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Clock Genes × Stress × Reward Interactions in Alcohol and Substance Use Disorders

Stéphanie Perreau-Lenz^{1,2} and Rainer Spanagel¹

¹Institute of Psychopharmacology, Central Institute for Mental Health, Medical Faculty of Mannheim/ Heidelberg University, Mannheim, Germany

²SRI International, Center for Neuroscience, Biosciences Division, Menlo Park, CA, USA

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.

Keywords

circadian clock; period genes; HPA axis; alcohol; heroin; cocaine; nicotine

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

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Corresponding author: Stéphanie Perreau-Lenz, Ph.D. Senior Research Scientist, SRI International, Biosciences Division – Center for Neuroscience, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA, Telephone: +1 650 859 4510, Fax: +1 650 859 3153, stephanie.perreau-lenz@sri.com.

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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 $\times$ 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. 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^{l9}*-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^{l9}*-mutant mice exhibiting increased ethanol sensitivity and consumption as compared to wild-type controls (Ozburn et al., 2013). On the other hand, *Clock^{l9}*-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; Sjöholm 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 peripheral tissue, the gene of interest, or even the time of the day during which they are applied. Table 1 summarizes the impact of alcohol and other drugs on the expression of clock genes in the SCN and mesocorticolimbic areas. Chronic exposure to opioids, psychostimulants, or alcohol strongly affects the rhythmic pattern of clock gene expression. For instance, the circadian expression of *Per* genes is shifted or even abolished by chronic opioid use and withdrawal in rats (Hood, Cassidy, Mathewson, Stewart, & Amir, 2011; Li et al., 2010) or in heroin-addicted patients (Li, Liu, Jiang, & Lu, 2009). Similarly reduced clock gene expression is observed in male

alcoholic patients (Huang et al., 2010). In rodents, prenatal alcohol exposure alters clock gene expression in the rat hypothalamus (Chen, Kuhn, Advis, & Sarkar, 2006).

Circadian clock control of the stress axis

The hypothalamo-pituitary-adrenal (HPA) axis, or stress axis, is under circadian regulation. Its key players, such as corticotropin-releasing hormone (CRH), CRH receptors, adrenocorticotrophic hormones, and glucocorticoids, all display endogenous circadian rhythms of release and synthesis controlled by the suprachiasmatic nucleus of the hypothalamus (Kalsbeek et al., 2012; Nader, Chrousos, & Kino, 2010). HPA-axis function is altered in several mutant clock gene mouse models. For instance, glucocorticoid production is impaired in mutant mice expressing deficiency in BMAL1 protein (*Arntl*-deficient mutant mice) (Leliavski, Shostak, Husse, & Oster, 2014). In addition, both *Per1^{Brdm1}*- and *Per2^{Brdm1}*-mutant mice show markedly increased levels of corticosterone across the light-dark cycle (Dallmann, Touma, Palme, Albrecht, & Steinlechner, 2006). *Per1^{Brdm1}*-mutant mice show constant high levels of corticosterone, while diurnal variation with peak release at the beginning of the dark phase could still be measured in *Per2^{Brdm1}*-mutant mice. In a separate study, Yang and colleagues have shown that corticosterone levels are lower in *Per2^{Brdm1}*-mutant mice, especially during the dark phase, and that these mutants do not show the typical daily rise of corticosterone observed before arousal as compared to their wild-type counterparts, but have intact glucocorticoid response to restrained stress (Yang et al., 2009). These data show the involvement of clock genes in the control of the HPA axis.

Interestingly, transcriptional activity of glucocorticoid receptors is also strictly controlled by circadian clock proteins (Charmandari et al., 2011; Han, Lee, Kim, Kim, & Cho, 2014). Recent research revealed that the protein CLOCK specifically represses glucocorticoid receptor activity through acetylation and epigenetic regulation when the circulating glucocorticoids are at the highest (Kino & Chrousos, 2011a, 2011b). Finally, *Crh* expression levels are increased in the paraventricular nucleus of the hypothalamus of *Per2*-mutant mice (Zhang et al., 2011). Altogether, these studies demonstrate the impact of the internal circadian state on the stress axis, and more specifically the potential impact of clock protein functions on gating stressful events by modulating glucocorticoid levels or receptor activity.

Dysregulation of the stress axis in AUD and SUDs

Chronic exposure to alcohol or other drugs of abuse also affects various components of the stress axis resulting in an overall increased HPA activity. In addicted patients, the daily rhythm of glucocorticoids is clearly affected. As repeatedly reported, levels of glucocorticoids display dampened rhythms and are often highly elevated throughout the day (Glavas, Ellis, Yu, & Weinberg, 2007; Loosen et al., 1993; Loosen, Chambliss, Pavlou, & Orth, 1991; Sarkar, 2012; Wong & Schumann, 2012). Thus, the rhythmic function of the adrenal gland is altered in abstinent alcoholics (Loosen et al., 1991, 1993; Uhart & Wand, 2009). Additionally, predictive correlation of enhanced cortisol levels and relapse could be found in abstinent alcoholics with cortisol levels measured at the beginning of the abstinence phase being significantly lower in long-term abstainers when compared to relapsers (Walter et al., 2006). Persisting elevated cortisol levels and altered ACTH diurnal rhythms were also

observed in 30-days abstinent heroin addicts (Li et al., 2009), and opioid therapy normalizes endocrine rhythms, including the endocrine rhythm of glucocorticoids (Brennan, 2013).

These long-term effects of alcohol and other drugs of abuse on HPA activity can be explained by the fact that key regulators of the stress axis are affected by drugs. Chronic alcohol exposure and alcohol bingeing in rodents influence the glucocorticoid feedback mechanism, mediated via glucocorticoid-responsive element (GRE) sites of the CRH promoter impairing CRH activity (Przybycien-Szymanska, Mott, & Pak, 2011; Przybycien-Szymanska, Mott, Paul, Gillespie, & Pak, 2011; Przybycien-Szymanska, Rao, & Pak, 2010; Spanagel et al., 2014). Intermittent access to alcohol also results in CRH increase in rhesus macaques (Schwandt et al., 2010), and functional variation of the *CRH* gene increases stress-induced alcohol consumption in non-human primates (Barr, 2013; Barr et al., 2009). In summary, HPA-axis function is strongly disrupted in alcohol-dependent subjects and other addicted patients (for further information see our recent review, Spanagel et al., 2014). In the following paragraph, we will next review convergent evidence that stress can profoundly affect clock gene function.

Stress affects clock genes

In rodents, chronic mild stress is known to alter circadian expression of various molecular clock genes. For example, acute and chronic physical stress elevates *mPer1* mRNA expression in mouse peripheral tissues (liver, heart, kidneys) (Takahashi et al., 2013; Yamamoto et al., 2005) and forced swim stress elevates *mPer1* in the paraventricular nucleus while failing to have any effect in the suprachiasmatic nucleus (Takahashi et al., 2001). The *Per1* gene is a target gene for glucocorticoids via a GRE promotor site. The effects of glucocorticoids on clock genes, and *Per1* in particular, has been shown in both mice (Yamamoto et al., 2005) and humans (Reddy, Gertz, Crawford, Garabedian, & Myers, 2012; Reddy et al., 2009). A similar impact of stress on *Per2* has also been demonstrated. In the mouse limbic areas, for instance, protein expression of PER2 is strictly controlled by glucocorticoids (Segall & Amir, 2010a, 2010b). Furthermore, glucocorticoid receptor (GR) function is necessary for the rhythmic expression of the clock protein PER2, because neuron-specific GR-inducible mutant mice do not display a daily rhythm of PER2 activity (Segall, Milet, Tronche, & Amir, 2009). Other stress conditions such as metabolic stress (e.g., restricted feeding) may also strongly affect the expression of clock genes in rodents (Angeles-Castellanos, Mendoza, & Escobar, 2007; Minana-Solis et al., 2009; Yoshida, Shikata, Seki, Koyama, & Noguchi, 2012). In summary, stress affects the expression and rhythmicity of clock genes in peripheral oscillators via a tight control of glucocorticoids on clock gene promotor function.

Stress increases risky alcohol consumption – gating role by clock genes?

Alcohol is frequently consumed for stress relief. The individual determinants and the temporal course of stress-induced alcohol consumption have been characterized by preclinical studies in rodents (Noori, Helinski, & Spanagel, 2014; Spanagel, Noori, & Heilig, 2014). Stress-induced alcohol consumption in rodents has a high genetic load and results, at least in part, from an interaction of clock genes with the stress axis and the reward

system. The latter statement is best exemplified by human genetic association studies with *Per* gene variants and stress-induced alcohol consumption. Hence, *PER2* allelic variation has been associated with high vs. low alcohol intake in adult alcoholics, with a significant association effect for SNP rs56013859 at the position 1071 base pairs downstream of the ATG site of *PER2* (Spanagel et al., 2005). The implication of the *Per2* gene in controlling drinking behavior has been further extended to stress effects. The same SNP of the *hPer2* gene also moderates the impact of severe stress on alcohol abuse (Blomeyer et al., 2013). Experienced alcohol users carrying a *G* allele haplotype at the same SNP rs56013859 and who experienced early young adult life stressful events (i.e., death of a loved one or relationship breakup) were found to drink to a lesser extent than homozygotes carrying the major *A* allele. Of note, no difference in *PER2*-Luciferase activity rhythm parameters could be observed in human fibroblasts from AUD patients from carriers of the rare *G* allele or homozygous carriers for the common *A* allele (McCarthy et al., 2013). We have also shown the specific functional implication of genetic variants in the promoter of the *hPer1* gene in increased alcohol consumption in adolescents that were exposed to severe adverse life events in early childhood (Dong et al., 2011). Psychosocial stress-induced alcohol drinking observed in young adults carrying the minor haplotype *C* in the *hPer1* SNP rs3027172 and who experienced severe adverse life events displayed higher frequency of heavy drinking as compared to homozygous carriers of the *T* allele. In the same study, we confirmed the specific implication of *Per1* on psychosocial stress-induced alcohol consumption in mice. *Per1^{Brdm1}*-mutant mice underwent various stressors such as social defeat stress and forced swim stress during voluntary alcohol home-cage drinking, which significantly increased their consumption as opposed to what was observed in wild-type control mice (Dong et al., 2011). Finally, the implication of the *Period* gene *Per3* has been recently unraveled as well. A variable number of tandem repeat polymorphism, based on 4-repeat or 5-repeat alleles, results in 3 *PER3* genotypes. Brower and colleagues showed that the three different *hPER3* genotypes are independent predictors of insomnia severity and that alcohol-dependent patients carrying the 4-repeat variant have the greatest severity of insomnia symptoms (Brower et al., 2012). In another study related to *Per3*, Wang and colleagues have recently shown that an insertion/deletion variant in a putative stress response element in the mouse *Per3* promoter causes local control of the transcript and shows a high correlation with alcohol- and stress-related traits (Wang et al., 2012).

As described above, the interaction of clock genes and glucocorticoids is well characterized. Clock genes such as *Per1* and *Per2* have been shown to be regulated by glucocorticoids in peripheral oscillators or other brain oscillators, such as mesocorticolimbic areas (Segall & Amir, 2010b). Taking into account their direct implication in drug-induced behaviors, this makes them potential candidates for conveying or gating the effects of stress onto alcohol intake and relapse. Interestingly, a more recent study has shown that clock genes are also gating glucocorticoids' maximal efficacy (Han et al., 2014). The authors have revealed that circadian clock proteins modulate the maximal glucocorticoids' receptor transactivation via the CLOCK-BMAL1 complex. Furthermore, they showed that BMAL1- and PERs-deficient fibroblast cells display hypersensitivity to glucocorticoids, suggesting an inhibitory influence of these clock proteins on GR activity. In parallel, recent studies have shown the implication of epigenetic mechanisms involved in the regulation of GR/clock genes

interaction (Kino & Chrousos, 2011a, 2011b). In the context of epigenetic modifications, Xydous and colleagues have demonstrated that dexamethasone treatment induces histone H3 posttranslational modifications at the *Per1* promoter, suggesting that chromatin remodeling occurs on the GRE binding site of the *Per1* promoter (Xydous, Prombona, & Sourlingas, 2014), supporting the idea of long-term epigenetic effects.

Even though the neurobiological mechanisms underlying the involvement of clock genes on the development of alcohol and drug abuse are still to be determined, our current knowledge shows that most neurotransmitter systems involved in drug abuse are influenced by clock genes. *Per2^{Brdm1}*-mutant mice hence show a hyper-glutamatergic state within the ventral striatum compared to littermate controls (Spanagel et al., 2005), and a hyper-dopaminergic striatal state throughout the light-dark cycle (Hampp et al., 2008). Of note, although a hyper-dopaminergic state could be observed in the striatum in *Per2*-mutant mice, these mice still displayed the same diurnal variations in dopamine levels as in wild-type, with a typical increase toward the dark phase (Hampp et al., 2008). Most interestingly, the serotonergic system is known to play a major role in driving the reaction of glucocorticoids to stress (Wyrwoll & Holmes, 2012). It is also closely related to circadian rhythms, by modulating photic input to the mammalian clock, the suprachiasmatic nucleus (SCN), in addition to playing a role in non-photoc input (Ciarleglio, Resuehr, & McMahon, 2011). Serotonergic adaptations have also shown to gate the transition from use to compulsive drug use and overlap with genetic risk factors for addiction (Müller & Homberg, 2014). Several studies indicate yet another potential mechanism of interaction of stress $\times$ clock genes on AUD and SUDs. This mechanism involves β endorphin. Prenatal alcohol exposure alters the expression of *Per2* expression in β -endorphin neurons of the hypothalamus (Chen et al., 2006), and *Per2* mutant mice fail to show ethanol-induced β -endorphin neuron activation (Agapito et al., 2010). Furthermore, mice exposed to alcohol prenatally show altered *Per2* driven β -endorphin release during stress exposure (Sarkar, Kuhn, Marano, Chen, & Boyadjieva, 2007). This indicates a likely role for clock genes in mediating β -endorphin regulation of stress-induced alcohol intake and will deserve further investigation (Sarkar, 2012).

Conclusion and perspective

Altogether, the findings presented above reveal a clear potential for clock genes in conveying the information of stress to different system levels and thereby influencing alcohol and drug use or triggering craving and relapse behavior. As represented in Fig. 1, stressors of different nature may hence influence this clock genes $\times$ environment interaction in the development of addictive behavior. Physical stress and psychosocial stress are well studied in this respect. However, more insidious stressors such as repeated light-shifts (night work) or chronic jet lags inducing internal desynchrony, physiological and metabolic dysregulations, or immune dysfunctions may be considered as serious stress factors as well. Increasing research evidence also shows the high relevance of individual chronotype (Roenneberg et al., 2007), as well as the importance of maintaining the integrity of stable rhythms of essential functions (i.e., feeding and sleep) to prevent disorders or diseases from developing, encompassing cancer, mental health disorders, and immune disorders. Being at the interface of numerous endocrine, physiological, and behavioral functions, clock genes,

as well as the different small molecules involved in their regulation, represent promising potential molecular targets for pharmacological intervention. With respect to alcohol abuse, this has recently been demonstrated by two different interventions: i) pharmacological inhibition of casein-kinase-1- ϵ/δ – one of the components of the circadian molecular clockwork critically involved in the stabilization of the Per complex – prevents relapse to alcohol drinking (Perreau-Lenz et al., 2012) and ii) targeting the melatonergic system in alcohol-dependent rats, with either melatonin or agomelatine, also reduces relapse behavior (Vengeliene, Noori, & Spanagel, 2015). These preclinical data support the idea that altered rhythmicity or sleep architecture in alcohol-dependent individuals can be reset pharmacologically and leads to a normalization of drinking behavior. To that end, special care should be given to maintain or reset patients' biological rhythms. Beside pharmacological treatments that target the clock gene machinery, behavioral therapy such as sleep deprivation and light therapy may well complement classical treatments already used in substance abusers and addicted patients in rehabilitation.

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Fig. 1. Influence of clock genes in the development of stress-induced alcohol use and abuse
 Clock genes are expressed in all mesocorticolimbic brain areas as well as in the HPA stress axis. Their expression displays daily rhythmic patterns that vary with the region and is thought to modulate the daily rhythm of neurotransmitter release or cell activity and metabolism. Alteration of clock genes has shown to potentiate alcohol use and abuse and to influence the development of other substance-use disorders. Taking into account their close relationship with the HPA axis, the glucocorticoid system, and the development of various SUD, they thus represent a potential key integrative gene $\times$ environment factor between stress and SUD.

Table 1

Effects of substance of abuse on clock gene expression

Drugs	Meso-corticolimbic Area	SCN
<i>Psychostimulants</i>	<ul style="list-style-type: none"> Cocaine shifts clock-gene rodents Per1 mRNA (Akhisaroglu, Ahmed, Kurtuncu, Manev, & Uz, 2004; Manev & Uz, 2009; Uz et al., 2005; Uz, Akhisaroglu, Ahmed, & Manev, 2003) Cocaine affects mPER1, mPER2, mPER3 protein expression and NPAS2 mRNA profile (Falcon, Ozburn, Mukherjee, Roybal, & McClung, 2013) Amphetamine shifts and reverses mPer1 and mPer2 mRNA patterns (Wongchitrat, Mukda, Phansuwan-Pujito, & Govitrapong, 2013) Amphetamine affects rPER1 protein diurnal pattern (Webb et al., 2009) Methamphetamine shifts mPer1 and mPer2 mRNA diurnal patterns (Iijima, Nikaido, Akiyama, Moriya, & Shibata, 2002) 	<ul style="list-style-type: none"> No effect in mice (Honma & Honma, 2009; Iijima et al., 2002; Masubuchi et al., 2000)
<i>Opioids</i>	<ul style="list-style-type: none"> Morphine withdrawal shifts mPer1 and mPer2 mRNA patterns (Li, Liu, Jiang, & Lu, 2009) Disruption of hPer1 and hPer2 in abstinent heroin addicts (Li, Shi, et al., 2009) 	<ul style="list-style-type: none"> No effect in mice (Li, Liu, et al., 2009)
<i>Alcohol</i>	<ul style="list-style-type: none"> Chronic alcohol alters clock genes and clock-controlled genes in liver (Filiano et al., 2013) 	<ul style="list-style-type: none"> No effect in mice (Filiano et al., 2013) Fetal alcohol exposure alters rPer1, rPer2 and rPer3 mRNA in the arcuate nucleus and SCN (Chen, Kuhn, Advis, & Sarkar, 2006)