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Impairment of interstrain social recognition during territorial aggressive behavior in oxytocin receptor-null mice

Tatsuya Hattori[a], Kouta Kanno[a], Miho Nagasawa[a], Katsuhiko Nishimori[b], Kazutaka Mogi[a,*], Takefumi Kikusui[a]

[a] Companion Animal Research, School of Veterinary Medicine, Azabu University, Sagamihara 252-5201, Japan
[b] Department of Molecular and Cell Biology, Graduate School of Agricultural Science, Tohoku University, Miyagi 981-8555, Japan

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ABSTRACT

In humans, oxytocin has been shown to be involved in in-group cooperative behaviors and out-group aggression. Studies have also demonstrated that oxytocin plays a pivotal role in social recognition. However, no empirical research has investigated the effect of oxytocin on in-group and out-group aggressiveness. We employed a resident-intruder paradigm to assess the ability of resident male mice to discriminate intruder male strain differences. We found that resident male mice exhibited higher frequencies of attack bites against intruders of different strains than against intruders of their own strain. Subsequently, we examined whether the interstrain recognition was regulated by the oxytocin system using oxytocin receptor (OTR)-null mice. OTR wild-type or heterozygous residents displayed higher aggression toward intruders of a strain different from their own (C57BL/6J). On the other hand, OTR-null residents exhibited greater aggression toward intruders of the same strain compared to OTR wild-type or heterozygous residents, and aggression levels were not different compared to those exhibited toward other strains. Our findings demonstrated that the oxytocin system contributes to interstrain social recognition in territorial aggression in male mice, implying that one function of oxytocin is to promote an in-group "tend-and-defend" response, such as in-group favoritism, which could be evolutionarily conserved in mammals.

1. Introduction

Out-group aggression, including war or ethnic cleansing, could be considered to be driven by the desire to increase the benefits to one's own group. Impressive findings have been reported in which oxytocin promoted in-group trust, cooperation, favoritism, and defensive aggression toward competing out-groups (De Dreu et al., 2010, 2011). These studies increased speculation regarding the neurological and evolutionary bases of out-group aggression. Many animal studies have shown that oxytocin is implicated in cooperative behaviors, such as maternal behavior and pair bonding, as well as in aggressive behaviors (Gimpl and Fahrenholz, 2001). Furthermore, experiments with transgenic mice have demonstrated that the oxytocin neural system is fundamental to social recognition. In rodents, a predominant information source for social recognition is encoded by olfactory or pheromonal signals, and such signaling plays a major role in kin recognition, territoriality, and mother–infant bond formation (Mogi et al., 2011). In addition to long-term social recognition, rodents are also known to form transient short-term memories of recently encountered individuals (Thor and Holloway, 1982). Since a pioneering study on oxytocin-knockout mice by Ferguson and colleagues (Ferguson et al., 2000), it has been revealed that oxytocin receptor (OTR) knockout mice fail to develop short-term memory (Macbeth et al., 2009; Takayanagi et al., 2005) as assessed by a habituation–dishabituation paradigm. In a series of these studies, it was shown that OTR-knockout mice could not distinguish between familiar and novel encounters with mice from the same strain, suggesting that the oxytocin system is involved in "fine" intrastrain recognition in mice (Macbeth et al., 2009). However, it was shown that OTR-knockout male mice could distinguish between familiar and novel females from different strains in the habituation–dishabituation paradigm, suggesting that the oxytocin system is less important in "broad" interstrain recognition in mice (Macbeth et al., 2009). Despite these advances in knowledge, the specific details of the contribution of oxytocin to social recognition and out-group aggressiveness remain unknown.

In this study, we examined whether the oxytocin system is involved in interstrain recognition and aggression in mice by using

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* Corresponding author. Tel.: +81 42 769 1673; fax: +81 42 850 2513.
E-mail address: mogik@azabu-u.ac.jp (K. Mogi).

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a resident-intruder paradigm. Normally, male mice display aggressive behavior toward other males to defend their own territory (Novotny et al., 1985; Miczek et al., 2001). First, we examined the effect of the intruder strain on the resident's aggressive behavior in male ICR, C57BL/6J, and BALB/c mice. We then investigated whether the effect of the intruder strain was the same in OTR-null residents.

2. Materials and methods

2.1. Animals

All animals were housed under a standard 12-h light/dark cycle (lights on from 6 a.m. to 6 p.m.), and the environment was maintained at a constant temperature (24 ± 1 °C) and humidity (50 ± 5%). Food and water were provided ad libitum. The study was approved by the Ethics Committee of Azabu University, Japan. For aggressive training, male ICR, C57BL/6J and BALB/c mice (ICR, $n = 10$; C57BL/6J, $n = 12$; BALB/c, $n = 12$) were purchased from Japan SLC (Hamamatsu, Japan) and housed as a "resident" paired with age-matched females of the same strain in test cages (175 mm × 245 mm × 125 mm) beginning at 8 weeks old. All genotypes of OTR mutant mice, which were generated from heterozygous mating, were also used as residents (OTR wild type, $n = 9$; OTR heterozygous, $n = 14$; OTR null, $n = 10$). The OTR mutant mice used were originally descended from 129/Sv and C57BL/6J backgrounds bred at Tohoku University (Takayanagi et al., 2005) and back-crossed with C57BL/6J for more than six generations after they were transferred to Azabu University. Genotype was confirmed using a polymerase chain reaction-based genotyping protocol, with DNA purified from pup tail clips. Pups were weaned at 28 days old and housed in same-sex groups until they were 8 weeks old, at which time each OTR mutant male was paired with an age-matched C57BL/6J female. As "intruders" for the resident-intruder test and aggression training, sexually naïve ICR, C57BL/6J, BALB/c, and A/J males, which were purchased from Japan SLC were housed in groups of six individuals per cage.

2.2. Aggression training

After the resident male mice had been housed with females for 2 weeks, they underwent aggression training using A/J males as intruders. Immediately after removal of the female from the resident's cage, an intruder was placed into the cage. The resident's behaviors were recorded for 5 min using a CCD camera connected to a video monitor, and then the intruder was removed and the female was returned to the cage. This training was conducted once a day during the light phase, for a total of 6 times with a 4-day interval. After the training, resident mice performed a stable number of attack bites (more than 20 times in 5 min). It has been shown that male mice do not show territorial aggression against female mice (Novotny et al., 1985; Miczek et al., 2001). Therefore, to confirm that males' territorial aggression was normal, the introduction of sexually naïve BALB/c females (Japan SLC, 10 weeks old) or BALB/c males (10 weeks old) that were castrated under ether anesthesia at 3 weeks old was further examined. Resident males that made few attack bites (less than 5 times during a 5-min observation period) toward females and castrated males were used as subjects for the following resident-intruder test (ICR, $n = 8$; C57BL/6J, $n = 10$; BALB/c, $n = 10$, OTR wild type, $n = 8$; OTR heterozygous, $n = 12$; OTR null, $n = 8$).

2.3. Resident-intruder test

The tests were conducted when residents were 10–11 weeks old. Each resident randomly encountered intruders of all strains, with at least 3 days between each encounter. An A/J, C57BL/6J, BALB/c, or ICR male (8–10 weeks old) was introduced into the resident's cage (ICR) as an intruder immediately after the female was removed, and the mice were observed during a 5-min test. Immediately after the test, the intruder was removed and the female was returned to the resident's cage. Similarly, an A/J, C57BL/6J, or BALB/c male was introduced into the cage of C57BL/6J, BALB/c, or OTR mutant mice. These intruders had not encountered a resident mouse prior to the resident-intruder test. That is, they were novel and unfamiliar to all residents in all tests. Each test was recorded using a CCD camera connected to a video monitor. The residents' behaviors were recorded and classified according to the following five definitions: sniffing the intruder's facial area, sniffing the intruder's anogenital area (each occurrence and total duration), tail rattle, attack bite (each occurrence), and latency to the first behavioral occurrence.

2.4. Statistical analysis

Each behavioral parameter in resident ICR, C57BL/6J and BALB/c males was analyzed by repeated measures analysis of variance (ANOVA) with intruder strains as a repeated factor, followed by Bonferroni post hoc comparisons. In the case of OTR mutants, each behavioral parameter was analyzed by two-way repeated measures ANOVA (repeated intruder strains × genotype), followed by Bonferroni post hoc tests.

3. Results

3.1. Resident-intruder tests in ICR, C57BL/6J, and BALB/c mice

When ICR males were residents, there was a significant difference only in the number of "attack bites" ($F_{[3,21]} = 41.325$, $P < 0.01$), but there were no significant differences in the other behavioral parameters (Table 1a). The post hoc test revealed that the number of attack bites by ICR residents against all other strains was higher compared with the number of bites against fellow ICR mice ($P < 0.01$, Fig. 1a). The number of attack bites against BALB/c was higher than that to either A/J or C57BL/6J ($P < 0.01$, Fig. 1a). Similarly with ICR residents, there was a significant difference only in the number of "attack bites," when the resident was either C57BL/6J ($F_{[2,8]} = 23.965$, $P < 0.01$) or BALB/c ($F_{[2,8]} = 64.671$, $P < 0.01$). There were no significant differences in the other behavioral parameters for either strain (Table 1b and c). Post hoc testing revealed that the number of attack bites against intruders of all other strains from C57BL/6J residents was higher compared with that against C57BL/6J intruders (Fig. 1b, $P < 0.05$). The number of attack bites against BALB/c was higher than the number of bites against A/J ($P < 0.01$). For BALB/c residents, post hoc testing revealed that the numbers of attack bites against A/J and C57BL/6J intruders were higher than the number of attack bites against BALB/c intruders (Fig. 1c, $P < 0.05$).

3.2. Resident-intruder test in OTR knockout mice

When the number of "attack bites" was used as the dependent variable, two-way repeated measures ANOVA reveled significant main effects of genotype ($F_{[2,26]} = 4.613$, $P < 0.05$) and intruder strain ($F_{[2,26]} = 18.993$, $P < 0.01$) and a significant interaction between genotypes and intruder strain ($F_{[4,23]} = 2.608$, $P < 0.05$). In both OTR wild-type and heterozygous residents, a post hoc comparison among intruder strains revealed that the number of attack bites against A/J or BALB/c was higher compared to that against C57BL/6J ($P < 0.05$, Fig. 2). In addition, the number of attack bites against BALB/c was higher compared with that against A/J ($P < 0.05$, Fig. 2). In OTR-null residents, there was no significant difference in the number of attack bites against the different intruder strains. With