Effect of Ethanol on Sleep-Awake State in Sleep-Disturbed Rats

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The present study was undertaken to investigate the effect of ethanol on the sleep-wake cycle in normal rats and sleep-disturbed rats. In normal rats, no significant difference was observed by ethanol in sleep latency, total awake time and total non-rapid eye movement (NREM) sleep time, except for total REM sleep time. On the other hand, in sleep-disturbed rats, ethanol at doses of 1 and 2 g/kg caused significant decreases in sleep latency and total awake time, and an increase in total NREM sleep time. In addition, ethanol showed a significant increase in delta activity in the sleep-disturbed model rat, different from triazolam. These results suggested that ethanol had not only a hypnotic but also a sleep-maintaining effect in sleep-disturbed rats at reasonable blood ethanol concentrations.

Key words ethanol; sleep-disturbed rat; sleep-wake pattern; sleep quality; blood ethanol concentration

It is well recognized that many people have sleep problems, especially in the last 20 years. Soldatos et al. reported that more than 20% of people suffering from insomnia drank alcohol as a sleep aid. Ethanol depresses the central nervous system and consequently shows antianxiety action or sedative action; however, it has been reported that ethanol disturbs the normal sleep pattern by altering the sleep-wake cycle. On the other hand, there are many reports about the effect of ethanol on sleep latency and sleep pattern in healthy subjects or normal animals. In connection with this, it is well known that many people who drink alcohol to help to fall asleep are not healthy but have trouble on sleeping. Therefore, there is a strong presumption that the effect of ethanol on insomniac patients is different from that in normal individuals. So far as we know, the work reported here is the first designed to elucidate the effect of ethanol on the sleep-wake cycle in sleep-disturbed animals.

In the present study, therefore, we studied the effect of ethanol on sleep latency, the sleep-wake cycle and delta activity in a sleep-disturbed model by placing rats on a grid, and compared with normal rats.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 220—300 g (Japan SLC, Shizuoka, Japan) were used. All animals were maintained in an air-conditioned room with controlled temperature (24±2 °C) and humidity (55±15%). They were housed in aluminum cages with sawdust and kept under a light–dark cycle (lights on from 7:00 to 19:00). The animals were allowed free access to food and water. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Surgery The animals were anesthetized with pentobarbital sodium (Nembutal® 35 mg/kg intraperitoneally (i.p.); Abbott Laboratories, North Chicago, IL, U.S.A.), and then fixed to a stereotaxic apparatus (SR-5N; Narishige, Tokyo, Japan). For electroencephalogram (EEG) recording, a stainless steel screw electrode (800 μm in diameter) was chronically implanted in the left frontal cortex (A: +1.0, L: −2.0) according to the atlas of Paxinos and Watson. A stainless steel screw fixed in the right frontal bone served as a reference electrode. To record the electromyogram (EMG), stainless steel wire electrodes (200 μm in diameter) were implanted into the dorsal neck muscle. The electrodes were connected to a miniature receptacle, and the whole assembly was fixed to the skull with dental cement. At least 7 d were allowed for recovery from the surgery.

EEG and EMG Recordings EEG and EMG were recorded with an electroencephalograph (Model EEG 7213; Nihon Kohden, Tokyo, Japan) for 6 h (9:30—15:30). The recording was carried out according to a method described previously. The signals were amplified and filtered (EEG, 0.5—30 Hz; EMG, 16—128 Hz), digitized at a sampling rate of 128 Hz, and recorded using the data acquisition program SleepSign ver.2.0 (Kissei Comtec, Nagano, Japan). EEG and EMG of the rat were measured in a cylindrical plastic cage (diameter, 26 cm; height, 31 cm) with its floor covered with sawdust or a stainless steel grid placed inside the plastic cage. The stainless steel rods of the grid (3 mm wide) were set 2 cm apart. The observation cage was placed in a sound-proof and electrically shielded box (70×60×60 cm).

Sleep-Wake State Analysis The sleep-wake states were automatically classified by 10-s epochs as awake, non-rapid eye movement (NREM) or rapid eye movement (REM) sleep by SleepSign ver.2.0, according to the previously described criteria. As a final step, the defined sleep-wake stages were examined visually and corrected, if necessary. Each state was characterized as follows: wakefulness, low-amplitude EEG and high-voltage EMG activities; NREM sleep, high-amplitude slow or spindle EEG and low-voltage EMG activities; REM sleep, low-voltage EEG and REM activities. Sleep latency was defined as the time from the start of the experiment up to the first 12 consecutive 10-s epochs of sleep.

Calculation of Delta Activity During NREM Sleep Delta activity during NREM sleep was determined using SleepSign ver.2.0. The power spectrum densities, integrated and averaged, could be divided into 4 frequency areas: delta wave (0.5—4 Hz), theta wave (4—8 Hz), alpha wave (8—13 Hz) and beta wave (13—30 Hz). Delta activity (%) was calculated using the following equation. Delta activity = (delta power / total power) × 100

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When rats were placed on a grid, a significant prolongation of sleep latency and significant increase in total wake time were observed in comparison with those of rats placed on sawdust. Total NREM and REM sleep times in rats placed on a grid were significantly shorter than those of rats placed on sawdust (Table 1).

**Blood Ethanol Concentration** Blood ethanol concentration was measured by the modified method of Tsuji et al. Blood samples (1 ml) were collected from the heart at 1, 2, 4 and 6 h after ethanol administration in a heparinized blood collection tube, and blood ethanol concentrations were determined by enzymatic assay (BML Inc., Saitama, Japan). A blood ethanol concentration of less than 0.09 mg/ml was considered to be 0.09 mg/ml, because low concentrations could not be assayed.

**Drugs** The following drugs were used: ethanol (Sigma, Tokyo, Japan) and triazolam (Halcion; Pfizer, NY, U.S.A.). Ethanol was diluted to 30% in distilled water. Triazolam at a dose of 0.2 mg/kg was used as a positive control, and suspensions of 0.5% carboxymethylcellulose solution. The drugs were administered orally at 9:30. EEG and EMG were measured for 6 h immediately after drug administration. Eight rats were used in each group, and the doses of each drug were administered using a crossover design. Drugs were administered at intervals of 7 d when the same rats were used for repeated experiments.

**Data Analysis and Statistics** Values shown are the means±S.E.M. A paired t-test was used for comparison of sleep parameters in rats placed on sawdust and on a grid. One-way analysis of variance (ANOVA) with Dunnett’s test was used to estimate the drug effects. A p-value 0.05 was considered significant.

### RESULTS

#### Comparison of the Sleep Parameters in Rats Placed on Sawdust or a Grid

When rats were placed on a grid, a significant prolongation of sleep latency and significant increase in total wake time were observed in comparison with those of rats placed on sawdust. Total NREM and REM sleep times in rats placed on a grid were significantly shorter than those of rats placed on sawdust (Table 1).

**Effect of Ethanol on Sleep Latency in Rats Placed on Sawdust or a Grid** In rats placed on sawdust, no significant effects were observed in sleep latency by ethanol at doses of 0.5—2 g/kg and triazolam at a dose of 0.2 mg/kg. On the other hand, in rats placed on a grid, ethanol at doses of 1 and 2 g/kg and triazolam at a dose of 0.2 mg/kg caused a significant shortening of sleep latency (Fig. 1).

### Table 1. Comparison of the Sleep Parameters in Rats Placed on Sawdust or a Grid

<table>
<thead>
<tr>
<th>Sleep parameters</th>
<th>Sawdust</th>
<th>Grid</th>
<th>Time (min)±S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep latency</td>
<td>49.4±7.7</td>
<td>121.8±11.2**</td>
<td></td>
</tr>
<tr>
<td>Total wake time</td>
<td>135.9±2.8</td>
<td>217.4±3.5**</td>
<td></td>
</tr>
<tr>
<td>Total NREM sleep time</td>
<td>187.8±1.8</td>
<td>132.9±3.4**</td>
<td></td>
</tr>
<tr>
<td>Total REM sleep time</td>
<td>36.4±1.4</td>
<td>9.7±0.8**</td>
<td></td>
</tr>
</tbody>
</table>

Data represent the means±S.E.M. (n=8). **Significantly different from rats placed on sawdust at p<0.01 (paired t-test).

#### Table 2. Effect of Ethanol on Hourly Wake Time in Rats Placed on Sawdust or a Grid

<table>
<thead>
<tr>
<th></th>
<th>Wake time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0—1 h 1—2 h 2—3 h 3—4 h 4—5 h 5—6 h</td>
</tr>
<tr>
<td>Sawdust</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>38.5±1.8 27.5±3.2 22.9±2.1 15.0±1.4 16.2±1.5 15.9±1.8</td>
</tr>
<tr>
<td>Ethanol (g/kg, p.o.)</td>
<td>41.0±1.9 22.8±1.8 15.1±1.6 20.5±2.2 18.9±2.2 22.8±3.2</td>
</tr>
<tr>
<td>0.5</td>
<td>37.6±2.0 32.5±2.8 20.4±3.3 20.6±2.9 17.3±1.5 21.8±1.8 23.0±1.1</td>
</tr>
<tr>
<td>1</td>
<td>39.9±2.2 31.0±1.6 20.6±2.3 17.3±1.5 21.8±1.8 23.0±1.1</td>
</tr>
<tr>
<td>2</td>
<td>32.3±2.6 22.8±2.9 19.9±2.1 18.0±1.6 18.3±1.3 25.1±3.2*</td>
</tr>
<tr>
<td>Triazolam (mg/kg, p.o.)</td>
<td>50.8±1.4 43.4±1.6 34.5±2.9 30.3±2.3 32.1±2.6 31.4±1.4</td>
</tr>
<tr>
<td>0.2</td>
<td>38.9±2.3 32.4±1.9 34.0±2.9 30.1±1.9 32.2±1.0</td>
</tr>
<tr>
<td>1</td>
<td>46.3±1.0 35.1±2.5 31.5±2.2 29.1±2.3 25.7±1.8 29.9±2.8</td>
</tr>
<tr>
<td>2</td>
<td>45.6±1.4 33.3±2.0* 26.7±1.2* 26.0±1.2 27.9±1.8 28.9±1.9</td>
</tr>
<tr>
<td>Triazolam (mg/kg, p.o.)</td>
<td>42.4±2.7** 37.8±2.9 29.2±2.0 29.2±2.8 29.8±1.8 28.0±2.7</td>
</tr>
</tbody>
</table>

Each value represents the means±S.E.M. of 8 rats. **Significantly different from the control group at p<0.05 and p<0.01, respectively. (Sawdust: F_{(4,35)}=2.564, 3.159, 1.512, 1.379, 1.479, 2.241; Grid: F_{(4,35)}=3.634, 2.841, 2.049, 1.439, 1.427, 0.688).
Effect of Ethanol on Hourly and Total Wake Time in Rats Placed on Sawdust or a Grid  
When rats were placed on sawdust, ethanol caused no significant difference compared with the control in hourly and total wake time at all doses used in the present study. Triazolam at a dose of 0.2 mg/kg caused a significant increase in wake time 5—6 h after administration (Table 2); however, triazolam at this dose showed no significant decrease in total wake time (0—6 h), (Fig. 2). On the other hand, in rats placed on a grid, ethanol showed a significant decrease in wake time at a dose of 2 g/kg (1—2 and 2—3 h). At 0—1 h after administration, triazolam at a dose of 0.2 mg/kg also caused a significant increase in NREM sleep time (Table 3). Total NREM sleep time (0—6 h) was also increased by ethanol (1, 2 g/kg) and triazolam (0.2 mg/kg) when rats were placed on a grid (Fig. 2).

Effect of Ethanol on Hourly and Total NREM Sleep Time in Rats Placed on Sawdust or a Grid  
Neither ethanol or triazolam had any remarkable effect on hourly and total NREM sleep time when rats were placed on sawdust (Table 3, Fig. 3); however, in rats placed on a grid, ethanol at a dose of 2 g/kg significantly increased the NREM sleep time (1—2 and 2—3 h). At 0—1 h after administration, triazolam at a dose of 0.2 mg/kg also caused a significant increase in NREM sleep time (Table 3). Total NREM sleep time (0—6 h) was also increased by ethanol (1, 2 g/kg) and triazolam (0.2 mg/kg) when rats were placed on a grid (Fig. 3).

Effect of Ethanol on Total REM Sleep Time in Rats Placed on Sawdust or a Grid  
In rats placed on sawdust, ethanol at a dose of 2 g/kg caused a significant decrease of total REM sleep time, although no significant effects were observed in total REM sleep time by triazolam at a dose of 0.2 mg/kg. On the other hand, when rats were placed on a grid, no remarkable effects were observed with not only ethanol but also triazolam on total REM sleep time (Fig. 4).

Effect of Ethanol on Delta Activity in Rats Placed on Sawdust or a Grid  
We calculated delta activity as an indi-

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**Table 3. Effect of Ethanol on Hourly NREM Sleep in Rats Placed on Sawdust or a Grid**

<table>
<thead>
<tr>
<th></th>
<th>NREM sleep time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0—1 h</td>
</tr>
<tr>
<td><strong>Sawdust</strong></td>
<td></td>
</tr>
<tr>
<td>Control Ethanol (g/kg, p.o.)</td>
<td>20.7±1.7</td>
</tr>
<tr>
<td>0.5</td>
<td>17.6±1.7</td>
</tr>
<tr>
<td>1</td>
<td>21.6±1.9</td>
</tr>
<tr>
<td>2</td>
<td>19.5±2.1</td>
</tr>
<tr>
<td>Triazolam (mg/kg, p.o.)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Grid</strong></td>
<td></td>
</tr>
<tr>
<td>Control Ethanol (g/kg, p.o.)</td>
<td>8.9±1.3</td>
</tr>
<tr>
<td>0.5</td>
<td>10.4±1.7</td>
</tr>
<tr>
<td>1</td>
<td>13.0±0.9</td>
</tr>
<tr>
<td>2</td>
<td>13.9±1.3</td>
</tr>
<tr>
<td>Triazolam (mg/kg, p.o.)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Each value represents the means±S.E.M. of 8 rats. *,** Significantly different from the control group at p<0.05 and p<0.01, respectively. (Sawdust: $F_{(4,35)}=2.555$, 2.716, 0.959, 2.056, 1.416, 0.971; Grid: $F_{(4,35)}=3.785$, 2.939, 2.683, 1.761, 1.941, 0.308).
The effect of ethanol on delta activity in rats placed on sawdust or a grid was studied. Blood ethanol concentrations were increased in rats treated with 1 g/kg ethanol at 1 and 2 h after administration (Fig. 5). Table 4 shows the effect of ethanol on delta activity in rats placed on sawdust or a grid. The results indicate that delta activity was significantly decreased by ethanol treatment, with a significant difference from the control group at p<0.01. Ethanol was administered orally.

**DISCUSSION**

In the present study, it was found that sleep latency was significantly prolonged by placing rats on a grid compared with rats placed on sawdust. In addition, an increase in total wake time and decreases in total NREM and REM sleep times were observed in this rat. In our present study, we used delta activity to estimate the drug effect in addition to sleep latency, wake time, NREM sleep time and REM sleep time.

Ethanol caused no significant sleep-inducing effect when rats were placed on sawdust. Almost identical findings were reported in diphenhydramine, chlorpheniramine16) and triazolam.10) It is generally recognized that rats are nocturnal; therefore, they are sleepy in the daytime especially when comfortable, such as on sawdust, which is why sleep-inducing drugs, including ethanol, showed no remarkable effect.
when rats were placed on sawdust. Ethanol caused a significant shortening of sleep latency in sleep-disturbed rats. On the other hand, it is reported that ethanol had no remarkable effect on sleep latency in healthy humans\(^2\), however, there is no report whether ethanol showed a sleep-inducing effect in insomnia patients. Our present data in rats suggested that ethanol has the possibility of improving the insomniac’s sleep. In addition, ethanol had no significant effect on the total wake time and total NREM sleep time in rats placed on sawdust. However, a significant decrease in total REM sleep time was observed. Accordingly, ethanol showed a significant decrease in the total wake time and a significant increase in the total NREM sleep time in rats placed on a grid, but no significant effect was seen in total REM sleep. In healthy subjects, Van et al.\(^1\) reported that no significant effect was observed by ethanol in total wake time and total NREM sleep time. Landolt et al.\(^1\) found that REM sleep time was reduced by ethanol in healthy subjects. On the other hand, in insomnia patients, Roehrs et al.\(^1\) described that ethanol at a dose of 0.9 g/kg caused an increase in total NREM sleep time. These findings clearly indicate that the same findings were observed in humans and rats after ethanol administration. In addition, Dettling et al.\(^1\) reported that when 0.9 g/kg ethanol was administered to humans, the maximum blood alcohol concentration reached 0.841 ± 0.155 mg/ml. In our present study, it was found that when rats were administered 1 g/kg and 2 g/kg of ethanol to rats, the blood ethanol concentrations were 0.86 ± 0.12 and 1.09 ± 0.11 mg/ml, respectively. From these findings, it was confirmed that the effect of ethanol at doses of 1 and 2 g/kg on the sleep-wake pattern in rats was almost the same as the findings when 0.9 g/kg ethanol was administered to humans.

As shown in the present results, ethanol caused a significant increase of delta activity in rats, whereas triazolam showed a significant decrease in delta activity. Shinomiya et al.\(^1\) have reported that delta activity is useful as an index of sleep quality. For instance, benzodiazepines such as triazolam, flunitrazepam and midazolam also decreased delta activity.\(^1\) Similar to our present results, Landolt et al.\(^1\) described that ethanol increased EEG power density in low delta frequencies in healthy middle-aged man. Roehrs et al.\(^1\) also emphasized the possibility that alcohol improved the quality of sleep in humans. These findings are in fair agreement with our present data, although there is a difference between rats and humans.

From the present results, it is concluded that ethanol had not only a hypnotic effect but also a sleep-maintaining effect in sleep-disturbed rats. In addition, ethanol showed an increase in delta activity as an indicator of sleep quality.

REFERENCES