Alcohol 49 (2015) 351–357

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Clock genes × stress × reward interactions in alcohol and substance use disorders

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ARTICLE INFO

Article history:
Received 13 April 2015
Accepted 13 April 2015

Keywords:
Circadian clock
Period genes
HPA axis
Alcohol
Cocaine
Nicotine

ABSTRACT

Adverse life events and highly stressful environments have deleterious consequences for mental health. Those environmental factors can potentiate alcohol and drug abuse in vulnerable individuals carrying specific genetic risk factors, hence producing the final risk for alcohol- and substance-use disorders development. The nature of these genes remains to be fully determined, but studies indicate their direct or indirect relation to the stress hypothalamo-pituitary-adrenal (HPA) axis and/or reward systems. Over the past decade, clock genes have been revealed to be key-players in influencing acute and chronic alcohol/drug effects. In parallel, the influence of chronic stress and stressful life events in promoting alcohol and substance use and abuse has been demonstrated. Furthermore, the reciprocal interaction of clock genes with various HPA-axis components, as well as the evidence for an implication of clock genes in stress-induced alcohol abuse, have led to the idea that clock genes, and *Period* genes in particular, may represent key genetic factors to consider when examining gene × environment interaction in the etiology of addiction. The aim of the present review is to summarize findings linking clock genes, stress, and alcohol and substance abuse, and to propose potential underlying neurobiological mechanisms.

Environmental stressors have a profound and durable impact on our general health by potentiating the risk for developing multiple diseases. In particular, stressors affect the development of alcohol- and substance-use disorders (AUD and SUDs). AUD is hence highly influenced by former experienced stressful life events, with epidemiologic surveys reporting that repeated stress correlates with doubling of alcohol binge drinking and multiplies by a factor of 6 the risk of developing AUD (Keyes, Hatzenbuehler, Grant, & Hasin, 2012; Spanagel, Noori, & Heilig, 2014). Furthermore, different stressors are affecting our behaviors: environmental stressors, physiological, psychological, or metabolic disease-driven stressors, but also an imbalance of the internal state, i.e., sleep deprivation and internal circadian desynchrony can be experienced as very stressful. Such stressors may interact with genetic factors to produce the final risk for AUD and SUDs. The mechanisms and nature of these interactions are not well understood but increasing evidence suggests that clock genes may be playing a critical gating role in this regard.

Here we will review findings that demonstrate that clock genes can influence the development of addictive behavior in laboratory animals and humans. Numerous other studies also show the impact of clock genes on stressful events by modulating the activity of the stress axis. On the other hand, alcohol and other drugs of abuse as well as different stressors influence the expression and rhythmicity of clock genes in central and peripheral oscillators. In conclusion, we will propose clock genes × stress interaction as one possible gating mechanism in the development of addictive behavior.

Clock genes – new key players in AUD and SUDs

During the preceding decade, a new family of genes has emerged as a major player in the addiction field: the so-called clock genes (Agapito, Barreira, Logan, & Sarkar, 2013; Agapito, Mian, Boyadjieva, & Sarkar, 2010; Falcón & McClung, 2009; Logan, Williams, & McClung, 2014; McCarthy, Fernandes, Kranzler, Covault, & Welsh, 2013; McClung, 2007; Perreau-Lenz & Spanagel, 2008; Rosenwasser, 2010). Clock genes, such as *Period* genes (*Per1-Per3*), *Cryptochrome* genes (*Cry 1-2*), *Circadian Locomotor Cycle Kaput- (Clock), Brain and Muscle ARNT-like protein 1 (Arntl1), Neuronal PAS domain protein 2 (NPAS2)*, or *D-box-binding protein (Dbp)* genes are molecular components of the circadian clockwork.

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http://dx.doi.org/10.1016/j.alcohol.2015.04.003
0741-8329/

These oscillatory proteins interact with each other in well-characterized but rather complex transcriptional-translational and post-translational feedback loops self-sustaining expression oscillations close to 24 h (Buhr & Takahashi, 2013; Ko & Takahashi, 2006; Lee & Kim, 2014). Their rhythmic expression and cell bioavailability is thereby influencing their own expression and the expression of clock-controlled genes. Clock-controlled genes are output genes whose transcription is subjected to circadian control by core clock proteins on specific RORE, E-Box, or D-Element promoter binding sites. These clock-controlled transcriptomes are largely tissue-specific and represent 10–15% of all transcripts (Bozek et al., 2009). They include various neuromodulators or neuropeptides (i.e., arginine vasopressin, pituitary adenylate cyclase-activating peptide 1), nuclear receptors, and cell metabolism modulators (Masri & Sassone-Corsi, 2013; Ripperger & Albrecht, 2012a, 2012b; Schmutz, Ripperger, Baeriswyl-Aebischer, & Albrecht, 2010; Zani et al., 2013). Clock genes are present in most brain areas and peripheral tissues or blood cells. Although oscillating ubiquitously, they are under the synchronizing control of the master circadian clock, located within the suprachiasmatic nucleus of the hypothalamus, which orchestrates the sustainability and synchronism of circadian activity of most of our biological functions (Bollinger & Schibler, 2014; Kalsbeek et al., 2011; Perreau-Lenz, Pévet, Buijs, & Kalsbeek, 2004). Desynchrony between these various oscillators in the body, from each other or from misalignment with the environment, may increase risk for the development of mental disorders and diseases (Baron & Reid, 2014; Hasler, Soehner, & Clark, 2014; Salgado-Delgado, Angeles-Castellanos, Buijs, & Escobar, 2008; Salgado-Delgado, Tapia Osorio, Saderi, & Escobar, 2011). Implication of the molecular components of these oscillators in the development of AUD and SUDs has been revealed as well over the years.

In the late 1990s, Andretic and colleagues first discovered the influence of clock genes on drug effects showing the inability of mutant *Drosophila* flies for the clock genes *period*, *clock*, *cycle*, and *doubletime* to express behavioral sensitization to repeated volatilized free-base cocaine exposure (Andretic & Hirsh, 2000). Shortly after this discovery, these findings were confirmed and extended in mice lacking functional *mPer1* and *mPer2* genes. Thus, cocaine-induced behavioral sensitization and conditioned place preference is absent in $Per1^{Brdm1}$-mutant mice, while tending to be increased in $Per2^{Brdm1}$-mutant mice (Abarca, Albrecht, & Spanagel, 2002). Although PER1 and PER2 proteins seem to produce similar effects on the control of circadian phenotypes, both being involved in the negative loop of the molecular circadian clockwork, they seem to produce rather specific (and even opposite) effects on drug-induced phenotypes.

Similarly, McClung and colleagues have recently revealed the differential effects of the protein CLOCK and NPAS2 (clock proteins known to be homologous in structure and function to CLOCK) on cocaine-induced behaviors in a tissue-specific manner. First, they demonstrated that $Clock^{\Delta 19}$-mutant mice, carrying a single point mutation inducing the protein CLOCK, are devoid of any transcriptional activity in all CLOCK-expressing cells, and display increased cocaine-induced conditioned place preference and self-administration when compared to wild-type mice (McClung, Nestler, & Zachariou, 2005; Ozburn, Larson, Self, & McClung, 2012). Most recently, they showed that, when applied specifically within the nucleus accumbens, adeno-associated virus-short hairpin RNA mediating knockdown of the gene *Clock* does not seem to affect cocaine-induced behaviors, whereas such expression knockdown of the gene *Npas2* decreases cocaine-induced conditioned place preference and self-administration (Ozburn et al., 2015).

In addition, the same clock gene may influence drug-induced behaviors differentially depending on the behavior assessed. Cocaine-induced self-administration, for instance, is interestingly not impaired in $Per1^{Brdm1}$-mutant mice, and these mutants display reinstatement of cocaine intravenous self-administration after extinction similar to their wild-type counterparts (Halbout, Perreau-Lenz, Dixon, Stephens, & Spanagel, 2011).

Clock has been recently implicated in alcohol behaviors as well, with the $Clock^{\Delta 19}$-mutant mice exhibiting increased ethanol sensitivity and consumption as compared to wild-type controls (Ozburn et al., 2013). On the other hand, $Clock^{\Delta 19}$-mutant mice do not differ in magnitude of the sensitized response to nicotine as compared to wild-type controls, demonstrate a similar preference for a nicotine-paired environment in the conditioned place-preference paradigm, and show a similar acquisition of nicotine self-administration (Bernardi & Spanagel, 2013).

Hence, each clock protein may have differential effects on different cell output messengers, effects that may vary depending on the tissue or brain structure and depending on the time of drug exposure. In conclusion, i) depending on the drug, clock genes may affect drug-related responses or not, and ii) clock genes (i.e., *Per1, Per2, Clock, Npas2*) seem to modulate specific drug-induced behaviors in different ways.

In agreement with the latter conclusion, $Per2^{Brdm1}$-mutant mice are less tolerant to the analgesic effects of morphine and exhibit attenuated precipitated withdrawal signs as compared to their control littermates (Perreau-Lenz, Sanchis-Segura, Leonardi-Essmann, Schneider, & Spanagel, 2010), while $Per1^{Brdm1}$-mutant mice do not differ from wild-type mice in tolerance to morphine and in the expression of naloxone-induced withdrawal symptoms (Perreau-Lenz, Zghoul, & Spanagel, 2007; unpublished data). When compared to their respective wild-type littermates, $Per2^{Brdm1}$-mutant mice show enhanced consumption of alcohol (Brager, Prosser, & Glass, 2011; Spanagel et al., 2005) and disruption of their daily rhythm of central alcohol sensitivity (Brager et al., 2011; Perreau-Lenz, Zghoul, de Fonseca, Spanagel, & Bilbao, 2009; Spanagel et al., 2005). However, these effects are yet again not observed in $Per1^{Brdm1}$-mutant mice, which do not differ from their wild-type counterparts in terms of alcohol consumption under home-cage basal conditions (Perreau-Lenz et al., 2009; Zghoul et al., 2007). Of note, these latter effects also seem to be strongly influenced by the genetic background. Hence, higher alcohol consumption is observed in *Per1*-mutant mice compared to their wild-type counterparts when backcrossed on the more alcohol-preferring background C57BL/6J (Gamsby et al., 2013).

In addition to the above-mentioned rodent studies, several human genetic studies have also revealed associations of certain polymorphisms of several clock genes with AUD and SUDs (Blomeyer et al., 2013; Brower, Wojnar, Sliwerska, Armitage, & Burmeister, 2012; Comasco et al., 2010; Dong et al., 2011; Kovanen et al., 2010; Malison, Kranzler, Yang, & Gelernter, 2006; Sjoholm et al., 2010; Spanagel et al., 2005; Surovtseva, Kudryavtseva, Voronina, Pronin, & Filipenko, 2012; Wang et al., 2012; Zou et al., 2008). Although associations of certain clock gene variants and AUD and SUDs could not always be replicated (Malison et al., 2006; Surovtseva et al., 2012), in sum these genetic studies link clock gene function and addictive behavior in humans (Partonen, 2015).

Alcohol and drugs of abuse influence the expression of clock genes

Reciprocally, the expression of clock genes as well as their rhythmic pattern of expression is also affected by alcohol and drugs of abuse (Logan et al., 2014; Perreau-Lenz & Spanagel, 2008). Various drugs of abuse when applied acutely have different effects on the expression of clock genes depending on the brain area, the