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The long-term abnormalities in circadian expression of Period 1 and Period 2 genes in response to stress is normalized by agomelatine administered immediately after exposure

Ori Koresh [a], Nitsan Kozlovsky [a], Zeev Kaplan [a], Joseph Zohar [b], Michael A. Matar [a], Hagit Cohen [a,*]

[a] *Beer-Sheva Mental Health Center, The State of Israel Ministry of Health, Anxiety and Stress Research Unit, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel*
[b] *Division of Psychiatry, The State of Israel Ministry of Health, The Chaim Sheba Medical Center, Ramat-Gan, and Sackler Medical School, Tel-Aviv University, Israel*

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Abstract

In mammals, the circadian and stress systems are involved in adaptation to predictable and unpredictable stimuli, respectively. A series of experiments examined the relationship between stress-induced posttraumatic stress (PTSD)-like behavioral response patterns in rats and brain levels of genes related to circadian rhythms. The effects of agomelatine, administered immediately after exposure, on stress-related behavior and on local expression of Per1 and Per2 were assessed. Animals were exposed to predator scent stress. The outcome measures included behavior in an elevated plus-maze (EPM) and acoustic startle response (ASR) 7 days after the exposure. Pre-set cut-off behavioral criteria classified exposed animals according to behavioral responses in EPM and ASR paradigms as those with 'extreme behavioral response' (EBR), 'minimal behavioral response (MBR),' or 'partial behavioral response' (PBR). Per1 and Per2 expression in hippocampal subregions, frontal cortex and suprachiasmatic nucleus (SCN) 8 days after exposure were evaluated using immunohistochemical and RT-PCR techniques at zeitgeber-times 19 and 13. The effects of agomelatine, on behavioral tests were evaluated on Day 8. Local brain expression of Per1 and Per2 mRNA was subsequently assessed. Data were analyzed in relation to individual behavior patterns. Animals with extreme behavioral response (EBR) displayed a distinct pattern of Per1 and Per2 expression in the SCN, which was the opposite of that observed in the control and MBR animals. In the DG, no variation in Per2 expression was observed in the EBR and PBR animals. Immediate post-exposure

* Corresponding author at: Anxiety and Stress Research Unit, Ministry of Health Mental Health Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, P.O. Box 4600, Beer-Sheva 84170, Israel. Tel.: +972 8 6401743; fax: +972 8 6401742.
E-mail address: hagitc@bgu.ac.il (H. Cohen).

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treatment with agomelatine significantly reduced percentage of extreme-responders and normalized the expression of Per1 and Per2 as compared to controls. Stress-induced alterations in Per genes in the EBR animals may represent an imbalance between normally precisely orchestrated physiological and behavioral processes and psychopathological processes. These findings indicate that these circadian-related genes play a role in the neurobiological response to predator scent stress and provide supportive evidence that the use of agomelatine immediately after traumatic experience may be protective against the subsequent development of PTSD.

1. Introduction

Frequent awakenings from sleep, exaggerated startle response, increased vigilance, and abnormal physiological reactivity are prominent symptoms of posttraumatic stress disorder (PTSD) and may indicate the maladaptive persistence of a normal response to threat. Among the heightened arousal symptoms in PTSD, sleep disturbances are particularly distressing and persistent. Anxiety, agitation, and excessive body movement have been found to characterize sleep complaints in combat-related PTSD patients compared to patients with primary insomnia. Moreover, abnormal circadian rhythms in a variety of bodily functions, including plasma cortisol, norepinephrine and corticotrophin releasing hormone, startle response, and blood pressure, have been found in PTSD patients (Laudenslager et al., 2009; Mellman et al., 2009; Miller and Gronfier, 2006; Pervanidou et al., 2007; Ulmer et al., 2009). It is entirely possible that alterations in the expression/functioning of the core circadian clock genes underlie these pathophysiological and mental features.

To thrive in a rhythmically changing environment, organisms have evolved endogenous clocks that synchronize their physiology to the outside world. In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus harbors the master circadian clock that coordinates the majority of diurnal aspects of behavior and physiology (Belle et al., 2009; Challet, 2007). The circadian oscillation of electrical activity of SCN neurons is driven by protein products of core clock genes. By serving as transcription–translation-based networks of positive and negative feedback loops, clock genes regulate their own expression by accumulating gene products over a 24-h period until the genes turn off and re-start their cycles anew (Feillet et al., 2008).

Seven clock genes play a role in the clockwork. CLOCK/BMAL1 heterodimers activate *Period* (*Per1-3*) and *Cryptochrome* (*Cry*) genes through a DNA sequence (E-Box) (Darlington et al., 1998; Gekakis et al., 1998; Jin et al., 1999; Reppert and Weaver, 2002; Takahashi et al., 2008). The PER and CRY proteins are translated in the cytoplasm and are phosphorylated by Casein Kinase Iε and δ and Glycogen Synthase Kinase 3β, leading to changes in their stability, association, and nuclear penetration (Harms et al., 2003; Iitaka et al., 2005; Kurabayashi et al., 2006). Upon entering the nucleus, they repress the actions of CLOCK/BMAL1, thus creating a negative feedback loop (McClung, 2007). A second feedback loop involves Rev-ERB-α and retinoid-related orphan receptor-α (ROR-α), which are responsible, respectively, for inhibition and activation of BMAL1 transcription, thus forming an interlocking regulatory loop. Built-in biochemical delays in the progression of these loops affect 24-h rhythmicity (Takahashi et al., 2008). Various post-translational modifications of the core circadian proteins provide an additional level of regulation of the molecular oscillator (Kondratov and Antoch, 2007).

Not limited to the SCN, circadian rhythmicity in clock gene expression has been detected in various peripheral tissues and in other brain regions (Ko and Takahashi, 2006; Stratmann and Schibler, 2006; Yamazaki et al., 2000; Yoo et al., 2004). Projections from the SCN to the locus coeruleus facilitate circadian regulation of noradrenergic activity and are important for transitions from focused attention to behavioral flexibility (Aston-Jones et al., 2001) and for contextual fear conditioning, circadian regulation of the sleep–wake cycle (Gonzalez and Aston-Jones, 2006), and synaptic plasticity including long-term potentiation (Chaudhury and Colwell, 2002; Chaudhury et al., 2005). Thus, the ability of clock genes to switch between acting as transcriptional activators and as repressors suggests that they could orchestrate circadian control of neuronal gene expression and neuronal activity by influencing the function of neurotransmitters and receptors involved in the regulation of emotion and cognition (Benca et al., 2009). Maintaining the phase-relation order of the various circadian rhythms, from the molecular to the behavioral levels, enables an organism to adapt, both behaviorally and physiologically, to temporal changes in the environment (Easton et al., 2003). Alterations or mutations in clock components or their regulators can lead to asynchrony from the cellular (cell cycle) to the organ-system level and can have profound consequences on mental functioning (emotions, cognition, behavior, and functioning) (Benca et al., 2009).

A large body of literature indicates that there is a link between clock gene functions (i.e., the circadian system) and mood regulation (Bunney and Potkin, 2008; Bunney and Bunney, 2000; Easton et al., 2003; Hampp et al., 2008; Mendlewicz, 2009; Turek, 2007; Yang et al., 2009). There is relatively little information regarding the role of clock genes in anxiety disorders and only a few studies have attempted to determine the effect of a dysfunctional circadian pacemaker on emotional behavior or stress responsiveness (Easton et al., 2003; Lamont et al., 2007). Of these studies, lesions of the SCN (Arushanian and Beier, 1999) or mutation of the cAMP-responsive element modulator (Maldonado et al., 1999), important for the output of the circadian clock, were both shown to alter levels of anxiety and locomotor activity. Moreover, Akiyama et al. (1999) reported that the expression of Per1 mRNA is rapidly reduced in the cerebellum by acute intraperitoneal injection of diazepam, triazolam and tandospirone, but not by clozapine and haloperidol.

This report summarizes a series of experiment studies which developed, stepwise, each based upon the results of its