

Acute ethanol impairs photic and nonphotic circadian phase resetting in the Syrian hamster

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Submitted 17 September 2008; accepted in final form 3 December 2008

Ruby CL, Prosser RA, DePaul MA, Roberts RJ, Glass JD. Acute ethanol impairs photic and nonphotic circadian phase resetting in the Syrian hamster. *Am J Physiol Regul Integr Comp Physiol* 296: R411–R418, 2009. First published December 10, 2008; doi:10.1152/ajpregu.90782.2008.—Disrupted circadian rhythmicity is associated with ethanol (EtOH) abuse, yet little is known about how EtOH affects the mammalian circadian clock of the suprachiasmatic nucleus (SCN). Clock timing is regulated by photic and nonphotic inputs to the SCN involving glutamate release from the retinohypothalamic tract and serotonin (5-HT) from the midbrain raphe, respectively. Our recent *in vitro* studies in the SCN slice revealed that EtOH blocks photic phase-resetting action of glutamate and enhances the nonphotic phase-resetting action of the 5-HT_{1A,7} agonist, 8-OH-DPAT. To explore the basis of these effects in the whole animal, we used microdialysis to characterize the pharmacokinetics of intraperitoneal injection of EtOH in the hamster SCN extracellular fluid compartment and then studied the effects of such EtOH treatment on photic and serotonergic phase resetting of the circadian locomotor activity rhythm. Peak EtOH levels (~50 mM) from a 2 g/kg injection occurred within 20–40 min with a half-life of ~3 h. EtOH treatment dose-dependently attenuated photic phase advances but had no effect on phase delays and, contrary to *in vitro* findings, markedly attenuated 8-OH-DPAT-induced phase advances. In a complementary experiment using reverse microdialysis to deliver a timed SCN perfusion of EtOH during a phase-advancing light pulse, the phase advances were blocked, similar to systemic EtOH treatment. These results are evidence that acute EtOH significantly affects photic and nonphotic phase-resetting responses critical to circadian clock regulation. Notably, EtOH inhibition of photic signaling is manifest through direct action in the SCN. Such actions could underlie the disruption of circadian rhythmicity associated with alcohol abuse.

suprachiasmatic nucleus; glutamate; serotonin; microdialysis; alcohol

ETHANOL (EtOH) is highly disruptive to mammalian circadian physiological and behavioral rhythms, including those for melatonin (43, 75), glucocorticoids (2, 70), thyroid-stimulating hormone (20), body temperature (5, 21, 22, 94), and sleep (11, 27, 42, 43, 72). Also, disrupted circadian timing of hypothalamic-pituitary-adrenal axis function is associated with increased EtOH preference in experimental animals, and in humans, may be a risk factor for developing alcoholism and relapsing after abstinence (16, 61, 98). In a related manner, circadian-based sleep problems are implicated in the development of alcoholism and in abstinent alcoholics' propensity to relapse (73, 74).

In mammals, the master circadian clock responsible for generating and maintaining physiological and behavioral rhythms is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (41, 56, 80). SCN clock-driven rhythms are entrained to the daily light-dark (LD) cycle via glutamate release from the retinohypothalamic tract in the SCN (34, 36, 54, 62, 79). Glutamatergic activation of the SCN *N*-methyl-D-aspartate (NMDA) receptor is essential for light-induced phase shifting to occur (1, 15, 23, 49, 50). In a recent report, we showed that EtOH can block such glutamate-induced phase shifts in the SCN *in vitro* (68). This finding fits with reports that EtOH inhibits glutamatergic signaling, both presynaptically and postsynaptically in other brain areas (46, 55, 57, 60, 84, 96) and that the NR2B subunit of the NMDA receptor complex involved in SCN photic phase resetting (57) is EtOH sensitive (3, 4, 58, 86). It is notable that EtOH's actions on the NMDA receptor are considered to be the basis for intoxication, dependence, tolerance, addiction, and neuronal damage during withdrawal (13, 14, 29, 33, 40, 88, 90, 91, 92), and thus disrupted photic signaling in the clock may also be related to EtOH effects on the NMDA receptor.

In addition to photic entrainment, the SCN receives a variety of nonphotic (behavior-induced) inputs that are important to circadian rhythm regulation. One such mediator of nonphotic input is serotonin (5-HT), which is supplied by a projection from the median raphe nucleus (49, 57). Serotonergic input also modulates photic signaling in the SCN (32, 47, 63, 64, 71, 82, 97) by inhibiting glutamatergic transmission (9, 12, 85, 87). Relevant to EtOH's disruptive effect on 5-HT-related circadian functions are observations that chronic EtOH increases the release of, and may decrease the reuptake of, central 5-HT (14, 76) and downregulates certain subtypes of 5-HT receptors (45, 59). Moreover, acute EtOH treatment potentiates serotonergic phase advances of the SCN *in vitro* (68). It is therefore apparent that EtOH effects on critical serotonergic and glutamatergic functions in the SCN could underlie the disordered circadian rhythmicity associated with chronic EtOH use.

To gain a better understanding of the actions of EtOH in the SCN clock, the present experiments were undertaken to characterize the pharmacokinetics of acute systemic EtOH administration in the SCN and to explore the *in vivo* effects of systemic and intra-SCN EtOH on photic and serotonergic phase-resetting processes in the Syrian hamster. Such information is important for determining the neural substrates of EtOH disruptive effects on circadian timing and whether the SCN represents a direct target for these actions.

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MATERIALS AND METHODS

Animals. Adult, male Syrian hamsters *Mesocricetus auratus* raised from breeders purchased from Harlan Sprague-Dawley (Madison, IL) were used in this study. Animals were maintained in a temperature-controlled vivarium (23°C) under a 14:10 LD photoperiod with light intensity of 270 lux with food (Prolab 3000; PMI Feeds, St. Louis, MO) and water provided ad libitum. The experiments were approved by the Kent State Institutional Animal Care and Use Committee and were conducted using the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

SCN pharmacokinetics of acute systemic EtOH administration. In vivo microdialysis was used to characterize the time course of EtOH in the SCN of freely behaving hamsters following intraperitoneal EtOH injection. Procedures for microdialysis of the SCN have been described previously (24). Concentrically designed microdialysis probes were constructed from a 26-gauge stainless-steel outer cannula into which was inserted 32-gauge fused silica tubing. Hemicellulose dialysis membrane tubing (12 kDa mol wt cutoff; 230 μ m outer diameter) was secured to the outer cannula with epoxy glue. The active dialysis length was 1.0 mm. Animals were anesthetized with pentobarbital sodium (Nembutal; 50 mg/kg), and Marcaine (0.25% bupivacaine, 0.10 ml) was injected in the scalp area before surgery. Animals received the antibiotic Combi-Pen-48 (15,000 units in 0.05 ml) subcutaneously after surgery. The microdialysis probe was targeted stereotactically to the SCN 2 days before experimentation, and the implantation site was confirmed histologically after experimentation. The 2-day recovery period was used, since this is considered optimal for tissue recovery (blood-brain barrier reestablishment) and probe function. Sampling was undertaken 1 h before, and extending 7 h after, the intraperitoneal injection (2.0 g/kg) at a flow rate of 1.0 μ l/min with a sampling interval of 20 min. EtOH in the microdialysate samples was measured using validated gas chromatography (GC) procedures (HP-5890A gas chromatograph; internal standard = secondary butanol, initial temperature = 40°C, purge time = 1 min, final temperature = 225°C, final time = 5 min). The standard curve for EtOH analysis was linear over the concentration range for SCN microdialysis measurements. The inter- and intra-assay coefficients of variability were 9.2 and 8.6%, respectively. To obtain an in vitro estimate of efficiency of EtOH delivery via our probes, EtOH was measured in dialysate samples collected from probes submerged in known EtOH standards maintained at 35, 37, or 39°C to determine if fluctuations in body temperature within this range would significantly affect probe efficiency. Mean probe efficiency was estimated to be ~12% and did not vary significantly between the tested temperatures ($t = 0$; $p = 1$). Results from this experiment were used to determine the optimal timing of EtOH injection before administration of the light pulses and to estimate an effective EtOH dosage in subsequent experiments.

Effects of acute systemic EtOH administration on photic phase resetting. Hamsters under LD were individually caged, and their general circadian locomotor activity rhythms were monitored over a 2-wk period before experimentation using infrared motion detectors interfaced with a computerized data acquisition system (Clocklab; Coulbourn Instruments, Whitehall, PA). Under constant darkness (DD), the onset of activity, designated as circadian time 12, was used as the phase reference point for the beginning of the subjective night. Activity onset was defined as the as the first 6 min that are 1) coincident with an intensity of activity that exceeded 10% of the maximum rate for the day; 2) preceded by a period of at least 4 h of inactivity; and 3) followed by a period of at least 30 min of sustained activity. Phase shifts were calculated as the difference between the projected times of activity onset on the day after stimulation as determined by 1) back extrapolation of the least-squares line through activity onsets on days 3–7 after treatment and 2) extrapolation of the least-squares line calculated from activity onset data collected during the 5 days before treatment.

On the day of the experiment, animals received an intraperitoneal injection of one of three doses of EtOH (0.5, 1.5, and 2.0 g/kg; diluted to 20% vol/vol in physiological saline) or saline vehicle preceding a 30-min phase-advancing light pulse (270 lux) at Zeitgeber time (ZT) 18.5 or a 30-min phase-delaying light pulse at ZT 14 (where ZT 12 is designated as the onset of the dark phase). Injections were undertaken in the dark using infrared goggles. Immediately after the light pulse, animals were released in DD for 2 wk to assess phase advances and delays using an Aschoff Type II procedure (19). The 'no light pulse' control animals received an intraperitoneal injection of saline or EtOH (2.0 g/kg) and did not receive a light pulse.

Effects of intra-SCN EtOH administration on photic phase resetting. In this experiment, EtOH was administered directly in the SCN via reverse-microdialysis perfusion. Hamsters under LD were individually caged, and their general activity rhythms were monitored over a 2-wk period before experimentation. Before experimentation, each animal received a microdialysis probe implant targeted to the lateral margin of the SCN. On the day of experimentation, the microdialysis probes were perfused with artificial cerebrospinal fluid (ACSF) alone or ACSF containing EtOH (500 mM; based on in vitro measurements of probe efficiency, this provided a theoretical outside tissue concentration of ~50 mM) from a syringe pump. The concentration of EtOH used was based on the pharmacokinetic data from systemic injections of EtOH in *experiment 1*. Continuous perfusion of the probes commenced 30 min before, and extended throughout and for 30 min after, a 30-min phase-advancing light pulse (270 lux) administered at ZT 18.5. Immediately after the light pulse, the animals were released into DD for 2 wk to assess phase-shifting responses using the Aschoff Type II procedure. The "no light pulse" controls received microdialysis perfusion of ACSF alone or containing EtOH, but remained in DD. In a separate group of animals, blood alcohol content (BAC) was measured after the 1.5-h perfusion of EtOH (500 mM). Animals were killed, and trunk blood was collected in a 1.5-ml microfuge tube containing heparin USP (1,000 U/ml) and centrifuged to isolate serum. BAC was measured using the Analox AM-1 Alcohol Analyzer (Lunenburg, MA). Location of the microdialysis probes was determined histologically at the end of the experiment from fixed frozen sections mounted and stained with cresyl violet.

Effects of acute systemic EtOH administration on nonphotic (serotonergic) phase resetting. Hamsters under LD were individually caged, and their general circadian locomotor activity rhythms were monitored over a 2-wk period before experimentation. On the day of the experiment, animals received an intraperitoneal injection of EtOH (2.0 g/kg) or saline vehicle preceding intraperitoneal injection of the serotonin 5-HT_{1A/7} agonist ($\pm$) 8-OH-DPAT (5.0 mg/kg) at ZT 6 (the time at which the phase-advance portion of the 8-OH-DPAT phase-response curve is maximal). Immediately after injection, animals were released into DD for 2 wk to assess phase advances using the Aschoff Type II procedure. Vehicle controls received intraperitoneal injection of dimethyl sulfoxide instead of 8-OH-DPAT.

Statistical analyses. Differences in behavioral phase shifts were assessed by ANOVA and subsequent Student-Newman-Keul's post hoc mean comparison test. The level of significance was set at $P < 0.05$.

RESULTS

Assessment of EtOH pharmacokinetics in the SCN. The pharmacokinetic profile of EtOH in the SCN extracellular fluid compartment following intraperitoneal injection of EtOH (2.0 g/kg) as assessed in freely behaving hamsters by microdialysis-GC analysis is presented in Fig. 1. Peak EtOH levels in the SCN occurred within 20–40 min postinjection, and the half-life of the absorbed EtOH ($t_{1/2}$ elimination) was ~3 h. Based on the in vitro calculation of microdialysis probe efficiency for EtOH (12%), the peak concentration of EtOH in the SCN was ~50 mM. Probe location along the lateral margin of the SCN

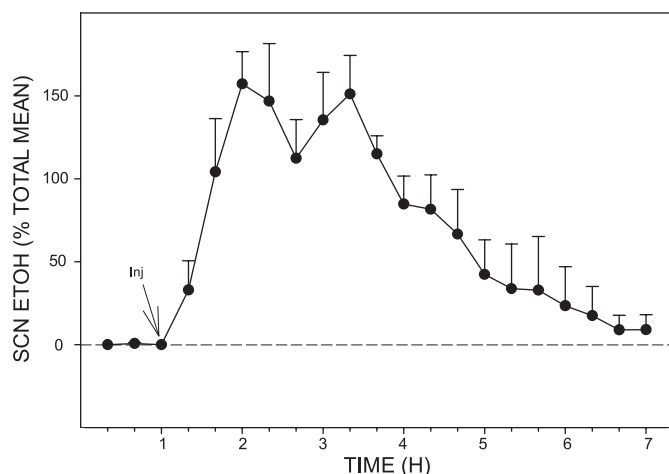


Fig. 1. Pharmacokinetics of ethanol (EtOH) in the suprachiasmatic nucleus (SCN) ($n = 5$ hamsters) following ip injection (Inj) of EtOH (2.0 g/kg). Peak levels (estimated at ~ 50 mM) occurred within 20–40 min of injection, and the half-life ($t_{1/2}$) for clearance was 3 h. Bars represent means $\pm$ SE.

was evaluated histologically as shown in the photomicrograph of Fig. 2, and the locations of probe tips for each animal used in this and subsequent experiments are shown diagrammatically in Fig. 3.

Acute systemic EtOH administration attenuates photic phase resetting in vivo. Photic phase-advance shifts at ZT 18.5 were significantly attenuated by intraperitoneal injection of EtOH in a dose-dependent manner [$F(3,28) = 14.6$; $P = 0.0001$]. Controls receiving intraperitoneal saline injection had phase advances averaging 1.90 ± 0.20 h ($n = 11$). Animals receiving intraperitoneal injection of EtOH at a dose range of 0.5 ($n = 11$), 1.5 ($n = 5$), and 2.0 ($n = 5$) g/kg showed diminishing phase advances averaging 2.00 ± 0.10 , 1.10 ± 0.20 , and 0.60 ± 0.20 h, respectively (Fig. 4). The two higher dosages significantly inhibited photic phase resetting (both $P < 0.05$ vs. saline controls). EtOH (2.0 g/kg) did not have a phase-resetting effect in the absence of a light pulse at ZT 18.5. No effect of EtOH (2.0 g/kg) was seen on photic phase delays. Controls receiving intraperitoneal saline injection showed phase delays of -0.15 ± 0.34 h ($n = 5$). Animals receiving intraperitoneal injection of EtOH showed phase delays of -0.67 ± 0.64 h [$n = 5$; $F(1,9) = 1.6$; $P < 0.24$]. Representative actograms for vehicle- and EtOH-treated (2.0 g/kg) animals receiving light pulses at ZT 18.5 are presented in Fig. 5.

The SCN is a direct target for EtOH inhibition of photic phase resetting. Reverse microdialysis perfusion targeting the SCN region with EtOH (50 mM estimated tissue concentra-

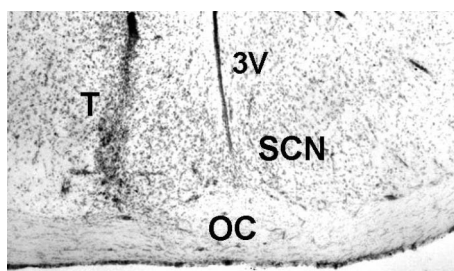


Fig. 2. Coronal section through the hypothalamus showing the probe tract (T) of a microdialysis probe targeted to the lateral margin of the SCN. 3V, third ventricle; OC, optic chiasm.

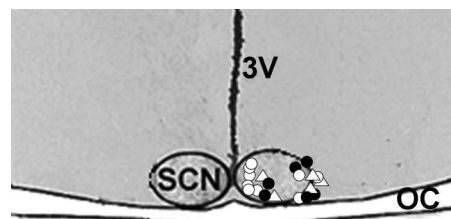


Fig. 3. Histologically verified probe tip locations relative to the SCN for each of the microdialysis experiments. Δ , Pharmacokinetics of EtOH after ip injection; $\bullet$, reverse dialysis of EtOH; $\circ$, reverse dialysis of vehicle.

tion) significantly attenuated photic phase-advance shifts at ZT 18.5. Animals receiving SCN ACSF perfusion had phase advances averaging 1.22 ± 0.14 h ($n = 6$; Fig. 6), whereas animals receiving SCN EtOH perfusion showed significantly smaller phase advances averaging 0.40 ± 0.25 h [$n = 4$; $F(1,8) = 9.70$; $P < 0.015$ vs. saline]. Neither ACSF nor EtOH SCN perfusion had a phase-resetting effect in the absence of a light pulse [0.06 ± 0.07 h ($n = 3$) and -0.17 ± 0.14 h ($n = 2$), respectively]. Representative actograms for vehicle- and EtOH-perfused animals are shown in Fig. 7. Reverse microdialysis perfusion of EtOH in the SCN did not produce a detectable BAC ($n = 2$), confirming that the EtOH from the SCN perfusion does not enter in the systemic circulation.

Acute systemic EtOH administration inhibits serotonergic phase resetting in vivo. Nonphotic phase-advance shifts caused by intraperitoneal injection of 8-OH-DPAT at ZT 6 were significantly inhibited by EtOH treatment (2.0 g/kg ip). Control animals receiving intraperitoneal saline injection instead of EtOH had 8-OH-DPAT-induced phase advances averaging 1.86 ± 0.28 h ($n = 6$). In contrast, animals receiving EtOH had diminished 8-OH-DPAT-induced phase-advance shifts averaging 0.50 ± 0.15 h [$F(3,20) = 13.95$; $P < 0.0001$ vs. saline; Fig. 8]. Other controls receiving EtOH or saline without 8-OH-DPAT did not have significant phase-resetting responses (0.31 ± 0.34 and -0.05 ± 0.12 h, respectively).

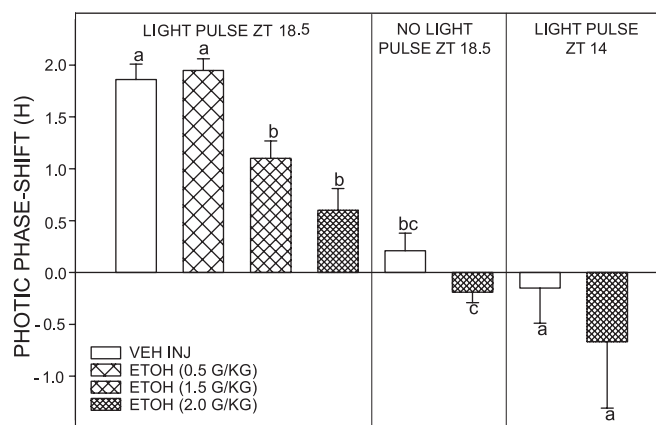
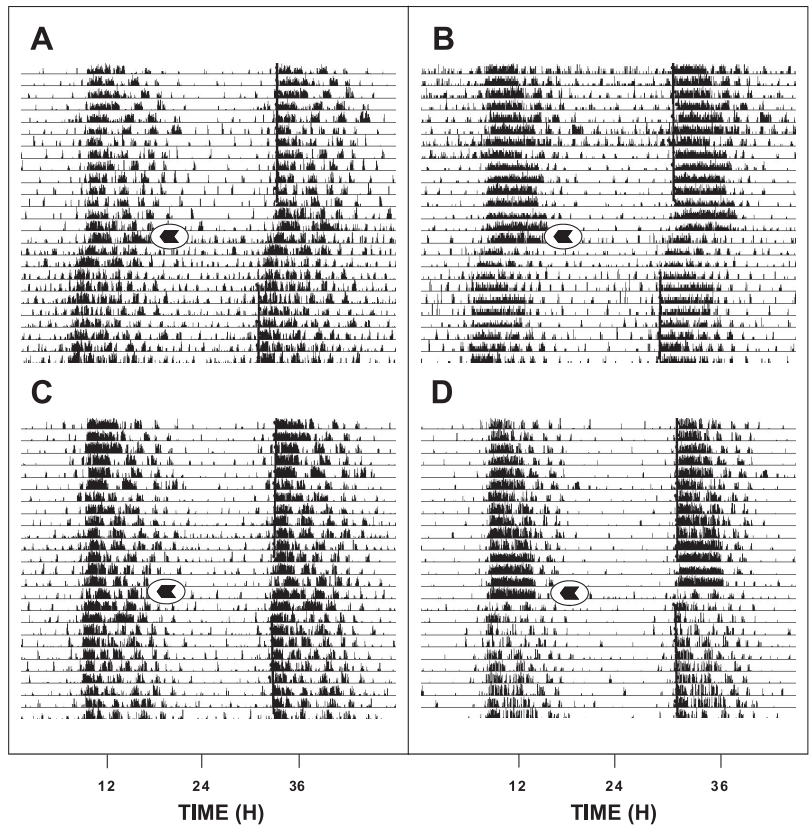


Fig. 4. EtOH dose dependently attenuates photic phase-advance shifts to a light pulse delivered late in the dark phase [Zeitgeber time (ZT) 18.5; left] but has no effect on phase-delay responses to a light pulse delivered at ZT 14 (right). The highest dose of EtOH (2.0 g/kg) had no phase-resetting effect of its own at ZT 18.5 in the absence of a light pulse (middle). For each time point, bars with different letters are significantly different ($P < 0.05$). Bars represent means $\pm$ SE.

Fig. 5. Representative double-plotted actograms of general locomotor activity showing EtOH attenuation of photic phase-advance responses to light pulses delivered at ZT 18.5. *A* and *B*: vehicle-injection. *C* and *D*: EtOH (2.0 g/kg) injection. Arrows denote the time of injection.



DISCUSSION

Alcohol abuse is associated with marked disturbances in the daily patterns of multiple circadian clock-regulated functions, including overt behavioral rhythms (e.g., the sleep-wake cycle; see Refs. 10, 11, and 44) and internal physiological and endocrine rhythms (28, 76). The circadian timing of these clock-generated rhythms is regulated by photic and nonphotic entrainment pathways to the SCN, and it is thus reasonable to speculate that the adverse chronobiological effects of EtOH

could involve some disruption of these pathways. Here we confirm this idea by showing that acute EtOH markedly attenuates the phase-resetting responses of the circadian clock to photic and nonphotic (serotonergic) signals. These inhibitory effects are induced in response to systemic (ip) administration of EtOH and for photic signaling, as well as also by direct reverse-microdialysis administration of EtOH to the SCN. This latter result strongly suggests that the SCN clock is a direct target for EtOH's disruption of photic phase resetting in vivo, which is consistent with our previous finding that acute EtOH blocks the phase-resetting effects of light-related (glutamate-induced) signaling in the isolated mouse SCN slice in vitro (68). Collectively, these results represent the first lines of evidence that acute EtOH can directly affect phase-resetting mechanisms of the circadian clock that regulate its physiological timekeeping activities.

Literature dealing with the circadian effects of EtOH in adult rodents is relatively sparse and is limited primarily to chronic drinking models. In particular, issues regarding site(s) and mechanism(s) of action have not been addressed. Progress in this area has also been hampered by inconsistencies in chronic EtOH drinking effects on various circadian endpoints within and between species. In hamsters, for example, chronic drinking is reported to have minor effects on circadian rhythm stability and period characteristics and does not impede the rate of reentrainment to a shifted LD cycle (51). On the other hand, chronic EtOH has been shown reduce light pulse-induced phase advances (but not phase delays) and inhibit phase advance shifts to triazolam (81). In rats, chronic EtOH alters the free-running period under DD but apparently has no effect on photic phase resetting (77, 78). Although species differences in

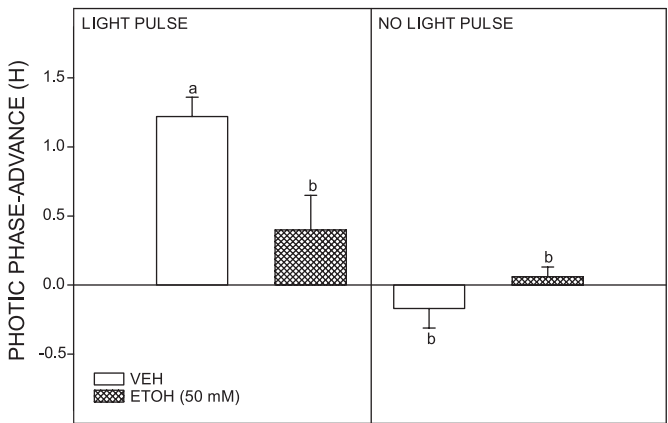


Fig. 6. Inhibition of photic phase-advance responses to a light pulse delivered late in the dark phase (ZT 18.5) by direct reverse-microdialysis perfusion of EtOH to the SCN. The EtOH perfusion had no phase-resetting effect at ZT 18.5 in the absence of a light pulse. Bars with different letters are significantly different ($P < 0.05$). Bars represent means $\pm$ SE.

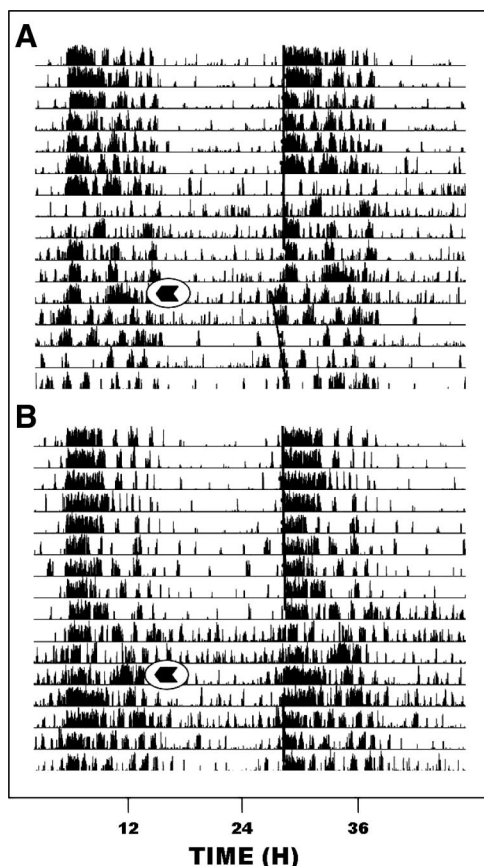


Fig. 7. Representative double-plotted actograms of general locomotor activity showing direct reverse-microdialysis perfusion of EtOH to the SCN attenuates photic phase-advance responses to light pulses delivered at ZT 18.5. *A*: microdialysis perfusion of vehicle [artificial cerebrospinal fluid (ACSF)]. *B*: reverse-microdialysis perfusion of EtOH (50 mM). Arrows denote the onset of the 1.5-h perfusion.

EtOH response are likely a major factor contributing to this variability, it is probable that methodological differences in the amount and duration of EtOH consumed could also be problematic. Another important consideration relating to chronic drinking is the development of compensatory responses and/or tolerance that could impact the effects of EtOH on circadian regulation. This is relevant to photic (glutamate-mediated) phase-resetting studies (i.e., light pulse phase shifting and reentrainment to LD shifts), since glutamate signaling pathways show compensation to chronic EtOH (30, 38, 39). Moreover, EtOH compensation could also factor into nonphotic phase-resetting responses because the activity of serotonergic pathways is significantly altered by chronic EtOH (45, 59). In this regard, the present experiments based on acute EtOH administration eliminate the potential confounds associated with compensation and thus may reveal a more defined picture of rapid neurophysiological actions of EtOH in the circadian system.

An interesting observation in the present study is that, while acute EtOH strongly inhibits photic phase-advance shifts during the late night, it does not affect photic phase-delay shifts during the early night. This result is largely consistent with a previous study in chronically drinking hamsters where EtOH inhibited photic advances but not delays (81). The basis of this

differential effect of EtOH on phase resetting is not clear but could reflect a distinct inhibitory action directed at a component of the intracellular photic clock-resetting pathway devoted to phase-advance shifting. Conceptually, such an action could be registered at a signaling step downstream from NMDA receptor activation and the subsequent generation of nitric oxide (NO) that are initial steps leading to photic phase advances and phase delays (23). Arguing against this, however, is the finding in the mouse SCN slice that EtOH may act upstream in this pathway to inhibit glutamate induction of NO (R. A. Prosser and H. M. Lee, unpublished observations). However, it must be noted that EtOH blocks glutamate-induced phase advances and phase delays in the mouse SCN *in vitro*, suggesting that there may be a species difference in the mode of EtOH inhibitory action in the SCN photic signaling pathway between hamsters and mice. Other possibilities are that there are EtOH actions outside the SCN that counter the inhibition or that there may be compensatory mechanisms *in vivo* that help with delays more than advances.

With respect to the effects of EtOH administered directly to the SCN via reverse-microdialysis perfusion, it is noteworthy that the estimated tissue concentration of EtOH outside the probe (~ 50 mM) attenuated photic phase advances to the same degree as when EtOH was administered intraperitoneally at 2.0 g/kg. These results are also consistent with those in the mouse SCN slice, where EtOH at a similar concentration range (20–50 mM) blocked glutamate-induced phase advances (68). Importantly, no differences in behavior (e.g., loss of balance, sedation, locomotor stimulation) other than the attenuation of photic phase advances was noted during or after the period of EtOH perfusion, and no detectable BAC was produced by this procedure. These observations, together with the *in vitro* evidence, strongly support the idea that EtOH effects on photic phase resetting are manifest within the SCN clock. In this regard, however, it is possible that EtOH inhibition of photic and nonphotic entrainment may be secondary to its hypothermic effects. This is unlikely, however, since EtOH does not induce hypothermia at room temperature at which the present experiments were undertaken (17, 31, 37, 94).

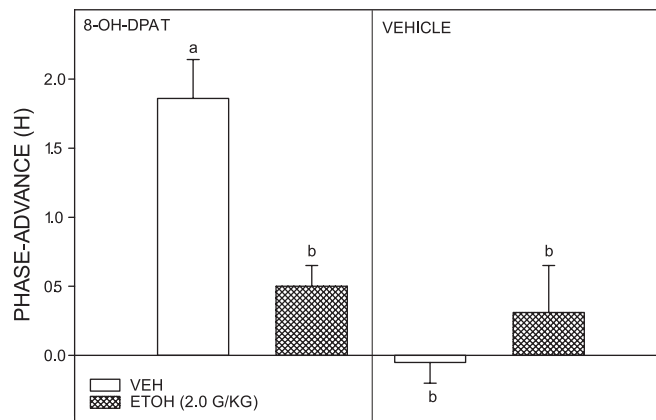


Fig. 8. EtOH (2.0 g/kg) inhibition of nonphotic phase-advance responses to the serotonin 5-HT_{1A,7} agonist ($\pm$)8-OH-DPAT (5.0 mg/kg) administered during the middle of the light phase (ZT 6; *left*). EtOH had no phase-resetting effect when administered before vehicle (dimethyl sulfoxide) injection at ZT 6 (*right*). Bars with different letters are significantly different ($P < 0.05$). Bars represent the means $\pm$ SE.

In addition to its effects on photic circadian clock resetting, EtOH also modulates the activity of neurotransmitters involved in mediating nonphotic signaling in the clock. Among these is 5-HT, which is strongly implicated in mediating behavioral phase resetting (reviewed in Ref. 52) and modulating photic input to the SCN (63, 71, 82, 97). Although the role of 5-HT in nonphotic shifting responses remains speculative, potent phase-resetting actions of 5-HT agonists are well documented. In particular, the 5-HT_{1A,7} agonist 8-OH-DPAT, used widely in circadian studies, has a phase-response curve (PRC) resembling behavioral PRCs and produces *in vivo* phase shifts of similar magnitude as nonphotic stimuli (~1.5 h) when administered systemically (8, 89), or in the SCN (25), and large shifts (~3 h) when administered to the SCN *in vitro* (69, 83). It is notable that acute EtOH affects central serotonergic activity by increasing the release and decreasing the reuptake of 5-HT (14, 76). In addition, chronic EtOH treatment downregulates 5-HT receptors (45, 59). The present finding that acute EtOH markedly diminished the phase-advancing action of 8-OH-DPAT (by 72%) appears inconsistent with these observations in that EtOH would be expected to increase serotonergic phase resetting. However, evidence of very rapid (~5 min) agonist-induced downregulation of 5-HT receptors has been seen *in vitro* (6), and pretreatments with 5-HT agonists have been shown to block 8-OH-DPAT-induced phase shifts in the SCN *in vitro* (67). Whereas no information on the dynamics of 5-HT_{1A} or 5-HT₇ receptor downregulation exists, it is conceivable that rapid internalization of these receptors may have occurred in the SCN or other circadian-related areas in response to an EtOH-induced increase in the availability of 5-HT in the synapse, causing the inhibition of serotonergic phase resetting seen here. In contrast with the present results, however, is our recent observation in the mouse SCN brain slice preparation that acute EtOH administration dose dependently enhances daytime 8-OH-DPAT-induced phase advances to a maximal ~30% increase (68). It was hypothesized that this enhancing effect of EtOH is manifest through its inhibition of glutamate (photic) signaling in the SCN. This idea is based on the concept that, since glutamate agonists inhibit nonphotic phase resetting (7, 65), suppression of glutamate response would lead to enhanced serotonergic shifting. The reason for the differential 8-OH-DPAT shifting response to acute EtOH between the *in vivo* and *in vitro* studies is unknown but could relate to species difference or more likely a strong shift-inhibiting action of EtOH on 5-HT pathways registered outside of the SCN in the *in vivo* experiment, which would not be seen in the deafferented SCN slice.

Another potential target for EtOH nonphotic effects is the γ -aminobutyric acid (GABA)-A/benzodiazepine receptor. GABA-A receptor activity is affected by EtOH (53), and central components of the circadian system express GABA and the GABA-A receptor subtype (26). Moreover, treatment of hamsters with the benzodiazepine triazolam produces phase-advance and phase-delay shifts thought to be mediated by the GABA-A receptor (93). It is notable in this regard that these phase-resetting actions of triazolam are inhibited by chronic EtOH, an effect that could be based on the well-documented desensitizing effects of chronic EtOH both on the GABA-A receptor and benzodiazepine action (30, 48). We also have preliminary data that treatment of the mouse SCN slice with a GABA-A antagonist (RO15-4513) blocks EtOH's inhibition of photic

(glutamate) phase-delay shifts and enhancement of 8-OH-DPAT phase-advance shifts (66), strengthening the argument for a role of the GABA-A receptor in mediating EtOH actions on photic and nonphotic phase-resetting pathways in the SCN.

The profile of EtOH in the SCN region following intraperitoneal injection of EtOH was assessed to characterize the central pharmacokinetics of EtOH in the Syrian hamster to offer a comparison with other rodents and to confirm in our light pulse experiment that the timing of the light pulse overlaps completely with sufficiently raised EtOH levels in the SCN. This was confirmed, since the 30-min light pulse was started ~20 min after EtOH injection, which coincided with near peak levels of SCN EtOH occurring 20–40 min postinjection, and lasting over an additional 40-min period. The estimated peak concentration of EtOH in the SCN extracellular fluid compartment was ~50 mM, which is within the effective dose range for blocking glutamate-induced phase-advance shifts in the mouse SCN slice (68). This pharmacokinetic profile of EtOH in the hamster SCN is similar to that seen in the rat nucleus accumbens (18, 95) and striatum (35) following intraperitoneal injection of the same dosage of EtOH as used here (2.0 g/kg), with peak levels (~60 mM) occurring within 15–30 min postinjection. The $t_{1/2}$ for clearance estimated from graphs from these papers ranged from ~1.5 to 3.0 h, which is similar to the 3-h period in the present study.

Perspectives and Significance

The present study is the first to demonstrate that acute EtOH dose dependently blocks *in vivo* light-induced phase-advance shifts. Such an effect, in theory, could severely impair photic entraining input to the circadian clock. We also provide evidence that serotonergic signaling in circadian pathways for nonphotic phase regulation is impaired by EtOH. This dual blockade of photic and nonphotic entrainment mechanisms could underlie the highly disruptive effects of alcohol abuse on rhythms such as the sleep-wake cycle and those of the hypothalamic-pituitary-adrenal axis associated with developing alcoholism. Notably, this study also provides a framework for future investigations by confirming that the SCN clock is a direct target for EtOH's effects in the circadian timing system.

ACKNOWLEDGMENTS

We thank Dr. Mahinda Gangoda for assistance in measuring EtOH with GC.

GRANTS

This work was supported by National Institute on Alcohol and Alcohol-Related Disorders Grant AA-015948 to R. A. Prosser and J. D. Glass.

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