Attenuation of Neophobia by Postweaning Individual Rearing in Mice

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It has been shown that individually reared mice showed marked deviations in behaviors and endocrinological functions as compared with group-reared mice. For example, isolated mice showed more aggressive behavior (2), lighter adrenal glands (3), and lower corticosterone level (1). In open field test, isolated mice showed greater ambulation and rearing score, and decreased defecation as compared with their grouped counterparts (6). These behavioral and endocrinological differences between isolated and grouped animals suggested that isolated mice react to novel environmental stimuli with less fear (4).

Recently, it has been suggested that neophobia to unexperienced food or fluid would be one of indices of fear to novel stimuli (7, 9). And, when nausea or sickness follows novel taste stimulus, animals show conditioned aversion to this stimulus. The present study was conducted to investigate the effects of social isolation for a prolonged period after weaning on neophobia and taste aversion learning in mice.

METHOD

Subjects and Apparatus

Thirty six experimentally naive male mice (*Mus musculus*) of CFW strain were used. At 21 days of age, they were weaned and divided into two groups; half were individually housed (isolated) in transparent plastic cage (175×245×125 mm, CL-0103, CLEA, Japan) and the other half were housed in groups of six animals (grouped) per cage of the same dimension for sixty days. Each cage was separated by a black painted woodboard. The mice were maintained with food and water ad libitum in a 12/12 h light/dark cycle (light, 7:00-19:00).

After sixty days of isolated and grouped housing, each mouse was housed individually throughout the days of behavioral examination. For measurement of amount of fluid consumption during experimental sessions, a stainless steel drinking tube was protruded into the cage, and was connected to vertically mounted 5 ml graduated cylinder.

Procedure

At eighty days old, activity was measured by photoelectric activity meter (Electric Motility Meter, Motron Products, Stockholm), where every 4-cm movement of animals was counted as activity score. Each isolated ($n=18$) and grouped ($n=18$) mouse was placed in a $310 \times 360 \times 175$ mm cage (CL-0106, CLEA, Japan), and activity was measured for 10 min.

After activity test, mice were subjected to water intake training schedule for three days, which was described previously (8). Briefly, on each day, mice were subjected to 23-h water deprivation and were given access to water through stainless steel drinking tube for 10 min in the experimental room. Then, they were returned to the breeding room, 50 min later, were given free access to water for 50 min.

On the treatment day, three subgroups were assigned to each group: experimental (E), saline control (S) and LiCl control (L) groups. The subjects of experimental and saline control groups were given access to 0.1% saccharin solution through stainless steel drinking tubes for 10 min. LiCl control mice were given access to water instead of saccharin solution. The fluid intake during 10-min session was recorded. The subjects of experimental and LiCl control groups received intraperitoneal injections of LiCl solution (0.15 M, 2% (v/w) of body weight). The subjects of saline control group were injected NaCl solution (0.15 M, 2% (v/w) of body weight).

During eight days of test sessions, animals were alternatively exposed to the 0.1% saccharin solution and water for 10 min on every two days in the experimental room under 23-h deprivation similar to the treatment and training days. All behavioral tests were conducted during 13:00 to 15:00 throughout the experiment.

RESULTS and DISCUSSION

At the beginning of behavioral examinations, the body weight was 50.6 ± 4.1 g (mean $\pm$ standard deviation) for isolated mice and 46.9 ± 2.6 g for grouped mice. The isolated mice were significantly heavier than the grouped mice ($t(34) = 3.23$, $p < 0.01$).

Total activity score in isolated mice was 987.8 ± 239.6 (mean $\pm$ standard deviation) and in grouped mice, 848.5 ± 171.1 . Although isolated tended to be more active, the difference between two groups was not statistically significant ($t(34) = 2.00$).

Three way analysis of variance on water consumption during training revealed that there was a significant effect of sessions ($F(2, 60) = 81.22$, $p < 0.01$), but no effect of isolation ($F(1, 30) = 1.61$), and no difference among subgroups before treatment ($F(2, 30) < 1$). Similarly, as to water consumption during the test sessions, statistical analysis revealed no significant effects of isolation and treatments of subgroups ($F(1, 30) = 4.05$, $F(2, 30) = 3.58$, respectively). Thus, there were no significant differences in water consumption between isolated and grouped mice throughout the present experiment.

Fig. 1 represents amount of first intake of saccharin solution for each group. LiCl injected groups (L) were given access to saccharin solution on the first day of test sessions, whereas the others were given on treatment day.

As shown in Fig. 1, isolated animals consumed more saccharin solution than the grouped animals. Statistical analysis showed that significant effects of postweaning rearing condition and the treatment of subgroups ($F(1, 30) = 4.81$, $p < 0.05$; $F(2, 30) = 5.73$, $p < 0.01$, respectively). That is, isolated animals less hesitated to consume novel tasting solution as compared with grouped mice. This tendency was apparent between LiCl control subgroups, although consumption of these subgroups was markedly less as compared with those of the experimental and saline control subgroups.

The consumptions of saccharin solution on test sessions were presented

Fig. 1. Effects of social isolation on fluid consumption of first access to saccharin solution. Dotted column: individually reared group, open column: group-reared group. E: experimental subgroup, S: saline injected control subgroup, L: LiCl injected control subgroup. Bars on top of columns depict the standard deviations of the means.

in Table 1. Both the isolated and grouped mice successfully acquired conditioned aversion to saccharin solution. Analysis of variance showed significant effects of treatment and trials of test sessions ($F(2, 30) = 54.2$, $p < 0.01$; $F(3, 90) = 17.54$, $p < 0.01$) and significant interaction of these two factors ($F(6, 90) = 4.28$, $p < 0.01$). However, there was no significant effect of isolation ($F < 1$) and no significant interactions with the other factors ($F < 1$ for all).

The results of the present study showed that mice housed individually for a prolonged period after weaning less hesitated to consume novel tasting solution as compared with their group-housed counterparts. This

Table 1 Effects of social isolation on consumption of saccharin solution during four test sessions.

Groups	Subgroups	n	1	2	3	4
Isolated	Experimental	6	0.04±0.08*	0.12±0.13	0.32±0.30	0.57±0.31
	Saline	6	1.80±0.63	1.78±0.67	2.05±0.43	2.11±1.01
	LiCl	6	0.85±0.53	1.82±1.03	1.62±0.85	2.16±1.20
Grouped	Experimental	6	0.04±0.06	0.12±0.18	0.37±0.48	0.64±0.63
	Saline	6	1.55±0.41	2.21±0.53	1.86±0.33	1.90±0.74
	LiCl	6	0.45±0.40	1.20±0.42	1.73±0.67	2.58±0.68

* Mean intake (ml) ± standard deviation

was consistent with the findings in rats (5), i.e. individual housing of rats over a prolonged period resulted in markedly suppressed intake of novel food and fluid. These findings together with the present results suggested that animals individually reared for a prolonged period show attenuated general neophobia to unexperienced environmental stimuli as compared with group-housed controls.

Individual rearing had no effect on the taste aversion learning in the present study. Presumably, it was because the conditioned aversion in the present study almost completely suppressed consumption of solution concealing the differences between rearing conditions.

SUMMARY

Thirty six male mice of CFW strain were housed in either isolated or grouped for sixty days after weaning, and were compared in an activity test, taste neophobia and taste aversion learning. The results showed that individual rearing appeared to attenuate neophobia to unexperienced tasting solution in mice. However, no effects of rearing condition were found in taste aversion learning. It is suggested that isolation for a prolonged period after weaning produced less reactivity to novel gustatory stimuli as well as to other environmental stimuli.

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