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Chronic Alcohol Treatment in Rats Alters Sleep by Fragmenting Periods of Vigilance Cycling in the Light Period with Extended Wakenings

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Abstract

Studies have shown that disturbed sleep produced by chronic alcohol abuse in humans can predict relapse drinking after periods of abstinence. How alcohol produces disturbed sleep remains unknown. In this study we used a novel analysis of sleep to examine the effects of alcohol on sleep patterns in rats. This analysis separates waking into multiple components and defines a period labeled vigilance cycling (VC) in which the rat rapidly cycles through various vigilance states. These VC episodes are separated by long duration wake periods (LDW). We find that 6 weeks of alcohol (6% in a liquid diet) caused fragmentation of extended VC episodes that normally occur in the light period. However, total daily amounts of slow-wave sleep (SWS) and rapid-eye movement sleep (REMS) remained constant. The daily amount of wake, SWS, and REMS remained constant because the alcohol treated rats increased the amount of VC in the dark period, and the sleep nature of VC in the dark period became more intense. In addition, we observed more wake and less REMS early in the light period in alcohol treated rats. All effects completely reversed by day 16 of alcohol withdrawal. Comparison of the effects of chronic alcohol to acute alcohol exposure demonstrated the effects of chronic alcohol are due to adaptation and not the acute presence of alcohol. The effects of chronic alcohol treatment in rats mimic the effects reported in humans (REMS suppression, difficulty falling asleep, and difficulty remaining asleep).

Keywords

long duration wake; brief wake; circadian rhythm; REMS suppression; sleep homeostasis; insomnia

INTRODUCTION

Although acute alcohol is a soporific agent, a frequent complaint of alcoholics is disturbed sleep. Specifically, alcoholics entering rehab programs have been found to have high pressure for rapid-eye-movement sleep (REMS), an increase in sleep onset latency, and inability to maintain sleep through the night [3,19,22]. Although REMS returns to normal after cessation of drinking, the insomniac effects can remain for years after recovery [1,5,8,12,13,34,39]. Furthermore, disturbances in sleep patterns in recovering alcoholics have been found to predict the probability of relapse drinking [4,6,8,12,13]. Why disturbed sleep should predict relapse

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to further alcohol consumption remains unknown, but at an even more fundamental level, the mechanistic basis for disturbed sleep patterns produced by alcohol exposure also remains unknown. An animal model of altered sleep induced by chronic alcohol would be helpful in addressing this issue.

A common animal model for both sleep and alcohol research is the rat. However, a difference between rat and human sleep is that rats are polyphasic sleepers whereas humans are monophasic sleepers. Thus when human alcoholics (or recovered alcoholics) complain of failure to maintain sleep throughout their monophasic sleep period, it becomes difficult to assess this parameter in the rat since rats normally have frequent awakenings throughout the rest period. Recently we developed an analysis of sleep patterns in rats that separates wake episodes into brief wake (BW) and long duration wake (LDW), and we used this division to identify a sleep unit that occurs between LDW events [33]. The term vigilance cycling (VC) was used as the label for this sleep period because of the rapid cycling between different vigilance states. In our prior study we suggested that periods of VC are analogous to the 8-h monophasic sleep period in humans. In the present study we used this analysis to examine the effects of chronic and acute alcohol consumption on sleep in rat.

We found that the long periods of VC that normally occur in the light period in untreated rats are fragmented into shorter periods by the chronic alcohol treatment. Further, since slow wave sleep (SWS) and rapid-eye movement sleep (REMS) occur within VC periods, there is also a drop in the overall amount of SWS and REMS in the light period. On the other hand, during the dark period VC takes on the more sleep intensive appearance of VC in the light period such that daily amounts of SWS and REMS are not altered by the chronic alcohol treatment. These effects of chronic alcohol are not mimicked by acute exposures to alcohol. These results document that the effects of chronic alcohol in rat (fragmentation of prolonged sleep episodes and suppression of REMS in the light period) are a result of adaptations to the presence of alcohol and closely resemble the pathologies observed in human alcoholics.

METHODS

Chronic alcohol treatment

Sixteen male Sprague-Dawley rats (250–275 g at the beginning of the treatment) were used; eight of them were assigned to the alcohol treated group and eight to the pair-fed control group. Beginning body weights were matched between the two groups. Rats were housed individually at 22 ± 2 °C ambient temperature with a 12h:12h light:dark cycle. Alcohol treated rats were treated with 6% alcohol (grain alcohol, Everclear) in liquid diet (Bio-serv) for 6 weeks. The alcohol treated rats consumed on average 11 ± 0.2 g alcohol/kg body weight/day. In a prior study we found that this protocol produces a blood alcohol level of ~0.12% when blood samples are taken at the end of the dark period [18]. Each pair-fed rat was given alcohol-free liquid diet of equal caloric content (calories from alcohol replaced with maltose dextrin) to that consumed by the weight-matched alcohol treated rat. All rats had *ad lib* access to water. These rats are the same rats for which previous results have been reported [23]. The focus of this previous study was to determine the optimum treatment protocol to produce alcohol-induced changes in sleep patterns. The results contained within the present report are a more detailed analysis from this group of animals and includes the data from the recovery period after cessation of alcohol treatment.

Acute alcohol treatment

Twenty male Sprague-Dawley rats (approx 350g) were used for dark onset alcohol administration study and nineteen rats of similar specifications for light onset alcohol administration. Housing, ambient temperature and light cycles used in these experiments were

the same as described in the chronic alcohol treatment study. Alcohol (Everclear) was administered within 30 min prior to a scheduled light change in a 30% solution in tap water (1 ml/100 g body weight) by gastric gavage (final dose 2.4 g/kg body weight). Control recordings were obtained after an equivalent water gavage (1 ml/100 g body weight). Rats had *ad lib* access to food and water at all times. Administration of alcohol at 3 g/kg produces a blood alcohol level around 0.153 % after 90 minutes and 0.1 % at three hour [37].

All experiments were done with the approval of the institutional animal care and use committee of Washington State University.

Instrumentation for sleep recordings

Surgical implantation of permanent EEG and EMG electrodes were as described previously [23]. A week of recovery from the instrumentation was allowed before sleep recordings commenced. In the chronic treated animals the instrumentation was done during the 5th week of the alcohol exposure. The alcohol diet was continued during the time of instrumentation.

Sleep recordings

During the period of data collection sleep recordings were made in enclosed environmental chambers as described previously [23]. Alcohol and pair-fed treatments continued in the environmental chambers through the first two days of sleep recordings after which the alcohol was removed from the diet of the alcohol treated animals over a two day period (6% to 3% to 0%). Recordings were interrupted for one hour each day (last hour of light period) for animal care. However since we analyze data in 2 h blocks we excluded data in the last 2 h block in the light period. After weaning from alcohol, the liquid diet was continued and additional sleep recordings were collected periodically throughout the recovery period. In the present study we report findings from recordings on the last 6% alcohol day and those obtained 2 days and 16 days after termination of the alcohol treatment. In the acute alcohol studies the animals were also gavaged daily during the 3 day habituation period.

Collection and analysis of EEG and EMG signals

We collected and analyzed the EEG and EMG signals as described previously [33] using an 8-sec epoch in the primary analysis of the sleep records. A 300 sec cutoff was used to separate BW from LDW. VC events were those events between LDW episodes. Further, as described previously [33] any isolated SWS events that were surrounded by LDW were not considered VC (because no cycling occurred), and such events were not used to divide periods of LDW. When examining the variation in slow-wave amplitude (SWA; average of amplitudes in the 0.5 to 4 Hz frequency bands only during SWS episodes) across the 22 h of recording, we averaged the SWA across the entire recording for each rat, and then the average SWA within each 2-h block was expressed as a percentage of the average across the entire recording.

Statistical Analysis

In the course of the experiment the instrumentation on two pair-fed animals failed before the first sleep recordings were made at the end of the alcohol treatment period. Thus the results reported from this phase of the treatment are from 8 alcohol treated animals and 6 pair-fed animals. During the recovery period (after day 2), the instrumentation on two of the alcohol treated animals failed. Thus the results for day 2 of recovery contain 8 alcohol treated and 6 pair-fed animals and results from day 16 of recovery from 6 animals in each group.

A two-way (light period versus dark period and alcohol treated versus pair-fed) analysis of variance (ANOVA) was used when comparing most parameters. However, because there was only 10 hours of recording in the light period versus 12 hours in the dark period, results that

scale with time of recording were either normalized to minutes of vigilance state per 2-h time period and the two-way ANOVA used or a t-test was used to compare alcohol treated versus pair-fed only within the dark or light period (number of LDW and VC episodes). When comparisons were made across 2-h time blocks, a two-way repeated measure ANOVA was used. Post-hoc pair-wise comparisons were done by the Holm-Sidak method and Bonferroni's method. We used repeated measures two-way ANOVA (time versus treatment) in the acute alcohol study. In all cases a p value <0.05 was considered statistically significant. All results are presented as averages $\pm$ SEM. All ANOVA were performed with SigmaStat version 3.5 software (SYSTAT Software, Richmond, CA), and t-tests were performed with Microsoft Office Excel 2003.

RESULTS

Effects of chronic alcohol treatment on sleep patterns

After 6 weeks of 6% alcohol in liquid diet rats show multiple alterations in the percentage of time they spend in various vigilance states as compared to the pair-fed control rats (Figure 1). Chronic alcohol treated rats show a suppression of wake and an increase of SWS and REMS in the dark period, but opposite changes (increased wake but decreases in SWS and REMS) in the light period (Figure 1B, 1D, and 1F). However, when one examines the total time spent in each state over the entire 22 hour recording period, there was no net change in the time spent in any one of the vigilance states (Figure 1G, 1H, and 1I). A more detailed analysis of the sleep pattern broken down into 2-h blocks reveals that significant differences are observed in wake, SWS and REMS at various times of the day even within the light and dark periods (Figure 1A, 1C, and 1E). Most notable are the increased amount of wake (Figure 1A) and suppression of REMS that occur during the first half of the light period (Figure 1E).

Shown in Figure 2 are representative hypnograms from two pair-fed animals (top two traces) and two alcohol treated animals (bottom two traces) with periods of VC indicated by the bars drawn above the hypnograms. The effects of the chronic alcohol treatment on sleep parameters are quantified and summarized in Table 1. In the light period, after chronic alcohol exposure the number of VC events was greater and there was a decrease in the average duration of individual VC episodes. The decrease duration of VC episodes resulted in a decrease in the percentage of time spent in VC. However, within VC in the light period, the average episode durations for BW, SWS, and REMS did not change significantly. The one change in these parameters was the percentage of REMS was reduced, which from the analysis presented in Figure 1 can be seen as due to a REMS suppression that occurred early in the light period (this is also apparent in the hypnograms in Figure 2). In addition to the lack of change in episode durations for BW, SWS, and REMS from the alcohol treatment, the duration of LDW episodes was also not significantly greater, although this parameter was on the borderline of significance (the p value for alcohol treatment in the two-way ANOVA was $p = 0.053$). The conclusion from these observations is the major effect of chronic alcohol treatment on the sleep patterns of rats in the light period is the alcohol exposure fragments the long periods of VC that normally occur in the light period, with little change in the characteristics of the events within the VC except for REMS suppression early in the light period.

In the dark period the chronic alcohol treatment caused the rats to spend more time in VC than the pair-fed controls. The average duration of the LDW episodes and the average duration of VC episodes were increased (the VC episode duration doubled), but neither change was statistically significant. Although the number of these events did decrease, the decreases were not statistically significant ($p = 0.106$ for VC and $p = 0.084$ for LDW). However, when analyzed by percentage of time spent in VC, the alcohol treated rats did spend significantly more time in VC than the pair-fed rats, indicating that there was a change in the distribution of VC. This

was due to the longer event durations of VC (a doubling), even if the doubling failed to reach statistical significance in the ANOVA of VC event duration.

Within the VC episodes of the dark period the average duration of REMS episodes was increased, and in fact became similar to the duration of these episodes in the light period. Furthermore, the percentage of REMS within VC also became similar to the percentage normally found in the light period. The average duration of SWS episodes increased by about 20 sec, but this increase was not found significant in the two-way ANOVA. However, this may be due to the fact that SWS duration did not change in the light period after alcohol treatment (the interaction term in the analysis almost reached significance; $p = 0.126$). By a straight *t*-test (alcohol vs pair-fed in the dark), the increase in SWS duration is easily significant ($p = 0.0206$). Finally, in the dark period the duration of BW events was not affected by the alcohol treatment, but the percentage of time spent in BW was significantly reduced compared to the pair-fed rats, and was approximately equal to that found in both the pair-fed and alcohol treated rats in the light period. The conclusion from these observations is the major effect of chronic alcohol treatment on the sleep patterns of rats in the dark period is the alcohol treatment causes the rats to spend more time in VC in the dark period, and the nature of the VC has characteristics of VC in the light period (generally longer durations episodes of VC, and within VC, longer episode durations of SWS and REMS, with a relatively less amount of BW).

Effects of alcohol on the slow-wave amplitude in the EEG

The analysis of chronic alcohol effects to this point indicated that alcohol fragments VC in the light period and that in the dark period, when the rats do sleep, the sleep appears to be more intense (i.e., dark period VC takes on more of the characteristics of light period VC). We next sought to determine if additional support for the latter conclusion could be found from the analysis of the EEG amplitude in the SWS periods. Increases in slow-wave amplitude (SWA) are typically interpreted as a sign of increase sleep intensity [2]. The SWA of the EEG varies over the course of the day with a peak that normally occurs soon after the onset of sleep in the normal rest period. This can be seen to occur in the pair-fed rats (Figure 3A, solid circles). However, in the alcohol treated rats the normal circadian variation of SWA was obliterated (Figure 3A, open circles). Since the SWA was normalized across the day, the loss of variation in SWA could be due to a suppression of SWA across the entire day, or an elevation of SWA across the entire day. In Figure 3B and 3C are the average power spectrum for the chronic alcohol treated rats and pair-fed rats shown at the first 2 h of the dark period (normally a low point) and the first 2 h of light period (normally the high point). The pair-fed rats have low amplitude in the delta band in the first 2 h of the dark period and high amplitude in the delta band in the first 2 h of the light period; whereas the chronic alcohol treated animals have high amplitude in the delta band in both periods. Thus the loss of variation was due to an elevation of SWA across the entire day. This observation is consistent with the conclusion that chronic alcohol treated rats are sleeping more intensely in the dark period compared to the pair-fed controls.

Recovery from the effects of chronic alcohol

Sleep patterns were collected over 20 days following termination of the alcohol treatment (results from day 16 and day 2 only are shown). The vigilance state diagrams for the animals 16 days after termination of the treatment is shown in Figure 4 and at this point there are absolutely no differences between the animals in the two groups. Further, analysis of LDW and VC, and analysis of behavior within VC, show complete recovery of every parameter that was altered at the end of the 6 week alcohol treatment (Table 2). Finally, analysis of SWA amplitude also showed a complete recovery of the variation of SWA across the day (Figure 5A). Also of note in Figure 5B and 5C is that the absolute power of the EEG in the alcohol treated animals also matched the control values. It is possible that absolute values could be

different between the two groups because of differences in the fidelity of the recording electrodes in the individual animals. However, in this set of animals, after recovery, the power spectra were almost identical. The observation that the SWA matched in the two groups after recovery supports the conclusion that the chronic elevation of the EEG power across the day at the end of the 6 week alcohol treatment (Figure 3B and 3C) was in fact due to an elevated EEG power spectra in the alcohol treated animals and not due to technical differences in the recordings.

A final issue is whether the changes observed after the 6 week alcohol treatment are due to adaptations to chronic treatment with alcohol or whether the changes are simply due to the acute presence of alcohol. We addressed this issue in two ways. First, we examined the sleep patterns of the chronic alcohol treated rats on day 2 after cessation of alcohol treatment, and second, we examined the effects of acute administration of alcohol at light and dark onset in a separate group of rats. On day 2 of recovery the animals should be virtually free of alcohol (taper to 3% alcohol in liquid diet on day 0 of recovery, alcohol completely removed at the end of the light period, day 1 is the first alcohol-free 24-h period, day 2 recordings thus started 24 hours after complete removal of alcohol). The vigilance state diagrams for the animals at this point are shown in Figure 6. It can be seen that the wake disturbance was still present (more wake in the light and less in the dark), and that some of the SWS and REMS disturbances were still present, but the alterations were not as great as those observed when the alcohol treatment was ongoing (Figure 1). It can also be seen in the more detailed analysis of LDW and VC, and behavior within VC, that some of the parameters were still altered similar to the last alcohol treatment day (Table 3). For example, the percentage of time in LDW and VC were still altered. In the light period the VC episode duration in the alcohol treated animals had begun to approach that of the pair-fed animals but was still shorter (this parameter was almost still significantly different than the pair-fed animals in that $p = 0.078$ for the interaction term in the two-way ANOVA), and in the dark period the characteristics within VC were still similar to the characteristics of VC in the light period (long durations of SWS and REMS episodes with a small percentage of BW and elevated percentage of REMS). Thus many facets of the sleep pattern disturbed by chronic alcohol were still present even after alcohol was no longer present, and thus the changes are likely due to adaptations to the chronic treatment and not the acute presence of alcohol.

Acute administration of alcohol

The second way we examined whether the effects of 6 weeks of alcohol treatment were due to adaptation to the presence of alcohol rather than the acute presence of alcohol was to examine sleep parameters in animals acutely exposed to alcohol. Acute dark onset administration of alcohol decreased wake and increased SWS throughout the entire dark period (Figures 7A–7D). Acute alcohol initially suppressed REMS which was followed by a rebound in REMS (Figure 7E, and top two hypnograms in Figure 8). No effect on wake, SWS, REMS was observed in the following light period when we analyzed the data in this manner (Figure 7A–7F). In regards to daily wake, SWS, and REMS, dark onset administration of alcohol caused a significant decrease in 22 h wake with a corresponding increase in SWS (wake: 729 ± 14 min for control versus 657 ± 13 min after alcohol, $p < 0.001$; SWS: 486 ± 14 min for control versus 553 ± 13 min for alcohol, $p < 0.001$) with no significant change in REMS (105 ± 3 min for control versus 110 ± 6 min for alcohol).

Examination of the VC behavior revealed additional alterations in sleep from the acute alcohol challenge. Dark onset administration of alcohol increased the percentage of time spent in VC in the dark period and within VC it decreased the percent and duration of BW events and increased the percent and duration of SWS events (Table 4). These effects within VC in the dark period are similar to the effects seen after the chronic alcohol treatment (Table 1).

However, in regards to REMS, there were significant differences. Whereas with chronic alcohol REMS was elevated throughout the dark period (Figure 1), with acute dark onset alcohol, REMS was initially suppressed followed by a rebound (Figure 7). Although the analysis of vigilance states suggested no changes occurred in the light period following dark onset administration of alcohol, analysis of VC and LDW event durations revealed that dark onset alcohol caused VC and LDW event durations to shorten without an overall change in the distribution of VC and LDW in the light period (Table 4). Thus, there was fragmentation of VC and LDW in the light period following alcohol administration at the onset of the preceding dark period, but the fragmentation that occurred was not as great as that seen after the chronic alcohol administration (Table 1). Further, the behavior of SWS and BW within VC in the following light period also revealed hangover effects. The percentage of time in BW within VC increased but BW episode duration decreased, whereas the percentage of time in SWS decreased and the SWS episode duration also decreased (Table 4). This increase in cycling within VC supports the conclusion that sleep was further compromised in the light period following dark onset administration of alcohol. A final bit of evidence that sleep was compromised at the beginning of the light period after dark period administration of alcohol was that SWA was suppressed during the first two hours of the light period after dark onset administration of alcohol (Figure 9A). In this set of animals we also observed an enhancement of SWA during the first two hours after alcohol (Figure 9A).

Acute alcohol administration at the beginning of the light period caused a paradoxical increase in wake and decrease in SWS during the first 2-h time block (Figure 7G and 7I). In addition, like dark onset acute administration of alcohol, REMS was initially suppressed followed by a REMS rebound; that in this case extended into the following dark period (Figure 7K and bottom two hypnograms in Figure 8). Consistent with the increase in wake, VC episodes, especially at the beginning of the period, were fragmented (Figure 8, bottom hypnogram, Table 5). In the following dark period the percentage of time in LDW was decreased with a corresponding increase in VC (Table 5). However, within VC there were no changes with regard to BW and SWS in the light period, or in the following dark period (Table 5). Thus the changes in wake and SWS distribution seen in the shaded portions of Figure 7G and 7I were due to changes in the distribution between VC and LDW and not the behavior within VC. The lack of change in BW and SWS behavior was further supported by the complete lack of change in SWA after light period onset alcohol (Figure 9B). REMS rebound appears to be due to increases in the percentage of time within VC spent in REMS and not changes in episode duration (Table 5). This suggests an increase in the number of REMS episodes, which we confirmed in a separate analysis (data not shown). The lack of effect on REMS episode durations by both light and dark onset administration of alcohol is in contrast to the increase in REMS episode duration observed in the dark period after chronic alcohol administration. Finally, light onset administration of alcohol caused no changes in daily wake or SWS (wake: 738 ± 2 min for control versus 717 ± 3 min for alcohol; SWS 470 ± 3 min for control versus 460 ± 3 min for alcohol) but did increase 22 h REMS (112 ± 2 min for control versus 144 ± 2 min for alcohol, $p < 0.001$).

DISCUSSION

Chronic alcohol treatment in rats caused a shift of sleep (SWS and REMS) from the light period to the dark period, with opposite shifts in wake, such that daily amounts of wake, SWS, and REMS did not change. In the light period these changes were mediated primarily by fragmentation of periods of VC with LDW episodes. In addition, in the early light period there was an overall decrease in wake and REMS. In the dark period there was a corresponding increased intensity of sleep as indicated by both an increase in the amount of VC in the dark period, and the VC that occurs in the dark period has a more intense sleep nature. The sleep disturbances in the light period (suppression of REMS and increase wake early in the period,

and fragmentation of VC) are very similar to the effects of chronic alcohol consumption in humans where difficulty falling asleep, REMS suppression, delayed REMS onset, and sleep fragmentation are frequent complaints [3].

Effects of chronic alcohol treatment requires adaptation

It is possible that the effects we observed in our chronic alcohol treatment are due to the acute consumption patterns of alcohol and not adaptations to the chronic presence of alcohol. For example, in a separate study we monitored light/dark differences in alcohol consumption during the 6% alcohol in liquid diet treatment and found that ~80% of the alcohol consumption occurs during the dark period (unpublished observation). Thus the increase sleep intensity in the dark period after chronic alcohol could be caused by the greater acute consumption of alcohol in this period. This could in turn cause less sleep pressure in the light period and hence the fragmentation of VC that we observe. Several lines of evidence argue against this interpretation. Although acute alcohol in naïve animals does cause an increase in SWS episode duration and percent time spent in SWS [18], and this study], this effect does not appear to be maintained after 2–3 weeks of chronic alcohol [18]. This early tolerance to the sleep inducing effects of alcohol is well supported in the literature [24]. Further, after only 2–3 weeks of alcohol, the entire spectrum of changes caused by prolonged alcohol treatment, particularly the changes in distribution of SWS between light and dark period, does not fully manifest [18,23].

In addition, although dark onset administration of alcohol did cause some fragmentation of VC in the following light period, the degree of fragmentation of VC in the light period is not anywhere near the degree seen with chronic alcohol treatment. The effects of chronic alcohol on VC in the light period are also not mimicked by light onset administration of alcohol. Although light onset did cause some fragmentation of VC early in the period, mainly due to the paradoxical increase in wake, after chronic alcohol the percentage time spent in LDW in the light period was doubled, an effect not seen with either acute dark onset or light onset alcohol administration. The reason for the paradoxical increase in wake after alcohol at light onset remains obscure. Alcohol-induced increases in locomotor activity following alcohol have been observed, but this phenomenon occurs robustly in mice, but not in rats [9,11,26].

Another argument that favors the interpretation that chronic effects of alcohol require adaptations is that although acute dark onset alcohol blunted the peak of the SWA in the early light period (as well as elevating SWA in the first 2 hours of the dark period), chronic alcohol treatment flattened any variation in SWA across the day. In total, these numerous observations support the interpretation that increase sleep intensity in the dark period after chronic alcohol exposure is a result of adaptation to light period changes (i.e., fragmentation of long periods of VC), not a cause of light period changes.

Recovery from chronic alcohol effects

By day 16 following stoppage of alcohol treatment the chronic alcohol treated animals had completely returned to normal sleep patterns. At day 2 of recovery, a time when no alcohol should be present, many of the disturbed sleep parameters were still present. This observation further supports our contention that the effects of chronic alcohol are due to adaptation and not the acute presence of alcohol. However, we were surprised by the rapidity of the recovery process. In humans, the alcohol-induced sleep disturbances can last for years [1,5,8,12,13,34, 39]. This suggests that our treatment did not fully manifest the adaptations that occur in humans when they adapt to chronic alcohol. This might be due to either the pattern by which the alcohol was consumed (for example, a more episodic alcohol exposure may produce more enduring changes [24]), or that the 6 week treatment was insufficient to produce the enduring changes

that might occur after decades of alcohol abuse in humans. This issue requires additional studies to be resolved.

Use of novel analysis to describe alcohol effects

A significant aspect of the present study is the use of the novel analysis that includes dividing wake into LDW episodes and BW episodes, which permits us to define a sleep unit in rats labeled VC [33]. First, this analysis allows us to describe effects of alcohol on the sleep patterns in rats that are not apparent in the traditional analysis by vigilance state distributions and analysis of vigilance state episode duration and number. For example, by the traditional analysis acute dark onset administration of alcohol had no hangover effects in the subsequent light period, but with the new analysis we could observe that sleep quality as measured by continuity of VC episodes and more rapid cycling between vigilance states within VC occurred. In the chronic exposure paradigm, the fragmentation of VC in the light period becomes obvious; an effect we conclude is similar to the inability of human alcoholics to maintain sleep throughout the night. Without some definition of an extended sleep unit in rats, such conclusions would not be possible.

Another aspect of the analysis is that the different components in the sleep patterns in rat are likely due to unique aspects of the neuronal circuits that control each component. Thus by identifying which components are altered by alcohol, it helps to identify where it is likely that chronic or acute alcohol is likely to be interacting with sleep regulatory mechanisms. In this novel analysis we find there are four components of sleep patterns that can be identified in such a manner. These include daily sleep homeostasis, the distribution between LDW and VC, the behavior of BW and SWS within VC episodes, and the behavior of REMS.

Total daily sleep in alcohol treated rats

We observed that after 6 weeks of alcohol that the homeostatic regulation of the amount of daily sleep (SWS and REMS) is intact. It has been proposed that the sleep homeostat may be mediated by accumulation of extracellular adenosine in the basal forebrain [27] or possibly cytokines [17], and that these substances eventually influence the flip-flop circuitry of sleep-wake transitions [31] at the level of the ventral lateral preoptic area (VLPO) [25]. The observation that after chronic alcohol the animals still had normal total daily sleep suggests that alcohol does not disrupt the mechanisms that underlie homeostatic regulation of sleep, but rather alcohol is likely to alter the mechanisms that control the timing and/or continuity of sleep. On the other hand, acute exposure to alcohol did alter the daily distribution of sleep states, with the effects of dark onset administration being different than light onset administration. This suggests that acutely alcohol does alter the manner in which the homeostatic signals are interpreted, and that the specific effects of acute alcohol in this regard are dependent upon the specific nature of the signal at particular times of the day, but that in the chronic exposure paradigm there is adaptation to bring total sleep back into balance.

Effects of alcohol on the distribution between LDW and VC and behavior within VC

We have previously [33] presented arguments that sleep-wake transitions within VC are likely the domain of the sleep-wake flip-flop switch [31], whereas control of LDW episodes is likely to be the domain of the hypothalamic integrator that balances circadian rhythms with sensory, cognitive, and emotional cues [32]. Thus our findings that the main effect of chronic alcohol was to alter the distribution of LDW and VC suggest that the main effect of chronic alcohol is to alter the function of the hypothalamic integrator and not the behavior (and by extension, the neurocircuitry) of state transitions driven by the sleep-wake flip-flop switch. Although the character of VC in the dark period did change (appears to be light period-like), this change is likely to be mediated by the balance between homeostatic drive for sleep with circadian (and possibly other) inputs onto the VLPO. For example, the loss of VC in the light period increases

the homeostatic drive for sleep and thus dark period adaptations need to occur to keep total daily sleep constant. On the other hand, the acute effect of alcohol given at dark onset does appear to alter the behavior within the flip-flop switch to favor SWS over BW, but the animal seems to adapt to these acute changes.

Another remarkable observation is the relative resistance of the BW and SWS behavior within VC in the light period to alcohol effects. This behavior was not altered by either chronic alcohol or acute light onset administration of alcohol. This suggests that the circuits that underlie the distribution of BW and SWS, which we speculate is the flip-flop switch, is not inherently sensitive to the acute presence of alcohol, at least not in the light period. However, dark onset administration of alcohol did cause decreases in duration of both BW and SWS in the following light period, an effect that occurs after alcohol has mostly cleared from the system. Again, this argues that within the switch (for example, the balance of GABAergic transmission between neuronal components) is relatively resistant to alcohol effects, but that this balance is upset by inputs onto the switch, which may be different at different times of day.

REMS suppression and rebound

Regardless of whether acute alcohol was given at light or dark onset, we observed an early REMS suppression followed by a profound REMS rebound. Interestingly, acute alcohol had no effect on REMS episode duration after either light or dark onset administration. Thus the acute presence of alcohol must increase the probability of entering into a REMS episode rather than alter the mechanisms that control REMS episode duration. With dark onset administration the REMS suppression and rebound balanced so there was no net change in total REMS, however with light onset administration, the REMS rebound was in excess of the suppression so there was a net increase in total REMS. These acute effects are in contrast to the effects observed after chronic alcohol treatment. After chronic alcohol the total amount of daily REMS remained normal, suggesting the homeostatic regulation of REMS was intact, but the distribution of REMS shifted from predominately in the light period to being about equally balanced between light and dark periods.

The chronic alcohol-induced alteration of REMS could be an action mediated within the REMS flip-flop switch in the brainstem or an action on the hypothalamic inputs onto the REMS flip-flop switch [20]. An argument in favor of the hypothalamic site of action is that durations of REMS episodes increased in the dark period but slightly decreased in the light period. If alcohol targeted mechanisms within the REMS flip-flop switch (e.g., enhanced suppression of REMS-on by REMS-off mechanisms) one would expect a similar change (suppression) in both light and dark periods. This suggests that after chronic alcohol the two components of the brainstem REMS flip-flop switch are functioning normally, but that the inputs onto the switch from the hypothalamus which control the circadian differences in REMS episode duration and number, have changed. Another important conclusion from these observations is further support that the effects of chronic alcohol require adaptation and is not mimicked by the acute effects of alcohol on REMS control mechanisms.

Circadian effects of alcohol

One clear effect of chronic alcohol on sleep is that the normal circadian pattern of sleep is disrupted. Both the normal distribution between VC and LDW was altered as was the behavior of VC in the dark period, which became more light-period like. The durations of BW episodes were not altered by alcohol treatment, suggesting alcohol has no effect on the factors that regulate this parameter. While most sleep models proposed to date do a good job explaining circuits that control wakefulness, SWS, and REMS, they have yet to address the mechanisms of circadian influences that adjust the distribution between LDW and VC, as well as the specific patterning of vigilance states within VC. Thus possible mechanisms that might underlie the

effects of alcohol on these patterns remain obscure. However, it is well documented that prolonged alcohol treatments can alter the circadian pattern of a variety of hormonal and behavioral rhythms [15,21,28,29,36]. Also mutations in the clock *Per2* gene have been shown to modulate alcohol consumption in both mice and humans [35]. How alcohol consumption alters circadian patterns and how clock genes influence alcohol consumption remain unknown.

Negative affect produced by chronic alcohol

In addition to circadian disruptions it is also possible that other actions of chronic alcohol exposure could impinge on sleep. In the hypothalamic integrator model of Saper et al. [32], the hypothalamus not only integrates circadian influences on sleep, but is also the site where other environmental cues, including emotional inputs from limbic structures, influence sleep. In the allostatic state model of drug addiction put forth by Koob and La Moal [16], compulsive drug seeking behavior is driven by both drug induced activation of brain reward systems but also negative affect due to drug induced upregulation of antireward systems. In the alcohol addicted state Helig and Koob [14] have put forth that this antireward system originates by excess actions of corticotropin-releasing factor (CRF) caused by CRF1 receptor upregulation in the amygdala. CRF is known to disrupt sleep [10,38] and CRF hyperactivity has been put forth as mediating the hyperarousal associated with primary insomnia [30]. These findings suggests that the negative affective state produced by chronic alcohol, perhaps mediated by CRF acting in the amygdala, may be a type of emotional input into the hypothalamic regulator that produces fragmentation of VC. Future studies will be needed to test this hypothesis.

Conclusion

Our analysis of sleep in rats that utilized the concepts of LDW and VC has enabled us to identify an extended sleep period in these polyphasic sleeping animals. Using this analysis we show that chronic alcohol treatment in rats also causes a disruption in the ability to maintain long sleep episodes (i.e., extended periods of VC in the light) similar to the insomniac effects observed in human alcoholics and recovered alcoholics [1,5,8,12,13,34,39]. In addition, we found that the normal circadian pattern of sleep was altered by alcohol. Another important aspect from this study is alcohol can have different actions on the distribution of LDW and VC versus the behavior of SWS and BW within VC. For example, in the light period VC is fragmented, but the behavior within VC is normal. This provides further support for our previous conclusion that LDW and BW are differently regulated [33] and demonstrates the usefulness of the analysis of sleep patterns used in this study. Additional studies will be needed to fully explore the nature of alcohol induced alterations in sleep, but our findings begin to define the nature of these alterations, and with an increase understanding of the neuronal mechanisms that underlie the different components of sleep, will point to mechanisms that account for the actions of alcohol on sleep.

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Figure 1.

Vigilance state diagram for alcohol treated (open symbols and bars; n = 8) and pair-fed (filled symbols and bars; n = 6) rats after 6 weeks of alcohol treatment. In this and all subsequent graphs shaded portion of graphs indicates dark period and unshaded area light period. Panels A, C, and E indicate percent of time spent in indicated vigilance state in two-hour time blocks. Panels B, D, and F summarize the distribution of vigilance states across dark and light periods. Panels G, H, and I illustrate the total amount of each vigilance state across the entire 22 recording period. Asterisks indicate that the alcohol treated value is statistically different from pair-fed value at the same time (*p<0.05, **p<0.005). Pound signs indicate the light period

value is significantly different from the corresponding dark period value within the treatment group ($p < 0.05$, $##p < 0.005$).

Figure 2. Representative hypnograms from two pair-fed (PF1 and PF2) and two alcohol treated (ET8 and ET9) rats after 6 weeks of alcohol treatment. R, S, and W on the vertical axis indicates REMS, SWS, and wake, respectively. The lines above the hypnogram (labeled VC on the vertical axis) indicate periods of vigilance cycling.

Figure 3.

Slow-wave amplitude (SWA) after 6 weeks of treatment for pair-fed (filled symbols, $n = 6$) and alcohol treated (open symbols, $n = 8$) animals are illustrated in panel **A**. Asterisks indicate data points from pair-fed animals that are significantly different from the peak value (data point with extra circle; one-way ANOVA; $p < 0.05$). In the lower panels are illustrated the averaged power spectra for the 1st 2 hours in the dark period (panel **B**) and 1st 2 hours of the light period (panel **C**). Above the data points are lines that indicate the δ and θ frequency ranges.

Figure 4.

Vigilance state diagram for alcohol treated (open symbols and bars; n = 6) and pair-fed (filled symbols and bars; n = 6) rats 16 days after complete cessation of alcohol exposure. Panels **A**, **C**, and **E** indicate percent of time spent in indicated vigilance state in two-hour time blocks. Panels **B**, **D**, and **F** summarize the distribution of vigilance states across dark and light periods. Panels **G**, **H**, and **I** illustrate the total amount of each vigilance state across the entire 22 recording period. Pound signs indicate the light period value is significantly different from the corresponding dark period value within the treatment group (##p<0.005).

Figure 5.

Slow-wave amplitude (SWA) on day 16 after cessation of alcohol treatment for pair-fed (filled symbols, $n = 6$) and alcohol treated (open symbols, $n = 6$) animals are illustrated in panel **A**. Asterisks indicate data points from pair-fed animals that are significantly different from the peak value (data point with extra circle; one-way ANOVA; $p < 0.05$). Pound signs indicate data points from alcohol treated animals that are significantly different from the peak value (data point with extra circle; one-way ANOVA; $p < 0.05$). In the lower panels are illustrated the averaged power spectra for the 1st 2 hours in the dark period (panel **B**) and 1st 2 hours of the light period (panel **C**). Above the data points are lines that indicate the δ and θ frequency ranges.

Figure 6.

Vigilance state diagram for alcohol treated (open symbols and bars; n = 8) and pair-fed (filled symbols and bars; n = 6) rats on day 2 after complete cessation of alcohol exposure. Panels A, C, and E indicate percent of time spent in indicated vigilance state in two-hour time blocks. Panels B, D, and F summarize the distribution of vigilance states across dark and light periods. Panels G, H, and I illustrate the total amount of each vigilance state across the entire 22 recording period. Asterisks indicate that the alcohol treated value is statistically different from pair-fed value at the same time (*p<0.05, **p<0.005). Pound signs indicate the light period value is significantly different from the corresponding dark period value within the treatment group (##p<0.005).

Figure 7.

Vigilance state diagram for dark onset administration of alcohol ($n = 20$, left two sets of panels) and light onset administration of alcohol ($n = 19$, right two sets of panels) after water gavage control (filled symbols and bars) and alcohol gavage (open symbols and bars). The shaded portion of graphs indicates dark period and unshaded portion of the graph indicates light period. Graphs **A**, **C**, and **E** (dark onset alcohol administration) and graphs **G**, **I**, and **K** (light onset alcohol administration) indicate percent of time spent in indicated vigilance state in two-hour time blocks. Panels **B**, **D**, and **F** (dark onset alcohol administration) and **H**, **J**, and **L** (light onset alcohol administration) summarize the distribution of vigilance states across dark and light period. Asterisks indicate that the alcohol treated value is statistically different from pair-fed value at the same time (* $p < 0.05$, ** $p < 0.005$). Pound signs indicate the light period value is significantly different from the corresponding dark period value within the treatment group (# $p < 0.05$, ## $p < 0.005$).

Figure 8.

Representative hypnograms from two rats from dark onset (top two hypnograms) and light onset (bottom two hypnograms) recordings. Control (water gavage) hypnograms are E6 co and C4 co and alcohol gavages are E6 et and C4 et. R, S, and W on the vertical axis indicates REMS, SWS, and wake, respectively. The lines above the hypnogram (labeled VC on the vertical axis) indicate periods of vigilance cycling.

Figure 9.

Slow-wave amplitude (SWA) after water gavage (filled circle) and alcohol gavage (open circle) after dark onset administration of alcohol (3A, $n = 20$) and after light onset administration of alcohol (3B, $n = 19$). Results were analyzed by two way ANOVA (treatment $\times$ time). After alcohol treatment the SWA increased in the first two hour in the dark period and decreased in the first two hours in the light period (** $p < 0.005$).

Table 1
Summary of the effect of 6 week treatment with alcohol on sleep parameters.

parameter	pair-fed		alcohol treated	
	dark period	light period	dark period	light period
<u>Long duration wake</u>				
No. of LDW episodes:	17.0±1.0	5.3±0.8	12.5±1.9	7.4±0.5 [*]
Duration of LDW:	1430±150	1020±160	1670±220	1480±110
% time in LDW:	55.3±4.2	14.9±2.5 ^{##}	40.9±3.9 [*]	30.0±2.4 ^{**#}
<u>Vigilance cycling</u>				
No. of VC episodes:	17.2±0.9	5.2±0.7	12.8±2.1	7.5±0.5 [*]
Duration of VC:	1130±100	6860±1090 ^{##}	2420±520	3590±300 ^{**}
% time in VC:	44.3±4.3	85.1±2.5 ^{##}	57.3±4.2 [*]	69.8±2.4 ^{*#}
<u>Brief wake</u>				
BW episode duration:	35.3±2.3	33.6±0.9	31.7±1.0	33.3±0.8
BW (% of VC):	35.2±3.6	22.9±1.7 ^{##}	25.3±2.6 [*]	23.9±1.0
<u>Slow-wave sleep</u>				
SWS episode duration:	55.7±9.4	89.3±11.8 [#]	75.3±6.3	84.5±3.9 [#]
SWS (% of VC):	55.2±2.7	58.7±2.5	58.8±2.8	61.2±0.8
<u>REMS</u>				
REMS episode duration:	48.0±6.6	76.8±5.9 ^{##}	69.5±7.0 [*]	65.3±2.1
REMS (% of VC):	9.6±1.3	18.4±1.1 ^{##}	16.0±0.8 ^{**}	14.9±1.0 [*]

Durations are reported in sec. No. of LDW episodes is the number of episodes assigned to LDW within the light or dark period. Duration of LDW is the average duration of LDW episodes within the period. Percent time in LDW and VC are relative to entire dark or light period. BW, SWS, and REMS (% of VC) are the percent of time within VC that is occupied by the respective state. BW, SWS, and REMS episode durations are the average duration of episodes that occurred within VC. Values are averages ±SE; n = 8 for alcohol treated animals and n = 6 for pair-fed animals.

[#] Pound sign: significantly different from dark period for same treatment p<0.05

^{##} p<0.005.

^{*} Asterisk: significantly different from pair-fed in same period p<0.05

^{**} p<0.005.

Table 2
Summary of sleep parameters on day 16 following after cessation of alcohol.

parameter	pair-fed		alcohol treated	
	dark period	light period	dark period	light period
<u>Long duration Wake</u>				
No. of LDW episodes:	18.7 ± 1.8	5.5±0.6	14.7±1.7	6.0±0.9
Duration of LDW:	1310 ± 100	1100±60##	1730±190	1050±60##
% time in LDW:	54.9 ± 2.6	17.1±2.6##	55.4±3.4	17.8±3.5##
<u>Vigilance Cycling</u>				
No. of VC episodes:	19.2 ± 1.8	5.3±0.6	15.0±1.8	6.5±1.0
Duration of VC:	1090 ± 150	6940±1720##	1470±330	5580±890##
% time in VC:	44.8 ± 2.7	82.8±2.6##	44.4±3.5	82.2±3.5##
<u>Brief wake</u>				
BW episode duration:	33.5 ± 2.2	30.6±1.4	32.6±1.3	31.2±1.5
BW (% of VC):	36.7 ± 2.0	24.2±1.6##	36.4±1.2	27.5±2.2##
<u>Slow-Wave Sleep</u>				
SWS episode duration:	48.4 ± 5.1	79.2±10.7##	46.0±2.5	68.1±7.0##
SWS (% of VC):	55.6 ± 1.9	60.1±2.5#	53.9±1.4	57.9±1.6#
<u>REMS</u>				
REMS episode duration:	50.4 ± 4.9	82.1±6.1##	53.1±4.7	72.4±4.2##
REMS (% of VC):	7.8 ± 0.9	15.7±1.2##	9.7±1.5	14.6±1.5##

See legend to Table 1 for explanation of parameters in table. Values are averages ±SE; n = 6 for alcohol treated animals and n = 6 for pair-fed animals.

Table 3
Summary of sleep parameters day 2 after cessation of alcohol treatment.

parameter	pair-fed		alcohol treated	
	dark period	light period	dark period	light period
<u>Long duration Wake</u>				
No. of LDW episodes:	20.5±2.1	4.7±0.7	13.9±2.1*	7.0±1.0
Duration of LDW:	1280±100	920±60#	1890±300*	1240±130*##
% time in LDW:	58.6±2.4	12.3±2.3##	49.7±2.7*	23.2±3.2*##
<u>Vigilance Cycling</u>				
No. of VC episodes:	21.0±2.1	5.0±0.8	14.4±2.3	7.3±1.0
Duration of VC:	930±140	8680±1790##	1760±260	5210±1490##
% time in VC:	41.3±2.5	87.7±2.3##	48.3±1.7	76.8±3.2*##
<u>Brief wake</u>				
BW episode duration:	30.2±2.2	30.1±1.8	28.5±1.3	28.1±0.9
BW (% of VC):	41.0±2.8	24.7±1.7##	26.7±2.1**	25.6±1.1
<u>Slow-Wave Sleep</u>				
SWS episode duration:	38.2±6.8	77.3±8.2##	63.5±6.5*	64.7±4.7
SWS (% of VC):	51.9±2.4	59.0±2.4#	60.4±2.1*	58.2±1.3
<u>REMS</u>				
REMS episode duration:	45.1±3.8	71.4±5.1##	61.4±3.5	70.5±4.5##
REMS (% of VC):	7.1±1.6	16.2±0.9##	12.9±0.7**	16.2±0.6#

See legend to Table 1 for explanation of parameters in table. Values are averages ±SE; n = 8 for alcohol treated animals and n = 6 for pair-fed animals.

Table 4
Summary of sleep parameters acute dark onset administration of alcohol.

parameter	control		acute alcohol	
	dark period	light period	dark period	light period
<u>Long duration wake</u>				
No. of LDW episodes:	18.5±0.9	5.0±0.4 ^{##}	17.3±1.0	5.8±0.4 ^{##}
Duration of LDW:	1360±70	1460±110	1100±80*	1220±110*
% time in LDW:	56.0±2.0	15.4±0.8 ^{##}	41.6±2.3**	15.0±0.7 ^{##}
<u>Vigilance cycling</u>				
No. of VC episodes:	18.2±0.9	5.1±0.3 ^{##}	17.4±0.9	6.4±0.3 ^{##}
Duration of VC:	1110±100	8100±600 ^{##}	1560±100	6430±310 ^{##} *
% time in VC:	43.8±2.0	84.5±0.8 ^{##}	58.3±2.3**	84.9±0.7 ^{##}
<u>BW</u>				
BW episode duration:	35.9±1.5	37.7±1.5	31.8±1.4*	34.2±1.3*
BW (% of VC):	37.1±1.6	23.7±0.7 ^{##}	30.2±1.7**	27.7±1.3*
<u>Slow-Wave Sleep</u>				
SWS episode duration:	53.7±4.0	96.0±4.5 ^{##}	64.2±3.9*	73.2±4.5 ^{##} **
SWS (% of VC):	56.8±1.4	59.4±0.7 [#]	62.1±1.5**	56.9±0.9 ^{##} *
<u>REMS</u>				
REMS episode duration:	53.3±3.7	88.6±3.6 ^{##}	53.2±3.3	77.7±3.2 ^{##}
REMS (% of VC):	6.2±0.7	17.0±0.6 ^{##}	7.8±0.7	15.4±0.9 ^{##}

See legend to Table 1 for explanation of parameters in table. Values are averages ±SE; n = 20. Statistical comparisons were by a 2-way repeated measures ANOVA. Pound signs and asterisks indicate significance as described for Table 1.

Table 5
Summary of sleep parameters after acute light onset administration of alcohol

parameter	control		acute alcohol	
	Light period	dark period	light period	dark period
<u>Long duration wake</u>				
No. of LDW episodes:	5.8±0.4	15.9±1.1 [#]	9.2±0.5 **	14.1±0.9 ^{##}
Duration of LDW:	1180±80	1660±110 ^{##}	99±600	1590±130
% time in LDW:	15.8±1.3	57.0±1.9	20.4±1.1	48.0±2.5**
<u>Vigilance cycling</u>				
No. of VC episodes:	5.9±0.4	16.1±1.1 ^{##}	9.3±0.5 **	14.7±1.0 ^{##}
Duration of VC:	6840±430	1310±140 ^{##}	4160±390 **	1650±130 ^{##}
% time in VC:	84.1±1.3	42.9±1.9 ^{##}	79.5±1.1	51.9±2.5 ^{##**}
<u>BW</u>				
BW episode duration:	30.3±1.1	33.2±0.9	32.4±2.6	31.2±1.1
BW (% of VC):	29.2±1.0	40.8±1.1 ^{##}	29.2±1.3	37.3±1.4 ^{##}
<u>Slow-Wave Sleep</u>				
SWS episode duration:	63.9±3.7	39.0±1.7 ^{##}	58.1±2.7	39.4±1.7 ^{##}
SWS (% of VC):	56.7±1.0	48.9±1.0 ^{##}	54.3±1.4	47.6±1.5 ^{##}
<u>REMS</u>				
REMS episode duration:	68.6±2.9	56.7±5.2 ^{##}	73.4±2.0	57.2±2.2 ^{##}
REMS (% of VC):	14.1±1.0	10.3±0.9 [#]	16.5±0.7 **	15.1±0.9 ^{#**}

See legend to Table 1 for explanation of parameters in table. Values are averages ±SE; n = 19. Statistical comparisons were by a 2-way repeated measures ANOVA. Pound signs and asterisks indicate significance as described for Table 1.