— INSTRUMENTATION & TECHNIQUES —

A new type of soil material for a burrowing chamber for mice

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We measure general activity of mice (*Mus musculus*) in a burrowing chamber from an ecological point of view. Natural topsoil or sand, however, is not suitable for long-term dwelling of mice, because burrows are likely to keep collapsing and to become rapidly rotten from being sprinkled with water to guard against collapse. For the present study, we devised a mixture of absorbent cotton and wood shavings as soil material. The mice burrowed in this soil just as they would in natural soil. About 10^4 cm³ of the cotton-shavings soil were durable for at least 40 days with 3 mice. These results suggest that this soil material can be a useful tool in not only the study of general activity but also other studies (e.g., the observation of maternal behavior of mice in burrows).

General activity or spontaneous activity of mice or rats is defined in terms of movements in the absence of any identifiable eliciting or reinforcing stimulus (Silverman, Chang, & Russell, 1988; Wong, 1979). It has been studied with stabilimeter cages or running wheels.

However, there is an ecological problem in the use of such apparatus. In nature, mice burrow and live in burrows. Thus, to study the behavior of mice experimentally, one would like to use a burrowing chamber and soil or other material in which mice can burrow.

Although natural topsoil and sand have been used as soil in previous studies of burrowing behavior, the use of these soil materials has some drawbacks. For example, the topsoil must be moistened to preserve burrows from collapse (Boice, 1977), and it is therefore likely to become rotten rather rapidly. Moreover, a chamber with topsoil is heavy, which makes handling difficult.

In the present study, we report on a new type of soil material that we have substituted for natural soil. This new type of soil material consists of absorbent cotton and wood shavings. Mice were tested in the burrowing chamber with the cotton-shavings soil material. We used 5 inbred mice strains to exclude hereditary bias.

METHOD

Subjects

Ninety mice—45 females and 45 males—were the subjects. They were derived from the BALB/c, CBA/Ca,

C3H/He, C57BL/6, and DBA/2J strains maintained at the University of Tsukuba. They were 90–110 days old when they were placed in the burrowing chamber.

Apparatus

A transparent plastic cage (top, 42×26 cm; bottom, 38×22 cm; height, 20 cm) was used as the burrowing chamber. The top was covered with a transparent acrylic board with many air holes.

The new type of soil was made as follows: First, 120 g of wood shavings were spread out evenly; next, 13 g of absorbent cotton were laid on top of the wood-shavings base. We laid these two materials alternately, filling half of the cage with the soil so that it consisted of five layers of shavings and four layers of absorbent cotton.

The wood shavings used in this study were White-flake from Charles River Japan, Inc. White-flake consists of fine chips (5×5×0.2 mm, typically) made from spruce fir; their specific gravity is 0.06, and 1 g of them can absorb 3.2 g of water.

The laboratory room was maintained on a 12:12-h light:dark cycle (lights on at 0800 h). The surface of the soil was illuminated brightly (150–200 lux) by fluorescent ceiling lamps.

We prepared three illumination conditions, depending on how the chambers were covered. The first cage was covered with opaque black paper on the side below the height of the soil surface and on the bottom (bottom-covered), the second cage was covered on the side above the height of the soil surface and on the top (top-covered), and the third cage was not covered at all (not covered).

The not-covered condition should be more useful for observing behavior of mice than the bottom-covered condition, which simulates the natural burrows best. So we

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Figure 1. Burrows in the cotton-shavings soil seen from above (upper panel: female BALB) and seen from below (lower panel: male CBA).

compared these variations to evaluate the not-covered condition for future use.

Procedure

Three mice of the same strain were placed on the soil surface at a time. Under the top-covered condition, the top was covered by opaque black cloth after placement. On the 40th day, the mice were removed and their burrows were recorded.

RESULTS

The mice that were placed in the apparatus ambulated and sniffed, and then engaged in cotton-loosening behavior. This consisted of a series of behaviors, which comprised the pressing down of lumps of cotton with the forepaws and the pulling of parts of the cotton with the mouth. It resembled gnawing or fraying (van Oortmerssen, 1971). Digging was observed after cotton-loosening. Diving or crawling under the cotton was observed in the last stage. These behaviors were found within 30 min after placement. Apart from the early characteristic behavior, the mice burrowed as they would under natural conditions.

Latency to burrow (in days), number of entrances and nests, and tunnel length (in meters) for each strain and sex under the three illumination conditions on the 40th day are shown in Table 1. Figure 1 shows the burrows on about the 30th day, seen from above (upper panel) and seen from below (lower panel). Analysis of variance (illumination condition $\times$ strain $\times$ sex) revealed neither significant main effects nor interactions for any measure.

The soil was still clean on the 40th day.

Table 1
Mean Latency to Burrow with SD (in days), Mean Number of Entrances and Nests, and Mean Total Tunnel Length (in meters), for Each Strain, Sex, and Illumination Condition

Factor/Level	Latency		Entrances		Nests		Length	
	M	SD	M	SD	M	SD	M	SD
Strain								
BALB	1.0	0.0	3.2	1.6	2.5	0.5	1.4	0.4
CBA	1.0	0.0	2.7	1.3	2.7	0.5	1.3	0.3
C3H	1.0	0.0	2.8	0.7	3.3	1.5	1.2	0.2
C57BL	1.0	0.0	2.2	0.4	2.8	0.7	1.3	0.3
DBA	1.0	0.0	1.7	0.8	2.7	1.3	1.3	0.1
Sex								
Female	1.0	0.0	2.4	1.1	3.1	1.0	1.3	0.3
Male	1.0	0.0	2.6	1.1	2.5	0.9	1.4	0.3
Illumination Condition								
BC	1.0	0.0	2.4	1.5	3.2	1.3	1.3	0.3
NC	1.0	0.0	2.7	1.2	2.6	0.8	1.4	0.3
TC	1.0	0.0	2.4	0.5	2.6	0.7	1.3	0.2

Note—BC = bottom covered; NC = not covered; TC = top covered.

DISCUSSION

The results showed that mice could burrow in the cotton-shavings soil as well as they might in natural soil.¹ The strength of burrows against collapse can be attributed to the plasticity of the cotton. This new soil is so handy and durable that its use is advantageous for the study of general activity of mice. If only 1 mouse were placed in it, the soil would be durable for at least 2 months before rotting. Burrows in the cotton shavings are parallel to the "maternal nest" referred to by Lisk, Pretlow, and Friedman (1969).

We found no difference among the burrows made by the different strains. This finding differs from the findings of Adams and Boice (1981) or Dudek, Adams, Boice, and Abbott (1983), who showed that the C57BL/6 burrowed more eagerly than the BALB/c. This discrepancy may be attributed to ease of digging actions in the cotton-shavings soil, which permitted BALB/c to burrow more.

We also found no difference among the illumination conditions. Thus, the not-covered condition (i.e., with the apparatus not covered by black paper) should be satisfactory for use in future work. This condition can be used for the observation of other behaviors (e.g., maternal behavior) in burrows made behind a transparent plastic wall.

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NOTE

1. We have recently found that the cotton-shavings soil is effective for other rodents: the rat (*Rattus norvegicus*), the golden hamster (*Mesocricetus auratus*), and the field mouse (*Apodemus speciosus*, *Apodemus argenteus*).