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A novel semi-automated apparatus for measurement of aggressive biting behavior in mice

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HIGHLIGHTS

- We developed an ARM (Aggression Response Meter) for evaluations of aggressiveness in mice.
- Aggressive biting behavior (ABB) toward inanimate objects was used as a paradigm.
- ARM can detect aggressiveness in the early stages of psychiatric disorders in mice.
- ARM can be used for the evaluation of ABB in both male and female mice.
- ARM can measure ABB repeatedly using the same individual over a long period of time.

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ABSTRACT

Background: Currently, behavioral research of aggressiveness is often conducted with intraspecific inter-male aggression tests. Intraspecific aggression is not detectable in early stages of psychiatric disorders or in female animals, except during the nursing period.
New method: We developed a semi-automated apparatus (ARM: Aggression Response Meter) for measurement of aggressive biting behavior (ABB) in mice. The apparatus is loaded with computer-controlled sticks that stimulate the mouse through touch, inducing irritation and anger. When the mouse bites the sticks in anger, a load sensor attached to the sticks detects ABB dynamically. Changes in ABB were assessed with isolation-reared/re-socialized mice using the ARM, and additional isolation-reared mice were tested using both the ARM and the resident-intruder test, and then buspirone, a serotonin 1A receptor agonist, was administered.
Results: ABB significantly increased during isolation rearing, and then significantly decreased throughout the re-socialization period; both changes were time-dependent. The ARM also detected ABB of female mice after 3 weeks of isolation rearing. Buspirone significantly inhibited aggressive behavior in both tests in a similar manner.
Comparison with existing method: The ARM detects aggressiveness in psychiatric disorders at an earlier stage and in both male and female mice.
Conclusions: ABB toward inanimate objects is a reliable paradigm that makes it possible to detect aggressiveness in the early stage of psychiatric disorders. The ARM is useful for the quantification of aggressiveness using the same individual repeatedly, and for objective evaluation of the effects of drugs on aggressiveness. The ARM can be used with both male and female mice.

1. Introduction

Aggressiveness is a common symptom in patients with psychiatric disorders and developmental disorders. Behavioral assessment of aggressiveness in laboratory animals is essential for the analysis of aggression mechanisms and evaluation of the action of psychotropic drugs.

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Currently, behavioral research of aggressiveness is often conducted with intraspecific intermale aggression tests using one set of male laboratory animals, such as a resident-intruder test (Blanchard and Blanchard, 1977; Malkesman et al., 2006; Mineur et al., 2003; Mucignat-Caretta et al., 2004; Vergnes et al., 1986). Generally, in intraspecific intermale aggression tests, an intruder mouse is introduced into a resident home cage and the behavior of the mice is observed. Then, evaluations of aggressiveness are carried out based on behavioral paradigms including biting attacks, wrestling, tail rattles, lateral threats and/or latency until the first attack of the intruder (Ibi et al., 2008; Koike et al., 2009; Sakaue et al., 2001).

Intraspecific aggression tests can be used only for male laboratory animals, since females typically do not display aggression toward strangers unless they have pups (Svare and Gandelman, 1976). It is conceivable that intraspecific aggression is dependent on the dynamic state of the male hormones. Thus, it is necessary to establish a method to measure aggressive behaviors unrelated to sexual hormones, since many psychiatric diseases accompanied by aggressiveness are unrelated to sex.

Certain psychiatric animal models are known to attack inanimate objects that touch their body or that move in front of their eyes (Sofia, 1969; Tsuda et al., 1988; Uchida et al., 2009). Indeed, when an experimenter touches such an aggressive animal with a stick repeatedly in its home cage, the animal often attacks the stick by biting it. This behavior is observed not only in males, but also in females. Because this aggressive biting behavior (ABB) is hardly observed in normal laboratory animals, it is considered that ABB is one sign of a psychiatric disorder. In the present study, we focused on this aggressive behavior. We attempted to use ABB as a behavioral paradigm of aggression.

We developed a semi-automated apparatus for the measurement of ABB to assess aggressiveness in mice without an intruder mouse. The apparatus functions as a mechanical touch stimulator and an aggressive biting response detector. It is loaded with sticks to give light touch stimulations to a mouse, and then to induce irritation and anger. When the mouse bites the sticks in anger, the load sensor attached to the sticks detects ABB dynamically. To evaluate the capacity and reliability of the apparatus, we measured changes in ABB under stress induced by social isolation. We also examined the influence of repeated tests using the same individual animal, and evaluated the effect of an antipsychotic drug on ABB using the apparatus. Moreover, we also measured ABB of female mice following long-term social isolation.

2. Materials and methods

2.1. Apparatus

The semi-automated apparatus for measuring ABB in mice, which we developed, is called the Aggression Response Meter (ARM); a schematic and photograph are shown in Figs. 1 and 2, respectively. The outer frame (23 cm × 27 cm × 16 cm) contains a load sensor (Figs. 1-*1, 2A-*1: Tec Gihan Co. Ltd., Kyoto, Japan), a pair of metal sticks for applying light touch or visual stimulation to a mouse (Figs. 1-*2, 2A-*2), a stick-driving unit for moving the sticks (Figs. 1-*3, 2A-*3), and a drive-sliding unit with a pair of rails (Figs. 1-*4b, 2A-*4b) and a knob (Figs. 1-*4a, 2A-*4a) for shifting the stick-driving unit. A transparent, acrylic, cylindrical animal chamber (Figs. 1-*5, 2A-*5, 2B) is set on the top of the frame. All parts, including the software but excluding the load sensor, were custom-made (Muromachi Kikai Co. Ltd., Tokyo, Japan).

The animal chamber is 95 mm in length and 35, 40 or 45 mm in inner diameter. A pair of slits is present in the floor of the chamber at an interval of 11 mm (15 mm in center-to-center distance; Fig. 2B-*6). Each slit is 4 mm in width and 90 mm in length. A pair of metal sticks is set on the stick-driving unit perpendicular to the floor of the chamber just below the slits at an interval of 12 mm (15 mm in center-to-center distance). Each stick is 50 mm in length and 3 mm in diameter with a dome-shaped head. The stick-driving unit is controlled automatically with a computer and makes the sticks move in an up-and-down motion through the slits, which is associated with the application of a light touch or visual stimulation to the mouse in the chamber. The stick-driving unit is loaded on the drive-sliding unit in order to be shifted by manual operation to the right and left. When the mouse bites the sticks, the dynamic strength of the biting behavior is detected three-dimensionally by the load sensor attached to the bases of the sticks, as well as the duration of the behavior. The detected data

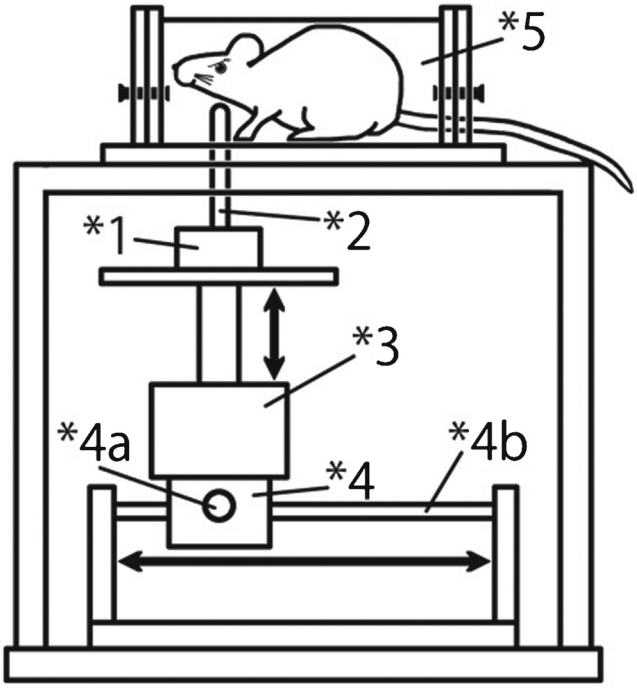

Fig. 1. Schematic illustration of the experimental system (ARM: Aggression Response Meter). The animal chamber (*5) is set on the top of the ARM. The stick-driving unit (*3) is controlled with a computer and makes a pair of sticks (*2) move in an up-and-down motion. The load sensor (*1) attached to the sticks detects the aggressive biting motion of the mouse. The stick-driving unit is shifted on the rails (*4b) of the drive-sliding unit by manual operation to the right and left. (*4) the body of the drive-sliding unit, (*4a) a knob attached to the drive-sliding unit.

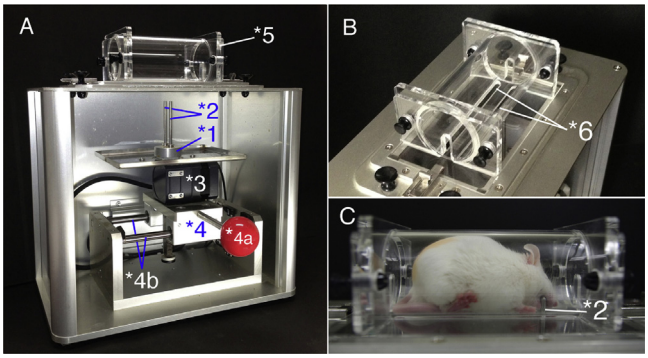

Fig. 2. Photographs of the ARM: (A) general view of the apparatus; *1, load sensor; *2, a pair of metal sticks; *3, stick-driving unit; *4, drive-sliding unit including a knob (*4a) and a pair of rails (*4b); *5, animal chamber. (B) animal chamber placed on the ARM, showing a pair of slits (*6). (C) Photograph showing a mouse exhibiting aggressive biting attack toward a stick (*2).

are inputted into a computer via an analog/digital converter. The mechanical noise and motions of mouse accompanying a life activity such as breathing movement are removed in programmable fashion. Because the vertical component of the data could be influenced by the body weight of the mouse, such data are eliminated from the final data. The values of intensity and incidence rate of ABB are displayed on a monitor graphically as well as numerically. Data of intensities are expressed in numerical values, as strength × time (Newton × millisecond: mNs).

2.2. Animals and drugs

The experiments were carried out on ddY strain and ICR strain mice (Sea:ddY and Sea:ICR; purchased from Kyudo Co. Ltd., Tosu, Japan). Normal male and female mice were housed together for mating to obtain offspring and a total of 116 offspring weighing 20–50 g (4–16 weeks old) were used in this study. One day after birth, each litter was adjusted to eight infants to ensure similar lactation and sufficient breast milk. Mice were housed in plastic cages lined with wood shavings. The size of cages for isolation-reared mice was 16 cm × 23 cm × 12 cm, while that for group-reared mice was 29 cm × 34 cm × 17 cm. The animal chamber used in the ABB tests (Figs. 1-*5, 2A-*5, 2B) was always placed in each home cage for habituation. All animals were housed under controlled conditions of temperature (22 ± 2 °C), humidity (55 ± 10%) and lighting (lights on 07:00, off at 19:00). Food and water were administered *ad libitum*. Cage exchange was performed every 10 days.

Buspirone hydrochloride, a serotonin 1A receptor agonist, was purchased from Sigma Chemical (St. Louis, MO) and fresh solutions were prepared daily. Saline was purchased from Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan).

All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and they were performed following approval by the Committee of Animal Experimentation, Kagoshima University and Kagoshima Immaculate Heart University. The experiments also complied with the current laws of Japan.

2.3. Measurement of ABB

Measurement of ABB was performed in the mouse-breeding room. The animal chamber was adjusted to the size of the animal so that the mouse could turn around in it, to avoid narrow space stress. The sticks were controlled with a computer to move at a rate of 100 mm/s, to extend 10 mm above the floor of the chamber and to remain for 1 s at the most extended position, which was set at 10-s intervals.

Prior to the measurement, the mouse was led into the animal chamber and left for a few minutes until its voluntary exploratory locomotion ceased, and then the behavioral test was initiated. The test consisted of two sessions. The first session was carried out to provoke the mouse and induce irritation and anger. The experimenter checked the position of the animal in the chamber, decided on the site of touch stimulation and shifted the stick-driving unit by hand to elevate the sticks to the hindlimb or abdominal level (Fig. 3: level A). At this level, the sticks went up through the slits of the floor and touched the hindlimb or abdomen. This touch stimulation was repeated 30 times in the first session. If the mouse exhibited aggressiveness, it became irritated and angry with the sticks, and then often exhibited kicking behavior, namely, it kicked the sticks away intensely with both hindlimbs throughout this session. Within a few minutes after the first session, the second session was started to measure the intensities and incidence rates of ABB. The experimenter shifted the stick-driving unit to elevate the sticks to head level (Fig. 3: level B). At this level, the sticks touched the mandible and/or whiskers or approached the face without touching it, and they were held at their most extended position. When the mouse bit them, the load sensor detected the dynamic strength and duration of biting behavior, and the experimenter clicked the "*response button*" on the display to record the number of biting behaviors. When the mouse paid no attention to the movement of the sticks, for example, it exhibited exploratory behavior or turned away, the experimenter clicked the "*invalid button*" to repeat the challenge while measuring. The measurement was performed 30 times in the second session, and the average intensity and number of responses were recorded in an Excel file together with all the other data detected by the load sensor.

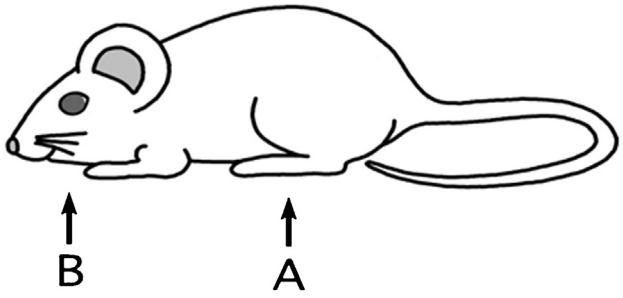

Fig. 3. Schematic drawing showing the sites of stimulation: (A), the hindlimb or abdominal level used in the first session for provoking the mice and (B) the head level used in the second session for measurement of aggressive biting behavior (ABB).

2.4. Socially isolated and re-socialized mice

A total of 26 male ddY strain mice and 20 ICR strain mice were used in this experiment. After weaning in postnatal week 3, male offspring were housed in same-litter groups for one additional week. At postnatal week 4, all mice were measured for ABB using the ARM. Just after the tests, they were divided into two groups according to housing conditions, that is, an isolation-housing group (one per cage; $n = 13$ in ddY and $n = 10$ in ICR mice) and a group-housing group (control group, four or five per cage; $n = 13$ in ddY and $n = 10$ in ICR mice). The ABB was measured at 1, 3, 5 and 7 weeks after the beginning of the isolation, namely, postnatal weeks 5, 7, 9 and 11. Long-term social isolation is known to cause stress in laboratory animals, and induces a variety of behavioral abnormalities including aggressive behavior (Hatch et al., 1963; Ibi et al., 2008; Miczek and O'Donnell, 1978; Valzelli, 1973; Wongwitdecha and Marsden, 1996).

Just after the last measurement at postnatal week 11, the isolated mice were re-socialized (4 or 5 per cage) and group-reared for an additional 2 weeks. The control mice were group-reared continuously. All mice were tested for ABB using the ARM 1 and 2 weeks from the beginning of the re-socialization (postnatal weeks 12 and 13). Experiments were conducted at the same time of day and the individual experimental order was fixed during these experiments.

2.5. Socially isolated female mice

To ascertain that long-term social isolation also induces and increases ABB in female mice, 14 female ddY mice were used in this experiment. At postnatal week 4, all mice were measured for ABB using the ARM. Just after the tests, they were divided into two groups according to housing conditions, that is, an isolation-housing group (one per cage; $n = 7$), and a group-housing group (control group; 7 per cage, $n = 7$). The ABB was re-measured 3 weeks after the beginning of the isolation at postnatal week 7. Experiments were conducted at the same time of day and the individual experimental order was fixed during these experiments.

2.6. Repeated tests in prolonged isolated mice and group-housed mice

To evaluate the influence of repeated tests on the ABB of each individual, the following tests were performed using both isolation- and group-housed ddY mice. A total of 20 male offspring were weaned at postnatal week 3, and the animals were either housed in groups (5 per cage: $n = 5$) or isolated ($n = 15$). The isolation-reared mice were divided into two groups. In the first isolation group ($n = 5$), a preliminary experiment was conducted prior to the subsequent repeated test. In this group, the ABB was measured twice on the days of postnatal weeks 13 and 16 (isolation rearing for 10 and 13 weeks), in order to assess whether the intensities and incidence rates of ABB no longer increased by the continuation of social isolation stress in this experimental period.

In the second isolation-reared group ($n = 10$) and group-housed mice, ABB was measured a total of seven times, every 3 or 4 days, in postnatal weeks 13–16 (isolation rearing for 10–13 weeks). Experiments were carried out at the same time of day and the individual experimental order was fixed during the experiments.

2.7. Administration of buspirone

Male ddY mice were weaned at postnatal week 3 and housed in isolation for 7 weeks. The isolated mice were prescreened for aggressive behavior a few days before the experiment. A group-housed intruder mouse was introduced into the home cages of isolated mice for a short time, and the isolated mice that showed aggressive behavior such as tail rattle and/or biting were used in this experiment ($n = 36$).

The intensities and numbers of ABB were measured using the ARM followed by resident-intruder tests described below. Just after the resident-intruder test, each mouse was placed on wire netting, picked up by its tail, and administered buspirone (2.5, 5.0 or 10.0 mg/kg i.p.; $n = 9$ each) or vehicle (0.9% saline; $n = 9$) intraperitoneally using a Terumo $29 \times 1/2''$ gauge syringe (Terumo Co., Tokyo, Japan) to control pain and fear minimally. Buspirone or vehicle was injected at a volume of 5 ml/kg to each mouse. Thirty minutes after the injection, ABB was re-measured using the ARM, and then the resident-intruder test was again performed 45 min after the injection.

The resident-intruder test, involving intraspecific aggression toward an animal of the same sex and age, was performed in the home cage of the resident, in which an unknown intruder male mouse was released, and the mice were videotaped for 15 min. The rating of aggressive behavior was scored on a 0–3 point scale: 0, no aggressive manifestations; 1, mild aggressive posture (tail rattle, lateral threat); 2, intermittent intensive attack of the other mouse, but no continuous fighting or biting, no vocalizations; and 3, continuous fighting or attempts to bite the intruder mouse, loud vocalizations. In the case of the development of the highest score of aggressive behavior, the test was immediately terminated to avoid injury.

2.8. Statistical analysis

The analyses were performed using log-transformation value (R programming language ver. 3.0.2 and SAS-JMP ver. 5.0.1, SAS Institute Inc., Cary, NC, USA). Analysis of variance was used to test for statistically significant differences among measured weeks and was interpreted using Tukey's HSD for multiple comparisons. Welch *t*-test (unpaired and paired) was used for comparisons between the isolation-reared mice and the group-housed mice. The level of statistical significance was $^{*}p < 0.05$.

3. Results

3.1. Changes of ABB during isolation and re-socialization periods

At postnatal week 4, prior to isolation rearing, no mice exhibited distinct ABB in response to the sticks in the ARM test, although a few very weak biting-like motions were observed in one session with 30 stimulus deliveries in some mice. As a rule, the behavior of the isolation-group mice was the same as the responses in the group-housed control mice in both strains. After 1 week of isolation rearing (postnatal week 5), most socially isolated ddY mice exhibited one or more distinct biting actions in response to the sticks in one session (Fig. 4A and B). In ICR strain mice, the biting-like actions were observed more frequently rather than in the ddY strain, although most actions were very weak (Fig. 4C and D). Overall, significant differences between the isolation-reared and group-housed mice were found in intensity ($p < 0.05$ in ddY and $p < 0.01$ in ICR) and in the numbers of ABB ($p < 0.01$ in both ddY and ICR). With isolation stress for 7 weeks (postnatal week 11), most isolated mice displayed intense biting responses many times in one session in both strains. On the other hand, in the group-housed control mice, obvious biting responses were not found.

When the isolated mice were re-socialized, the ABB then began to decrease (Fig. 4). Although all mice exhibited biting behavior after 1 week of re-socialization (postnatal week 12), most mice exhibited no biting responses after 2 weeks of re-socialization (postnatal week 13) in the ARM test in both strains. After 2 weeks of re-socialization, no significant differences were found in the intensities and numbers of ABB between the isolated/re-socialized and group-housed mice in the ARM test.

Supplementary Videos 1 and 2 show typical biting responses in the ddY mice socially isolated for 7 weeks and no response in the control mice, respectively.

3.2. Changes of ABB in female ddY mice

At postnatal week 4, prior to isolation rearing, no female mice exhibited distinct ABB in response to the sticks in the ARM test, although one or more weak biting-like motions were observed in one session. As a rule, the behavior of the isolation group was the same as the responses in the group-housed control mice. After 3 weeks of isolation rearing (postnatal week 7), most socially isolated mice exhibited distinct biting actions in response to the sticks many times in one session. Overall, significant differences between the isolation-reared and the group-housed mice were found in the intensity and the number of ABB in the ARM test after 3 weeks of isolation-rearing (Fig. 5, $p < 0.01$).

3.3. Influences of repeated tests on ABB

In all ddY mice isolated for more than 10 weeks and group-housed mice, the ABB was tested using the ARM. In the preliminary experiment, in the first isolation group, no significant differences were found between isolation of 10 weeks and that of 13 weeks in terms of intensity and incidence rates (intensity, 22.0 ± 1.0 and 22.2 ± 2.2 mNs; number of responses in one session, 29.2 ± 1.0 and 25.0 ± 1.4 [mean $\pm$ S.E.M.], respectively), indicating that the ABB of mice isolated for 10–13 weeks did not change in this period in the ARM test. In other words, ABB no longer increased with the continuation of social isolation stress in this period.

In the second group of isolation-reared mice tested seven times, every 3 or 4 days for 3 weeks (10–13 weeks after the beginning of isolation), the intensity and incidence rate of ABB neither increased nor decreased significantly during the experimental period. In the group-housed mice, ABB did not significantly change among the seven trials during the 3-week experimental period (Fig. 6).

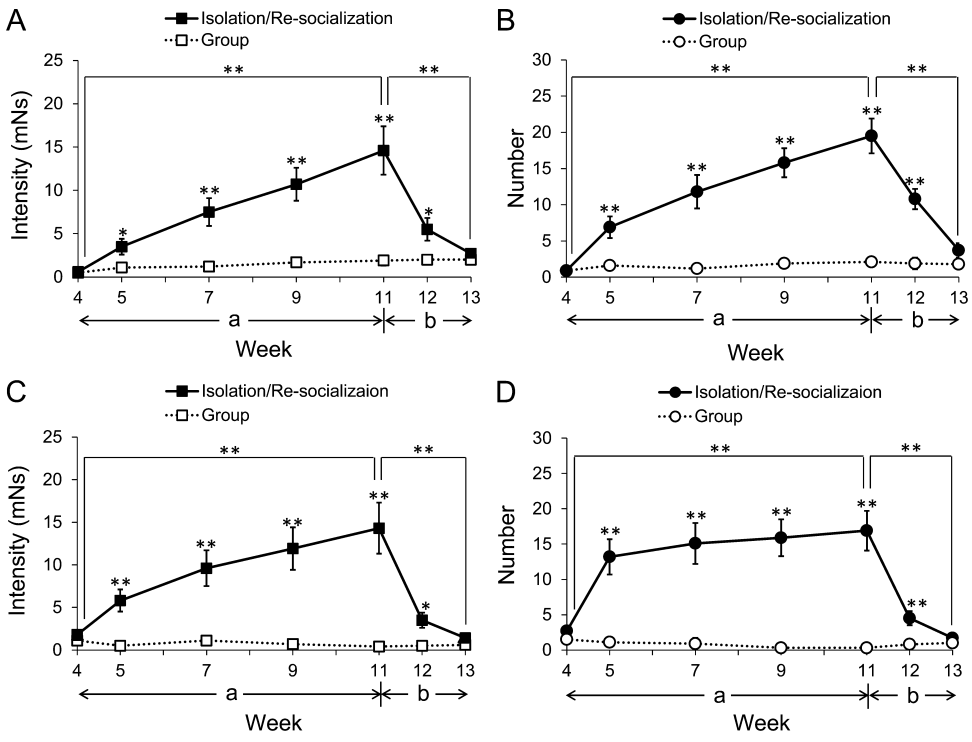

Fig. 4. Changes of aggressive biting behavior (ABB) during socially isolated and re-socialized periods. (A and B) ddY strain mice; (C and D) ICR strain mice. Both intensity (A and C) and number of responses in one session (B and D) were significantly increased during the 7 weeks of isolation rearing (A-a, B-a, C-a, D-a; postnatal weeks 4–11, $^{**}p < 0.01$), and then significantly decreased during the 2 weeks of re-socialization (A-b, B-b, C-b, D-b; postnatal weeks 11–13; $^{**}p < 0.01$) in isolated/re-socialized mice in both strains ($n = 13$ in ddY mice, $n = 10$ in ICR mice). Both intensity and number of responses were significantly different between the isolated/re-socialized mice and the control group-housed mice ($n = 13$ in ddY and $n = 10$ in ICR strain mice) at postnatal weeks 5–12 (1, 3, 5, 7 weeks from the beginning of the isolation and 1 week from the beginning of the re-socialization). Meanwhile, no significant differences between the isolated/re-socialized and control mice were found at postnatal week 4 or 13 (before the isolation rearing or 2 weeks from the beginning of re-socialization). $^{*}p < 0.05$, $^{**}p < 0.01$, compared with control group. Intensity data are expressed in numerical values, as strength × time (Newton × millisecond: mNs). Values shown are mean ± S.E.M.

3.4. Effects of buspirone

Before the administration of buspirone or vehicle, all isolation-reared mice used in this experiment exhibited ABB in terms of intensity and incidence rate in the ARM trials, and violent aggressive behavior against an intruder mouse in the resident-intruder tests. After the administration of buspirone, the intensity and incidence rate of ABB significantly decreased in a dose-dependent manner (Fig. 7A and B). The aggressiveness scores in the resident-intruder test also decreased in a dose-dependent manner (Fig. 7C). It was considered that the patterns of reduction of ABB in the ARM test were similar to those of intermale aggression. In the control mice that received an injection of vehicle, neither ABB nor intermale aggression changed significantly.

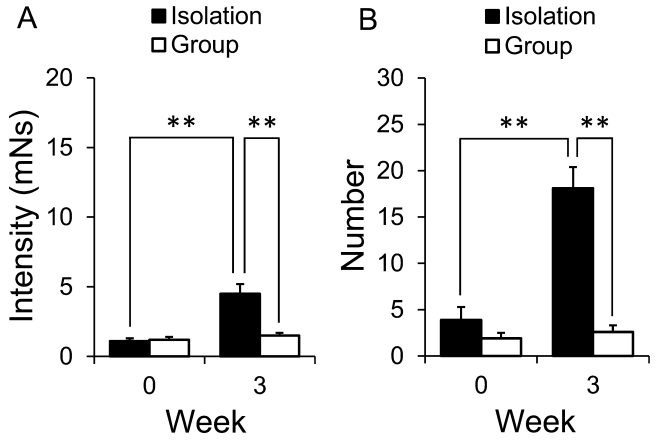

Fig. 5. Aggressive biting behavior (ABB) induced by social isolation in female ddY strain mice: (A) intensity of ABB and (B) number of ABB in one session. ABB was measured before social isolation (week 0, postnatal week 4) and re-measured after 3 weeks from the beginning of isolation (postnatal week 7). The control mice were group-housed throughout the experiment. In the isolation-reared mice ($n = 7$), the intensity and the number of ABB increased significantly after 3 weeks of isolation rearing ($^{**}p < 0.01$), whereas the behaviors of the control mice ($n = 7$) did not significantly change in either intensity or number of responses in one session. Both intensity and number of responses were significantly different between the isolated mice and the control group-housed mice after 3 weeks from the beginning of isolation ($^{**}p < 0.01$). Intensity data are expressed in numerical values, as strength × time (Newton × millisecond: mNs). Values shown are mean ± S.E.M.

4. Discussion

Protracted social isolation in laboratory animals causes stress, which induces a variety of behavioral abnormalities including increased anxiety-related behavior, cognitive deficits, hyperlocomotion, and deficit in pre-pulse inhibition of the acoustic startle reflex, as well as increased aggressiveness (Hatch et al., 1963; Ibi et al., 2008; King et al., 2009; Miczek and O'Donnell, 1978; Roncada et al., 2009; Valzelli, 1973; Voikar et al., 2005; Wei et al., 2007; Wongwitdecha and Marsden, 1996). Thus, isolated animals have been used for the analysis of aggression mechanisms and evaluation of drug actions on aggressiveness (Cai et al., 1993; Koike et al., 2009; Matsuda et al., 2001; Uchida et al., 2009; Wongwitdecha and Marsden, 1996; Yen et al., 1959), and have been used as models of psychiatric disorders including stress disorder, schizophrenia and/or depression (Brenes and Fornaguera, 2009; Day-Wilson et al., 2006; Fone and Porkess, 2008; King et al., 2009; Koike et al., 2009; Leng et al., 2004; Miura et al., 2002; Weiss and Feldon, 2001).

In the present study, we developed a new apparatus to evaluate aggressiveness by measuring ABB toward inanimate objects

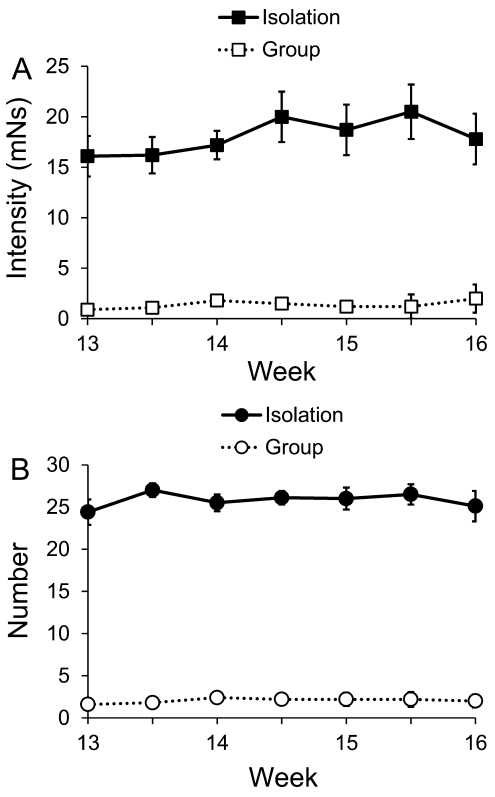

Fig. 6. Changes of aggressive biting behavior (ABB) in the prolonged isolated mice and group-housed mice: (A) intensity of ABB and (B) number of ABB in one session. ABB was measured during postnatal weeks 13–16 (isolated for 10–13 weeks), a total of 7 times, every 3 or 4 days. The intensity and number of ABB in one session did not significantly change during the 3 weeks of the experimental period in either isolation-reared ($n = 10$) or group-housed mice ($n = 5$). Intensity data are expressed in numerical values, as strength × time (Newton × millisecond: mNs). Values shown are mean ± S.E.M.

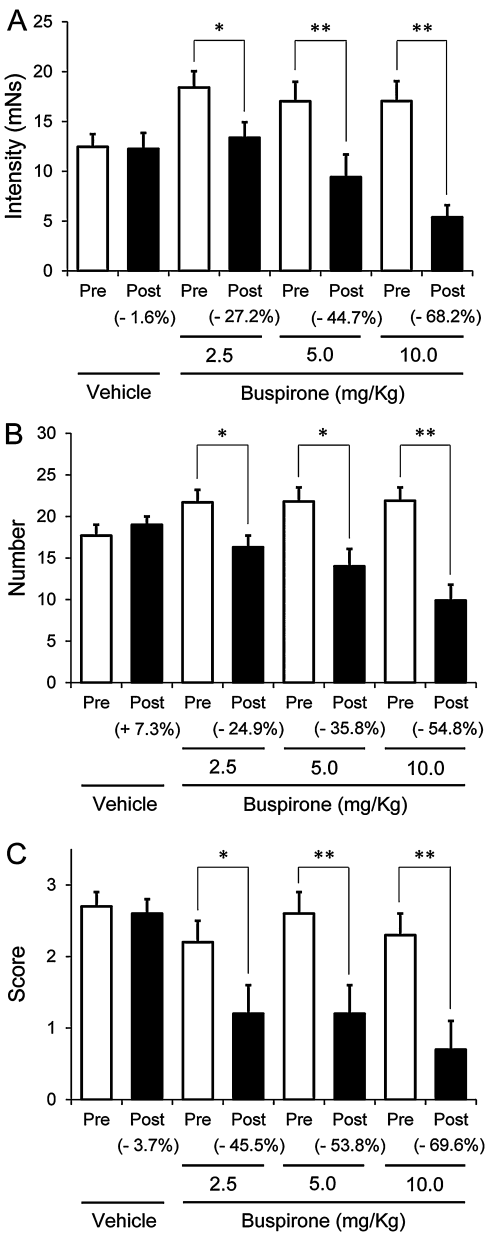

Fig. 7. Changes of aggressive biting behavior (ABB) and intraspecific intermale aggression after the administration of buspirone. The intensity and number of ABB were measured using the ARM followed by resident-intruder tests. Just after the resident-intruder test, each mouse was administered buspirone (2.5, 5.0 or 10.0 mg/kg i.p.; $n = 9$ each) or vehicle (0.9% saline; $n = 9$) intraperitoneally. Thirty minutes after the injection, ABB was re-measured, and then the resident-intruder test was performed 45 min after the injection. (A) Intensity of ABB. Data are expressed in numerical values, as strength × time (Newton × millisecond: mNs), (B) number of ABB in one session, and (C) the scores of the resident-intruder test. The test was scored on a 0–3 point scale. Both ABB and intermale aggression dose-dependently decreased in similar manners. $^{*}p < 0.05$, $^{**}p < 0.01$. Values shown are mean ± S.E.M.

in mice. The present results of this animal experiment suggest that the measurement of ABB is suitable for the evaluation of aggressiveness; responses were significantly increased in a period-dependent fashion during the isolation-rearing periods and decreased significantly throughout the re-socialization. To the best of our knowledge, no studies have reported continuous changes of aggression in the same individual mice during isolation and re-socialization periods for a long period. In the present study, social isolation increased ABB both in ddY- and ICR-strain mice similarly in the ARM test, suggesting that the ARM is useful for evaluation of aggressiveness in two or more strains of mice. Moreover, the present study revealed that social isolation also increased ABB of female animals in the ARM test, suggesting that the ABB test is effective in the evaluation of female mouse aggressiveness.

Currently, for the evaluations of aggressive symptoms in psychiatric animal models, intraspecific intermale aggression tests such as a resident-intruder test have been widely conducted. On the other hand, there are only a few studies that examine ABB for evaluation of aggressiveness (Sofia, 1969; Uchida et al., 2009). In the present study, we propose a new behavioral protocol for evaluating aggressiveness using the ABB paradigm.

It is considered that the triggers and neural mechanisms of ABB and intraspecific intermale aggression are not identical. Intermale aggression is usually induced toward animals of the same sex in order to protect territory, food and/or females (Blanchard and Blanchard, 1977). This type of aggression is not always exhibited in female mice. Intraspecific aggression in female animals appears exclusively in the nursing period (Svare and Gandelman, 1976). On the other hand, ABB may be triggered by emotional responses toward trivial stimuli such as a light touch or an object moving in front of the eyes. It is natural that female mice also exhibit ABB.

Intraspecific aggressive behavior against animals of the same sex is not clearly exhibited in the early stages of isolation (Yen et al., 1959), whereas ABB is detectable in mice isolated for only 1 week in the ARM test. It is conceivable that the measurement of ABB is more suitable for detecting the early stages of aggressiveness in psychiatric disorders than conventional methods. ABB is a valuable behavioral index in studies of the pathogenesis of psychiatric diseases accompanied by aggressiveness.

Meanwhile, both ABB and intermale aggression increase in response to social isolation and decrease in response to re-socialization (Valzelli, 1969). In addition, ABB and intermale aggression were inhibited by buspirone, a serotonin 1A receptor agonist, in a dose-dependent manner. It is conceivable that ABB is suitable as a behavioral paradigm for the evaluation of drug actions on aggressiveness in psychiatric model mice as well as intermale aggression tests. It has been reported that serotonin 1A receptor agonists inhibit aggressive behaviors in laboratory animals (Matsuda et al., 2001; Matto et al., 1998). The findings of the present study concur well with these reports.

In the present study, the animal chamber as used in the test was always placed in each home cage for habituation, and mice entered it regularly. Since each mouse could turn around in the chamber, restraint stress and discomfort appeared to be minimal. Moreover, the stimulation sticks were thick, the heads of which were dome-shaped and moved relatively slowly, so it is thought that the sticks did not inflict pain on the mice. Indeed, the group-housed mice hardly responded to the stick stimuli. Moreover, it is considered that the mechanical noises did not affect the behavior of the testing mice, because the mice did not respond to sticks that moved behind them unless the sticks touched them. Thus, it appears likely that the ARM tests themselves were either not very stressful or did not result in an additional increase in behavioral symptoms. Furthermore, in the present study, ABB did not change significantly in either the prolonged isolated mice or the control group-housed mice, in spite of repeated tests for 3 weeks. These results suggest that ABB is not markedly changed in repeated tests, at least with an experimental period of 3 weeks, and indicate that biting behavior is advantageous for long-term evaluation of symptoms of aggression using the same individual repeatedly.

When isolated mice are re-socialized in the same cage, fights often occur with other mice and rank order is determined in the cage (Grant and Chance, 1958). In the present study, although fights were observed among the re-socialized mice, ABB was markedly reduced during the re-socialization period, even in the mice ranked lowest in the hierarchy. Previous studies reported that chronic mild stress-induced behavioral abnormalities decrease after releasing animals from the stress (Einon and Morgan, 1977; Gentsch et al., 1988; Hatch et al., 1963; Hellemans et al., 2004; Lu et al., 2003). The findings of the present study concur with these previous reports.

The aggressive biting test using the ARM consists of a 5-min provoking session followed by a 5-min measuring session in each animal. The entire test simply involves these components. Data processing is finished simultaneously with the end of the second session. The ARM saves both time and labor in evaluating aggressiveness. Moreover, the ABB test using the ARM can be performed under relatively uniform conditions in each laboratory, and experimenters can carry out the test with less potential subjective bias.

5. Conclusion

We concluded that ABB toward inanimate objects was a reliable paradigm that made it possible to detect aggressiveness in the early stages of psychiatric disorders. Furthermore, it is conceivable that this paradigm is valuable for drug evaluation of psychotropic agents using the same individual repeatedly over a long period. The ARM can be used to detect aggressiveness quantitatively in two or more strains of mice, and in both male and female mice.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jneumeth.2014.02.017.

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