Differential effects of oxytocin receptor antagonist on social rank and other social behavior in male mice

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Abstract

Social rank within a group is essential for survival in many animals. Rank in the community helps to avoid unnecessary conflicts and establish stable relationships with others. Oxytocin has received increasing attention for its function in social behavior. However, the causal relationship between oxytocin levels and social rank has remained unclear. Here, we examined the effects of intraperitoneal injection of the oxytocin receptor antagonist L-368-899 on (1) social rank, (2) sex preference, (3) social preference, and (4) dyadic interaction in male mice. In the tube test, injection of the oxytocin receptor antagonist had no effect on first-rank mice, but caused fluctuation of the rank in second-rank mice, suggesting that the function of oxytocin in the maintenance of the social rank is rank-dependent. Second, injection of the oxytocin receptor antagonist dose-dependently impaired the sex preference of the male mice, confirming that oxytocin is essential in sexual behavior. Third, injection of the oxytocin receptor antagonist had no effect on social preference and dyadic interaction between the male mice, suggesting that oxytocin is not necessary in direct social interaction. Taken together, these results demonstrate that the role of the oxytocin in male mice is limited to a specific context of social behavior.

Highlights

- The stable social rank of the male mice in their cages was clearly observed in the tube test.
- Administration of an oxytocin receptor antagonist to first-rank mice caused no change in social rank.
- Administration of an oxytocin receptor antagonist to second-rank mice caused fluctuation of social rank.
- Sex preference, but not social preference and dyadic interactions, were impaired after the injection of the oxytocin receptor antagonist.

Introduction

Social rank is formed through interactions with other individuals and experiences in the community. The existence of social rank helps animals to avoid unnecessary conflicts, reduce energy costs spent in competing for limited resources, and secure comfortable living spaces. Thus, social rank has a profound impact on an individuals' survival, reproductive success, health, and other behaviors.[1]

Despite their importance to societies and individuals, the psychological and neurobiological mechanisms underlying social rank remain unclear. Tube tests have been used to examine the social rank of rodents.[1] In the tube test, two mice are inserted from either end of a thin tube and face each other at the center of the tube. The rank in rodents can be measured by examining the performance of the mice in a round-robin match within a cage, where the dominant mouse pushes the opponent backward in the tube and pushes him out of the tube. Mice with the highest rank in the tube test are known to groom lower-rank mice excessively and remove the whiskers and/or body hair of lower-rank mice, suggesting that there is a strong correlation between the rank in the tube test and the frequency of dominant behavior over other individuals.[2] In addition, dominant mice tend to exhibit more courtship behavior towards female mice by omitting ultrasonic vocalization than subordinate male mice.[2,3] In a hot spot task, a behavioral task in which four mice were placed on a cold floor and placed to compete for a single warm spot, higher rank mice in the tube test spent more time dominating the hot spot.[4] These studies have demonstrated the utility of the tube test as a reliable behavioral assay for examining social rank in rodents.

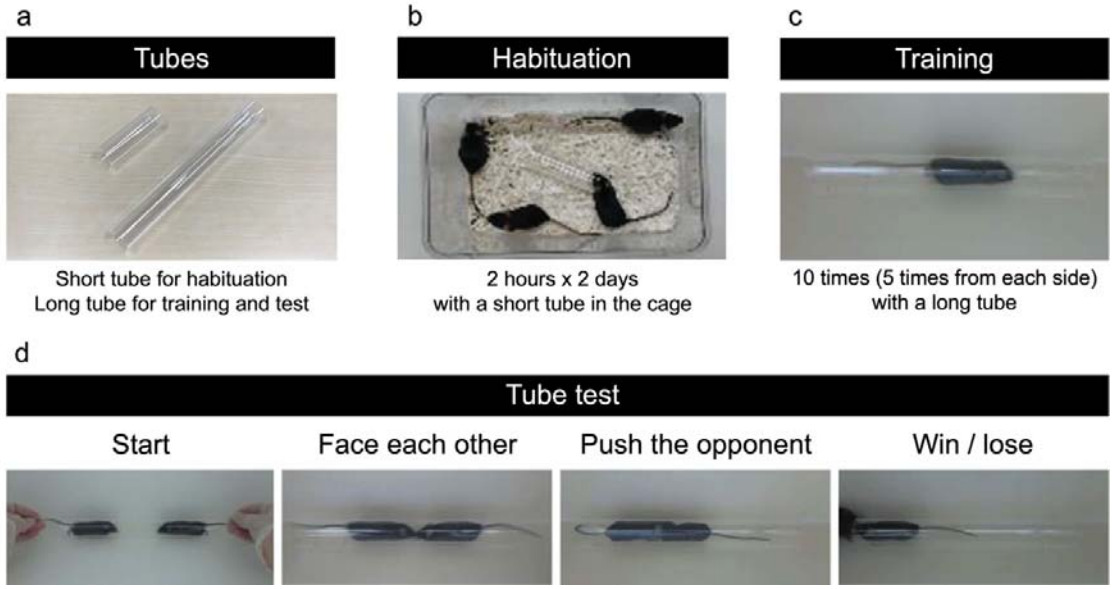
Oxytocin has received increasing attention for its role in social behaviors.[5] Oxytocin has been shown to be involved in a variety of social behavior, including pair-bonding,[6] maternal behavior,[7] aggression,[8] social decision making,[9] observational fear conditioning,[10] and social recognition[11] in many species. Oxytocin is emerging as a candidate pharmacological target for the treatment of mental disorders, especially those with social dysfunction, such as autism spectrum disorder,[12,13] social anxiety disorder,[14] and schizophrenia.[15] A recent study indicated that oxytocin receptor expression in the brain is different in mice with different social ranks.[16] However, the causal relationship between oxytocin levels and social rank remains unclear.

Here, we examined the effects of intraperitoneal administration of a blood–brain barrier penetrating oxytocin receptor antagonist[17], L-368,899, on various social behaviors. First, we examined the effect of oxytocin receptor antagonist on the tube test to examine the causal relationship between oxytocin and social rank. Second, we examined the effect of the oxytocin receptor antagonist on sex preference to validate

the effect of the oxytocin antagonist on the discrimination of other individuals and on social interaction, and to rule out the possibility that the oxytocin receptor antagonist is not working. Third, we examined the effect of oxytocin antagonists on a social preference task with unfamiliar male mice and direct dyadic interactions between familiar male mice in an open-field box.

Results

In experiment 1, we examined whether oxytocin antagonists affected the social rank of first- and second-rank mice in the tube test. Following habituation (Supplementary Movie 1) and training in the tube (Supplementary Movie 2), we started the tube test (Figure 1, Supplementary Movie 3). When all ranks of the four mice in the cage were kept stable for three consecutive days, we defined that the rank in the cage was stabilized. It took a minimum of 3 days and a maximum of 15 days to stabilize a rank, with an average of 9.4 days (standard error of the mean = 2.05) to inject the oxytocin receptor antagonist to the first-rank individual and with an average of 6.2 days (standard error of the mean = 1.66) to inject the oxytocin receptor antagonist to the second-rank individual. All cages tested in this experiment showed a linear social rank structure (rank 1, 2, 3, and 4). Total time spent for the match during the stable three consecutive days was different between the rank pairing (Figure 1f, F (2.111, 99.20) = 39.20, $p < 0.0001$, repeated measures one-way ANOVA). The pair between first- and second-rank mice took a longer time until the winner/loser was determined. The pairs including fourth-rank mice took significantly shorter time until the winner/loser was determined. There was no correlation between body weights and ranks (Figure 1g, r^2 = 0.0017, N = 60, p = 0.7556, simple linear regression).



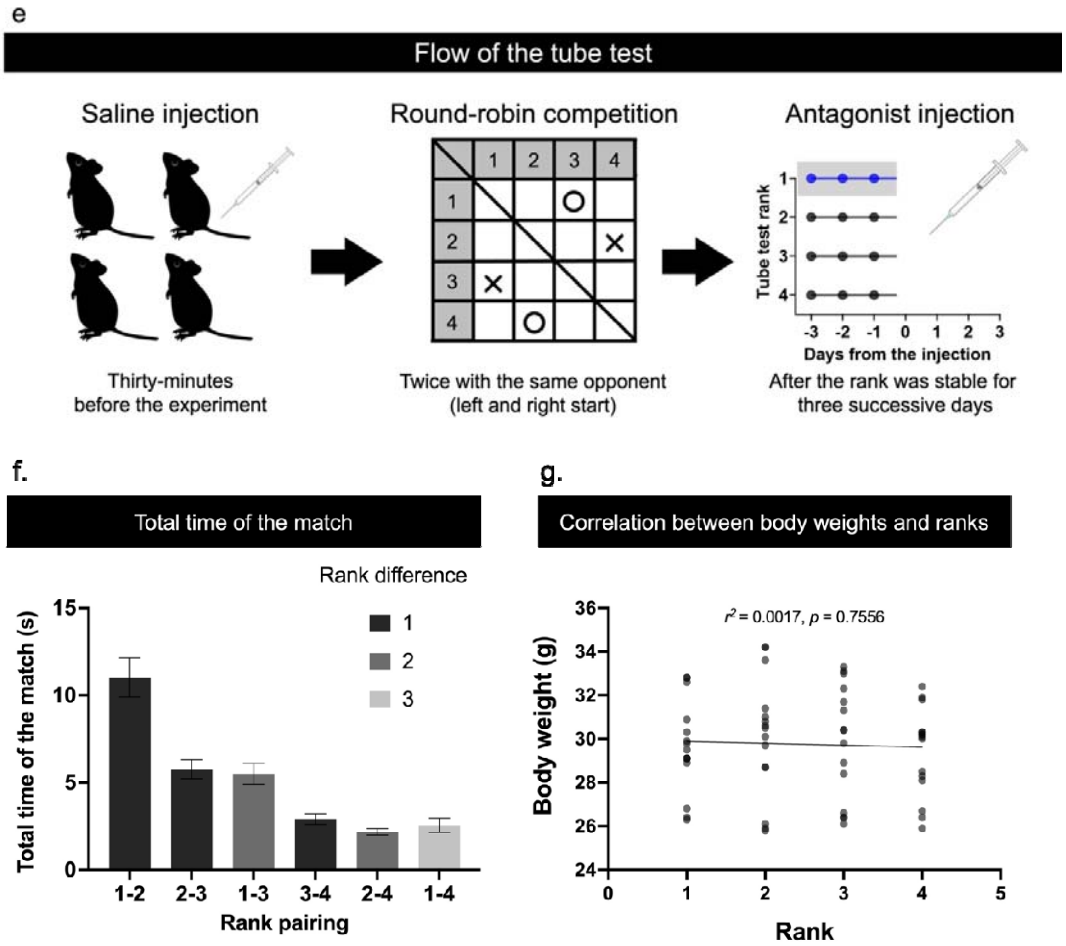


Figure 1. Procedure and results of the tube test

a. A short tube for habituation (35 mm diameter, 2 mm thickness, 30 cm long, acrylic) and a long tube for the tube test (35 mm diameter, 2 mm thickness, 10 cm long, acrylic). **b.** Habituation for the tube in the home cage. Mice were group-housed with four individuals. The short tube was placed in the cage, and the mice were allowed to have free contact with the tube for two hours on two days. **c.** Training for the tube test. During the training phase, the same tube as used in the test was used, and each mouse was trained to pass through the tube 10 times. Mice were started from each entrance on the left and right side five times each, for two days. **d.** After all the ranks in the cage had stabilized for three consecutive days, the first- or second-rank individual was given intraperitoneal injections of 10 mg/kg of oxytocin receptor antagonist on the next day. The other individuals were given intraperitoneal injections of saline. **f.** Total time spent in the match of each rank pair is shown. Total time spent for the match during the stable three consecutive days was different between the rank pairing (F (2.111, 99.20) = 39.20, p < 0.0001, repeated measures one-way ANOVA). The pair between first- and second-rank mice took longer time until the winner/loser was determined. Matches including fourth-rank mice took significantly shorter

until the winner/loser was determined. Error bars indicate standard error of mean. g. Correlation between ranks and weights of the mice is shown. We measured body weights of the subjects every time we ran the experiment. We examined the correlation between body weights and ranks based on the body weights of mice in three stable days before the injection of the oxytocin receptor antagonist to the first-rank mouse and the ranks of each mouse. There was no correlation between body weights and ranks (r^2 = 0.0017, N = 60, p = 0.7556, simple linear regression).

The first- or second-rank individual was administered intraperitoneal injections of 10 mg/kg of the oxytocin receptor antagonist L-368,899, which crosses the blood-brain barrier and selectively antagonizes central oxytocin receptors[17], on the next day. When the oxytocin receptor antagonist was administered to first-rank mice, there was no change in the rank of all five groups tested (Figure 2a). When oxytocin receptor antagonists were administered to the second-rank mice, two groups of second-rank mice increased in rank on the day of oxytocin receptor antagonist administration and one group of second-rank mice decreased in rank on the day following administration, while the other two groups of second-rank mice showed no effect (Figure 2b). We compared the effect of the oxytocin receptor antagonist on the rank change after the injection. There was a significant difference of the effects of the oxytocin receptor antagonist on social rank in between first- and second-rank mice ($\chi^2(1) = 4.286$, p = 0.038, Chi-square test). In both first- and second-rank mice, injection of the oxytocin receptor antagonist did not change obvious aggressive and affiliative behavior toward the opponent. In addition, there was no change of the body weights after the injection of the oxytocin receptor antagonist in both first- and second-rank mice (Supplementary figure 1a-b, first-rank mice: t (14) = 1.916, p = 0.076, paired t-test, second-rank mice: t (14) = 0.7433, p = 0.470, paired t-test).

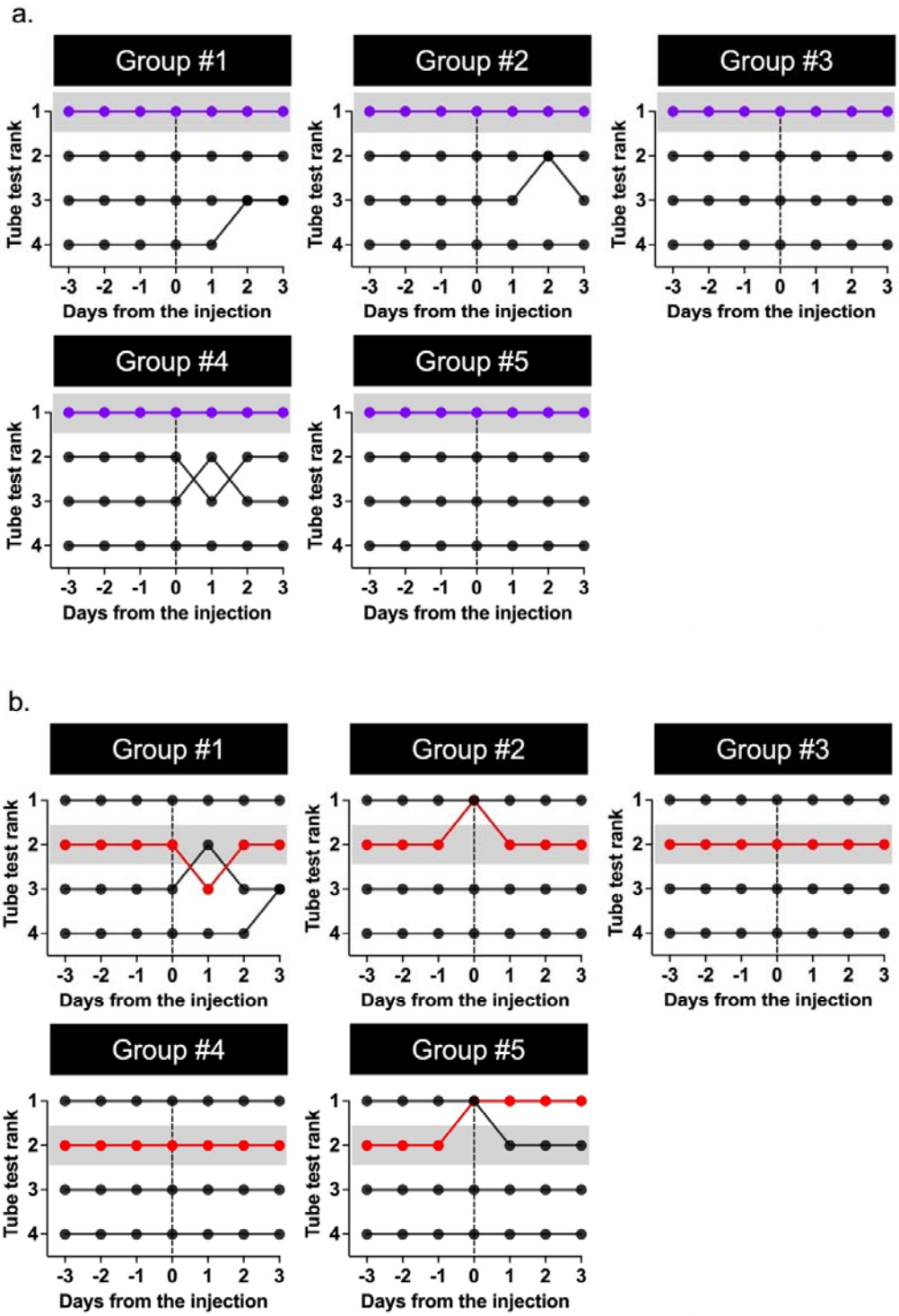


Figure 2. Rank change of first- and second-rank individuals after oxytocin receptor antagonist administration

The results of the tube test before and after intraperitoneal injection of the oxytocin receptor antagonist L-368,899 to the first- and second-rank mice. a. The results of three days before and after the injection of the oxytocin receptor antagonist on first-rank mice are shown. b. The results of three days before and after the injection of the oxytocin receptor antagonist on second-rank mice are shown. N =5 for each condition.

To examine whether oxytocin receptor antagonists affect sex preference in male mice, we used a sex preference task in experiment 2 (Figure 3a). Mice were intraperitoneally injected with saline or 3 or 10 mg/kg of oxytocin receptor antagonists and then placed in a box 30 min after the injection. After 15 min of habituation, the mice were temporally removed from the box, and the stimulus individuals, male and female C57BL/6J mice, were placed in each basket. We did not conduct ovariotomy or manipulation of the estrous cycle for the stimulus female mouse. The subject mice were then placed again in the center of the box and allowed to explore freely in the box for 10 min. We found a dose-dependent impairment of the preference for female mice by the oxytocin receptor antagonist (Figure 3b-d). There was a significant difference in preference for females in the saline group (t (7) = 3.242, p = 0.0142, paired t-test), whereas there was no significant difference in preference for males and females in the oxytocin receptor antagonist 3 mg/kg group (t (7) = 2.133, p = 0.0703, paired t-test) and 10 mg/kg group (t (7) = 0.6733, p = 0.5223, paired t-test). We found no significant effect of the oxytocin receptor antagonist on the total time spent with male and female mice between the saline, 3 mg/kg, and 10 mg/kg oxytocin receptor antagonist conditions (F (2,21) = 1.861, p = 0.180, one-way ANOVA).

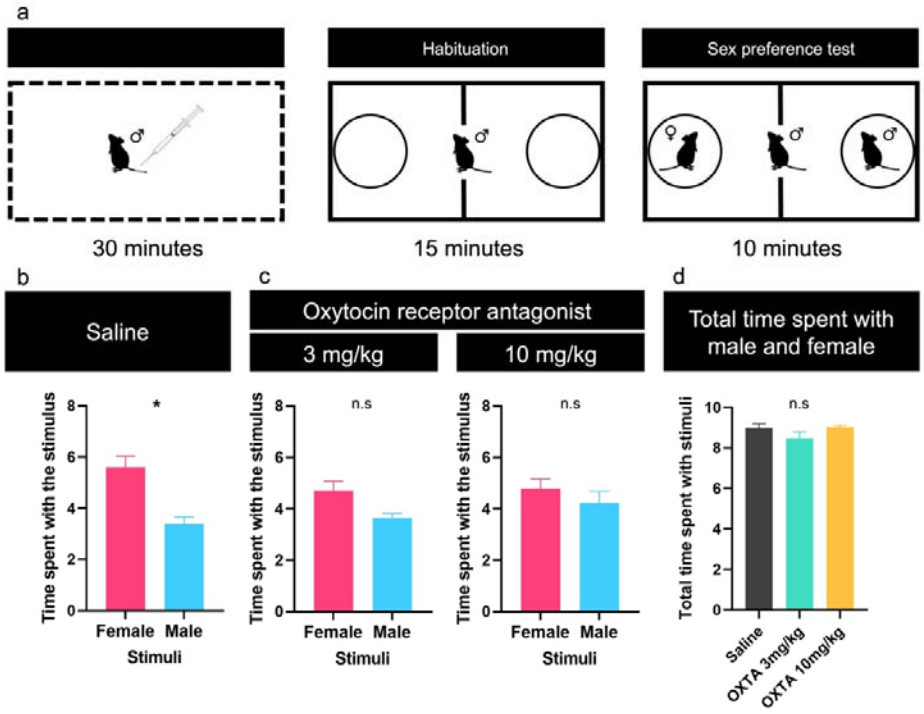


Figure 3. Effect of the oxytocin receptor antagonist on the sex preference task.

a. The apparatus used for the test was a custom-made white vinyl chloride box (25 cm length × 50 cm width × 30 cm height), with a basket containing the stimulus individuals placed at the center of the 25 cm long box and at both ends of the 50 cm wide box. A wall was attached to the center of the 50 cm width to separate the space on the left and right sides. Mice were intraperitoneally injected with 3 or 10 mg/kg of oxytocin receptor antagonist or saline and then placed back to their home cage for 30 min. In their home cage, they were allowed to interact with a cagemete. After 15 min of habituation, the mice were temporally removed from the box, and the stimulus individuals were placed in each basket. The subject mice were then placed in the center of the box and allowed to move freely for 10 min. White noise (75 dB) was presented throughout the experiment to mask the external noise. **b.** Time spent with the male and female stimuli in male mice 30 minutes after the injection of saline. Male mice preferred to spend more time with female mice. There was a significant difference in time spent in female and male mice (t (7) = 3.242, p = 0.0142, paired t-test). **c.** Time spent with the male and female stimuli in male mice 30 minutes after the injection of oxytocin receptor antagonist. Preference of the male mice to the female mice was impaired in the condition of the oxytocin receptor antagonist at the dose of 3 and 10 mg/kg. There was no significant difference in time spent in female and male mice in the dose of 3 mg/kg (t (7) = 2.133, p = 0.0703, paired t-test) and 10 mg/kg (t (7) = 0.6733, p = 0.5223, paired t-test). **d.** Total time spent with male and female mice between saline, 3 mg/kg, and 10 mg/kg oxytocin receptor antagonist

conditions. There was no significant difference in total time spent with the stimuli between the conditions (F (2,21) = 1.861, p = 0.180, one-way ANOVA). Error bars indicate standard error of mean. N = 8 for each condition.

To examine whether oxytocin receptor antagonists affect social preference in male mice, we used a social preference task in experiment 3 (Figure 4a). Mice were intraperitoneally injected with saline or 10 mg/kg of oxytocin receptor antagonists and then placed in a box 30 min after the injection. After 15 min of habituation, the mice were temporally removed from the box, and stimuli, a male C57BL/6J mouse and a novel object, were placed in each basket. The subject mice were then placed again in the center of the box and allowed to explore freely in the box for 10 min. We found no impairment of the preference for the male mouse by the oxytocin receptor antagonist (Figure 4c). There was a significant difference in preference for females in the saline group (t (8) = 5.649, p = 0.0005, paired t-test) and oxytocin receptor antagonist 10 mg/kg group (t (9) = 4.668, p = 0.0012, paired t-test).

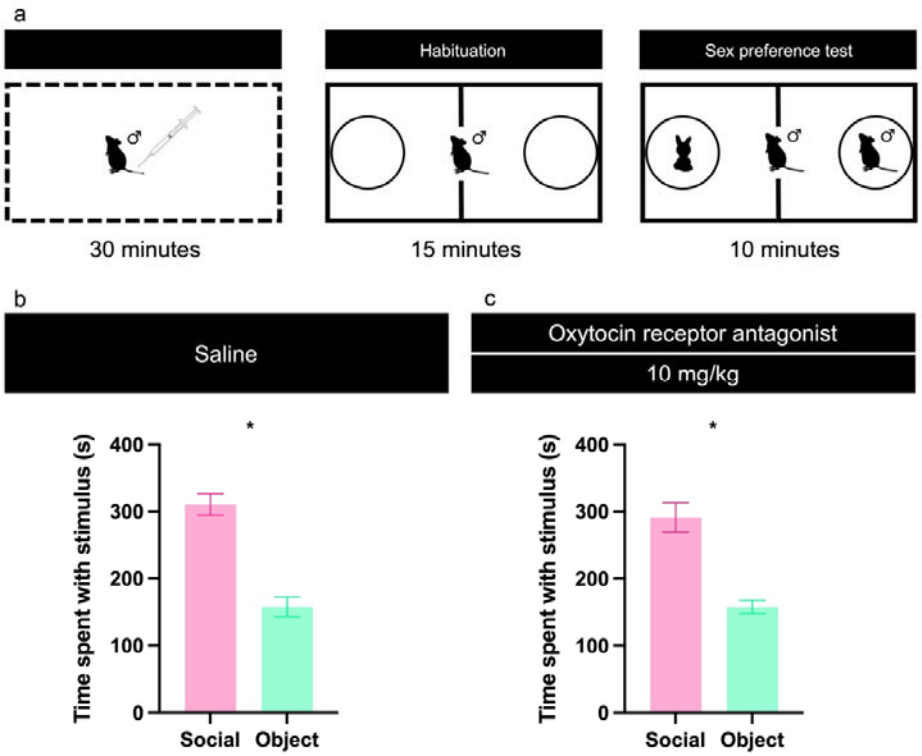
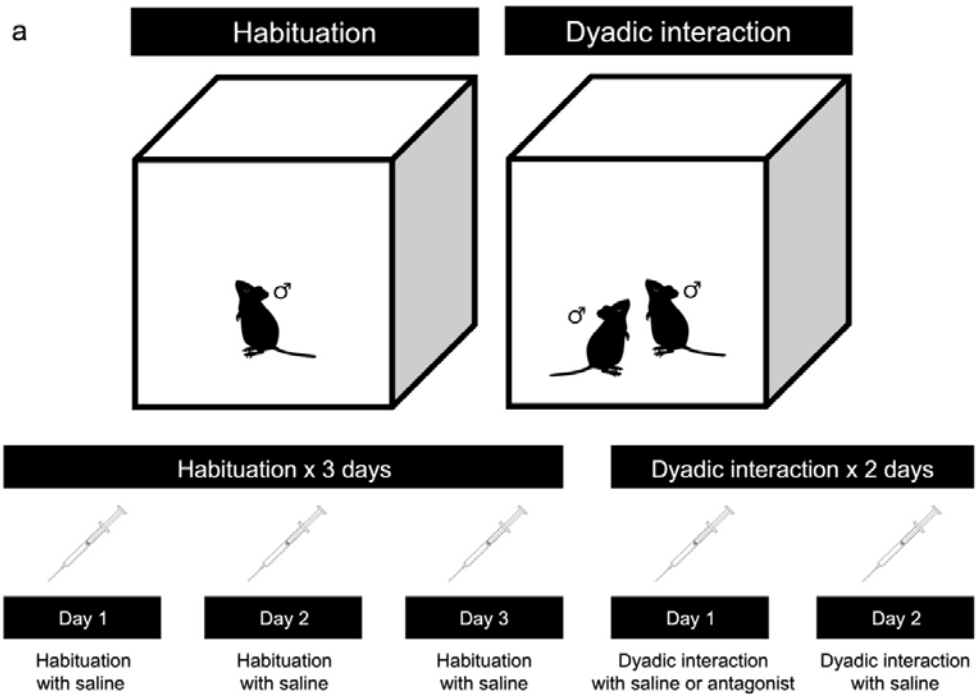


Figure 4. Effect of the oxytocin receptor antagonist on the social preference task.

a. The apparatus used for the test was a custom-made white vinyl chloride box (25 cm length × 50 cm width × 30 cm height), with baskets containing a novel male mouse or a novel object placed at the center of the 25 cm long box and at both ends of the 50 cm wide box. A wall was attached to the center of the 50 cm width to separate the space on the left and right sides. Mice were intraperitoneally injected with saline or 10 mg/kg of oxytocin receptor antagonist and then placed in the box 30 min after the injection. After 15 min of habituation, the mice were temporally removed from the box, and the novel male mouse and the novel object were placed in each basket. The subject mice were then placed in the center of the box and allowed to move freely for 10 min. White noise (75 dB) was presented throughout the experiment to mask the external noise. **b.** Time spent with the novel social and non-social object stimuli in male mice 30 minutes after the injection of saline. Male mice preferred to spend more time with a novel male mouse. There was a significant difference in time spent in novel social stimulus and novel non-social object (t (8) = 5.649, p = 0.0005, paired t-test). **c.** Time spent with the novel social and non-social object stimuli in male mice 30 minutes after the injection of oxytocin receptor antagonist 10 mg/kg. Male mice preferred to spend more time with a novel male mouse after the oxytocin receptor antagonist, suggesting that the oxytocin receptor antagonist had no effect on social preference in male mice. There was a significant difference in time spent in novel social stimulus and novel non-social object (t (9) = 4.668, p = 0.0012, paired t-test). N = 8 for saline condition, N = 9 for oxytocin receptor antagonist condition.

To examine the effects of an oxytocin receptor antagonist on dyadic interactions between two male mice, we observed dyadic interactions in an open-field box in experiment 4 (Figure 5a, Supplementary Movie 4). In this experiment, we injected the oxytocin receptor antagonist or saline into both male cagemate mice 30 min before the experiment, and examined the time spent in dyadic interaction and the average distance between the two mice (Figure 5b-c). Intraperitoneal injection of oxytocin receptor antagonist had no effect on the contact duration (F (1,8) = 0.1425, p = 0.7156, two-way repeated measures ANOVA) or distance between the individuals (F (1,8) = 0.0888 p = 0.7732, two-way repeated measures ANOVA). We found a significant decrease in the contact duration (F (1,8) = 11.21, p = 0.0101, two-way repeated-measures ANOVA) and the distance between the mice (F (1,8) = 6.216, p = 0.0373, two-way repeated-measures ANOVA) from day 1 to day 2. The experimental day × drug interaction had no significant effect on the contact duration (F (1,8) =

0.03277, p = 0.8609, two-way repeated-measures ANOVA) or distance between mice (F (1,8) = 2.332, p = 0.1653, two-way repeated-measures ANOVA).



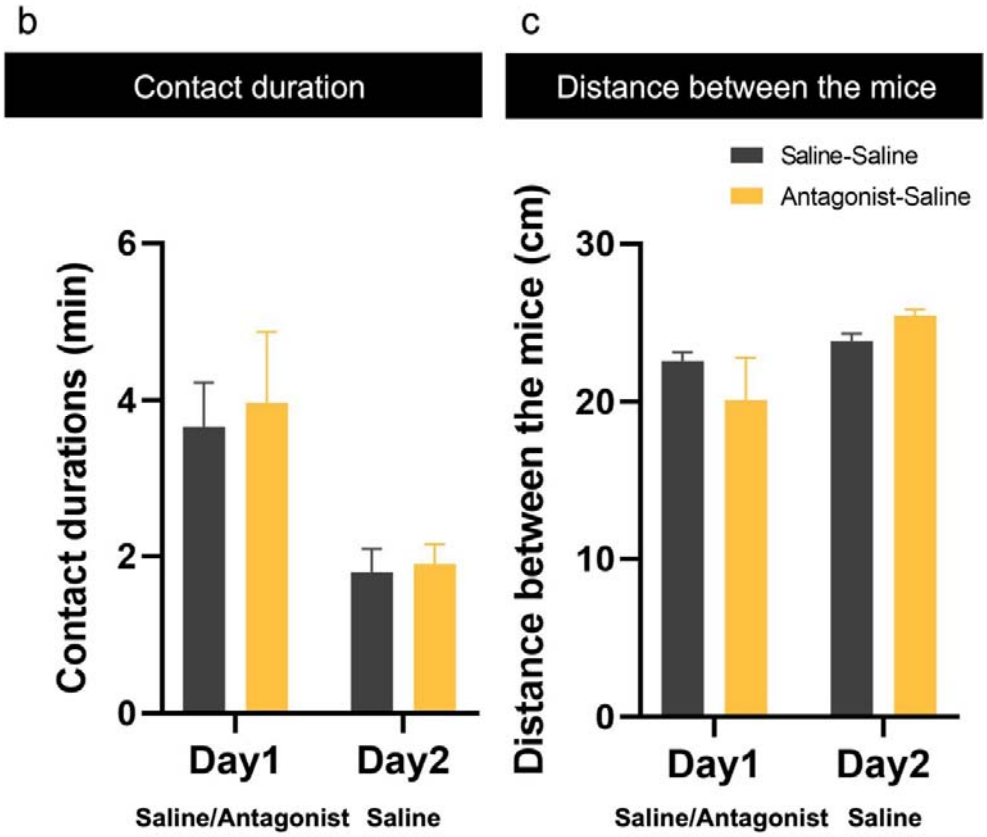


Figure 5. Effects of the oxytocin receptor antagonist on contact time and distance between individuals in dyadic interaction in the open-field box

a. Habituation to the injection and apparatus was performed for 30 min per day for three days prior to the start of the experiment. The apparatus was a custom-made white vinyl chloride box measuring 50 cm in length × 50 cm in width × 50 cm in height. The cameras (Logicool HD Webcam C270n, Logicool) were placed 106 cm above the bottom of the box. The video was recorded on a Windows PC. During habituation, the mice were placed in an open-field box 30 min after receiving an intraperitoneal injection of saline and allowed to explore freely. The experiment was conducted over two successive days. On day 1, an oxytocin receptor antagonist or saline was injected intraperitoneally 30 min prior to the experiment. On day 2, saline was injected intraperitoneally 30 minutes before the experiment. The duration of the experiment was 30 minutes. White noise (75 dB) was presented throughout the experiment to mask the external noise. **c.** We examined the effects of intraperitoneal injection of an oxytocin receptor antagonist on dyadic interactions in an open field box. In this experiment, we injected the oxytocin receptor antagonist or saline into male cagemate mice 30 min before the experiment, and then examined the contact duration and average distance between the two mice. The intraperitoneal injection of oxytocin receptor antagonist had no effect on

the contact duration (F (1,8) = 0.1425, p = 0.7156, two-way repeated-measures ANOVA) or distance between the individuals (F (1,8) = 0.0888 p = 0.7732, two-way repeated-measures ANOVA). The experimental day had a significant effect on the contact duration (F (1,8) = 11.21, p = 0.0101, two-way repeated-measures ANOVA) and distance between the mice (F (1,8) = 6.216, p = 0.0373, two-way repeated-measures ANOVA). The experimental day × drug interaction had no significant effect on the contact duration (F (1,8) = 0.03277, p = 0.8609, two-way repeated-measures ANOVA) or distance between mice (F (1,8) = 2.332, p = 0.1653, two-way repeated-measures ANOVA). Error bars indicate standard error of mean. N = 10 (5 pairs) for each condition.

Discussions

To clarify the role of oxytocin receptors in social rank and other social behaviors, this study examined the effects of intraperitoneal injection of the oxytocin receptor antagonist L-368,899, which crosses the blood-brain barrier and selectively antagonizes central oxytocin receptors in male mice. In experiment 1, we obtained a stable social rank within a cage of four C57BL/6J mice using the tube test after habituation and training. Following the determination of social rank in the group for three consecutive days, we systemically administered the oxytocin receptor antagonist to first- and second-rank mice. Intraperitoneal injection of the oxytocin receptor antagonist had no effect on first-rank mice but had an effect on second-rank mice in the social rank, suggesting that the role of oxytocin in the maintenance of the social rank is rank-dependent. In experiment 2, sex preference was impaired without affecting social preference by the intraperitoneal injection of oxytocin receptor antagonist. In experiment 3, intraperitoneal injection of oxytocin receptor antagonist had no effect on the social preference, supporting the existing finding that oxytocin receptor. These results in experiment 2 & 3 support the existing finding that oxytocin plays an important role in sexual behavior or motivation[17-20] but not in social interactions.[11,19,21,22] In experiment 4, contact time and distance between the male mice in dyadic interaction in the open-field situation were not affected by the intraperitoneal injection of the oxytocin receptor antagonist, confirming that oxytocin is not important in direct social interaction. Taken together, our results demonstrate that the role of oxytocin in the social behavior of male mice is limited to specific contexts.

In experiment 1, stable social rank was obtained using a tube test. Total time spent for the match during the stable three consecutive days was different between the rank pairing. This result is consistent with previous findings using a tube test.[1,2,4,23] In addition, there was no correlation between the body weight and the ranks. The C57BL/6J mice used in this experiment were inbred, and all mice were almost genetically identical. Because the mice were reared in the same cage from birth, their environmental factors throughout development were almost identical. Nevertheless, a stable and linear social rank relationship was demonstrated for all the cages. This result suggests that social rank is generated even if there are no genetic or inherited differences in the given situation. In this sense, social rank is a product of learning, a long-term change in behavior caused by experiences in their environment.

In the tube test, there was no change in the rank of the first-rank mice after injection of the oxytocin receptor antagonist, demonstrating that oxytocin receptor antagonist administration had no effect on social dominance. Meanwhile, the oxytocin

receptor antagonist affected the rank of second-rank mice. In three of the five groups of oxytocin receptor antagonist-injected second-rank mice, the rank fluctuated on the day or day after the oxytocin receptor antagonist injection. The body weight of the second-rank mice after the oxytocin receptor antagonist was not different from the body weight before the injection, ruling out the possibility that the gain or loss of the body weight affected the fluctuated rank. One possible explanation for this result is the difference in the neurobiological states between dominant and subdominant mice. In a study that utilized autoradiography to determine the degree of oxytocin and vasopressin receptor binding in many brain regions in mice of different social ranks, the differential expression of oxytocin receptors in the brain was observed in dominant, subdominant, and subordinate mice.[16] Different baseline expression levels of oxytocin receptors might explain the differential effects of oxytocin antagonists on dominant and subdominant mice in the present study.

Another possible explanation for the rank-dependent effects of oxytocin receptor antagonists is the difference in the necessity of individual discrimination between first- and second-rank mice. For first-rank mice, there is little need for individual discrimination, since every mouse to compete with was a subordinate for the first-rank mice. For second-rank mice, however, they need to change their behavior according to whether the opponent is dominant or subordinate, so individual recognition is important. If individual discrimination or response selection based on information is impaired, the rank of second-rank mice fluctuates. The difference in the necessity of individual discrimination might explain the differential effects of the oxytocin receptor antagonist on first- and second-rank mice.

In the tube test, the oxytocin receptor antagonist was administered after the social rank of the mice stabilized. The finding that the oxytocin receptor antagonist has no effect on first-rank mice indicates that oxytocin receptors do not play an important role in the "maintenance" of social dominance. The role of the oxytocin receptors on the “acquisition” of social dominance is still unknown. Oxytocin receptor antagonists inhibit the acquisition of partner preference in female prairie voles.[24] According to the social salience hypothesis of oxytocin,[25] the critical role of oxytocin may be related to the acquisition but not the maintenance of social signals. Further studies are needed to clarify the effect of oxytocin receptor manipulation on the acquisition of social rank.

In experiment 2, we examined the role of oxytocin receptors in sex preference in male mice. A significant preference for mice of the opposite sex was observed in the saline group, confirming that male mice prefer to spend more time with females. The preference for females in the male mice decreased in a dose-dependent

manner in the oxytocin receptor antagonist group. In particular, there was no significant preference for female mice in the dose of the oxytocin receptor antagonist at 3 and 10 mg/kg. In addition, there was no significant difference in the total time spent with male and female mice, implying that social preference, irrespective of sex preference, was not impaired by injection of the oxytocin receptor antagonist. We also demonstrated that sex preference was impaired with the oxytocin receptor antagonist at 3 and 10 mg/kg. This result replicated the finding by the study conducted by Haskal de la Zerda et al.[19]. These results of impaired social preference after the blockade of the oxytocin receptors is in contrast to the results obtained from oxytocin knockout mice[11,21,22] but are consistent with the oxytocin receptor knockout mice.[26,27]

In experiment 3, the intraperitoneal injection of the oxytocin receptor antagonist had no effect on the social interaction with an unfamiliar mouse. In experiment 4, the intraperitoneal injection of the oxytocin receptor antagonist to two familiar cagemate mice had no effect on the contact duration and distance between the male mice in the dyadic interaction in the open-field box. These results indicate that oxytocin is not important for social interactions between male mice. This result confirmed that the impaired sex preference of male mice in experiment 2 was not due to an increased preference for the same sex in male mice. Both the contact duration and distance between the male mice decreased on the day after injection of the oxytocin receptor antagonist in experiment 4. This result suggests that habituation to the existence of the other in the open-field context occurred in both oxytocin receptor antagonist and saline injection groups. Habituation is also a type of learning, and the subject mice are well habituated to the box and experimental room before the dyadic interaction. The result indicates that the oxytocin receptor is not critical in learning the context of the dyadic interaction in the open-field box.

Taken together with the results found in experiments 1, 2, 3 and 4, intraperitoneal injection of the oxytocin receptor antagonist might affect the response selection based on individual recognition. In experiment 1, the second-rank mice need to choose their response based on the information about the opponents, but such complicated response selection is not necessary in experiment 2, 3, and 4. Compared to primates and birds, mice and other rodents have not developed vision during evolution. In the perceptual world of mice, olfaction likely plays an important role in discriminating between individuals. Indeed, oxytocin receptors are known to be distributed in many olfaction-related brain regions,[28] and inhibition of oxytocin in the anterior olfactory nucleus impairs the olfactory perception of individuals without affecting non-social odor discrimination.[29] A previous important finding suggests that

sensory signals, such as olfactory and somatosensory cues, may not play a critical role in determining social rank in the tube test.[29] Altered individual discrimination induced by the impairment of olfactory perception contributes to impaired sex preference by the oxytocin receptor antagonist, but not to rank-dependent social rank alternation.

Recently, many laboratories have reported clear behavioral differences between dominant and subordinate mice in the same cage. Chou et al.[30] further showed that the most dominant mice spent more time in the tube even if the material in the tube prevented further forward movement to the exit of the tube with no opponent, implying that one of the critical factors that determines the social rank in a cage may not be the condition of the dominant mice, but the conditions of the subordinate mice. Kunkel and Wang[23] reported an increased social preference in dominant mice. A recent finding suggests that social dominance and mnemonic abilities are correlated in young human children and mice.[31] These results demonstrate that the behavior of the dominant mouse is different from that of other cagemates, implying that various social and nonsocial behaviors are modulated by social rank.

The relationship between oxytocin and social memory has been repeatedly demonstrated in oxytocin knockout mice[11] and in microinjections of the oxytocin receptor antagonist in the medial amygdala and lateral septum.[32] However, the result of the dyadic interaction indicates that individual recognition of familiar mice might not be impaired by the oxytocin receptor antagonist because there was no impairment in the contact time and distance between the male mice in the dyadic interaction on the day and day after the injection of the oxytocin receptor antagonist. In this experiment, we used familiar mice in the cages of two mice. In this situation, there are only dominant and subordinate mice in the cage, which is different from the cage of four mice that have a more complicated social structure. For mice in a large social network, the necessity of individual discrimination and response selection based on this information is critical and complicated. The difference in the size of the social structure might cause differential effects of the oxytocin receptor antagonist on rank-dependent fluctuations in social rank. Our results extend the previous finding by demonstrating that the oxytocin receptor antagonist may play an essential role in the complicated social context that requires response selection based on individual recognition. Further studies are necessary to elucidate the psychological and neurobiological mechanisms underlying social ranking.

The concentrations of 3 or 10 mg/kg of the oxytocin receptor antagonist used in this study were higher than those used in previous studies on rodents[19,33,34] and primates.[17] A concentration of 10 mg/kg was chosen as it had clear effects in

experiment 2 and in a study that examined the effects of the oxytocin receptor antagonist on observational fear conditioning in mice.[10] A non-human primate study with rhesus monkeys validated that peripherally administered L-368,899 entered the cerebrospinal fluid (CSF) and accumulated in the hypothalamus, septum, orbitofrontal cortex, amygdala, and hippocampus, but not in other areas.[17] In that study, the authors also demonstrated that peripherally administered L-368,899 at 1 and 3 mg/kg reduced or eliminated interest in infant and sexual behavior. Studies in mice have also shown that an oxytocin receptor antagonist dose of 3 mg/kg affects sex preference and other tasks.[19] Despite the strong oxytocin receptor blockade, oxytocin receptor antagonist administration had no effect on social dominance in the first-rank mice, confirming that oxytocin is not important for dominant behavior.

There are some limitations in this study. We did not investigate the effect of the oxytocin receptor antagonist on third- and fourth-rank mice because we intended to examine the mechanism of social dominance when we started this research project. In the future study, manipulating the oxytocin level in both dominant and subordinate mice will be an important step to understand the mechanism of social rank. We also did not conduct ovariotomy or manipulation of the estrous cycle for the stimulus female mouse. To control the condition of the stimulus, manipulating the sexual condition of the female mouse may be important. In addition, we did not use oxytocin receptor ligand, oxytocin, in our experiments. In the future, manipulating the level of oxytocin by oxytocin itself or oxytocin agonist in central and peripheral nervous systems will be an important step to understand the role of oxytocin level in social behavior including the social rank.

In general, research on oxytocin has focused a great deal of attention on its role in social behavior. However, the majority of the research has been based on findings from studies in which oxytocin itself is administered to alter social behavior.[40-50] Few studies have examined the effects of centrally acting selective antagonists of oxytocin receptors on behavior, compared to the attention given to oxytocin itself or oxytocin agonists. The experimental results of the present study, in which the effects of oxytocin receptor antagonists were limited to specific situations, suggest that oxytocin receptors do not play an important role in various social behaviors. This evidence is supported by the "social salience hypothesis" of oxytocin,[24] which suggests that oxytocin may play an important role in the salience of social stimuli by way of its relationship with the neurotransmitter dopamine.

Conclusions

In this study, we examined the effect of systemic administration of the oxytocin receptor antagonist L-368,899, which crosses the blood-brain barrier and selectively antagonizes central oxytocin receptors, on (1) the social rank of the mice using a tube test, (2) sex preference with place preference task, and (3) dyadic interaction between the mice in the open-field task. We obtained a stable rank for mice reared in the same cage. Intraperitoneal injection of the oxytocin receptor antagonist to first-rank mice had no effect on social rank, suggesting that oxytocin is not critical for the maintenance of social rank in the highest rank individual. On the other hand, the injection of the oxytocin receptor antagonist to second-rank mice caused fluctuations in social rank, suggesting that the role of oxytocin in social rank is rank-dependent. Second, intraperitoneal injection of the oxytocin receptor antagonist impaired the sex preference of male mice in the place preference task, confirming that oxytocin plays an essential role in sexual behavior. Third, intraperitoneal injection of the oxytocin receptor antagonist had no effect on the dyadic interaction between male mice in the open-field box, suggesting that oxytocin is not essential for social interactions between male mice in a semi-naturalistic social context. Taken together, these results indicate that the role of oxytocin in male mice is limited to a specific context of social behavior. We have only begun to learn how the brain represents social rank.[35-37] Future studies need to specify the psychological factors that determine social rank in depth, and the neurobiological causal and correlational relationship between social rank and its underlying neurobiological mechanisms need to be elucidated.

STAR Methods

Subjects

All data were collected from 2-6 month old adult male wild-type C57BL/6J mice. The mice were maintained on a 12:12 light cycle. All the experiments were conducted during the dark phase of the light cycle. The mice had unrestricted access to food and water in their cages. Their weights were monitored daily. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Committee of Keio University. This study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Procedure

Experiment 1: Tube test

In experiment 1, we examined the effects of intraperitoneal administration of the oxytocin antagonist L-368,899 on social rank in mice. We used 3-6 month old 24 adult male C57BL/6J mice. All the mice were reared and group-housed with four individuals in their home cages. All mice were born to the same parents in each cage. The procedure of the tube test followed the protocol described by Fan et al.,[38] with some modifications. The tube test experiment was divided into three phases: habituation, training, and testing. In the habituation phase, a short tube (35 mm in diameter, 2 mm thickness, 10 cm long, acrylic, Figure 1a) was placed in the cage, and the mice were allowed free contact with the tube for two hours for two days (Figure 1b, Supplementary movie 1). During the training phase, a long tube (35 mm diameter, 2 mm thickness, 30 cm long, acrylic, Figure 1a) was used, and each mouse was trained to pass through the tube 10 times in total (Figure 1c, Supplementary movie 2). Mice were started from each entrance on the left and right sides five times each for two days. In the testing phase, the mice competed in a round-robin competition for the tube test (Figure 1d). We conducted the tube test in the 63.5 cm length x 40.0 cm width x 60.0 cm height box (MED Associates) with the door kept opened. A long tube (35 mm diameter, 2 mm thickness, 30 cm long, acrylic, Figure 1a) was used in the test phase. White noise (75 dB) was presented throughout the experiment to mask the external noise. Cameras (Logicool HD Webcam C270n, Logicool) were placed on the side of the tube and were located 30 cm apart from the tube. While the mice performed the tube test, a movie was recorded on a Windows PC. Because there were left and right entrance conditions, the mice had to compete against the same opponent twice from each starting location. The order of matches was set randomly using a random number generator. Mice are ranked in order of number of wins. If the mouse won and lost to

the same opponent, it was considered as a draw in this experiment. To eliminate potential order fluctuations induced by the effects of needle penetration and injection, saline was administered intraperitoneally to all four mice 30 min before the start of every experiment. After the injection of the saline, we placed them back into their cage with their cage mate. The tube test was initiated when the experimenter placed the mice in the tube and simultaneously released their hands from their tails (Supplementary Movie 3). In the test, the winner was determined when either the mouse pushed the other mouse out of the tube or stepped back and out of the tube. The mouse whose hind leg went outside the tube was the loser, and the mouse that stayed in the tube longer than the opponent was the winner. The winner/loser was determined in all competitions without setting a time limit. When all ranks of the four mice in the cage were kept stable for three consecutive days, we determined that the rank in the cage was stabilized. Obtaining a stable rank in the cage took at least three days. Before the injection of the oxytocin receptor antagonist to the first-rank individual, it took 1-2 weeks (mean = 9.4 days, standard error of the mean = 2.05 days). Before the injection of the oxytocin receptor antagonist to the second-rank individual, it took about a week (mean = 6.2 days, standard error of the mean = 1.66 days). After the rank had stabilized for three consecutive days in the test, the first- or second-rank individual was administered intraperitoneal injections of 10 mg/kg oxytocin receptor antagonist on the next day. The other mice were administered an intraperitoneal injection of saline. On the day after oxytocin receptor antagonist administration, all four mice received saline, and the experiment continued until the third day after oxytocin receptor antagonist administration (Figure 1e). After the injection of the saline or oxytocin receptor antagonist, we placed them back into their cage with their cage mate.

Experiment 2: Sex preference task

To examine whether oxytocin receptor antagonists affect sex preference in male mice, we used a place preference task (Figure 3). We used 2-4 month old 24 adult male C57BL/6J mice and 2-4 month old 6 adult male and female C57BL/6J mice as stimuli. All mice were reared and single- or group-housed with 2-4 individuals in their home cages. A custom-made white vinyl chloride box (25 cm length × 50 cm width × 30 cm height) was used for the test. Male and female mice were placed in a white cylindrical pen stand (8 cm diameter × 10 cm height). The pen stands containing the stimulus individuals were placed at the center of both ends of a 50 cm wide box. A 500 mL empty glass bottle was placed on top of the pen stand to prevent the subject mouse from climbing. Two partition walls (5 cm length × 5 mm thickness × 30 cm height) were attached to both sides of the center of the 50 cm width box to separate the spaces on

the left and right sides. The cameras (Logicool HD Pro Webcam C920r, Logicool) were placed 80 cm above the bottom of the box. While the mice performed the sex preference test, a movie was recorded on a Windows PC. Mice were intraperitoneally injected with 3 or 10 mg/kg of oxytocin receptor antagonist or saline and then placed in the box 30 min after the injection. After 15 min of habituation, the mice were temporally removed from the box, and the stimulus individuals, male and female C57BL/6J mice, were placed in the basket. The subject mice were then placed again in the center of the box and allowed to explore freely in the box for 10 min. White noise (75 dB) was presented throughout the experiment to mask the external noise.

Experiment 3: Social preference task

To examine whether oxytocin receptor antagonists affect social preference in male mice, we used a social preference task (Figure 4). We used 2-4 month old 24 adult male C57BL/6J mice and 2-4 month old 17 adult male and female C57BL/6J mice as stimuli. All mice were reared and single- or group-housed with 2-4 individuals in their home cages. A custom-made white vinyl chloride box (25 cm length × 50 cm width × 30 cm height) was used for the test. Male and female mice were placed in a white cylindrical pen stand (8 cm diameter × 10 cm height). The pen stands containing the stimulus individuals were placed at the center of both ends of a 50 cm wide box. A 500 mL empty glass bottle was placed on top of the pen stand to prevent the subject mouse from climbing. Two partition walls (5 cm length × 5 mm thickness × 30 cm height) were attached to both sides of the center of the 50 cm width box to separate the spaces on the left and right sides. The cameras (Logicool HD Pro Webcam C920r, Logicool) were placed 80 cm above the bottom of the box. While the mice performed the sex preference test, a movie was recorded on a Windows PC. Mice were intraperitoneally injected with 3 or 10 mg/kg of oxytocin receptor antagonist or saline and then placed in the box 30 min after the injection. After 15 min of habituation, the mice were temporally removed from the box, and the stimulus individuals, male and female C57BL/6J mice, were placed in the basket. The subject mice were then placed again in the center of the box and allowed to explore freely in the box for 10 min. White noise (75 dB) was presented throughout the experiment to mask the external noise.

Experiment 4: Dyadic interaction in the open-field box

To examine the effects of an oxytocin receptor antagonist on dyadic interactions between two male mice, we observed dyadic interactions in an open-field box (Figure 4, Supplementary Movie 4). We used 2-3 month old 20 adult male C57BL/6J mice. All the mice were group-housed with two individuals in their home cages. Habituation to the saline injection and apparatus was performed for 30 min per day for three days prior to

the start of the experiment. The apparatus was a custom-made white vinyl chloride box measuring 50 cm in length × 50 cm in width × 50 cm in height. The cameras (Logicool HD Webcam C270n, Logicool) were placed 106 cm above the bottom of the box. While the mice performed the dyadic interaction in the open-field box, a movie was recorded on a Windows PC. During habituation, the animals were placed in an open-field box immediately after receiving an intraperitoneal injection of saline and were allowed to behave freely. The experiment was conducted over two successive days. On day 1, an oxytocin receptor antagonist or saline was injected intraperitoneally 30 min prior to the experiment. On day 2, saline was injected intraperitoneally 30 minutes before the experiment. The duration of the experiment was 30 minutes. White noise (75 dB) was presented throughout the experiment to mask the external noise.

Drug

The oxytocin receptor antagonist L-368,899 (CAS 160312-62-9, Tocris Bioscience) was dissolved in saline, divided into small aliquots, and stored in a deep freezer at -80°C until further use. Prior to the experiment, we defrosted the oxytocin receptor antagonist solution and administered it intraperitoneally at a dose of 3 mg/kg in experiment 2 or 10 mg/kg in experiments 1, 2, 3, and 4. For habituation and control conditions, saline was administered intraperitoneally, as in the drug condition. The concentration of the oxytocin receptor antagonist used in this study (3 or 10 mg/kg) was relatively higher than that used in previous studies (1 or 3 mg/kg), which reported the behavioral effects of such administration.[17,19,32,33] A concentration of 10 mg/kg was chosen because it yielded the maximal effect in experiment 2 and a previous study in mice.[10]

Analysis

In all experiments, RStudio and GraphPad Prism were used for data analysis. In experiments 2, 3, and 4, we used an open-source visual programming framework, Bonsai[39] to track the activities of mice using computer vision analysis. The videos were converted to grayscale, smoothened, and monochrome inverted. The mice were identified by setting a contrast threshold. The locomotor activity of the mice in the box was quantified by measuring the changes in the central coordinates of the mice. In experiment 2 and 3, we defined the mice as spending time with the stimulus if they were located within 7.5 cm from the edge of the box containing stimulus mice. In experiment 4, we determined the distance between two male mice. If the distance between the two mice was within 5 cm, we defined the mice as being in contact with each other.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability

The original codes written for the analyses are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

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Author contributions

D.N. and K.T. designed all the experiments. D.N. collected all the tube test data. D.N. and K.T. collected data on sex preference, social preference, and dyadic interaction tasks with the help of H.H., S.Y., and K.H.. H.H., R.T., and Y.U. helped to set up the experimental apparatus. D.N. and K.T. analyzed the data. K.T. wrote the manuscript. D.N. and K.T. created all the figures. D.N., K.Y., Y.U., K.H., and K.T. discussed the data and commented on the manuscript accordingly. K.T. revised the manuscript.

References

1. Wang F, Kessels HW, Hu H. (2014). The mouse that roared: neural mechanisms of social hierarchy. Trends in Neuroscience, 37, 674-682.
2. Wang F, Zhu J, Zhu H, Zhang Q, Lin Z, Hu H. (2011). Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. *Science*, 334, 693-697.
3. Nyby J, Dizinno GA, Whitney G. (1976). Social status and ultrasonic vocalizations of male mice. *Behavioral Biology*, 18, 285-289.
4. Zhou T, Zhu H, Fan Z, Wang F, Chen Y, Liang H, Yang Z, Zhang L, Lin L, Zhan Y, Hu H. (2017). History of winning remodels thalamo-PFC circuit to reinforce social dominance. *Science*, 357, 162-168.
5. Fan S, Weinberg-Wolf H, Piva M, Dal Monte O, Chang SWC. (2020). Combinatorial Oxytocin Neuropharmacology in Social Cognition. *Trends Cogn Sci*, 24, 8-12.
6. Young LJ, Wang Z. (2004). The neurobiology of pair bonding. *Nat Neurosci*, 7, 1048-54.
7. Insel TR. (1990). Oxytocin and maternal behavior. In N. A. Krasnegor & R. S. Bridges (Eds.), Mammalian parenting: Biochemical, neurobiological, and behavioral determinants (pp. 260–280). Oxford University Press.
8. Bosch OJ, Meddle SL, Beiderbeck DI, Douglas AJ, Neumann ID. (2005). Brain oxytocin correlates with maternal aggression: link to anxiety. *J Neurosci*, 25, 6807-6815.
9. Chang SW, Barter JW, Ebitz RB, Watson KK, Platt ML. (2012). Inhaled oxytocin amplifies both vicarious reinforcement and self reinforcement in rhesus macaques (*Macaca mulatta*). *Proc Natl Acad Sci U S A.*, 109, 959-964.
10. Pisansky MT, Hanson LR, Gottesman II, Gewirtz JC. (2017). Oxytocin enhances observational fear in mice. *Nat Commun*, 8, 2102.
11. Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. (2000). Social amnesia in mice lacking the oxytocin gene. *Nat Genet*, 25, 284-288.
12. Donaldson ZR, Young LJ. (2008). Oxytocin, vasopressin, and the neurogenetics of sociality. *Science*, 322, 900-904.
13. Andari E, Nishitani S, Kaundinya G, Caceres GA, Morrier MJ, Ousley O, Smith AK, Cubells JF, Young LJ. (2020). Epigenetic modification of the

oxytocin receptor gene: implications for autism symptom severity and brain functional connectivity. *Neuropsychopharmacology*, 45, 1150-1158.

14. Neumann ID, Slattery DA. (2016). Oxytocin in General Anxiety and Social Fear: A Translational Approach. *Biol Psychiatry*, 79, 213-221.
15. Shilling PD, Feifel D. (2016). Potential of Oxytocin in the Treatment of Schizophrenia. *CNS Drugs*, 30, 193-208.
16. Lee W, Hiura LC, Yang E, Broekman KA, Ophir AG, Curley JP. (2019). Social status in mouse social hierarchies is associated with variation in oxytocin and vasopressin 1a receptor densities. *Horm Behav*, 114, 104551.
17. Boccia ML, Goursaud AP, Bachevalier J, Anderson KD, Pedersen CA. (2007). Peripherally administered non-peptide oxytocin antagonist, L368,899, accumulates in limbic brain areas: a new pharmacological tool for the study of social motivation in non-human primates. *Horm Behav*. 52, 344-351.
18. Carter CS. (1992). Oxytocin and sexual behavior. *Neurosci Biobehav Rev*, 16, 131-144.
19. Haskal de la Zerda S, Netser S, Magalnik H, Wagner S. (2020). Impaired sex preference, but not social and social novelty preferences, following systemic blockade of oxytocin receptors in adult male mice. *Psychoneuroendocrinology,* 116, 104686.
20. Oti T, Satoh K, Uta D, Nagafuchi J, Tateishi S, Ueda R, Takanami K, Young LJ, Galione A, Morris JF, Sakamoto T, Sakamoto H. (2021). Oxytocin Influences Male Sexual Activity via Non-synaptic Axonal Release in the Spinal Cord. Curr Biol, 31, 103-114.
21. Choleris E, Gustafsson JA, Korach KS, Muglia LJ, Pfaff DW, Ogawa S. (2003). An estrogen-dependent four-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-alpha and -beta knockout mice. *Proc Natl Acad Sci U S A*, 100, 6192-6197.
22. Crawley JN, Chen T, Puri A, Washburn R, Sullivan TL, Hill JM, Young NB, Nadler JJ, Moy SS, Young LJ, Caldwell HK, Young WS. (2007). Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. Neuropeptides, 41, 145-163.
23. Kunkel T, Wang H. (2018). Socially dominant mice in C57BL6 background show increased social motivation. *Behav Brain Res*, 336, 173-176.

24. Insel TR, Hulihan TJ. (1995). A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behav Neurosci*, 109, 782-789.
25. Shamay-Tsoory SG, Abu-Akel A. (2016). The Social Salience Hypothesis of Oxytocin. *Biological Psychiatry*, 79, 194-202.
26. Sala M, Braida D, Donzelli A, Martucci R, Busnelli M, Bulgheroni E, Rubino T, Parolaro D, Nishimori K, Chini B. (2013). Mice heterozygous for the oxytocin receptor gene (Oxtr(+/-)) show impaired social behaviour but not increased aggression or cognitive inflexibility: evidence of a selective haploinsufficiency gene effect. *J Neuroendocrinol*, 25, 107-118.
27. Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, Yanagisawa T, Kimura T, Matzuk MM, Young LJ, Nishimori K. (2005). Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc Natl Acad Sci U S A*, 102, 16096-16101.
28. Vaccari C, Lolait SJ, Ostrowski NL. (1998). Comparative distribution of vasopressin V1b and oxytocin receptor messenger ribonucleic acids in brain. *Endocrinology*, 139, 5015-5033.
29. Oettl LL, Ravi N, Schneider M, Scheller MF, Schneider P, Mitre M, da Silva Gouveia M, Froemke RC, Chao MV, Young WS, Meyer-Lindenberg A, Grinevich V, Shusterman R, Kelsch W. (2016). Oxytocin Enhances Social Recognition by Modulating Cortical Control of Early Olfactory Processing. *Neuron*. 90, 609-621.
30. Chou YJ, Lu YH, Ma YK, Su YS, Kuo TH. (2021). The decisive role of subordination in social hierarchy in weanling mice and young children. *iScience*, 24, 102073.
31. Chou YJ, Ma YK, Lu YH, King JT, Tasi WS, Yang SB, Kuo TH. (2022). Potential cross-species correlations in social hierarchy and memory between mice and young children. *Commun Biol*, 5, 230.
32. Lukas M, Toth I, Veenema AH, Neumann ID. (2013). Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: male juvenile versus female adult conspecifics. *Psychoneuroendocrinology*, 38, 916-926.
33. Blitzer DS, Wells TE, Hawley WR. (2017). Administration of an oxytocin receptor antagonist attenuates sexual motivation in male rats. *Horm Behav*, 94, 33-39.

34. Olszewski PK, Waas JR, Brooks LL, Herisson F, Levine AS. (2013). Oxytocin receptor blockade reduces acquisition but not retrieval of taste aversion and blunts responsiveness of amygdala neurons to an aversive stimulus. *Peptides*, 50, 36-41.
35. Dwortz MF, Curley JP, Tye KM, Padilla-Coreano N. (2022). Neural systems that facilitate the representation of social rank. *Philos Trans R Soc Lond B Biol Sci*, 377, 20200444.
36. Lee W, Dowd HN, Nikain C, Dwortz MF, Yang ED, Curley JP. (2021). Effect of relative social rank within a social hierarchy on neural activation in response to familiar or unfamiliar social signals. *Sci Rep*, 11, 2864.
37. Padilla-Coreano N, Batra K, Patarino M, Chen Z, Rock RR, Zhang R, Hausmann SB, Weddington JC, Patel R, Zhang YE, Fang HS, Mishra S, LeDuke DO, Revanna J, Li H, Borio M, Pamintuan R, Bal A, Keyes LR, Libster A, Wichmann R, Mills F, Taschbach FH, Matthews GA, Curley JP, Fiete IR, Lu C, Tye KM. (2022). Cortical ensembles orchestrate social competition through hypothalamic outputs. *Nature*, 603, 667-671.
38. Fan Z, Zhu H, Zhou T, Wang S, Wu Y, Hu H. (2019). Using the tube test to measure social hierarchy in mice. *Nat Protoc*, 14, 819-831.
39. Lopes G, Bonacchi N, Frazão J, Neto JP, Atallah BV, Soares S, Moreira L, Matias S, Itskov PM, Correia PA, Medina RE, Calcaterra L, Dreosti E, Paton JJ, Kampff AR. (2015). Bonsai: an event-based framework for processing and controlling data streams. *Frontiers in Neuroinformatics*, 9, 7.
40. Tan O, Musullulu H, Raymond JS, Wilson B, Langguth M, Bowen MT. (2019). Oxytocin and vasopressin inhibit hyper-aggressive behaviour in socially isolated mice. *Neuropharmacology*, 156, 107573.
41. Cherepanov SM, Gerasimenko M, Yuhi T, Furuhara K, Tsuji C, Yokoyama S, Nakayama KI, Nishiyama M, Higashida H. (2021). Oxytocin ameliorates impaired social behavior in a Chd8 haploinsufficiency mouse model of autism. *BMC Neurosci*, 22, 32.
42. Takemoto T, Baba M, Yokoyama K, Kitagawa K, Nagayasu K, Ago Y, Seiriki K, Hayata-Takano A, Kasai A, Mori D, Ozaki N, Takuma K, Hashimoto R, Hashimoto H, Nakazawa T. (2022). Oxytocin ameliorates impaired social behavior in a mouse model of 3q29 deletion syndrome. *Mol Brain*, 15, 26.

43. Zhang X, Li Q, Zhang M, Lam S, Sham PC, Bu B, Chua SE, Wang W, McAlonan GM. (2015). The Effect of Oxytocin on Social and Non-Social Behaviour and Striatal Protein Expression in C57BL/6N Mice. *PLoS One*, 10, e0145638.
44. Sakamoto T, Sugimoto S, Uekita T. (2019). Effects of intraperitoneal and intracerebroventricular injections of oxytocin on social and emotional behaviors in pubertal male mice. *Physiol Behav*, 212, 112701.
45. Mahalati K, Okanoya K, Witt DM, Carter CS. (1991). Oxytocin inhibits male sexual behavior in prairie voles. *Pharmacol Biochem Behav*, 39, 219-222.
46. Kohli S, King MV, Williams S, Edwards A, Ballard TM, Steward LJ, Alberati D, Fone KCF. (2019). Oxytocin attenuates phencyclidine hyperactivity and increases social interaction and nucleus accumben dopamine release in rats. *Neuropsychopharmacology*, 44, 295-305.
47. Yoshida M, Takayanagi Y, Inoue K, Kimura T, Young LJ, Onaka T, Nishimori K. (2009). Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J Neurosci*, 29, 2259-2271.
48. Lukas M, Toth I, Reber SO, Slattery DA, Veenema AH, Neumann ID. (2011). The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology*, 36, 2159-2168.
49. Arakawa H, Blanchard DC, Blanchard RJ. (2015). Central oxytocin regulates social familiarity and scent marking behavior that involves amicable odor signals between male mice. *Physiol Behav*, 146, 36-46.
50. Choe KY, Bethlehem RAI, Safrin M, Dong H, Salman E, Li Y, Grinevich V, Golshani P, DeNardo LA, Peñagarikano O, Harris NG, Geschwind DH. (2022). Oxytocin normalizes altered circuit connectivity for social rescue of the Cntnap2 knockout mouse. *Neuron*, 110, 795-808.