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Intracranial mast cells contribute to the control of social behavior in male mice

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ABSTRACT

Mast cells (MCs) exist intracranially and have been reported to affect higher brain functions in rodents. However, the role of MCs in the regulation of emotionality and social behavior is unclear. In the present study, using male mice, we examined the relationship between MCs and social behavior and investigated the underlying mechanisms. Wild-type male mice intraventricularly injected with a degranulator of MCs exhibited a marked increase in a three-chamber sociability test. In addition, removal of MCs in Mast cell-specific Toxin Receptor-mediated Conditional cell Knock out (Mas-TRECK) male mice showed reduced social preference levels in a three-chamber sociability test without other behavioral changes, such as anxiety-like and depression-like behavior. Mas-TRECK male mice also had reduced serotonin content and serotonin receptor expression and increased oxytocin receptor expression in the brain. These results suggested that MCs may contribute to the regulation of social behavior in male mice. This effect may be partially mediated by serotonin derived from MCs in the brain.

1. Introduction

Mast cells (MCs) originate from hematopoietic stem cells and play an important role in allergic reactions and immune responses in the peripheral system [1–3]. MCs circulate in the blood as immature cells and are terminally differentiated in the tissue where they reside [4,5]. In humans and rodents, including mice, MCs are known to exist not only in peripheral tissue but also in intracranial tissue. In intracranial tissue, MCs are present in the dura mater, the pia mater, the ventricles around the hippocampus, and the area surrounding the hypothalamus; however, intracranial MC numbers depend on the animal species [6–9]. MCs contain many mediators, such as monoamines, cytokines, and growth factors [10]. MC activation induces degranulation and the release of cellular contents, thereby modulating blood-brain barrier permeability and affecting neuronal activity [11]. Furthermore, brain MCs regulate central nervous inflammation caused by microglial activation [12].

The population of intracranial MCs has been reported to be altered by stress conditions or various stimuli [13–16]. MCs have been implicated in a variety of emotional behaviors in previous studies using various experimental models, such as MC-deficient or MC-overexpressing mice, and pharmacological manipulation of intracranial MC levels. MC-deficient $Kit^{W-sh/W-sh}$ (Wsh/Wsh) mice showed alterations in anxiety-like behavior, and cromolyn (disodium cromoglycate), an MC stabilizer, injected into wild-type mice intracerebroventricularly (i.c.v.) showed similar effects [17]. We have also reported that MC-deficient $Kit^{W/W-v}$ (W/Wv) mice exhibited alterations in anxiety-like behavior and depression-like behavior [18]. In addition, some researchers have indicated that monoamines released from brain MCs, such as serotonin or histamine, affect higher brain function. For example, serotonin derived from brain MCs affects learning and memory in the hippocampus [6], and histamine residing in intracranial MCs contributes to the sleep-wake system [18].

Brain monoamine levels have been reported to play a key role in controlling social behavior [19–21]. Modulation of brain serotonin levels or manipulation of serotoninergic neurons can regulate social behaviors, and dopamine levels or dopaminergic neurons can also regulate social behaviors. For example, optogenetic activation or inhibition of target cells, such as serotoninergic or dopaminergic neurons, can alter social behaviors [22–25]. In addition to monoamines, oxytocin and oxytocin receptors have been reported to be involved in social behavior [22–28], and central MCs may also regulate social behavior in association with oxytocin since peripheral MCs express oxytocin

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receptors on the surface of the cell [29].

However, the role of monoamines derived from MCs in social behavior is unknown, although cromolyn treatment suppresses restraint stress-induced behavior, including social behavior [30]. Furthermore, prenatal allergen exposure increases MC activation and thereby affects social behavior, including sexual behavior, in adult mice [31]. Thus, MCs are likely involved in the regulation of social behavior, but the mechanisms underlying this relationship remain unknown. Therefore, we investigated the role of MCs in emotional behavior, including social behavior, using a new model of MC-deficient mice in which MCs can be selectively depleted by administration of diphtheria toxin (DT) [32].

2. Materials and methods

2.1. Animals

The experimental mice consisted of C57BL/6 male mice (Japan SLC, Shizuoka, Japan) and mast cell-specific enhancer-mediated Toxin Receptor-mediated Conditional cell Knockout (Mas-TRECK) mice [32] aged 8∼10 weeks at the beginning of the experiment. Mas-TRECK mice were provided by Dr. M. Kubo (RCAI, RIKEN, Yokohama, Japan). Food and water were available *ad libitum*. A 24 -h light-dark cycle (lights on for 12 h, off for 12 h) was maintained throughout the study (lights on at zeitgeber time (ZT) = 0 at 08:00 am). Room temperature was maintained at 24 ± 1 °C throughout the experiment. The Animal Study Committee of Tokushima University approved these experiments (License No. T30−68), and we performed them in accordance with Guidelines for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

2.2. Pharmacological treatments and injection procedure

Cannulas were implanted i.c.v. at the time of the surgery. A guide cannula (25 gauge) was implanted at 0.5 mm posterior to the bregma, 1.2 mm lateral to the midline, and 2.0 mm below the surface of the skull into the lateral ventricle through stereotactic surgery [33]. Screw electrodes were used to fix the cannula to the skull. To maintain the patency of the cannula, a 31-gauge dummy cannula was inserted and did not extend beyond the end of the guide cannula. Before the start of experimentation, a two-week recovery time was provided by individually caging the mice, thereby ensuring that their body weight and food intake reverted to normal. Cannula potency was carefully checked after 1 week of postsurgical recovery by checking the water intake subsequent to i.c.v. angiotensin II (100 ng/μl; Sigma-Aldrich, St. Louis, MO, USA) injection in each mouse. The mice used in the experiments were only those that showed an immediate drinking response after injection. Compound 48/80 (C48/80; 10 μg dissolved in 1.0 μl saline; Sigma-Aldrich), an MC degranulator, cromolyn sodium salt (cromolyn; 30 μg dissolved in 1.0 μl saline; Sigma-Aldrich), an MC stabilizer, or vehicle (saline) was injected i.c.v. slowly over the course of 1 min using a Hamilton microsyringe. We evaluated social behavior using a three-chamber sociability test, and each drug was administered 30 min before habituation to the testing environment.

2.3. DT treatment

The Mas-TRECK mouse was newly established as a selective MC-deficient mouse [32]. The TRECK system relies on the fact that the mouse DT receptor (DTR) binds DT very weakly compared to the human DTR. Therefore, MCs can be conditionally depleted in a selective manner by injecting DT into Mas-TRECK mice.

DT was diluted in phosphate-buffered salts (PBS) according to a reference paper [32]. Mas-TRECK mice and wild-type (WT) mice were intraperitoneally (i.p.) injected with DT (Calbiochem, San Diego, CA) (250 ng/250 μl per mouse) or PBS on 5 consecutive days. After DT administration, we conducted sampling. Behavioral tests were conducted within 14 days as a previous paper reported that the duration of MC depletion was at least approximately 14 days. Each group was given PBS or DT *via* intraperitoneal injections for 5 consecutive days.

2.4. Behavioral testing

2.4.1. Three-chamber sociability test (3-CST)

The 3-chamber box (42 × 60 × 25 cm) was made of acrylic and each chamber measured 42 × 20 × 25 cm. In the first session, the test mice were placed in the corner of the center chamber to habituate to the 3 chambers and two empty cups for 10 min. In the second session (sociability test), novel male mice of the same age were placed into the cup in the right chamber, and the social behavior of the test mice in the 3-chamber was monitored for 10 min. The novel mice were habituated to the cup before the sociability test. The 3-chamber box was cleaned with 70 % ethanol between sessions, and after each test mouse, the two cups were also changed. The total distance traveled in the 3-chamber box and the time spent in each chamber zone were recorded using an automated image analysis system (Ohara & Co., Ltd., Tokyo, Japan) derived from ImageJ (National Institutes of Health, Bethesda, MD, USA). The time spent in the contact zone was analyzed using recorded video. The contact zone was considered to be a 2-cm distance from each cup, and the time spent in the contract zone was manually scored to determine the amount of time that the test mouse spent in the contact zone for both the novel mouse cup and the empty cup. The social preference level was calculated as [contact novel mouse cup – contact empty cup] / [contact novel mouse cup + contact empty cup].

2.4.2. Open field (OF) test

The chamber was an open-top box (45 × 45 × 30 cm) made of acrylic. The test mice were placed in the chamber and allowed to habituate for 10 min before the test. The locomotor behavior and anxiety-like behavior of the mice in the chamber were monitored for 10 min. The OF chamber was cleaned with 70 % ethanol between sessions and after each test mouse. The total distance traveled and time spent in the central (30 % of the box) and peripheral areas were recorded using an automated image analysis system (Ohara & Co., Ltd., Tokyo, Japan).

2.4.3. Light-dark transition (LD) test

A chamber (21 × 42 × 25 cm) divided equally into two compartment boxes was used for the LD test. The light box consisted of a white floor, walls and lid. The dark box consisted of a black floor, walls and lid and was completely enclosed except for a small door (4 × 8 cm) opening to allow movement between the dark box and the light box. The mice were placed in the dark box, and the amount of time spent in each box and the number of transitions were recorded for 10 min and analyzed automatically (Ohara & Co., Ltd., Tokyo, Japan). The chambers were cleaned with 70 % ethanol between sessions and after each test mouse.

2.4.4. Elevated plus maze (EPM) test

The mice were placed in the closed arm of an apparatus consisting of 2 opposing runways. One of the runways consisted of the closed arms (5 × 30 cm) of the maze and had walls (15-cm high). The other runway consisted of the open arms (5 × 30 cm) of the maze and had no walls. The maze was elevated to 140 cm above the floor. The behavior of the mice in the maze was monitored for 10 min. The time spent in the open arms was recorded and automatically analyzed (Ohara & Co., Ltd., Tokyo, Japan). The apparatus was cleaned with 70 % ethanol between sessions and after each test mouse.

2.4.5. Forced swimming test (FST)

The mice were subjected to swim sessions in individual acrylic cylinders (20-cm diameter × 40-cm height) containing water, 15 cm deep, at 25 °C. The mice were tested for 7 min, and immobility was measured during the last 5 min of the test. The total freeze time was recorded and analyzed using an automated image analysis system (Ohara & Co., Ltd.,

Tokyo, Japan). The water was changed after each test mouse.

2.4.6. Tail suspension test (TST)

The mice were exposed to the TST in which the tips of their tails were fixed with adhesive tape to wires that dangled from the ceiling. The mice were tested for 7 min, and immobility was measured during the last 5 min of the test. The percentage of time that the mice spent immobile was measured for 5 min, and decreased immobility time indicated reduced depressive-like behavior. Data were recorded using an automated image analysis system (Ohara & Co., Ltd., Tokyo, Japan).

2.4.7. Marble burying test

The box (25 × 40 × 17 cm) was made of acrylic and filled with flat bedding 5 cm deep. In the habituation session, the mice were placed in the corner of the box and explored without marbles for 10 min. In the test session, 24 marbles were arranged approximately 4 cm apart on the bedding. The compulsive-like behavior of the mice in the box was monitored for 30 min. The number of marbles covered with bedding (to 2/3 their depth) was counted.

2.4.8. Grip strength test

The mice gripped a wire. The grip strength of each mouse was measured 3 times using IMADA digital force gauges. The strength presented was the average calculated from 3 tests.

2.4.9. Wire hanging test

The mice were placed on a wire mesh surface. The wire mesh (21 × 15 cm) was made of iron. The wire hanging test was monitored for 3 min. The time to fall was recorded and presented as the mean latency.

2.5. High-performance liquid chromatography (HPLC)

Sampling was performed 24 h after DT administration for molecular analysis (HPLC and real-time RT-PCR), and the whole brain was removed and dissected into 9 regions (medial prefrontal cortex, lateral septum, nucleus accumbens, thalamus, hypothalamus, hippocampus, amygdala, cerebellum and brain stem). All brain tissues were frozen in liquid nitrogen and stored at −80 °C until use. The samples were weighed and homogenized in a buffer of 0.2 M perchloric acid containing 100 μM disodium ethylene diamine tetra acetic acid (EDTA 2Na) (5 μl/mg tissue) and incubated for 30 min on ice. The samples were then centrifuged at 14,500 rpm at 4 °C for 15 min, and the supernatant was transferred to new tubes. All samples were adjusted to pH 3 with CH3COONa and filtered with a 0.2 μm filter (Minisart RC4 filter, Sartorius AG, Goettingen, Germany). A mobile phase composed of a mixture of 0.1 M citric acid and 0.1 M sodium acetate anhydrous (pH 3.9) containing 0.65 mM sodium 1-octanesulfonate, 13.4 μM EDTA 2Na, and MeOH (83:17 %, w/v) was used at a flow rate of 0.23 mL/min for analysis of the samples. Separation was performed using an SC-50DS column (3.0 × 150 mm, EICOM, Kyoto, Japan). Samples (30 μl) were injected onto the column, and norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and serotonin (5-HT) were detected using an electrochemical detector fitted with a graphite cell (WE-3 G, EICOM, Japan) set at +0.75 V *versus* the *in situ* Ag/AgCl reference electrode. Data were collected using PowerChrom software (ADInstruments, Australia). DOPAC + HVA/DA and 5-HIAA/5-HT ratios were used to estimate the metabolic ratio.

2.6. Real-time RT-PCR analysis

We used predesigned, gene-specific TaqMan probes and primer sets (Applied Biosystems, Foster City, CA) to investigate the expression of the following genes: MAO-A (monoamine oxidase A, Mm00558004_m1), Tph2 (tryptophan hydroxylase 2, Mm00557715_m1) Slc6a4 (solute carrier family 6 member 4, Mm00439391_m1), 5htr1a (5-hydroxytryptamine receptor 1A, Mm00434106_s1), 5htr1b (5-hydroxytryptamine receptor 1B, Mm00439377_s1), and oxytocin receptor (Mm01182684_m1). Real-time RT-PCR was carried out using an Applied Biosystems 7900 H T real-time RT-PCR system and TaqMan universal PCR Master Mix (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. We normalized the values to those for the housekeeping gene actin-beta (Mm02619580_g1) for endogenous quantity control.

2.7. Tissue processing

For MC staining, brains were postfixed in 4% paraformaldehyde (Nacalai Tasque, Kyoto, Japan) overnight after the mice were perfused. After overnight incubation, the solution was replaced with 30 % sucrose solution. The brains were cut into 40-μm-thick coronal sections on a cryostat (Leica CM1850 UV, Leica Microsystems, Inc., Buffalo Grove, IL). The sections were stored at 4 °C in 0.05 % sodium azide/PBS until processing.

2.8. MC staining

All sections were stained with toluidine blue (TB) (Sigma, St. Louis, MO). TB-stained brain MCs were analyzed in every section taken throughout the brain, starting approximately 2.0 mm posterior of bregma to approximately 4.0 mm posterior of bregma (bregma -2.06 mm to -4.06 mm). Fifty sections were counted for each brain. The dura mater was softly removed from the skull and stained with TB. Regarding the TB staining, slides were washed in water for 5 min, before staining with TB (4 mg/ml TB in 2% NaCl, pH 2.3∼2.5), washed briefly in distilled water 3 times, dehydrated through a series of ethanols (2 × 95 % for 10 s, 100 % for 15 s) and xylene for 3 min, and coverslipped with a soft mount (Wako pure chemical industry, Ltd., Osaka, Japan).

2.9. Statistical analysis

The results are as the means ± SE (standard error). Statistical analysis was carried out using the software package SPSS (SPSS Inc., Chicago, IL, USA). Regarding drug administration to WT mice, behavioral test results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test and Student's *t*-test. In the behavioral tests, these results were analyzed by two-way ANOVA followed by Tukey's post hoc test and Student's *t*-test. MC counts were analyzed using one-way ANOVA followed by Tukey's post hoc test. Monoamines and real-time RT-PCR data were analyzed using Student's *t*-test. $P < 0.05$ was assumed to indicate statistical significance.

3. Results

3.1. Behavioral effects of MCs on social behavior after compound 48/80 or cromolyn injection into the brains of wild-type (WT) mice

We investigated the effect of manipulating brain MCs by degranulation (compound 48/80) or stabilization (cromolyn) drugs on social behavior using WT mice. In the three-chamber sociability test, the compound 48/80-injected group showed increased total distance ($F_{2, 27} = 5.447$, $p = 0.008$ for saline *vs* compound 48/80) and social preference levels compared to the saline group and cromolyn-injected group ($F_{2, 27} = 12.322$, $p = 0.028$ for saline *vs* compound 48/80, $p < 0.001$ for compound 48/80 *vs* cromolyn) (Fig. 1b–d). The cromolyn-injected group exhibited similar results to the control group. However, no differences in the number of entries were observed (Fig. 1a). Our results indicated that overactivation of brain MCs elevated social preference levels (Fig. 1d) and that brain MCs were involved in the regulation of social behavior. To prove these findings more conclusively, we next used transgenic mice, which were the Mas-TRECK mice.

We also conducted an additional experiment with intraperitoneal

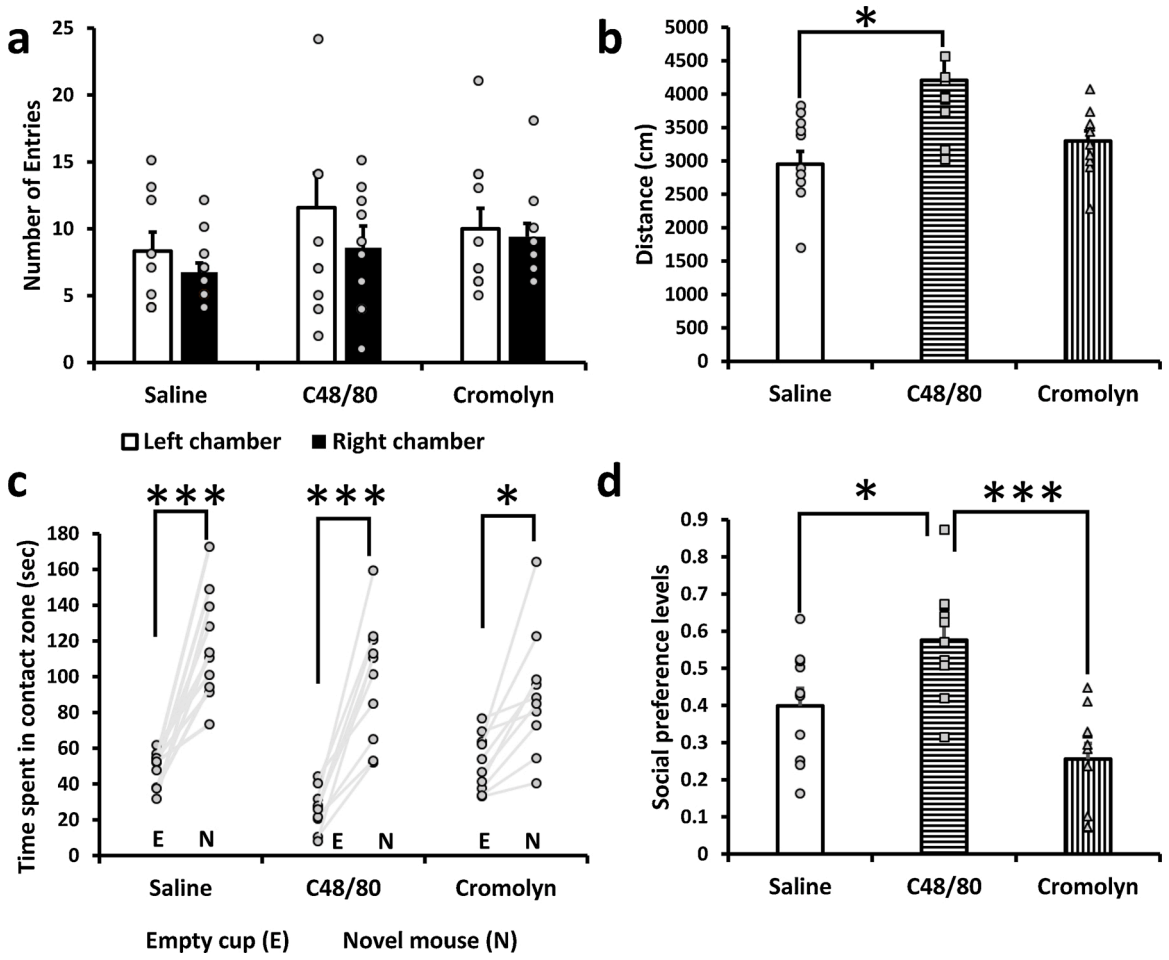

Fig. 1. Effect of i.c.v. reagent administration to wild-type (WT) mice in the three-chamber sociability test. The WT mice were injected with i.c.v. saline, 10 μg/μl compound 48/80 (C48/80) and 30 μg/μl cromolyn. Number of entries to right or left chamber (a), total distance traveled during the test (b), time spent in either the contact zone of the empty cup (E time) or the contact zone of the novel mouse cup (N time) (c), social preference levels (N time - E time) / (N time + E time) (d). All data are shown as the means ± SE (n = 10/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, analyzed by one-way ANOVA followed by Tukey's post hoc tests (b, d) and Student's *t*-test (a, c).

injection of compound 48/80 to investigate behavioral changes caused by systemic degranulation. We performed a three-chamber sociability test similar to the test performed when WT mice were injected i.p. (Supplemental Fig. 1). The time spent in the contact zone was longer in compound 48/80-injected mice than in vehicle-injected mice, suggesting that systemic degranulation of MCs tends to increase social interaction behaviors, similar to the results seen when the reagent was administered i.c.v. However, the social preference level was not statistically significant.

3.2. Depletion of systemic MC after DT administration in Mas-TRECK mice

We confirmed that the Mas-TRECK system can selectively remove MCs by the administration of DT. We injected DT (250 ng/mouse) intraperitoneally (i.p.) for 5 consecutive days into the Mas-TRECK mice or WT mice, the expression of MCs was investigated using TB staining (Fig. 2a). In the peripheral nervous system, *i.e.*, in the ears of the DT-injected Mas-TRECK mice, MCs were completely depleted ($F_{3,\ 8} = 22.866$, $p < 0.001$) (Fig. 2b). In the central nervous system, MCs exist in the meninges (the dura mater and pia mater) and brain, including the ventricles, in WT mice. The MC pictures in Fig. 2 show the granulation status. In the area from approximately 2.0 mm posterior to the bregma to approximately 4.0 mm posterior to the bregma (bregma -2.06 mm to -4.06 mm), we found that MCs are present mainly in the ventricles around the hippocampus. In the dura mater of the DT-injected Mas-TRECK mice, MCs were completely depleted ($F_{3,\ 8} = 5.081$, $p = 0.035$ for WT DT *vs* Mas-TRECK DT) (Fig. 2c). In the brain, MCs were significantly decreased in the DT-injected Mas-TRECK mouse group (MC-depletion group) compared to the control group ($F_{3,\ 8} = 6.241$, $p = 0.019$ for WT DT *vs* Mas-TRECK DT) (Fig. 2d). Since the number of MCs can be recovered after a certain period, we investigated MC recovery at 45 days and 60 days after DT injection. Forty-five days after DT injection, MC expression was equivalent to that of the control group in the brain (Fig. 2d). However, the expression of MCs in both the ears and dura mater was not recovered (Fig. 2b–c). Sixty days after DT injection, MC expression recovered in the dura mater of the MC-depletion group; however, it did not recover in the ear (Fig. 2b–c).

3.3. Monoamine content and gene expression after DT administration in the brains of Mas-TRECK mice

We investigated the monoamine content and gene expression in several brain regions of the Mas-TRECK mice (Fig. 3a). The DA content in the lateral septum increased in the MC-depletion group compared to the control group ($t_{13} = -2.819$, $p = 0.015$) (Fig. 3b). In the hypothalamus, the DA content tended to decrease, while DA turnover tended to increase in the MC-depletion group (Supplemental Fig. 2). The 5-HT content significantly decreased in several regions, such as the thalamus ($t_{13} = 2.356$, $p = 0.035$) and cerebellum ($t_{13} = 4.715$, $p < 0.001$),

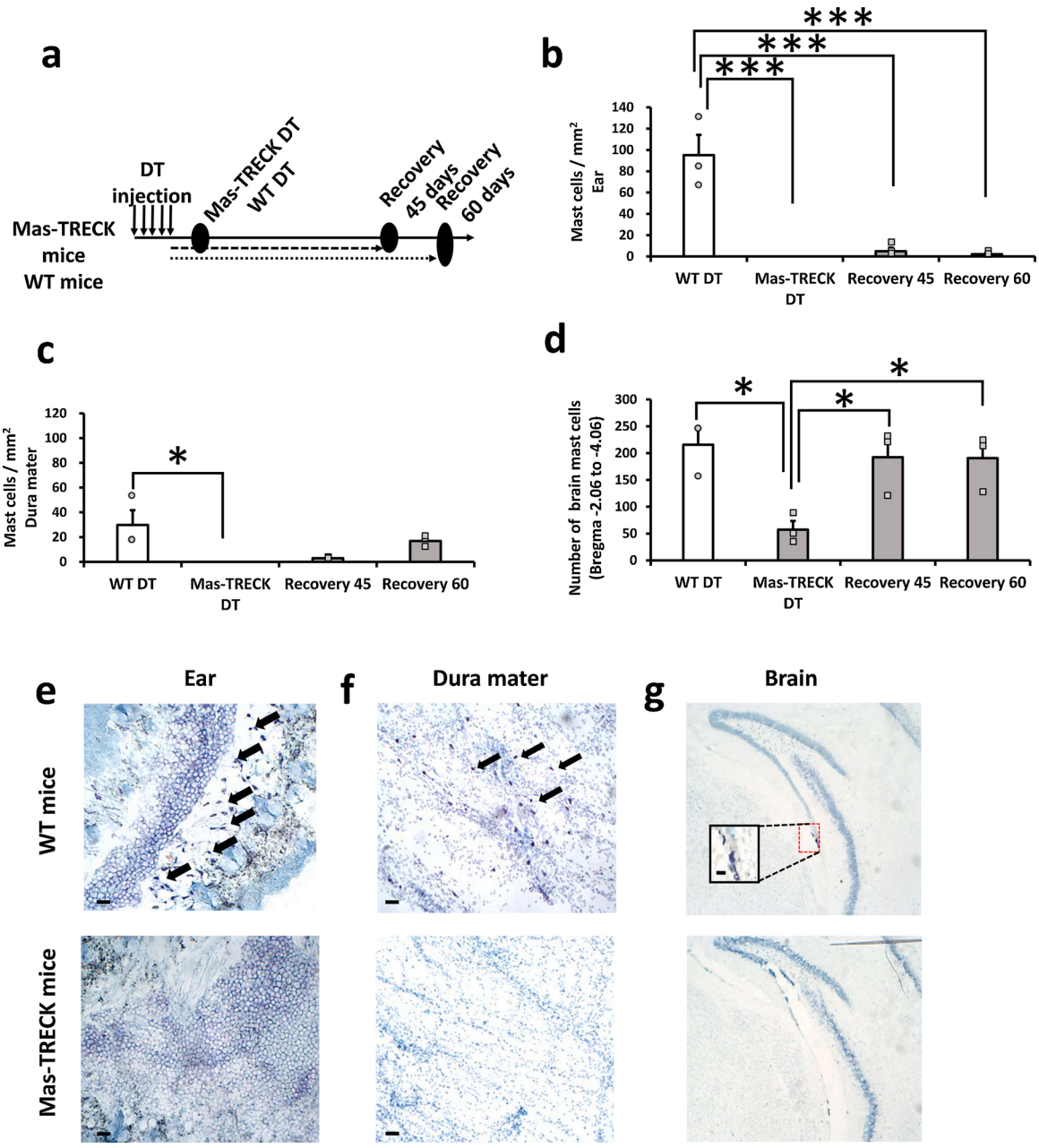

Fig. 2. Confirmation of the presence of mast cells using toluidine blue staining in intracranial and peripheral tissues of wild-type (WT) and Mas-TRECK mice injected with diphtheria toxin (DT). These samples were collected at three time points: at the end of the DT injections and 45 (Recovery 45) or 60 (Recovery 60) days after 5 consecutive days of DT injections (a). Numbers of mast cells per ear area (b), dura mater area (c), and serial section (50 sections per mouse) of brain (bregma -2.06 mm to -4.06 mm) around the hippocampus (d). All data are shown as the means ± SE (n = 3/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, analyzed by one-way ANOVA followed by Tukey's post hoc tests.
Arrows show the distribution of mast cells. Black scale bars indicate 100 μm (ear) (e), 100 μm (dura mater) (f), and 40 μm (brain) (g). The small black box shows mast cells in the brain.

and tended to decrease in the hypothalamus of the MC-depletion group ($t_{13} = 2.062$, $p = 0.060$) (Fig. 3c). Moreover, 5-HT turnover in the MC-depletion group increased in the nucleus accumbens ($t_{13} = -2.994$, $p = 0.010$), thalamus ($t_{13} = -2.554$, $p = 0.024$) and cerebellum ($t_{13} = -3.345$, $p = 0.005$) (Fig. 3d). NE content showed no significant differences between the control and MC-depletion groups (data not shown).

Regarding the gene expression related to monoamine metabolism and social behavior, the mRNA levels of the 5-HT1b receptor in the nucleus accumbens decreased in the MC-depletion group compared to the control group ($t_{13} = 2.797$, $p = 0.015$), and the mRNA levels of the 5-HT1a receptor in the medial prefrontal cortex also tended to decrease in the MC-depletion group ($t_{10} = 2.158$, $p = 0.056$) (Fig. 3e and f). In the thalamus, the mRNA levels of MAO-A increased in the MC-depletion group, although there were no differences in 5-HT transporter (Slc6a4) or synthase (Tph2) mRNA expression (Fig. 3g, h and i). In the amygdala and hippocampus, no differences in mRNA levels related to monoamines or their metabolites were noted. In the thalamus ($t_{13} = -2.537$, $p = 0.025$) and hypothalamus ($t_{13} = -2.379$, $p = 0.033$), the mRNA levels of the oxytocin receptor increased in the MC-depletion group (Fig. 3j).

3.4. Behavioral changes after DT administration in Mas-TRECK mice

We evaluated muscle strength, locomotor activity, anxiety-like

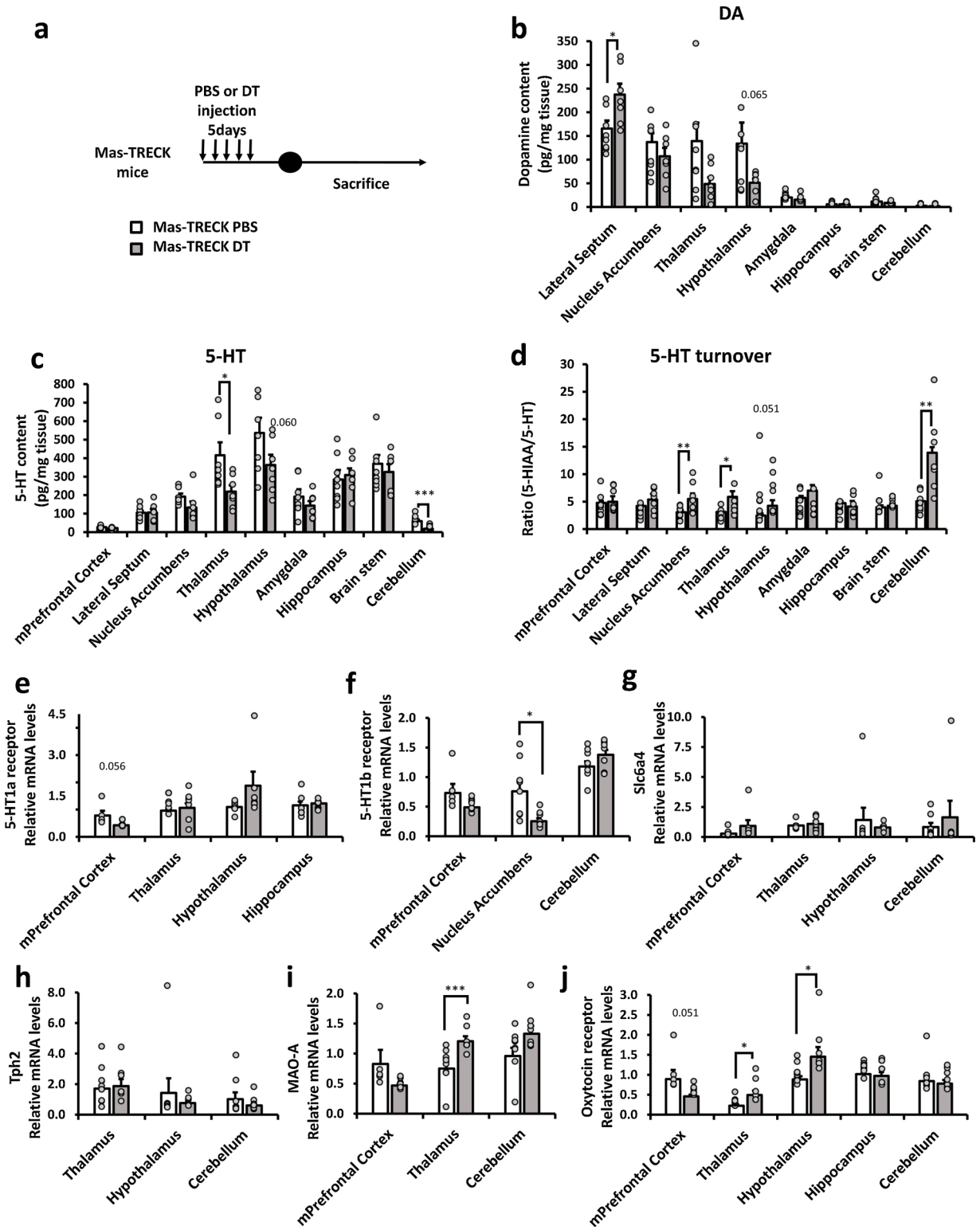

Fig. 3. Monoamine content and mRNA expression in several areas of the brain of Mas-TRECK mice injected with PBS or DT. These mice were sacrificed after 5 consecutive days of DT injections (a). Brain levels of dopamine (DA) (b), serotonin (5-HT) (c), 5-HT turnover (5-HIAA/5-HT) (d), 5-HT 1a receptor (e), 5-HT 1b receptor (f), 5-HT transporter (Slc6a4) (g), tryptophan hydroxylase 2 (Tph2) (h), monoamine oxidase A (MAO-A) (i), and oxytocin receptor (j). All data are shown as the means ± SE (n = 7-8/group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, analyzed by Student's *t*-test in the PBS- *versus* DT-injected Mas-TRECK mice.

behavior, depression-like behavior and obsessive-compulsive-like behavior by behavioral tests, including the wire hanging test, grip strength test, OF test, LD test, EPM test, FST, TST, and marble burying test using WT mice and Mas-TRECK mice (Fig. 4a). However, there were no differences in these behavioral tests among the 4 groups (WT mice and Mas-TRECK mice injected with PBS or DT) (Fig. 4b-e, Supplemental Fig. 3). Next, we investigated social behavior using a three-chamber sociability test. The MC-depletion group showed a significant decrease in social preference level compared to WT mice ($F_{3,\ 26} = 6.179$, $p = 0.006$) (Fig. 5c). Sixty days after DT injections, when the number of MCs

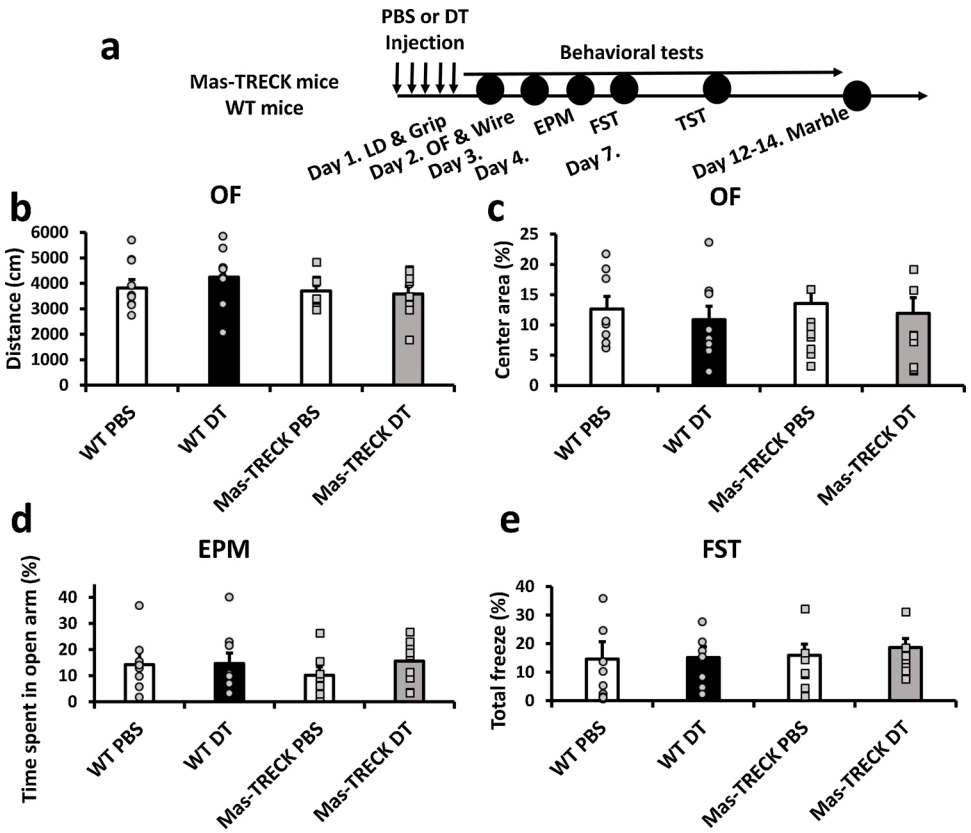

Fig. 4. Wild-type (WT) and Mas-TRECK mice injected with PBS or DT. Behavioral tests performed within 14 days after 5 consecutive days of DT injections (a). Total distance during open field (OF) test (b), percentage of time spent in the center area in the OF (c), percentage of time spent in the open arms in elevated plus maze (EPM) (d), and percentage of freezing time during forced swim test (FST) (e). All data are shown as the means ± SE (n = 9/group) and analyzed by two-way ANOVA followed by Tukey's post hoc tests.

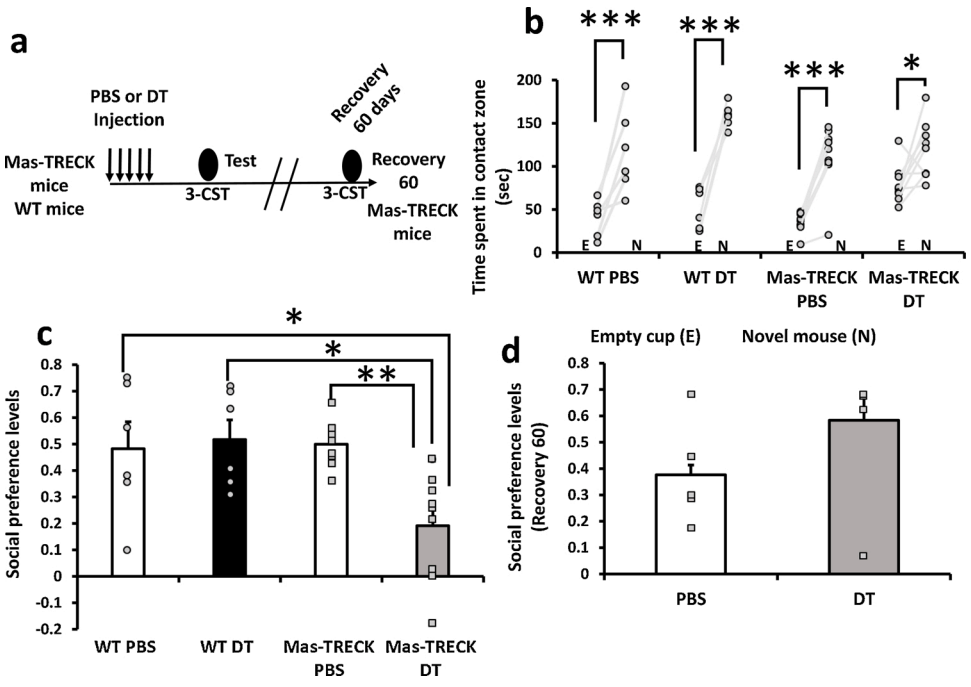

Fig. 5. Effects of mast cell depletion in Mas-TRECK mice in the three-chamber sociability test (3-CST). Wild-type (WT) and Mas-TRECK mice injected with PBS or DT. Behavioral tests were performed after PBS or DT injection, and only Mas-TRECK mice were retested in the three-chamber sociability test 60 days (Recovery 60) after 5 consecutive days of DT injections (a). Time spent in either the contact zone of the empty cup or the contact zone of the novel mouse cup (b) and social preference levels (c). Social preference levels in Mas-TRECK mice 60 days after DT administration (d). All data are shown as the means ± SE [n = 6-9/group in (b) and (c), n = 5/group in (d)]. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, analyzed by two-way ANOVA followed by Tukey's post hoc tests (c) and Student's *t*-test (b, d).

in the brain had recovered, the social preference level in Mas-TRECK mice had returned to the levels observed in the control group (Fig. 5d). These results suggested that the Mas-TRECK mice exhibited changes in social behavior without changes in anxiety-like and depression-like behavior.

4. Discussion

In the current study, we examined the relationship between MCs and social behavior using only male mice. The interpretation of experimental results is more complicated in female mice because their emotional and social behaviors differ depending on the menstrual cycle. Therefore, this study has the limitation that the results apply only to

males and different results would be obtained when targeting females. Since there may be sex differences in the effects of MCs on brain function, studies using female mice should also be carried out in the future. As a main finding in our experiments, WT mice injected i.c.v. with an MC degranulator exhibited a marked increase in social behavior, while the MC-depletion group showed social deficits without anxiety-like and depression-like behavior.

Recent reports have indicated that MCs were involved in social behavior. For example, acute stress induced a decrease in social interaction, but these behavioral changes were ameliorated by MC stabilizers [30]. In addition, early life allergic exposure modulated sociosexual behavior in later life by underlying effects on brain-resident MCs [31]. However, these experiments involved exposure to stress or allergens, and the role of MCs themselves in the regulation of social behavior and the underlying mechanisms have not been revealed. Therefore, in our experiments, an MC stabilizer or MC degranulator was injected into the brains of WT mice to confirm the direct involvement of brain-resident MCs in social behavior. In our three-chamber sociability test, the results showed that social behavior was enhanced in mice centrally injected with an MC degranulator. These mice were less interested in the empty cups, so the social preference levels were higher than those of the controls and mice administered the MC stabilizer (Fig. 1). These results suggest that, overactivation of brain MCs may cause excessive social behavior. Although the exact percentage of MCs that have been degranulated is unknown at this point, our previous study showed that i.c.v. administration of compound 48/80 increased histamine release in WT mice by 250 % compared to baseline [18]. This result indicates that the degranulation agent used in this study sufficiently activates MCs.

On the other hand, systemic degranulation of MCs also tended to increase social interaction behaviors, although it did not reach statistical significance (Supplemental Fig. 1). Systemic degranulation of MCs causes allergic reactions and various immune reactions, so more careful consideration may be required for evaluating effects on social behavior. We are now planning to investigate whether MCs in the brain or periphery contribute more to the regulation of social behavior by administering PEGylated diphtheria toxin, which does not cross the blood-brain barrier, to Mas-TRECK mice [34]. Thus, MC homeostasis (probably mainly intracranial MC homeostasis) may regulate social behavior even without exposure to stress or allergens.

Previous experiments using MC-deficient Kit mice reported that these mice had higher anxiety-like behavior than WT mice [17]. However, Kit mice do not innately express MCs, and whether these results were direct effects of MCs is unclear because c-kit is nonspecific for MCs expressed in the brain. In this study, the Mas-TRECK mice that we used involve the specific and conditional removal of MCs in mice by administering DT. We confirmed that MCs were eliminated in the brain and periphery of Mas-TRECK mice after the administration of DT (Fig. 2). Regarding the results of the behavioral tests, the MC-depletion group showed no changes in anxiety and depression levels. On the other hand, these MC-depleted mice exhibited a social deficit (Fig. 5c). Sixty days after DT injection, the Mas-TRECK mice showed recovered intracranial MC numbers, while the number of peripheral MCs had not yet recovered in 60 days (Fig. 2b). Regarding the difference in the recovery period between brain and peripheral MCs, the number of brain MCs after the DT injections was not completely depleted, and it is possible that recruitment from the periphery may have occurred. During the 60 days after the DT injections, social preference levels in the three-chamber sociability test also recovered (Fig. 2, Fig. 5d). These results suggested that the social deficits observed in the MC-depletion group was caused by the depletion of intracranial MCs rather than peripheral MCs. Since the MC lifespan is reported to be approximately 40 days to 6 months [35], our results showed that the MC number started to recover 40–60 days after the complete removal of MCs (Fig. 2). Combined with the results of the pharmacological experiments mentioned above, our results suggested that intracranial MCs and granules in the intracranial MCs are certainly involved in the regulation of social behavior (Fig. 1, Fig. 5).

To study the mechanisms of MC-mediated social behavior, we analyzed the monoamine content and related mRNA expression. MCs store a number of different chemical mediators, including histamine, interleukins, serotonin, and dopamine [10,36]. Among them, serotonin is particularly known to be involved in social behavior [37]. However, almost all reports examining the relationship between 5-HT and social behavior refer to 5-HT from serotonin neurons in the raphe nucleus [37,38]. For example, activation of dorsal raphe neurons using optogenetics increases sociability in a three-chamber sociability test [39]. In the present study, the 5-HT content in the MC-depletion group was reduced in the thalamus (Fig. 3c), where MCs mainly exist in the brains of humans and rodents (Supplemental Fig. 4) [6,8,9]. The 5-HIAA content, a serotonin metabolite, was not changed (Fig. 3), although the expression of MAO-A mRNA was increased in the thalamus (Fig. 3i). These results indicated that brain serotonin levels in the MC-depletion group were reduced by deficiencies in MCs in the brain. In addition, activation of the 5-HT1b receptor in the nucleus accumbens has been shown to rescue social deficits in an autism mouse model [39], and the 5-HT1a receptor plays a critical role in the normal development of social behavior in an autism mouse model [40]. Our results also indicated that 5-HT1b receptor expression in the nucleus accumbens was decreased (Fig. 3f) and that 5-HT1a receptor expression in the medial prefrontal cortex tended to be decreased (Fig. 3e) in the MC-depletion group, which also showed social deficits (Fig. 5c). Since mouse bone marrow-derived MCs present multiple 5-HT receptor mRNAs, including 5-HT1a and 1b [41], depletion of intracranial MCs may have affected the decreased expression of 5-HT1a and 1b receptors in our study (Fig. 3e and f). Combining these data, serotonin derived from intracranial MCs may be able to regulate social behavior in addition to the 5-HT derived from serotonergic neurons in the raphe nucleus.

In our study, we found some region-specific changes in DA and 5-HT content in the brain whether or not MCs were present. Therefore, region-specific changes in monoamine content or turnover in regions where MCs are absent are considered indirect changes caused by MC depletion. To solve this problem, we think it is necessary to use micro-dialysis to analyze region-specific monoamine release in the future. Furthermore, as previously reported [6,42], there are many times more MCs in the meninges than in the brain parenchyma. In fact, the mouse brain has only 200 MCs; however, the number of MCs in the dura mater of mice is at least twice that in the brain (Fig. 2c). The presence of MCs in the meninges may also regulate social behavior *via* neurotransmitters such as serotonin.

In addition to monoamines, oxytocin and oxytocin receptors also have been reported to be involved in social behavior [22,26]. Oxytocin receptor is known to express on the surface of MCs in the colon, although there are no reports on the relationship between intracranial MCs and oxytocin. Therefore, we first predicted that the expression of the oxytocin receptor would be decreased in MC-depleted mice. However, contrary to our expectations, the expression of the oxytocin receptor was increased in these mice in the thalamus and hypothalamus, although the medial prefrontal cortex tended to have lower expression (Fig. 3j). Although it is unclear why the oxytocin receptor showed such changes in MC-depleted mice, region-specific changes in the oxytocin receptor were also observed in autism model mice [43]. We are now investigating the mechanism underlying the alterations in oxytocin receptor expression in both MC-depleted animals and autism model mice.

In conclusion, the presence of intracranial MCs, including those in the meninges and brain, may contribute to social behaviors, and monoamines derived from MCs, such as serotonin, may control behavioral changes. However, these behaviors could also be affected by other factors, such as cytokines derived from MCs. In future studies, selective MC depletion in the brain parenchyma and meninges in the skull and the influences of sex and age must be evaluated. Our laboratory has previously reported that brain MCs are involved in sleep-wake behavior; furthermore, this study indicated that intracranial MCs control social behavior. Patients with mental illness have been reported to exhibit both

sleep disorders and social deficits. We hope that our findings will be useful in elucidating the pathological mechanisms of social deficits in mental or neurological disorders such as autism spectrum disorders.

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CRediT authorship contribution statement

Daisuke Tanioka: Conceptualization, Data curation, Funding acquisition, Investigation, Writing - original draft. **Sachiko Chikahisa:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing. **Noriyuki Shimizu:** Methodology. **Tetsuya Shiuchi:** Resources. **Noriaki Sakai:** Conceptualization, Methodology. **Seiji Nishino:** Writing - review & editing. **Hiroyoshi Séi:** Conceptualization, Project administration, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bbr.2021.113143.

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