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Ethanol consumption in mice: relationships with circadian period and entrainment

Jennifer L. Trujillo[a,b], David T. Do[a,b], Nicholas J. Grahame[c], Amanda J. Roberts[d], Michael R. Gorman[a,b,*]

[a]*Department of Psychology, University of California, San Diego, CA, USA*
[b]*Center for Chronobiology, University of California, San Diego, CA, USA*
[c]*Department of Psychology, Indiana University Purdue University, Indianapolis, IN, USA*
[d]*Molecular and Integrative Neurosciences Department, The Scripps Research Institute, La Jolla, CA, USA*

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Abstract

A functional connection between the circadian timing system and alcohol consumption is suggested by multiple lines of converging evidence. Ethanol consumption perturbs physiological rhythms in hormone secretion, sleep, and body temperature; and conversely, genetic and environmental perturbations of the circadian system can alter alcohol intake. A fundamental property of the circadian pacemaker, the endogenous period of its cycle under free-running conditions, was previously shown to differ between selectively bred high- (HAP) and low- (LAP) alcohol preferring replicate 1 mice. To test whether there is a causal relationship between circadian period and ethanol intake, we induced experimental, rather than genetic, variations in free-running period. Male inbred C57Bl/6J mice and replicate 2 male and female HAP2 and LAP2 mice were entrained to light:dark cycles of 26 or 22 h or remained in a standard 24 h cycle. On discontinuation of the light:dark cycle, experimental animals exhibited longer and shorter free-running periods, respectively. Despite robust effects on circadian period and clear circadian rhythms in drinking, these manipulations failed to alter the daily ethanol intake of the inbred strain or selected lines. Likewise, driving the circadian system at long and short periods produced no change in alcohol intake. In contrast with replicate 1 HAP and LAP lines, there was no difference in free-running period between ethanol naïve HAP2 and LAP2 mice. HAP2 mice, however, were significantly more active than LAP2 mice as measured by general home–cage movement and wheel running, a motivated behavior implicating a selection effect on reward systems. Despite a marked circadian regulation of drinking behavior, the free-running and entrained period of the circadian clock does not determine daily ethanol intake.

Keywords: Circadian; Entrainment; Alcohol; Tau; C57 mice; Alcohol preferring mice

Introduction

In all of the mammalian species in which it has been studied, the suprachiasmatic nuclei (SCNs) of the anterior hypothalamus function as a daily, or circadian, clock that exerts a marked influence on myriad aspects of physiology and behavior (Liu et al., 2007). At the cellular level, circadian rhythms are generated by interacting with transcriptional and translational feedback loops of a few dozen genes, including three homologs of the period (*per*) gene, so named because point mutations in Drosophila altered the circadian cycle length (i.e., period) under constant environmental conditions. Although circadian rhythmicity driven by clock gene expression can be seen in both the SCN and in tissues throughout the brain and body, only the rhythms in the SCN are self-sustaining. This master pacemaker thus sits atop a hierarchy where it orchestrates the circadian organization of multiple physiological systems below (Albrecht, 2006; Liu et al., 2007; Yamazaki et al., 2000). The circadian timing system has proven relevant to a wide array of health conditions (Maywood et al., 2006). For example, shift work that requires people to time their sleep and activity counter to the preferred phase of their circadian clock has been recently classified by the World Health Organization as a probable carcinogen (International Agency for Research on Cancer, 2008). Conversely, incorporation of circadian timing considerations can improve cancer treatment outcomes of chemotherapy by optimizing therapeutic and minimizing toxic actions of drugs (Hrushesky, 1993; Rivard et al., 1985).

The biology of alcohol consumption, likewise, displays a pronounced circadian organization (Rosenwasser, 2001;

* Corresponding author. Department of Psychology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0109. Tel.: +1-858-822-2466; fax: +1-858-534-7190.
E-mail address: mgorman@ucsd.edu (M.R. Gorman).

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Spanagel et al., 2005b; Wasielewski and Holloway, 2001). Alcohol consumption in the general human population, for instance, peaks early in the evening, whereas alcohol-dependent subjects report greatest cravings in the morning (Arfken, 1988; Danel et al., 2003). Ethanol acts on numerous physiological systems that are strongly rhythmic (e.g., sleep, body temperature, melatonin) (Danel et al., 2001; Landolt et al., 1996; Rupp et al., 2007) and produces different effects as a function of time of day (Danel et al., 2001). Furthermore, the chronotype of humans, morning ''larks'' versus evening ''owls,'' predicts alcohol intake, with greater consumption reported by evening types (Adan, 1994; Wittmann et al., 2006). Shift workers too have been reported to have increased alcohol consumption or risk for heavy drinking, although not consistently across studies (Hermansson et al., 2003; Webb et al., 1990). Among abstinent alcoholics, relapse is predicted by the degree of persistent disruption of the sleep/wake cycle (Drummond et al., 1998). Finally, a single nucleotide polymorphism in the *per2* gene reportedly associates with elevated alcohol intake among a population of human alcoholic subjects (Spanagel et al., 2005a).

Important aspects of the temporal organization of human alcohol consumption are reproduced in rodents, making them ideal subjects for experimental assessment of causal relationships between circadian function and ethanol biology. Mice and rats express pronounced daily rhythms in voluntary alcohol intake and time-dependent responses to ethanol (Baird et al., 1998; Freund, 1970; Trujillo et al., 2009). Repeated shifting of the rat circadian pacemaker can alter voluntary alcohol consumption (Clark et al., 2007). A null mutation of the *per2* clock gene likewise increases ethanol consumption in mice (Spanagel et al., 2005a). In both rats and mice, artificial selection for high versus low alcohol preference has produced line differences in circadian period measured by wheel running under constant environmental conditions (Hofstetter et al., 2003; Rosenwasser et al., 2005b). Because of its hierarchical nature, however, it is difficult to know at which level of physiological organization that the circadian system is implicated in these effects. The genetic studies raise the possibility of a direct causal relationship between fundamental mechanisms of circadian pacemaker function and an alcohol consuming phenotype. Alternatively, effects on alcohol consumption could occur downstream of the pacemaker on, for example, reward or arousal processes, that have a circadian character (McClung, 2007). Finally, altered entrainment or perturbation of the circadian system may act as a chronic nonspecific stressor (i.e., introduce a general allostatic load; Boulos and Rosenwasser, 2004) that could induce changes in drinking behavior.

Circadian biologists have a number of analytical tools with which they assess the nature of circadian influence on physiology and behavior (Daan and Aschoff, 2001; Dunlap et al., 2004). In the absence of temporal cues from the environment, circadian rhythms ''free run'' with an endogenous period, tau (τ). The light:dark cycle, however, typically synchronizes (i.e., entrains) the endogenous rhythm to match the 24 h day by resetting the clock daily to offset any discrepancy between τ and 24 h. Because light can reset the clock earlier or later depending on when it falls in the endogenous cycle, animals can entrain to a range of environmental periods both somewhat longer and shorter than 24 h using so called T cycles, where T indicates the period of the entraining environmental cycle (e.g., T26 indicates alternating 13 h of light and 13 h of dark). The phase dependence of light's actions further implies that the phase of the entrained rhythm can be varied systematically: as T lengthens, the endogenous rhythm adopts a progressively earlier alignment with the lighting cycle (i.e., animals become more like ''larks'') expressed in circadian terminology as a ''phase angle of entrainment'' (see Materials and methods section for precise definition). T cycles may also be used to influence the endogenously expressed free-running period, τ. Transfer to constant conditions from an entraining long T cycle produces a τ that is longer than observed after transfer from an entraining short T cycle. Such period aftereffects may persist for at least a month in rodents (Pittendrigh and Daan, 1976).

Using T cycles to induce long-term changes in the functional organization of the circadian system of mice, we tested two hypotheses suggested by epidemiological and correlational studies in humans and rodents: first, that there is a causal relationship between the period of the free-running circadian pacemaker and ethanol intake in C57BL/6J mice; second, that there is a causal relationship between the phase angle of entrainment and alcohol consumption in C57BL/6J mice. Finally, we assessed whether aspects of circadian rhythmicity in addition to the free-running period and phase angle of entrainment distinguished high-alcohol preferring (HAP) and low-alcohol preferring (LAP) mice (Grahame et al., 1999, 2003) not yet studied from a circadian perspective. We provide strong evidence against a direct connection between circadian period or entrainment phase and alcohol intake in mice. Instead, we confirm an association between high alcohol preference and activity levels in these genetically distinct mice.

Materials and methods

Subjects and housing

Male C57BL/6J mice (Jackson Laboratories, Sacramento, CA) and male and female HAP2 and LAP2 mice were acquired and housed in standard shoebox cages with food (Mouse diet 5015; Purina Mills) and water available ad libitum. The latter lines were selected for differences in alcohol drinking from the same progenitor population, and using the same phenotype (free-choice consumption of 10% ethanol over a 4-week period) as replicate 1 HAP and LAP mice that showed a difference in free-running period (Hofstetter et al., 2003). Subjects were group housed before circadian rhythm or ethanol intake measurement but