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Chronobiology of ethanol: Animal models

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ABSTRACT

Clinical and epidemiological observations have revealed that alcohol abuse and alcoholism are associated with widespread disruptions in sleep and other circadian biological rhythms. As with other psychiatric disorders, animal models have been very useful in efforts to better understand the cause and effect relationships underlying the largely correlative human data. This review summarizes the experimental findings indicating bidirectional interactions between alcohol (ethanol) consumption and the circadian timing system, emphasizing behavioral studies conducted in the author's laboratory. Together with convergent evidence from multiple laboratories, the work summarized here establishes that ethanol intake (or administration) alters fundamental properties of the underlying circadian pacemaker. In turn, circadian disruption induced by either environmental or genetic manipulations can alter voluntary ethanol intake. These reciprocal interactions may create a vicious cycle that contributes to the downward spiral of alcohol and drug addiction. In the future, such studies may lead to the development of chronobiologically based interventions to prevent relapse and effectively mitigate some of the societal burden associated with such disorders.

Introduction

Chronic alcoholism is associated with dramatic disruptions in sleep and other circadian biological rhythms (e.g., Brower, 2001; Conroy et al., 2012; Kuhlwein, Hauger, & Irwin, 2003). These disruptions can sometimes persist over extended periods of abstinence, and appear to increase the risk of relapse (Brower, 2003; Landolt & Gillin, 2001). Similarly, chronobiological disruption may be a risk factor for excessive alcohol intake in non-dependent populations, such as adolescents, shift-workers, and frequent transmeridian travelers (Rogers & Reilly, 2002; Trinkoff & Storr, 1998; see also Hasler, Soehner, & Clark, 2015). Together, these clinical and epidemiological observations suggest that linkages between circadian disruption and excessive drinking reflect bidirectional causal interactions (Danel & Touitou, 2004; Hasler, Smith, Cousins, & Bootzin, 2012; Rosenwasser, 2001; Spanagel, Rosenwasser, Schumann, & Sarkar, 2005). Such reciprocal interactions could lead to a vicious cycle phenomenon and possibly contribute to the downward spiral from alcohol use to abuse to addiction.

Of course, it is very difficult to infer causal relationships from clinical and epidemiological data, and even more difficult to isolate and identify controlling variables. Thus, as is the case for other psychiatric disorders, animal models have proven to be extremely useful in this regard. As described in this review, substantial data have now accumulated to indicate that alcohol (ethanol) alters fundamental properties of the circadian clock. In turn, disruptions or alterations in the circadian system induced by either environmental or genetic manipulations can alter voluntary ethanol intake in experimental animals.

This review summarizes the experimental findings supporting these conclusions, emphasizing studies performed in the author's laboratory. In the first two sections of the review, I describe the evidence that alcohol consumption and/or administration alters both the endogenous free-running period of the circadian clock as well as its phase-shifting response to environmental light signals. These studies demonstrate that alcohol acts directly on the circadian clock system to affect circadian rhythms. Next, I discuss the evidence that manipulation of the circadian clock via exposure to atypical environmental lighting regimens can alter voluntary alcohol intake. While the results of such studies are complex and include some inconsistent findings, they do demonstrate that chronobiological "stressors" may influence alcohol consumption. Finally, I review the evidence for reciprocal genetic linkages

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between the circadian rhythmicity and alcohol intake, and show that circadian clock genes influence alcohol drinking, while in turn, genes associated with ethanol responsiveness contribute to circadian regulation.

Effects of chronic ethanol treatment on free-running circadian period

My laboratory initially became interested in the chronobiology of ethanol because of a fortuitous observation made in the course of an experiment conducted to determine the effects of neonatal treatment with the serotonin-selective tricyclic antidepressant, clomipramine, on free-running circadian rhythms in adult Wistar rats (Dwyer & Rosenwasser, 1998). In this and other experiments, we frequently examine free-running period because it is thought to reflect a quantitative property of the underlying circadian pacemaker that can be evaluated at the level of physiology and behavior. Neonatal clomipramine treatment was known to produce a spectrum of affective-behavioral and neurobiological effects in adulthood, including increasing ethanol preference in 2-bottle free-choice drinking tests. As a manipulation check, we offered the animals free access to 10% v/v ethanol and plain water for several weeks under constant darkness while monitoring free-running circadian drinking rhythms via lickometers. Both male and female rats showed significant shortening of free-running period during ethanol access, and a gradual return toward baseline after termination of ethanol access.

At that time, only one previously published study had examined the effects of ethanol on circadian rhythms in a whole-animal model (Mistlberger & Nadeau, 1992). In that experiment, male hamsters were housed in running-wheel cages and maintained on either 28% ethanol or plain water as their sole drinking fluid. While no effects of ethanol treatment were seen under steady-state light–dark (LD) entrainment or during re-entrainment following LD phase-shifts, ethanol treatment significantly lengthened free-running circadian period under constant dim light, opposite in direction to the effect observed by Dwyer and Rosenwasser (1998). While these two studies differed in choice of species, housing conditions, light intensities, ethanol concentrations, and ethanol access conditions, it has continued to be the case that the reported effects of ethanol on free-running period are subtle and somewhat variable between and within experiments. As discussed elsewhere, similar variability also characterizes the reported effects of antidepressants and other mood-altering drugs on free-running period (Klemfuss & Kripke, 1994; Rosenwasser, 2001; Wollnik, 1992).

In a subsequent experiment (Rosenwasser, Fecteau, & Logan, 2005), we maintained male Long-Evans rats in running-wheel cages and provided either plain water or 10% or 20% ethanol as the sole drinking fluid. Free-running period was evaluated before, during, and following 3–5 weeks of ethanol treatment. While most animals showed discernible alterations in free-running period during ethanol treatment relative to baseline conditions, responses were idiosyncratic and included clear examples of both period shortening and period lengthening. Further, ethanol-induced period change was significantly correlated with individual differences in baseline period, such that rats with the shortest baseline periods displayed the most robust period lengthening under ethanol. Finally, when ethanol treatment was terminated, most animals in the 10% ethanol treatment group showed a return toward baseline periods, as in Dwyer and Rosenwasser (1998), but many animals in the 20% ethanol group actually showed an exacerbation of the original ethanol effect during ethanol "withdrawal."

In a later experiment, we examined the effects of both free-choice and forced intake of 10% ethanol on free-running circadian period in male C57BL/6 mice housed in running wheels

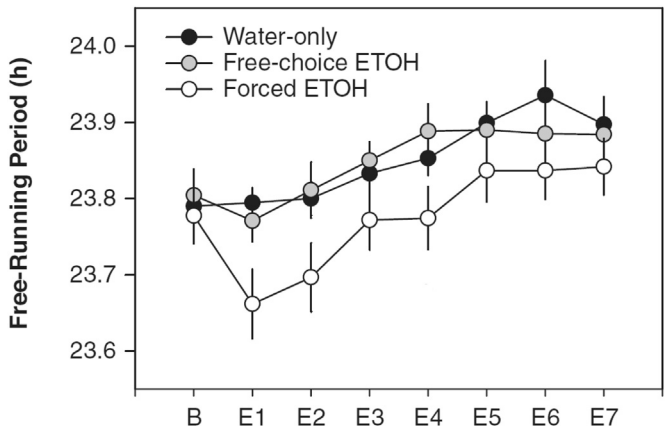

Fig. 1. Mean (±SEM) free-running circadian period in male C57BL/6 mice housed in continuous darkness. Water-only mice were maintained on plain water and served as controls; free-choice ETOH mice were allowed continuous access to plain water and 10% v/v ethanol solution via separate drinking tubes; forced ETOH mice had 10% ethanol solution as their only drinking fluid. B indicates a 3-week baseline during which all groups had only water available; E1–E7 indicate successive 3-week epochs during which ethanol was presented to the relevant groups while the controls were continued on plain water only. Modified from Seggio et al., 2009.

(Seggio, Fixaris, Reed, Logan, & Rosenwasser, 2009). This experiment employed a between-groups design, in which the forced- and free-choice ethanol groups were maintained on ethanol for 21 weeks, while a control group was maintained on plain water. Forced ethanol intake resulted in a persistent shortening of free-running period relative to controls, but no significant effect was seen in the free-choice group (Fig. 1).

Most recently, we monitored free-running circadian period under long-term ethanol access in male and female rats of the selectively bred high-drinking P ("Preferring") and HAD2 ("High Alcohol Drinking, replicate 2") lines (Rosenwasser, McCulley, & Fecteau, 2014). In this experiment, animals were maintained on either continuous free-choice access to 10% ethanol or on an intermittent ethanol-access schedule consisting of two weeks of access alternating with two weeks of ethanol deprivation. While this study revealed subtle effects of sex, genotype, and access schedule, the major effect was that animals displayed shortening of free-running period during ethanol access.

While animals consumed considerable amounts of ethanol via their drinking water in the studies just discussed, there is little evidence that intoxication or dependence occur under such conditions, even in high-drinking genotypes. In order to focus on possible effects of ethanol dependence and withdrawal on circadian period, we assessed circadian activity rhythms in male C57BL/6 and C3H/He mice following chronic-intermittent exposure to ethanol vapor using a protocol known to induce signs of dependence (Logan, McCulley, Seggio, & Rosenwasser, 2012; Logan, Seggio, Robinson, Richard, & Rosenwasser, 2010). In these experiments, we employed both 4-day and 16-day vapor exposure regimens, but significant shortening of free-running period was seen only in C3H mice, and only following the longer exposure regimen.

Taken together, these experiments indicate that chronic ethanol exposure can modify free-running circadian period. Nevertheless, these effects are typically modest, and may vary by species and strain. Further, while most of our studies revealed ethanol-induced shortening of circadian period, examples of period lengthening were also observed (Rosenwasser, Fecteau, & Logan, 2005). Finally, it appears that the most consistent effects are seen during and following ethanol treatments that expose animals to levels of