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Abstract

An exclusively milk formula diet stunted the growth of mice immediately following weaning. Milk-fed mice displayed a low-frequency profile of exploratory behavior, while pellet-fed mice showed high-frequency exploration. In contrast to exploratory behavior, feeding behavior did not differ significantly between milk- and pellet-fed mice. Despite showing low-frequency exploratory behavior, mice on an exclusively milk formula diet showed no difference in behavioral activities analyzed by an automatic hole-board apparatus compared to pellet-fed mice. These results suggest that the growth stunt caused by an exclusively milk formula diet retards the acquisition of active exploratory behavior without affecting the emotional state of mice.

Key words: feeding, exploratory behavior, growth, weaning, milk formula

Introduction

Milk formula is widely utilized as a substitute for mother's milk in all mammals. The influence of milk formula on growth and development during the suckling period has been studied using artificially reared rat pups (Smart et al., 1983, 1984; Auested et al., 1990; Kanno et al., 1997). Some differences in behavior and brain development were observed between artificially reared and mother-reared rat pups (Diaz et al., 1982; Smart et al., 1984; Moore et al., 1990; Kaneko et al., 1996). These studies suggested that the nutritional composition of milk formula and/or maternal separation strongly affects behavior and brain development. No information is currently available concerning the effect of a prolonged post-weaning milk formula diet on brain development. The present study investigates the effects of an exclusively milk formula post-weaning diet on the feeding and exploratory behavior of mice. Feeding and exploratory behavior were analyzed using a food-search-compulsion-apparatus (FSCA), which was designed to distinguish these two behaviors under the living conditions of mice. We further analyzed various exploratory activities using an automatic hole-board apparatus to examine emotionality and anxiety of mice in an unfamiliar environment. We demonstrated that an exclusively milk formula diet stunts the growth of mice immediately following weaning and prevents the acquisition of active exploratory behavior without affecting the emotional state of the mice.

Materials and Methods

Animals, diet, and food-search-compulsion-apparatus (FSCA)

Male *ddY* mice were maintained under controlled temperature and lighting conditions with a 12 h light/dark cycle (lights on at 0600). Mice were isolated from the mother at 20 d of age and fed either milk or food pellets until 10.5 wk of age. Constituents of milk formula and food pellets used in this study are shown in Table 1. The milk formula has lower protein and higher carbohydrate content, but is isoenergetic with mouse milk (Sensui et al., 1996). Feeding and exploratory behavior were monitored using an FSCA (Fig. 1A). The FSCA was an acrylic cage equipped with two separate vertical cylinders (180 cm high) of stainless steel wire, the tops of which had a chamber containing either food (milk or pellets) or nothing. Mice needed to climb up to the chamber to seek and obtain food. The number of entries and the duration of time spent in each chamber were monitored using detectors attached to the chambers. Mice had access to water *ad libitum* without climbing the cylinders.

Feeding and exploratory behavior under mice's living conditions

The training task was conducted by placing mice in the FSCA for 2 wk (from 5 - 7 wk of age). A food chamber containing milk or pellets and an empty chamber were connected to the left and right cylinders, respectively. Following training, the mice were returned to normal plastic cages and maintained for 2 wk. After a 2-wk recovery period, mice were transferred back to the FSCA. We designated entries into the food chamber as feeding behavior. Entries into the empty chamber were designated exploratory behavior, although whether mice explored their territory with a specific aim was not determined. Feeding and exploratory behavior were recorded at 10 wk of age.

Behavioral activities in unfamiliar environments

Exploratory activities, i.e., total locomotor activity, numbers and duration of rearing and head-dipping, and latency to the first head-dipping, were recorded using the automatic hole-board apparatus (model ST-1 Muromachi Kika, Japan) consisting of a gray wooden box

(50 x 50 x 50 cm) with four 3 cm equidistant holes in the floor. An infrared beam sensor was installed on the wall to detect the number and duration of rearing and head-dipping behaviors and the latency to the first head-dipping. Other behavioral parameters such as locus, distance and speed of movement of mice in the hole-board were recorded by an overhead color CCD camera; the heads of the mice were painted yellow and the color CCD camera followed their center of gravity. Data from the infrared beam sensor and the CCD camera were collected through a custom-designed interface (CAT-10, Muromachi Kika, Japan) as a reflection signal. Head-dipping behaviors were double-checked via an infrared sensor and the overhead color CCD camera. Thus, only when both the head intercepted the infrared beam and the head was detected at the hole by the CCD camera was head-dipping behavior counted. All data were analyzed and stored on a personal computer using analytical software (Comp ACT HBS, Muromachi Kika, Japan). Mice were placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min while exploratory activities were automatically recorded.

Statistical methods

Statistical significance was determined using Student's unpaired *t* test.

Animal care and ethical standards

All procedures for the care and use of experimental animals were approved by the Animal Research Committee at Obihiro University of Agriculture and Veterinary Medicine and were conducted under the Guidelines for Animal Experiments of Obihiro University of Agriculture and Veterinary Medicine and the Guiding Principles in the Use of Animals in Toxicology that were adopted by the Society of Toxicology in 1989. The animals were humanely killed by an overdose of anesthetic ether at the end of the experiment.

Results

Profiles of feeding and exploratory behavior in milk- and pellet-fed mice

Milk-fed mice had lower body weight gain than pellet-fed mice during the first 2 wk post weaning. The body weight of milk-fed mice increased shortly afterward and reached that of pellet-fed mice at 8 wk of age. The mean body weight of milk-fed mice was significantly lower than that of pellet-fed mice between 4 and 7 wk of age; however, after 8 wk of age the difference was no longer statistically significant (Fig. 1B). Thus, an exclusively milk formula diet stunted the growth of mice only immediately following weaning.

We analyzed both feeding and exploratory behavior in milk-and pellet-fed mice at 10 wk of age. Milk-fed and pellet-fed mice had different exploratory behavior profiles. Pellet-fed mice displayed high-frequency exploratory behavior and repeatedly entered the empty chamber (Fig. 2A). In contrast, milk-fed mice displayed low-frequency exploratory behavior; either seldom re-entering the empty chamber or never entering it at all (Fig. 2B). The number of entries into the empty chamber (exploratory behavior) in milk-fed mice was significantly lower than pellet-fed mice, whereas the number of entries into the food-containing chamber (feeding behavior) was not significantly different (Fig. 2C, D).

Various exploratory activities in milk- and pellet-fed mice analyzed by an automatic hole-board apparatus in unfamiliar environments

An exclusively milk formula diet markedly inhibited exploratory behavior in an FSCA (Fig. 2B&C). This low-frequency exploratory behavior in milk-fed mice may have been caused by emotional depression; therefore, analysis and comparison of various exploratory activities in milk- and pellet-fed mice under unfamiliar environments were conducted. An automatic hole-board apparatus offers a simple method for measuring the response of an animal to an unfamiliar environment (Boisser and Simon, 1964; Rodriguez Echandia et al., 1987) and is a useful tool for objectively estimating various emotional states of animals (Takeda et al., 1998; Tsuji et al., 2000; Tsuji et al., 2001). The effect of an exclusively milk formula diet on various exploratory activities, i.e., total locomotor activity, number and duration of rearing and head-dipping, and latency to the first head-dipping, is shown in Fig. 3. Results of these exploratory activities in milk-fed mice, however, were not significantly different from those of pellet-fed mice, suggesting that an exclusively milk formula diet after weaning does not affect the emotional state of mice.

Discussion

In the present study, we demonstrated that an exclusively milk formula diet stunts the growth of mice immediately following weaning and prevents the acquisition of active exploratory behavior without affecting emotional state. In contrast to exploratory behavior, feeding behavior did not differ significantly between milk- and pellet-fed mice. These results suggest that exclusively milk-fed mice fail specifically in the expression of active exploratory behavior. The stunting of growth, caused by an exclusively milk formula diet after weaning, might affect brain development or function and lead to the failure in acquisition of active exploratory behavior.

Feeding and exploratory behavior were recorded at 10 wk of age. Body weight gain of milk-fed mice was significantly lower than that of pellet-fed mice for the first 2 wk post weaning, which is likely to be caused by the different nutritional compositions and/or different diet quality between milk formula and pellet food. As a consequence of the lower body weight gain, the mean body weight of milk-fed mice was significantly lower than that of pellet-fed mice between 4 and 7 wk of age, but the difference was no longer statistically significant after

8 wk of age (Fig. 1B), indicating that an exclusively milk formula diet stunted the growth of mice only immediately following weaning. Therefore, growth and/or brain development in the weeks following weaning appear to be the most important for the acquisition of active exploratory behavior. Conversely, feeding behavior and other exploratory activities, analyzed using the automatic hole-board apparatus, did not differ significantly between milk- and pellet-fed mice (Fig. 2D & 3). These results suggest that low-frequency exploratory behavior in milk-fed mice is not caused by emotional depression or a decrease in spontaneous activity, and also appears to be unrelated to the motivation to obtain food.

The influence of milk formula during the suckling period on growth and development has been studied (Smart et al., 1983, 1984; Auested et al., 1990; Kanno et al., 1997). Several investigators have reported the importance of the nutritional composition of milk formula and/or maternal separation in behavior and brain development (Diaz et al., 1982; Smart et al., 1984; Moore et al., 1990; Kaneko et al., 1996). The present report is the first to demonstrate the effect of a prolonged post-weaning milk formula diet on behavior and brain function. After physiologic weaning periods, mice begin to seek food independent of their mother. The acquisition mechanisms of this self-sufficiency in obtaining food, however, remain unknown. The acquisition of active exploratory behavior, which was not observed in mice fed an exclusively milk formula diet, might relate to the acquisition of the self-sufficiency in obtaining food. Thus, the growth stunt caused by an exclusively milk formula diet may retard the development of brain function involved in the motivation to explore their territory, resulting in low-frequency exploration. Our results suggest that growth and/or development of the brain in the several weeks following weaning are critical for the acquisition of active exploratory behavior. Further studies are required to elucidate what nutritional compositions are lacking in a prolonged milk formula diet and to determine the brain area involved in the acquisition of active exploratory behavior.

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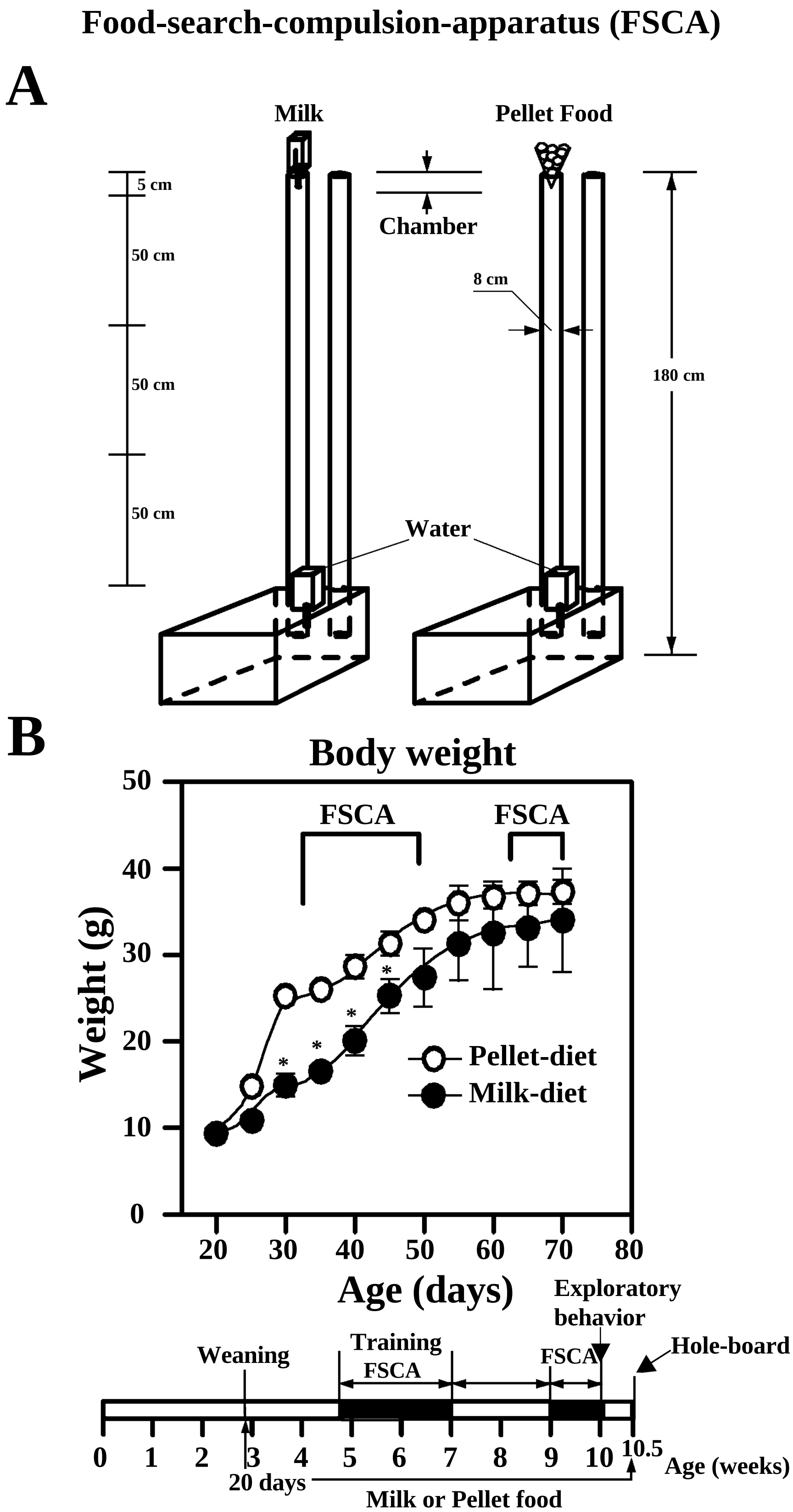
Figure legends

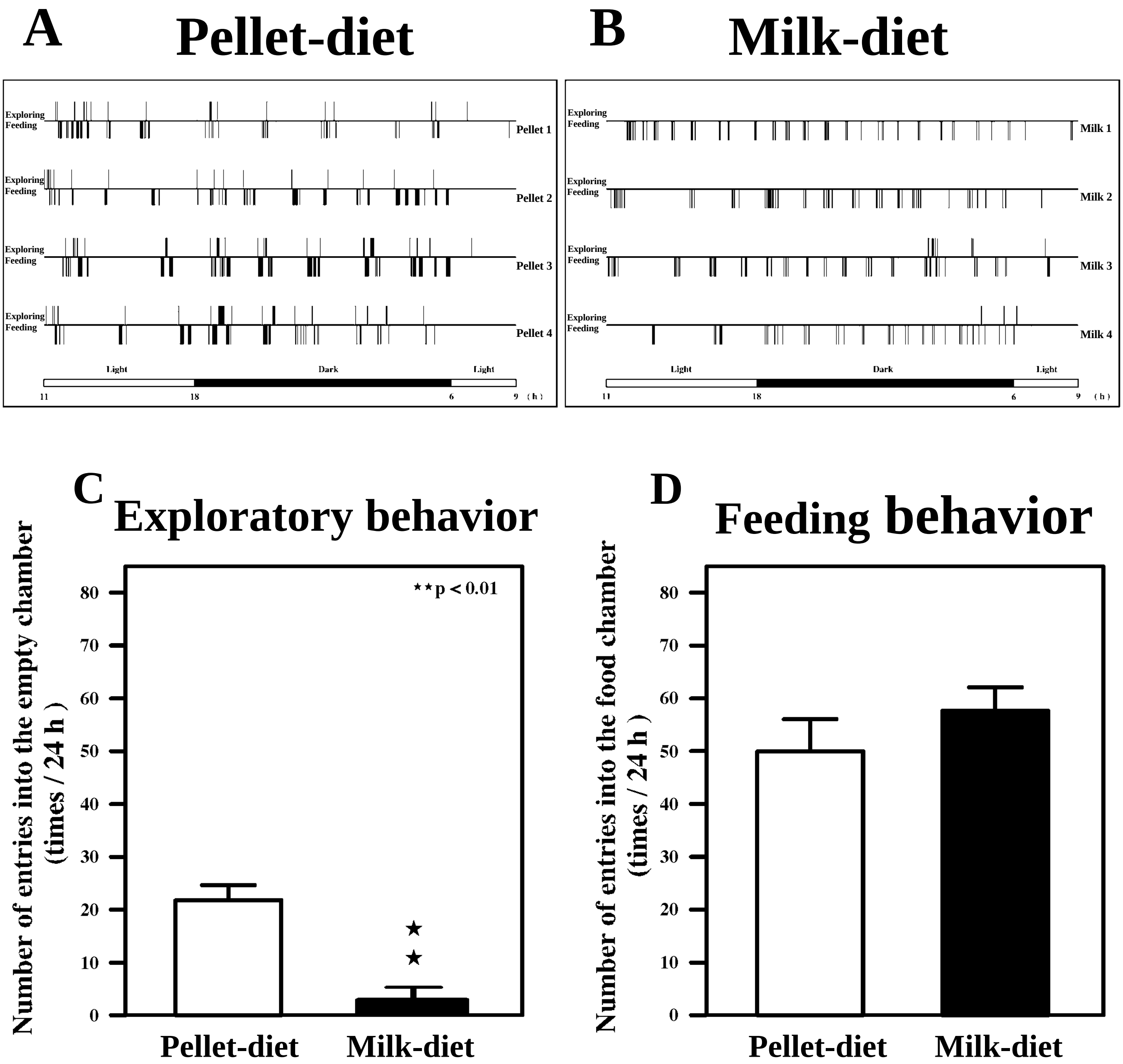
Fig. 1. Experimental apparatus and changes in body weight. (A) Illustration of the food-search-compulsion-apparatus (FSCA). The FSCA is an acrylic cage equipped with two separate vertical stainless steel wire cylinders, the top of which has a chamber containing either food (milk or pellets) or nothing. Entries into the empty chamber were termed exploratory behavior and those into the food chamber were termed feeding behavior. (B) Changes in body weight of milk-fed mice (n = 7) and pellet-fed mice (n = 7) for 7 wk after weaning at 20 d of age. Except for training and the analysis of exploratory behavior, the mice were maintained in normal plastic cages and fed milk or food pellets and water *ad libitum*. The mean body weight of milk-fed mice was significantly less compared to pellet-fed mice between 4 and 7 wk of age. After 8 wk of age, however, there was no significant difference between groups. Vertical bars represent SD. Asterisk indicates $p < 0.05$. Statistical significance was determined using Student's *t* test.

Fig. 2. Profiles of feeding and exploratory behavior of pellet- and milk-fed mice housed in an FSCA. (A and B) Profiles of feeding and exploratory behavior in pellet-fed mice (A) and milk-fed mice (B) at 10 wk of age. Pellet 1-4 and Milk 1-4 represent the profiles of four different mice in each group (n = 7). (C) Comparison of exploratory behavior between milk- and pellet-fed mice. Exploratory behavior in milk-fed mice (n = 7) was significantly lower compared to pellet-fed mice (n = 7). (D) Comparison of feeding behavior between milk- and pellet-fed mice. Feeding behavior was not significantly different. Vertical bars represent SD. Double asterisk indicates $p < 0.01$. Statistical significance was determined using Student's *t* test.

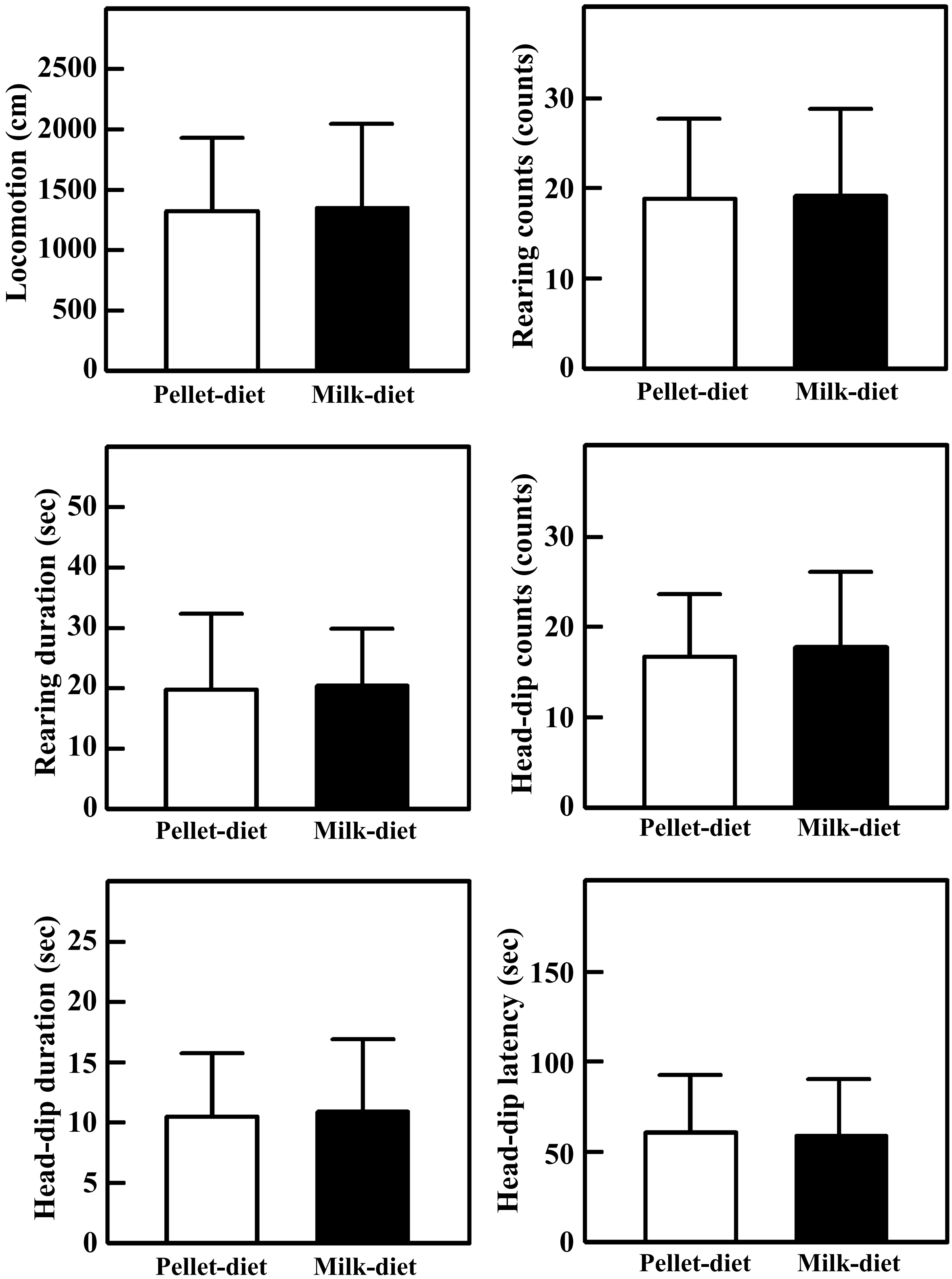
Fig. 3. Exploratory activities in milk-fed and pellet-fed mice analyzed by the automatic hole-board test under unfamiliar environments. Exploratory activities on the hole-board, i.e., total locomotor activity, number and duration of rearing and head-dipping, and latency to the first head-dipping were measured for 5 min. Vertical bars represent SD.



Ishii et al. Figure 1



Ishii et al. Figure 2



Ishii et al. Fig.3

Table 1 Constituents of formula and food pellets (%)

	Water	Protein	Lipid	Carbohydrate	Ash	Crude fiber
Food pellets	8.9	25.4	4.4	50.3	6.9	4.1
Formula	87.6	1.6	3.5	7.0	0.3	—