A Successful Method for Development of Voluntary Alcohol Intoxication in Mice

SETSUO KOMURA, MASAO UEDA* and TOSHIKOY KOYAYASHI*

Department of Legal Medicine, Yamaguchi University School of Medicine, Ube and Department of Legal Medicine*, Faculty of Medicine, Kyoto University, Kyoto


Eight strains of male mice, C57BL, C3H SWM, SW, KK, KSB, KR and DBA, were fed on a standard pelletized diet and offered a choice of water or 10% sake solution (sake containing 10% alcohol). Both young (3 months of age) and old (8 months of age) groups were studied simultaneously. The degree of intoxication was measured by recording the drinking behavior on a pulse recorder, by calculating gaschromatographically the blood alcohol concentration, by taking depth electroencephalogram readings and so on. Intoxication, shown by lack of coordination such as grossly impaired gait, was observed only in the older mice of strain with a moderate natural alcohol preference such as C3H, SWM, SW, KK and KSB. In general, the intoxicated mice were over 9 months of age, tended to consume fluids regardless of the time of day or night and suffered a loss of body weight. The blood alcohol levels of them were over 4.6%. The threshold elevation of the ascending reticular activating system on electroencephalogram in a intoxicated mouse reached about 167%. The present study provides a successful method for the development of voluntary alcohol intoxication in mice.

MATERIALS AND METHODS

Eight strains of male mice, C57BL, C3H, SWM, SW, KK, KSB, KR and DBA, were used. All of them came from Kyoto University and were fed on a standard pelletized laboratory diet. In addition to water, the mice had access to sake, Japanese rice wine, that had been diluted with water so that its alcohol content is 10% and its saccharide content is 3.3% (hereafter referred to as 10% sake solution).

Received for publication, September 29, 1975.
In a preliminary experiment, we accidentally observed two old mice, about 9 months of age, showing signs of drunkenness. Consequently we thought that the effect of age was a factor which could not be overlooked in producing intoxicated mice. For comparative purposes both young and old groups were studied simultaneously; the young adult mice were approximately 3 months of age and the old mice were 8 months of age, at the beginning of this experiment. They were offered a choice of water or 10% sake solution in general, for about 3 months and over.

The degree of intoxication was measured by observing the behavior, by recording the drinking behavior on a pulse recorder, by calculating gaschromatographically the blood alcohol concentration (Ueda 1967) and by taking depth electroencephalogram readings (Komura et al. 1973).

Both A (young) and B (old) groups were divided into two subgroups: those mice without implanted electrodes and those with. The electrodes were implanted stereotaxically into the motor cortex and midbrain reticular formation.

The animals were kept on a 12-hr light and 12-hr dark schedule.

RESULTS AND DISCUSSION

Comparisons were first made between the preference ratio for this sake solution in these eight strains of mice and their preference for alcohol. The ordering of the strains according to alcohol preference was the same ordering after offering them a choice between water and 10% sake solution (Table 1). However, it appears that the sake preference was higher than that for alcohol, probably because of the saccharide contained in sake (Komura et al. 1972b).

Intoxication was observed only in the older mice of strains with a moderate natural alcohol preference, such as C3H, SWM, SW, KK and KSB (Table 2). For instance, in C3H mice, three of the eight animals in non-electrode subgroup and two of the four in electrode subgroup were intoxicated. In both SWM and SW strains, one intoxicated mouse was found in each subgroup, and in both KK and KSB strains, one was observed in the subgroup without electrodes. The mice in all other strains, C57BL, KR and DBA, were never intoxicated.

In general, the intoxicated mice were over 9 months of age, tended to consume fluids regardless of the time of day or night and suffered a loss of body weight. Thus it seems possible to predict in advance whether or not a mouse will become intoxicated by observing the drinking behaviors and the changes of body weight.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ratio† (%)</th>
<th>Strain</th>
<th>Ratio† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL</td>
<td>81.8±11.5</td>
<td>KK</td>
<td>46.6±12.0</td>
</tr>
<tr>
<td>C3H</td>
<td>57.8±12.3</td>
<td>KSB</td>
<td>45.1±8.9</td>
</tr>
<tr>
<td>SWM</td>
<td>56.7±9.1</td>
<td>KR</td>
<td>23.0±7.2</td>
</tr>
<tr>
<td>SW</td>
<td>50.0±10.7</td>
<td>OBA</td>
<td>12.0±4.5</td>
</tr>
</tbody>
</table>

* Sake (Japanese rice wine) containing 10% (v/v) alcohol.
† The ratio of consumption of 10% sake solution over water, in per cent.

Results are given as the mean±s.d. of 5 mice.
### Table 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>Group of 3 months of age</th>
<th>Group of 8 months of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No electrode</td>
<td>Electrode</td>
</tr>
<tr>
<td>C57BL</td>
<td>0 (10)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>C3H</td>
<td>0 (10)</td>
<td>0 (4)</td>
</tr>
<tr>
<td>SWM</td>
<td>0 (4)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>SW</td>
<td>0 (4)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>KK</td>
<td>0 (6)</td>
<td>0 (6)</td>
</tr>
<tr>
<td>KSB</td>
<td>0 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>KR</td>
<td>0 (10)</td>
<td>0 (4)</td>
</tr>
<tr>
<td>DBA</td>
<td>0 (10)</td>
<td>0 (4)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Preference ratio for 10% sake solution and age in weeks in a C3H mouse.\[s/s+w=ml of 10\% sake solution/ml of total fluid × 100.**

E.E.G. means taking electroencephalograms. The arrow of solid line shows the time when the mouse was undoubtedly intoxicated and the arrow of dotted line indicates when the mouse seemed to be intoxicated by observing only the drinking behavior on a pulse recorder.

The blood alcohol levels of intoxicated mice were over 4.6%. All of the intoxicated or drunken mice showed lack of co-ordination such as grossly impaired gait and movements, or falling to side. Some of them developed tremors and/or drank themselves to death within a few days after the drunken state occurred. For example, a C3H mouse with implanted electrodes was intoxicated at least twice from 10 to 19 months of age (Fig. 1). During the drunken state, the body weight decreased and the sake preference increased. The details of the behavior before and after the drunken state that occurred at 43 weeks of age are shown in Figs. 2–6. Fig. 2 shows the consumption of 10% sake solution and water two days before the drunken state. Fig. 3 shows the drinking behaviors one day before the drunken state appeared. Ten % sake solution and water were consumed alternately regardless of the time of day or night. Fig. 4 shows that on the day when the drunken state occurred, the sake preference ratio was 100% and the body weight decreased. The blood alcohol concentration reached 6.1%. 
Fig. 2. Consumption of 10% sake solution and water during a day in a C3H mouse (43 weeks old; body weight, 27.5 g) 2 days before the drunken state. The abscissa indicates the time of day. The sake solution and water consumed during a day were 5.82 and 1.83 ml, respectively. The preference ratio for 10% sake solution was 76%. o, 10% sake; ●, water.

Fig. 3. Consumption of 10% sake solution and water during a day in a C3H mouse (43 weeks old; body weight, 27 g), one day before the drunken state. The sake solution and water consumed during a day were 4.77 and 3.55 ml, respectively. The preference ratio was 58%. o, 10% sake; ●, water.

at 11 o'clock in the morning. The electroencephalograms of cortex and reticular formation, as shown in Fig. 5, had dominant slow waves with high voltages in comparison with that of the normal state. Fig. 6 shows that the mouse drinks much more water on the first day after the drunken state.

Next example is a SWM mouse with implanted electrodes, which was intoxicated at least three times from 10 to 15 months of age, as shown in Fig. 7.
Alcohol Intoxication in Mice

Fig. 4. Consumption of 10% sake solution and water during a day in a C3H mouse (43 weeks old; body weight, 25g), on the day when the drunken state occurred. The sake solution and water consumed during a day were 7.12 and 0.0 ml, respectively. The preference ratio was 100%. ○, 10% sake.

![Graph showing consumption of sake and water over time](image)

Fig. 5. Electroencephalographic patterns of the cortex and the midbrain reticular formation (RF) at the normal and drunken states in a C3H mouse.

During the drunken state which occurred at 42 weeks of age, the sake preference increased, the body weight decreased and the blood alcohol concentration reached 5.8%. At that intoxicated state, the electroencephalograms were recorded and the threshold elevation of the ascending reticular activating system reached about 167%, as shown in Fig. 8. This drunken SWM mouse consumed about 0.26 ml of 10% sake solution per day per g of body weight, or about 20 g of alcohol per day per kg of body weight.

Since it could be argued that a 16% alcohol solution should have been used instead of 10% sake solution in this study, another experiment was performed with twelve C3H mice, 14 months of age. Offering a choice between water and 10% alcohol solution caused a decrease in the number of drunken mice and the intoxication occurred at a later stage. Three of the mice were distinctly drunken...
Fig. 6. Consumption of 10% sake solution and water during a day in a C3H mouse (43 weeks old; body weight, 24 g), on the first day after the drunken state. The sake solution and water consumed during a day were 0.18 and 7.0 ml, respectively. The preference ratio was 3%. ○, 10% sake; ●, water.

Fig. 7. Preference ratio for 10% sake solution and age in weeks in a SWM mouse. $s/s+w=ml$ of 10% sake solution/ml of total fluid $\times 100$. E.E.G. means taking electroencephalographs. The arrow shows the time when the mouse was undoubtedly intoxicated.

Fig. 8. Electroencephalographic arousal response of the cortex induced by the stimulation of the midbrain reticular formation at the drunken and normal states in a SWM mouse (42 weeks old; body weight, 33 g).
at some point after 15 months of age (Komura et al. 1972a). This study will be reported later.

Based on these results, it is suggested that the possible important factor in producing the drunken and intoxicated mice by voluntary alcohol intake is to use old mice, 8 months of age or more, with moderate natural alcohol preference. The drunken state seems to be accompanied by a reduction of the body weight, a rapid increase in the preference for alcohol beverage and a consumption regardless of the time of day or night.

References