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ENVIRONMENTAL MODULATION OF ALCOHOL INTAKE IN HAMSTERS: EFFECTS OF WHEEL-RUNNING AND CONSTANT LIGHT EXPOSURE

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Abstract

BACKGROUND—Alcohol abuse leads to marked disruptions of circadian rhythms, and these disturbances in themselves can increase the drive to drink. Circadian clock timing is regulated by light, as well as by nonphotic influences like food, social interactions, and wheel-running. We previously reported that alcohol markedly disrupts photic and nonphotic modes of circadian rhythm regulation in Syrian hamsters. As an extension of this work, we characterize the hedonic interrelationship between wheel-running and ethanol intake and the effects of environmental circadian disruption (long-term exposure to constant light [LL]) on the drive to drink.

METHODS—First, we tested the effect of wheel running on chronic free-choice consumption of a 20% (v/v) ethanol (EtOH) solution and water. Second, the effect of this alcohol drinking on wheel running in alcohol-naïve animals was investigated. Third, we assessed the influence of LL, known to suppress locomotor activity and cause circadian rhythm disruption, on EtOH consumption and wheel-running behavior.

RESULTS—Inhibitory effects of wheel running on EtOH intake and vice versa were observed. Exposure to LL, while not affecting EtOH intake, induced rhythm splitting in 75% of the animals. Notably, the splitting phenotype was associated with lower levels of EtOH consumption and preference prior to, and throughout, the period of LL exposure.

CONCLUSIONS—These results are evidence that exercise may offer an efficacious clinical approach to reducing EtOH intake. Also, predisposition for light-induced (or other) forms of circadian disruption may modulate the drive to drink.

Keywords

circadian; ethanol; exercise; constant light; rhythm splitting

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INTRODUCTION

It is now well established that ethanol (EtOH) is highly disruptive to circadian behavioral and physiological rhythms and that such circadian timing problems lead to alcohol abuse and may prompt relapse behaviors in abstinent alcoholics. Chronic drinking delays and dampens circadian rhythms of melatonin (Kuhlwein et al., 2003; Rojdmarm et al., 1993), glucocorticoids (Rasmussen et al., 2000), thyroid stimulating hormone (Danel and Touitou, 2003), and body temperature (Baird et al., 1998; Danel et al., 2001; Devaney et al., 2003; Wasielewski and Holloway, 2001). Alcohol abuse is also associated with decreases in rapid eye movement and slow-wave sleep and general fragmentation of sleep and activity rhythms (Brower, 2001; Brower et al., 2001; Ehler and Slawecki, 2000; Kubota et al., 2002; Landolt et al., 1996; Roehrs et al., 1996). Likewise, individuals with circadian-related sleep disorders (Roehrs and Roth, 2001a&b) or hypothalamic-pituitary-adrenal axis dysfunction (Costa et al., 1996; O'Callaghan et al., 2005; Zimmerman, 2003) and those that experience chronic circadian rhythm perturbations induced by shift work or repeated jetlag (Gordon et al., 1986; Smart, 1979) have a much greater risk of developing alcoholism. The result is a self-perpetuating cycle of circadian rhythm deterioration and reinforced alcohol use.

Circadian timing in mammals is generated and maintained by the suprachiasmatic nucleus (SCN; Klein et al., 1991; Moore, 1983; Rusak and Zucker, 1979). The SCN clock receives a variety of regulatory signals (Zeitgebers) that synchronize internal rhythms to the external environment. Photoc information entrains the SCN clock to the daily light-dark cycle via a direct, glutamatergic projection from the retina (Hendrikson et al., 1972; Johnson et al., 1988; Moga and Moore, 1997; Rusak, 1977) and also by an indirect pathway through the intergeniculate leaflet (IGL; Card and Moore, 1982; Johnson et al., 1989). Nonphotic information, such as food and water availability, social interactions, and exercise, coordinates clock timing to other important environmental influences. The IGL and the midbrain raphe nuclei mediate nonphotic signaling to the SCN via neuropeptide-Y and serotonin (5-HT) release, respectively (Albers and Ferris, 1984; Biello et al., 1994; Glass et al., 2003; Marchant et al., 1997; Meyer-Bernstein and Morin, 1996). A complex, reciprocal interaction exists between photic and nonphotic stimuli which serves to optimize the timing of circadian rhythms (Challet and Pevet, 2003).

Many nonphotic circadian Zeitgebers, including wheel running, are reinforcing stimuli, and brain systems involved in circadian regulation are linked to the mesocorticolimbic reward pathway (see Webb et al., 2009). EtOH and other drugs of abuse are thought to override the reward circuitry (Kelley, 2004) such that the drug is perceived as having a greater value than natural rewards. Moreover, evidence suggests that such agents can act as circadian Zeitgebers (Kosobud et al., 2007), and thus, the interaction of addictive drugs with these linked systems may underlie the habitual nature of drug abuse. Central to the maladaptation of brain and behavior to drugs is hedonic substitution, whereby EtOH or drugs are chosen by addicted humans or animals in lieu of natural rewards. Notably, hedonic substitution implies that natural rewards can also replace drug rewards. This is supported by recent evidence that wheel running decreases cocaine self-administration in cocaine-dependent animals (Smith et al., 2008), and that environmental enrichment reduces heroin reward (El Rawas et al., 2009).

Our previous work has focused on the effects of EtOH on circadian clock regulation. EtOH was found to markedly attenuate photic and nonphotic phase-resetting responses and disrupt circadian activity patterns. To date, however, little is known about how perturbations of the circadian system affect EtOH consumption. The present study was therefore undertaken to examine: 1) the interaction between wheel running and EtOH consumption; and 2) how disruption of the photic environment by constant light exposure (LL) affects drinking. Given the emerging evidence supporting a key role of the circadian system in alcohol abuse,

understanding circadian pathways that modulate alcohol consumption is fundamental to the development of strategies for preventing alcoholism and relapse in abstinent alcoholics.

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 h light: 10 h dark photocycle (LD) 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 University Institutional Animal Care and Use Committee and were conducted under the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Effect of wheel running on EtOH consumption

This experiment was designed to test whether wheel running affects EtOH consumption and/or preference (calculated as the percentage of volume EtOH solution consumed over total volume of fluid consumed) in free-choice chronic drinkers. Hamsters under LD were individually housed in cages with locked running wheels. Animals received free-choice between tap water containing 20% EtOH (v/v) and tap water alone for at least 2 wks prior to experimentation (phase I). After the 2 wk EtOH acclimation period, wheels were unlocked for animals in the experimental group, while wheels remained locked for controls (phase II). After 4 wks of access to running wheels, wheels were locked for the experimental group, and remained locked for controls (phase III). Wheels were interfaced with a Clocklab computerized data acquisition system (Coulbourn Instruments, Whitehall, PA) to monitor wheel running. Revolutions per minute (RPMs), distances run, and daily EtOH and water consumption were calculated over the course of the experiment.

Effect of EtOH consumption on wheel running

This experiment was undertaken to examine the effect of EtOH on wheel-running activity. A separate group of hamsters under LD were individually caged and their circadian locomotor activity rhythms and wheel running activity were monitored as described. After 2 wks, animals in the experimental group received free-choice 20% EtOH, while controls received water only. RPMs, distances run, and daily EtOH and water consumption were calculated over the 50 day course of the experiment.

Effect of constant light on EtOH consumption

This experiment was undertaken to determine the effect of disruption of the photic environment by long-term LL exposure on chronic free-choice EtOH consumption and preference. Animals were placed on free-choice 20% EtOH for 2 wks prior to experimentation. After the 2 wk EtOH acclimation period, the LD photocycle was changed to LL for 60 days. Assessments of circadian activity were undertaken using activity bout analysis. A wheel-running bout was defined as a period of continuous wheel running activity (regardless of duration) separated by at least 20 min of activity quiescence. Total wheel-running activity was calculated as the product of the number of activity bouts times bout duration across the 24 h day. These measurements were averaged over a 3 day period for each animal beginning 5 days prior to the transition to LL and 3 days immediately following transition to LL. In animals that exhibited rhythm splitting, activity bouts were averaged over 3 day periods beginning 5 days prior to rhythm splitting and immediately after rhythm splitting. Daily EtOH and water consumption was measured for each animal over the course of the experiment.

Statistical Analyses

Combined effects of wheel running and EtOH were assessed using a repeated measures ANOVA and then by a subsequent Newman-Keuls post hoc mean comparison test when appropriate. LL vs. LD effects on EtOH and locomotor activity were similarly analyzed. Split rhythm vs. non-split rhythm effects were analyzed using a randomized one-way ANOVA with a subsequent Newman-Keuls test. Correlational determinations between wheel running and EtOH were undertaken using a Pearson's correlational analysis. The level of significance was set at $p < 0.05$.

RESULTS

Wheel running decreases EtOH consumption

In animals maintained under LD, access to unlocked running wheels produced a significant decrease in free-choice 20% EtOH consumption ($F_{(1,70)}=14.6$, $p < 0.001$), but not in EtOH preference ($F_{(1,70)}=0.3$, $p=0.608$; Fig. 1). During phase I (all wheels locked), EtOH consumption in the designated experimental (running) group ($n=9$) was 15.1 ± 1.0 g/kg/day, with a $80.1 \pm 3.3\%$ preference for the EtOH solution over water. EtOH consumption in the designated non-running control group ($n=9$) was 17.6 ± 1.0 g/kg/day with a $75.4 \pm 3.3\%$ EtOH preference. During phase II (wheels unlocked for the experimental group), EtOH consumption for this group dropped to 12.8 ± 0.8 g/kg/day, with a $79.5 \pm 3.6\%$ EtOH preference. Control EtOH consumption during phase II was 17.4 ± 0.8 g/kg/day, with an EtOH preference of $82.1 \pm 3.6\%$. During phase III (wheels relocked), EtOH consumption in the experimental group increased to 14.3 ± 0.7 g/kg/day, with an EtOH preference of $76.2 \pm 3.2\%$. Controls consumed 16.7 ± 0.7 g/kg/day, with an EtOH preference of $80.7 \pm 3.2\%$.

EtOH consumption decreases wheel running

In animals maintained under LD with unlocked wheels, free-choice 20% EtOH consumption significantly reduced wheel running (Fig. 2). During the 10 day period prior to EtOH introduction, hamsters in the designated experimental (EtOH-drinking) group ($n=9$) averaged 14.5 ± 1.2 km/day, at 10.0 ± 0.8 rpm. Wheel running for the designated control (water-drinking) group ($n=9$) was not significantly different, averaging 13.2 ± 1.2 km/day at 9.2 ± 0.8 rpm. During the subsequent 3 wk presentation of EtOH, experimental animals ran significantly less than controls, averaging 11.3 ± 0.3 km/day at 7.8 ± 0.2 rpm vs. 12.3 ± 0.3 km/day at 8.6 ± 0.3 rpm, respectively ($F_{(1,68)}=4.8$, $p < 0.02$). After this time the suppression was lost.

Constant light effects on wheel running and EtOH intake and preference

Hamsters placed under LL ($n=9$) exhibited a free-running circadian locomotor activity rhythm with a characteristic long tau (Fig. 3). This treatment caused a marked decrease in wheel-running distance compared to LD (3.9 ± 0.5 km/day vs. 7.5 ± 0.3 km/day; $F_{(1,8)}=98.4$, $p < 0.001$; Fig. 5). This decrease was evident soon (~2 days) after LL onset, and persisted throughout the duration of LL. This effect of LL was reflected in a significant decrease in the number of activity bouts measured by wheel running across the circadian day compared to initial bout number under LD (2.1 ± 0.2 vs. 2.7 ± 0.2 bouts, respectively; $F_{(1,34)}=4.3$, $p < 0.05$). Multiplying these bout numbers by the average daily bout duration (80.7 ± 18.6 vs. 164.2 ± 18.2 , respectively; $F_{(1,34)}=10.3$, $p < 0.01$) confirmed the suppressive effect of LL on total locomotor activity as well as on wheel-running distance (Fig. 4).

Of the 9 of hamsters maintained in LL for 60 days, 6 exhibited rhythm splitting and 3 did not. For all of the animals, there were no LL-associated changes in either EtOH consumption or preference (Fig. 5). In marked contrast, animals without split rhythms consumed significantly more EtOH than their splitting counterparts ($F_{(1,60)}=116.7$, $p < 0.001$)

and showed greater preference for the 20% EtOH solution over the duration of the experiment ($F_{(1,60)}=101.8$, $p<0.001$; (Fig. 5&Fig. 6). Notably, these differences were apparent under LD *prior* to the onset of LL, with the non-splitting animals consuming 15.4 ± 0.7 g/kg/day EtOH and showing an EtOH preference of $74.7\pm2.7\%$ vs. 11.9 ± 0.5 g/kg/day and $60.8\pm1.9\%$ for splitting animals, suggesting an underlying phenotypic predisposition for rhythm splitting linked to EtOH intake. In terms of activity, splitters exhibited a persistent and more pronounced reduction in daily wheel-running distance vs. non-splitters under LL (Fig. 5&Fig. 6). There was no phenotypic difference in this activity under LD prior to LL exposure.

DISCUSSION

The central reward pathways underlying alcoholism are closely and reciprocally linked to the circadian timing system. This interplay is evidenced by the ability of reinforcing stimuli to serve as strong nonphotic circadian Zeitgebers, which in turn, can influence the circadian modulation of reward-seeking behaviors and responsiveness to reward stimulation (Murray et al., 2009). We have shown previously that EtOH disrupts circadian activity patterns and impairs phase-resetting of circadian rhythms (Brager et al., 2010; Ruby et al., 2009a&b). Here, we extend this work to show that rewarding and disruptive environmental manipulations that affect circadian timing can dramatically impact EtOH intake. First, access to a running wheel (a nonphotic circadian Zeitgeber *and* a rewarding stimulus) reduces EtOH consumption, indicating that a reinforcing behavioral influence can compete with the drive for EtOH reward (hedonic substitution). Notably, this effect is reciprocal, as EtOH presentation reduces daily wheel-running distance. The link between pathways for reward and circadian regulation is underscored in the third experiment where EtOH consumption and preference are far lower in animals susceptible to the rhythm splitting effect of LL than for those whose rhythms remain intact. Thus, a factor intrinsic to SCN circadian clock phase-regulation is likely involved in the drive for EtOH. Collectively, these results provide evidence coupling the circadian-related phenomenon of rhythm splitting to an inherent difference in EtOH self-administration and offer further support for the role of the circadian timing system in the predisposition for alcohol abuse.

Reciprocative interactions between exercise and EtOH

Access to a running wheel decreased EtOH drinking and access to EtOH decreased wheel-running. This effect could be due to a reinforcing effect of wheel running substituting in part for EtOH reward, and is consistent with previous studies where running wheel access decreased EtOH intake in other high alcohol-preferring species including the C57BL/6j mouse (Ehringer et al., 2009) and the P (but not NP) rat (McMillan et al., 1995). In a similar manner running wheel access suppresses cocaine self-administration in cocaine-dependent rats (Smith et al., 2008). These observations support the hypothesis that the rewarding effects of exercise may substitute for the hedonic effects of drug and alcohol abuse. Another explanation relates to the proposed role of central ghrelin signaling in modulating alcohol reward. Stimulation of ghrelin receptors located in the ventral tegmental area (VTA) is required for reward stimulation by EtOH (Jerlhag et al., 2009). Vigorous exercise markedly suppresses blood levels of the acylated (active) form of ghrelin that crosses the blood-brain barrier (Broom et al., 2007), and thus suppression of ghrelin could decrease the incentive value of EtOH, as reflected in the present results. A third possibility is that animals running on a wheel for much of the night (averaging 11–12 km) would have less time and/or inclination to drink. Arguing against this explanation, however, is our concomitant activity measurements from overhead motion detectors showing that the extent of non-wheel-related locomotor activity (including drinking behavior) is equivalent for the locked and unlocked wheel groups. Regardless of the mechanism behind the wheel-running effect, the present

data supports exercise as a natural reinforcer, and as such, it may substitute for the hedonic aspects of drug and alcohol abuse.

It should be noted that the wheel-running-induced suppression in EtOH intake reported in the studies above contrasts somewhat with results from a study in C57BL/6J mice, where EtOH consumption was largely unaffected by running wheel access, although there was an increase in wheel-running when EtOH was withdrawn, suggestive of a reward substitution effect (Ozburn et al., 2008). Also, it has been reported that wheel-running has a stimulatory effect on EtOH intake and preference in Lewis rats (Werme et al., 2002). These discrepancies could be related to procedural differences, as for example, the mouse study employed repeated, alternating 6-day exposures to locked and unlocked wheels with no constant locked-wheel control group. Here, hamsters had continuous access to unlocked or locked wheels for 30 days starting after an initial 10-day EtOH acclimation period. Another difference is that the Lewis rats exhibit a uniquely high level of addiction and excessive wheel-running behavior that could possibly produce an additive, rather than substitutive reward effect. It is also important to note that despite the exercise-induced decrease in free-choice EtOH consumption, the hamsters in the present study showed little change in EtOH preference over water. In other words, runners drank less of both fluids. This result is surprising, given that vigorous exercise should increase thirst, and therefore elevate water consumption. A likely explanation is that the unchanged EtOH preference may be secondary to the decrease in alcohol consumption, in that EtOH inhibits the antidiuretic effects of vasopressin, causing dehydration (Treistman et al., 1991). Thus, animals drinking less alcohol should be less dehydrated, and therefore drink less water.

A prediction from the hedonic substitution hypothesis presented above is that EtOH intake should displace the reinforcing effect of wheel running. Such was the case for hamsters with free-choice EtOH, who ran ~1 km/day less than water controls. It may be argued that the decreased wheel running was due to the sedative or vestibular-related effects of EtOH. This is unlikely, however, as discussed above, general locomotor levels measured using overhead motion detectors were comparable between the EtOH and water groups. Moreover, hamsters and other EtOH-preferring species are not particularly sensitive to the sedative effects of EtOH, nor do they drink to the point of sedation (Kurtz et al., 1996; Schuckit, 1994; Theile et al., 1998, 2000).

Circadian disruption and EtOH: Differential effects of constant light

Exposure of hamsters and rats to LL is disruptive to circadian timing because it suppresses locomotor activity and splits the activity rhythm into two antiphase components in the majority (~75%) of animals (de la Iglesia et al., 2000). In addition, LL induces depression-like behaviors (Fonken et al., 2009), and abolishes circadian rhythmicity in corticosterone (Claustrat et al., 2008) and melatonin secretion (Wideman and Murphy, 2009; Wurtman et al., 1963). Given these widespread circadian-related disruptions and their association with augmented alcohol drinking, it was hypothesized that the LL-induced reduction in locomotor activity would be accompanied by increased drive for EtOH reward. Paired comparisons between initial LD levels of activity and EtOH intake and subsequent LL levels in the group as a whole, however, revealed that, although locomotor activity was suppressed under LL, there was little change in EtOH consumption or preference. This latter effect differs from that of rats, where LL has a suppressive effect on EtOH intake (Geller, 1971).

Retrospective analysis of the present LL data revealed marked differences between animals with split rhythms and those which maintained intact rhythms. Although EtOH consumption and preference were not affected by LL in either of the phenotypes, there was strikingly less locomotor activity in the split group in addition to their significantly lower EtOH intake at all time points in comparison to the non-splitters. Notably, these differences in EtOH drive

were expressed under LD prior to the LL exposure, revealing the existence of an underlying predisposition for subdued EtOH intake and activity linked to rhythm splitting. A tie-in between a propensity for EtOH intake and rhythm splitting under LL is further evidenced by results from a previous study in rats, where (in contrast to hamsters) there was a higher degree of rhythm splitting in selectively bred ethanol-preferring (HAD2) rats compared to other lines of rats tested (Rosenwasser et al., 2005). Regardless of these differential effects of rhythm splitting propensity on EtOH intake, it is evident from these studies that underlying genetic and/or physiological components of the SCN clock can contribute in a substantial way to the propensity for EtOH intake.

It is unclear why some animals undergo rhythm splitting under LL while others do not, but this may reflect individual differences in the intrinsic sensitivity of the circadian system to light. Another possibility is that the absence of rhythm splitting in some animals may be a direct result of EtOH intake itself. We have previously shown that EtOH impairs photic regulation of circadian rhythms in a dose- and concentration-dependent manner (Brager et al., 2010; Ruby et al., 2009a&b). Thus, the higher alcohol consumption by nonsplitters under LD and LL may have rendered their clocks less sensitive to light in general. This may also explain the lesser degree of LL-induced activity suppression in nonsplitters. Also, the LL photocycle may have unmasked greater sensitivity to EtOH-induced stimulation of locomotor activity among non-splitting animals, and exposed an innately higher hedonic threshold in this group. A third possibility is that it may be due to inherent instability of bilateral communication pathways between both SCNs, as splitting is associated with their anti-phase oscillatory clock gene expression (de la Iglesia et al., 2000). This relates to a fourth possibility whereby the dissimilarities in circadian activity and alcohol drinking patterns between splitters and non-splitters may directly involve clock genes. Given the emerging role of *Per2* in alcohol consummatory behavior, inherent differences in the expression or function of this clock gene in particular may underlie our observations. Mice lacking *mPer2* consume more alcohol (Spanagel et al., 2005), yet show less EtOH-related activity suppression than wildtypes (Ruby et al., 2009c). Interestingly, *mPer2*^{-/-} mice show robust circadian rhythmicity under LL (Steinlechner et al., 2002) which is similar to non-splitters. It is thus possible that *Per2* expression is decreased more in non-splitters relative to splitters. Consistent with this model are observations that *Per2* expression in the SCN is significantly reduced by LL exposure (Sudo et al., 2003). While the basis for the observed differences between these groups remains to be elucidated, these data further support the existence of a link between the circadian timing system and EtOH intake and suggest that hamsters resistant to LL-induced rhythm splitting may be a useful animal model for circadian activity-related aspects of alcoholism in future studies. Moreover, exploring the differences between the hamster splitting vs. non-splitting phenotypes at the cellular and molecular levels would be useful to understanding the neurophysiological mechanisms underlying the role of the circadian system in modulating EtOH consumption.

Perspectives and Significance

We are just beginning to understand the roles of the circadian timing system in alcohol and drug abuse, and conversely, the impact of substance abuse on the circadian clock. In the present study, we show an inverse relationship between wheel running and EtOH consumption, suggestive of reward-based hedonic substitution. In addition, we show that the phenotype for LL-induced rhythm splitting is linked to a predisposition for subdued EtOH intake and greater activity suppression. This may be clinically relevant, as the circadian internal dissociation and rhythm fragmentation induced by environmental photic shifts in humans is variable, and largely dependent upon an individual's genetically-determined tau (Wever, 1979;1983). An inborn predisposition to circadian rhythm disruption may thus be a causative factor determining the propensity for alcohol abuse. Together, these results

suggest that exercise may be an effective treatment strategy for alcoholics, and that predisposition to circadian rhythm instability affects alcohol consumption.

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Figure 1.

Wheel running suppresses EtOH consumption. *Top graph*: mean daily ethanol consumption for all animals (dashed line; these animals were considered statistically as a single group, as they were not randomly separated into running and non-running groups until the start of Phase II), runners (solid line), and non-runners (dotted line). Phase I: ethanol acclimation period (wheels locked). Phase II (shaded): wheels unlocked for experimental group and locked for controls. Phase III: all wheels locked. *Bottom graph*: ethanol consumption and preference for runners and non-runners averaged over each phase. Bars represent means $\pm$ SEM. Among all groups and phases, bars with different letters are significantly different ($p < 0.05$).

Figure 2.

EtOH consumption suppresses wheel running. *Top graph:* daily distance run by animals given free-choice between 20% ethanol and water (solid line) or water only (dotted line). Shaded area represents wheel acclimation period; EtOH introduction occurred at the beginning of the non-shaded region. *Bottom graph:* distance run by drinkers (EtOH) and non-drinkers (water) during the initial 3 wks of EtOH drinking. Bars represent means $\pm$ SEM. Bars with different letters are significantly different ($p<0.05$).

Figure 3. Representative double-plotted actograms of wheel-running activity of free-choice 20% EtOH drinking hamsters showing a free-running non-split rhythm (*top*) and split rhythm (*bottom*). The shaded area designates the initial LD photocycle; the unshaded area designates LL.

Figure 4.

Suppression of general daily locomotor activity (expressed as mean daily bout number $\times$ mean daily bout duration) by exposure to LL. Locomotor activity is maximally suppressed compared to LD levels during the initial 3 days of LL treatment assessed in all hamsters. This activity was also suppressed in hamsters of the rhythm splitting phenotype immediately prior to and during splitting. Bars represent means $\pm$ SEM. Bars with different letters are significantly different ($p < 0.05$).

Figure 5.

Wheel-running distance (*top panel*), free-choice 20% EtOH consumption (*middle panel*) and EtOH preference (*bottom panel*) for hamsters under LL exhibiting non-split and split rhythms (middle and right side, respectively) and averaged for all hamsters (Group; left side). Gray bars represent the averages measured under the initial LD photocycle. White bars represent the first and last 5 days of LL. Bars represent means $\pm$ SEM. For each parameter, bars with different letters are significantly different ($p < 0.05$).

Figure 6. Mean daily EtOH consumption (*top panel*) and wheel-running distance (*bottom panel*) for splitting (solid line) and non-splitting (dotted line) hamsters on free-choice 20% EtOH under LL.