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# Effects of the Acute Administration of Ethanol on the Sleep of the Rat: A Dose-Response Study[1]

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MENDELSON, W. B. AND S. Y. HILL. *The effects of the acute administration of ethanol on the sleep of the rat: A dose-response study.* PHARMAC. BIOCHEM. BEHAV. 8(6) 723–726, 1978. – Seven-hr sleep recordings were performed on rats following intraperitoneal injection of saline or one of four doses of ethanol (1.1, 1.5, 2.0 or 2.5 g/kg). Total minutes of REM sleep and percentage REM sleep were decreased in a dose-dependent manner. Percentage nonREM sleep increased with progressively higher doses. The decrease in REM sleep appeared to be related to a decrease in the number of REM sleep episodes and an increase in the length of the REM-nonREM cycle. Other variables such as mean length of REM sleep episodes and REM sleep efficiency were unchanged. An analysis of the first and second 3.5 hr of the recording showed that ethanol continued to have marked effects on REM and nonREM sleep during the second 3.5 hr, when blood levels were declining. Ethanol produced decreases in sleep latency, but total sleep time was unchanged.

Ethanol Sleep REM sleep

ALTHOUGH clinical experience and systematic studies suggest that ethanol may have a variety of effects on sleep, their nature is surprisingly poorly defined. Data from animal and human studies tend to agree that rapid eye movement (REM) sleep decreases after acute administration [1, 7, 9]. It is not clear, however, whether the decrease in REM sleep is related to changes in REM sleep latency, number of REM sleep episodes, REM-nonREM cycle length, or to other factors. It is also unclear whether these effects are dose-related. An extensive search of the literature on animals, for instance, found only one study dealing with this problem [1], and even there only two doses were administered. For these reasons we have performed a dose-response study of the acute effects of ethanol on the sleep of rats.

## METHOD

Forty-six male Sprague-Dawley rats weighing 200–250 g were anaesthetized with 40 mg/kg subcutaneous pentobarbital, and then were surgically implanted with electrodes for recording the electroencephalogram (EEG) and electromyogram (EMG) as described in a previous publication [4]. In summary, size 0–80, stainless steel screws, 3.2 mm long, were anchored in the skull such that their tips rested on the dura. The EEG was recorded from screw electrodes placed in the right frontal and parietal areas. An electrode in the left frontal area served as ground. The two EMG electrodes consisting of 0.13 mm teflon-coated stainless steel wire were embedded in posterior neck muscles. One week after electrode implantation, the rats were randomly assigned to one of five groups, and received an intraperitoneal injection of either saline or ethanol in doses of 1.1, 1.5, 2.0 or 2.5 g/kg, in concentrations of 10, 13, 15, 18 and 15 percent respectively. These were prepared by diluting 95 percent (v/v) ethanol in sterile normal saline. (The highest dose, 2.5 g/kg, was administered as a slightly larger volume of 15 percent solution, in order to avoid possible peritoneal irritation which is sometimes associated with solutions greater than 20 percent.) Rats were fed on Purina Lab Chow ad lib throughout the study.

Seven-hr recordings, performed on a Grass Model 7 polygraph with a paper speed of 15 mm/sec, were started immediately after injection at 9:00 a.m. Lights were on in the recording chamber from 8:00 a.m. to 8:00 p.m. Records identified by a randomly assigned code number were read in 20 sec spochs, which were determined to be waking, nonREM or REM sleep. In an awake record, the EEG showed low amplitude mixed frequency, containing various amounts of theta waves, and the EMG was relatively high. NonREM sleep was characterized by high amplitude, slower waves. REM sleep was scored in the presence of low

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amplitude EEG containing mixed frequencies or predominantly theta activity, with a low EMG. These stages are illustrated in earlier publications from this [4] and other [5] laboratories. Data were analyzed by a one-way analysis of variance (ANOVA). In those cases in which the ANOVA was found to be significant ($p<0.05$), a Neuman-Keuls test was performed to determine significance of differences between individual doses.

## RESULTS

As seen in Table 1 and Fig. 1, there were significant decreases in both REM sleep time ($p<0.01$) and percentage REM sleep ($p<0.005$). In both cases the values for placebo and 1.1 g/kg dose animals were significantly higher than those for the 2.5 g/kg group. NonREM sleep percentage increased ($p<0.005$), such that the placebo and 1.1 g/kg groups had significantly lower values than the 2.5 g/kg group ($p<0.01$ in both cases; see Fig. 2). As might have been expected from the apparent behavioral sedation of the animals, sleep latency (the time from the beginning of the recording until sleep onset) was decreased ($p<0.02$), with the shortest sleep latency for the 2.0 g/kg group. Total sleep time during the recording was not significantly altered by ethanol. Similarly, intermittent waking time and number of waking episodes were unaltered.

The sleep data were also examined to compare the first and second 3.5 hr of the recording (Fig. 1). Analysis of the data demonstrates that percentage REM sleep decreased to a degree approaching statistical significance in the first 3.5 hr ($p<0.08$) and then decreased significantly in the second 3.5 hr ($p<0.02$). Similarly, percentage nonREM sleep increased to a degree approaching significance in the first 3.5 hr ($p<0.08$) and rose significantly in the second 3.5 hr ($p<0.02$).

In order to determine the source of decreased REM sleep, a variety of variables were examined; a significant reduction in the number of REM sleep episodes ($p<0.001$) was found. The 2.5 g/kg group values were significantly less than those for the saline or 1.1 g/kg groups ($p<0.01$ in both cases). REM sleep latency, length of the first REM sleep episode, mean length of all REM sleep episodes, and REM sleep efficiency (a measure of the amount of interruptions of each REM episode) were unchanged. REM-nonREM cycle length (the mean time from the beginning of one REM episode to the beginning of the next) was increased by ethanol ($p<0.01$), such that the value for the 2.5 g/kg group was greater than those of the saline ($p<0.01$) or 1.1 g/kg ($p<0.01$) groups.

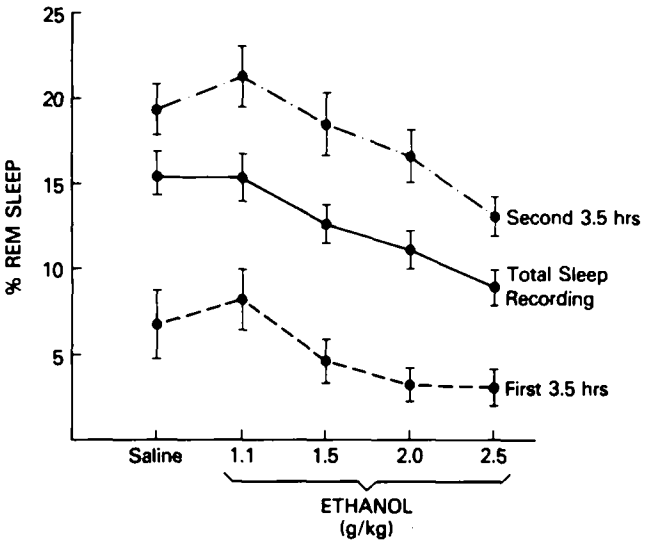


FIG. 1. The effect of four doses of ethanol on percentage REM sleep in the rat.

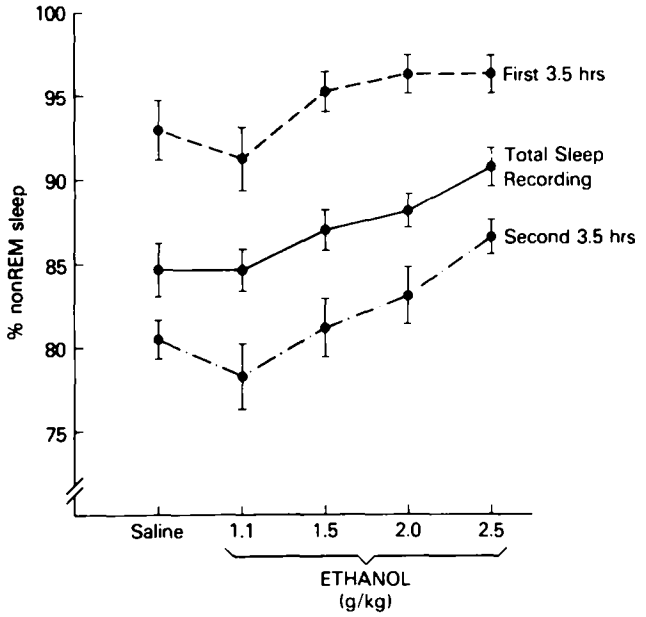


FIG. 2. The effect of four doses of ethanol on percentage nonREM sleep in the rat.

## DISCUSSION

These data suggest that the ethanol-induced decrease in REM sleep is dose-related. The lack of change over the entire seven-hr recording at low doses (1.1, 1.5 and 2.0 g/kg), and the decrease at the highest dose (2.5 g/kg), may help explain inconsistencies in the literature on normal humans. Most studies on normal humans have been at doses of approximately 1.0 g/kg. They have shown either decreased REM sleep in the first half of the recording, with no effect on the entire recording [3,7], or decreased REM sleep for the entire eight hours [2,10]. As some of these authors [3] have commented, the difference in these studies may be dose-related, as a dose of 1.0 g/kg may be slightly below that which is needed to consistently decrease REM sleep for the entire night. The results of our study are similar to those of Branchey, Begleiter and Kissin [1], whose lowest dose (0.5 g/kg) failed to affect REM sleep, although we required a higher dose to achieve consistent decreases. As in their two-dose study, we have confirmed that the increase in percentage nonREM sleep is dose-related.

The analysis of the first and second 3.5 hr periods was designed to determine whether a single dose of ethanol would produce an initial decrease, followed by an increase in REM sleep, as has been reported in humans [3,7]. What we found instead was that lower doses had no significant effect on total REM sleep time, and that higher doses affected both parts of the recording. This result is similar to a report that a decrease in REM sleep occurred in two

TABLE 1

SLEEP EEG DATA (MEAN±SEM) FOR RATS GIVEN A SINGLE INTRAPERITONEAL INJECTION OF SALINE OR ETHANOL

| | Saline (n=10) | 1.1 g/kg (n=9) | 1.5 g/kg (n=10) | 2.0 g/kg (n=10) | 2.5 g/kg (n=7) | Significance* |
|---|---|---|---|---|---|---|
| Total Recording Period (min) | 426.8± 3.4 | 437.4± 2.4 | 429.5± 4.7 | 435.5± 3.6 | 425.2± 3.7 | NS |
| Total Sleep Time (min) | 266±16.1 | 298.3±12.8 | 272.8±18.3 | 292.1±16.3 | 272.0±16.9 | NS |
| Intermittent Walking Time (min)** | 127.4±12.6 | 123.9±12.2 | 140.4±15.9 | 130.1±14.8 | 131.0±10.9 | NS |
| NonREM Sleep Time (min) | 224.4±14.6 | 251.3±13.0 | 236.9±15.4 | 258.4±14.5 | 248.1±16.4 | NS |
| REM Sleep Time (min) | 41.6± 4.6 | 47.0± 3.7 | 35.9± 4.6 | 33.8± 4.0 | 23.9± 3.2 | $p<0.01$ |
| % Intermittent Awake Time | 32.6± 3.4 | 29.4± 2.9 | 34.2± 4.0 | 30.9± 3.6 | 32.8± 3.2 | NS |
| % nonREM Time | 84.3± 1.5 | 84.1± 1.4 | 87.0± 1.2 | 88.5± 1.0 | 91.1± 1.1 | $p<0.005$ |
| % REM Time | 15.7± 1.5 | 15.9± 1.4 | 12.9± 1.2 | 11.5± 1.0 | 8.9± 1.1 | $p<0.005$ |
| Sleep Latency (min)† | 35.1± 6.4 | 17.4± 4.6 | 18.2± 3.7 | 14.7± 2.3 | 22.5± 4.7 | $p<0.02$ |
| REM Latency (min)‡ | 99.1±12.4 | 86.3±12.8 | 120.6±24.1 | 136.6±18.4 | 150.4±28.2 | NS |
| No. REM Episodes§ | 19.3± 1.8 | 21.8± 1.6 | 15.9± 1.9 | 15.3± 1.6 | 9.8± 1.6 | $p<0.001$ |
| Mean Length of REM Episodes (min) | 2.1± 0.1 | 2.1± 0.1 | 2.3± 0.1 | 2.2± 0.2 | 2.4± 0.1 | NS |
| REM Efficiency¶ | 95.8± 0.9 | 96.3± 0.8 | 95.4± 0.9 | 96.8± 1.0 | 97.1± 1.0 | NS |
| REM-nonREM Cycle Length (min)# | 16.4± 1.3 | 15.5± 1.0 | 21.3± 3.1 | 21.3± 2.5 | 28.2± 3.4 | $p<0.01$ |
| Length of 1st REM Episode (min) | 1.8± 0.2 | 1.4± 0.3 | 1.5± 0.2 | 1.0± 0.2 | 1.2± 0.3 | NS |
| No. of Intrmittent Waking Episodes | 21.9± 2.4 | 24.0± 2.4 | 24.4± 2.0 | 25.4± 1.6 | 25.4± 1.9 | NS |

Significance of comparison of individual values (Neuman Keuls test):
REM Sleep Time: Saline > 2.5 ($p<0.05$); 1.1 > 2.5 ($p<0.01$)
% nonREM Time: 2.5 > saline ($p<0.01$); 2.5 > 1.1 ($p<0.01$)
% REM Time: Saline > 2.5 ($p<0.01$); 1.1 > 2.5 ($p<0.01$)
Sleep Latency: Saline > 2.0 ($p<0.05$); saline > 1.5 ($p<0.05$); saline > 1.1 ($p<0.05$)
No. REM Episodes: Saline > 2.5 ($p<0.01$); 1.1> 2.5 ($p<0.01$)
REM Cycle Length: 2.5 > saline ($p<0.01$); 2.5 > 1.1 ($p<0.01$)

* One-way analysis of variance.

** Waking time following sleep onset.

† Time from beginning of recording to sleep onset (first occurrence of a non-waking sleep stage lasting at least 40 sec continuously).

‡ Time from sleep onset until the beginning of the first REM episode.

§ A REM sleep episode is defined as at least 40 sec of REM sleep, either continuous or separated by no more than 40 sec of a stage other than REM.

¶ The REM efficiency of each REM episode is the amount of actual REM sleep time in a REM episode divided by the total length of the episode, expressed as a percentage. The REM efficiency for the recording represents the means of the REM efficiency values for all REM episodes.

# Time from the beginning of one REM episode to the beginning of the next.

consecutive 3.0 hr periods in rats [1], although in the present study higher doses were required to produce this action. The source of this difference between studies is not clear, although it should be noted that the rats in the present study were of approximately half the weight of those observed by Branchey, Begleiter and Kissin [1]. It has been reported that an intraperitoneal dose of 2.5 g/kg requires 6.3 hr to be completely metabolized in the rat [6]. An implication may be that ethanol produces changes in the regulation of sleep in the rat which persist even when blood concentrations are declining. A study in cats also reported decreases in percentage REM sleep in consecutive 3.5 hr periods, once again at a lower dose [9]. The source for the discrepancy in effective doses is not clear, aside from the differing species and route of administration.

Previous studies have differed on the issue of which REM sleep variable is associated with the decrease in REM sleep. Our study suggests that the main effect is related to a decrease in the number of REM sleep episodes and an increase in REM-nonREM cycle length; the decrease in number of REM episodes is in agreement with the unpublished data from this laboratory on the effects of somewhat higher doses of ethanol given by intragastric intubation to rats. There are no other data on rats available on this point. A study in cats found decreases in length of REM periods to be associated with the decrease in percentage REM sleep [9]. Data on normal humans tends to agree that there is no systematic change in REM sleep latency [10,11]. One study, however, suggested that the decrease in REM sleep is due to a decreased mean length of REM sleep episodes [11] and two studies found a decrease in the length of the first REM sleep spisode [7,8]. We also observed that values for the latter parameter decreased by 30%, but (possibly due to large variability) this was not significant. Besides the obvious difference in species, the cause of these differing results is unclear.

Finally, it is interesting that in our study, as in a variety of studies of normal humans [3,11], total sleep time is generally unchanged, although sleep latency is often shortened. This would seem to reflect not only the rapid metabolism of ethanol, but also the efficiency of homeostatic mechanisms regulating the architecture of sleep.

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