated by oxygen consumption. So even though specific cells may have more mitochondrial DNA, these particular mitochondria are impaired. The mechanism by which this occurs is currently unknown, but, interestingly, steroid injections can replicate the increase in mtDNA in saliva. Overall, this study observed a marker of metabolic features of MD: namely changes in the DNA of the mitochondrion, the organelle responsible for oxidative phosphorylation in all cells in the body.

Next, the results of genome-wide association studies (GWAS) from these data were presented. Only one locus was found to be close to significance in primary MD, near the gene *SIRT1* (data presented in a poster session at the INSPIRE Conference, 2014, Authors et al.). *SIRT1* is known to be involved in mitochondrial biogenesis. It was proposed that a

switch mechanism might exist to explain these data. Once a patient develops MD (through a yet-to-be discovered genetic mechanism) there are changes in metabolic functions and these are detectable in the saliva and indicated by the increase in mtDNA. One of the “take-home” messages from this talk was the importance of designing genetic studies of MD to take into account possible sources of heterogeneity, even though doing so requires time-consuming clinical phenotyping. While progress with the genetics of schizophrenia was made by cutting down on clinical phenotyping and increasing sample size, for MD there may not be any shortcuts.

Genetics of bipolar disorder and related endophenotypes

Nelson Freimer (UCLA) described his research elucidating the genetics of bipolar disorder (BD-I) using endophenotypes in both human and non-human primate populations. As there are multi-level component phenotypes in BD qualifying BD as a complex disorder, multiple approaches are needed to give traction to understanding the genetic causes of symptoms in BD, from behaviors to genotypes.

The study discussed in this session uses large pedigrees in Costa Rica and Colombia that were chosen because these populations, which originated from admixture between Europeans and Native Americans, are closely related genetic isolates. Comprehensive phenotyping and genotyping (using both single nucleotide polymorphisms (SNPs) and whole genome sequencing) were performed in ~850 individuals. The comprehensive phenotyping uncovered a large diversity of phenotypic information (Fears et al., 2014), particularly with regard to behavior and structural MRI. The genetic approach included linkage and association analysis of heritable traits, statistical and bioinformatics analyses of annotated variants, and analysis of variants with predicted deleterious effect. Some traits identified as both heritable and associated with disease, which are the most interesting to study further. In general, the traits measured by the MRI are the most heritable. The volume of the amygdala was found to be a trait with one of the greatest LOD scores after the screen. Such a finding related to the amygdala might not have been that unexpected given the biology of BD; decreased amygdala size is an important and reproducible measure of BD. However, since this screen was undirected, the amygdala finding supports the power of this approach of unbiased endophenotype screening.

In the region of amygdala linkage over 60,000 genetic variants were identified in these pedigrees. Given this number of variants there is a need to screen and filter these data to look specifically at variants with predictions of deleterious function, novel versus rare alleles, and variants related to gene regulation (e.g. variants in areas of active chromatin).

Next, the role of sleep and activity measures as endophenotypes of BD-I (bipolar 1 disorder) in the Costa Rican and Colombian pedigrees were discussed. Three approaches for assessing these endophenotypes were carried out: chronotype from individual self-report measures (not discussed in detail in the presentation), behavioral measurements using actigraphy, and molecular phenotypes from the assessment of fibroblasts obtained from the cohort. Two-week actigraphy measurements from over 600 pedigree members (both BD-I and controls) were assessed. Such measurements included assessments of rest, sleep, activity, and circadian rhythms. Numerous sleep and activity traits were found to be heritable and

significantly associated with BD-1. The most interesting might be the traits related to circadian variation in activity. Finally, the in vitro molecular assays were discussed. Circadian activity was measured in fibroblasts taken from skin biopsies using a viral luciferase construct driven by the *Bmal* promoter. Currently, the study includes biopsies from 175 donors (89 BD-1 and 86 controls) and circadian parameters such as phase and period length can be ascertained using this method. The cells are grown under different conditions to assess responses to entrainment and free-running conditions. Next, Freimer discussed how comparative analysis across species can be used to understand the human data. Some limitations of human studies include environmental variability, availability of tissues, ability to do controlled longitudinal studies, or implementation of an invasive study. Rodents are often the animal models of choice for comparative studies; however, rodents have significant sequence divergence, poor synteny, functional divergence, significant brain and behavior differences, and evolved ecological niche compared to humans. In contrast, primates have low sequence divergence and there is ecological conservation with humans. Moreover, we can control the environment in which the primates live. The goal of such a study, using a primate animal model, is to integrate genetics, genomics, and behavior at multiple levels.

Thus, the final segment of this presentation focused on data using vervet monkeys, or African green monkeys, as a model system. Whole genome sequencing has been undertaken in 700 vervet monkeys that reside in a large research colony and a catalog of deleterious variants with human interest has been assembled. The phenome of these monkeys has been collected with regard to brain, behavior, and metabolism phenotypes. Some examples of the analyzed phenotypes include: MRI, CSF (cerebral spinal fluid) biomarkers, blood biomarkers, actigraphy, metabolism and growth (lipids, glycemic measures, gut microbiome), and multi-tissue transcriptome analysis from more than 100 tissues in over 90 monkeys with data from blood and fibroblasts from over 500 monkeys. More than 400 monkeys have had actigraphy recorded for a week on average. Using just this single measure, one can identify those traits related to actigraphy that are heritable in both monkeys and humans such as phase, mean activity during day or night, number of awakenings during sleep, etc (see Figure 3).

In summary, sleep and activity measures provide promising endophenotypes for understanding the genetics of BD; however, identifying causative variants will be complex. Thus the analysis of human phenotypes in non-human primates will ultimately be useful for understanding the biologic mediators of these endophenotypes.

Genetics of narcolepsy – Evidence for an autoimmune pathogenesis

Birgitte Kornum (Glostrup Hospital, Denmark) detailed how understanding the genetics of narcolepsy has provided insights into the molecular mechanisms underlying this disorder. Narcolepsy type 1 (NT1) is defined by severe sleepiness, rapid transition to REM sleep and abnormal dreaming, and cataplexy. NT1 has a moderate prevalence of 1:3000. However, as NT1 is determined by very specific phenotypes that can be clearly delineated in the clinic, genome-wide genetic variants can be found even with a smaller number of patients. NT1 is believed to be caused by a lack of the neuropeptide, hypocretin, through a very specific loss

of neurons in the hypothalamus. This cellular feature is specific to narcolepsy patients; it is not found in the brains of patients with insomnia or other sleep disorders.

Genetic studies of NT1 have focused on three approaches: 1) *HLA* studies investigating whether the loss of hypocretin is a result of autoimmune dysfunction; 2) family based studies including linkage analysis and exome sequencing (not discussed in this presentation); and 3) population studies i.e. GWAS. The *HLA* findings regarding NT1 have focused on the *HLA* locus on chromosome 6 where there is strong association of the *DQB1* allele with NT1. This is one of the strongest *HLA* associations ever found. *HLA* genes encode for MHC molecules that present peptides from the inside of cells to the outside where T cells can interact with them. Other *HLA-DQ* haplotypes influence narcolepsy susceptibility, with some of them even decreasing narcolepsy risk. GWAS has implicated the T cell receptor alpha (*TCRA*) chain (Hallmayer et al., 2009; Hor et al., 2010), and interestingly these cells interact with MHC molecules. The SNP with the strongest association in *TCRA* is in the antigen binding pocket, which if functionally relevant could implicate dysregulated antigen binding in NT1. A smaller effect size has also been found in the purinergic receptor subtype (*P2RY11*), an ATP G-protein coupled receptor (Kornum et al., 2011). The most significant SNP in *P2RY11* is in the 3' UTR. The "A" variant of this SNP leads to a decrease in receptor expression, whereas the "G" variant is correlated with higher expression and is thought to be a "protective" variant. The function of the P2RY11 protein is thought to be important for the survival of lymphocytes. Furthermore, a separate study using immunochip GWAS has found significant association with *HLA*, *TCRA*, *TNFSF4/OX40L*, and *CTSH* (Faraco et al., 2013), and GWAS of a Chinese cohort has implicated *TCRA*, *TCRB*, *P2RY11/DNMT1*, *ZNF365*, and *IL10RB* (Han et al., 2013). Taken together, the genetic studies of NT1 appear to converge on genes encoding proteins involved in lymphocyte and immune function.

Using this convergent genetic data can provide hypotheses regarding a potential autoimmune pathology in NT1. An autoimmune model was presented by which some unknown factor in the periphery (e.g. H1N1 influenza) triggers a response by mimicking hypocretin or another target in the hypocretin cells through presentation in the periphery to a CD4+ T cell that will then be activated. Microglia in the brain have also been found to be able to present epitopes to CD4+ T cells. It is possible that there is some stress that makes the hypocretin neurons vulnerable. The CD4+ T cells activated by influenza can enter the brain and become reactivated by microglia cells and/or help B cells produce antibodies that can enter the brain if the blood-brain barrier permits this. It was hypothesized that CD8+ T cells are attracted to the hypocretin-producing neurons and are involved in their death. Within two weeks of exposure to a trigger, a person can get narcolepsy symptoms suggesting a rapid pathogenesis, which would be supported by an autoimmune response. Together, these data demonstrate how genetic studies can be combined with known biological processes to delineate mechanisms of pathogenesis. This appears to be feasible in the case of narcolepsy because of the well-defined phenotype and pathology of this disorder.

Research on narcolepsy, a disorder of disturbed homeostasis, thus provides optimism that by creating classes of patients based on phenotype and a well-known HLA association can alter

clinicians' thinking about a disease and its mechanisms. This, in turn, can lead to an entirely new approach to intervention for that disease.

How to manipulate sleep

Optogenetic and pharmacosynthetic deconstruction of sleep/wake circuitry in the brain

Gary Aston-Jones (Medical University of South Carolina) reviewed the role of the locus-coeruleus (LC) norepinephrine (NE) in circadian regulation of arousal and described cutting-edge optogenetic and pharmacogenetic techniques currently employed in his laboratory to selectively control LC-NE neurons in rodents.

The LC is a small pontine nucleus that contains mostly NE-releasing neurons which project throughout all levels of the neuraxis (Aston-Jones & Cohen, 2005). LC neurons follow a circadian pattern of activity with most activity during waking, less during slow-wave sleep, and virtually no activity during paradoxical sleep (Aston-Jones & Bloom, 1981). This circadian pattern of LC activity is controlled by indirect inputs from the circadian oscillator, the suprachiasmatic nucleus (SCN; Aston-Jones, Chen, Zhu, & Oshinsky, 2001) that are relayed in part via hypocretinergic neurons within the dorsomedial hypothalamus (Gompf & Aston-Jones, 2008). The role of the LC in circadian behavior is underscored by the discovery that neurotoxic lesions of the LC disrupt the circadian pattern of sleep and waking without perturbing the overall amount of sleep and waking (González & Aston-Jones, 2006), and this relationship between LC and circadian behavior is reciprocal, as light deprivation induces apoptosis in LC-NE neurons, resulting in symptoms of depression (Gonzalez & Aston-Jones, 2008).

The important role of the LC-NE system in circadian regulation of arousal, as well as in the clinical disorders associated with disruptions of circadian processes, provide a strong rationale for higher-resolution studies of these roles. For example, it would be advantageous to selectively modify LC activity or specific projections from LC in a temporally discrete manner in intact animals using new technologies, e.g., optogenetics or designer receptors. Such studies could provide unambiguous evidence of LC involvement in circadian processes and a detailed account of which LC projections mediate specific aspects of these processes. Techniques such as electrical stimulation of LC enable high-precision temporal control of LC activity but lack regional and circuit specificity; conversely, NE-specific neurotoxins can be used to selectively target LC neurons but lack temporal control. Optogenetic and designer-receptor techniques offer a solution that is both pathway and temporally specific.

Optogenetics is a technique in which opsin-based light-sensitive ion channels and pumps are selectively expressed in a specific subtype of neurons (Zhang, Aravanis, Adamantidis, de Lecea, & Deisseroth, 2007). These can be either excitatory (e.g., channelrhodopsin-2 (ChR2) conducts cations) or inhibitory (e.g., halorhodopsin is a chloride pump), and they are gated in response to specific wavelengths of light. Thus, intracranial fiber optics may be used to illuminate areas of brain that express these opsins to activate or inhibit selectively specific neural populations or projections of these populations. Opsins respond with high fidelity to brief pulses of light and can be used to drive neurons on a millisecond timescale.

Many approaches can be used to induce expression of opsin genes in specific neural populations. Aston-Jones' laboratory targets the LC-NE system using replication-deficient lentiviral and adeno-associated viral vectors to deliver the opsin genes. These vectors are injected directly into the LC using a "double-barrel" pipette, i.e., an electrode fused to an injector pipette. The characteristic electrical signature of the LC is used to ensure that the pipette is within the LC during vector injection. Although tissue surrounding LC is also exposed to the vector, the opsin genes are expressed only within LC-NE neurons, because gene expression is under the control of the synthetic dopamine beta-hydroxylase (DBH) promoter PRSx8 (Hwang, Carlezon, Isacson, & Kim, 2001). DBH catalyzes the final step of NE synthesis and is expressed only in NE neurons. Expression of ChR2 in LC was shown to be both restricted to LC and robust. Using an optotrode (an electrode fused to an optical fiber), 1-s laser pulses into LC were shown to elicit robustly bursts of action potentials in an isoflurane-anesthetized animal with ChR2 expressed in LC. Further, during LC photostimulation the cortical and hippocampal electroencephalogram (EEG) power spectra were shifted to higher frequencies with both lower delta and higher theta power. Several seconds of LC photostimulation was sufficient to wake a sleeping animal, both functionally validating this approach and indicating an important role of the LC-NE system in waking.

Although optogenetics provides a temporally precise method to selectively inhibit/drive LC-NE activity, it also requires an optical indwelling fiber to be attached to an external laser. This cumbersome setup is technically challenging and limits neural manipulations to small volumes of tissue proximal to the optical fiber. A pharmacogenetic solution known as designer receptors exclusively activated by designer drugs (DREADDs) obviates these issues, as no external hardware is required. DREADDs are modified versions of human muscarinic G-protein-coupled receptors (GPCR) that have been re-engineered to respond to the otherwise inert ligand clozapine N-oxide (CNO) and not the native ligand acetylcholine (Armbruster, Li, Pausch, Herlitze, & Roth, 2007). DREADDs are available in Gi, Gs, and Gq-coupled variants, all of which couple to the native GPCR intracellular signaling cascades and are transported to native locations throughout the soma, dendrites, and terminals. They have no constitutive activity, and thus, only modulate neural activity when CNO is systemically or locally applied. The Gq-coupled DREADD hM3Dq was shown to be expressed robustly and selectively in the LC under the PRSx8 promoter, and local application of CNO in the LC was shown to increase significantly LC spike rate (Vazey & Aston-Jones, 2014). Further, systemic CNO injection was shown to result in cortical EEG activation in an isoflurane-anesthetized animal, and this activation hastened emergence from anesthesia (Vazey & Aston-Jones, 2014). This effect of DREADD-mediated LC activation was found to be dependent on both alpha and beta NE receptors; when animals were pre-treated with a combination of the alpha-1 antagonist prazosin and the beta antagonist propranolol, which would block the effects of activation of the LC on its target cells, emergence from anesthesia was retarded, and CNO administration had no compensatory effect (Vazey & Aston-Jones, 2014)., CNO administration could, however, ameliorate the retarding effects of either propranolol or prazosin alone, indicating that both alpha and beta receptors play a major role in emergence from isoflurane anesthesia.

Aston-Jones concluded his presentation by considering the clinical potential of DREADDs. Unlike pharmacological interventions, DREADDs target a specific cell type within a

restricted region, and can even be used to target specific projections within that pathway, e.g., if retrogradely transported canine adenovirus is used as the vector. Also, because the CNO ligand does not have known activity at other receptors, the number of side effects is expected to be much lower than with traditional pharmacotherapies, which have numerous non-therapeutic and dose-limiting effects. Also, DREADD-based therapies would be developed rationally from the neuroscience knowledge base as opposed to serendipitous observations of drug effects. Moreover, unlike deep brain stimulation, DREADD-based therapies would not require implanted hardware and the logistical challenges associated, e.g. battery maintenance and device failure. In light of the central role of the LC in circadian processes and pathology as well as the robust and selective activation of LC with hM3Dq described in this presentation, treatment of circadian pathology is a particularly promising potential application of DREADDs. Using these methods to maintain homeostasis could substantially reduce the burden of allostatic load in patients with circadian pathology, alleviating the high levels of associated morbidity and treatment costs.

A human pharmacogenetic perspective on sleep and neuronal plasticity

Hans-Peter Landolt (University of Zurich) described how mediators of activity-dependent, synaptic plasticity such as brain-derived neurotrophic factor (BDNF), adenosine A_{2A} receptors (A_{2A}R), and metabotropic glutamate receptors of subtype 5 (mGluR5) contribute to neurophysiologic markers of sleep homeostasis in humans. EEG delta activity in non-rapid-eye-movement (NREM) sleep is the best-established physiological marker of sleep homeostasis. It is tightly regulated and provides a precise marker of the prior history of sleep and wakefulness. It is highest at sleep onset both during baseline and recovery sleep, and is consistently increased with prolonged wakefulness. Murine studies have indicated that the increase in delta activity in NREM sleep with prolonged wakefulness is under genetic regulation and is strongly associated with genotype and expression of genes associated with synaptic plasticity (Maret et al., 2007). Specifically, studies employing high-density microarray analysis have revealed that wakefulness is associated with expression of *Arc*, *BDNF*, *Homer/Vesl*, and *NGFI-A* (Cirelli, Gutierrez, & Tononi, 2004).

BDNF is a primary mediator of synaptic plasticity (Lu, Nagappan, Guan, Nathan, & Wren, 2013). In animals *BDNF* expression varies as a function of both sleep state as well as sleep deprivation (Guzman-Marin et al., 2006; Huber, Tononi, & Cirelli, 2007), and it is causally involved in sleep homeostasis, i.e. intracerebral microinjections of BDNF during waking result in increased delta activity during NREM sleep (Faraguna, Vyazovskiy, Nelson, Tononi, & Cirelli, 2008). A known functional Val66Met polymorphism in pro-BDNF results in diminished activity-dependent secretion of BDNF in humans (Duman & Aghajanian, 2012); hence, Landolt hypothesized that humans heterozygous for the Val66Met polymorphism (Val/Met) would display reduced EEG delta activity in NREM sleep compared to Val/Val homozygotes.

Eleven individuals with Val/Met heterozygosity and eleven individuals with Val/Val homozygosity matched for age, sex, education, BMI, and chronotype were kept awake for 40 hours. During baseline sleep, before sleep deprivation, higher EEG delta activity was observed in the first NREM sleep episode in the Val/Val group compared to the Val/Met

group ($p < 0.01$), supporting the hypothesis that increased BDNF expression results in increased delta activity in NREM sleep. As expected, both groups displayed greater delta activity during the first NREM episode, and delta activity was significantly increased in both groups in NREM recover sleep following 40 hours of wakefulness. There was a trend towards increased NREM delta activity in the Val/Val group during recovery sleep, although this difference was not significant. The overall difference between genotypes supports the notion that synaptic plasticity and its related processes contribute to regulation of delta activity in NREM sleep, and BDNF is involved in this regulation.

Landolt then addressed how BDNF may mediate these changes in NREM delta activity. One possible mediator is the adenosine A_{2A} receptor. Animal studies have shown that BDNF-enhancement of long-term potentiation (LTP) is dependent upon signaling of adenosine A_{2A} receptors (Fontinha, Diógenes, Ribeiro, & Sebastião, 2008). Further, A_{2A} receptor-knockout animals fail to display an increase in NREM sleep following sleep deprivation (Hayaishi, Urade, Eguchi, & Huang, 2004). Hence, the A_{2A} receptor (ADORA_{2A}) genotype was determined in several participants, and 8 single nucleotide polymorphisms (SNPs) were catalogued across the gene. Eight haplotypes of these SNPs were observed in the study participants, and 5 of these haplotypes (HT1-5) that occurred with a frequency beyond 10% were studied further. Notably, although individuals with the HT4 haplotype ($n = 14$) showed similar baseline NREM delta activity compared to non-HT4 individuals ($n = 31$), HT4 individuals showed greater NREM delta activity after 40 hrs of wakefulness ($p < 0.02$). To determine whether these differences reflected differences in A_{2A} receptor signal transduction, participants were treated with 200mg caffeine, an A_{2A} receptor antagonist, once at 11 hours and once at 23 hours during the 40-hr period of wakefulness using a randomized, placebo-controlled, double-blind, cross-over design. Caffeine treatment significantly attenuated increases in NREM delta activity in non-HT4 individuals ($n = 17$, $p < 0.002$), but not in individuals with the HT4 haplotype ($n = 6$), a haplotype with a SNP known to result in caffeine insensitivity. These findings indicate that A_{2A} receptor signaling contributes to NREM delta activity and sleep homeostasis.

Adenosine A_{2A} receptors are G_s-coupled and stimulate gene expression via CREB, and CREB-mediated expression of plasticity-associated genes such as *Arc* and *BDNF* is synergistically augmented by signaling at co-localized mGluR5 (Conn, Battaglia, Marino, & Nicoletti, 2005). Additionally, murine studies have indicated that the increase in delta activity in NREM sleep with prolonged wakefulness is strongly associated with genotype-dependent over-expression of the mGluR5-associated protein *Homer1a*, a core mediator of synaptic plasticity and sleep homeostasis (Maret et al., 2007). Thus, the role of mGluR5 receptors in NREM delta activity was assessed in humans. The mGluR5-selective radioligand ¹¹C-ABP688 and positron emission tomography were used to measure mGluR5 receptor availability in participants ($n = 23$) after 9 and 33 hours of wakefulness, and mGluR5 availability was increased at 33 hours of wakefulness compared to 9 hrs ($p < 0.006$) (Hefti et al., 2013). Moreover, the increase in mGluR5 availability was positively correlated with increase in NREM delta activity following sleep deprivation, indicating mGluR5 signaling likely plays a significant role in sleep homeostasis. Taken together, these studies support a major role for mediators of activity-dependent, synaptic plasticity in human sleep

homeostasis. Thus synaptic plasticity exerts its effects on homeostasis via numerous pathways, making the body's attempts to self-regulate akin to an orchestra conductor, directing various elements into a cohesive whole.

Behavioral interventions to improve sleep

Anne Germain (University of Pittsburgh) described behavioral interventions and associated mechanisms that improve sleep. High quality sleep was subjectively defined as regular, predictable, consolidated, restorative, and sufficient, and objectively defined as alignment of homeostatic and circadian processes. Poor quality sleep results from many inciting factors; in this presentation brain activation through mental activity (e.g., worrying in bed, rumination, anxiety, etc.) was submitted as a major contributor to poor sleep. Mental activity, in this context, sometimes stems from affective disorders such as mood disorders, anxiety disorders, substance dependence and cognitive deficits, and, in turn, sleep and circadian disorders contribute to the etiology of these affective disorders in predisposed individuals. Thus, behavioral interventions that improve sleep are hypothesized to concomitantly alleviate symptoms of these disorders, and conversely, treatment of affective disorders is expected to improve sleep quality, thus contributing to both sleep/wake and emotional homeostasis.

Behavioral treatments of sleep and circadian disorders share four common elements: 1) monitoring of sleep-wake patterns and episodes that are known to affect sleep quality 2) establishing a regular sleep-wake schedule 3) reinforcing associations between bed and restorative sleep 4) using voluntary behavior to influence involuntary physiological/psychological processes. Likewise, cognitive-behavioral therapy for insomnia (CBTI) typically employs four basic rules that adhere to these elements with the intention of aligning homeostatic and circadian processes. Patients are instructed to reduce their time in bed to match their sleep time, to only go to bed when sleepy, to only stay in bed when asleep, and to wake up the same time everyday regardless of sleep quantity and quality. Supporting the hypothesis that treating insomnia will concurrently alleviate symptoms of comorbid disorders, in a pilot study of patients with concurrent depression and insomnia, CBTI combined with escitalopram was shown to be more efficacious than control behavioral interventions combined with escitalopram for treatment of insomnia, and there was a trend towards better depression outcomes (Manber et al., 2008). Similarly, CBTI was found to reduce insomnia severity significantly more than control behavioral intervention in recovering alcohol dependent patients (Arnedt, Conroy, Armitage, & Brower, 2011). However, both the active and control groups showed reductions in drinking, obviating whether CBTI played a meaningful role in alcohol use.

Nightmares are a form of sleep disturbance that is often comorbid with trauma-related psychiatric illnesses such as post-traumatic stress disorder (PTSD). Imagery rehearsal therapy (IRT) is a behavioral therapeutic approach predicated on nightmares being a learned behavior that can be subverted by practicing a desired behavior. Patients are instructed to write down nightmares, re-script them into more benign dreams, and then mentally rehearse the new scripts. In a meta-analysis IRT was shown to increase sleep quality while reducing nightmare frequency and other symptoms of PTSD (Casement & Swanson, 2012), providing

more evidence that concurrent mood disorders may respond to treatments that target sleep quality. However, individual studies do not always confirm significant effects; e.g., IRT combined with a brief behavioral treatment of insomnia (BBTI) was found to significantly reduce symptoms of insomnia, but only trended towards reducing nightmare frequency and other PTSD symptoms (Germain et al., 2012).

Psychiatric disorders such as bipolar disorder are marked, in part, by disturbances in circadian and sleep/wake rhythms. Behavioral interventions that aim to re-entrain the circadian rhythm by either appropriately synchronized light exposure or by scheduling of regular daily routines (Frank et al., 1997) have lead to significant prophylaxis of affective episode recurrence (Frank et al., 2005). Taken together, evidence within this presentation shows a general and sometimes significant trend of sleep-targeting therapies leading to reductions in affective disorder severity. These findings lend support to the idea that the alignment of circadian and homeostatic processes is critical for emotional regulation, in addition to sleep regulation.

To enhance the efficacy of future behavioral treatments to improve sleep, it will be important to identify candidate brain circuits that mediate treatment response. Candidate circuits include arousal-related pathways such as the LC described in Aston-Jones' presentation, reward pathways, and threat-detection systems. For example, neuroimaging data suggest that neural activity during NREM sleep within the amygdala, a structure with a well-established role in fear response, was reduced after patients were treated with CBTI (Milgrom et al., 2011). Further, brief treatments for insomnia were shown to reduce amygdala response to threatening stimuli, indicating that the amygdala may be a primary target for treatments of insomnia, which could explain why CBTI reduces symptoms of PTSD. Many of the presented studies of sleep-targeting behavioral interventions showed non-significant trends toward evidence of concomitant effects on affective disorder severity. However, effect sizes were notable and studies were often underpowered for these secondary outcomes. Given the neurobiological evidence of attenuated amygdala activation, this presentation provided a strong rationale for larger investigations of the role sleep-targeting therapies in treating affective disorders.

Overall, this session demonstrated the potential of new methodologies to activate specific brain circuits, how genetic variants related to plasticity interact with sleep homeostasis and how this may explain variation in treatment response and finally, that behavioral interventions targeting homeostatic and circadian processes are effective.

The INSPIRE 2014 Debate RESOLVED: We know how light and wake therapy work in the treatment of mood disorders

Overview—An entertaining and thought-provoking feature of the second INSPIRE conference was a debate chaired by Michael Hastings (MRC Laboratory of Molecular Biology, Cambridge). Samer Hattar (Johns Hopkins University) proposed the motion that “We understand the mechanistic basis of the relationship between light and mood disorders,” while Russell Foster (Oxford University) opposed it.

Affirmative

Hattar presented recent research from his laboratory which suggests that abnormal light cycles can directly influence mood in rodents, independent of circadian arrhythmicity or sleep disturbances (LeGates et al., 2012). This contradicts the prevailing view that sleep and/or circadian rhythm disruption is a necessary intermediate step between aberrant light input and altered mood. These experiments were inspired by the discovery that intrinsically photosensitive retinal ganglion cells (ipRGCs) – which project to the suprachiasmatic nucleus (SCN) and have an established role in circadian photoentrainment – also project to areas of the brain involved in mood regulation, such as the lateral habenula and medial amygdala (Hattar et al., 2006).

T7 light/dark cycles do not disrupt sleep or circadian rhythms—Wildtype mice were exposed to a T7 light/dark cycle (3.5 hours of light followed by 3.5 hours of dark) for 2 weeks, while a control group was housed under a standard T24 light/dark cycle. Circadian period length was extended under the T7 cycle, but the mice remained rhythmic; this was established through measurements of core body temperature, general activity levels, and the expression of the core clock gene PER2. Likewise, T7 cycles do not affect sleep architecture or total sleep time in mice (Altimus et al., 2008).

T7 mice display altered mood and impaired cognition—Despite an apparent lack of sleep or circadian rhythm disruption, mice housed under a T7 cycle displayed striking behavioral alterations. Relative to T24 mice, depression-like behavior was increased in T7 mice, as evidenced by reduced sucrose preference, and greater immobility in the forced swim test. In addition, their performance was impaired in two cognitive tasks: the Morris water maze and a standard object recognition memory paradigm. Elevated corticosterone was highlighted as a potential contributor to their depression-like phenotype, while compromised hippocampal long-term potentiation (LTP) could underlie their cognitive deficits. Impaired cognition in T7 mice may be a direct consequence of their depression-like phenotype, as chronic administration of the antidepressant fluoxetine rescued their deficits in object recognition memory and hippocampal LTP.

Which retinal circuits are responsible for altered mood?—There are five different types of ipRGC with distinct morphological and electrophysiological properties. M1 ipRGCs project to non-image forming brain regions, while non-M1 ipRGCs project to brain areas involved in image formation (Ecker et al., 2010; Schmidt et al., 2011). In a further experiment, mice in which M1 ipRGCs had been genetically ablated were exposed to T7 cycles (LeGates et al., 2010). The increased depression-like behavior, cognitive impairments and deficient hippocampal LTP witnessed in wildtype mice exposed to T7 cycles were not observed in these mice. This suggests that M1 ipRGCs mediate the effect of abnormal light cycles on mood in wildtype mice.

To add a further layer of complexity, there are two subtypes of M1 ipRGC; those that express the transcription factor Brn3b and those that do not. Brn3b-positive M1 ipRGCs project to the olivary pretectal nucleus and govern the pupillary light reflex, while Brn3b-negative M1 ipRGCs project to the SCN and regulate circadian rhythms (Chen et al., 2011).

Hence, the selective ablation of Brn3b-positive M1 ipRGCs impairs the pupillary light reflex, but does not affect circadian photoentrainment. Crucially, these mice are immune to the depression-inducing effects of T7 cycles, highlighting Brn3b-positive M1 ipRGCs as a key player in the relationship between light and mood and implying that the lengthened period in T7 and the SCN are not sufficient to impair mood and cognition under this irregular light cycle. Consistent with this, these cells provide the principal retinal input to the lateral habenula, a brain region implicated in depression (Ranft, 2010; Sartorius, 2010). Thus, Hattar concluded that the retinal circuits implicated in the relationship between light exposure and mood have been sufficiently elucidated.

Negative

Foster argued that the relationship between light and mood is likely mediated by multiple mechanistic pathways that are not mutually exclusive. He evaluated the evidence for several putative pathways, concluding that the existing data are limited, at times contradictory, and mostly correlational rather than causal. He also predicted that different mechanisms – and therefore different treatments – will prove most relevant to different mood disorders.

Sleep and Mood—Disruption of the sleep/wake cycle is a common feature of several mood disorders. Sleep disruption could make a causal contribution to altered mood in patients with mood disorders, although at present there is little direct evidence for this hypothesis. A wide range of neurotransmitters is implicated in the generation and maintenance of sleep, so this pathway may be highly complex. The picture is complicated further by the diverse range of inputs to the systems that govern sleep, including light information from the eye, the rhythmic circadian output of the SCN, sleep pressure from the sleep homeostat, and social influences on sleep timing.

Circadian Rhythms and Mood—Bright light therapy has proven relatively effective as a treatment for seasonal affective disorder (SAD), although it is not understood why. One suggestion is that SAD patients are prone to spontaneous internal desynchrony, whereby circadian rhythms in different peripheral tissues become misaligned. In this context, a bright light pulse may serve to re-align circadian rhythms throughout the body. In favor of this hypothesis, the timing of light exposure appears to influence the effectiveness of bright light therapy in SAD patients (Eastman et al., 1998). Mood is, however, also improved by exposure to natural light throughout the day, which precludes a purely circadian explanation (Grandner et al., 2006). Moreover, although forced desynchrony protocols lead to depressed mood in healthy individuals (Boivin et al., 1997), there is no empirical evidence of internal desynchrony in SAD, either before or after light therapy.

It has also been argued that ipRGCs are critically involved in SAD, and indeed, a weak association has been reported between the disorder and a missense mutation in melanopsin (Roecklein et al., 2009). This hypothesis implies that ‘blue’ light should be maximally effective in the treatment of SAD, but as yet there is no definitive evidence to support this (Anderson et al., 2009; Gagne et al., 2011). In response to this claim, Samer Hattar drew attention to human imaging studies that have reported activation in limbic brain regions such as the amygdala during exposure to ‘blue’ wavelength light (Vandewalle et al., 2007; 2010).

The inconsistent evidence on this point suggests that more research is needed to determine the specific effects of ipRGCs on SAD.

Serotonin and Mood—Another hypothesis about the effect of light on mood is that light exposure promotes serotonin synthesis. As serotonin levels and mood are positively associated, this could explain the effectiveness of bright light therapy in the treatment of SAD. Consistent with this account, serotonin production is lowest during winter (Lambert et al., 2002), when natural light exposure is attenuated. Moreover, serum levels of bipterin – an essential co-factor in serotonin synthesis – are elevated after light therapy in individuals with SAD (Hoekstra et al., 2003). Finally, the ablation of dorsal raphe nucleus (DRN)-projecting retinal ganglion cells in gerbils leads to a reduction in serotonin levels in the DRN, and an increase in depression-like behavior (Ren et al., 2013). Hattar noted that these projections do not exist in mice.

Melatonin and Mood—In humans, exposure to a light pulse during the subjective night causes an elevation in mood (Badia et al., 1991), but if exogenous melatonin is administered at the same time as the light pulse, there is no such alteration. These results provide causal evidence that light can influence mood via the suppression of melatonin. Interestingly however, exposure to a light pulse during the subjective day can also elevate mood (Rueger et al., 2006). Since there is no melatonin to suppress at this time, melatonin cannot be the only mediator of the relationship between light and mood.

Cortisol and Mood—In mice, it has been demonstrated that the magnitude of corticosterone release is dependent upon light levels (Ishida et al., 2005). Moreover, depression is associated with increased serum cortisol levels in humans (Nestler et al., 2002). Thus, glucocorticoids could mediate the relationship between light and mood. Indeed, mice housed under T7 cycles show elevated serum corticosterone levels and increased depression-like behavior (LeGates, 2012). Samer Hattar also remarked that there is seasonal variation in glucocorticoid concentration, which may be relevant to SAD, since levels are highest in winter (Persson et al., 2008).

Wake Therapy and Mood—Wake therapy (i.e. sleep deprivation) has an immediate antidepressant effect in 60% of patients with affective disorders, but this effect disappears immediately after recovery sleep (Wirz-Justice et al., 2005). The mechanistic basis of this effect is largely unknown, although recent studies in mice point to an up-regulation of adenosine signalling (Hines et al., 2013).

In conclusion, the specific mechanism of light and wake therapy for mood disorders remains an open question. While there are several lines of evidence that may prove to have a common cause, there is insufficient evidence to provide a parsimonious theory.

Summary—Hattar showed how a melanopsin-mediated effect of light can modulate mood, whereas Foster argued that no-one has yet shown that light does modulate mood by a defined mechanism. The contrast is one of “can do” and “does do.” The possibility therefore remains open to further research, and the secret to successful resolution of the question may

be judicious selection of patient populations, the contextual, environmental settings and the specific mechanism of interest.

What are the intervening circadian variables in psychosocial/behavioral and pharmacologic interventions for mood and anxiety disorders?

Circadian and sleep homeostatic modulation of fMRI assessed brain responses during cognitive performance

Pierre Maquet (University of Liege) introduced his talk with the background setting that cognitive performance depends on circadian and homeostatic influences. In addition, brain activity, whether measured by EEG or other techniques, also depends on these factors (Dijk & Lockley, 2002). What is not well understood, however, is how different parts of the brain respond to cognitive tasks in the setting of increasing sleep pressure or an oscillating circadian rhythm. Although there is a growing literature on human neuroimaging under sleep deprivation (Dang-Vu et al., 2010), little is known about the interplay of homeostatic and circadian systems in this regard. Maquet's group has explored this topic by taking advantage of individual differences arising from polymorphisms in the period3 gene. Per3 polymorphisms have been shown to be involved in sleep homeostasis, but not circadian rhythms (Archer et al., 2003; Viola et al., 2007). Individuals homozygous for the long allele (5/5) are more likely to be morning (lark) types, show more slow-wave activity during the first NREM cycle of baseline sleep as well as recovery sleep after deprivation. In contrast, individuals homozygous for the short allele (4/4) are more likely to be evening (owl) types. The 5/5 allele is associated with greater sensitivity to sleep deprivation, while the 4/4 demonstrates more resilience, under various cognitive performance tests especially working memory tasks (Viola et al., 2007; Groeger et al., 2008).

Maquet then presented data on his laboratory's experiments on subjects with these per3 polymorphisms, who underwent testing in morning and evening on two days, with or without intervening sleep deprivation. This allowed for assessment of altered homeostatic pressure versus circadian influence. Melatonin levels confirmed the lack of circadian rhythm differences between groups, while slow wave activity (SWA) was elevated in the 5/5 group, suggesting increased baseline sleep pressure occurring at baseline. They evaluated fMRI responses during the 3-back working memory task in morning sessions with or without deprivation (Vandewalle et al., 2009). Deactivation of brain areas involved in working memory occurred only in the 5/5 group after deprivation. Areas of preferential activation after deprivation were only seen in the 4/4 group, such as frontal cortex, thalamus, cerebellum, parahippocampal gyri, suggesting recruitment of these areas perhaps in a compensatory manner to maintain performance.

Maquet further explained results from individuals with different behaviorally-defined chronotypes, noting that these groups differ not only in circadian physiology but also in homeostatic features, including higher SWA, and faster dissipation, in morning versus evening types (Mongrain, Carrier, & Dumont, 2006). Psychomotor vigilance testing (PVT) was performed in these subjects during scans occurring either 1.5 or 10.5 hours after awakening following a normal night of sleep. They confirmed the results of circadian

differences and increase SWA with faster dissipation in the morning types (Schmidt et al., 2009). fMRI showed larger activation in evening types in the locus coeruleus and hypothalamus related to the fastest (“optimal”) reaction times from the PVT. These reaction times were similar between the groups, but the fMRI correlate differed. This hypothalamic region was near the SCN but its exact anatomical substrate remains uncertain. One interpretation of this difference is that altered sleep pressure impacted the brain differently. Consistent with this, they showed an inverse relation of SWA in the first NREM cycle with the BOLD signal in this hypothalamic region. Thus, the lower BOLD signal in the morning types might be from either increased homeostatic pressure, or reduced circadian pressure, although the mechanisms mediating this effect remain unknown.

Maquet discussed ongoing work to explore this topic in 5/5 and 4/4 individuals. Assessments included pre-lab actigraphy phase, lab adaptation, DLMO, MSLT, baseline sleep, 42-hour constant routine, and recovery sleep. Twelve fMRI sessions were obtained (morning and evening) during the constant routine and after recovery sleep. Again using the 3-back working memory task, they showed deactivation of default mode network regions (as expected) during the task. However, as sleep pressure builds, the extent of this expected task-dependent decrease of the default network was reduced. Further work investigating these findings is underway.

PTSD: Golden hours and circadian rhythms

Joseph Zohar (Sheba – Academic Medical Center) began his presentation by explaining that the most recent update to the Diagnostic and Statistical Manual (DSM-5) involved important restructuring in regard to anxiety disorder categorization, from a descriptive approach to an endophenotype approach. Thus, anxiety disorders are sub-categorized into three families of anxiety, stress, and obsessive-compulsive disorders (OCD), with post-traumatic stress disorder falling in the second category. He pointed out that there are several advantages to be leveraged in the study of PTSD, including the fact that the disorder has an identifiable inciting event. Although there are treatments for chronic phase of PTSD, a key research and clinical topic relates to the need for early drug options for preventing the development of this chronic phase of PTSD.

The relationship of PTSD with cortisol as a physiological marker of stress is not clearly delineated. Cortisol levels could be a consequence of traumatic experience, but the baseline HPA axis activity could also influence susceptibility to development of PTS after trauma. To explore this question, experiments were conducted on rats that differed in HPA axis activity. Specifically, the Lewis strain exhibit impaired HPA axis response to stress compared to the Fisher strain, which are relatively hyper-responsive. Moreover, in an animal model of experimental stress and PTSD-like behavior, Fisher and Lewis rats showed lower and higher, respectively, PTSD-like response compared to Sprague-Dawley rats (Cohen et al., 2006), suggesting that HPA axis hypo-function may be causally related to PTSD pathophysiology.

If this hypothesis is correct, then pre-treatment with cortisol would mitigate the PTSD-like response to stress. Along this line, Lewis rats were given a single dose 1 hour before experimental stress, which dramatically reduced PTSD like behavior (Cohen 2006).

However, the critical question is whether treatment after a traumatic event could mitigate the development of PTSD-like response, because this mimics the clinical setting in which providers encounter patients after the traumatic event has occurred. Indeed, rodent experiments demonstrated that acute steroid administration 1 hour after experimental stress mitigated the PTSD-like response (Cohen et al., 2008), while administration in the chronic phase had no effect.

From a clinical perspective, the most common medications administered acutely after a traumatic event are benzodiazepines. These drugs actually suppress HPA axis activity, which may be counter-productive if cortisol levels are protective against PTSD development. Indeed, some evidence exists that early exposure to benzodiazepines is associated with greater subsequent development of PTSD (Gelpin et al., 1996).

This encouraging animal work was then extended to humans. Patients presenting to the emergency department after motor vehicle accident were randomized to receive saline versus hydrocortisone infusion within 6 hours of exposure to trauma (n=24). In this pilot work (unpublished), with 1 month and 3 month follow-up, reduced rates of acute and chronic PTSD symptoms were evident in the steroid arm (Zohar et al., 2011).

In regards to the potential importance of the circadian system, Dr Zohar went on to discuss the time of day of exposure to stress. Circadian timing is predicted to be important, with the hypothesis that less PTSD-like behavior would occur after experimental stress delivered at times of higher endogenous cortisol levels (i.e., in the morning hours). This turned out to be true in their most recent work (Cohen et al., 2014) in which stress exposure during the inactive phase (low cortisol) was associated with higher PTSD-like behavior than exposure during the active phase (high cortisol). Parallel work looking at this in humans is now underway.

Lessons Learned

Throughout the conference there was an emphasis on the importance of communication between basic and clinical scientists. The conference appropriately ended with take-home messages for each group of scientists and some comments from attendees on those messages.

Clinical science take-home messages for basic scientists

Michael Hastings (MRC Laboratory of Molecular Biology, Cambridge) emphasized the enormous opportunities presented by the greatly enlarged scope and increased refinement of technology-based approaches being used in clinical settings to monitor and, in some cases, to interact with patients and subjects. For example, mobile technology (including hardware and software applications, e.g. EMA techniques, smartphone apps, data loggers) is being utilized by several groups. There remain ethical and financial questions about how to use these technologies and how to determine which approach is best to answer which question, but combined with good software, these technologies bring access in real-time in the relevant contexts to clinical/patient groups. They provide a “window into the life” of a

subject/patient as it happens, and also offer an opportunity simultaneously to intervene, diagnose, and assess.

Furthermore, these advances in technology can be combined with more extensive and previously less accessible patient populations (e.g, those in China, Costa Rica, Colombia), making them newly available for research purposes. Because we have massively enlarged capacity, scope and refinement, we can start to make a virtue of individual differences, which likely are pivotal to understanding mental disorders.

The opposite side of this brave “individualism” coin is a remaining nagging doubt. This is best exemplified by comparison with neurodegenerative diseases where some conditions can have 100% penetrance: the patient population is at one level homogeneous and the genetic basis can be determined with absolute certainty. In contrast, because of the contribution of experiential events, is it the case that every occurrence of mental illness in humans is a unique event, arising from a unique combination of genes, environment and life-history? After all, there is an infinite genetic mix and infinite possibilities of experiences. If these interact, we have ‘infinity squared’ possibilities. As a counterpoint, we must hope that as we continue to move toward more refined definitions of clinical conditions, and enhanced power in GWAS studies an anatomisation of mental health will become possible. Until we achieve that, however, the nagging doubt remains.

Having heard what the clinicians are up to, what should basic biologists do? We ought to be clear about what we can and cannot offer. Animal models are about mechanisms--either the natural mechanism of disease or as a putative target existing as a natural mechanism. However, it would be misleading to pretend that animal models recapitulate the complete human condition. Basic biologists should embrace the precision in what they do, but also recognize that alone it is not the answer to human clinical questions. There is complexity in seeking to apply the knowledge. How do the two approaches best meld? Neuroimaging in animals represents a promising approach, but we must be thoughtful about its use. With animal-based models we can start to do things that are relevant, and this methodology also brings promise to the future of therapies. We therefore need to find a balance between precision and resolution of approaches with the precision of application.

Basic science take-home messages for clinical scientists

Guy Goodwin (University of Oxford) then summarized what clinical scientists should take away from the conference by reviewing the conference content hierarchically; from molecular research to the cellular investigations and concluding with system-wide considerations. He argued that the future of research in psychiatry is to attract the best of basic scientists to try to solve our messy but interesting clinical problems.

The *molecular* research take-away messages for clinicians were to be aware of how genetics can give insight to pathology, how the characterization of diseases affects research and how fortunate pharmacological findings can open up new opportunities in disease mechanism research. Narcolepsy research was used as an example of how creating classes of patients based on phenotype and a well-known HLA association changed clinicians’ approach to this disease and led to an autoimmune hypothesis of its pathology and a detailed account of

disease mechanism involving hypocretin. This is now leading to a new class of hypnotic drugs like almorexant (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3907703/>). The HLA associations of schizophrenia are not yet understood but could provide a parallel starting point. Narcolepsy is also. This is an example of how characterizing a disease using classes of patients can be advantageous compared to continuum models.

Continuing with the molecular findings, Goodwin also discussed how serendipity in clinical psychopharmacology can be informed by identifying the molecular targets of effective drugs. The dopamine hypothesis of psychosis was an old example which followed the discovery of effective antipsychotic drugs. Recent new examples of this phenomenon include the discovery of ketamine as an antidepressant and agomelatine as an anxiolytic. Their efficacy linked to their pharmacology offered a clue to pathology that should not be ignored.

Moving on to the *cellular* level, Goodwin highlighted the presence of clock mechanisms in all cells. Research in mood and anxiety disorders needs to overcome the major challenge in examining the biology behind the behavior in psychological disorders: the non-availability of brain tissue from living patients. Fibroblasts and pluripotent human cells now offer new cellular insight into psychiatric disorders, as they can be manipulated to have more neuron-like or glial properties. They represent innovative tools for investigating the role of circadian and homeostatic clocks in mood and anxiety disorders *in vitro*. The data related to stress and mitochondria presented by Flint is an unexpected finding. The role of stressful life events in either causing vulnerability to depression from early in life or precipitating illness later on remains elusive. If correct a mitochondrial explanation could also have far-reaching, multi-system implications.

Finally, Goodwin discussed take-home messages with *system-wide* implications. One aim repeated throughout the conference was the importance of finding biomarkers related to mood and anxiety disorders. In relation to the circadian system, its homeostatic nature is a challenge because determining cause and effect has proved difficult. So is it sleep, activity or reward that drives and so best predicts mood variability or the risk of depression? In principle, homeostatic systems can be analysed by controlled interventions that open their feedback loops or perturb them in a systematic way. In the case of the circadian system light is a clear candidate to be used in this way to analyse clinical states. The intriguing finding that blue light gives us an unconscious measure of the dark/light cycle has yet to be fully incorporated into clinical studies. and understanding what drives these biomarkers (e.g. sleep, activity, emotions). In addition genetic dissection of the homeostat, using function modifying polymorphisms in genes with known actions is of great potential interest.

Finally, our measures of human behavior are still largely limited to subjective reporting of experience. The ubiquitous spread of smartphones and the growing commercial interest in health monitoring represents a major opportunity to move circadian studies of sleep and circadian function into the world of big data. Clinicians will need to rely less on subjective reporting of behavior and much more on objective measures that can be used as feedback to drive behavioral change. Examples presented during the poster presentations included

experience sampling methodology. Examples included EMA, actigraphy, and a variety of cell phone applications.

Audience Comments on the Take-Home Messages

David Kupfer (University of Pittsburgh)—The opportunity to communicate with one another may inspire us to improve our framing of questions. There is still a need around issues of standardization and taking advantage of large datasets. It will require more methodology. It has to take place to continue the communication between basic and clinical science. We are in an era in which the technological breakthroughs and availability of techniques offer golden opportunities, and we must take advantage of them. Although we focused primarily on mood and anxiety disorders, what we have covered is applicable to all disease processes and all of health. These techniques will be applicable to a host of broader questions.

Kathleen Merikangas (NIMH)—The entire meeting has been characterized by rich interaction between basic and clinical—a rare experience. This may be attributable to the context: “Prevent and treat” makes us come together to think about systems, identify targets and the people for whom these interventions work. Environmental inputs into the brain and cross-talk between brain systems and environment—the interaction was highlighted.

Marion Leboyer (University of Paris)—Direct communication between basic and clinical scientists as well as between senior and junior investigators will help to prepare the next generation of scientists. This is a period of hope and of paradigmatic shift. We need to bring this message to industry, as well, to see that industry remains engaged in psychiatry, convincing them that understanding of disorders will inform treatment.

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References

1. Borbely AA, Achermann P. Sleep Homeostasis and Models of Sleep Regulation. *Journal of Biological Rhythms*. 1999; 14:559–570.
2. Maywood ES, et al. Analysis of core circadian feedback loop in suprachiasmatic nucleus of mCry1-luc transgenic reporter mouse. *Proceedings of the National Academy of Sciences*. 2013; 110:9547–9552.
3. Bae K, et al. Differential Functions of mPer1, mPer2, and mPer3 in the SCN Circadian Clock. 2001; 30:525–536.
4. Maywood ES, et al. The Tau Mutation of Casein Kinase 1 ϵ Sets the Period of the Mammalian Pacemaker via Regulation of Period1 or Period2 Clock Proteins. *Journal of Biological Rhythms*. 2014; 29:110–118. [PubMed: 24682205]
5. Anand, S.; Edwards, J.; Nolan, P. Generation of mouse mutants as tools in dissecting the molecular clock.. In: Kalsbeek, A., et al., editors. *The Neurobiology of Circadian Timing*. Vol. 199. Elsevier; Amsterdam: 2012. p. 247-265.

6. Welsh DK, et al. Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Current Biology*. 2004; 14:2289–2295. [PubMed: 15620658]
7. Maywood ES, et al. A diversity of paracrine signals sustains molecular circadian cycling in suprachiasmatic nucleus circuits. *Proceedings of the National Academy of Sciences*. 2011; 108:14306–14311.
8. Brancaccio M, et al. A Gq-Ca²⁺ Axis Controls Circuit-Level Encoding of Circadian Time in the Suprachiasmatic Nucleus. 2013; 78:714–728.
9. Reddy AB, et al. Circadian orchestration of the hepatic proteome. *Current Biology*. 2006; 16:1107–1115. [PubMed: 16753565]
10. Lamia KA, Storch K-F, Weitz CJ. Physiological significance of a peripheral tissue circadian clock. *Proceedings of the National Academy of Sciences*. 2008; 105:15172–15177.
11. Li JZ, et al. Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proceedings of the National Academy of Sciences*. 2013; 110:9950–9955.
12. Hastings MH, Maywood ES, Reddy AB. Two Decades of Circadian Time. *Journal of Neuroendocrinology*. 2008; 20:812–819. [PubMed: 18601704]
13. Kyriacou CP, Hastings MH. Circadian clocks: genes, sleep, and cognition. *Trends in Cognitive Sciences*. 14:259–267. [PubMed: 20418150]
14. Klei L, et al. Heritability of morningness-eveningness and self-report sleep measures in a family-based sample of 521 hutterites. *Chronobiology international*. 2005; 22:1041–1054. [PubMed: 16393707]
15. Vink JM, et al. Genetic analysis of morningness and eveningness. *Chronobiology International*. 2001; 18:809–822. [PubMed: 11763988]
16. Hickie IB, et al. Manipulating the sleep-wake cycle and circadian rhythms to improve clinical management of major depression. *BMC Medicine*. 2013; 11:79. [PubMed: 23521808]
17. Hickie I. Evidence for separate inheritance of mania and depression challenges current concepts of bipolar mood disorder. *Molecular psychiatry*. 2014; 19:153–155. [PubMed: 24365866]
18. Merikangas K, et al. Independence of familial transmission of mania and depression: results of the NIMH family study of affective spectrum disorders. *Molecular psychiatry*. 2013
19. Lamers F, Hickie I, Merikangas KR. Prevalence and correlates of prolonged fatigue in a US sample of adolescents. *American Journal of Psychiatry*. 2013; 170:502–510. [PubMed: 23632835]
20. Sletten TL, et al. Genetic and environmental contributions to sleep-wake behavior in 12-year-old twins. *Sleep*. 2013; 36:1715–1722. [PubMed: 24179306]
21. Robillard R, et al. Delayed sleep phase in young people with unipolar or bipolar affective disorders. *Journal of affective disorders*. 2013; 145:260–263. [PubMed: 22877966]
22. Robillard R, et al. Sleep-wake cycle and melatonin rhythms in adolescents and young adults with mood disorders: Comparison of unipolar and bipolar phenotypes. *European Psychiatry*. 2013; 28:412–416. [PubMed: 23769680]
23. Robillard R, et al. Sleep-wake cycle in young and older persons with a lifetime history of mood disorders. *PLoS ONE*. 2014:9.
24. Sivertsen B, et al. Sleep problems in children with autism spectrum problems: a longitudinal population-based study. *Autism*. 2012; 16:139–150. [PubMed: 21478225]
25. Constantino JN, et al. Sibling recurrence and the genetic epidemiology of autism. *American Journal of Psychiatry*. 2010; 167:1349–1356. [PubMed: 20889652]
26. Huguet G, Ey E, Bourgeron T. The genetic landscapes of autism spectrum disorders. *Annual review of genomics and human genetics*. 2013; 14:191–213.
27. Moss J, Howlin P. Autism spectrum disorders in genetic syndromes: implications for diagnosis, intervention and understanding the wider autism spectrum disorder population. *Journal of Intellectual Disability Research*. 2009; 53:852–873. [PubMed: 19708861]
28. Pagan C, et al. Mutation screening of ASMT, the last enzyme of the melatonin pathway, in a large sample of patients with Intellectual Disability. *BMC medical genetics*. 2011; 12:17. [PubMed: 21251267]

29. Gabriele S, Sacco R, Persico AM. Blood serotonin levels in autism spectrum disorder: A systematic review and meta-analysis. *European Neuropsychopharmacology*. 2014; 24:919–929. [PubMed: 24613076]
30. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic review and meta analysis. *Developmental Medicine & Child Neurology*. 2011; 53:783–792. [PubMed: 21518346]
31. Pagan C. Disruption of the serotonin-N-acetylserotonin-melatonin pathway may be a large subgroup biomarker for autism and is associated with sleep problems. *Translational Psychiatry*. In press.
32. Fears SC, et al. Multisystem component phenotypes of bipolar disorder for genetic investigations of extended pedigrees. *JAMA Psychiatry*. 2014; 71:375–387. [PubMed: 24522887]
33. Hallmayer J, et al. Narcolepsy is strongly associated with the T-cell receptor alpha locus. *Nature genetics*. 2009; 41:708–711. [PubMed: 19412176]
34. Hor H, et al. Genome-wide association study identifies new HLA class II haplotypes strongly protective against narcolepsy. *Nature genetics*. 2010; 42:786–789. [PubMed: 20711174]
35. Kornum BR, et al. Common variants in P2RY11 are associated with narcolepsy. *Nature Genetics*. 2011; 43:66–71. [PubMed: 21170044]
36. Faraco J, et al. ImmunoChip study implicates antigen presentation to T cells in narcolepsy. *PLoS genetics*. 2013; 9:e1003270. [PubMed: 23459209]
37. Han F, et al. Genome wide analysis of narcolepsy in China implicates novel immune loci and reveals changes in association prior to versus after the 2009 H1N1 influenza pandemic. *PLoS genetics*. 2013; 9:e1003880. [PubMed: 24204295]
38. Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu. Rev. Neurosci.* 2005; 28:403–450. [PubMed: 16022602]
39. Aston-Jones G, Bloom F. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *The Journal of Neuroscience*. 1981; 1:876–886. [PubMed: 7346592]
40. Aston-Jones G, et al. A neural circuit for circadian regulation of arousal. *Nature neuroscience*. 2001; 4:732–738.
41. Gompf HS, Aston-Jones G. Role of orexin input in the diurnal rhythm of locus coeruleus impulse activity. *Brain research*. 2008; 1224:43–52. [PubMed: 18614159]
42. Gonzalez M, Aston-Jones G. Circadian regulation of arousal: role of the noradrenergic locus coeruleus system and light exposure. *Sleep*. 2006; 29:1327–1336. [PubMed: 17068987]
43. Gonzalez M, Aston-Jones G. Light deprivation damages monoamine neurons and produces a depressive behavioral phenotype in rats. *Proceedings of the National Academy of Sciences*. 2008; 105:4898–4903.
44. Zhang F, et al. Circuit-breakers: optical technologies for probing neural signals and systems. *Nature Reviews Neuroscience*. 2007; 8:577–581.
45. Hwang D-Y, et al. A high-efficiency synthetic promoter that drives transgene expression selectively in noradrenergic neurons. *Human gene therapy*. 2001; 12:1731–1740. [PubMed: 11560767]
46. Armbruster BN, et al. Evolving the lock to fit the key to create a family of G protein-coupled receptors potentially activated by an inert ligand. *Proceedings of the National Academy of Sciences*. 2007; 104:5163–5168.
47. Vazey EM, Aston-Jones G. Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. *Proceedings of the National Academy of Sciences*. 2014; 111:3859–3864.
48. Maret S, et al. Homer1a is a core brain molecular correlate of sleep loss. *Proceedings of the National Academy of Sciences*. 2007; 104:20090–20095.
49. Cirelli C, Gutierrez CM, Tononi G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron*. 2004; 41:35–43. [PubMed: 14715133]
50. Lu B, et al. BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nature Reviews Neuroscience*. 2013; 14:401–416.

51. Guzman-Marin R, et al. Suppression of hippocampal plasticity-related gene expression by sleep deprivation in rats. *The Journal of physiology*. 2006; 575:807–819. [PubMed: 16825295]
52. Huber R, Tononi G, Cirelli C. Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep*. 2007; 30:129–139. [PubMed: 17326538]
53. Faraguna U, et al. A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. *The Journal of Neuroscience*. 2008; 28:4088–4095. [PubMed: 18400908]
54. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. *science*. 2012; 338:68–72. [PubMed: 23042884]
55. Fontinha B, et al. Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A_{2A} receptor activation by endogenous adenosine. *Neuropharmacology*. 2008; 54:924–933. [PubMed: 18384819]
56. Hayaishi O, et al. Genes for prostaglandin d synthase and receptor as well as adenosine A_{2A} receptor are involved in the homeostatic regulation of nrem sleep. *Archives italiennes de biologie*. 2004; 142:525–531. [PubMed: 15493553]
57. Conn PJ, et al. Metabotropic glutamate receptors in the basal ganglia motor circuit. *Nature Reviews Neuroscience*. 2005; 6:787–798.
58. Hefti K, et al. Increased metabotropic glutamate receptor subtype 5 availability in human brain after one night without sleep. *Biological psychiatry*. 2013; 73:161–168. [PubMed: 22959709]
59. Manber R, et al. Cognitive behavioral therapy for insomnia enhances depression outcome in patients with comorbid major depressive disorder and insomnia. *Sleep*. 2008; 31:489. [PubMed: 18457236]
60. Arnedt JT, et al. Cognitive-behavioral therapy for insomnia in alcohol dependent patients: a randomized controlled pilot trial. *Behaviour research and therapy*. 2011; 49:227–233. [PubMed: 21377144]
61. Casement MD, Swanson LM. A meta-analysis of imagery rehearsal for post-trauma nightmares: Effects on nightmare frequency, sleep quality, and posttraumatic stress. *Clinical psychology review*. 2012; 32:566–574. [PubMed: 22819998]
62. Germain A, et al. Placebo-controlled comparison of prazosin and cognitive-behavioral treatments for sleep disturbances in US military veterans. *Journal of psychosomatic research*. 2012; 72:89–96. [PubMed: 22281448]
63. Frank E, et al. Inducing lifestyle regularity in recovering bipolar disorder patients: results from the maintenance therapies in bipolar disorder protocol. *Biological Psychiatry*. 1997; 41:1165–1173. [PubMed: 9171907]
64. Frank E, et al. Two-year outcomes for interpersonal and social rhythm therapy in individuals with bipolar I disorder. *Archives of general psychiatry*. 2005; 62:996–1004. [PubMed: 16143731]
65. Milgrom, O., et al. Effects of CBT for insomnia on relative regional cerebral metabolic rate of glucose during NREM sleep and morning wakefulness in adults from primary insomnia.. In: Dinges, DF., editor. 25th Anniversary Meeting of the Associated Professional Sleep Societies, LLC; Journal of Sleep and Sleep Disorders Research. Minneapolis, MN. 2011; p. A173
66. LeGates TA, et al. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature*. 2012; 491:594–598. [PubMed: 23151476]
67. Hattar S, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *Journal of Comparative Neurology*. 2006; 497:326–349. [PubMed: 16736474]
68. Altimus C, et al. Rods-cones and melanopsin detect light and dark to modulate sleep independent of image formation. *Proceedings of the National Academy of Sciences*. 2008; 105:19998–20003.
69. Ecker JL, et al. Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. *Neuron*. 2010; 67:49–60. [PubMed: 20624591]
70. Schmidt TM, Chen S-K, Hattar S. Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends in neurosciences*. 2011; 34:572–580. [PubMed: 21816493]
71. Chen S-K, Badea T, Hattar S. Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. *Nature*. 2011; 476:92–95. [PubMed: 21765429]
72. Ranft K, et al. Evidence for structural abnormalities of the human habenular complex in affective disorders but not in schizophrenia. *Psychol Med*. 2010; 40:557–567. [PubMed: 19671211]

73. Sartorius A, et al. Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biological psychiatry*. 2010; 67:e9–e11. [PubMed: 19846068]
74. Eastman CI, et al. Bright light treatment of winter depression: a placebo-controlled trial. *Archives of General Psychiatry*. 1998; 55:883–889. [PubMed: 9783558]
75. Grandner MA, Kripke DF, Langer RD. Light exposure is related to social and emotional functioning and to quality of life in older women. *Psychiatry research*. 2006; 143:35–42. [PubMed: 16725207]
76. Boivin DB, et al. Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. *Archives of General Psychiatry*. 1997; 54:145. [PubMed: 9040282]
77. Roeklein KA, et al. A missense variant (P10L) of the melanopsin (OPN4) gene in seasonal affective disorder. *Journal of affective disorders*. 2009; 114:279–285. [PubMed: 18804284]
78. Anderson J, et al. Lux vs. wavelength in light treatment of Seasonal Affective Disorder. *Acta Psychiatrica Scandinavica*. 2009; 120:203–212. [PubMed: 19207131]
79. Gagné A-M, et al. Impact of blue vs red light on retinal response of patients with seasonal affective disorder and healthy controls. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2011; 35:227–231. [PubMed: 21094670]
80. Vandewalle G, et al. Brain responses to violet, blue, and green monochromatic light exposures in humans: prominent role of blue light and the brainstem. *PLoS One*. 2007; 2:e1247. [PubMed: 18043754]
81. Vandewalle G, et al. Functional magnetic resonance imaging-assessed brain responses during an executive task depend on interaction of sleep homeostasis, circadian phase, and PER3 genotype. *The Journal of Neuroscience*. 2009; 29:7948–7956. [PubMed: 19553435]
82. Lambert G, et al. Effect of sunlight and season on serotonin turnover in the brain. *The Lancet*. 2002; 360:1840–1842.
83. Hoekstra R, et al. Effect of light therapy on biopterin, neopterin and tryptophan in patients with seasonal affective disorder. *Psychiatry Research*. 2003; 120:37–42. [PubMed: 14500112]
84. Ren C, et al. Direct retino-raphe projection alters serotonergic tone and affective behavior. *Neuropsychopharmacology*. 2013; 38:1163–1175. [PubMed: 23370156]
85. Badia P, et al. Bright light effects on body temperature, alertness, EEG and behavior. *Physiology & behavior*. 1991; 50:583–588. [PubMed: 1801013]
86. Rüger M, et al. Time-of-day-dependent effects of bright light exposure on human psychophysiology: comparison of daytime and nighttime exposure. *American Journal of Physiology-regulatory, integrative and comparative physiology*. 2006; 290:R1413–R1420.
87. Ishida A, et al. Light activates the adrenal gland: timing of gene expression and glucocorticoid release. *Cell Metabolism*. 2005; 2:297–307. [PubMed: 16271530]
88. Nestler EJ, et al. Neurobiology of Depression. *Neuron*. 2002; 34:13–25. [PubMed: 11931738]
89. Persson R, et al. Seasonal variation in human salivary cortisol concentration. *Chronobiology International*. 2008; 25:923–937. [PubMed: 19005896]
90. Wirz-Justice A, et al. Chronotherapeutics (light and wake therapy) in affective disorders. *Psychological medicine*. 2005; 35:939–944. [PubMed: 16045060]
91. Hines D, et al. Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. *Translational Psychiatry*. 2013; 3:e212. [PubMed: 23321809]
92. Dijk D-J, Lockley SW. Invited Review: Integration of human sleep-wake regulation and circadian rhythmicity. *Journal of applied physiology*. 2002; 92:852–862. [PubMed: 11796701]
93. Dang-Vu TT, et al. Functional neuroimaging insights into the physiology of human sleep. *Sleep*. 2010; 33:1589. [PubMed: 21120121]
94. Archer SN, et al. A length polymorphism in the circadian clock gene *Per3* is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep*. 2003; 26:413–415. [PubMed: 12841365]
95. Viola AU, et al. *PER3* Polymorphism Predicts Sleep Structure and Waking Performance. *Current Biology*. 2007; 17:613–618. [PubMed: 17346965]

96. Groeger JA, et al. Early morning executive functioning during sleep deprivation is compromised by a PERIOD3 polymorphism. *Sleep*. 2008; 31:1159. [PubMed: 18714788]
97. Mongrain V, Carrier J, Dumont M. Circadian and homeostatic sleep regulation in morningness–eveningness. *Journal of Sleep Research*. 2006; 15:162–166. [PubMed: 16704571]
98. Schmidt C, et al. Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area. *Science*. 2009; 324:516–519. [PubMed: 19390047]
99. Cohen H, et al. Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biological psychiatry*. 2006; 59:1208–1218. [PubMed: 16458266]
100. Cohen H, et al. Early post-stressor intervention with high-dose corticosterone attenuates posttraumatic stress response in an animal model of posttraumatic stress disorder. *Biological psychiatry*. 2008; 64:708–717. [PubMed: 18635156]
101. Gelpin E, et al. Treatment of recent trauma survivors with benzodiazepines: a prospective study. *The Journal of clinical psychiatry*. 1996; 57:390–394. [PubMed: 9746445]
102. Zohar J, et al. High dose hydrocortisone immediately after trauma may alter the trajectory of PTSD: interplay between clinical and animal studies. *European Neuropsychopharmacology*. 2011; 21:796–809. [PubMed: 21741804]
103. Cohen S, et al. Diurnal Fluctuations in HPA and Neuropeptide Y (NPY) ergic Systems Underlie Differences in Vulnerability to Traumatic Stress Responses at Different Zeitgeber Times. *Neuropsychopharmacology*. 2014

Figure 1.

Local brain pacemakers, local transcriptomes, sleep, and memory. Adapted from Ref. 13.

Figure 2.

Using new family studies to model the BD concept. This model depicts the potential relationships between the three independent dimensions (mania activation, psychosis, and depression) evident in new family studies of common mood disorders and observed clinical syndromes. The shared floor of this model is no activation, no depression, and no psychotic symptoms. Clinically, peak A represents the mania–hypomania (high activation, moderate psychosis, and low depression) syndrome; peak B represents the mixed states (moderate activation, low psychosis, and moderate depression); peak C represents atypical depression (low activation/retardation, low psychosis, and moderate depression); and peak D represents psychotic depression (very low activation/retardation, moderate psychosis, and high depression). In this model, each clinical syndrome can occur independently. Over the life course, an individual may experience one or more of these specific clinical syndromes but does not necessarily experience both low- and high-activation states.

Figure 4. Homeostatic mechanisms and their relationships to circadian mechanisms, sleep–wake regulation, brain activity, and mood and anxiety disorders: a simplified summary of the INSPIRE 2014 conference. Adapted from Ref. 91.