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Effects of post-weaning social isolation on social behaviors and oxytocinergic activity in male and female rats

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ABSTRACT

Aims: Post-weaning social deprivation is known to induce behavioral and neuronal alterations associated with anxiety and stress responses in adulthood. However, the effects of social deprivation on the development of sociability are poorly understood. We examined the effects of social deprivation on subsequent social behaviors and oxytocinergic activity using socially-isolated (approximately two months post-weaning) male and female rats.

Main methods: The behaviors were analyzed using a social preference test and a social approach test. Immunohistochemical investigations were conducted in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) to examine the effects of social isolation on oxytocinergic activity in these regions. Oxytocinergic activity was measured by quantifying the number of oxytocin neurons expressing Fos following exposure to a novel conspecific. In all of the experiments of this study, ovariectomized females were used for social stimuli.

Key findings: The behavioral results show that isolation-reared females, but not males, displayed impaired social preference and decreased social approach towards ovariectomized females, compared with the pair-reared group, suggesting low priority of processing social versus non-social stimuli and low motivation for contact with a stranger, respectively. The immunohistochemical results show that social isolation decreased both the number and the ratio of Fos-positive cells in oxytocin neurons in the PVN in females, but not in males, following exposure to ovariectomized females. In the SON, the Fos-positive ratio was decreased in isolation-reared females, but not in males, compared with the pair-reared group.

Significance: Post-weaning social isolation changed social behaviors and oxytocinergic activity in female rats, suggesting that in female rats post-weaning social experiences contribute to the development of sociability. These findings could impact the treatment of social dysfunction in humans.

1. Introduction

Stressful social experiences during early-life increase the risk of developing neuropsychiatric disorders related to anxiety, mood, and personality in humans [1, 2, 3], although the mechanisms remain unclear. Secretory regulation of oxytocin (OT), a mammalian peptide hormone, is one of the physiological functions affected by the early-life environment [4]. For example, children who experience high levels of early-life neglect exhibit lower peripheral levels of OT following physical contact with their mothers than control children reared in a typical home environment [5]. OT synthesized in the hypothalamic neurons is delivered via axonal transport to the posterior pituitary and secreted into the blood stream where it promotes uterotonic action and milk secretion. In addition, OT is known to regulate social behaviors (affiliative, aggressive, and parental behaviors) through brain receptor activation [6]. In this instance, the peptide acts as a neuromodulator via synaptic transmission or by diffusion into cerebrospinal fluid in discrete brain regions such as the amygdala, septum, and nucleus accumbens [7, 8].

Tanaka et al. previously reported that post-weaning social isolation impairs social recognition performance in rats [9]. Inducing isolation is an experimental paradigm to deprive animals of social experiences during development. In post-weaned laboratory rats, physical contact with cage-mates, called "social play," is generally observed. Levels of social play display an inverted U-shaped curve across development, peak levels occur at around 30 days old before declining following sexual maturation [10]. Deprivation of social play, accompanied by isolation, may influence

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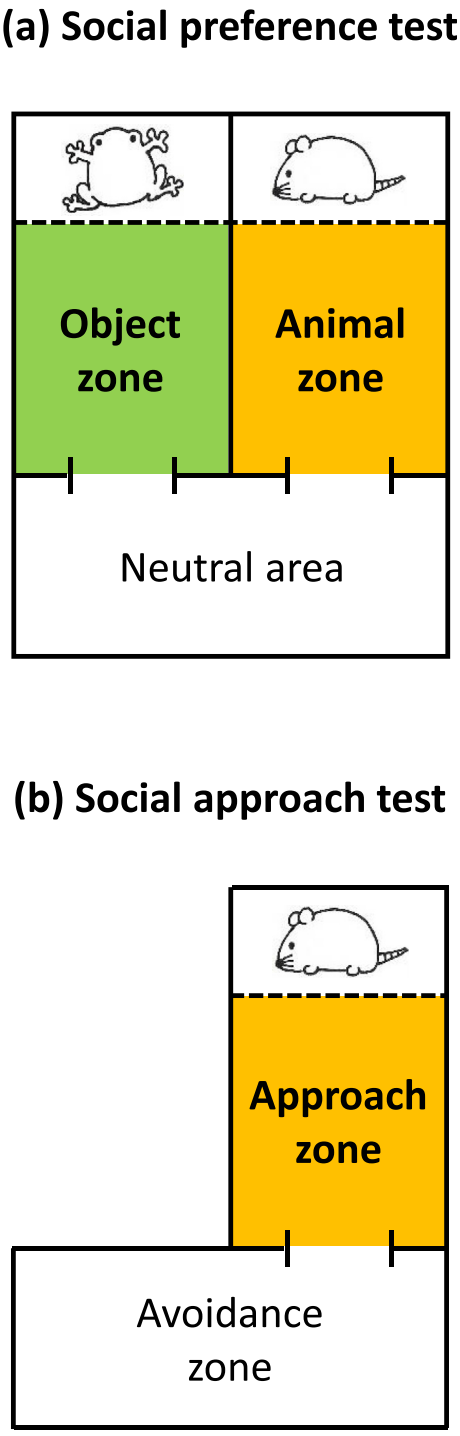

Fig. 1. Apparatuses for the social preference and the social approach tests. (a) The apparatus for the social preference test was composed of a neutral area and two zones (object and animal). A test animal can enter the object or the animal zone from the neutral area. Each zone contains a small chamber separated by a clear punch-out board. An object (rubber frog) and a stimulus animal were put in the object and animal chambers, respectively. (b) The apparatus for the social approach test was composed of avoidance and approach zones. The approach zone contained a small chamber separated by a clear punch-out board. A stimulus animal was put in the chamber of the approach zone.

endogenous OT effects on development because OT release is promoted by non-noxious sensory inputs such as touch, vibration, and warmth [11, 12]. The effects of social isolation on social behaviors and the non-apeptide (OT and vasopressin) system has been examined in studies of the prairie vole, a rodent model of social monogamy and biparental care [13, 14, 15]. In prairie voles, pair-bonding and parental care are promoted by OT and vasopressin [16]. However, in non-monogamous, uniparental species such as rats, the peptides regulate other social behaviors without affecting pair-bonding and male parental care, suggesting a different evolution of sociability behaviors and the nonapeptide system in these species. In this study, we examined the effects of post-weaning social isolation on social behaviors and oxytocinergic activity in adulthood using male and female rats. Social behaviors were analyzed in two tests, social preference and social approach, in order to assess the priority for processing of social versus non-social stimuli and the motivation for contact with a novel conspecific, respectively. Immunohistochemical investigations were conducted in the main OT production sites, the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus, to examine the effects of post-weaning social isolation on oxytocinergic activity. Following exposure to a stranger, the OT activity level was assessed by quantifying the number of OT neurons expressing the neuronal activation marker, Fos. In all of the experiments of this study, stimulus animals were ovariectomized females, which were same- and opposite-sex for female and male subjects, respectively.

2. Materials and methods

2.1. Animals

Long-Evans rats born from nulliparous females (SLC, Shizuoka, Japan) were used. The animals were bred in plastic cages (25 × 40 × 20 cm) containing sawdust in a controlled light and temperature environment (12 h light-dark cycle, 23 °C) with food and water provided *ad libitum*. All procedures were conducted in compliance with the guiding principles for the care and use of laboratory animals, approved by Kochi University as well as the National Institute of Health Guidelines (NIH Publication No. 8023, revised 1978).

The rats were weaned at 23 days old. The animals were then randomly divided into two rearing conditions, paired (two same-sex siblings) or isolated. Pups from the same dam were evenly divided into the two rearing condition groups to avoid any confounding factor of litters; for example, a litter composed of seven males and eight females was divided into two paired males, two isolated males, four paired females, and four isolated females (the remaining three males were not used in this study). The paired and isolated animals were housed in a room with olfactory and auditory but no visual cues from other cages. All groups received minimal handling, with weekly cleaning consisting of changing the cage before experimental manipulations. Conditions were sustained for approximately two months until the beginning of behavioral testing or histological analysis. Twelve female rats (purchased from SLC) were ovariectomized at 7–8 weeks old and used as stimulus animals in the experiments. Each stimulus animal was used 6–8 times for behavioral tests and 4 times for social exposure before brain collection, balanced across each rearing group. The stimulus animals were always female, regardless of the sex of the test animal.

2.2. Social preference test

A social preference test was conducted on 10–12-week old rats (n = 46). The rats were tested in an acrylic apparatus (illustrated in Fig. 1a) composed of a neutral area (20 × 48 cm) and two compartments, object and animal zones (40 × 24 cm each). The neutral area and the object and animal zones were separated by opaque walls with a passage (12 cm square) to allow a test animal to enter the object or animal zone from the neutral area. Each zone contained a small chamber (12 × 24 cm) separated by a clear board (H 35 cm × W 23 cm × T 0.5 cm), in which holes (5 mm in diameter) were drilled at intervals of 1 cm.

The test was conducted in the light phase, 2–4 hours after lights were turned on. Animals were transferred to the test room two hours before

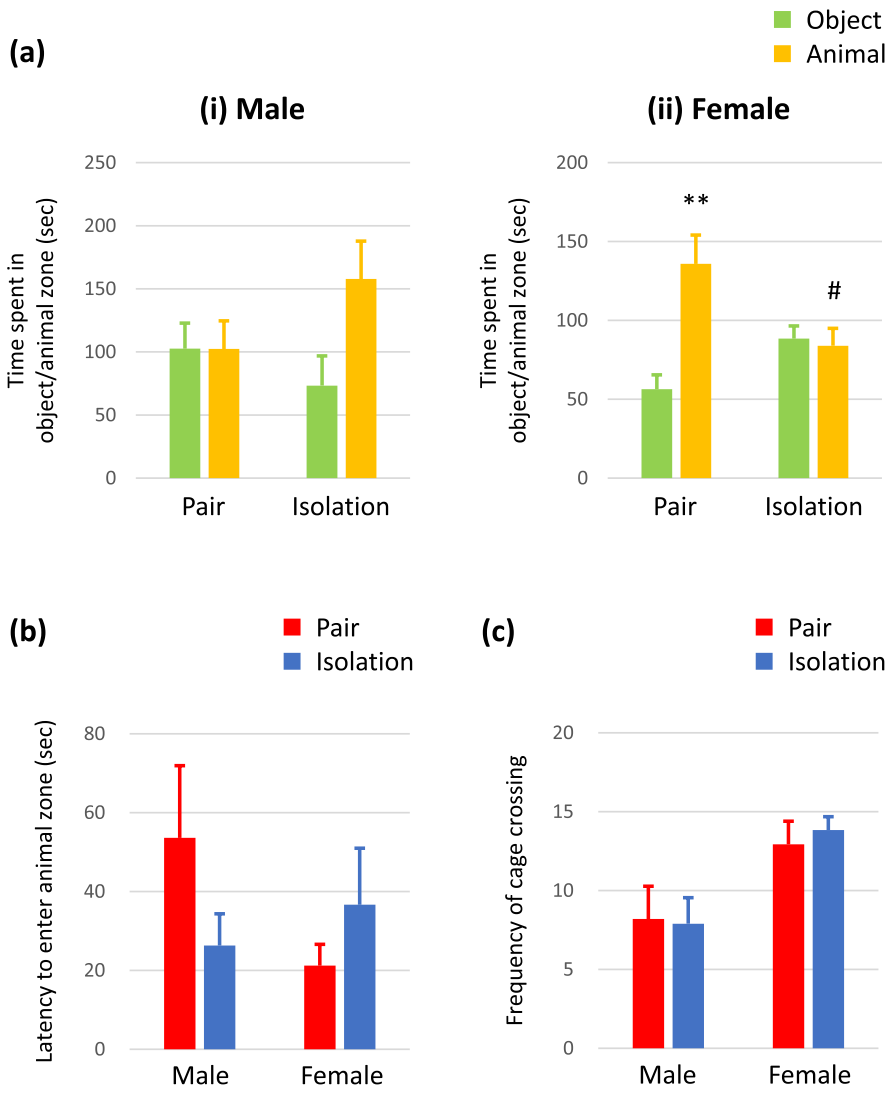

Fig. 2. Social preference test. (a) Histogram illustrating time spent in the object and animal zones by paired and isolated groups of males (i) and females (ii). Results were analyzed by a two-way analysis of variance followed by a Bonferroni post hoc test. (b) Histogram illustrating latency to enter the animal zone. Results were analyzed by Student's or Welch's *t*-test. (c) Histogram illustrating frequency of cage crossing. Results were analyzed by Student's or Welch's *t*-test. Values are expressed as mean ± standard error of mean. **: $p < 0.01$, animal zone vs. object zone; #: $p < 0.05$, isolated group (male, n = 10; female, n = 14) vs. paired group (male, n = 10; female, n = 12).

the tests begun. The test animal was put in the apparatus 5 min prior to testing for habituation to the apparatus, before being returned to the cage. Subsequently, a novel object (rubber frog) and a novel animal (age-matched, ovariectomized, conspecific female) were put in the object and the animal chambers, respectively, and the test animal was placed in the middle of the neutral area. The test animal was allowed to access both the object and animal zones. The object and animal zones were counter-balanced. The behavior of the test animal was recorded with a video camera (HDR-PJ670, SONY, Tokyo, Japan) for 5 min. Time spent in the object and the animal zones was measured using the video analysis software, Image J (ver. 1.50b; National Institute of Mental Health, Bethesda, MD). The latency to enter the approach zone and the frequency of cage crossing were manually measured by an investigator blinded to experimental conditions. The animals were returned to the breeding room after testing was completed.

2.3. Social approach test

A social approach test was conducted on 10–12-week old rats (n = 32), which were different to those animals used for the social preference test. The rats were tested in an acrylic apparatus (illustrated in Fig. 1b) composed of avoidance (20 × 48 cm) and approach (40 × 24 cm) zones. A test animal was allowed to enter the approach zone from the avoidance zone through a passage (12 cm square). The approach zone contained a small chamber (12 × 24 cm) separated by a clear punch-out board. The test was performed in the same manner as the social preference test with the exception of the following points: 1) a novel stimulus animal (age-matched, ovariectomized, conspecific female) was put in the chamber of the approach zone, and 2) a test animal was put in the middle of the avoidance zone and time spent in the approach zone was measured.

2.4. Immunohistochemistry

Immunohistochemical analysis was conducted on 12–13-week old rats (n = 24), randomly selected from animals used for the social preference test. The test animal and a novel stimulus animal (age-matched, ovariectomized, conspecific female) were moved from their home cages to the same model of cage containing fresh sawdust. Each subject was exposed to a different stimulus animal from that exposed in the social preference test. The brains were collected two hours after the move to the test cage, based on the evidence that Fos expression peaks two hours after neuronal activation [17]. During brain collection, animals were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused through the left cardiac ventricle with 0.1 M phosphate-buffered saline (pH 7.4) followed by 400 ml fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were immediately removed, postfixed overnight in the same fixative, equilibrated in phosphate buffer containing 20% sucrose at 4 °C, and sliced in the coronal plane on a freezing cryostat at a thickness of 20 μm. The brain sections were stored in cryoprotectant containing 30% sucrose and 30% ethylene glycol in

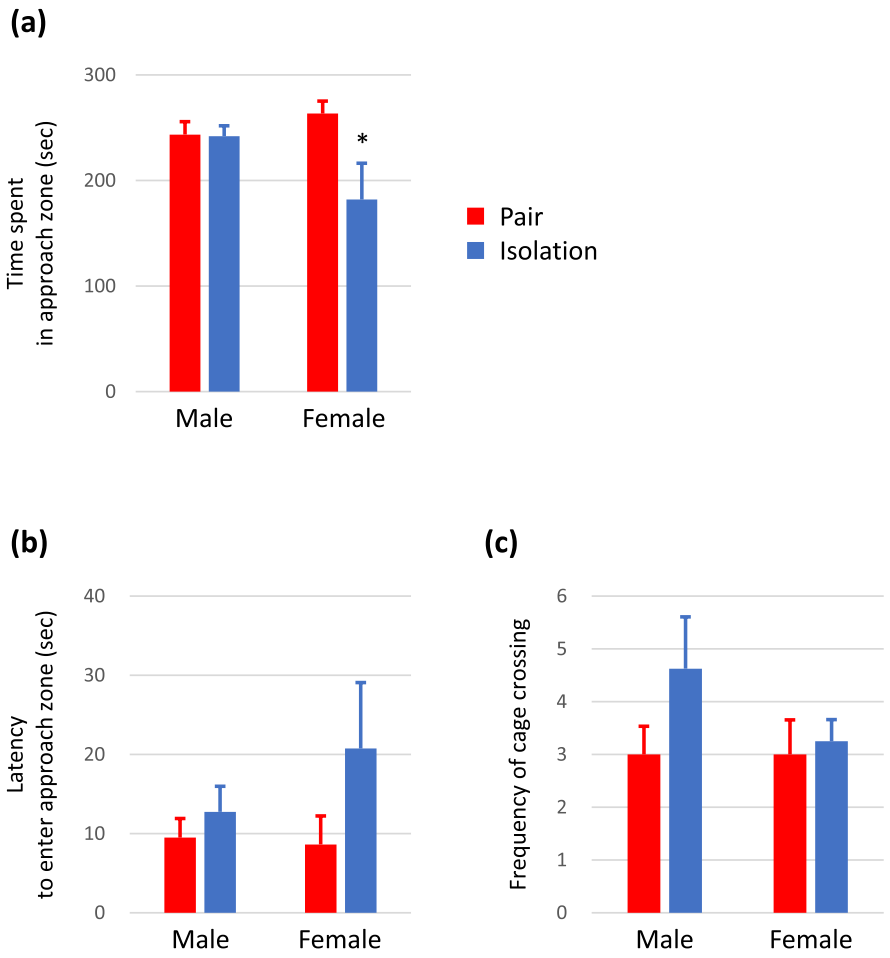

Fig. 3. Social approach test. (a) The histogram illustrates time spent in the approach zone by the paired and isolation reared groups of males and females. (b) The histogram illustrates latency to enter the approach zone. (c) The histogram illustrates frequency of cage crossing. Results were analyzed by Student's or Welch's *t*-test. Values are expressed as mean ± standard error of mean. *: $p < 0.05$, isolated group vs. paired group (n = 8 in each group).

phosphate buffer at -20 °C until analyzed.

Following a wash in 0.05 M Tris-buffered saline (TBS, pH 7.4) to thoroughly remove cryoprotectant, free-floating sections were incubated in a mixed diluent of rabbit polyclonal anti-OT (1:10000; ImmunoStar, Hudson, WI) and mouse monoclonal anti-Fos (1:1000; sc-271243, Santa Cruz Biotechnology, CA) antibodies with 2% normal donkey serum for 48 hours at 4 °C. The sections were then incubated in a mixed diluent of fluorescein isothiocyanate-conjugated donkey anti-rabbit immunoglobulin G (IgG) and Cy3-conjugated donkey anti-mouse IgG antibodies (1:1000, respectively; Jackson ImmunoResearch Laboratories, West Grove, PA) for two hours at room temperature in the dark and then washed in 0.05 M TBS. The sections were then mounted on silane-coated slides with VECTASHIELD® mounting medium (H-1200; Vector Laboratories, Burlingame, CA). All antisera were diluted in TBS containing 0.25% Triton X-100 and 0.3% bovine serum albumin.

Photographs were captured using a microscope (ECLIPSE 80i; Nikon, Tokyo, Japan) with a lighting device and a CCD camera (VB-7000; KEYENCE, Osaka, Japan). The number of positive cells for OT and Fos were counted in four sections (600 × 800 μm/section) per animal throughout the rostral-caudal extent of the PVN and the SON. The Fos-positive ratio in OT-positive cells was calculated by dividing the number of OT and Fos double-positive cells by the total number of OT-positive cells. The OT-positive ratio in Fos-positive cells was calculated by dividing the number of OT and Fos double-positive cells by the total number of Fos-positive cells. Anatomically matched sections (PVN, Bregma -1.80 to -1.88 mm; SON, Bregma -1.40 to -1.60) were used for the analysis. Nuclei classification was identified according to the rat brain atlas [18].

2.5. Statistical analysis

A two-way repeated measure analysis of variance (ANOVA) (2 rearing conditions × 2 zones) was conducted for the social preference test, followed by Bonferroni post hoc tests. In all other analyses, differences between the paired and isolated rearing conditions were assessed using Student's or Welch's *t*-test. Significance was set at $p < 0.05$. These analyses were conducted by using the statistical software, Kaleida Graph (ver. 4.5; HULINKS, Tokyo, Japan).

3. Results

3.1. Social preference test

Results of the social preference test were analyzed to assess the effects of two factors, zones and rearing conditions, on time spent in the animal and the object zones. There was no significant effect of rearing conditions on time spent in either the object or the animal zone in males ($F_{1,18} = 3.02$, $p = 0.091$; Fig. 2a (i)). However, in females, an interaction was observed ($F_{1,24} = 10.90$, $p < 0.01$; Fig. 2a (ii)). Post hoc analyses showed no significant difference in time spent in either the object or the animal zone for the isolated group, while the paired group spent more time in the animal zone than in the object zone ($p < 0.01$). Time spent in the animal zone was also less in the isolated group than in the paired group ($p < 0.05$). There were no significant differences in the latency to enter the

Table 1

Quantitative immunohistochemical data for OT and Fos levels in the PVN

	Male		Female	
	Pair	Isolation	Pair	Isolation
OT-positive cells				
Overall cell number	23.4 (±1.26)	23.6 (±3.62)	23.3 (±2.45)	23.4 (±1.87)
Fos-positive ratio (%)	71.7 (±6.32)	71.9 (±4.23)	71.4 (±2.53)	45.6 ** (±4.11)
Fos-positive cells				
Overall cell number	101.0 (±5.54)	87.2 (±9.51)	78.7 (±7.31)	86.8 (±5.58)
OT-positive ratio (%)	16.7 (±1.88)	19.4 (2.99)	22.5 (±3.78)	12.1 * (±0.90)
Double-positive cells	17.0 (±2.11)	16.8 (±2.45)	16.8 (±2.30)	10.6 * (±1.03)

Quantitative immunohistochemical data for OT and Fos levels in the SON

	Male	Female	Male	Female
	Pair	Isolation	Pair	Isolation
OT-positive cells				
Overall cell number	21.3 (±1.45)	22.4 (±1.68)	18.6 (±1.87)	17.8 (±1.62)
Fos-positive ratio (%)	83.2 (±3.24)	79.2 (±2.99)	81.9 (±1.47)	67.2 * (±4.76)
Fos-positive cells				
Overall cell number	75.9 (±4.01)	74.3 (±4.67)	49.7 (±5.65)	60.7 (±7.12)
OT-positive ratio (%)	23.9 (±2.68)	23.8 (1.06)	31.1 (±2.04)	19.8 ** (±0.90)
Double-positive cells	17.9 (±1.65)	17.8 (±1.60)	15.4 (±1.45)	12.4 (±1.54)

Results were analyzed by Student's or Welch's *t*-test. Values are expressed as mean ± standard error of mean. *: $p < 0.05$, **: $p < 0.01$, isolated group vs. paired group (n = 6 in each group). OT, oxytocin; PVN, paraventricular nucleus; SON, supraoptic nucleus.

approach zone between paired and isolated groups of males ($t_{18} = 2.08$, $p = 0.06$; Fig. 2b) and females ($t_{24} = 1.01$, $p = 0.33$; Fig. 2b). There was also no significant difference in the frequency of cage crossing between the two rearing conditions in males ($t_{18} = 0.11$, $p = 0.91$; Fig. 2c) and females ($t_{24} = 0.53$, $p = 0.60$; Fig. 2c).

3.2. Social approach test

In the social approach test, time spent in the approach zone was compared between the paired and isolated groups. There was no difference in time spent in the approach zone between the paired and isolated groups in males ($t_{14} = 0.10$, $p = 0.92$). However, in females, the isolated group spent less time in the approach zone compared with the paired group ($t_{14} = 2.24$, $p < 0.05$) (Fig. 3a). The latency to enter the approach zone was not significantly different between paired and isolated groups of males ($t_{14} = 0.81$, $p = 0.43$; Fig. 3b) and females ($t_{14} = 1.34$, $p = 0.21$; Fig. 3b). There was also no significant difference in the frequency of cage crossing between the two rearing conditions in males ($t_{14} = 1.45$, $p = 0.17$; Fig. 3c) and females ($t_{14} = 0.32$, $p = 0.75$; Fig. 3c).

3.3. Fos expression in OT neurons after social stimuli

Double-immunohistochemical staining for OT and Fos was conducted in the PVN and the SON. Immunoreactivities of OT and Fos were detected in the cell soma and the nucleus, respectively. The quantitative data is outlined in Table 1. There were no significant differences in OT-positive cell numbers in both male and female, paired and isolated groups in the PVN (male; $t_{10} = 0.05$, $p = 0.96$: female; $t_{10} = 0.01$, $p = 0.99$) and the SON (male; $t_{10} = 0.47$, $p = 0.65$: female; $t_{10} = 0.32$, $p = 0.76$). There were also no significant differences in the number of Fos-positive cells in paired and isolated groups in the PVN (male; $t_{10} = 1.26$, $p = 0.24$: female; $t_{10} = 0.89$, $p = 0.40$) and SON (male; $t_{10} = 0.25$, $p = 0.80$: female; $t_{10} = 1.20$, $p = 0.26$).

A comparison of the number of OT and Fos double-positive cells between the paired and isolated groups is presented graphically in Fig. 4a. There was no significant difference between paired and isolated male groups in the number of double-positive cells in the PVN ($t_{10} = 0.06$, $p = 0.95$) and the SON ($t_{10} = 0.04$, $p = 0.97$). In females, however, the isolated group had a lower number of double-positive cells than the paired group in the PVN ($t_{10} = 2.48$, $p < 0.05$; Fig. 4d for representative photographs of the female PVN), but not in the SON ($t_{10} = 1.44$, $p = 0.18$). A similar tendency was observed for the Fos-positive ratio in total OT-positive cells (Fig. 4b) and OT-positive ratio in total Fos-positive cells (Fig. 4c). There were no significant differences in the Fos-positive ratios in paired and isolated male groups in the PVN ($t_{10} = 0.02$, $p = 0.98$) and the SON ($t_{10} = 0.90$, $p = 0.39$). In females, the isolated group had lower Fos-positive ratios in the PVN ($t_{10} = 5.35$, $p < 0.01$) and the SON ($t_6 = 2.95$, $p < 0.05$) compared to the paired group. There were no significant differences in OT-positive ratios in the PVN ($t_{10} = 0.75$, $p = 0.47$) and the SON ($t_{10} = 0.05$, $p = 0.96$) in paired and isolated male groups. In females, the isolated group had lower OT-positive ratios in the PVN ($t_{10} = 2.67$, $p < 0.05$) and the SON ($t_6 = 5.05$, $p < 0.01$) compared to the paired group.

4. Discussion

The primary finding of this study is that post-weaning social isolation changes social behaviors in female rats. We first conducted the social preference test, using apparatus designed to assess priority for processing of social versus non-social stimuli [19, 20, 21]. In this test, the pair-reared female rats spent more time in the animal zone than in the object zone. The isolation-reared group spent less time in the animal zone than pair-reared animals, and there was no significant difference between the time spent in the object and the animal zones. This result suggests that social isolation reduces the preference for social novelty observed in pair-reared animals. The preferential processing of social over non-social stimuli is commonly observed in healthy adult humans, while it is disrupted in schizophrenia, autism, and the rodent models of these disorders [22, 23, 24]. This bias is thought to result from a difference in reward values between social and non-social stimuli [25, 26]. Additionally, we investigated a simple approach behavior toward a stranger using the social approach test. In this test, the isolation-reared females spent less time in the approach zone compared with the pair-reared group, suggesting decreased motivation for contact with a novel individual. No differences were found between pair- and isolation-reared groups in the latencies to enter the animal zone in the social preference test or the approach zone in the social approach test, although any effect may have been masked by the large differences between individuals. The isolation-induced alterations shown in the female social preference and approach behaviors are indicative of a low level of sociability (the tendency to underestimate the value of social stimuli), and are not secondary effects of social isolation on locomotor activity, because there was no difference in the frequency of cage crossing between paired and isolated groups.

It is well known that social cues evoke OT release [27]. We examined the effects of social isolation on the activity of hypothalamic OT neurons following exposure to an unfamiliar female conspecific. We found that oxytocinergic activity was decreased in response to the stimulus female in the isolation-reared females. The social stimuli were applied in the breeding cage, not the apparatus for behavioral testing, to minimize the effects of environmental changes. The level of oxytocinergic activity was determined by counting the number of Fos-positive OT neurons using an immunohistochemical method. The isolation-reared females exhibited lower Fos-positive cell number and ratio in OT neurons in the PVN following exposure to an unfamiliar female conspecific than the pair-reared group. In addition, in the SON, the Fos-positive ratio of OT neurons was lower in isolation-reared females than pair-reared females. These results suggest that social isolation decreased the oxytocinergic

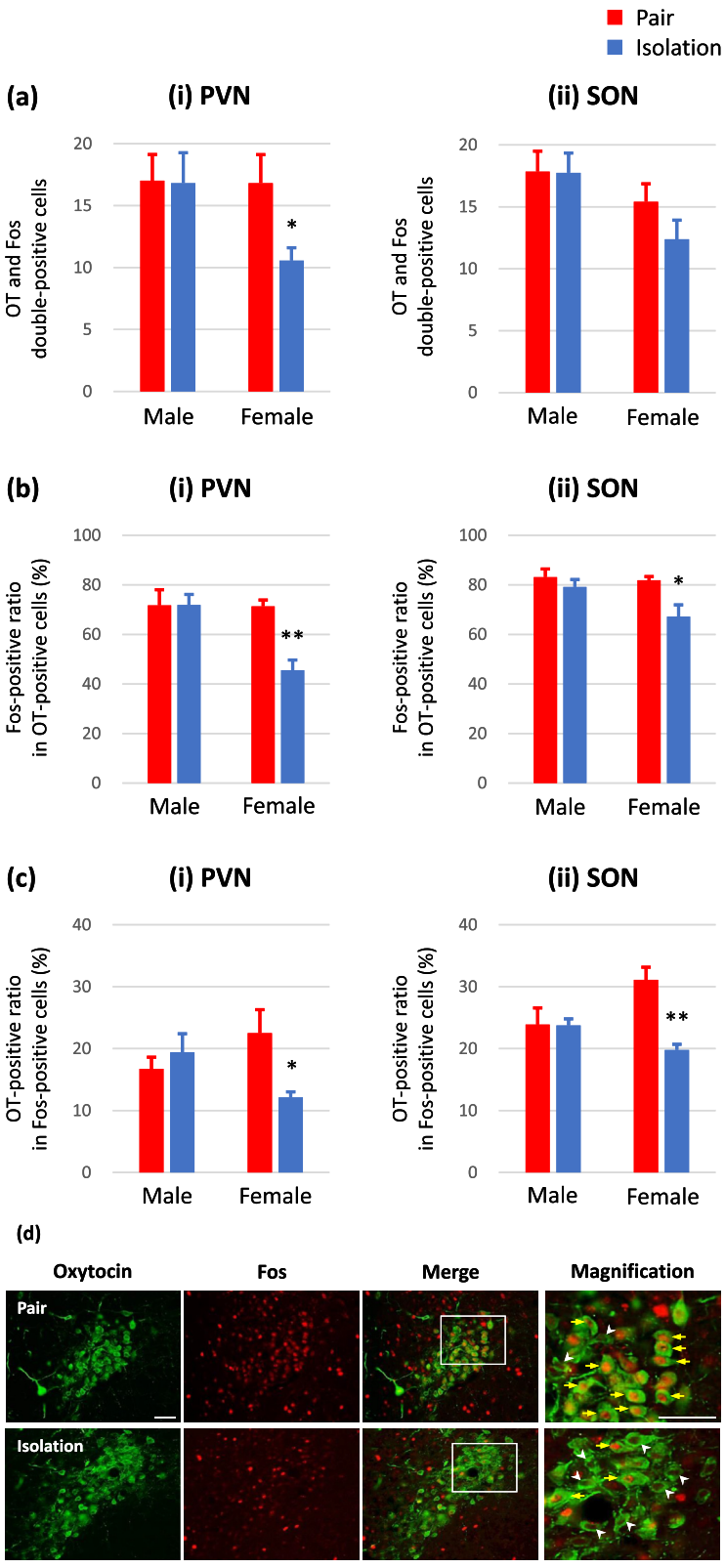

Fig. 4. Immunohistochemical staining for oxytocin (OT) and Fos. (a) Histograms illustrating OT and Fos double-positive cell numbers in the paraventricular nucleus (PVN, (i)) and the supraoptic nucleus (SON, (ii)) of paired or isolated males (n = 6 in each group) and females (n = 6 in each group). (b) Histograms illustrating Fos-positive ratios in total OT-positive cells in the PVN (i) and the SON (ii) of each group. (c) Histograms illustrating OT-positive ratios in total Fos-positive cells in the PVN (i) and the SON (ii) of each group. Results were analyzed by Student's or Welch's *t*-test. Values are expressed as mean ± standard error of mean *: $p < 0.05$, **: $p < 0.01$, isolated group vs. paired group. (d) OT, Fos, and the merged images of the PVN of the paired (upper) and isolated (lower) females are shown. Magnifications of the merged views are shown in the rightmost column. Arrows indicate OT and Fos double-positive cells. Arrowheads indicate Fos-negative, OT-positive cells. Bar = 50 μm.

response to social stimuli in female rats. This explanation was further supported by the isolation-induced decrease in the OT-positive ratio in total Fos-positive neurons in the PVN and SON. OT synthesized in the PVN and the SON regulates social behaviors through axonal projection to, primarily, the forebrain [28, 29]. There is growing evidence that the modulatory effects of brain OT facilitate social preference and approach behaviors [30, 31, 32, 33]. Although the isolation-reared females exhibited a deficit in social preference and a reduction in social approach, it is unclear whether decreased oxytocinergic activity contributed to their behavioral profile in the present study. Future research is required to clarify the mechanism of changes in social behaviors in these animals.

Previous studies have reported that post-weaning social isolation

induces social impairments in male rats [34, 35, 36]. Nevertheless, there were no demonstrable effects of isolation on male social preference and approach behaviors in the current study. However, in the social preference test, even male control rats did not display greater preference for the social stimulus over the non-social stimulus, which has been shown previously in normal rodents [37, 38] and healthy humans [23]. Although there is no clear explanation for the disagreement of our results with previous findings, differences in experimental conditions, such as the species and strain of subjects, testing apparatus, and social stimuli, might be responsible. In particular, it should be noted that stimulus animals were female in all the experiments in this study. The stimulus females were sexually non-receptive, because they had been ovariectomized. However, exposing male subjects to female stimuli is likely to produce a reproductive context, which can influence male social behaviors [39]. Additionally, differences in the effects of social isolation on male and female animals should also be interpreted in consideration of the above limitation. While social isolation altered female, but not male, social behaviors and oxytocinergic activity, the biological valence of the stimulus animals was not comparable between male and female subjects. Reproductive and non-reproductive social behaviors arise from different motivations and can be modulated differently by the nonapeptide system [40, 41, 42]. Therefore, differences between male and female behavioral and histological data in this study do not prove the existence of sex differences in the effects of social isolation.

OT promotes the development of social behaviors [43]. Administration of synthetic OT into adolescent (33–42 days old) rats increases social interaction levels in adulthood [44]. The same research group has demonstrated that OT exposure in adolescent rats enhanced plasma OT concentrations and hypothalamic OT receptor mRNA in adulthood [21], suggesting that OT exposure has organizational effects on the brain OT system during adolescence. The social isolation procedure in the present study deprived animals of social experiences during the developmental period and resulted in alterations of social behaviors and oxytocinergic activity in adult females. Although the critical period of social development remains unclear, adolescent rats are likely to be in the process of maturation. Early social deprivation has been shown to influence social behaviors and the OT system in adulthood. For example, maternal separation has long-lasting effects on social recognition performance [45], intermale aggression [46], maternal care [47], and brain OT receptor binding [48], although there are differences among species and sexes. These findings suggest that mother-infant interactions contribute to developmental alterations of the brain system that is responsible for social behaviors during infancy. Furthermore, post-weaning social experiences, typified by social play with cage-mates, are expected to be important for the new developmental stage of social competence. The isolation-induced behavioral and histochemical abnormalities might be indicative of disruption of the common post-weaning changes in sociability.

5. Conclusion

Post-weaning social isolation impaired social preference and reduced social approach in female rats. Additionally, social isolation decreased female oxytocinergic activity in a social context. Overall, these findings suggest that in female rats, post-weaning social experiences contribute to the development of sociability. This study could have implications for the treatment of social dysfunction associated with neuropsychiatric disorders in humans.

Declarations

Author contribution statement

Kenjiro Tanaka: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yoji Osako: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Kou Takahashi: Analyzed and interpreted the data; Wrote the paper.

Chiharu Hidaka: Performed the experiments; Analyzed and interpreted the data.

Koichi Tomita: Analyzed and interpreted the data.

Kazunari Yuri: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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