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Alcohol Consumption and the Body's Biological Clock

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This review summarizes new findings on the bidirectional interactions between alcohol and the clock genes, underlying the generation of circadian rhythmicity. At the behavioral level, both adult and perinatal ethanol treatments alter the free-running period and light response of the circadian clock in rodents; genetic ethanol preference in alcohol-preferring rat lines is also associated with alterations in circadian pacemaker function. At the neuronal level, it has been shown that ethanol consumption alters the circadian expression patterns of *period (per)* genes in various brain regions, including the suprachiasmatic nucleus. Notably, circadian functions of β-endorphin–containing neurons that participate in the control of alcohol reinforcement become disturbed after chronic alcohol intake. In turn, *per2* gene activity regulates alcohol intake through its effects on the glutamatergic system through glutamate reuptake mechanisms and thereby may affect a variety of physiological processes that are governed by our internal clock. In summary, a new pathologic chain has been identified that contributes to the negative health consequences of chronic alcohol intake. Thus, chronic alcohol intake alters the expression of *per* genes, and, as a consequence, a variety of neurochemical and neuroendocrine functions become disturbed. Further steps in this pathologic chain are alterations in physiological and immune functions that are under circadian control, and, as a final consequence, addictive behavior might be triggered or sustained by this cascade.

Key Words: Alcohol, Opioid Peptide Rhythm, NK Cell Rhythm, Circadian Activity Rhythm, Period Genes, Genetic Variation of *Per 2* Gene.

INTRODUCTION

CIRCADIAN CLOCKS REPRESENT an adaptation to daily alterations in the environment, and they enable cells (and organisms) to anticipate and prepare for these changes. The synchronization of an organism with both its external and internal environments is critical for its health and well-being. Recently, it has been shown that disruption of normal circadian rhythmicity is associated with various pathologies, including cancer, sleep disorders and depression. Alcohol consumption and abuse interferes with transmission processes in the central nervous system, affects the activity of a number of biological systems, and leads to serious health problems. During drinking and withdrawal, an alcoholic often has problems falling asleep and a decrease in total sleep time as well as disruptions in other daily biological rhythms. Many alcoholics also have depression. To identify the relationship between alcohol-induced alteration of the body's biological clocks and alcohol drinking-related pathologies, this review evaluates the effects of prenatal and adult alcohol exposure on the core molecular components of the circadian clock mechanism. It also examines the alcohol-induced changes in endogenous rhythmic output signals from the suprachiasmatic nucleus (SCN) and the regulation of neuroendocrine and immune functions, circadian behavior, and alcohol drinking.

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CIRCADIAN RHYTHMS AND CELLULAR CLOCKS

Circadian rhythms (from the Latin *circa dies*, meaning approximately one day) describe biological phenomena that oscillate within a 24-hour cycle. These rhythms provide a temporal framework necessary for adequate homeostasis. By anticipating both environmental and internal changes, cells (and organisms) can efficiently program their physiological tasks and optimize survival. At the cellular level, circadian rhythms are originated by genetic elements (clock genes) organized in autoregulatory transcription-translation feedback loops that form the cellular core oscillator. This oscillating machinery controls expression of the so-called clock-controlled genes and ultimately generates circadian rhythms in physiology and behavior. The current mammalian clockwork model involves three *Per* genes (*Per1*, *Per2*, and *Per3*), two *cryptochrome* genes (*Cry1*

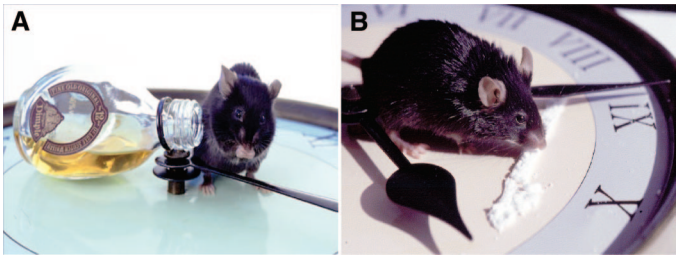

Fig. 1. The reinforcing properties of alcohol and cocaine are altered in mice lacking functional *per* genes. In particular, *mPer2* mutant mice have a 3-fold higher alcohol intake compared to wild-type control mice (**A**). Cocaine-induced reward is also altered in *mPer1* mutant mice. These mice show a complete loss of cocaine-induced conditioned place preference (**B**).

and *Cry2*), the *clock* gene (*Clock*), the *Rev-erb* gene, and the gene encoding brain-muscle Arnt-like protein 1 (*Bmal1*). Heterodimeric complexes encoded by *Clock* and *Bmal1* genes drive the expression of *Per* and *Cry* during the light phase, leading to the accumulation of *period* protein (PER)/*cryptochrome* protein (CRY) complexes that enter the nucleus and suppress transcription of *Clock* and *Bmal1*, thereby completing a negative feedback loop. A positive feedback is established by the rhythmic expression of *Rev-erb*, which represses *Bmal1* transcription, but is itself inhibited by PER. For a thorough review of the molecular oscillating mechanisms, see Reppert and Weaver (2002). Circadian oscillations in mammalian peripheral tissues are conducted by autonomous clock mechanisms and synchronized by a central pacemaker located in the SCN of the hypothalamus (Albrecht and Oster, 2001; Holzberg and Albrecht, 2003). This hypothalamic master clock is entrained by external cues called zeitgebers. The main external zeitgeber is the photic input, coming from the eyes to the SCN through the retinohypothalamic tract carrying information from the light/dark cycle. However, SCN entrainment can be complemented by internal signals such as melatonin (Stehle et al., 2003). In higher organisms, external cues are unable to reach the peripheral cellular oscillators, therefore the SCN has to entrain the peripheral clocks through neural and endocrine pathways (Buijs et al., 2003). Peripheral oscillators are believed to orchestrate tissue-specific circadian physiology and metabolic programs (Hastings et al., 2003).

Circadian clocks represent an adaptation to daily alterations in the environment, and they enable cells (and organisms) to anticipate and prepare for these changes. The synchronization of an organism with both its external and internal environments is critical to its health and well-being.

In humans, chronic alcoholism is associated with dramatic disruptions in sleep and other circadian biological rhythms, including body temperature, blood pressure, metabolism, hormone secretion, and EEG topography (Brower, 2001; Devaney et al., 2003; Fonzi et al., 1994; Imatoh et al., 1986; Kawano et al., 2002; Kodama et al., 1988; Liu et al. 2000, Mukai et al., 1998; Numminen et al., 2000; Sano et al., 1993; Schmitz et al., 1996). These widespread chronobiological disruptions are similar to those associated with mood disorders (Rosenwasser and Wirz-Justice, 1997), and, indeed, mood dysregulation is a hallmark of alcohol withdrawal (Driessen et al., 2001). Disruptions in sleep and circadian rhythms can persist through extended periods of abstinence, and persisting chronobiological disruptions may in turn promote relapse to drinking (Brower, 2003; Drummond et al., 1998; Gillin et al., 1994; Kuhlwein et al., 2003; Landolt and Gillin, 2001). These effects are probably a direct consequence of chronic excessive ethanol intake, since similar alterations in sleep and circadian rhythms (Ehlers and Slawecki, 2000; El-Mas and Abdel-Rahman, 2000; Kakihana and Moore, 1976; Rajakrishnan et al., 1999; Rouhani et al., 1990) and in mood regulation (Kliethermes et al., 2002; Overstreet et al., 2002) occur in experimental animals during chronic ethanol treatment and/or ethanol withdrawal.

In this review, new findings are summarized demonstrating a strong influence of alcohol intake on the central clock and its clock genes as well as on behavioral, endocrinological, and immunologic processes underlying circadian rhythmicity. Furthermore, the surprising finding that the activity of clock genes modulates acute and chronic alcohol effects further shows that the interplay of alcohol and our internal clock and vice versa provide fundamental mechanisms in understanding the pathologic consequences of alcohol drinking and abuse.

EFFECTS OF ALCOHOL INTAKE ON CIRCADIAN ACTIVITY RHYTHMS

Rosenwasser and his colleagues have recently analyzed the effects of both chronic ethanol intake and genetic ethanol preference on circadian behavioral activity rhythms in rats. In one experiment (Rosenwasser, 2004; Rosenwasser et al., 2005a), a free-running circadian activity (wheel-running) rhythms were monitored for several weeks before, during and after forced exposure to either 10% or 20% (v/v) ethanol solution as the only drinking fluid. Alterations in free-running period in response to both ethanol concentrations, and across individuals, both lengthening and shortening of free-running period were observed. These effects are similar to the bidirectional effects on free-running period seen with other mood-altering drugs, including antidepressants and benzodiazepines (Rosenwasser and Wirz-Justice, 1997; Subramanian and Subbaraj, 1996; Wollnik, 1992). When treatment was terminated, those animals previously consuming 10% ethanol generally showed a return toward the baseline, pretreatment period activity; but, in contrast, several animals that had been consuming 20% ethanol instead showed an exacerbation of the original ethanol effect on treatment termination. These results indicate that both chronic ethanol intake and ethanol withdrawal may be associated with changes in a fundamental parameter of the circadian pacemaker, its free-running period, and are consistent with previous

preliminary results in both rats (Dwyer and Rosenwasser, 1998) and hamsters (Mistlberger and Nadeau, 1992). It should also be noted that alterations in free-running circadian period have also recently been described in rats after perinatal ethanol treatment (Marchette et al., 2003; Sei et al., 2003).

In addition to their effects on free-running period, chronic treatment with antidepressants and anxiolytics can also modify the response of the circadian pacemaker to light signals (Rosenwasser and Wirz-Justice, 1997; Subramanian and Subbaraj, 1996). Rosenwasser and his group (Rosenwasser, 2004; Rosenwasser et al., 2005b) recently found altered light response in ethanol-treated adult rats. Male rats were housed in running-wheel cages under standard 12:12 light-dark cycles and maintained on 20% (v/v) ethanol solution as the only drinking fluid, whereas parallel control rats were maintained on plain water. On repeated occasions, the light-dark cycle was replaced temporarily with constant darkness for several days. During the first complete cycle of constant darkness, a brief (15-minute) light pulse was presented, either during the early or the late subjective night, or no pulse was presented. Analysis of free-running period expressed during the epochs of constant darkness revealed that relative to the other conditions, light pulses delivered during the late subjective night resulted in consistent light-induced period shortening but only in control, not in ethanol-treated animals. Similar alterations in the photic sensitivity of the rat circadian pacemaker have recently been described after perinatal ethanol exposure (Farnell et al., 2004; Sei et al., 2003).

In addition to the role of chronobiological disruption in promoting relapse in withdrawn alcoholics, recent evidence suggests that individual differences in sleep quality and possibly circadian organization may also predict initial susceptibility to alcohol abuse and alcoholism (Brower, 2001; Crum et al., 2004; Wong et al., 2004). Thus, long-term chronobiological disruption may serve as a chronic stressor (Boulos and Rosenwasser, 2004), predisposing affected individuals to excessive drinking. Alternately, these observations may reflect genetic linkages between the mechanisms regulating the expression of the circadian and ethanol-preference phenotypes.

To better understand these relationships, Rosenwasser and colleagues examined free-running circadian activity rhythms in ethanol-naive rats of the selectively bred ethanol-preferring (P, HAD2) and nonpreferring (NP, LAD2) lines (Murphy et al. 2002). When maintained in constant light, both ethanol-preferring lines (P, HAD) displayed shorter free-running periods than did the corresponding low-preference lines (NP, LAD) (Rosenwasser et al., 2004; Rosenwasser et al., 2005). In contrast, when housed in constant darkness, only HAD rats but not P rats displayed shorter free-running periods than did the relevant comparison line. In addition, most HAD rats showed "splitting" of the circadian activity pattern into two distinct daily bouts when maintained in long-term constant light, but this was not seen in any of the other three lines. Thus, although some differences (short period in constant light) were seen in both ethanol-preferring lines when compared with their corresponding nonpreferring lines, other differences (short period in constant darkness, splitting in constant light) were seen only between the HAD and LAD lines. Similarly, shortened free-running period under constant darkness was recently reported in selectively bred ethanol-preferring mice, compared with low-preference mice (Hofstetter et al., 2003). Taken together, the results of these experiments indicate that selective breeding for ethanol preference is associated with alterations in circadian pacemaker function in both rats and mice and suggest strong genetic linkages between circadian organization and ethanol preference.

To summarize, both chronic ethanol treatment and genetic ethanol preference are associated with alterations in the free-running period and light response of the circadian pacemaker in rats. These results suggest that the chronobiological disruptions seen in human alcoholics are partially due to ethanol-induced disruption of fundamental biological timing processes and partially due to genetic associations between the circadian and ethanol-preference phenotypes. The fact that chronic ethanol treatment and genetic ethanol preference has a strong impact on circadian activity rhythms further implies that physiological effects that are under the control of our internal clock are also affected by alcohol intake.

ALCOHOL EFFECTS ON CENTRAL CLOCKS GOVERNING NEUROENDOCRINE FUNCTIONS

Sarkar and his colleague have recently investigated alcohol effects on the circadian functions of -endorphin–containing neurons that participate in the control of the reward and reinforcement of alcohol drinking (Chen et al., 2004). These studies have shown that administration of ethanol, via a liquid diet paradigm for a period of two weeks, abolishes the circadian rhythm of proopiomelanocortin mRNA expression of β-endorphin neurons in the arcuate nucleus of the hypothalamus. The circadian expression of the clock-governing rat *Period* genes (*rPeriod1* mRNA and *rPeriod2* mRNA) in the arcuate nucleus was significantly altered, suggesting that ethanol administration disrupted the internal clock. Moreover, ethanol consumption altered the circadian rhythms of *rPeriod2* and *rPeriod3* mRNA levels in the SCN, suggesting that ethanol also affected the function of the central pacemaker. These data identified the vulnerability of the body's clock machinery and its opioidergic system to chronic alcohol drinking. The detrimental effect of ethanol on pro-opiomelanocortin (POMC) rhythm observed in this study is consistent with the previous observation that biological and behavioral sensitivity to ethanol varies throughout the light and dark cycles (Brick et al., 1984; Ryabinin et al., 2003). Ethanol's disruption of the circadian rhythm of the hypothalamic POMC system

may have significant physiological consequences. The POMC system is functionally integrated with both the hypothalamic-pituitary-adrenal axis and the mesolimbic dopamine system, which have central roles in mediating the behavioral, neuroendocrine, and pathologic responses to ethanol (Amalric et al., 1987; Froehlich et al., 2001; Gianoulakis, 2001; Herz, 1997; Kiefer and Wiedemann, 2004). The peptide products of the POMC system have been implicated in analgesia, reproduction, thermo-regulation, cardiovascular, respiratory, and neuroendocrine regulation as well as in consummatory, locomotor, and aggressive behaviors (Morley, 1983). Furthermore, it has been shown that -endorphin is involved in psychiatric disorders such as schizophrenia and depression (Baker et al., 1996; Schreiber et al., 2002; Watson et al., 1985). Clinical studies have shown a disruption in the circadian rhythm of the internal process in patients with depression (Iverson et al., 2002; Nestler et al., 2002); therefore, alcohol disruption of POMC rhythm may have pathologic consequences, including depression.

In summary, the neuroendocrine studies demonstrated that in control rats, POMC mRNA levels in the arcuate nucleus and *Per* gene mRNA levels in the arcuate nucleus and SCN displayed robust circadian rhythms. Ethanol administration significantly altered the circadian patterns of POMC in the arcuate nucleus and *rPer1* and *rPer2* in the arcuate nucleus and the SCN. These results suggest that chronic alcohol administration significantly alters central and internal clocks governing neuroendocrine functions.

ALCOHOL EFFECTS ON CLOCKS GOVERNING IMMUNE FUNCTIONS

Recent studies show a connection between altered circadian rhythms and cancer (Bovbjerg, 2003, Fu and Lee, 2003). The immunosuppressive effect of chronic ethanol is well documented. There is a strong association between ethanol consumption and both increased cancer risk and infection morbidity (Garro et al., 1992; Imhof and Koenig, 2003; Watson et al., 1994). Sarkar's laboratory has recently reported that mRNA and protein levels of the cytolytic factors Granzyme B and perforin, as well as the cytokine interferon (IFN)-γ, follow a physiological circadian rhythm that concurrently drives the circadian rhythm in natural killer (NK) cell cytolytic activity (Arjona et al., 2004). Furthermore, chronic ethanol consumption is able to suppress NK cell activity by directly disrupting the circadian rhythm of Granzyme B, perforin and IFN-γ. NK cells with an altered rhythm in Granzyme B and perforin are not able to anticipate the accumulation of critical cytotoxic components. Thus, the killing capacity of these NK cells is seriously compromised. These findings demonstrate that even though splenic NK cells from alcoholic rats are able to maintain a circadian pattern, their cytolytic activity is significantly diminished. Hence, disrupted rhythms of Granzyme B, perforin, and IFN-γ in alcoholic individuals may underlie the higher incidence of infections and cancer within this population. Likewise, disrupted rhythms in the synthesis and accumulation of cytolytic factors and IFN-γ may be the cause of the higher incidence of cancer that has been discovered lately in people, such as night-shift workers and flight crews, with altered daily rhythms (Bovbjerg, 2003). It has been shown in mice, a novel experimental model of chronic jet lag disrupts circadian gene expression and accelerates tumor growth (Filipski et al., 2004). Similarly, a disruption of the molecular clock machinery by mutation of *Per2* increases the susceptibly of mice to the development of tumors (Fu et al., 2002). In conclusion, alcohol intake and abuse significantly alters the activity of our central clock and thereby also influences immune functions.

INTERPLAY OF CLOCK GENES AND ALCOHOL

A link between the activity of clock genes and drugs of abuse was recently established by Jay Hirsh and his coworkers (Andretic et al., 1999). They developed a new model of repeated cocaine administration in *Drosophila* flies. Repeated intermittent administration of cocaine usually leads to behavioral sensitization—a phenomenon that has been implicated in drug craving. Flies mutant in the *Per* gene did not behaviorally sensitize after repeated exposure to volatilized free-base cocaine (Andretic et al., 1999, Hirsh, 2001). This finding was further substantiated in mutant mice. Thus, after repeated cocaine injections, a sensitized behavioral response was absent in *mPer1* mutant mice. In contrast, *mPer2* mutant mice exhibited a hypersensitized response to cocaine (Abarca et al., 2002). Conditioned place preference experiments revealed similar behavioral reactions: *mPer1* knockout mice showed a complete lack of cocaine reward, whereas *mPer2* mutants showed a strong cocaine-induced place preference (Abarca et al., 2002). In summary, these data suggest an opposing role of *mPer1* and *mPer2* gene activity on cocaine-induced behavioral sensitization and reward processes. Because these processes are involved in the development of cocaine addiction, it would be important also to know how *mPer1* and *mPer2* gene activity affects responses to acute and chronic alcohol intake.

Recently, Spanagel and his collaborators have shown that *mPer2* mutant mice exhibit significantly enhanced alcohol intake and preference when pharmacologically relevant concentrations of 8% to 16% ethanol are offered in a two-bottle, free-choice test (Spanagel et al., 2005). It was further demonstrated that neither the caloric value, taste differences, or variations in alcohol elimination can account for the enhanced alcohol intake in *mPer2* mutant mice (Spanagel et al., 2005). Rather, alterations in the brain reinforcement systems of the mutant mice might drive an enhanced incentive motivation to consume more alcohol than control animals. Indeed, *mPer2* mutant mice exhibit a hyperglutamatergic state in their brain reinforce-

ment systems due to a down-regulation of the glutamate transporter GLAST. It is suggested that a hyperglutamatergic state contributes to enhanced alcohol consumption (Tsai and Coyle, 1998). Direct evidence for this hypothesis has been achieved by acamprosate treatment. Acamprosate is used in the clinic for relapse prevention, and it is suggested that acamprosate acts mainly on a hyperglutamatergic state yet has only little effect on a "normal" glutamatergic state (Dahchour and De Witte, 2003; Spanagel and Zieglgänsberger, 1997). Therefore, acamprosate should be more effective in reducing alcohol consumption in *mPer2* mutant mice than in wild-type mice. Indeed, after repeated acamprosate treatment, mutant mice showed decreased alcohol consumption along with a normalization of extracellular glutamate levels. After discontinuation of acamprosate treatment, glutamate levels went up again, and, in parallel, alcohol consumption increased again significantly compared with wild-type animals (Spanagel et al., 2005).

In preliminary studies, *mPer1* mutant mice were also examined with respect to alcohol reinforcement. In these experiments, *mPer1* mutant mice did not differ in voluntary alcohol home cage consumption from wild-type littermate control animals, nor did they differ in an operant ethanol self-administration paradigm (unpublished observation). This is surprising, as cocaine reinforcement is strongly influenced by *mPer1* gene activity. Therefore, it has to be concluded that the transcription factors mPer1 and mPer2 have the ability to control distinct sets of target genes, further supporting the notion that alcohol and cocaine reinforcement processes are regulated by different neurochemical pathways.

In summary, these are intriguing new findings in the alcohol field, providing for the first time a potential molecular basis for the phenomenon of enhanced alcohol consumption in shift workers (Trinkoff and Storr, 1998) and people suffering from jet lag, as observed in the case of aircraft personnel (Rogers and Reilly, 2002). Thus, rotating shift work and the traveling over time zones alter the pattern of *Per* gene expression profiles and subsequently influence their alcohol drinking behavior. Whether this will have an impact on enhanced rates of alcohol dependence and addiction is not yet known. Therefore, future animal research ought to address the question of whether *per* genes are also directly implicated in alcohol sensitivity, tolerance, withdrawal and, most importantly, relapse behavior.

GENETIC VARIATIONS OF HUMAN CLOCK GENES ARE ASSOCIATED WITH ALCOHOL INTAKE

As described above, recent animal work has shown the effect of alcohol on circadian rhythmicity. Results in humans also point toward an interaction of alcohol with different human physiological systems that are subject to circadian rhythmicity. For example, recent electrophysiological recordings observed time-of-day effects of ethanol consumption on EEG topography. In a study investigating the effects of ethanol on the P300 event-related potential, Liu et al. (2000) could show a decrease of the α2-amplitude in the morning but not in the evening. Conversely, the P300 amplitude was lowered in the evening but not in the morning. Although these findings provide neurophysiologic evidence for a biphasic alteration of circadian rhythmicity by alcohol, other studies also describe a biphasic effect of alcohol on body temperature, blood pressure, and the hemostatic system (Devaney et al., 2003; Kawano et al., 2002; Numminen et al., 2000). These findings point toward an interaction of alcohol with different human physiological systems that are subject to circadian rhythmicity. As the findings in animals indicate, it is reasonable to hypothesize that effects of alcohol in humans may be regulated by clock genes. In particular, the animal work suggests *Per2* as a candidate gene in this respect, and the importance of the *Per2* gene in regulating circadian rhythms in humans is evidenced by a finding of Toh et al. (2001), who showed that a functional polymorphism in the casein kinase Ie binding region of the human *Per2* gene results in familial advanced sleep phase syndrome.

Schumann and his collaborators carried out an exploratory association analysis of the human *Period2* gene (*hPer2*) and phenotypes relevant to alcohol dependence (Spanagel et al., 2005). They performed a search for single nucleotide polymorphisms (SNPs), based on sequencing the exons, exon-intron boundaries, and regulatory domains of the *hPer2* gene, and selected six informative SNPs with a minor allele frequency >0.05 for genotyping. Although they did not observe an association of the *hPer2* gene with alcohol dependence (Apanagel et al., 2005). they found a significant association of a haplotype that comprised four tagging SNPs of the *hPer2* gene with high (300 g/day) versus low ($<$300 g/day) alcohol intake in a sample of 215 alcohol-dependent patients (Spanagel et al., 2005). To assess the individual contribution of each SNP to the phenotype observed, a stepwise regression analysis was performed, which identified one SNP as the only relevant covariable. Phylogenetic footprint analyses revealed that this SNP is embedded in a C*A*T TTT motif, which is preserved in humans, chimpanzees, and rats. They also found that the SNP associating with alcohol intake in humans is located in an enhancer-like structure in intron 3, containing transcription factor binding sites known to be expressed in the human brain. In a sequence four bases upstream and 14 bases downstream of the SNP, Schumann and his colleagues found transcription factor binding motifs for nuclear factor-B, Sp1, c-myb, E47, and IL-6 RE-BP. The SNP associated with amount of drinking altered the binding motifs for Sp1, c-myb, and nuclear factor-B, suggesting a possible regulatory function of this SNP in transcriptional activation of *Per2* (also see Prokunina et al., 2002).

To confirm the above results, replication studies assessing an association of this specific *hPer2* genotype with alcohol drinking patterns are necessary. Despite the fact that *hPer2* does not seem to be associated with alcohol

dependence per se, the analysis of the association of *hPer2* with drinking behavior is clinically relevant. Amount of alcohol intake is one important risk factor for the development of alcohol dependence (Russell et al., 2004) and a crucial component for the definition of binge drinking in juveniles (Kuntsche et al., 2004). To address these issues, studies analyzing drinking behavior in general population samples of adults and appropriate adolescent samples are required.

One crucial finding of the animal studies of alcohol drinking behavior in *Per2* knockout mice was the functional interaction of *Per2* and the glutamate-aspartate transporter (GLAST), which leads to a downregulation of *GLAST* in *Per2* knockout mice and an increase in synaptic glutamate concentrations. This hyperglutamatergic state is thought to induce the increased amount of alcohol intake observed in these mice (Spanagel et al., 2005). Evidence for a genetic basis of a hyperglutamatergic state in humans is provided by the differential response to acamprosate. Compared with placebo treatment, acamprosate treatment increases abstinence rates only by 10% to 20% (Mann et al., 2004). Thus, genetic variations of the *hPer2* gene as well as the *GLAST* gene may be predictors of the response to acamprosate treatment. This hypothesis needs to be tested in pharmacogenetic studies. It could be strengthened by an investigation of the biological effects of genetic variations implicated in alcohol drinking behavior and, possibly, the response to therapy. Therefore, the hypothesis of a genotype-specific transcriptional activation of *Per2* needs to be addressed in humans on a mechanistic level. Here, interindividual differences in circadian rhythmicity and transcriptional regulation by signaling pathways, which are not part of the circadian clockwork, may obscure or erroneously create quantitative differences in the expression observed in ex vivo material. One possibility to circumvent this methodical problem is to propagate genotype specific (immortalized) cells in culture and assess their transcriptional activation under controlled in vitro conditions. Such analyses may provide causal explanations of the results of human association studies and thus may identify new treatment options for alcohol dependence.

CONCLUSION

Recent studies indicate that exposure to ethanol has long-term effects on the body's internal clocks and on circadian-modulated behavioral, neuroendocrine, and immune functions. In turn, circadian and sleep-wake disruptions have been linked to numerous disorders including depression, alcoholism, compromised immune function, and increased incidence of cancer in the adult. Moreover, altered circadian function and sleep disturbances may exacerbate disorders associated with aging and impair performances in shift workers. Therefore, alcohol-dependent adults could experience a multitude of problems associated with the disturbance of the body's internal clocks. Hence, further studies are needed to determine the effects of both adult and prenatal alcohol exposure on the molecular machinery governing the clock mechanisms in the central clock in the SCN neurons and in the internal clocks of ethanol-targeted neurons and peripheral organs. These studies should advance our understanding of the long-term effect of ethanol on the body's internal clocks and sleep-wake cycle and of the negative health consequences of alcohol abuse. Furthermore, continued analysis of the role of *per* genes in alcohol sensitivity, tolerance, withdrawal, and relapse behavior may provide significant information on how these clock genes affect alcohol-seeking behaviors.

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