Neuroscience and Biobehavioral Reviews 34 (2010) 1249–1255

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Review

Circadian clock genes: Non-circadian roles in sleep, addiction, and psychiatric disorders?

Alan M. Rosenwasser[a,b,c,*]

[a] Department of Psychology, University of Maine, Orono, ME 04469-5742, United States
[b] School of Biology and Ecology, University of Maine, Orono, ME 04469-5742, United States
[c] Graduate School of Biomedical Sciences, University of Maine, Orono, ME 04469-5742, United States

ARTICLE INFO

Article history:
Received 21 May 2009
Received in revised form 13 March 2010
Accepted 15 March 2010

Keywords:
Circadian
Clock gene
Sleep disorders
Drug addiction
Alcoholism
Affective disorder

ABSTRACT

Elucidation of the cellular and molecular mechanisms of the circadian clock, along with the realization that these mechanisms are operative in both central and peripheral tissues, has revolutionized circadian biology. Further, these observations have resulted in an explosion of interest in the health implications of circadian organization and disorganization at both molecular and physiological levels. Thus, recent research has implicated mutations and polymorphisms of circadian clock genes in diabetes and obesity, cardiovascular disease, and cancer. At the neuro-behavioral level, circadian clock genes have also been implicated in sleep disorders, drug and alcohol addiction, and other psychiatric conditions. While such findings are frequently described as revealing "non-circadian" effects of clock genes, it remains possible that most of these non-circadian effects are in fact secondary to the loss of cellular and systemic rhythmicity. This review summarizes the evidence linking circadian clock genes to biobehavioral dysregulation, and considers criteria for defining a pleiotropic clock gene effect as non-circadian.

Contents

1. Introduction

Identification of the cellular and molecular bases of the circadian clock has revolutionized circadian biology. While the detailed elucidation of molecular clock components and their precise functions is an ongoing endeavor, there is now broad agreement that the cellular circadian clock comprises a set of interlocking positive and negative transcription-translation feedback loops, temporally modulated and stabilized by posttranslational processes (Allada et al., 2001; Ko and Takahashi, 2006; Lowrey and Takahashi, 2004). Further, the circadian clock is based on highly conserved molecular mechanisms, organized according to similar logic, and incorporating overlapping (though not identical) molecular elements throughout the animal kingdom. Operation of this clock network results in the rhythmic expression of both "core" circadian clock genes (i.e., genes whose products are essential to keeping the clock running, and which represent state variables of the clock), as well as "clock-controlled genes" (i.e., genes whose products transfer rhythmic molecular signals from the core clock loop to other cellular processes). In mammals, the most well-studied clock genes are those comprising the core negative feedback loop, including the *period* (*Per*) genes, *Per1* and *Per2*, the *cryptochrome* (*Cry*) genes, *Cry1* and *Cry2*, and the *Clock*, *Bmal1*, and *Npas2* genes. In brief, the Clock or Npas2 protein (depending on tissue) dimerizes with the Bmal1 protein to exert positive transcriptional drive on the *Per* and *Cry* genes, while in turn, the Per and Cry proteins dimerize, translocate

* Correspondence address: Department of Psychology, University of Maine, 5742 Little Hall, Orono, ME 04469-5742, United States. Tel.: +1 207 581 2035; fax: +1 207 581 6128.
E-mail address: alanr@maine.edu.

0149-7634/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neubiorev.2010.03.004

to the nucleus, and inhibit Clock/Bmal1 or Npas2/Bmal1 transcriptional activity via protein–protein interactions. Despite the conceptual simplicity of this scheme, the definition of a core clock gene, and the distinction between clock genes and clock-controlled genes, is not always completely straightforward (Lakin-Thomas, 2006; Roenneberg and Merrow, 1998).

The functional significance of circadian clock genes has been investigated primarily through (a) analysis of the level or temporal pattern of clock gene expression under a variety of physiological conditions and in response to various chronobiotic stimuli, and (b) examination of the physiological and behavioral consequences of spontaneous, chemically induced or targeted clock gene disruption in animal models, or in association with human genetic polymorphisms. Such studies have amply confirmed the relevance of the molecular circadian clock for the normal expression of circadian rhythms at the behavioral and physiological levels, while in turn, detailed analyses of circadian phenotype in clock gene mutant animals have informed our understanding of aspects of clock gene and protein functions at the cellular level.

One of the most important outcomes of this work has been the recognition that circadian clock genes and functional-molecular clock loops are expressed in a wide variety of cell types, not only within previously identified circadian pacemaker loci of the central nervous system (i.e., the suprachiasmatic nucleus (SCN) in mammals; the dorsal and ventral-lateral pacemaker cells in *Drosophila*), but also within a variety of other central and peripheral tissues and organs. This realization provided firm physiological and genetic bases for the hierarchically coupled multiple-oscillator circadian timing system, as originally inferred from phenomenological observations (Rosenwasser and Adler, 1986), and spurred a virtual explosion of research into the functions of both central and peripheral circadian clocks in health and disease (Chen-Goodspeed and Lee, 2007; Hastings et al., 2003; Haus and Smolensky, 2006; Laposky et al., 2008; Maywood et al., 2006; Preuss et al., 2008; Scheer et al., 2009; Takahashi et al., 2008; Turek et al., 2005). In particular, recent research tying the molecular circadian clock to both cellular and systems-level metabolic processes and to cell-cycle control have led to an appreciation of the role of disrupted circadian timing in diabetes and obesity, cardiovascular disease, and cancer.

In addition, circadian clock genes have also been implicated in a wide variety of neuro-behavioral processes. Thus, disruption of normal clock gene function in experimental animals alters sleep-wake regulation, drug responses, and affective behavior, while genetic polymorphisms and familial clock gene mutations are associated with sleep disorders, drug and alcohol addiction, and other psychiatric problems in human populations (Barnard and Nolan, 2008; Falcon and McClung, 2009; Franken and Dijk, 2009; Germain and Kupfer, 2008; Lamont et al., 2007; Manev and Uz, 2006; McClung, 2007a, 2007b; Perreau-Lenz and Spanagel, 2008; Spanagel et al., 2005b; Turek, 2007). Naturally, such behavioral observations serve to focus attention on the possible functions of rhythmic clock gene expression in non-SCN regions of the central nervous system, including limbic and striatal regions associated with sensory-motor and emotional regulation (Amir and Stewart, 2009; Guilding and Piggins, 2007; Uz et al., 2005). Nevertheless, an important conceptual question remains largely unaddressed: does the extensive evidence for pleiotropic effects of circadian clock genes in diverse physiological and behavioral processes imply functions for these genes *outside* their role in cellular circadian time-keeping, or do the widespread effects of clock gene activity depend directly on the maintenance of normal cellular and genomic rhythms? In other words, do these findings highlight the critical importance of the circadian clock for health and well-being, or rather, reveal only that so-called circadian clock genes have a multitude of cellular and physiological functions besides their critical roles in circadian time-keeping.

The present review will outline the evidence that clock genes influence basic behavioral processes relevant to sleep disturbances, drug and alcohol addiction, and psychiatric disorders, and will consider whether these effects provide evidence for non-circadian functions of the circadian clock genes.

2. Sleep regulation

According to the highly influential two-process model (Borbely, 1982; Daan et al., 1984), the sleep–wake cycle is controlled jointly by a circadian process regulating the timing of sleep and by a homeostatic process regulating sleep drive (and/or sleep "intensity"). While both processes contribute to the overt day–night sleep–wake pattern, they have generally been viewed as operating independently and additively (Dijk and Czeisler, 1995; Dijk and von Schantz, 2005). Thus, the circadian timing of mammalian sleep and wakefulness, like other neuro-behavioral functions, is controlled by the SCN circadian pacemaker, while sleep homeostasis is regulated by a distributed network of forebrain and brainstem sleep-promoting and wake-promoting systems (Fuller et al., 2006; Mistlberger, 2005). According to this view, neural or genetic manipulations that disrupt circadian clock function might be expected to alter the *timing* of sleep within the circadian day, but not the homeostatic regulation of sleep.

Nevertheless, several studies suggest that circadian clock genes may contribute to the homeostatic regulation of sleep, separate from their role in circadian clock function. Thus, arrhythmic mice bearing double-deletion of the *Cry 1* and *Cry2* genes or a null mutation of the *Bmal1* gene exhibit increased non-rapid-eye-movement (NREM) sleep time, increased NREM sleep intensity (assayed by EEG delta power), and attenuation of the normal post-deprivation NREM rebound (Laposky et al., 2005; Wisor et al., 2002). In contrast, deletion of only the *Cry1* or *Cry2* gene, which results in a much less dramatic circadian phenotype than does the double-deletion, fails to alter sleep homeostasis (Wisor et al., 2008). The lack of post-deprivation compensation in *Cry1*/*Cry2* and *Bmal1* knock-out mice probably indicates that NREM sleep drive is already maximal in these animals, even under non-deprived baseline conditions. Mice with mutations of either the *Clock* gene (Naylor et al., 2000) or *Npas2* gene (Dudley et al., 2003; Franken et al., 2006) show the opposite baseline sleep phenotype, characterized by decreased total NREM sleep time. While *Npas2* mutants also show an attenuated response to sleep deprivation, this has been attributed to reduced sleep drive, rather than to a ceiling effect as in the *Cry* mutants. In marked contrast to these findings, neither single nor double-mutation of the *Per1* and/or *Per2* genes altered sleep homeostasis (Kopp et al., 2002; Shiromani et al., 2004). Despite the implication that specific clock genes may increase, decrease, or have no effect on sleep drive, sleep deprivation has been shown to broadly increase cortical clock gene expression in wild-type mice of several inbred strains (Franken et al., 2007; Wisor et al., 2008). Nevertheless, different clock genes showed different response kinetics during and following sleep deprivation, both within and between strains.

While the rest-activity cycles of insects and other invertebrates have long been a major focus of circadian rhythm research, these species have historically been considered to not display "true sleep". Recently, however, it has been argued that the long-standing exclusion of invertebrates from sleep research may have been due primarily to researchers' use of restrictive electrophysiological markers for sleep, such as EEG, that may not be applicable to invertebrate species. Indeed, careful behavioral analysis indicates that behavioral rest in *Drosophila* – like mammalian sleep – is associated with increased arousal thresholds, is influenced by both