Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 87 (2007) 297–305
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Chronic ethanol intake modulates photic and non-photic circadian phase responses in the Syrian hamster

Joseph A. Seggio [a], Ryan W. Logan [b], Alan M. Rosenwasser [a,b,*]

[a] Department of Biological Sciences, University of Maine, Orono, ME 04469, USA
[b] Department of Psychology, University of Maine, Orono, ME 04469, USA

Received 1 February 2007; received in revised form 1 May 2007; accepted 2 May 2007
Available online 10 May 2007

Abstract

Chronic alcohol intake disrupts sleep and other circadian biological rhythms in both human alcoholics and in experimental animals. Recent studies from our laboratory indicate that these effects may be due, in part, to ethanol-induced alterations in fundamental properties of the circadian pacemaker. The present study explored the effects of chronic voluntary ethanol intake (25% v/v) on circadian phase responses to both photic and non-photic stimuli in Syrian hamsters. Hamsters were used in these experiments because they are a popular model organism in behavioral chronobiology research, and are characterized by unusually high levels of voluntary ethanol intake. Relative to controls, ethanol-exposed animals showed attenuation of circadian phase responses and wheel running activity following acute administration of the benzodiazepine, triazolam, a non-photic phase-shifting stimulus. In addition, ethanol-exposed animals displayed reduced phase advances, but normal phase delays, in response to brief light pulses. While the mechanisms underlying these effects remain to be elucidated, we hypothesize that ionotropic GABA and glutamate receptors may be involved, since these proteins serve as important targets for the neurobiological effects of ethanol, and are also known to be critically involved in the modulation of photic and non-photic circadian phase responses.

Keywords: Circadian; Wheel running; Ethanol; Alcohol; Benzodiazepine; Triazolam; Hamster

1. Introduction

Chronic alcohol intake results in dramatic disruptions in sleep and other circadian biological rhythms in both human alcoholics and in experimental animals. Nevertheless, since these studies have generally been conducted under naturalistic conditions (in humans) or during synchronization to laboratory light–dark cycles (in animals), the extent to which such disruptions reflect pharmacological effects on the circadian pacemaker is largely unexplored. The effects of light, drugs and other stimuli on the circadian pacemaker are most readily identified through studies of free-running circadian rhythms, as expressed in continuous darkness or in constant light. Under such conditions, the phase and period of an overt free-running rhythm are thought to reflect the phase and period of the underlying circadian pacemaker (Rosenwasser, 2001; Turek, 1987).

Previous studies in our laboratory showed that chronic ethanol intake alters the free-running period of circadian activity rhythms in both unselected rats (Dwyer and Rosenwasser, 1998; Rosenwasser et al., 2005a) and selectively bred ethanol-preferring rats (Fecteau et al., 2006), thus confirming earlier preliminary findings in the Syrian hamster (Mistlberger and Nadeau, 1992). In addition, chronic ethanol intake attenuates the period-altering aftereffects of brief light pulses presented during late subjective night (but not early subjective night) (Rosenwasser et al., 2005b). While the period and phase of the circadian pacemaker may be influenced by both spontaneous and evoked activity (Mistlberger et al., 1998; Mrosovsky, 1996b, 1999; Van Reeth and Turek, 1989), changes in daily wheel running during ethanol intake in these studies were modest and did not appear to be correlated with effects on free-running rhythms (Mistlberger and Nadeau, 1992; Rosenwasser et al., 2005a). Thus, taken together, these results indicate that the chronobiological effects of ethanol are mediated

* Corresponding author. Department of Psychology, 5742 Little Hall, University of Maine, Orono, ME 04469-5742, USA. Tel.: +1 207 581 2035; fax: +1 207 581 6128.
E-mail address: alanr@maine.edu (A.M. Rosenwasser).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.05.001

in part by alterations in the period of the circadian pacemaker and its response to perturbation.

The present experiments were designed to examine the effects of chronic ethanol intake on the response of the circadian pacemaker to both photic and non-photic phase-shifting stimuli. These experiments utilized Syrian hamsters because hamsters express very "accurate" free-running rhythms, characterized by low cycle-to-cycle variability in activity onset time under free-running conditions, and are therefore generally the species of choice in behavioral studies of circadian phase responses. In addition, Syrian hamsters are known to display very high levels of free-choice ethanol consumption, exceeding that seen in selectively bred ethanol-preferring rats or in high ethanol-preferring strains of inbred mice, providing a possible model for excessive alcohol consumption in humans (Kulkosky and Cornell, 1979; McMillan et al., 1977; Piercy and Myers, 1995).

Thus, we examined the effects of chronic voluntary ethanol intake on the circadian phase-shifting response to photic stimulation (light pulses) and to a well characterized non-photic (pharmacological) phase-shifting stimulus — the benzodiazepine, triazolam. The response to light pulses was examined because light pulses are the most widely studied circadian phase-shifting stimulus, and because of our earlier finding that chronic ethanol intake modifies the period-altering effects of light pulses in rats (Rosenwasser et al., 2005b). The response to triazolam injections was examined because triazolam and other benzodiazepines produce large amplitude circadian phase responses thought to be mediated by benzodiazepine binding to GABA-A receptors in the SCN and other neural components of the circadian system (Smith and Turek, 1989; Turek and Losee-Olson, 1986). Indeed, GABA and GABA-A receptors are densely localized within the circadian system (Gao et al., 1995; Michels et al., 1990; Moore and Speh, 1993), and given the extensive evidence for GABA-A receptor modulation by ethanol (Davies, 2003; Faingold et al., 1998), provide a likely target for the chronobiological effects of ethanol. Indeed, cross-tolerance between ethanol and benzodiazepines has been widely reported in both humans and rodents (Mihic et al., 1992), and a preliminary report indicates that chronic ethanol may reduce circadian phase responses to triazolam in the hamster (Joy and Turek, 1989).

Both brief light pulses and acute triazolam injections result in phase-dependent phase responses of the circadian pacemaker, and the phase-response curves (PRCs) characterizing these responses are well known (Rosenwasser and Dwyer, 2001; Smith et al., 1992). Thus light pulses result in phase delays during early subjective night and phase advances during late subjective night, while triazolam injections result in phase advances during mid-subjective day and phase delays during late subjective night. In the present experiments, both stimuli were tested at phases expected to yield both maximal phase advances and maximal phase delays.

2. Methods

2.1. General procedures

Twenty-three male Syrian hamsters (*Mesocricetus auratus*, LVG strain), approximately six weeks of age, were obtained from Charles River Laboratory (Wilmington, MA) and maintained individually in running wheel cages (Lafayette Instruments, wheel diameter: 35 cm) with food (Prolab 3000) and fluids (water and/or ethanol solution; see below) provided ad libitum. Running wheel activity was recorded and analyzed using the ClockLab interface system (Actimetrics), and fluid intakes were assessed at irregular intervals (approximately every 7 to 10 days). The hamsters were initially maintained in a light–dark (LD) 14:10 cycle, and following the establishment of stable, light-entrained rhythms, were divided randomly into two groups: a control group, which received water only ($N=12$), and an experimental group offered 25% (v/v) ethanol solution in free choice with water (2-bottle test) ($N=11$). After allowing a period of 21 days for ethanol intake to stabilize in the experimental group, all animals were tested for their circadian phase-shifting responses to the benzodiazepine, triazolam and to light pulses, using a version of the Aschoff Type II protocol (Mistlberger, 1996; Mrosovsky,

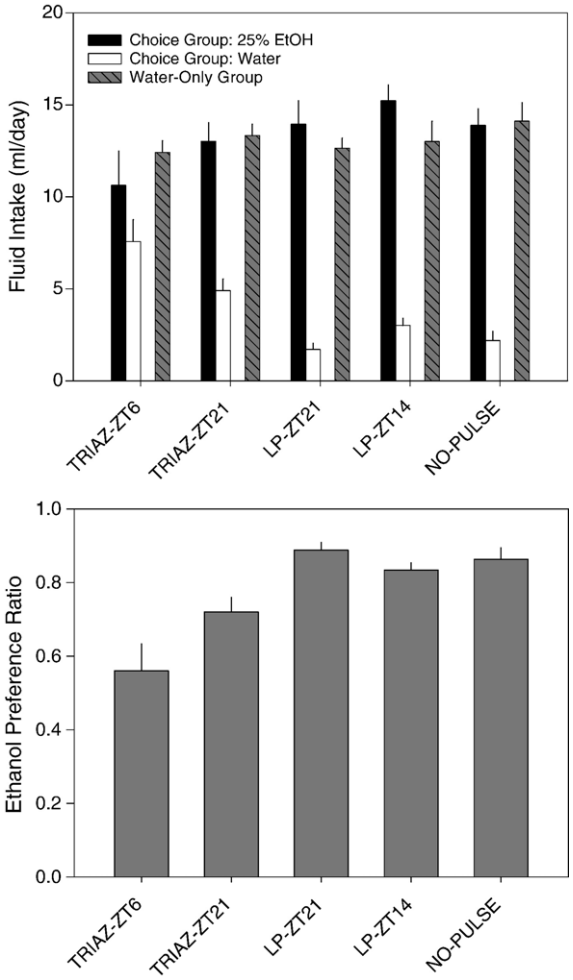

Fig. 1. Top panel: mean (+SEM) daily fluid intakes in ethanol-exposed and control groups of hamsters. Data represent the last intake assessment prior to each of the 5 successive phase-shift tests indicated along the *x*-axis. Bottom panel: mean (+SEM) ethanol preference ratios (i.e., daily intake of 25% ethanol solution divided by total daily fluid intake) in ethanol-exposed hamsters assessed immediately prior to each testing condition.

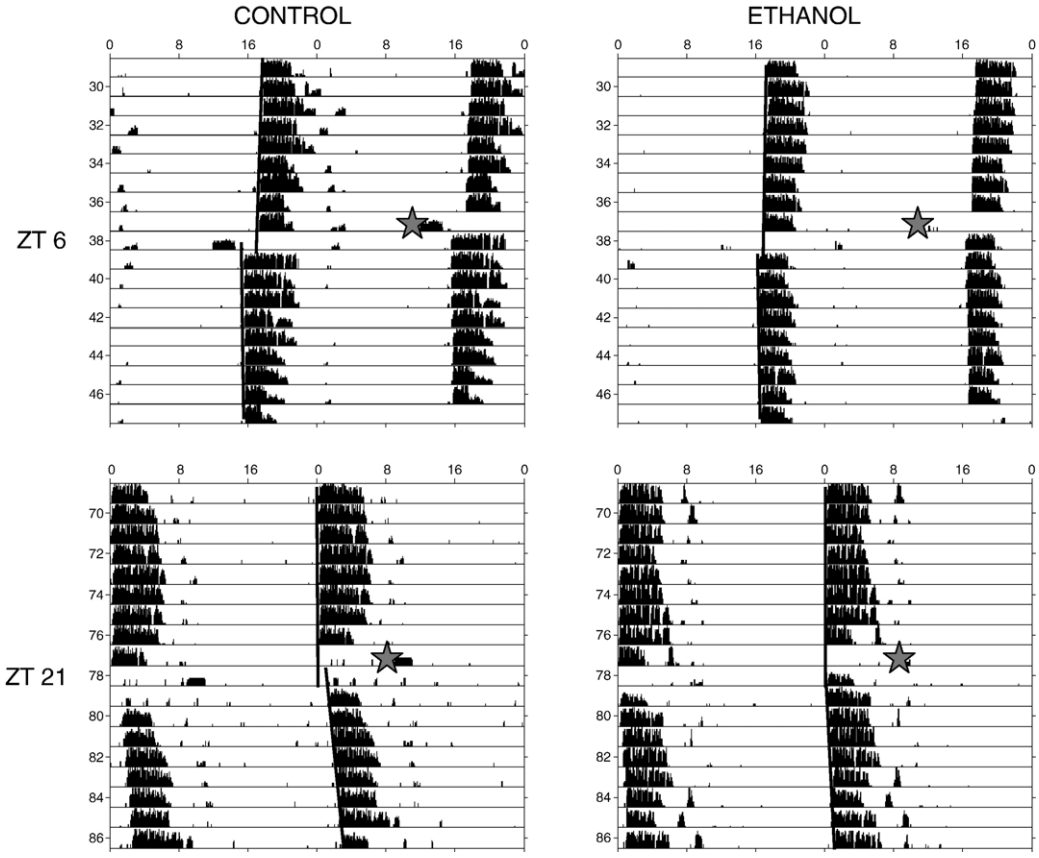

Fig. 2. Double-plotted actograms showing representative responses to triazolam injections administered at ZT 6 (top panels) or ZT 21 (bottom panels) in control (left panels) and ethanol-exposed (right panels) hamsters. Vertical lines superimposed on one side of each double-plot show the approximate times of daily activity onsets, determined separately for pre- and post-treatment data samples, and stars show approximate times of triazolam administration.

1996a). In this protocol, animals are kept under an entraining LD cycle until the presentation of the phase-shifting stimulus, and then maintained subsequently for several cycles in constant darkness (DD) for assessment of the phase of the free-running rhythm. Each stimulus was tested at two different "zeitgeber" times (ZT, with scheduled dark onset defined as ZT 12), chosen to yield maximal phase advances and phase delays (see below). Finally, a "no-pulse" control condition, in which animals were simply transferred from LD to DD beginning at the normal time of dark onset, was also tested for comparison to the effects of light pulses. Thus, each animal was tested a total of 5 times in the same sequence (triazolam-induced phase advances, triazolam-induced phase delays, light-induced phase advances, light-induced phase delays, and no-pulse control). Each phase response test was separated from the preceding test by two to three weeks of re-exposure to the LD cycle, ensuring stable entrainment prior to the delivery of all phase-shifting stimuli. Finally, blood alcohol levels were measured in trunk blood at the time of sacrifice in half the ethanol-exposed animals using an Analox Instruments AM-1 alcohol analyzer. Blood samples were collected at the approximate middle of the subjective night, at a time when near-maximal blood alcohol levels would be expected, due to the normal nocturnal patterns of ethanol intake (cf., Hiller-Sturmhofel and Kulkosky, 2001).

2.2. Triazolam-induced phase responses

Triazolam-induced circadian phase responses were examined at ZT 6 and at ZT 21, phases expected to result in maximal phase advances and phase delays, respectively (Turek and Losee-Olson, 1986). For injections at ZT 6, hamsters were removed from their cages during the light phase, 6 h before the next scheduled light-to-dark transition, gently restrained manually, and injected with triazolam (5 mg/kg, i.p. dissolved in DMSO). Immediately following injection, the animals were placed back into their cages, at which time the lights were turned off and the animal was then allowed to free-run in DD for at least seven days. For injections at ZT 21, the lights remained on for an extra 9 h beyond scheduled dark onset on the day of the injection (i.e., until the normal time of ZT 21), and were turned off immediately following the injection, whereupon the animals were maintained for several days in DD. This protocol allowed the hamsters to be injected in the light at both ZT 6 and ZT 21, and also ensured that triazolam injections were followed immediately by a light-to-dark transition at both test times.

2.3. Light-induced phase responses

Light-induced circadian phase responses were examined at ZT 21 and at ZT 14, expected to yield maximal phase

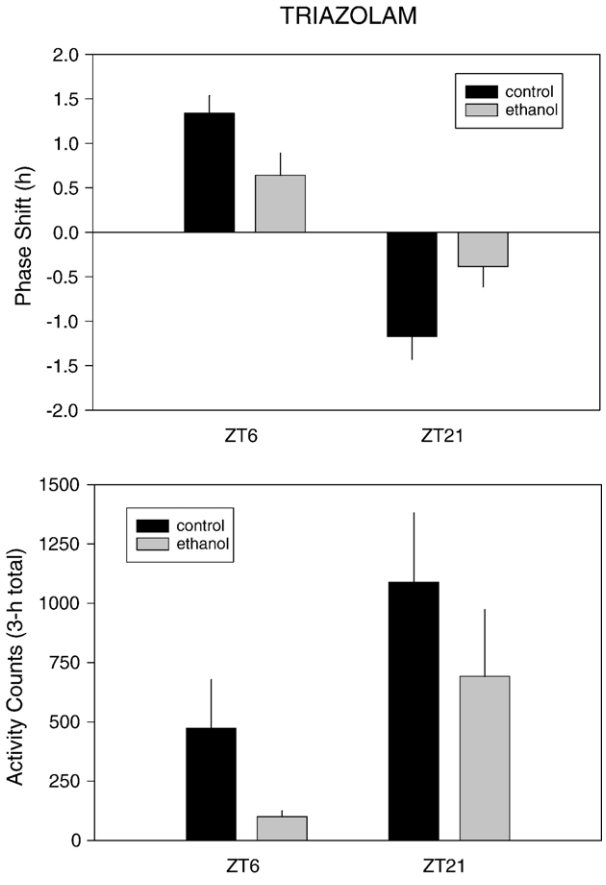

Fig. 3. Top panel: mean (+SEM) phase responses to triazolam injections at ZT 6 and ZT 21 in ethanol-treated (grey bars) and control (black bars) hamsters. Phase advances are plotted as positive numbers and phase delays as negative numbers. Bottom panel: mean (+SEM) activity (wheel-turns) recorded during the 3 h immediately following triazolam injections.

advances and phase delays, respectively. Light pulses (15 min, about 50 lx, incandescent white light) were scheduled at 9 (ZT 21) or 2 (ZT 14) h after the last light-to-dark transition, and were followed by at least seven days of DD to allow for assessment of free-running circadian rhythms. Finally, animals were tested for their phase-shifting response to simple transfer from LD to DD conditions ("no-pulse" control) by leaving the lights off for several days following the last light-to-dark transition.

2.4. Data analysis

Raster-style actograms were generated using ClockLab software for visual inspection of activity rhythms. The magnitude and direction (advance vs. delay) of circadian phase responses were determined using ClockLab's automated activity onset algorithm, as follows: Pre-stimulus phase was estimated as the mean time of activity onset over the last four days of LD entrainment, while post-stimulus phase was estimated by a regression line fit to activity onsets over the 5–7 free-running cycles immediately following the test stimulus, except that, when necessary, the first 1–2 activity onsets following the stimulus were discarded from the analysis due to the occurrence of "transients" prior to the establishment of a steady-state free-running phase. Phase responses were then determined as the difference between these two phase estimates extrapolated to the first post-stimulus cycle. In addition, since triazolam-induced phase responses are associated with induced locomotor activity (Van Reeth and Turek, 1989), we determined the total number of wheel-turns occurring in the 3-hour window following triazolam injections.

Mean phase responses for each of the five test conditions were compared between ethanol-treated and control groups using *t*-tests. Because activity-level distributions display positive skew, means of both raw and log-transformed post-triazolam activity levels were compared using *t*-tests, but since the statistical results were not affected by this transformation, raw-score results are presented here. Finally, Pearson's correlation coefficient and linear regression were used to examine the relationship between phase responses and log-transformed wheel running activity across individuals.

2.5. Ethical considerations

The experimental procedures described in this report were reviewed and approved by the University of Maine's Institutional Animal Care and Use Committee (IACUC).

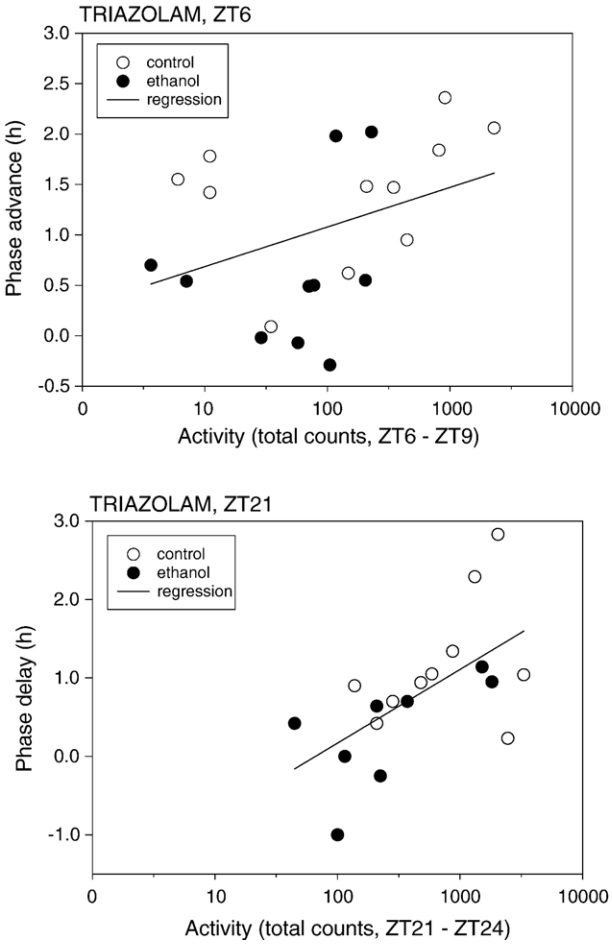

Fig. 4. Relationship between triazolam-induced phase responses and post-injection activity levels in ethanol-exposed (filled circles) and control hamsters (open circles) at ZT 6 (top panel) and ZT 21 (bottom panel). Best-fit linear regression lines are also presented.

3. Results

3.1. Fluid intake

As expected, hamsters offered 25% ethanol in free choice with water voluntarily consumed large quantities of ethanol (Fig. 1). Indeed, ethanol-exposed animals consumed 25% ethanol solution in quantities (10–15 ml/day) that were very similar to the water volumes consumed by water-only control animals. While ethanol intake was already well established by the time of the first phase-shift test (triazolam at ZT 6), further increases in ethanol intake as well as decreases in water intake occurred over the course of this long-term experiment. Thus, ethanol preference (i.e., the intake of 25% ethanol solution as a percentage of total daily fluid intake) was about 50–60% at the time of the initial phase-shift test, but increased to 80–90% during subsequent tests with light pulses. While animals were not weighed until the end of the experiment (terminal body weight: mean=148.4 g, SEM=3.80), daily ethanol intake over the course of the experiment may be estimated at about 15 g/kg/day, or about twice that typically reported for selectively bred ethanol-preferring rats. On the other hand, terminal blood alcohol levels were very variable (mean=36.1 mg/dl, SEM=16.1, range=4.7–75.6), and generally below levels considered to be intoxicating in rats and mice.

3.2. Triazolam-induced phase responses

Most animals showed triazolam-induced phase responses of between 0 and 2.0 h, depending on treatment group and ZT of testing, but four animals (two each in the ethanol-exposed and the control groups) displayed aberrant, very large (>8-hour) phase-shift responses to triazolam at ZT 21; these aberrant responses were excluded from the main analyses, and are discussed separately below. In addition, one ethanol-treated animal produced an uninterpretable activity record following triazolam injection at ZT 6, and was also excluded from the statistical analysis.

Ethanol-treated animals displayed significantly reduced phase responses to triazolam at both ZT 6 ($t_{20}=2.23$, $p=.038$) and ZT 21 ($t_{17}=2.28$, $p=.036$) (Figs. 2 and 3). Several triazolam-injected animals displayed substantial bursts of wheel running activity immediately following treatment (Fig. 2), and while ethanol-treated animals were generally less active following triazolam injections (Fig. 3), these differences were not significant. Despite the lack of significant differences in triazolam-induced activity, however, circadian phase responses and wheel running activity following triazolam were significantly positively correlated at ZT 6 ($r_{20}=.496$, $p=.022$) and showed a similar but non-significant trend at ZT 21 ($r_{17}=.436$, $p=.062$), when considering both ethanol-exposed and control animals (Fig. 4). (Similar relationships were not seen within the ethanol-exposed or control groups, presumably due to the smaller N in these samples.)

As mentioned above, four animals (two from each group) showed very large, 8- to 12-hour phase responses in response to triazolam administration at ZT 21 (Fig. 5). Similar responses to other non-photic phase-shifting stimuli have been reported recently in animals pre-exposed to constant light for one or more cycles prior to phase-shift testing (Knoch et al., 2004, 2006; Landry and Mistlberger, 2005). Thus, these responses may have been related to our decision to delay the onset of darkness from the normal ZT 12 until ZT 21 on the day of treatment in order to facilitate triazolam administration during the night phase of the LD cycle, resulting in exposure to 23 h of continuous light. In any case, these large shifts clearly represent outliers in our data set, and since similar responses were seen in both groups, are not related to chronic ethanol intake.

3.3. Light-induced phase responses

As expected, light pulses generally resulted in phase delays at ZT 14 and in phase advances at ZT 21 (Figs. 6 and 7). Further, the transition from LD to DD yielded small phase advances in the no-pulse control condition, as has been reported previously (Mrosovsky, 1996a,b) (Fig. 7). Circadian phase responses to

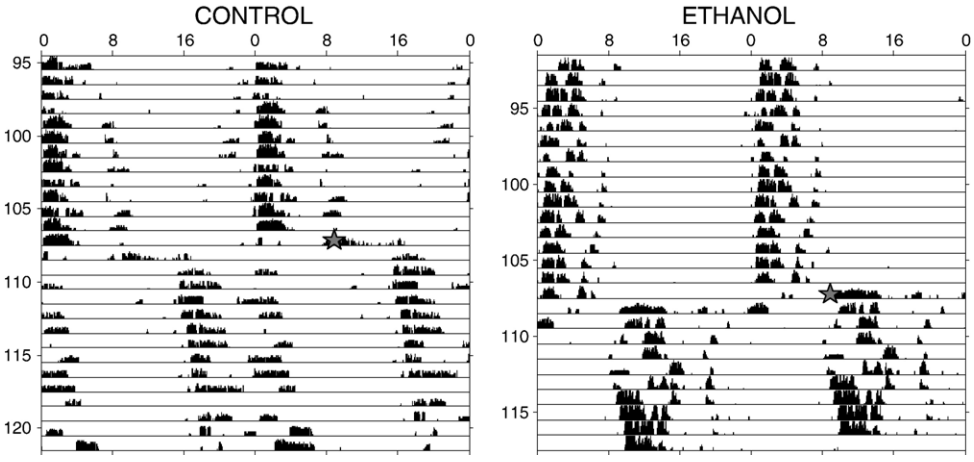

Fig. 5. Double-plotted actograms showing examples of very large apparent “type-0” circadian phase responses in one control (left panel) and one ethanol-exposed (right panel) hamster. Other conventions as in Fig. 2.

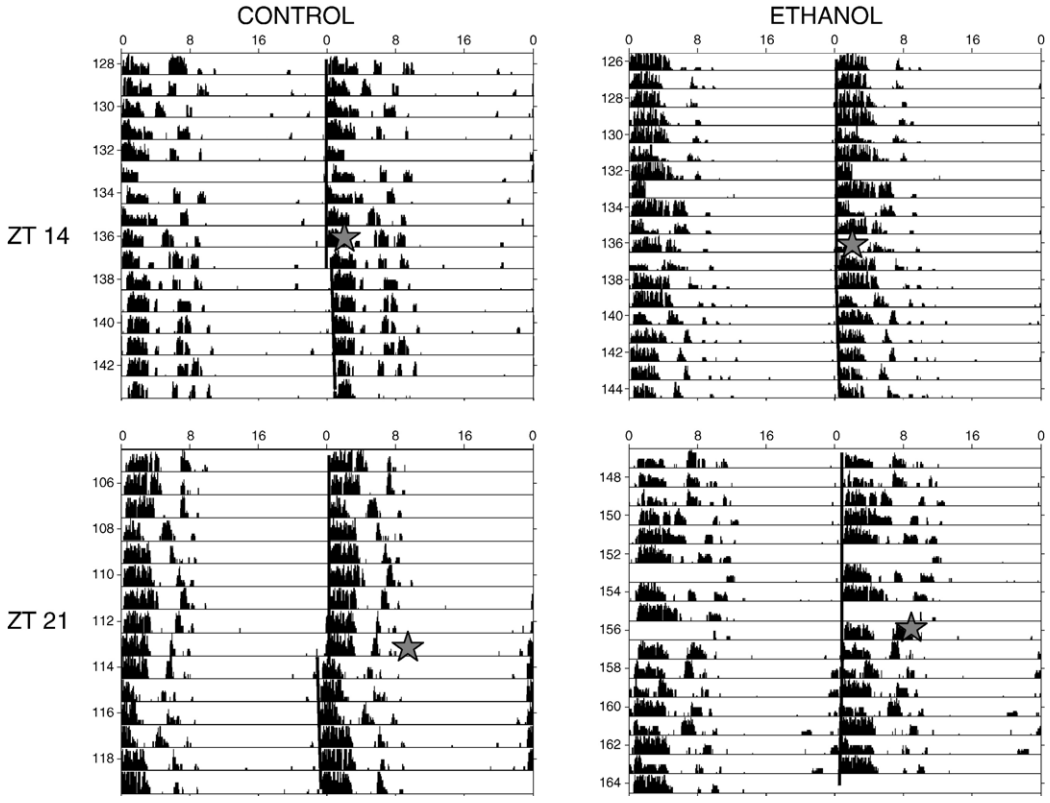

Fig. 6. Double-plotted actograms showing representative light-induced phase responses to light pulses presented at ZT 14 (top panels) or ZT 21 (bottom panels) in control (left panels) and ethanol-exposed (right panels) hamsters. Other conventions as in Fig. 2.

light pulses were significantly reduced in ethanol-treated animals at ZT 21 (t_{21}=2.46, p=.023) but not at ZT 14, nor did ethanol-treated and control animals differ in their phase responses under the no-pulse control condition. Further, since ethanol-treated animals showed essentially identical responses to the ZT 21 light pulse and to the no-pulse condition, the phase-shifting effect of light appears to have been blocked completely at this phase.

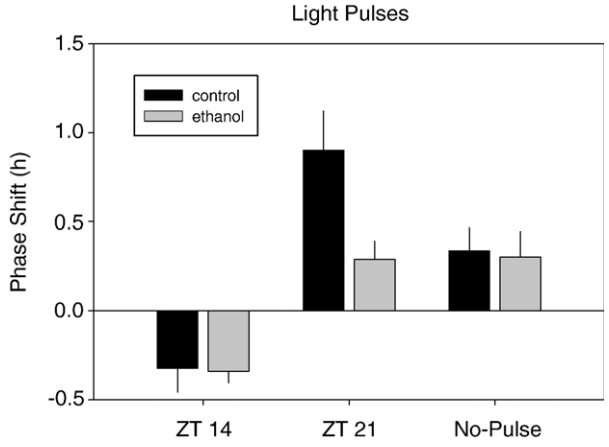

Fig. 7. Top panel: mean (+SEM) light-induced phase responses to light pulses presented at ZT 14 and ZT 21, and under "no-pulse" control conditions, in ethanol-treated (grey bars) and control (black bars) hamsters.

4. Discussion

The results of this study indicate that chronic ethanol intake modulates both photic and non-photic phase responses in the Syrian hamster. These results confirm and extend our previous work on the chronobiological effects of ethanol intake in rats (Dwyer and Rosenwasser, 1998; Fecteau et al., 2006; Rosenwasser et al., 2005a,b), and provide additional evidence that ethanol alters fundamental properties of the underlying circadian pacemaker.

At the cellular level, the effects of ethanol on the circadian pacemaker could be mediated by either non-specific effects on fundamental cellular processes or by specific receptor-mediated effects on neurotransmission. The specific effects of ethanol on central neurotransmission are thought to be largely dependent on ethanol modulation of ionotropic neurotransmitter receptors — most prominently, the GABA-A receptor and the NMDA-type glutamate receptor (Faingold et al., 1998), and a substantial body of evidence indicates that GABA-A and NMDA receptors play critical roles in phase-control of the suprachiasmatic nucleus (SCN) circadian pacemaker by both photic and non-photic stimuli (Rosenwasser, 2003).

The SCN and other neural components of the circadian timing system are richly endowed with both GABAergic neurons and with GABA-A receptors (Michels et al., 1990; Moore and Speh, 1993) that mediate the circadian phase-shifting effects of benzodiazepines and other GABA-A agonists (Smith and Turek, 1989). Like benzodiazepines, ethanol exerts positive allosteric

modulation of the GABA-A receptor, and chronic ethanol treatment is well known to result in GABA-A down-regulation (Faingold et al., 1998) accompanied by cross-tolerance to benzodiazepines (Mihic et al., 1992). Thus chronic ethanol-induced GABA-A down-regulation within the circadian system could account for the attenuation of triazolam-induced circadian phase responses seen in this study and reported previously in abstract form by Joy and Turek (1989). It should be acknowledged, however, that since this study did not include saline-injected controls, we cannot rule out the possibility that apparent triazolam-induced phase responses seen in the present study were actually due – in whole or in part – to aspects of the injection procedure itself, perhaps in combination with the alterations in photoperiodic conditions that accompanied the injections, which differed between the ZT 6 and ZT 21 treatment times.

Previous studies reveal that the PRC characterizing the circadian phase-shifting effect of triazolam is essentially identical to that seen for induced locomotor activity and/or behavioral arousal (Antle and Mistlberger, 2000; Mrosovsky, 1996b; Rosenwasser, 2003). Indeed, since triazolam-induced phase responses can be prevented by post-injection restraint, it has been suggested that drug-induced locomotor activity may mediate the effects of triazolam on the circadian pacemaker (Van Reeth and Turek, 1989). This suggestion is controversial, however, since benzodiazepines can evoke circadian phase responses in the absence of induced locomotor activity (Biello and Mrosovsky, 1993; Marchant and Morin, 1999), and since triazolam- and activity-induced phase responses are affected differentially by several neural and pharmacological interventions (Rosenwasser, 2003). While the present study does not fully resolve these issues, we found that chronic ethanol intake reduced both the locomotor and the circadian phase-shifting responses to triazolam, and that the two responses were correlated across animals. Of course, it is possible that the two responses depend on independent populations of GABA-A receptors that are affected similarly by chronic ethanol intake.

The effects of light on the SCN circadian pacemaker are mediated largely by light-evoked glutamate release from retinal efferents to the SCN and by NMDA receptor-dependent signaling within SCN neurons (Gillette and Mitchell, 2002). In addition, photic signaling in SCN neurons is modulated by GABAergic mechanisms, and both GABA-A and GABA-B agonists generally inhibit the circadian phase-shifting effects of light (Gillespie et al., 1997; Mintz et al., 2002; Ralph and Menaker, 1986, 1989). Despite discrepant findings in the literature regarding *direct* GABA-A agonists that may be related to central vs. peripheral sites of administration (Gillespie et al., 1997; Ralph and Menaker, 1989), *peripheral* administration of *indirect* GABA-A agonists (i.e., benzodiazepines) selectively blocks the phase-advancing but not the phase-delaying effects of light (Ralph and Menaker, 1986), similar to the effects of chronic ethanol intake seen here and in our previous study using rats (Rosenwasser et al., 2005b). On the other hand, since chronic ethanol treatment is known to up-regulate NMDA receptors (Faingold et al., 1998), one might have predicted that this treatment would actually potentiate light-induced phase responses, in contrast to the observed phase-specific blocking of photic phase responses. It must be remembered, however, that phase responses were tested during ongoing ethanol intake, which could have masked any NMDA-receptor up-regulation. We are planning to test this hypothesis by examining the effects of chronic ethanol intake on photic phase responses during acute ethanol withdrawal.

Despite the extensive evidence for chronic ethanol-induced changes in the expression of GABA-A and NMDA receptors in other brain areas, the effects of chronic ethanol on these receptor systems within the circadian timing system have not been examined, and thus their possible role in mediating the present results remains speculative. On the other hand, chronic ethanol exposure has been reported to alter both neuropeptide (vasopressin, vasoactive intestinal peptide) (Madeira et al., 1997) and circadian clock gene (*per1*, *per2*, *per3*) (Chen et al., 2004) expression within the SCN, and while a specific role for these elements in modulating the response of the pacemaker to phase-shifting stimuli has not been as clearly established as that for GABA and glutamate receptors, such effects could certainly contribute to ethanol-induced alterations in circadian phase-shifting as well. Reciprocally, mutation of the *per2* isoform of the mouse per gene alters both glutamatergic tone and voluntary ethanol intake (Spanagel et al., 2005), further highlighting the complex interactions between ethanol intake, neurotransmitter systems, and chronobiological regulation.

We unexpectedly observed a small number of cases of near-12-hour phase responses in animals of both groups treated with triazolam at ZT 21. These responses resemble the so-called "type-0" resetting that has been associated with very strong phase-shifting stimuli and/or with low-amplitude circadian pacemakers (cf., Knoch et al., 2004). In order to use the Aschoff type-II approach – in which potential phase-shift stimuli are delivered near the transition from LD entrainment to a DD free-run (Mistlberger, 1996; Mrosovsky, 1996a) – to test the response to triazolam at ZT 21, it was necessary to either inject the animals in darkness or to extend the light phase of the LD cycle from ZT 12 to ZT 21 on the day of the test. We chose the latter strategy, both to equate lighting conditions for the ZT 6 and ZT 21 injections, and for experimental convenience. However, recent studies have shown that pre-exposure to even relatively short periods of continuous light (i.e., 1–3 circadian cycles) can dramatically potentiate the response to other varieties of non-photic stimuli (Knoch et al., 2004, 2006; Landry and Mistlberger, 2005). Thus, these observations suggest that similar light-induced potentiation of non-photic phase responses may also occur for triazolam-induced responses in some animals.

Finally, the present results also confirm previous observations that Syrian hamsters will voluntarily consume very high levels of ethanol. The estimated ethanol intake of about 15 g/kg/day in the present study is very similar to previously reported values for this species (Harris et al., 1979; Kulkosky and Cornell, 1979; McMillan et al., 1977; Piercy and Myers, 1995), and about twice that typically reported for various lines of selectively bred ethanol-preferring rats (e.g., Murphy et al., 1986). One possible complication in the interpretation of the present study is that, while substantial ethanol intake was already established by the time of the first phase response test, further increases in ethanol preference occurred over the course of the study. Thus, since

blood alcohol was assessed only at the end of the study, blood levels could have differed somewhat across the different phase response tests. On the other hand, previous studies show that even prolonged exposure to ethanol results in little or no metabolic or functional tolerance, and does not produce signs of dependence or a physical withdrawal syndrome, in this species (Harris et al., 1979; Kulkosky and Cornell, 1979; McMillan et al., 1977; Piercy and Myers, 1995). Indeed, the greater ethanol intake in hamsters relative to rats appears to be largely due to the much higher rate of ethanol metabolism in the former species (Kulkosky and Cornell, 1979), so it is not surprising that blood alcohol levels seen in the present study were modest and variable, and very similar to mid-dark-phase levels seen in previous studies of 24-hour free-choice intake in ethanol-preferring rats (Aalto, 1986; Agabio et al., 1996; Murphy et al., 1986) and mice (Kakihana and Moore, 1976; Millard and Dole, 1983).

In conclusion, this study revealed that chronic ethanol intake modulates photic and non-photic circadian phase responses in the Syrian hamster, a species characterized by very high levels of voluntary ethanol intake. These effects are likely to be related to modulation of specific ethanol-sensitive neurotransmitter receptors and clock genes within the SCN and/or other components of the circadian timing system, but these hypotheses remain to be tested directly.

Acknowledgements

This work was supported by NIAAA grant AA013893 to AMR.

References

Aalto J. Circadian drinking rhythms and blood alcohol levels in two rat lines developed for their alcohol consumption. Alcohol 1986;3:73–5.

Agabio R, Cortis G, Fadda F, Gessa GL, Lobina C, Reali R, et al. Circadian drinking pattern of Sardinian alcohol-preferring rats. Alcohol Alcohol 1996;31:385–8.

Antle MC, Mistlberger RE. Circadian clock resetting by sleep deprivation without exercise in the Syrian hamster. J Neurosci 2000;20:9326–32.

Biello SM, Mrosovsky N. Circadian phase-shifts induced by chlordiazepoxide without increased locomotor activity. Brain Res 1993;622:58–62.

Chen CP, Kuhn P, Advis JP, Sarkar DK. Chronic ethanol consumption impairs the circadian rhythm of pro-opiomelanocortin and period genes MRNA expression in the hypothalamus of the male rat. J Neurochem 2004;88:1547–54.

Davies M. The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. J Psychiatry Neurosci 2003;28:263–74.

Dwyer SM, Rosenwasser AM. Neonatal clomipramine treatment, alcohol intake and circadian rhythms in rats. Psychopharmacology (Berl) 1998;138:176–83.

Faingold CL, N'Gouemo P, Riaz A. Ethanol and neurotransmitter interactions—from molecular to integrative effects. Prog Neurobiol 1998;55:509–35.

Fecteau ME, Miura Y, Turner LM, Rosenwasser AM. Effects of repeated cycles of ethanol access and deprivation on free-running circadian activity rhythms and voluntary ethanol intake in ethanol-preferring (p, had-2) rats. Alcohol Clin Exp Res 2006;30:84A [abstract].

Gao B, Fritschy JM, Moore RY. GABAA-receptor subunit composition in the circadian timing system. Brain Res 1995;700:142–56.

Gillespie CF, Mintz EM, Marvel CL, Huhman KL, Albers HE. GABA(A) and GABA(B) agonists and antagonists alter the phase-shifting effects of light when microinjected into the suprachiasmatic region. Brain Res 1997;759:181–9.

Gillette MU, Mitchell JW. Signaling in the suprachiasmatic nucleus: selectively responsive and integrative. Cell Tissue Res 2002;309:99–107.

Harris RA, Krause W, Goh E, Case J. Behavioral and biochemical effects of chronic consumption of ethanol by hamsters. Pharmacol Biochem Behav 1979;10:343–7.

Hiller-Sturmhofel S, Kulkosky P. Chronobiological regulation of alcohol intake. Alcohol Res Health 2001;25:141–8.

Joy JE, Turek FW. Effects of alcohol and triazolam on the circadian activity rhythm of the golden hamster. Soc Neurosci Abstr 1989;15:727.

Kakihana R, Moore JA. Circadian rhythm of corticosterone in mice: the effect of chronic consumption of alcohol. Psychopharmacologia 1976;46:301–5.

Knoch ME, Gobes SM, Pavlovska I, Su C, Mistlberger RE, Glass JD. Short-term exposure to constant light promotes strong circadian phase-resetting responses to nonphotic stimuli in Syrian hamsters. Eur J Neurosci 2004;19:2779–90.

Knoch ME, Siegel D, Duncan MJ, Glass JD. Serotonergic mediation of constant light-potentiated nonphotic phase shifting of the circadian locomotor activity rhythm in Syrian hamsters. Am J Physiol Regul Integr Comp Physiol 2006;291:R180–8.

Kulkosky PJ, Cornell NW. Free-choice ethanol intake and ethanol metabolism in the hamster and rat. Pharmacol Biochem Behav 1979;11:439–44.

Landry GJ, Mistlberger RE. Differential effects of constant light on circadian clock resetting by photic and nonphotic stimuli in Syrian hamsters. Brain Res 2005;1059:52–8.

Madeira MD, Andrade JP, Lieberman AR, Sousa N, Almeida OF, Paula-Barbosa MM. Chronic alcohol consumption and withdrawal do not induce cell death in the suprachiasmatic nucleus, but lead to irreversible depression of peptide immunoreactivity and MRNA levels. J Neurosci 1997;17:1302–19.

Marchant EG, Morin LP. The hamster circadian rhythm system includes nuclei of the subcortical visual shell. J Neurosci 1999;19:10482–93.

McMillan DE, Ellis FW, Frye GD, Pick JR. Failure of signs of physical dependence to develop in hamsters after prolonged consumption of large doses of ethanol. Pharmacol Biochem Behav 1977;7:55–7.

Michels KM, Morin LP, Moore RY. GABAA/benzodiazepine receptor localization in the circadian timing system. Brain Res 1990;531:16–24.

Mihic SJ, Kalant H, Liu JF, Wu PH. Role of the gamma-aminobutyric acid receptor/chloride channel complex in tolerance to ethanol and cross-tolerance to diazepam and pentobarbital. J Pharmacol Exp Ther 1992;261:108–13.

Millard WJ, Dole VP. Intake of water and ethanol by C57BL mice: effect of an altered light–dark schedule. Pharmacol Biochem Behav 1983;18:281–4.

Mintz EM, Jasnow AM, Gillespie CF, Huhman KL, Albers HE. Gaba interacts with photic signaling in the suprachiasmatic nucleus to regulate circadian phase shifts. Neuroscience 2002;109:773–8.

Mistlberger RE. Aschoff type II method: commentary. Chronobiol Int 1996;13:393–4.

Mistlberger RE, Nadeau J. Ethanol and circadian rhythms in the Syrian hamster: effects on entrained phase, reentrainment rate, and period. Pharmacol Biochem Behav 1992;43:159–65.

Mistlberger RE, Bossert JM, Holmes MM, Marchant EG. Serotonin and feedback effects of behavioral activity on circadian rhythms in mice. Behav Brain Res 1998;96:93–9.

Moore RY, Speh JC. GABA is the principal neurotransmitter of the circadian system. Neurosci Lett 1993;150:112–6.

Mrosovsky N. Methods of measuring phase shifts: why I continue to use an Aschoff type II procedure despite the skepticism of referees. Chronobiol Int 1996a;13:387–92.

Mrosovsky N. Locomotor activity and non-photic influences on circadian clocks. Biol Rev Camb Philos Soc 1996b;71:343–72.

Mrosovsky N. Further experiments on the relationship between the period of circadian rhythms and locomotor activity levels in hamsters. Physiol Behav 1999;66:797–801.

Murphy JM, Gatto GJ, Waller MB, McBride WJ, Lumeng L, Li TK. Effects of scheduled access on ethanol intake by the alcohol-preferring (P) line of rats. Alcohol 1986;3:331–6.

Piercy KT, Myers RD. Female Syrian golden hamster: drinking of high concentrations of ethanol aversive to other mammals. Alcohol 1995;12:207–11.

Ralph MR, Menaker M. Effects of diazepam on circadian phase advances and delays. Brain Res 1986;372:405–8.

Ralph MR, Menaker M. GABA regulation of circadian responses to light. I. Involvement of GABAA-benzodiazepine and GABAB receptors. J Neurosci 1989;9:2858–65.

Rosenwasser AM. Alcohol, antidepressants, and circadian rhythms. Human and animal models. Alcohol Res Health 2001;25:126–35.

Rosenwasser AM. Neurobiology of the mammalian circadian system: oscillators, pacemakers and pathways. In: Fluharty SJ, Grill HJ, editors. Progress in psychobiology and physiological psychology, vol. 18. San Diego: Elsevier-Academic Press; 2003. p. 1–38.

Rosenwasser AM, Dwyer SM. Circadian phase shifting: relationships between photic and nonphotic phase-response curves. Physiol Behav 2001;73:175–83.

Rosenwasser AM, Fecteau ME, Logan RW. Effects of ethanol intake and ethanol withdrawal on free-running circadian activity rhythms in rats. Physiol Behav 2005a;84:537–42.

Rosenwasser AM, Logan RW, Fecteau ME. Chronic ethanol intake alters circadian period-responses to brief light pulses in rats. Chronobiol Int 2005b;22:227–36.

Smith RD, Turek FW. Beta-methyl carboline, a benzodiazepine inverse agonist, attenuates the effect of triazolam on the circadian rhythm of locomotor activity. Experientia 1989;45:334–7.

Smith RD, Turek FW, Takahashi JS. Two families of phase-response curves characterize the resetting of the hamster circadian clock. Am J Physiol 1992;262:R1149–53.

Spanagel R, Pendyala G, Abarca C, Zghoul T, Sanchis-Segura C, Magnone MC, et al. The clock gene *per2* influences the glutamatergic system and modulates alcohol consumption. Nat Med 2005;11:35–42.

Turek FW. Pharmacological probes of the mammalian circadian clock: use of the phase-response curve approach. Trends Pharmacol Sci 1987;8:212–7.

Turek FW, Losee-Olson S. A benzodiazepine used in the treatment of insomnia phase-shifts the mammalian circadian clock. Nature 1986;321:167–8.

Van Reeth O, Turek FW. Stimulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. Nature 1989;339:49–51.