Digging Behavior of ddY Mouse

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Abstract: In the present studies, the behavior of ddY mice digging wood chips was carefully observed. When mice were individually placed on new 5 cm-thick wood chips, their behavior was found to be the same irrespective of their age or sex. The behavior was not prevented by non-noxious 5 black steel rods which were used to measure digging ability, and was not related to habituation or learning. But moist or dirty chips remarkably weakened digging ability. These findings strongly suggest that the digging behavior is a natural and instinctive one, but not an expression of anxiety as previously reported.

Key words: ddY mouse, digging behavior

Some researchers previously reported that mice and rats “bury” not only noxious but also non-noxious objects in wood chips [4, 7]. Others interpreted the behavior of “burying” non-noxious objects as an index of anxiety because anxiolytic agents affect the behavior [2, 3]. But, when estimating changes in behavior, the nature of the behavior must be well recognized. Experimental animal researchers dealing with mice have already noticed that mice dig in new wood chips in the dwelling cage, and it is easy to imagine that scattered wood chips will cover the objects. Before considering the “burying” behavior as an index of anxiety, the nature of the behavior must be clarified, but careful observations of the behavior in different situations had not been reported. In this study, we observed digging behavior of ddY mice and investigated the differences between the behavior and the previously reported “burying” behavior.

Male and female ddY mice (Japan SLC, Inc., Tokyo), 4, 6, 8, 10 and 12 weeks old, were used. They were housed in groups of five of the same age and the same sex in plastic cages (338 × 140 × 225 mm) with wood chips (2–1.2 cm square, 0.3–0.7 mm thick, made of white wood; Dou’ou Rika Sangyo Co., Sapporo, Japan) on the bottom, with free access to food (F2: Funabashi Farm Co., Tokyo, Japan) and water. The animal room was kept at 21–25°C with 50–60% humidity and lights on from 7:00 to 19:00. For identification, each mouse in a cage was painted on the tail with a different colored paint. The following experiments were performed from 15:00 to 17:00. All of the experiments were carried out under the control of the Ethics Committee for Animal Experiments of Akita University School of Medicine, Japan.

In order to establish suitable observation conditions, pre-trials were performed on the number of non-noxious objects and observation duration. In these trials, we have found that 5 evenly placed steel rods (covered with black vinyl tape: 5 cm long, 1.3 cm in diameter and weighing 25 g) were the most suitable for measuring mouse digging ability, and that the ability did not increase over 30 min observation. Ten mice of each

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age and each sex were individually placed in plastic cages (the same size as above) with a 5 cm thickness of the new wood chips (the experiment cage), and their behavior was observed for 30 min. After the observation, the mice were returned to the domestic cages. Twenty-four hr after the previous observations, all of the mice were individually placed in experiment cages, which 5 rods were evenly spaced, and their behavior was observed for 30 min. The digging ability of the mice was indicated by the number of rods completely covered with wood chips.

In order to get information on habituation or learning, 10 other 8-week-old male mice prepared. The mice were individually placed in the experiment cages with 5 rods, and after 30 min the number of rods completely covered with wood chips was counted. Twenty-four, 48 and 72 hr after the first observation, the same observations were performed on the experiment cage with new wood chips.

To detect the effects of wood chips condition, moist wood chips (no water oozed out even when tightly squeezed) or dirty wood chips (collected from two dwelling cages occupied by the other 10 6-week-old male mice at 4 day intervals) were prepared. The 10 male mice, which had produced the dirty chips and were 8-weeks old at the experiment day, were individually placed in the experiment cage with new chips and 5 rods, and after 30 min the number of rods completely covered with chips was counted. Twenty-four hr later this, the observation in the experiment cage with a 5 cm thick layer of the moist chips and 5 rods was performed, and 24 hr after the second observation, the observation in the experiment cage with a 5 cm thick layers of the dirty chips and 5 rods was also performed.

Even in the experiment cage without rods, all of the mice of each age and each sex dug the new wood chips. The chips were scattered in front of and behind the mice. In the experiment cage with rods, the rods were covered with the scattered chips. Although the mice frod on, sniffed and bit the rods, they only dug the chips as in the experiment cage without rods, and did not intentionally scatter the chips onto the rods. Digging ability did not differ with age or sex (Table 1). Other 8-week-old male mice showed signs of the ability as in every observation period for three days (Table 2, upper part). The other 8-week-old male mice also had the same ability with the new chips, but with the moist and dirty chips the ability was remarkably weakened (Table 2, lower part).

The present findings clearly indicate that the “burying” behavior previously reported is the digging behavior presented in our studies. As the digging behavior is found in mice of every age and both sexes in situations without obstacles, the behavior can be regarded as a natural and instinctive one. Furthermore, when considering that the behavior has no relation to habituation or learning, it will not be induced by anxiety. This is supported by the previous report indicating that the behavior has no relation to mouse aggression [6]. Although the purpose of the behavior has not yet been made clear in the present studies, the finding that the moist and the dirty chips weaken digging ability

### Table 1. The digging ability of mice of different ages and sex

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td>2.8 ± 0.84</td>
<td>3.4 ± 1.06</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>3.0 ± 0.89</td>
<td>3.2 ± 1.00</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>3.6 ± 1.02</td>
<td>3.4 ± 0.89</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>3.4 ± 1.09</td>
<td>3.8 ± 0.83</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>3.6 ± 1.20</td>
<td>3.4 ± 1.02</td>
</tr>
</tbody>
</table>

The values indicate the mean ± SE of the numbers of rods completely covered with wood chips by the 10 mice.

### Table 2. The digging ability of 8-week-old male mice

1) in repeated trials

<table>
<thead>
<tr>
<th>Trial (at intervals of 24 hours)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.2 ± 1.07</td>
<td>3.2 ± 1.12</td>
<td>3.6 ± 1.02</td>
<td>3.4 ± 1.02</td>
</tr>
</tbody>
</table>

2) with different kinds of wood chips

<table>
<thead>
<tr>
<th>Wood Chip Condition</th>
<th>New</th>
<th>Moist</th>
<th>Dirty</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.3 ± 0.89</td>
<td>0.4 ± 0.72*</td>
<td>0*</td>
</tr>
</tbody>
</table>

The values in the tables indicate the mean ± SE for the numbers of the rods completely covered with wood chips by the 10 mice. *p<0.01 compared to the new wood chips (Mann-Whitney U test).
suggests that the behavior may be that of adaptation to a new environment [1]. Previous neuropharmacological studies on the behavior [2, 3, 5] should therefore be considered as approaches to investigating the adaptation mechanisms of mice.

References