The mesencephalic trigeminal sensory nucleus is involved in acquisition of active exploratory behavior induced by changing from a diet of exclusively milk formula to food pellets in mice

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The number of text pages: 21
The number of figures: 4

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Abbreviations

Me5, mesencephalic trigeminal nucleus; VMH, ventromedial hypothalamus; FSCA, food search compulsion apparatus.

Abstract

Post-weaning mice fed exclusively milk display low-frequency exploratory behavior (Ishii, T., Itou, T., and Nishimura, M. (2005) Life Sci. 78, 174-179) compared to mice fed a food pellet diet. This low-frequency exploratory behavior switched to high-frequency exploration after a switch from exclusively milk formula to a food pellet diet. Acquisition of the high-frequency exploratory behavior was irreversible. Recently, we demonstrated that the mesencephalic trigeminal nucleus (Me5) is involved in the control of feeding and exploratory behavior in mice without modulating the emotional state (Ishii, T., Furuoka, H., Itou, T., Kitamura, N., and Nishimura, M. (2005) Brain Res. 1048, 80-86). We therefore investigated whether the Me5 is involved in acquisition of high-frequency exploratory behavior induced by the switch in diet from an exclusively milk formula to food pellets. Mouse feeding and exploratory behaviors were analyzed using a food search compulsion apparatus, which was designed to distinguish between the two behaviors under standard living conditions. Immunohistochemical analysis of immediate early genes indicated that the Me5, which receives signals from oral proprioceptors, is transiently activated after the diet change. The change from low-frequency to high-frequency exploratory behavior was prevented in milk-fed mice by bilateral lesion of the Me5. These results suggest that the Me5 is activated by signals associated with mastication-induced proprioception and contributes to the acquisition of active exploratory behavior.

Section: Cognitive and Behavioral Neuroscience
Themes: Neural Basis of Behavior
Topics: Neuroethology
Keywords: exploratory behavior, Me5 lesions, weaning

1. Introduction

The mesencephalic trigeminal nucleus (Me5) receives proprioceptive sensory afferents of the trigeminal nerve from the jaw-closing muscle spindles and the periodontal ligaments, and it innervates the motor trigeminal nucleus, which participates in the jaw-jerk reflex (Corbin and Harrison, 1940; Harrison and Corbin, 1942). The Me5 fibers also project into the tuberomammillary nucleus of the posterior hypothalamus, where the cell bodies of histamine neurons are localized (Ericson, 1989). Histamine neurons in the tuberomammillary nucleus project to the ventromedial hypothalamus (VMH) and to the Me5 (Inagaki et al., 1987; Inagaki et al., 1988; Sakata et al., 2003). Fujise et al. (1993) suggested that the oral proprioceptive signals induced by mastication might modulate hypothalamic histamine neurons through the ascending pathway from the Me5. Moreover, Fujise et al. (1998) demonstrated that the Me5 is involved in mastication-induced modulation of satiation and eating parameters. Their study suggests that the Me5 receives signals relating to mastication-induced proprioception and modulates satiation via a satiety center in the VMH. To investigate how the Me5 is involved in the control of feeding and exploratory behavior, we previously examined the effect of bilateral electrolytic lesions of the Me5 on feeding and exploratory behavior in mice using a food search compulsion apparatus (FSCA) designed to distinguish between the two behaviors under standard living conditions (Ishii et al., 2005a; Ishii et al., 2005b). We found that mice with bilateral Me5 lesions have unique feeding and exploratory behavior profiles in the FSCA compared with sham-operated mice. Me5-lesioned mice spent more time in the food chamber during each trial in the FSCA, but the number of entries into the food chamber was decreased by 40% compared with sham-operated mice. Moreover, Me5 lesions markedly inhibited exploratory behavior, manifested as low-frequency exploration (Ishii et al., 2005b). In spite of the low-frequency exploration in the FSCA, Me5 lesions had no effect on various behavioral

activities analyzed in an automatic hole-board apparatus (Ishii et al., 2005b), the apparatus of which 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 Me5, therefore, controls exploratory behavior other than feeding behavior in mice through its ascending neuronal pathways without modulating the emotional state.

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). To investigate the effect of a prolonged post-weaning milk formula diet on brain development, we previously analyzed the feeding and exploratory behavior of mice fed either milk or food pellets until 10 wk of age and compared them between exclusively milk- and pellet-fed mice (Ishii et al., 2005a). 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 the hole-board apparatus compared to pellet-fed mice (Ishii et al., 2005a). Thus, a prolonged post-weaning milk formula diet prevents the acquisition of active exploratory behavior in mice without affecting the emotional state.

In the present study, we examined the effect of changing mice from an exclusively milk formula diet to food pellets at 10 wk of age on feeding and exploratory behavior using an FSCA. To determine whether signals induced by a diet change from milk formula to food pellets are transmitted via the Me5, we examined the expression of Fos B and c-Fos in the Me5 neurons of milk-fed mice after switching to the pellet diet. Moreover, to investigate whether the Me5 is involved in acquisition of active exploratory behavior, we examined the effect of bilateral electrolytic lesions of the Me5 on feeding and exploratory behavior in milk-fed mice.

2. Results

Acquisition of active exploratory behavior after a diet change from an exclusively milk formula to food pellets

Recently, we demonstrated that an exclusively milk formula diet prevents the acquisition of active exploratory behavior in mice without affecting their feeding behavior or their emotional state (Ishii et al., 2005a). Namely, milk-fed mice displayed low-frequency exploratory behavior, either seldom re-entering the empty chamber or never entering it at all. In contrast, pellet-fed mice showed high-frequency exploratory behavior and repeatedly entered the empty chamber. 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. In spite of the low-frequency exploration, an exclusively milk formula diet had no effect on various behavioral activities analyzed in the hole-board apparatus, i.e., total locomotor activity, frequency and duration of rearing and head-dipping, and latency to the first head-dipping (Ishii et al., 2005a). In the present study, to investigate whether a diet change from exclusively milk formula to food pellets causes a switch from low-frequency to high-frequency exploratory

behavior, we examined the effect of changing mice from an exclusively milk formula diet to food pellets at 10 wk of age on feeding and exploratory behavior using an FSCA. We found that the diet change caused a switch from low-frequency to high-frequency exploratory behavior. This change to an active high-frequency exploratory behavior occurred at least 3 d after the diet change (Fig. 2A). The number of entries into the empty chamber (exploratory behavior) in milk-fed mice significantly increased 5 days after a diet change to food pellets ($p < 0.01$, by Wilcoxon signed-ranks test) (Fig. 2B, left). The increased number of entries into the empty chamber, which corresponds to that in mice fed a food-pellet diet post weaning (Ishii et al., 2005a), did not change even after the diet was switched back to milk formula ($p > 0.05$ vs. before switching back to milk formula, by paired *t*-test; $p < 0.01$ vs. before the diet change to food pellets, by Wilcoxon signed-ranks test) (Fig. 2B, left). Moreover, Welch's *t*-test indicated that significant differences in exploratory behavior between milk-fed mice after a series of diet change and age-matched milk-fed control mice (between 5 d after a diet change to pellet food, 27.4 ± 5.2, and age-matched milk-fed control mice at 10 wk + 5 d of age, 2.1 ± 1.6 times / 24 h, $p < 0.01$; between 5 d after a return to a milk-diet, 30.6 ± 9.0, and age-matched milk-fed control mice at 10 wk + 10 d of age, 3.5 ± 1.4 times / 24 h, $p < 0.01$). On the other hand, Student's *t*-test indicated that no significant differences in feeding behavior between milk-fed mice after a series of diet change and age-matched milk-fed control mice (between 5 d after a diet change to pellet food, 52.3 ± 6.4, and age-matched milk-fed control mice at 10 wk + 5 d of age, 49.1 ± 5.3 times / 24 h, $p > 0.05$; between 5 d after a return to a milk-diet, 57.7 ± 6.7, and age-matched milk-fed control mice at 10 wk + 10 d of age, 50.3 ± 7.3 times / 24 h, $p > 0.05$). Thus, once the mice acquired high-frequency exploratory behavior, they maintained it, even if the diet was switched back to milk formula. Feeding behavior did not change after altering the diet in either direction ($p > 0.05$, by paired *t*-test) (Fig. 2B, right).

Changes in Fos expression in the Me5 following diet change from an exclusively milk formula to food pellets

The Me5 receives signals from oral proprioceptors (Harrison and Corbin, 1942) and projects to higher brain regions (Ericson et al., 1991). Moreover, the Me5 is involved in mastication-induced modulation of satiation and feeding (Fujise et al., 1998; Sakata et al., 2003). We hypothesized that the Me5 receives signals related to a diet change from an exclusively milk formula to food pellets and transmits these signals to higher brain regions associated with exploratory and feeding-related behaviors. We therefore investigated whether the expression of the immediate early genes Fos B and c-Fos (Sagar et al., 1988; Zerial et al., 1989) in the Me5 is enhanced during acquisition of high-frequency exploratory behavior following a change from an exclusively milk formula diet to a food pellet diet. We found that the number of both Fos B- and c-Fos-expressing cells in the Me5 significantly increased 1, 2, and 3 d after the diet change [c-Fos, $F(4,15) = 125.67$, $p < 0.001$; Fos B, $F(4,15) = 316.37$, $p < 0.001$, one-way ANOVA] ($p < 0.05$, by Tukey-Kramer post hoc test) (Fig. 3A, B). Thus, Fos B and c-Fos were transiently expressed in Me5 neurons 1 to 3 d after switching to the pellet diet. Maximal expression of c-Fos was observed 2 d after the diet change and then rapidly declined (Fig. 3A, B). The time course of Fos B expression in the Me5 was similar to c-Fos. Moreover, the time course of Fos B and c-Fos expression in the Me5 preceded the change from low-frequency to high-frequency exploratory behavior (Fig. 2 and 3).

Feeding and exploratory behavior in milk-fed mice with Me5 lesions

To investigate whether the Me5 is involved in the transmission of signals related to diet change and whether it is required for acquisition of active exploratory behavior, we examined

the effect of bilateral electrolytic lesions of the Me5 on exploratory behavior after changing the diet. There was no significant difference in exploratory behavior between bilateral Me5-lesioned and sham-operated milk-fed mice prior to the change to pellet food ($p > 0.05$, by Student's *t*-test) (Fig. 4B). After the switch to pellet food, however, the exploratory behavior of sham-operated mice changed from low-frequency to high-frequency exploration ($p < 0.01$, by Wilcoxon signed-ranks test) (Fig. 4B, left). In contrast, Me5-lesioned milk-fed mice maintained low-frequency exploratory behavior following the change in diet ($p > 0.05$, by paired *t*-test) (Fig. 4A, B, left). Thus, Me5 lesions prevented the acquisition of active exploratory behavior in milk-fed mice following a change from an exclusively milk formula to a food pellet diet. In contrast, the feeding behavior of both Me5-lesioned and sham-operated mice did not change after the switch to the food pellet diet ($p > 0.05$, by paired *t*-test) (Fig. 4B, right). Moreover, there was no significant difference in the mean body weight between the Me5-lesioned and sham-operated mice ($p > 0.05$, by Student's *t*-test) (Fig. 1B).

3. Discussion

In the present study, we demonstrated that the Me5 contributes to the acquisition of active exploratory behavior in mice after a diet change from exclusively milk formula to food pellets. Exclusively milk-fed mice display a low-frequency of exploratory behavior, whereas pellet-fed mice show high-frequency exploration. This low-frequency of exploratory behavior in exclusively milk-fed mice is maintained without regard to age (Ishii et al., 2005a). However, the low-frequency exploratory behavior in milk-fed mice changed to high-frequency exploration after switching them to a food pellet diet. The change in exploratory behavior occurred at least 3 d after the diet change, and mice continued the high-frequency behavior even if their diet was switched back to milk. Thus, the change from low-frequency to

high-frequency exploratory behavior is irreversible under these conditions. On the other hand, age-matched milk-fed control mice without the diet change kept the low-frequency exploratory behavior. These results suggest that signals induced by a diet change from milk formula to food pellets are transmitted to the brain, triggering a permanent change from low-frequency to high-frequency exploratory behavior.

Recently, we demonstrated that the Me5 is involved in the control of feeding and exploratory behavior in mice without modulating the emotional state (Ishii et al., 2005b). The Me5 receives proprioceptive sensory input from periodontal ligaments (Harrison and Corbin, 1942) and from the masseteric muscle spindles through the trigeminal sensory nerve (Corbin and Harrison, 1940). Fujise et al. (1993, 1998) suggest that the Me5 receives signals relating to mastication-induced proprioception and modulates satiation via a satiety center in the VMH. To determine whether signals induced by a diet change from milk formula to food pellets are transmitted via the Me5, we examined the expression of Fos B and c-Fos in Me5 neurons of milk-fed mice after a diet change at 10 wk of age. We found that Fos B and c-Fos in Me5 neurons are transiently expressed 1 to 3 d after the switch to the food pellet diet. Maximal expression of Fos B and c-Fos was observed 2 d after the diet change and then rapidly declined. Thus, the time-course of Fos B and c-Fos expression in the Me5 preceded the change in exploratory behavior. These results suggest that a signal from oral proprioceptors after the diet change activates the Me5.

Neurons express c-Fos following synaptic excitation by sensory stimulation (Friauf, 1992). Immunohistochemical analysis indicates that Fos B and c-Fos immunoreactivity is rapidly increased several hours after the onset of stimulation (Miyata et al., 2001; Morgan and Curran, 1990; Sagar et al., 2003). Therefore, the induction of immediate early genes is considered an early and sensitive marker of neuronal activation (Herrera and Robertson, 1996;

Liu et al., 1998; Miyata et al., 2001; Nakazato et al., 2001; Sagar et al., 1998). In the present study, however, Fos B and c-Fos expression in the Me5 reached maximal levels 2 d after a diet change from milk formula to food pellets. Compared to other studies, Fos B and c-Fos expression in the Me5 took substantially longer to reach maximal levels after the onset of stimulation. It is not clear why Fos activation is delayed under these conditions.

To further examine whether the Me5 is involved in acquisition of active exploratory behavior, we produced bilateral Me5 lesions in milk-fed mice. Sham-operated milk-fed mice underwent a change from low-frequency to high-frequency exploratory behavior after a diet change to food pellets. In contrast, Me5-lesioned mice retained low-frequency exploratory behavior after changing to a food pellet diet. These results suggest that the Me5 is involved in the transmission of signals to the brain relating to diet change and that activation of the Me5 is required for acquisition of high-frequency exploratory behavior. On the other hand, there was no difference in the feeding behavior between Me5-lesioned and sham-operated milk-fed mice. We previously reported that, in pellet-fed mice, Me5 lesions inhibit not only exploratory but also feeding behavior (Ishii et al., 2005b). The reason for this different effect of Me5 lesions on feeding behavior between milk-fed and pellet-fed mice, however, remains unclear.

The development and excitability of the central nervous system are modulated by signals from sensory afferent neurons that are generated in response to dietary composition (Fujise et al., 1998; Liu et al., 1998; Masumoto et al., 1998; Sakata et al., 2003). Moreover, a soft diet after the weaning period reduces synaptic formation in the cerebral cortex and impairs spatial learning ability in adulthood (Yamamoto and Hirayama, 2001). Furthermore, there is a possible link between reduced mastication and hippocampal neuron loss in senile impairment of spatial memory (Onozuka et al., 1999; Yamamoto and Hirayama, 2001). The Me5 projects to the posterior hypothalamus (Ericson et al., 1991) and is involved in mastication-induced

modulation of satiation (Fujise et al., 1998; Sakata et al., 2003). Thus, it seems that sensory signals from oral proprioceptors transmit to higher brain regions via the Me5 and affect brain function and development. A liquid diet, such as milk formula, results in decreased mastication compared to a food pellet diet (Liu et al., 1998). Therefore, we suspect that changes in mastication induced by a diet change from milk formula to food pellets is transmitted to higher brain regions via the Me5 through its ascending neuronal pathways, resulting in the high-frequency exploratory behavior. Further studies are required to elucidate which neuronal pathways are involved in the acquisition of active exploratory behavior and to determine the physiologic significance of high-frequency exploratory behavior.

4. Experimental Procedures

Animals and FSCA

Male *ddY* mice were maintained under controlled temperature and lighting conditions with a 12-h light/12-h dark cycle (lights on at 0600). Mice were isolated from the mother at 20 d of age and fed milk formula until 10 wk of age (Ishii et al., 2005a). The diet was then changed from exclusively milk formula to food pellets. 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) (Ishii et al., 2005a; Ishii et al., 2005b). 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 or left empty. Mice had to climb up to the chamber through the intra-cylinders 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 could obtain water *ad libitum* without climbing the cylinders.

Feeding and exploratory behavior in milk-fed mice before and after diet change

The training task was conducted by placing mice in the FSCA for 2 wk (from 4 to 6 wk of age). A food chamber containing milk 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. Mice were then 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 (Ishii et al., 2005a; Ishii et al., 2005b). General feeding and exploratory behavior were recorded at 10 wk of age. After this first recording of these behaviors, the diet was changed from exclusively milk formula to food pellets. A second recording of feeding and exploratory behavior was conducted 5 d after the diet change from milk formula to food pellets. After the second recording of these behaviors, the diet was changed back to milk formula, and a third recording of feeding and exploratory behavior was conducted after 5 d.

Me5 lesions

Bilateral electrolytic Me5 lesions were produced in 6-wk-old mice anesthetized with Avertin® (0.36 g kg^{-1}). Using a stereotaxic apparatus, a 0.2-mm-diameter stainless steel electrode was positioned 5.3 mm posterior to the bregma, 0.9 mm lateral to the midsagittal suture, and 3.2 mm below the surface of the skull. As described in our previous report (Ishii et al., 2005b), anodal electrolytic lesions were produced by passing a 1.3-mA current through the electrode three times for 1 s each. Out of 12 mice that received lesions, 6 had successful bilateral Me5 lesions, 3 had unilateral Me5 lesions, and 3 had lesions in peri-Me5 regions. All

successful Me5 lesions were restricted to the caudal level of the Me5. Sham-operated mice (6 mice) underwent an identical operation but without application of a current. Histologic analysis was performed by light microscopy. After fixation with 10% neutral-buffered paraformaldehyde solution and embedding in paraffin, serial brain sections (4 μm thick) were stained with hematoxylin-eosin to reveal the extent of damage to the Me5. Only data from the mice with successful Me5 lesions were used.

Feeding and exploratory behavior in Me5-lesioned mice

The training task was conducted by placing mice in the FSCA for 2 wk (from 4 to 6 wk of age). Bilateral electrolytic Me5 lesions or sham operation was carried out at 6 wk of age. The mice were returned to normal plastic cages and maintained for 2 wk. After this 2-wk recovery period, mice were transferred back to the FSCA. General feeding and exploratory behaviors were recorded at 10 wk of age. After this first recording of these behaviors, the diet was changed from exclusively milk formula to food pellets. A second recording of feeding and exploratory behavior was conducted 5 d after the diet change from milk formula to food pellets.

Immunohistochemistry

Milk-fed mice before, and 24, 48, 72, and 96 h after a diet change to food pellets were anesthetized with Avertin® (0.36 g kg^{-1}) and transcardially perfused with 15 ml of 4% neutral-buffered paraformaldehyde solution following heparinized phosphate-buffered saline. All mice were maintained in the same condition as the mice analyzed feeding and exploratory behavior in the FSCA. The brains were dissected out and postfixed with 10% neutral-buffered paraformaldehyde solution. After embedding in paraffin, serial brain sections (4 μm thick) were prepared. Brain sections were pretreated with pepsin and then incubated for 2 h with

1:1000 rabbit anti-c-Fos polyclonal antibody (Ab-2; Oncogene Research Products, Boston, MA) or with 1:1000 rabbit anti-Fos B polyclonal antibody (sc-48; Santa Cruz Biotechnology, Santa Cruz, CA), and bound antibodies were detected using an ABC kit (Vector Laboratories, Burlingame, CA). Stereologic analysis of the total number of c-Fos- expressing cells in the caudal level of the Me5 was conducted using a series of sections obtained from the region as follows: 5 sets of 8 sections in each mouse were obtained from every tenth section picked from a series of 75 sections (4 μm thick) from the Me5 region, and the total number of cells expressing Fos B and c-Fos in each set was counted and averaged.

Statistical methods

To evaluate the changes of Fos expression in the Me5 following diet change, data were analyzed by one-way ANOVA after Bartlett test. Tukey-Kramer was used as a post hoc test. In comparison of feeding and exploratory behavior in milk-fed mice before and after a series of diet change and also in milk-fed mice with and without Me5 lesions before and after diet change, data were analyzed by either paired *t*-test or Wilcoxon signed-ranks test after F-test. In comparison of feeding and exploratory behavior in milk-fed mice after a series of diet change with those in age-matched milk-fed control mice, data were analyzed by either Student's *t*-test or Welch's *t*-test after F-test. A p value of less than 0.05 was considered statistically significant.

Animal care and ethical standards

All procedures for the care and use of experimental animals were approved by the Animal Research Committee in Obihiro University of Agriculture and Veterinary Medicine and were conducted under the Guidelines for Animal Experiments in 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.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research (B) (to T.I.) from the Japan Society for the Promotion of Science, The President Discretionary Budget of Obihiro University of Agriculture and Veterinary Medicine (to T.I.), and a Grant-in-aid for the 21th Century Centers of Excellence from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (E-1) (to T.I.).

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Figure legends

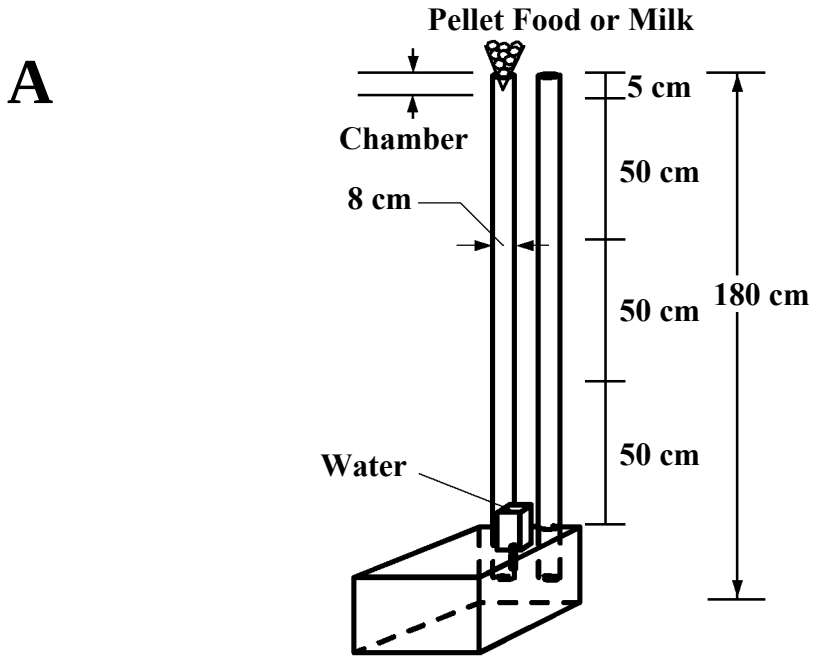
Figure 1. FSCA and changes in body weight after bilateral Me5 lesions. (A) Illustration of the FSCA. The FSCA is an acrylic cage equipped with two separate vertical stainless steel wire cylinders, the top of which has a chamber containing food (milk formula or food pellets) or left empty. Entries into the empty and the food-containing chambers were termed exploratory and feeding behaviors, respectively. (B) Changes in body weight of bilaterally Me5-lesioned mice (n=6) and sham-operated mice (n=6) after surgery at 6 wk of age. Except for the analysis of feeding and exploratory behaviors in the FSCA, the mice were maintained in normal plastic cages and fed food and water *ad libitum*. The mean body weight of Me5-lesioned mice was not significantly different from that of sham-operated mice. Results represent the means ± SD.

Figure 2. Time course of the change from low-frequency to high-frequency exploratory behavior and comparison of feeding and exploratory behavior after a series of diet changes. (A) Representative example of the change in exploratory behavior of a milk-fed mouse after changing to a pellet diet at 10 wk of age (n = 8). (B) Comparisons of feeding and exploratory behavior after a series of diet changes. Feeding and exploratory behavior of milk-fed mice (n = 8) were examined before and 5 d after a diet change to pellet food. The diet of the mice was returned to milk, and feeding and exploratory behavior were examined after 5 d. Results represent means ± SD. $^{**}p < 0.01$ *vs.* exclusively milk-fed mice.

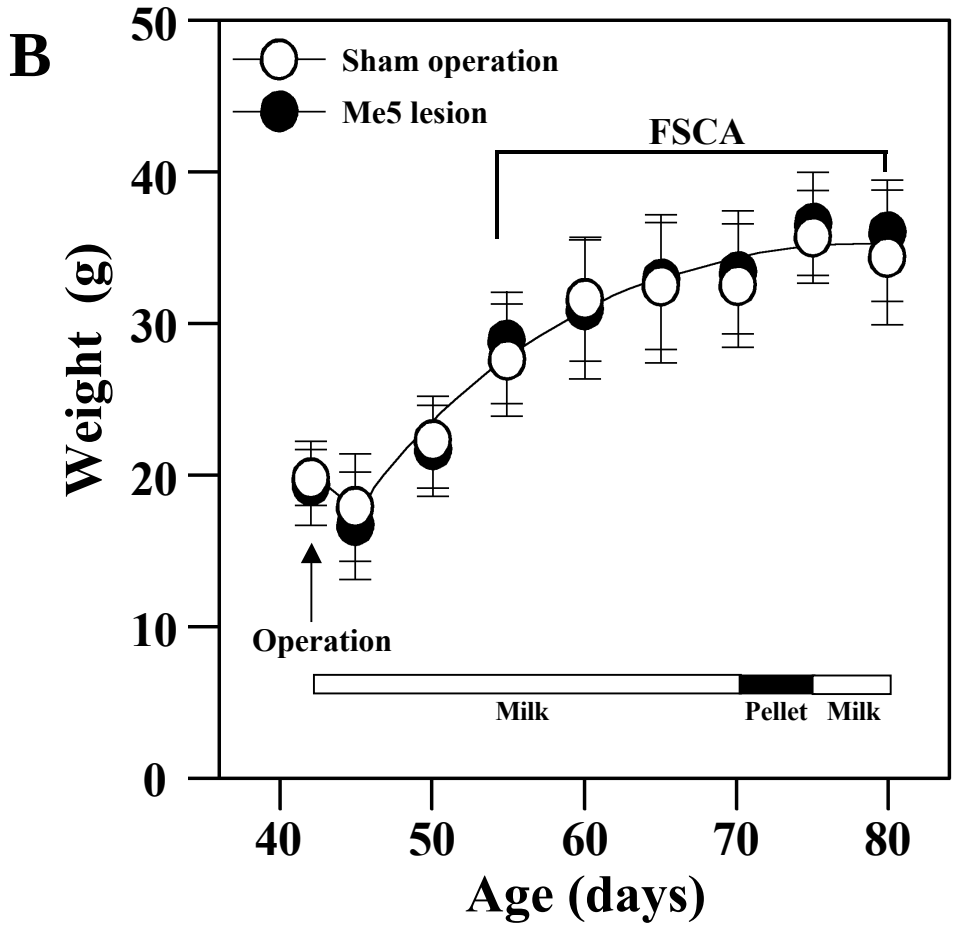
Figure 3. Fos B and c-Fos expression in the Me5 after a diet change from exclusively milk to food pellets. (A) Time course of Fos B and c-Fos expression in the Me5 of milk-fed mice after changing to a food pellet diet at 10 wk of age. Fos B- and c-Fos-immunostained sections before (0 h), and 24, 48, and 72 h after changing to a pellet diet. Controls lacking the primary antibody

are labeled as "- 1st Ab control". Scale bar, 100 μm. (B) The number of Fos B- and c-Fos-expressing cells in the Me5. Stereologic analysis of the total number of Fos B- and c-Fos-expressing cells in the caudal level of the Me5 was conducted using a series of brain sections of milk-fed mice before (0 h) (n = 4), and 24 (n = 4), 48 (n = 4), 72 (n = 4), and 96 h (n = 4) after changing to a pellet diet. Statistical analysis was performed by one-way ANOVA followed by Tukey-Kramer post hoc test. Results represent means ± SD. *$p < 0.05$.

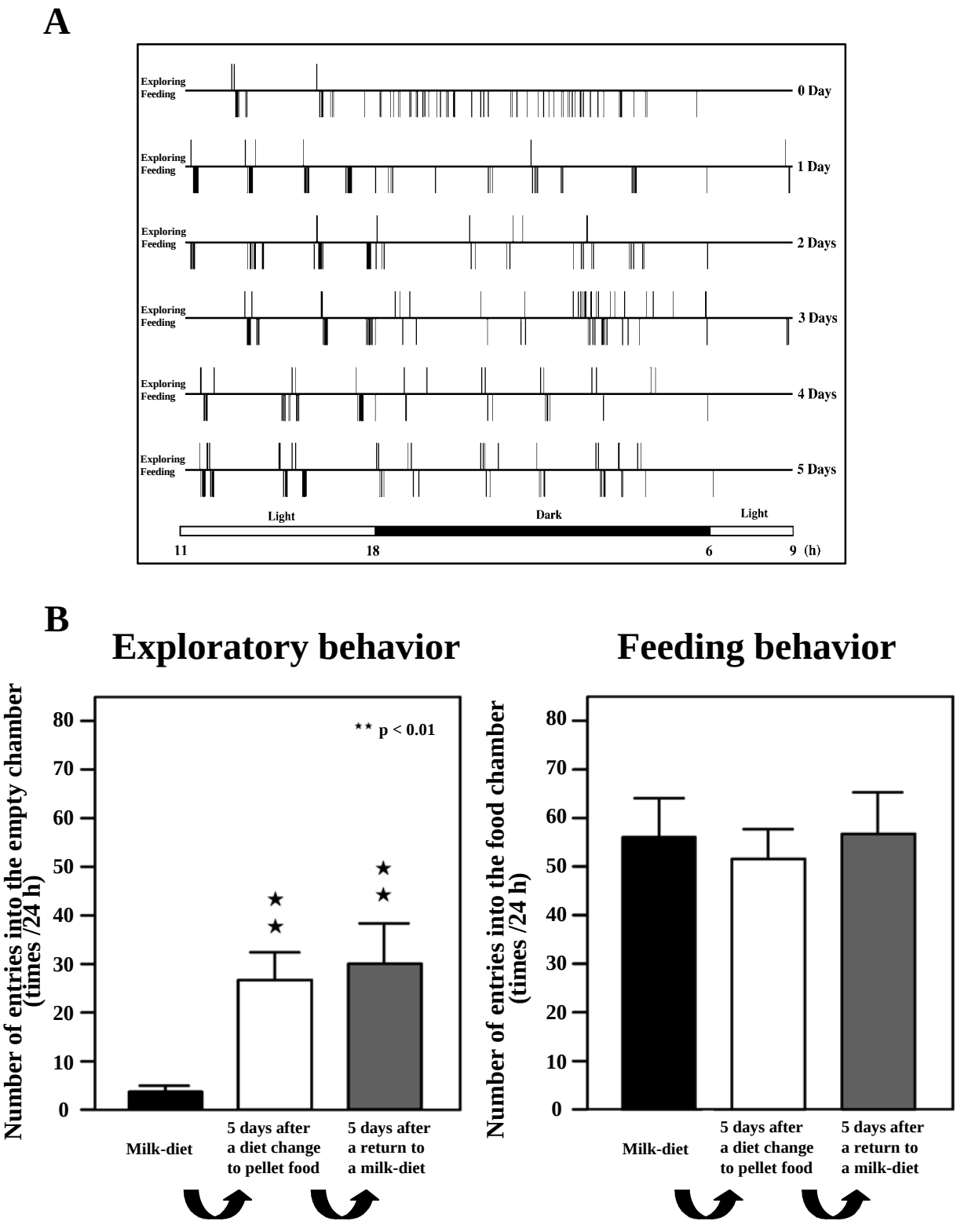
Figure 4. Effect of bilateral Me5 lesions on feeding and exploratory behavior in milk-fed mice. (A) Representative profile of feeding and exploratory behavior in a bilateral Me5-lesioned milk-fed mouse (top) and in the same mouse 5 d after changing to a food pellet diet (bottom). *Inset*, histologic analysis of a hematoxylin-eosin-stained brain section from a Me5-lesioned mouse. Scale bars, 1 mm (left) and 100 μm (right). Asterisks (*) show the sites of the electrolytic lesions in the Me5. (B) Comparison of feeding and exploratory behavior of sham-operated (n = 6) and Me5-lesioned milk-fed mice (n = 6) before and 5 d after a diet change to food pellets. Low-frequency exploratory behavior of sham-operated milk-fed mice (n = 6) but not of Me5-lesioned milk-fed mice (n = 6) changed to active high-frequency exploration 5 d after switching to a food pellet diet (left). Feeding behavior of both Me5-lesioned (n = 6) and sham-operated milk-fed mice (n = 6) did not change after a switch to a food pellet diet (right). Results represent means ± SD. **$p < 0.01$ *vs.* before diet change.



Food-search-compulsion-apparatus (FSCA)



Ishii et al. Fig. 1.



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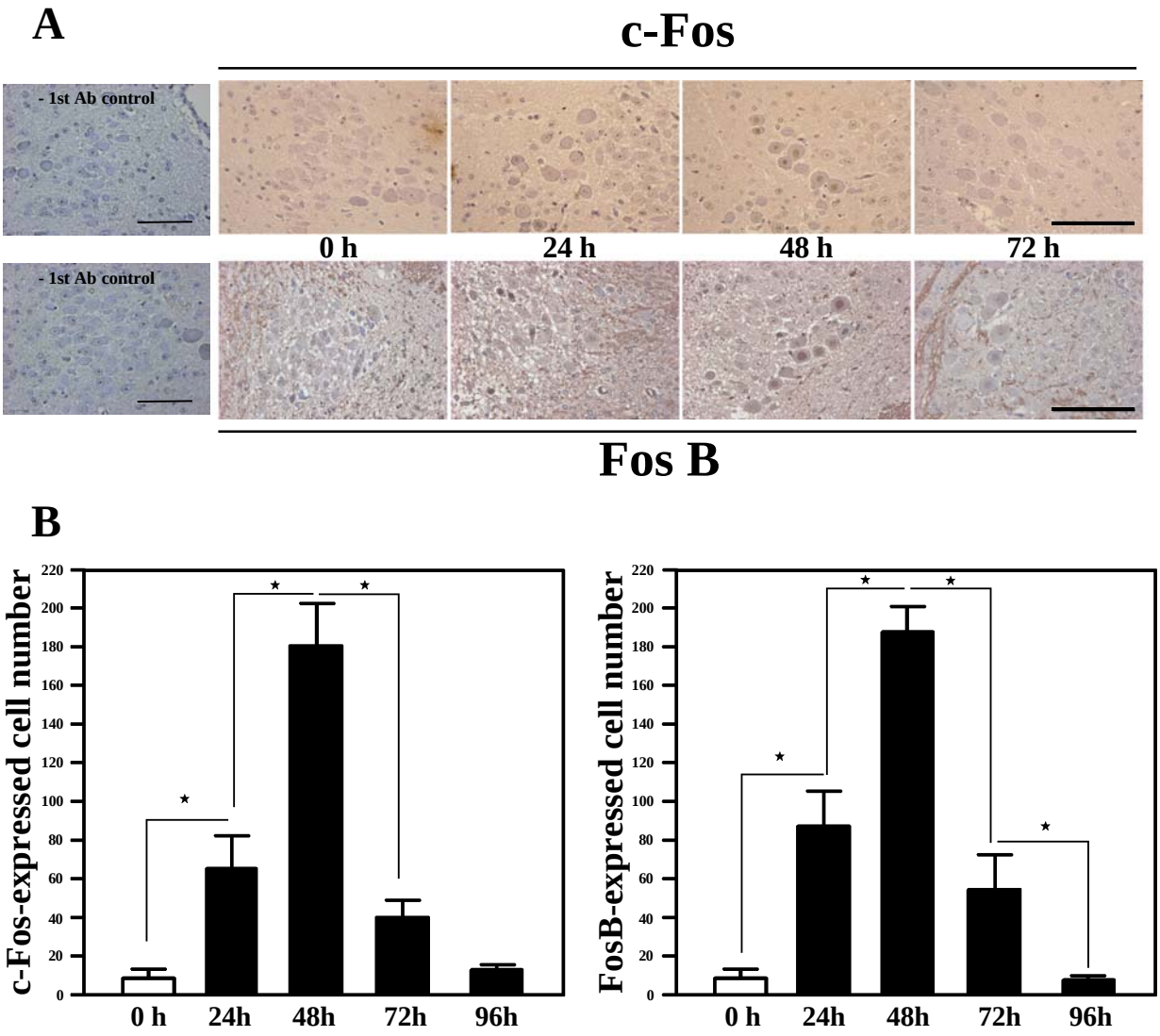

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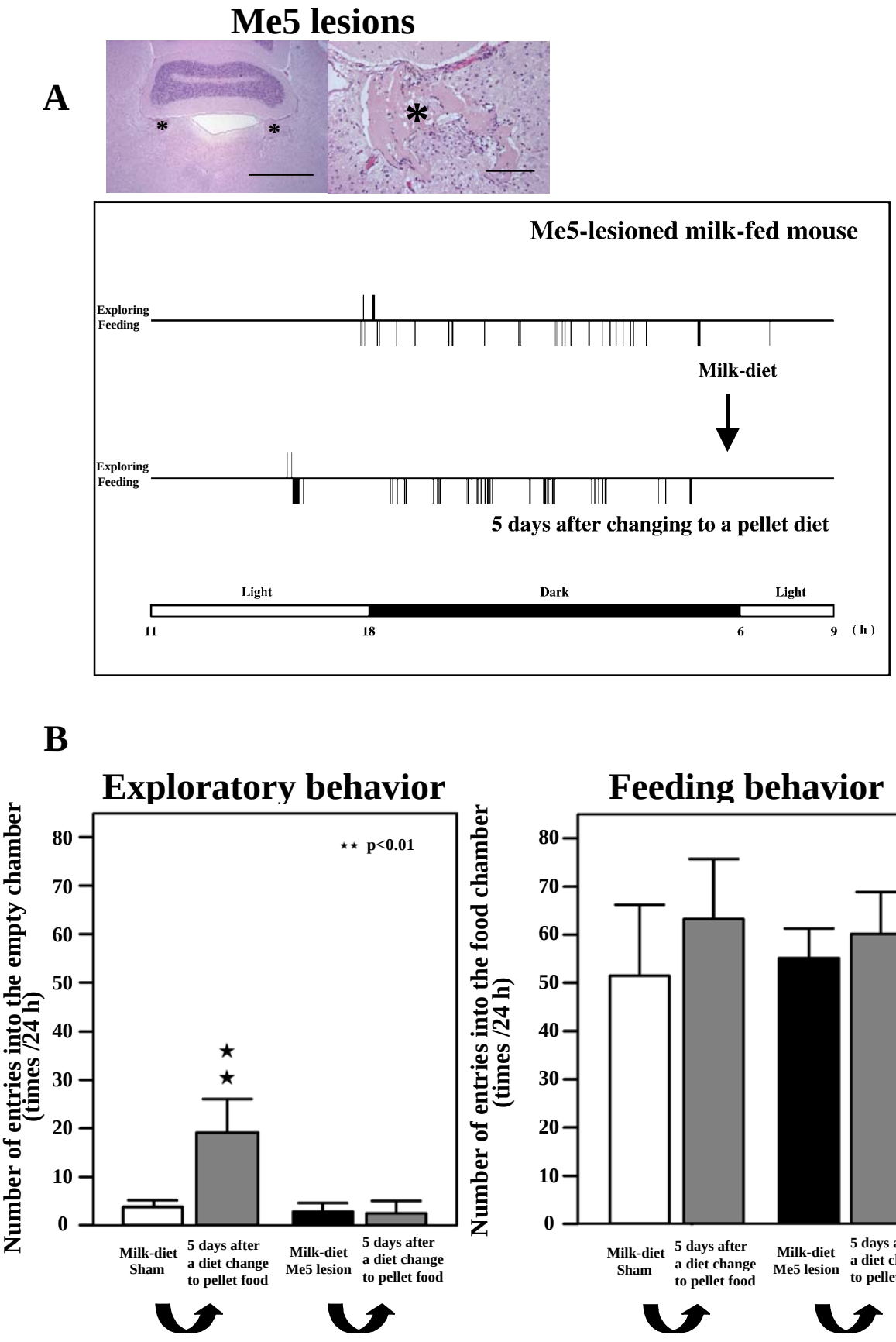

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