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Phase–Response Curve for Ethanol: Alterations in Circadian Rhythms of Temperature and Activity in Rats

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BAIRD, T. J., R. J. BRISCOE, M. VALLETT, S. A. VANECEK, F. A. HOLLOWAY AND D. V. GAUVIN. *Phase–response curve for tthanol: Alterations in circadian rhythms of temperature and activity in rats.* PHARMACOL BIOCHEM BEHAV **61**(3) 303–315, 1998.— Circadian rhythms of core body temperature and general activity in Sprague–Dawley rats were monitored for 21 days using remote radiotelemetry to examine acute and sustained effects of 0 (saline) 1.0, and 2.0 g/kg ethanol injections administered at four different times of day. Ethanol produced dose-dependent and statistically significant hypothermia and hypoactivity when injected at 0100, 0700, 1300, and 1900 h; however, the magnitude of the hypothermic effect was greatest at the 1900-h injection time. Cosinor analyses revealed persistent alterations in both activity and temperature rhythms, which lasted for at least 48 h postinjection. Ethanol significantly shortened the period of activity rhythms when injected in either 1.0 or 2.0 g/kg doses at 0700 and 1300 h, and produced similar period-shortening effects on temperature rhythms at 1300 and 1900 h. The acrophase of the activity rhythm was significantly phase delayed by 1.0 g/kg ethanol at 0700 h, while the acrophase of temperature was significantly phase advanced by 2.0 g/kg ethanol at 0100 h, but significantly phase delayed by the same dose administered at 1300 h. A statistically significant and dose-dependent reduction in the amplitude of the body temperature rhythm was observed at the 1900-h administration time. There were no differences in the MESOR (Midline Estimating Statistic of Rhythm; i.e., rhythm-adjusted mean value) of either temperature or activity circadian rhythms as a function of ethanol treatment at any dose. © 1998 Elsevier Science Inc.

Ethanol Circadian rhythm Body temperature Locomotor activity Chronopharmacology Cosinor analysis Phase shift Hangover

IT is increasingly recognized that organisms are differentially sensitive to the pharmacological effects of many drugs as a function of their time of administration within the light/dark (L/D) cycle. Chronopharmacology describes both a scientific discipline concerned with basic research on the mechanisms mediating time dependency in the pharmacological actions of drugs, as well as the applied practice of timing drug treatment so as to effect maximum therapeutic efficacy, while minimizing the magnitude or probability of adverse drug reactions (23). The term, chronotoxicity, alludes to the fact that many organisms are more sensitive to the toxic and lethal effects of drugs and environmental or other poisons as a function of the time of day at which exposure occurs (6,17,24,27,34). Chronopharmacology research indicates that many drugs of abuse display differential potency and/or efficacy as a function of the circadian phase at which they are administered (19,27,30).

Circadian phase-dependent effects of ethanol on temperature regulation and behavior have been observed in laboratory animals (2,45). Ethanol toxicity also has been shown to express circadian variability in rodents (5,18,34). A circadian rhythm in the development of tolerance and sensitization to the hypothermic effect of ethanol has been reported by Williams et al. (45), suggesting that, in addition to modulating the acute effects of drugs, circadian rhythms may also influence the development of long-term adaptive responses to repeated drug administration. A recent report from this laboratory (10)

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has replicated a reliable finding that ethanol self-administration in laboratory rats is also characterized by a circadian pattern favoring greater relative intakes in the dark phase of the light/dark cycle (8,10,15,16,22,35).

Findings of time-of-administration dependency in the effects of ethanol are not limited to laboratory animals, but have also been documented in studies with human subjects. Several investigators have demonstrated, for example, that ethanol has differential sedative, hypnotic, performance, and subjective effects in humans according to the time-of-day of its administration (31,33,46). The circadian pattern of self-administration in humans is characterized by increased consumption of ethanol as the day progresses, a finding that appears to be strongly related to availability, accessibility, and sociocultural influences (4). Circadian variability in self-administration and acute responses to ethanol's multiple effects is also accompanied by time-of-day differences in ethanol elimination kinetics in humans and laboratory animals (25,36,40-42,47).

Circadian changes in the pharmacodynamics and pharmacokinetics of ethanol, in addition to changes in tissue sensitivity, may account for a large share of the observed circadian variability in response to ethanol (23,31), and may also influence 24-h patterns of ethanol self-administration in humans and laboratory animals. An examination of the temporal relation between consumption levels, circadian pharmacodynamics, pharmacokinetics, and circadian susceptibility to pharmacologic and toxic effects of ethanol reveals that the interrelationships among these variables are complex. Peak intake in rats occurs during the early phase of the dark cycle (10), and coincides with onset of the phase of increased general activity, relatively slow ethanol elimination rates (41), and enhanced susceptibility to stimulatory effects of low-dose (0.5 g/kg) ethanol on locomotor activity (2). In humans, peak intake occurs later in the light phase (4), and corresponds to the approximate end of the activity period, intermediate values for ethanol elimination rate (25,42), and heightened sensitivity to ethanol's interoceptive cues and performance-impairing effects (31).

Numerous interactions between ethanol and circadian, ultradian, and infradian rhythms have been reviewed by el-Guebaly (5). It is important to note that ethanol's acute, as well as its delayed effects (i.e., hangover, withdrawal), result in disruptions across a wide range of physiological and behavioral systems that are characterized by regular, cyclic variations across an approximate 24-h period. Numerous reports have revealed an association between chronic ethanol consumption and dysregulation in the circadian patterns of several physiological systems (1,7,22,32). It has recently been hypothesized that disruption of circadian rhythms may be a key etiologic factor in the pathogenesis of hangover (12). Gauvin, Cheng, and Holloway (12) have reviewed literature conceptualizing the hangover state as 1) a physiologic rebound or opponent process (37,38) from the initial drug effect (39), driven by the need to maintain homeostasis; 2) a toxic reaction to congeners or metabolites of ethanol (21); and 3) a disruption in the natural circadian variation of multiple physiological systems (9,12,20). There is evidence that all three of these interpretations may be valid, indeed, it has been suggested that they may not be mutually exclusive, but interactive in generating the hangover state (12).

Some recent reports from this laboratory suggest that circadian rhythm desynchronosis may represent a pertinent factor in the pathogenesis of hangover. First, there appears to be subjective similarity between interoceptive states engendered by photoperiod phase shifts and by acute ethanol withdrawal (11). In this study, rats trained to discriminate hangover, induced by 18-h pretreatments of 4.0 g/kg ethanol, from normal homeostasis in a drug discrimination paradigm completely generalized a photoperiod phase-advance of 8 h to the hangover training cue. Second, acute injections of 4.0 g/kg ethanol produced alterations in the circadian rhythms of core body temperature, general activity, and electrocortical activity in delta, theta, alpha, and beta frequency bands (12). Though this data was not analyzed to specifically identify which circadian parameters may have been affected, the temporal parameters of the circadian rhythm disruption in these dependent variables appeared to correspond closely with the time course of hangover discriminability established in previous drug discrimination experiments (13,14), and agrees well with the emergence and resolution of hangover symptomatology in humans (12). Other reports suggesting that ethanol may cause sustained alterations in circadian rhythms of laboratory animals (9,20,26,29,43) lend some degree of validity to the desynchronosis hypothesis.

The purpose of the current project was to examine the chronopharmacology of ethanol, in terms of the acute and delayed effects of differentially timed injections on circadian rhythms of temperature and activity in rats. The effects of acute ethanol injections on four circadian rhythm parameters, including mesor, amplitude, acrophase, and period, were evaluated to determine the extent to which hangover might be related to an ethanol-induced disruption of circadian rhythms. Partial evidence supporting this hypothesis would be obtained if the temporal patterns of any alterations in these parameters correlate with the temporal onset and decay of the hangover state. It has been previously noted that alterations in circadian rhythms, such as those produced secondary to ethanol and L/D shifts, engender similar subjective states and symptoms. Analysis of the dose- and time-dependent effects of ethanol on circadian rhythms will allow evaluation of the validity of opponent processes and circadian disruptions as potential mechanisms in the pathogenesis of hangover. The general hypotheses were that experimental subjects would demonstrate increased susceptibility to ethanol-induced hypothermia and hypoactivity early in the dark phase of the L/D cycle, and that ethanol would differentially alter parameters of circadian temperature and activity rhythms, including the period, acrophase, amplitude, and mesor as a function of drug dose and time-of-day at which it was administered.

METHOD

Subjects

Ninety-nine male Sprague–Dawley rats (225–275 g) were sequentially purchased from Sasco, Inc. (Omaha, NE) and given 1 week to acclimate to the laboratory environment before any experimental procedures were performed. Each group of animals was 3 months of age (±10 days) when surgeries and subsequent radiotelemetry recordings began. Remote radiotelemetry transmitters failed in three subjects during the course of recording. Three additional experimentally naive rats were subsequently implanted with transmitters and substituted for these losses. Animals were individually housed in standard plastic "shoe-box" cages within a temperature- and humidity-controlled colony room having a 12 L:12 D cycle, with lights on at 0600 and off at 1800 h, and were given standard laboratory rat chow and water ad lib. The American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited colony room was maintained by trained technicians and veterinarians from the Department of

Animal Resources at the University of Oklahoma Health Sciences Center throughout the course of the study.

Surgical Procedures

Following the 1-week acclimation period, each rat was implanted intraperitoneally with a remote radiotelemetry transmitter (Model XMFH, Minimitter Co., Inc., Sunriver, OR) calibrated for core body temperature and activity monitoring. Surgeries were performed under sodium pentobarbital (35 mg/kg IP), and ketamine (30 mg/kg IP) anesthesia with a 15-min pretreatment of 0.1 ml atropine SC. (0.54 mg/ml). Rats were given 1 week for recovery postsurgery, and allowed ad lib access to food and water for the remainder of the experimental procedures. Upon completion of recording, rats were euthanized with an overdose (100 mg/kg) of pentobarbital. Death was insured by cardiac transection. The radiotelemetry transmitters were subsequently removed, washed with soap and water, rinsed, and chemically sterilized in 5% v/v benzalkonium chloride (Zephiran chloride, Winthrop Laboratories, NY). Transmitters were surgically reimplanted in subsequent groups of rats for the duration of the life of the internal battery (5 months). Battery replacements and recalibrations were completed by Minimitter, Inc. (Sunriver, OR).

Remote Radiotelemetry

Activity and temperature were recorded continuously (24 h/day) in eight rats over the 21 days in which experimental procedures were implemented. Temperature and activity data were acquired serially in 10-min bins by Dataquest III software interfaced with a Data Sciences (St. Paul, MN) radiotelemetry data collection system. Data were collected and stored on a 386DX IBM-clone personal computer system (American Neuroscience Research Foundation, Yukon, OK), interfaced with eight Dataquest (RA1010) radio receivers through a BCM100 consolidation matrix and DQ1088 interface card (Minimitter Co., Inc., Sunriver, OR). Temperature was sampled once for each 10-min recording bin. Raw frequency data were transmitted from each implanted telemetry disc to the computer, and converted to temperature readings from a standard calibration curve by the Dataquest software. General activity was transmitted to the computer in the form of digital pulses, marking either horizontal or vertical movement of the rat within the shoebox cage. Individual "events" were recorded continuously over each 10-min recording bin, and the total number of counts per bin was then tabulated by the computer and stored at the end of each sampling interval.

Experimental Design

A mixed factor within- and between-groups design was utilized to provide data on the effects of three doses [0 (saline), 1, and 2 g/kg] of ethanol, administered at circadian times 0100, 0700, 1300, and 1900 h, on daily rhythms of activity and temperature, as detailed in Table 1. The design of the experiment required 12 groups (three doses × four time points) of rats, with $n = 8$/group (total $n = 96$) for the application of parametric statistics. The first 7 days of each 21- day recording epoch provided a baseline recording against which the effects of both saline and ethanol injections could be assessed. One saline injection was given on day 8 in all groups, and one injection of either 0 (saline), 1.0, or 2.0 g/kg ethanol was administered on day 16 according to dose–group designations. Thus, saline and ethanol treatments were separated by 7 days within each dose group. This series of injections was repeated across each of the four times of day noted above. The within-subjects component was added to the design for two reasons: 1) to control for potential circannual shifts in the homeostatic set points of the dependent variables, which would be expected to produce significant shifts in between-group baselines due solely to the sequence of monitoring across the 15 months of recording required by this study; and 2) drugs, handling, and injections may serve as "setting" events for the endogenous biological clock(s) themselves. The independent group design would control for these contingencies.

Ethanol Injections

Ethanol injections, 1.0 or 2.0 g/kg, were administered intraperitoneally from a 10% w/v stock solution. The stock solution was made by mixing appropriate volumes of pure ethanol (95%; Quantum Chemical Co., Tuscola, IL) in normal (0.9%) saline (44). Saline injections, isovolumetric to the ethanol treatments, were also administered IP.

Data Analysis

Core body temperature and activity data for each rat were recorded for 21 consecutive days. Consecutive periods were selected from each of the 7-day baseline, saline, and treatment epochs (O_1, O_2, O_3 see Table 1) to examine the acute effects of ethanol on activity and temperature. Multivariate analyses

TABLE 1
EXPERIMENTAL DESIGN

	Time of Day			
Dose ETOH	0100	0700	1300	1900
0.0 g/kg (saline)	O_1, O_2, O_3	O_1, O_2, O_3	O_1, O_2, O_3	O_1, O_2, O_3
1.0 g/kg	O_1, O_2, O_3	O_1, O_2, O_3	O_1, O_2, O_3	O_1, O_2, O_3
2.0 g/kg	O_1, O_2, O_3	O_1, O_2, O_3	O_1, O_2, O_3	O_1, O_2, O_3

The time of day at which groups received ethanol (ETOH) injections is expressed on a 24 h clock (0100 = 1:00 a.m., 1300 = 1:00 p.m.). Doses of ethanol administered are expressed in grams of drug per kilogram body weight. In each group, observation 1 (O_1) represents the 7-day baseline condition, observation 2 (O_2) represents the 7-day epoch immediately following one saline injection, and observation 3 (O_3) corresponds to the 7-day epoch immediately following one ethanol injection.

of variance (MANOVAs) were used to test for significant differences in temperature- and activity-dependent measures as a function of ethanol dose and circadian time (relative to isovolumetric saline). Circadian time of injection (four levels), ethanol dose (three levels), and observation epoch condition (three levels) were loaded as between-subjects factors, and time was loaded as a within-subjects factor. Once significant MANOVA effects were found, further univariate ANOVAs were conducted within each individual (dose- and time-of-administration) group for activity and temperature data, and post hoc Tukey HSD tests were used to test for significant differences between baseline, saline, and ethanol epochs.

Cosinor analyses were performed on raw temperature and activity data files. Four circadian rhythm parameters, including period, mesor, amplitude, and acrophase, were calculated for the baseline, saline, and ethanol treatment epochs of each group for both activity and temperature data. MANOVAs were run on all groups on each of these four dependent variables. Circadian time, ethanol dose, and observation epoch were again loaded as between-subjects factors. One-way ANOVAs were used to test for significant differences between baseline, saline, and ethanol treatment conditions within each dose and time group on all four dependent measures. Individual comparisons were conducted with Tukey HSD post hoc tests.

RESULTS

Acute Ethanol Chronopharmacology

An analysis of the full experimental design was first conducted with MANOVAs on temperature and activity data. Due to their large size, temperature records of each 7-day recording epoch were broken down into five smaller files covering approximately 33.6 h (1.4 days) each. Activity records were broken down in the same manner, and subsequently both temperature and activity files were analyzed with omnibus MANOVAs. Significant main time-of-day, $F(3, 252) = 9.08$, $p < 10^{-5}$, main dose, $F(2, 252) = 8.36$, $p < 0.001$, main observation epoch, $F(2, 252) = 10.78$, $p < 10^{-4}$, and main time, $F(199, \infty) = 5.83$, $p < 10^{-6}$, effects were found for activity in the first (33.6 h) epoch. However, main dose effects were nonsignificant in the last four activity epochs. Significant main group and main time effects were found throughout all five temperature periods analyzed, but main dose effects were all nonsignificant.

Given that the main dose effects were not statistically significant for the entire temperature record, and were significant only for the first 33.6 h of the activity record, the dose-dependent effects of ethanol on general activity and temperature appeared to be relatively transient. The hypothermic effects of ethanol at each of the four different administration times of day, 0100, 0700, 1300, and 1900 h, are depicted in Figs. 1–4, respectively. The effects of ethanol, at these same four injection times, on general activity are displayed in Figs. 5–8. Because ethanol's acute effects on both temperature and activity were of relatively short duration, and essentially limited to a 2–4-h period after the injections, a more restricted analysis was conducted on temperature and activity readings for the first 3 h postinjections. Significant main time-of-day, $F(3, 252) = 21.38$, $p < 10^{-6}$, main dose, $F(2, 252) = 6.53$, $p < 0.01$, main observation epoch, $F(2, 252) = 6.20$, $p < 0.01$, and main time, $F(17, \infty) = 12.16$, $p < 10^{-6}$, effects were observed in the overall MANOVAs conducted on the truncated temperature records. Main time-of-day, $F(3, 252) = 13.64$, $p < 10^{-6}$, main dose, $F(2, 252) = 16.95$, $p < 10^{-6}$, main observation epoch, $F(2, 252) = 26.81$, $p < 10^{-6}$, and main time, $F(17, \infty) = 78.26$, $p < 10^{-6}$, effects were statistically significant for activity, as well.

Due to significant baseline differences between groups, univariate ANOVAs were used as simple-effects tests to analyze within-group (i.e., baseline epoch vs. saline epoch vs. ethanol epoch) differences in the 1.0 and 2.0 g/kg ethanol treatment groups at 0100, 0700, 1300, and 1900 h administration times. All within-group comparisons were thus made for baseline, saline, and ethanol treatment epochs within each of eight dose and administration time groups for activity and temperature. Injections of 1.0 g/kg ethanol did not produce a statistically significant hypothermic effect at any of the four injection times, while the 2.0 g/kg dose produced significant hypothermia at 1300 h, $F(2, 21) = 7.50$, $p < 0.004$, and 1900 h, $F(2, 21) = 3.60$, $p < 0.05$, only. Post hoc tests revealed that the hypothermia was specific to ethanol, when compared to saline, treatment epochs at both 1300 h, ($p < 0.006$), and 1900 h ($p < 0.046$). Relative to saline, 1.0 g/kg ethanol produced significant hypoactivity, $F(2, 21) = 4.61$, $p < 0.022$, at the 0100-h administration time ($p < 0.017$), whereas 2.0 g/kg ethanol produced significant decreases in activity at the 0100 h, $F(2, 21) = 16.21$, $p < 10^{-4}$, 0700 h, $F(2, 21) = 6.53$, $p < 0.007$, 1300 h, $F(2, 21) = 13.89$, $p < 10$), and 1900 h, $F(2, 21) = 11.16$, $p < 0.0005$, administration times. Post hoc tests on the 2.0 g/kg ethanol injection groups revealed significant differences between the ethanol and saline observation epochs at 0100 h ($p < 0.0003$), 0700 h ($p < 0.02$), 1300 h ($p < 0.0006$), and 1900 h ($p < 0.0005$).

Figure 9 illustrates the differential magnitude of change in core body temperature (hypothermia) and activity (hypoactivity) in the 1.0 and 2.0 g/kg ethanol dose groups as a function of injection time. Temperature change scores were calculated by identifying the lowest temperature reading within 3 h postinjection for each individual rat in each group, and subtracting this value from its own baseline temperature, taken as the mean value over the 1 h immediately prior to ethanol injection. Activity change scores were calculated by subtracting the group mean activity count for the first 3-h postethanol injection from each rat's individual baseline activity for the 1 h immediately preceding injection. The group average activity for the 3 h following ethanol administration was used to calculate individual activity change scores because, in contrast to the temperature records, the "peak" or maximum effect of ethanol on activity was not as easily identifiable in individual subject records, but is clearly evident within the first 3 h postinjection in the group-averaged data. The individual activity and temperature change scores were then averaged to yield the values depicted in Fig. 9. Overall MANOVAs conducted on the change scores for all conditions revealed significant main time-of-day, $F(3, 56) = 2.98$, $p < 0.04$, and main dose effects, $F(1, 56) = 21.84$, $p < 10^{-4}$, for temperature, but only a significant main time-of-day effect, $F(3, 56) = 3.67$, $p < 0.02$, for activity. Univariate ANOVAs were run within each ethanol dose condition to test for significant differences as a function of the four different administration times over the 24-h cycle. The magnitude of hypothermia produced by 1.0 g/kg ethanol was significantly different across the four injection periods, $F(3, 28) = 5.45$, $p < 0.005$. Hypothermia was greatest at 1900 h, relative to all other injection times ($p < 0.05$ for all post hoc comparisons). Similarly, the magnitude of the hypothermia induced by 2.0 g/kg dose was variable across injection times, $F(3, 28) = 3.77$, $p < 0.03$; the hypothermic response was greatest at the 1900 h injection time relative to the 0100 h ($p < 0.05$) and 0700 h ($p < 0.04$), but not the 1300 h ($p = 0.58$) injection time. Hypothermia at 1300 h, following the 2.0 g/kg dose was not significantly different from values observed at

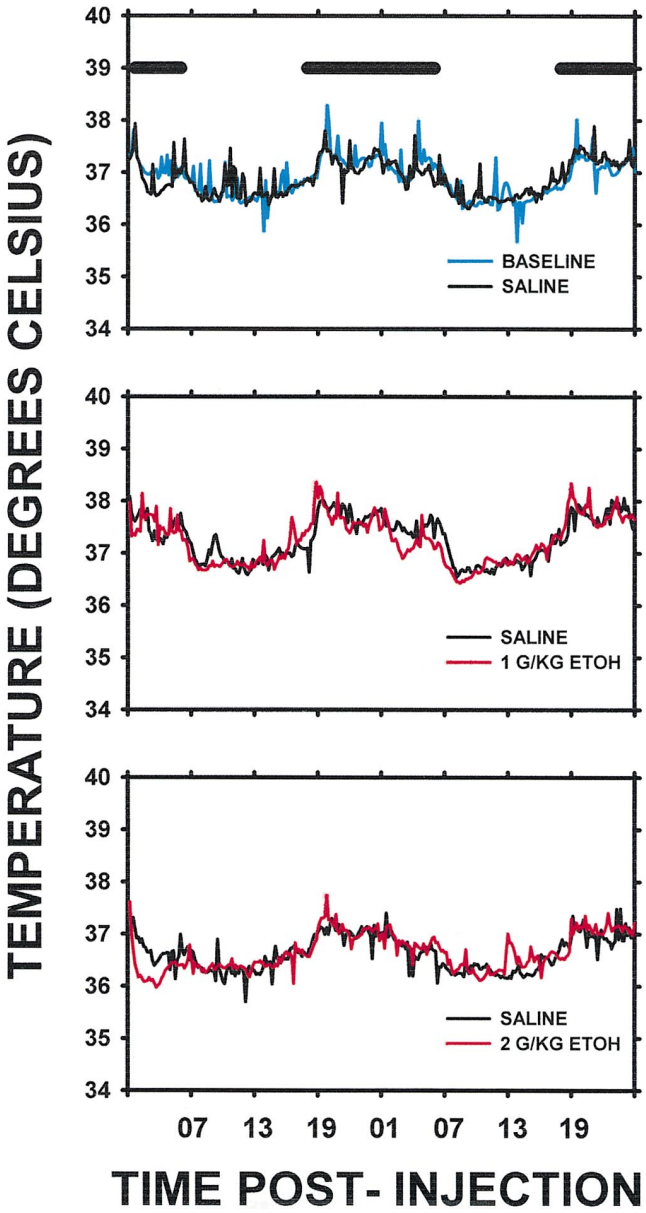

FIG. 1. Ethanol hypothermia at 0100 h. Group mean temperature values (°C) beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on core body temperature are displayed. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

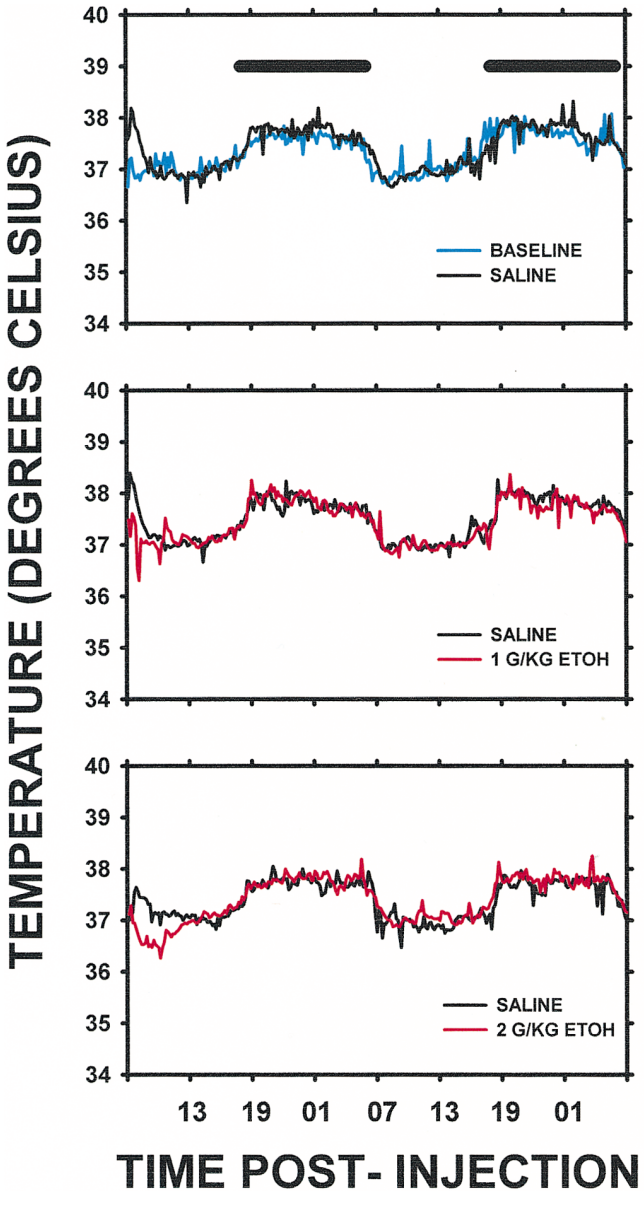

FIG. 2. Ethanol hypothermia at 0700 h. Group mean temperature values (°C) beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on core body temperature are displayed. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

0100, 0700, or 1900 h (all $ps > 0.05$). Concordant with the significant hypothermic response, a group-dependent hypoactivity was observed, $F(3, 28) = 3.14$, $p < 0.05$. The hypoactivity was significantly greater at the 1900-h injection time, relative to 0700 h, for the 2.0 g/kg ethanol dose, only ($p < 0.04$). There were no statistically significant differences in ethanol's effect on general activity between the four different times of administration at the 1.0 g/kg dose $F(3, 28) = 0.94$, $p = 0.43$.

Cosinor Analyses

Cosinor analyses were used to generate several circadian rhythm parameters, including period, mesor, amplitude, and acrophase, to examine any sustained effects of 1.0 and 2.0 g/kg ethanol injections on activity and temperature rhythms. Cosinor data were compiled on 72-h long temperature and activity epochs, beginning at 2400 h (midnight) for each of the baseline, saline, and ethanol treatment epochs examined. Tables 2 and 3 display group mean values for the four parameters (± standard errors) calculated on activity and temperature records, respectively. Segments of the activity data records from two rats in the 1300 h, 2.0 g/kg group were lost, and as a result, reliable cosinor parameters could not be generated for these subjects. Data from these subjects were therefore excluded from the analysis.

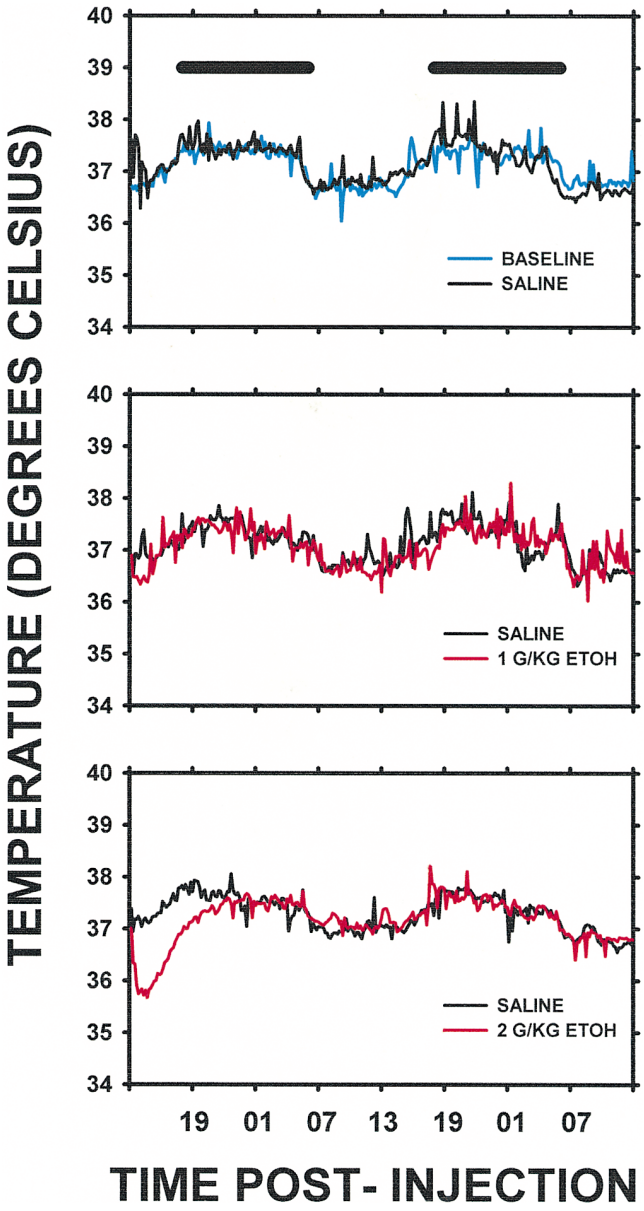

FIG. 3. Ethanol hypothermia at 1300 h. Group mean temperature values (°C) beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on core body temperature are displayed. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

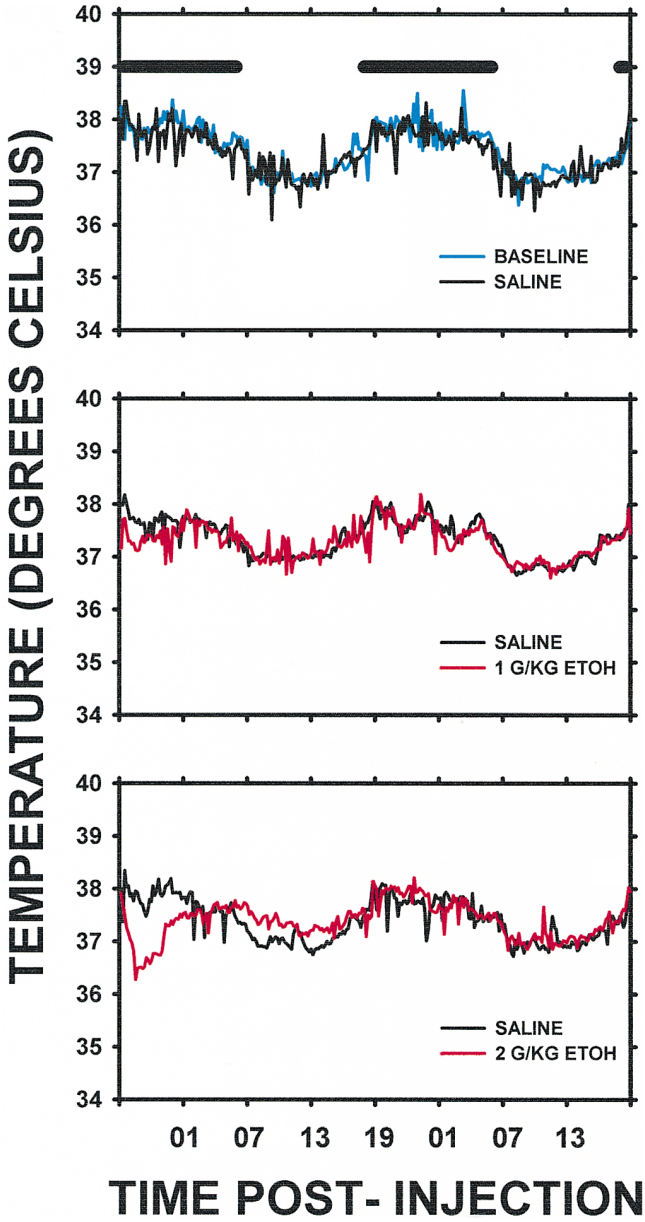

FIG. 4. Ethanol hypothermia at 1900 h. Group mean temperature values (°C) beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on core body temperature are displayed. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

A significant main group effect was observed in the overall MANOVA on the cosinor-derived period, $F(3, 83) = 7.81$, $p < 0.001$, of the temperature rhythm. Univariate tests revealed that the period of the body temperature rhythm was significantly shortened, $F(2, 21) = 23.00$, $p < 10^{-5}$, by 1.0 g/kg ethanol at 1900 h ($p < 0.001$), and by 2.0 g/kg ethanol at 1300 h, $F(2, 21) = 8.17$, $p < 0.003$, and 1900 h, $F(2, 21) = 5.30$, $p < 0.02$ (see Table 3). Post hoc tests revealed that these changes were significantly different from saline treatment at both 1900 h ($p < 0.04$) and 1300 h ($p < 0.004$). Cosinor analyses demonstrated that the mesor of the body temperature rhythm was unaffected by any dose of ethanol at any administration time. Alterations in the amplitude of the temperature rhythm were produced by ethanol administration, as corroborated by a significant main dose effect in the overall MANOVA $F(2, 83) = 3.88$, $p < 0.03$. The temperature rhythm amplitude was dose dependently attenuated by ethanol at 1900 h; the 1.0 g/kg dose decreased, $F(2, 21) = 8.42$, $p < 0.003$, the rhythm amplitude by 25.2% ($p < 0.002$), relative to saline, while the 2.0 g/kg dose was associated, $F(2, 21) = 5.26$, $p < 0.02$, with a 38.2% amplitude reduction ($p < 0.02$), again, relative to the saline

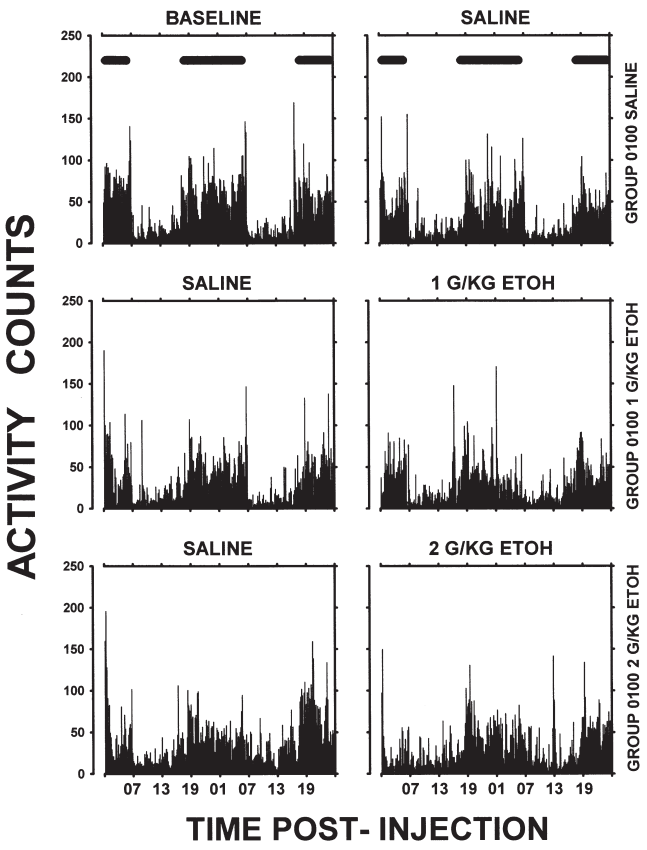

FIG. 5. Ethanol hypoactivity at 0100 h. Group mean values for general activity beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on general activity are displayed. Each bar represents the average of activity counts accumulated for eight rats per 10-min bin. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

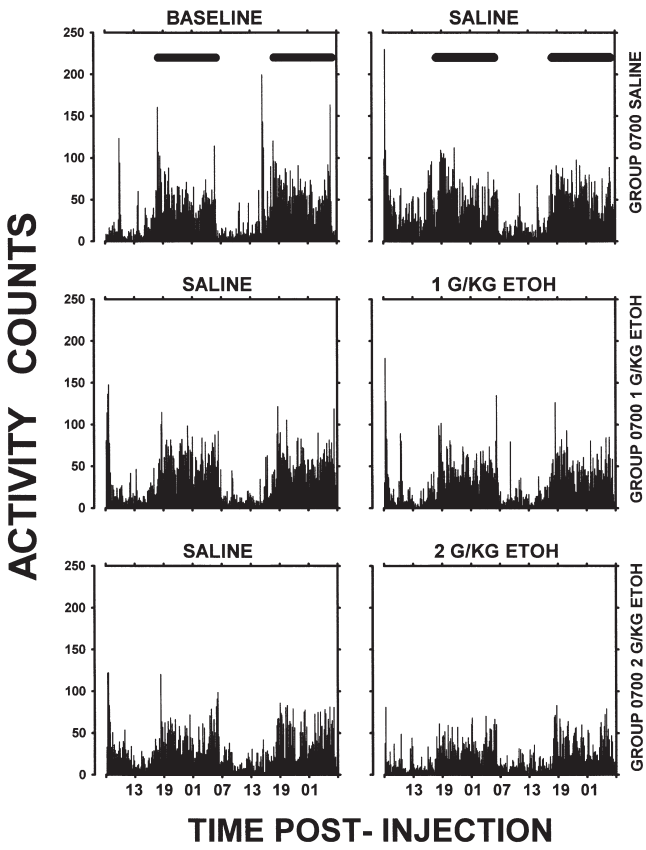

FIG. 6. Ethanol hypoactivity at 0700 h. Group mean values for general activity beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on general activity are displayed. Each bar represents the average of activity counts accumulated for eight rats per 10-min bin. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

epoch. Overall MANOVAs revealed significant main group, $F(3, 83) = 4.09$, $p < 0.01$, and main dose, $F(2, 83) = 4.77$, $p < 0.01$, effects on the acrophase of the core body temperature rhythm. Significant shifts in the acrophase of the temperature rhythm were observed at 0100 h, $F(2, 21) = 3.81$, $p < 0.04$, and 1300 h, $F(2, 21) = 3.61$, $p < 0.05$, for the 2.0 g/kg ethanol dose only. Post hoc tests indicated that the acrophase was advanced by 114 min at 0100 h ($p < 0.05$), and delayed by 207 min at 1300 h ($p < 0.04$).

Two activity rhythm parameters, period and acrophase, were altered by acute ethanol injections (see Table 2). Significant main group, $F(3, 83) = 9.90$, $p < 10^{-4}$, and main dose effects, $F(2, 83) = 6.09$, $p < 0.01$, were found for period, and a significant main group effect was also found for the activity acrophase, $F(3, 83) = 7.08$, $p < 0.001$, in overall MANOVAs conducted on all dose and injection time conditions. The period of the circadian rhythm was significantly affected by both 1.0, $F(2, 21) = 12.77$, $p < 0.001$, and 2.0 g/kg ethanol, $F(2, 19) = 14.56$, $p < 0.001$. Specifically, the period of the general activity rhythm was shortened by injections of 1.0 g/kg at 0700 h ($p < 0.001$), and by 2.0 g/kg of ethanol administered at 1300 h ($p < 0.001$). The mesor of general activity was not affected by any dose of ethanol at any time of day. The amplitude of the activity rhythm was likewise unaffected by ethanol treatment; however, the acrophase of activity was significantly shifted, $F(2, 21) = 4.73$, $p < 0.03$, by 1.0 g/kg ethanol at 0700 h ($p < 0.04$), indicating a phase delay of 126 min.

DISCUSSION

The present study describes the chronopharmacologic profile of ethanol administered acutely at four different time points within the circadian cycle of a normal 12 L:12 D cycle. Ethanol, at 2.0 g/kg, produced significant hypothermia relative to both baseline values and following isovolumetric saline injections when administered at 1300 and 1900 h. When temperature changes were evaluated in terms of the absolute deviation from baseline, the groups injected at 1900 h displayed the most pronounced hypothermic effect at both 1.0 and 2.0 g/kg doses. Activity was significantly decreased by 1.0 g/kg ethanol in the 0100 h group, and in all four injection times by the 2.0 g/kg dose. However, when change scores were analyzed, the only significant difference in ethanol-induced hypoactivity noted was between the 0700 and 1900h administration times for 2.0 g/kg dose. Nevertheless, the overall pattern of the activity change scores at the four administration times approximated that of the temperature data. One reason for the overall lack of statistical significance in activity change scores may

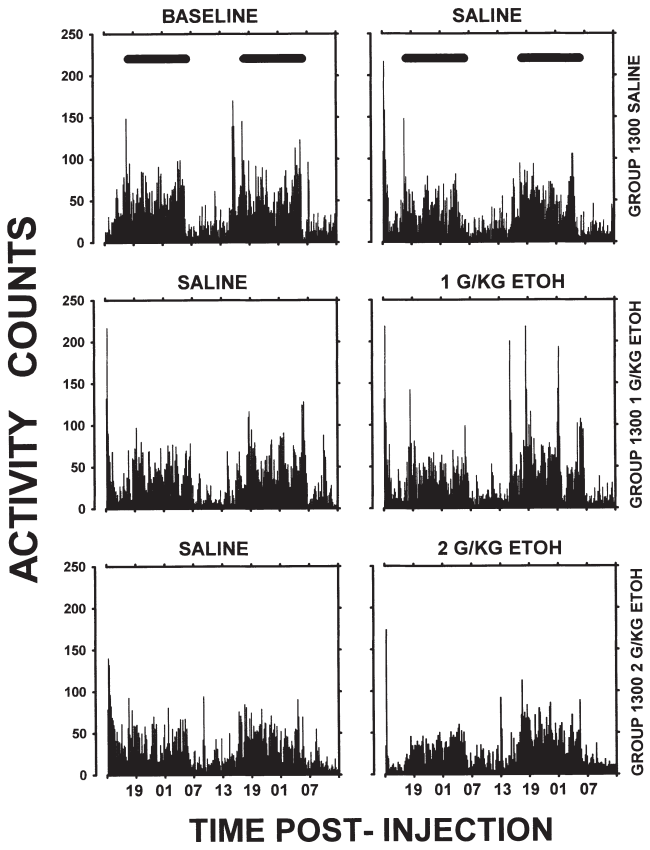

FIG. 7. Ethanol hypoactivity at 1300 h. Group mean values for general activity beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on general activity are displayed. Each bar represents the average of activity counts accumulated for eight rats per 10-min bin. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

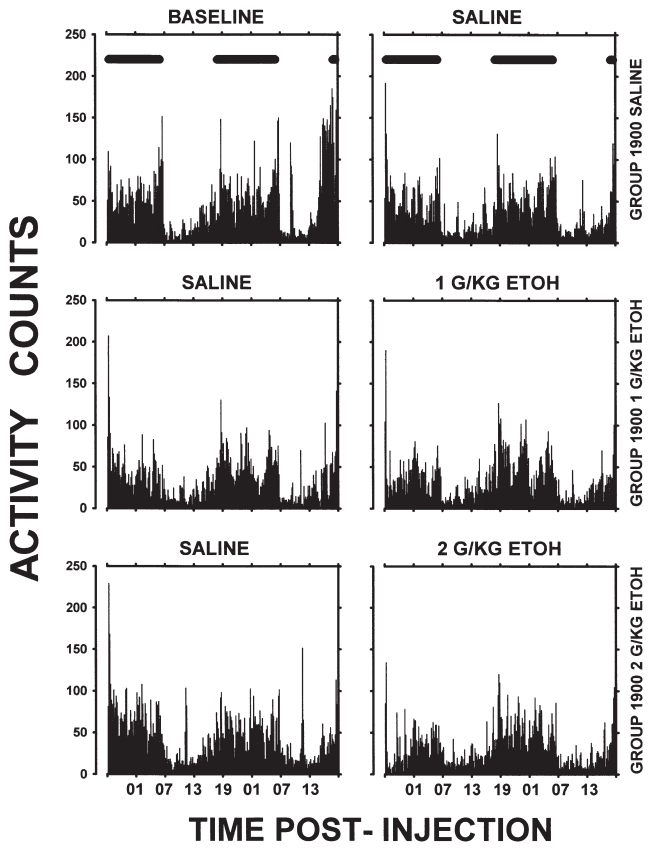

FIG. 8. Ethanol hypoactivity at 1900 h. Group mean values for general activity beginning at the time of injection, and continuing for 48 h postinjection. The effects of 0 g/kg (saline), 1.0 g/kg, and 2.0 g/kg ethanol on general activity are displayed. Each bar represents the average of activity counts accumulated for eight rats per 10-min bin. Dark bars indicate the dark phase of the L/D cycle. Standard error values are not shown for clarity.

be related to the tendency of all injection groups to exhibit a brief period of hyperactivity immediately postinjection, related to handling and/or the stress of the large bolus injections. This may have contributed additional measurement error to the relatively marked intra- as well as interindividual patterns observed in the activity data at baseline.

The results obtained in this experiment agree well with several studies that have systematically examined acute responses to ethanol in laboratory animals as a function of time of day. Williams et al. (45) found a circadian rhythm in the development of tolerance and apparent sensitization to ethanol hypothermia in rats. Tolerance was observed in those groups injected in the light phase of the L/D cycle, while sensitization was reported in rats injected in the dark phase. The greatest hypothermic response after one acute injection of 3 g/kg ethanol was observed at 1800 h, lowest at 0200 and 0600 h, and intermediate at 1400 h. Sauerbier reported significant increases in fetal toxicity (embryonic reabsorptions, anatomical malformations, etc.) when ethanol was administered in the early to intermediate portion of the dark phase (34). There was an orderly increase in the number of dead fetuses, from 0700 h (fewest) to 0100 h (most), and a similar orderly decrease in body weight of fetuses at death, from 0700 h (highest) to 0100 h (lowest). This agrees well with data demonstrating peak susceptibility to ethanol toxicity in mature mice in the early portion of the dark phase (5,18,28). Brick et al. (2) found that the greatest hypothermic response to ethanol in rats occurred when they were injected at 0600 h; however, low-dose ethanol induced significant elevations in activity at 2400 h, and significantly augmented the magnitude of a startle response, while a higher dose produced hypoactivity comparable to all other groups (0600, 1200, and 1800 h). The reason for the discrepancy in the circadian pattern of ethanol-induced hypothermia between our study and that of Brick et al. (2) is not clear, but may be related to differences in the L/D cycle and/or the time of injections. Nevertheless, it is interesting to note that Brick et al. (2) obtained the lowest blood alcohol levels (BALs) and observed peak values of aldehyde dehydrogenase at 2400 h. A recent study from our laboratory showed significantly lower BALs and faster ethanol elimination rates at 0100 h relative to 0900 and 1700 h in rats subjected to a cycling shift work schedule (10). Overall, the pattern of results obtained in the current study are consistent with prior work demonstrating the time-dependent pharmacology and toxicity of ethanol.

Ethanol altered circadian body temperature and activity rhythms according to the time of day of its administration.

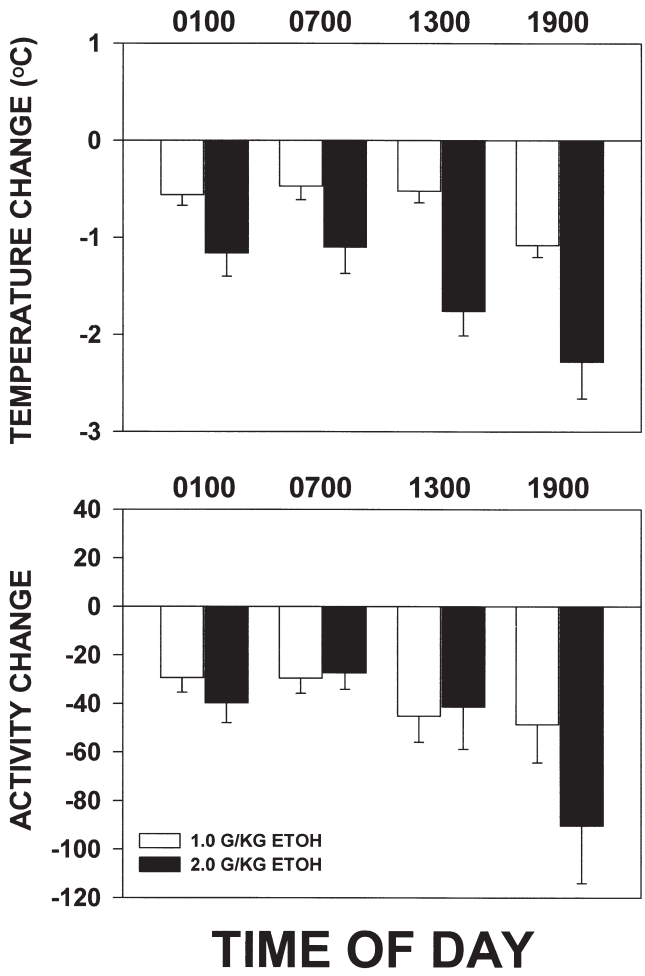

FIG. 9. Group-dependent activity and temperature changes. Magnitudes of change in core body temperature (top panel) and general activity (bottom panel) after injections of 1.0 g/kg and 2.0 g/kg ethanol are depicted. Bars represent group means (± SE).

Cosinor analyses revealed significant effects of ethanol treatment on three circadian rhythm parameters, period, acrophase, and amplitude, but the mesor of both temperature and activity was unaffected. Shortening of the period of activity and temperature rhythms was observed, and this effect was both dose and time dependent. At the 1.0 g/kg dose, the period of activity was shortened at the 0700 and 1900 h administration times, while at the 2.0 g/kg dose, the period of both temperature and activity rhythms was attenuated by injection at 1300 h. The 2.0 g/kg ethanol dose also shortened the period of temperature rhythm when administered at 1900 h. The effects on period appeared to be most consistent at the 1300 and 1900 h injection times, as period shortening was observed in both temperature and activity data at 1300 h, and both doses of ethanol effected period shortening at 1900 h. These results suggest a possible relationship between circadian susceptibility to the acute hypothermic and hypoactive effects of ethanol and the potential for altering the period of the circadian rhythm.

Ethanol significantly, and dose dependently, attenuated the amplitude of the temperature rhythm only at the 1900 h injection time. Interestingly, this amplitude reduction appears to underlie a slight "hyperthermic rebound" observed after 2.0 g/kg ethanol administration at 1900 h (Fig. 4). The onset of a "hyperthermic" rebound episode coincided with the normal circadian decline in temperature values observed around 0700 h, and its termination was coincident with the normal circadian rise in temperature observed around 1900 h. In other words, the entire period of apparent hyperthermia rebound, suggested to be a marker of ethanol hangover states, was contained within a time of day at which temperature values are normally at their lowest. Gallaher and Egner (9) have reported similar rebound effects after acute injections of 2.0, 4.0, and 6.0 g/kg ethanol. Effects such as these have been interpreted as reflecting a phase shift in the temperature rhythms of ethanol-treated animals, relative to that of saline treated controls. According to this line of reasoning, the apparent hyperthermia is really the result of either a phase advance, or a phase delay in the circadian rhythm of ethanol treated animals. Therefore, ethanol-treated groups are out of phase with saline groups, and only appear to exhibit a hyperthermic rebound.

The findings of this study suggest another possible interpretation. Attenuation of the temperature rhythm amplitude, such as that observed at 1900 h, may account for apparent hyperthermic rebounds. Accordingly, attenuation of the rhythm amplitude around its mean value would be expected to result in absence of the normal magnitude of decrease observed at the rhythm's nadir (trough value). However, due to the bidirectional nature of circadian temperature rhythms, hypothermia would also be predicted to occur at some point following a high-dose ethanol administration, due to the absence of the normal magnitude of temperature increase observed relative to normal conditions. Because ethanol also exhibited effects on period and phase in this study, it is also possible that some combination of phase shifting, amplitude attenuation, and period alterations may produce these results. These findings suggest that apparent rebound effects in body temperature following an acute dose of ethanol, in ethanol-naive subjects, may be more closely associated with sustained alterations of circadian rhythms than with the activation of compensatory opponent-processes governing temperature regulation.

The acrophase of activity was delayed by 1.0 g/kg ethanol at 0700 h, while the temperature rhythm was phase advanced by 2.0 g/kg ethanol at 0100 h, but phase delayed by the same dose at 1300 h. The delay of the temperature acrophase produced by ethanol at 1300 h may be related to the initial acute ethanol hypothermia in a straightforward manner. The magnitude of the hypothermia appears to have prevented the normal and gradual increase in temperature that is normally observed from 1300 to 1900h so that by the time the rhythm recovered, a significant phase delay was evident, relative to baseline and saline pretreatment conditions. The phase advance in temperature at 0100 h, and the phase delay in activity at 0700 h, are more difficult to explain in such simple terms. Some drugs appear to exert their actions on circadian rhythms indirectly, by modifying the characteristics of entraining stimuli influencing the pacemaker cells in the suprachiasmatic nucleus (SCN) of the hypothalamus, or by causing acute alterations in feedback to the SCN from peripheral sites at which a drug is active (3). In contrast, chronobiotics are defined as drugs that affect circadian rhythms through their direct actions on pacemaker function (3). The phase advance in temperature at 0100 h and the phase delay in activity at 0700 h produced by ethanol injections are not as obviously related to an acute effect on these variables as was observed at the 1300 h injection time. In fact, hypothermia and hypoactivity were

TABLE 2
COSINOR PARAMETERS CALCULATED ON ACTIVITY DATA

			Period	Cosine of Acrophase	Mesor	Amplitude
		Baseline	1409 ± 17	0.97 ± 0.02	46.6 ± 10.1	28.5 ± 5.7
	0 g/kg	Saline	1412 ± 10	0.91 ± 0.03	41.7 ± 9.2	26.0 ± 4.9
		Saline	1389 ± 13	0.89 ± 0.05	37.0 ± 6.0	22.7 ± 4.6
		Baseline	1468 ± 17	0.89 ± 0.05	51.4 ± 5.7	28.0 ± 3.0
0100h	1 g/kg	Saline	1429 ± 16	0.97 ± 0.04	42.5 ± 5.5	24.5 ± 3.3
		Etoh	1431 ± 16	0.94 ± 0.02	38.3 ± 5.2	21.1 ± 3.6
		Baseline	1469 ± 14	0.81 ± 0.07	40.0 ± 6.4	19.0 ± 4.6
	2 g/kg	Saline	1424 ± 20	0.92 ± 0.03	43.0 ± 5.4	24.5 ± 4.4
		Etoh	1444 ± 21	0.81 ± 0.08	35.9 ± 5.4	19.0 ± 4.0
		Baseline	1383 ± 11	0.65 ± 0.08	59.2 ± 5.6	43.7 ± 3.52
	0 g/kg	Saline	1415 ± 9	0.53 ± 0.12	40.1 ± 5.1	20.7 ± 1.8
		Saline	1451 ± 34	0.66 ± 0.14	42.3 ± 5.0	19.2 ± 2.0
		Baseline	1348 ± 17	0.93 ± 0.03	40.3 ± 4.7	27.1 ± 4.0
0700h	1 g/kg	Saline	1379 ± 10	0.94 ± 0.03	37.0 ± 4.4	25.2 ± 4.0
		Etoh	1283 ± 14	0.66 ± 0.12	35.2 ± 4.7	22.2 ± 3.9
		Baseline	1410 ± 12	0.92 ± 0.04	35.8 ± 5.0	23.0 ± 5.0
	2 g/kg	Saline	1366 ± 25	0.67 ± 0.16	32.9 ± 4.1	18.8 ± 2.6
		Etoh	1331 ± 8	0.61 ± .10	26.7 ± 4.5	16.7 ± 3.3
		Baseline	1342 ± 12	0.93 ± 0.06	39.7 ± 3.7	20.8 ± 2.2
	0 g/kg	Saline	1418 ± 18	0.76 ± 0.13	41.3 ± 5.6	16.2 ± 2.6
		Saline	1423 ± 25	0.66 ± 0.14	38.5 ± 4.3	17.8 ± 1.8
		Baseline	1391 ± 10	0.90 ± 0.04	45.2 ± 6.2	27.4 ± 5.0
1300h	1 g/kg	Saline	1408 ± 21	0.91 ± 0.04	38.2 ± 4.6	19.3 ± 2.9
		Etoh	1457 ± 19	0.76 ± 0.07	40.5 ± 4.5	21.1 ± 2.9
		Baseline	1429 ± 10	0.87 ± 0.05	40.6 ± 6.0	23.9 ± 5.0
	2 g/kg	Saline	1431 ± 11	0.81 ± 0.08	35.2 ± 4.6	17.1 ± 2.0
		Etoh	1341 ± 11	0.87 ± 0.05	31.3 ± 5.0	17.6 ± 2.8
		Baseline	1399 ± 13	0.45 ± 0.12	62.5 ± 7.2	32.9 ± 4.0
	0 g/kg	Saline	1442 ± 22	0.93 ± 0.02	42.2 ± 5.5	23.5 ± 4.2
		Saline	1430 ± 12	0.91 ± 0.05	39.3 ± 3.5	22.0 ± 3.2
		Baseline	1376 ± 8	0.69 ± 0.10	38.0 ± 7.4	22.9 ± 5.0
1990h	1 g/kg	Saline	1316 ± 16	0.65 ± 0.09	40.2 ± 6.6	20.7 ± 2.9
		Etoh	1351 ± 16	0.78 ± 0.10	36.9 ± 5.1	19.2 ± 3.8
		Baseline	1441 ± 9	0.76 ± 0.05	43.0 ± 6.7	24.0 ± 4.6
	2 g/kg	Saline	1456 ± 15	0.89 ± 0.05	43.3 ± 5.7	23.9 ± 2.7
		Etoh	1424 ± 15	0.81 ± 0.06	34.8 ± 5.3	16.4 ± 3.3

Group mean values (± SE) are shown for all four cosinor parameters, period, acrophase, mesor, and amplitude, calculated from raw activity data.

relatively blunted at these times. This may suggest that ethanol is not simply acting on end-organs and target tissues to alter SCN feedback, but is demonstrating direct actions on the pacemaker itself. This possibility may be further validated by the finding that ethanol's effects on the phase, period, and amplitude of temperature and activity rhythms were sustained for at least 48 h postinjection, and therefore, may be less likely to be attributable to a transient "masking" effect.

The results of this study, indicating phase delays of temperature and activity rhythms at 0700 and 1300 h are in partial agreement with prior work demonstrating phase delays in the locomotor activity rhythm associated with acute ethanol injections administered during the light phase of the L/D cycle (43). Other studies, employing different administration schedules and modes of ethanol administration, have also revealed phase delays in general activity and wheel running of rodents. Mistleberger and Nadeau (26) reported phase delays and period lengthening in the circadian rhythm of wheel running in hamsters chronically exposed to ethanol in their drinking water. Interestingly, when examining the rate of reentrainment after a 6-h photoperiod phase shift, these investigators saw no evidence that the chronic ethanol regime impaired the rate of reentrainment of the activity rhythm. Motohashi et al. (29) found phase delays in the rhythm of general activity on the next day following multiple intubations of 2.0 g/kg ethanol, administered at 2-h intervals between 0900 and 1900 h. In the current study, lengthening of the activity period was not observed in any group. On the contrary, activity periods were shortened by ethanol injections at both 0700 and 1300 h. Due to numerous differences in the experimental design of this study, relative to those employed in prior work, any number of factors, including ethanol dose, time of administration,

TABLE 3
COSINOR PARAMETERS CALCULATED ON BODY TEMPERATURE DATA

			Period	Cosine of Acrophase	Mesor	Amplitude
		Baseline	1438 ± 9	0.95 ± 0.02	37.4 ± 0.15	0.50 ± 0.04
	0 g/kg	Saline	1410 ± 17	0.97 ± 0.01	37.4 ± 0.13	0.48 ± 0.02
		Saline	1428 ± 9	0.88 ± 0.12	35.6 ± 0.68	0.42 ± 0.03
		Baseline	1431 ± 8	0.98 ± 0.01	37.2 ± 0.13	0.48 ± 0.05
0100 h	1 g/kg	Saline	1450 ± 13	0.96 ± 0.01	37.3 ± 0.13	0.52 ± 0.05
		Etoh	1418 ± 8	0.94 ± 0.03	37.2 ± 0.12	0.54 ± 0.03
		Baseline	1445 ± 9	0.85 ± 0.08	36.6 ± 0.12	0.47 ± 0.04
	2 g/kg	Saline	1439 ± 10	0.91 ± 0.02	36.6 ± 0.12	0.45 ± 0.03
		Etoh	1505 ± 17	0.60 ± 0.12	36.7 ± 0.11	0.45 ± 0.04
		Baseline	1396 ± 17	0.97 ± 0.01	37.3 ± 0.08	0.42 ± 0.03
	0 g/kg	Saline	1359 ± 24	0.83 ± 0.08	37.4 ± 0.08	0.45 ± 0.03
		Saline	1423 ± 19	0.97 ± 0.02	37.4 ± 0.07	0.50 ± 0.04
		Baseline	1405 ± 9	0.89 ± 0.05	37.4 ± 0.09	0.52 ± 0.03
0700 h	1 g/kg	Saline	1393 ± 7	0.98 ± 0.01	37.5 ± 0.09	0.50 ± 0.03
		Etoh	1400 ± 15	0.94 ± 0.03	37.4 ± 0.09	0.51 ± 0.02
		Baseline	1428 ± 10	0.98 ± 0.01	37.4 ± 0.26	0.60 ± 0.03
	2 g/kg	Saline	1419 ± 12	0.94 ± 0.03	37.4 ± 0.25	0.48 ± 0.03
		Etoh	1425 ± 11	0.95 ± 0.03	37.4 ± 0.27	0.53 ± 0.03
		Baseline	1418 ± 11	0.94 ± 0.07	37.1 ± 0.06	0.42 ± 0.03
	0 g/kg	Saline	1421 ± 29	0.80 ± 0.04	37.1 ± 0.11	0.40 ± 0.03
		Saline	1434 ± 9	0.81 ± 0.14	37.1 ± 0.08	0.48 ± 0.05
		Baseline	1435 ± 9	0.96 ± 0.04	37.0 ± 0.13	0.50 ± 0.06
1300 h	1 g/kg	Saline	1446 ± 19	0.90 ± 0.05	37.0 ± 0.14	0.47 ± 0.05
		Etoh	1429 ± 11	0.91 ± 0.02	37.1 ± 0.15	0.44 ± 0.05
		Baseline	1428 ± 7	0.86 ± 0.05	37.1 ± 0.11	0.47 ± 0.04
	2 g/kg	Saline	1439 ± 15	0.73 ± 0.07	37.3 ± 0.08	0.48 ± 0.04
		Etoh	1375 ± 13	0.94 ± 0.03	37.1 ± 0.10	0.46 ± 0.03
		Baseline	1415 ± 11	0.96 ± 0.02	37.3 ± 0.08	0.48 ± 0.02
	0 g/kg	Saline	1449 ± 9	0.94 ± 0.02	37.4 ± 0.07	0.55 ± 0.03
		Saline	1453 ± 8	0.92 ± 0.03	37.4 ± 0.06	0.54 ± 0.02
		Baseline	1461 ± 10	0.65 ± 0.10	37.3 ± 0.10	0.40 ± 0.04
1900 h	1 g/kg	Saline	1447 ± 10	0.88 ± 0.04	37.3 ± 0.09	0.48 ± 0.03
		Etoh	1379 ± 8	0.94 ± 0.02	37.3 ± 0.10	0.35 ± 0.03
		Baseline	1430 ± 12	0.86 ± 0.04	37.4 ± 0.17	0.49 ± 0.05
	2 g/kg	Saline	1425 ± 10	0.94 ± 0.03	37.4 ± 0.19	0.54 ± 0.06
		Etoh	1381 ± 12	0.88 ± 0.03	37.4 ± 0.12	0.33 ± 0.02

Group mean values (± SE) are shown for all four cosinor parameters, period, acrophase, mesor, and amplitude, calculated from raw core body temperature data.

route of administration, etc., may potentially account for the discrepant findings. Validation of these results will await future studies employing similar methodology.

To our knowledge, this study is the first to describe circadian phase dependence in the disruption of several key parameters (period, acrophase) of circadian rhythms of general activity and core body temperature following a single acute dose of ethanol. Alterations in rhythm period, phase, and amplitude were sustained for at least 48 h, and exhibited a temporal pattern congruent with the time course of ethanol hangover. These findings highlight another point of similarity between interoceptive states engendered by ethanol and by photoperiod phase shifts in that both conditions are associated with circadian rhythm disruptions. Circadian desynchronosis, produced by shifts of the photoperiod, has been shown to engender increased consumption of ethanol in laboratory rats (10). The time-dependent increases in self-administered ethanol found in this previous study occurred during the circadian period associated with the greatest hypothermic effect in the present study. Whether circadian desynchronosis represents a "setting condition" for human ethanol consumption is currently an open question. It is well known that alcoholics will consume ethanol to avoid withdrawal (12). In view of recent findings from this laboratory, and those of other studies, which indicate pathological alterations in multiple circadian rhythms in alcoholics, the possibility exists that circadian rhythm desynchronosis, related to ethanol's acute and aftereffects on the circadian system may represent the basis for interoceptive states that contribute to sustained drinking patterns in these individuals.

There is currently a lack of information about ethanol's actions on putative biological components of the mammalian

circadian pacemaker. Questions thus remain concerning the specific mechanisms by which ethanol might exert effects on circadian rhythms like those observed in this study. Given ethanol's ubiquitous actions throughout the nervous system, this will no doubt prove to be a fertile area for future research. On another level, studies are in progress in this laboratory to continue to evaluate ethanol's potential to serve as a pharmacological zeitgeber. Ethanol appears to have robust effects on circadian rhythms, given that rats in this study were under the constant influence of one of the most powerful zeitgebers, the L/D cycle, yet still exhibited alterations of period and phase of temperature and activity rhythms. An experiment in progress in our laboratory will use a more traditional chronobiometric design to explore zeitgeber characteristics of ethanol under free-running conditions in constant dark to further delineate its effects on rhythm entrainment.

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