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ショートレポート

Voluntary Wheel Running Activity Induced Food Avoidance Among Laboratory Mice: A Further Study Using C57BL Mice

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Introduction

Since the time of Lett and Grant (1996)'s discovery of the conditioned taste aversion paradigm based on voluntary wheel running among rats, various studies have been conducted on this topic (Boakes and Nakajima, 2009). However, many of these reports have been on rats and have not yet been clarified in other species.

Recently, Nakajima (2019a) demonstrated voluntary wheel running based on conditioned flavor avoidance (CFA) among laboratory mice (Institute of Cancer Research [ICR] mice) using flavored food. Nakajima (2019b, 2021) reported the replication of running-based CFA in ICR mice. Additionally, Nagaishi et al. (2021) reported the CFA-based wheel running with another strain of mice (C57BL mice). However, their experimental design was inadequate and needed to be set up with a proper experimental design. For instance, no control group was established for the conditioned and unconditioned stimuli unpaired treatment. Therefore, the present study modified the experimental design of Nagaishi et al. (2021)'s study and conducted a replication study using the same strain of mice.

Method

Subjects and Apparatus. At the beginning of the adaptation treatment, eight experimentally naive, seven-week-old male C57BL mice had a mean weight of 23.0 g (range:21.4–25.0 g). They were individually housed in clear plastic cages to avoid interactions with conspecific mice in their home cages, a feature that would reduce taste aversion (cf. Hishimura, 2015). In particular, each of the two clear plastic home cages (CL-2003-02, CLEA Japan, Tokyo) was divided into four compartments using a metal plate. The mice were individually housed in compartments, 12.5 cm wide, 20.5 cm long, and 15.0 cm high. Additionally, the floor was covered with a 3 cm wood chip layer. The woodchip floor was changed every week. The chow pellets (MF diet; Oriental Yeast, Tokyo, Japan) were placed in a metal container hooked to a metal plate for partitioning in each compartment. Fresh tap water was provided in each compartment using a bottle with a metal nozzle tube positioned 6.5 cm above the floor. The animals were adapted to this condition for one week before beginning the adaptation treatment. Further, their body weights were maintained at 85–90% with *ad libitum* feeding throughout the experiment. The vivarium was maintained on a light-dark cycle of 12 h (lights on at 0800 h), with controlled temperature (23 °C) and humidity (60%).

The experiment was conducted in a conventionally illuminated experimental room with four feeding translucent plastic cages (purchased from Seria corporation; 10.5 cm wide, 16 cm long, 10.5 cm high) and four commercial wheels with a counter (MR-758, Marukan corporation; 15 cm wide, 38 cm in diameter). Each translucent plastic cage was covered with a translucent plastic lid with four air holes, and a ceramic vessel for food stimuli was placed on the floor of the case. Each wheel was made of spherical plastic, and one side of the wheel was removable, which was the animal's entrance. The number of wheel rotations was automatically measured using a counter attached to the product.

Procedure. Before the experiment, the mice were assigned to one of two groups of four mice each and matched for body weight the day before the experimental phase. All mice were placed in plastic cages on the first day with a 7.5 g cubic piece of processed cheese (QBB Baby Cheese Original, Rokko Butter, Hyogo) for 20 min. Adaptation to the wheels was omitted from this experiment.

In the subsequent 12 days, the conditioning phase comprised six cycles, with one cycle every two days. On the day of each cycle, mice in the paired group were given 20 min of access to cheese in the feeding cages, immediately followed by 40 min confinement to the wheels. On the other day, the mice spent 60 min in their home cages in the experimental room. Mice in the unpaired group were given 20 min access to cheese in the feeding cages, followed

by 40 min holding in the home cages on the day of each cycle. On the other day, the mice spent 20min holding in their home cages in the experimental room, immediately followed by a 40 min confinement to the wheels. These two-day procedures were counterbalanced within each group.

Ethical considerations. The Animal Ethics Committee of Tezukayama University (Approval No. 2021-03) approved all treatments in this study.

Results

Cheese intake. Figure 1 shows that both groups initially consumed very little cheese, but the mice in the unpaired group gradually increased their consumption over time. The little or no increase in cheese consumption among the paired mice suggests that taste avoidance was established by wheel running. A 2 (group) × 6 (conditioning day) mixed-design ANOVA yielded significant main effects of group (F [1, 6] = 7.29, p<.05) and conditioning day (F [5, 30] = 18.20, p<.001), and the interaction term was also significant (F [5, 30] = 9.42, p<.001). A post-hoc simple main effect analysis of the interaction revealed a marginal group difference on the third day, F (1, 36) = 9.30, p<.005, on the fourth day, F (1, 36) = 13.67, p<.001, the fifth day, F (1, 36) = 13.67, p<.001, and on the sixth day, F (1, 36) = 9.30, p<.005. The simple day effect was significant for the paired (F [5, 30] = 4.36, p<.005) and unpaired (F [5,30] = 23.26, p<.001) groups.

Wheel turns. The number of wheel turns gradually increased during the conditioning phase in both groups of mice. The average (± standard error) of the paired group was 940±115, 1288±458, 1946±106, 2011±191, 2011±211, and 1938±184 turns per day in six running days. The means (± standard errors) of the unpaired group were 1152±173, 1403±135, 1919±19, 1918±76, 1966±155, and 2250±94 turns per day. A 2 (group) × 6 (conditioning day) mixed-design ANOVA revealed significant main effects of conditioning day (F [5, 30] = 10.22, p<.001). The main effects of group (F [5, 30] = 0.46, *n.s.*) and the interaction term (F [5, 30] = 0.36, *n.s.*) weren't also significant.

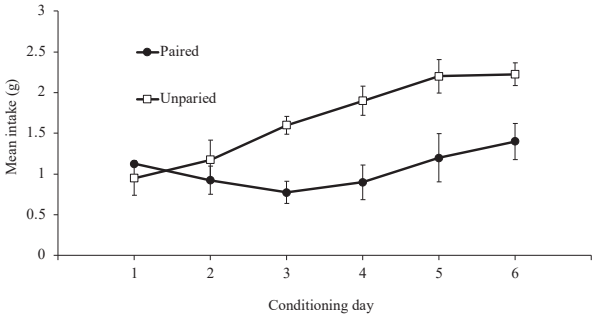

Figure 1. Mean intake of two groups (each n=4) on the conditioning phase. Error bars indicate the standard errors of means.

Discussion

This study demonstrated wheel-running-based conditioned flavor avoidance in C57BL mice. This result verifies the robustness and generality of previous studies (Nakajima, 2019a; Nakajima, 2019b; Nakajima, 2021) in running-based CFA and is consistent with the results of Nagaishi et al. (2021). However, the number of subjects in this study was four in each group, which may lead to reliability issues. Considering recent animal ethical issues (especially the 3Rs), we believe that a small number of subjects may demonstrate more robustness and generality.

Future steps will require replicating the various associative learning phenomena (e.g., latent inhibition and overshadowing) observed in running-based CFA in mice.

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