Oxytocin and CD38 in the paraventricular nucleus play a critical role in paternal aggression in mice.

室傍核のオキシトシンとCD38がマウスの父親の攻撃行動に重要な役割を果たす

大阪大学大学院

大阪大学・金沢大学・浜松医科大学・千葉大学・福井大学

連合小児発達学研究科

小児発達学専攻

SHABALOVA　Anna

２０２０年３月　博士学位論文

Hormones and Behavior 120 (2020) 104695

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Oxytocin and CD38 in the paraventricular nucleus play a critical role in paternal aggression in mice

Anna A. Shabalova[a,b,1], Mingkun Liang[a,c,1], Jing Zhong[a,d], Zhiqi Huang[a,e], Chiharu Tsuji[a], Natalia A. Shnayder[a], Olga Lopatina[a,f], Alla B. Salmina[a,f], Hiroshi Okamoto[g], Yasuhiko Yamamoto[g], Zeng-Guo Zhong[a,h], Shigeru Yokoyama[a], Haruhiro Higashida[a,f,*]

[a] Department of Basic Research on Social Recognition and Memory, Research Center for Child Mental Development, Kanazawa University, Kanazawa 920-8640, Japan
[b] Department of Socioneurosciences, United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University and University of Fukui, Kanazawa Campus, Kanazawa 920-8640, Japan
[c] Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine, Nanning, Guangxi 530011, China
[d] Department of Physiology, Guangxi University of Chinese Medicine, Xianhu Campus, Nanning, Guangxi 530200, China
[e] Pharmaceutical Sciences, Guangxi University of Chinese Medicine, Xianhu Campus, Nanning, Guangxi 530200, China
[f] Laboratory for Social Brain Studies, Research Institute of Molecular Medicine and Pathobiochemistry, Department of Biochemistry, Krasnoyarsk State Medical University named after Prof. V. F. Voino-Yasenitsky, Krasnoyarsk 660022, Russia
[g] Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Sciences, Kanazawa 920-8640, Japan
[h] Center of Research & Development of New Drugs, Guangxi Traditional Chinese Medical University, Guangxi Zhuang Autonomous Region, Nanning 530001, China

ARTICLE INFO

Keywords:
Parental behavior
Paternal aggression
Oxytocin
CD38
c-Fos
Hypothalamus
Paraventricular nucleus

ABSTRACT

In mammals, the development of healthy offspring requires maternal care. Behavior by lactating mothers toward other individuals is an important component of maternal aggression. However, it is unclear whether fathers display aggression primed by pups (an external factor), and the protection mechanism is poorly understood. To address this question, we examined paternal aggression in the ICR mouse strain. We found that sires exposed to cues from pups and lactating dams showed stronger aggression toward intruders than did sires that were deprived of family cues or exposed to nonlactating mates. c-Fos immunohistochemistry showed that cells in both the paraventricular and supraoptic nuclei (PVN and SON, respectively) in the hypothalamus of sires exposed to any cues were highly activated. However, c-Fos activation in oxytocinergic neurons was increased only in sires exposed to pup cues and solely in the PVN. In *Cd38*-knockout sires, the presence of pups induced no or reduced parental aggression; however, this phenotype was recovered, that is, aggression increased to the wild-type level, after intraperitoneal administration of oxytocin (OT). Specific c-Fos activation patterns induced by pup cues were not found in the PVN of knockout sires. These results demonstrate that the PVN is one of the primary hypothalamic areas involved in paternal aggression and suggest that a CD38-dependent OT mechanism in oxytocinergic neurons is critical for part of the behavior associated with the protection of offspring by nurturing male mice.

1. Introduction

Mammals demonstrate parental behaviors that increase the survival and normal development of their offspring (Dulac et al., 2014; Feldman et al., 2019; Finkenwirth and Burkart, 2018; Lonstein and Fleming, 2002; Lonstein and Gammie, 2002; Perry et al., 2015). Parental behavior targets both biological and foster offspring (Abraham et al., 2014; Abraham and Feldman, 2018; Dulac et al., 2014; Finkenwirth and Burkart, 2018) and is mediated by sensory inputs, neurotransmitters, and hormones, including serotonin, dopamine, prolactin and oxytocin (OT) (Abraham and Feldman, 2018; Bridges, 2015; Bridges and Grattan, 2018; Dulac et al., 2014; Heiming et al., 2013; Jin et al., 2007; Kohl et al., 2018; Kohl and Dulac, 2018; Lei et al., 2017; Numan and Young, 2016). The neurocircuits involved in parental behavior are referred to as the parental (social) brain (Abraham et al., 2014; Donaldson and Young, 2008; Dulac et al., 2014; Heiming et al., 2013).

* Corresponding author at: Department of Basic Research on Social Recognition and Memory, Center for Child Mental Development, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-8640, Japan.
E-mail address: haruhiro@med.kanazawa-u.ac.jp (H. Higashida).
[1] These authors equally contributed.

https://doi.org/10.1016/j.yhbeh.2020.104695
Received 22 August 2019; Received in revised form 21 January 2020; Accepted 21 January 2020

Parental care experiences change both maternal and paternal brains (Abraham et al., 2014; Feldman et al., 2019; Rogers and Bales, 2019).

One obvious parental behavior, parental aggression, occurs when animals attack other individuals to protect their offspring (Dulac et al., 2014; Rogers and Bales, 2019). This type of aggression is a reactive-impulsive subtype of behavior that is more dependent on the hypothalamus and limbic systems than is controlled-instrumental aggression (Nelson and Trainor, 2007). It has been shown that the intensity of maternal aggression is very high during early lactation, that is, the first week postpartum, and then declines until weaning (Giovenardi et al., 1998; Lonstein and Gammie, 2002; Palanza and Parmigiani, 1994). Aggressive behavior in rodents is frequently examined using the resident-intruder test, in which attacks by a resident mouse against an intruder are observed (Kowalczyk et al., 2018; Paterson and Vickers, 1985; Yoshimura et al., 1988). This behavior is based on the protection of biological pups from both male and female strangers (Consiglio et al., 2005; Palanza and Parmigiani, 1994) and on the defense of territory (Liang et al., 2014). The level of aggression demonstrated by new fathers (sires) against a male intruder as part of paternal aggression and the mechanism that mediates the display of paternal aggression by males are not yet fully understood (Trainor et al., 2008).

It has been reported that among the extrinsic factors related to aggressive behavior, pups are powerful stimuli for rodents' social behavior and maternal aggression (Nelson and Trainor, 2007). As intrinsic factors, the (pro)social neuropeptides oxytocin (OT) and arginine vasopressin play important roles in social interaction, memory and maternal aggression (Bosch, 2013; Bridges, 2015; Consiglio, 1996; de Jong and Neumann, 2017; Donaldson and Young, 2008; Finkenwirth et al., 2016). Consistently, brain injection of OT facilitated pup-directed maternal nurturing behavior and increased maternal aggression in lactating rats (Bosch and Neumann, 2012; Consiglio et al., 2005; Jin et al., 2007). OT is a nonapeptide synthesized in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus. We have shown that the release of OT, an intrinsic factor, in the brain is regulated by intracellular calcium and that it depends on CD38, which catalyzes cyclic ADP-ribose production from NAD, and on TRPM2 cation channels, which are warm-temperature sensors (Higashida et al., 2018; Jin et al., 2007; Zhong et al., 2016). Interestingly, in *Cd38*-knockout (CD38 KO) mice, social and parental behaviors, including pup retrieval, are impaired, but external administration of OT rescued these impaired behaviors under the pup retrieval paradigm (Akther et al., 2013). Among the extrinsic factors, the pup cues are more important than the cues from mates in paternal nurturing behavior (Liang et al., 2014). In addition, the male's establishment of territory, which is lost in CD38 KO mice, is critical for sires to retrieve nurturing pups (Liang et al., 2014). However, three types of mice with gene deletions of OT (*Oxt*), OT receptor (*Oxtr*) or *Cd38* exhibit equal degrees of social amnesia (Higashida et al., 2012, 2019; Jin et al., 2007; Modi and Young, 2012; Takayanagi et al., 2005). Male, aggressive behavior is increased in *Oxt* KO mice (Winslow et al., 2000) and in *Oxtr* KO mice (Sala et al., 2011; Takayanagi et al., 2005), while the opposite effect was reported in *Oxt* KO mice (DeVries et al., 1997). However, it has been shown that mice with *Oxtr* haplodeficiency do not display gene-dosage-dependent increases in aggression (DeVries et al., 1997; Sala et al., 2013). To assess this inconsistency in OT-dependent nurturing and parental aggression and because we have not yet carefully measured aggression in CD38 KO mice (Liang et al., 2014), we examined aggression in CD38 KO mice, studied it in relation to OT, and compared it with aggression in *Oxt* KO and *Oxtr* KO mice.

Male nurturing (paternal) behavior is less obvious than female nurturing behavior (Kohl and Dulac, 2018; Liang et al., 2014; Saltzman and Ziegler, 2014). One reason for this difference is that most male animals are not monogamous, the social system most often associated with male parenting. However, we have previously shown that the ICR male mouse displays intensive parental behavior, even though it is not monogamous (Jin et al., 2007; Liu et al., 2013). Our previous results showed that the neural circuitry mediating this type of paternal nurturing behavior includes the medial preoptic area (mPOA), the nucleus accumbens (NAcc), and the ventral pallidum (VP; Akther et al., 2014), suggesting that paternal nurturing behavior is mediated by the same brain regions that mediate maternal nurturing behavior, as proposed by Numan and his colleagues (Numan, 2015; Numan and Stolzenberg, 2009; Numan and Young, 2016). Therefore, the principal aim of this study was to determine whether the mechanism or the neurocircuits for paternal aggression are similar to those for maternal aggression. Here, we tested our hypothesis that the display of maternal and paternal aggression requires offspring cues and is regulated by OT within the PVN and the SON, both of which are located in the hypothalamic aggression area (Kruk, 1991, 2014; Numan, 2015; Numan and Young, 2016), in ICR wild type (WT) and *Cd38* KO (CD38 KO) mice (Jin et al., 2007; Kato et al., 1999). We compared aggression in virgin males and sires who lived with their families or were deprived of such cues. We examined the activation of oxytocinergic neurons in the hypothalamus by c-Fos staining under different interactive conditions, such as males alone and/or males kept with dams and pups, according to Bosch (2005).

2. Materials and methods

2.1. Animals

WT male and female Slc:ICR mice (Institute of Cancer Research of the Charles River Laboratories, Inc., Wilmington, MA, USA) were obtained from Japan SLC, Inc. (Hamamatsu, Japan) through a local distributor (Sankyo Laboratory Service Corporation, Toyama, Japan). The ICR mice were originally obtained from Charles River Laboratories in 1965 and, since then, have been bred in Japan under the alternative name Swiss CD1. The offspring of the WT (ICR) and CD38 KO mice (Jin et al., 2007) were born in our laboratory colony. The pups were weaned at 21–28 days of age and housed in same-sex groups of 3–5 animals until pairing. WT males were paired with WT females, and KO males were paired with KO females. The animals were kept in a nursing cage in our laboratory under standard conditions (24 °C; 12-h light/dark cycle, lights on at 08:00) with food and water provided *ad libitum*. All of the animal experiments were performed in accordance with the Fundamental Guidelines for the Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan and were approved by the Committee on Animal Experimentation of Kanazawa University.

2.2. Behavioral tests

2.2.1. Resident-intruder test

The resident-intruder test is based on animals' territorial defensive behavior against unfamiliar intruding conspecifics (Koolhaas et al., 2013). This test can also be used to examine maternal aggression, as previously described (de Jong et al., 2014). Each resident male (virgin, paired male or sire) was confronted in its home cage by a group-housed (five mice per cage) intruder mouse (ICR or CD38 KO virgin male) for 10 min. Each intruder mouse was used only once to avoid submission/dominance effects after the first interaction. Behavioral interactions during each confrontation were recorded and subsequently scored by an observer. The latency to the first attack, total number of attacks and cumulative duration of aggression were analyzed.

2.2.2. Anxiety-related behavior

Sires with different reproductive experience were evaluated in the open field (OF) and elevated-plus maze (EPM) tests for the assessment of anxiety-related behavior. The animals were moved to the experimental room 30 min prior to the test to allow habituation. The tests

were performed on different days with at least 1 day of rest between the tests. OF and EPM were performed as previously described (Lopatina et al., 2014).

2.2.3. Open field test

Three groups of sires (sires alone, sires exposed to dams and sires exposed to pups) were tested for anxiety-related behaviors as described above. The open field test measures locomotion and anxious behaviors as previously described (Lopatina et al., 2014). The open field box consisted of a square box (600 × 600 × 200 mm) covered with polypropylene sheets inside the wooden box. The center arena (300 × 300 mm) was outlined. Each animal was placed in the box for 10 min. The overall activity in the box was measured, and the amount of time spent and the distance traveled in the center arena were noted. The distance traveled in the field was recorded using a digital video system and ANY-maze software (Lopatina et al., 2014). This paradigm is based on the idea that mice naturally prefer to be near a protective wall rather than in open areas where they may be exposed to danger. After each trial, the test chambers were cleaned with a damp towel and 1% sodium hypochlorite followed by 70% alcohol (Liu et al., 2013).

2.2.4. Elevated plus maze

The elevated plus maze is widely used in the study of anxiety-like behavior (Lopatina et al., 2014). The maze apparatus was elevated to 50 cm above the floor and consisted of a central platform (5 × 5 cm) from which two open arms (5 × 25 cm) and two closed arms (5 × 25 cm with 15-cm high transparent walls) extended in opposite directions. At least 30 min prior to the behavioral test, the mice were brought to the testing room for habituation. At the initiation of each session, the mice were placed on the central platform facing an open arm and were allowed to explore the maze freely for 5 min.

2.3. Aggression of male mice with different reproductive experience

WT and CD38 KO male mice with different types of reproductive experience were assessed for aggression behaviors. (1) Virgin male mice were kept in new individual cages for 7 days ("no reproductive virgin males" group). (2) One male was paired with a female, who was allowed to deliver their pups. Soon after parturition, the pups were removed so that the dam was unable to nurse the pups. The sire was isolated in a new cage for 7 days and exposed to the nonlactating dam every day for 6 h. (3) Another male was paired with a female; after the female delivered their pups, the sire was isolated in a new cage and exposed to the mate dam, which nursed their pups in a separate cage, for 6 h/day for 7 days. (4) Another male was paired with a female; after the female delivered their pups, the sire was kept in a new cage, the dam and pups were kept in another cage, and only the pups were placed in the sire's cage for 6 h every day for 7 days. On the 7th day, (30 min before the resident-intruder testing, the female or/and pups were removed), an unfamiliar male mouse (ICR; virgin male) was introduced into the cage of the experimental resident male, which had already established the territory, for 10 min. The resident-intruder test used here has been previously described (Veenema et al., 2007; Yoshimura et al., 1988). The tests were videotaped for subsequent behavioral scoring, which was performed by two researchers who were blinded to the treatment conditions. The attack latency, the number of attacks and the duration of attacks per male over three days were scored and averaged. Cross-genotype experiments were performed using the above-described combinations of resident and intruder mice of different genotypes.

2.4. Immunohistochemistry

All mice were anesthetized 1 h after the resident-intruder test. The mice were intracardially perfused with cold phosphate-buffered saline (PBS) followed by cold 4% paraformaldehyde (PFA) in PBS solution. The brains were removed and postfixed in a 4% PFA solution overnight at 4 °C. Brain regions were cut into two to four larger blocks. The blocks were sliced by a microtome into 40-μm-thick sections. The sections were preincubated in blocking solution (3% bovine serum albumin and 0.3% Triton X-100 in PBS) for 1 h and then incubated with an anti-OT monoclonal antibody (1:500; PS38, MABN844, Merck Millipore, Merck KGaA, Darmstadt, Germany) and/or a rabbit anti-c-Fos polyclonal antibody (sc-52, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in blocking solution for 12 h at 4 °C. After three washes with washing buffer, the sections were incubated with a goat anti-rabbit IgG antibody coupled to Alexa Fluor 488 (Invitrogen, Carlsbad, CA, USA) in blocking solution for 1 h at room temperature. Images were obtained using an Olympus IX71 inverted microscope equipped with a cooled CCD camera (Cool SNAP HQ2; Roper Scientific, Tucson, AZ, USA). The number of c-Fos immunopositive nuclei in each brain section was recorded and analyzed using Metamorph software (Molecular Devices, Downingtown, PA, USA), as previously described. Only the c-Fos-positive nuclei within a specific size range were counted, and a constant threshold level of staining was used; only c-Fos-positive cells with a fluorescence diameter < 13.5 μm and an intensity above 445.6 (arbitrary units) were counted. The number of OT-immunoreactive neurons in the PVN of the hypothalamus was analyzed.

2.5. Oxytocin administration

Mice received intraperitoneal (i.p.) injections (1 mL/100 g of body weight) of either OT or phosphate-buffered saline (PBS). OT was dissolved in PBS at 100 ng/mL and used at 30 ng (30 pM)/mouse. Sires received a single injection of 30 ng OT (30 pM)/mouse 30 min prior to the resident-intruder test.

2.6. Statistical analysis

The effect size was estimated by eta squared for ANOVAs and Cohen's d for pair-wise comparisons. We use the online effect size calculator at http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-SMD1.php for Cohen's d. Student's two-tailed *t*-test was used for single comparisons between two groups. The percent of male mice showing aggression was analyzed by Fisher's exact test for single comparisons between two groups. Analysis of variance (ANOVA) for repeated measures was used to analyze the attack latency, the number of attacks and the duration of aggression during the resident-intruder test. Bonferroni's *post hoc* test was used for further analysis. When appropriate, ANOVA was followed by Bonferroni *post hoc* testing. For all tests, the software package SPSS (version 12) was used. The data are presented as the mean ± SEM. Significance was considered at $P < 0.05$.

3. Results

3.1. Aggression of WT male mice of the ICR strain with different reproductive experience

We first tested whether adult ICR mice display aggression in the resident-intruder test paradigm. We tested two groups of WT mice exposed to different reproductive experiences: 10–12-week-old young virgin males singly housed or sires with family experience after delivery of pups by their paired mates (dams; Fig. 1A, lower panel). Compared with WT virgin males of the ICR strain, the WT sires started to attack the WT male virgin intruder approximately 3-fold more quickly, and the difference was significant (Fig. 1B; two-tailed Student's *t*-test, $n = 10$ and 10, $P < 0.001$, Cohen's d = 1.76). This result led us to conduct further analysis on paternal aggression because the ICR sires, as a useful model, resemble male California mice (*Peromyscus californicus*) in their paternal aggression (Trainor et al., 2008).

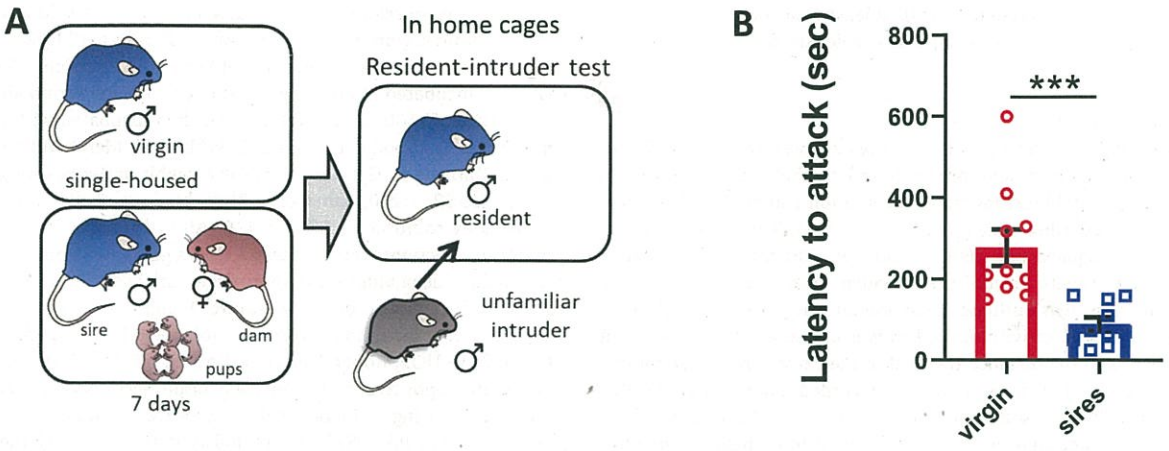

Fig. 1. Aggression in WT mice with different types of reproductive experience. (A) Scheme of the experiment. Wild-type ICR mice (virgin adult males and sires) were introduced to unfamiliar intruders in their home cages (dams and pups were removed from the cages 30 min prior to testing). (B) Latency to attack (s) in the resident-intruder test of sires (n = 10) and virgin males (n = 10). The data are presented as the mean ± SEM. Two-tailed Student's t-test, ***$P < 0.001$.

3.2. Aggressive behavior of sires stimulated by different family cues

To elucidate which family cues (extrinsic factors) are most important in triggering sire aggression, we subjected sires to four different living conditions for 7 days as described in the methods section and illustrated in Fig. 2A and subjected them to the resident-intruder test.

Fig. 2B shows the average latency of attack of the male intruder by the resident mouse. The latency of aggression by sires that had been exposed to lactating dams (dam) and nurtured pups (pups) was significantly shorter than the latency of aggression by sires that were not exposed to a family cue (alone; one-way ANOVA, $F_{3,38} = 8.93$, $P < 0.001$, $\eta^2 = 0.041$). Furthermore, the number of attacks (Fig. 2C, one-way ANOVA, $F_{3,38} = 8.12$, $P < 0.001$, $\eta^2 = 0.39$) and the attack duration (Fig. 2D, one-way ANOVA, $F_{3,37} = 7.63$, $P < 0.001$, $\eta^2 = 0.38$) in these groups were significantly larger than in the group of sires living separately. There was no difference in the attack behavior parameters between sires of the first (alone) and second (mate) groups. The time course of attack behavior during the 3 consecutive postpartum days is illustrated for each parameter (latency, number and duration) in Supplementary Fig. 1. On all days, sires exposed to pups with or without dams were significantly aggressive to the intruder, showing that lactating dams provided pup cues directly or indirectly.

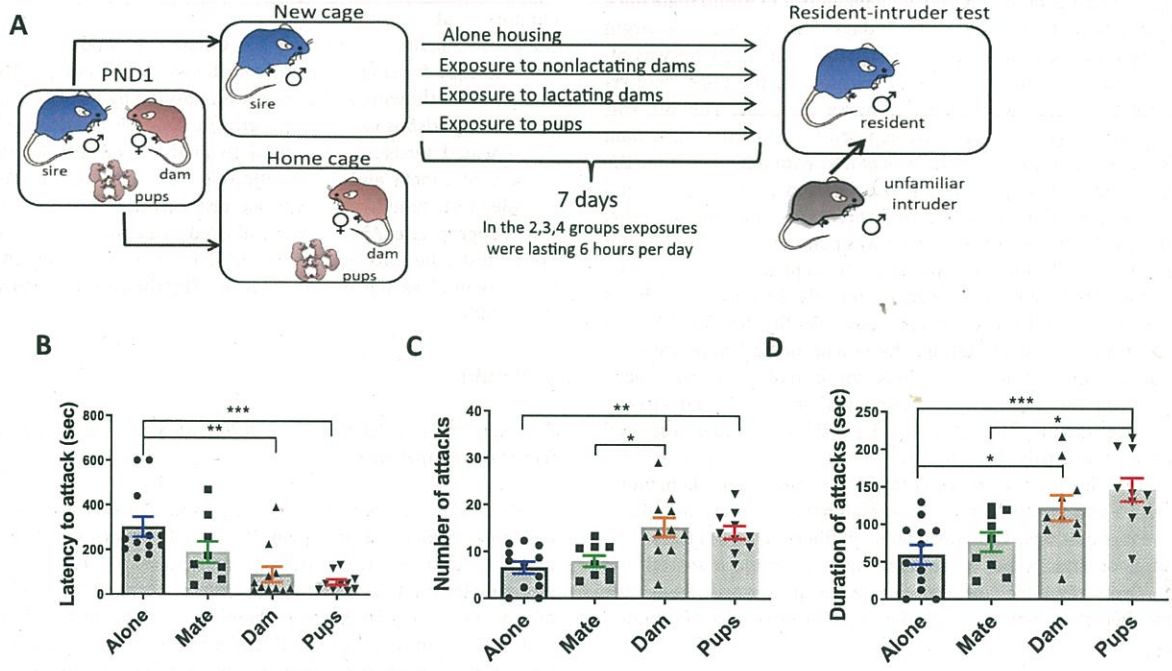

Fig. 2. Aggressive behavior of WT sires after different types of family-cue stimulation. (A) Scheme of the experiment. After the delivery of pups on PND1, the sires were moved to new individual cages and divided into four groups; each group received a different type of social stimulation for 1 week. One week later, the sires were tested in the resident-intruder test on 3 consecutive days [Alone, n = 12; with nonlactating mates (Mate), n = 9; with lactating dams (Dam), n = 11; with pups (Pups), n = 10]. (B) Latency to attack (s). (C) Number of attacks. (D) Duration of attacks (s). The data are presented as the mean ± SEM of the values obtained on 3 successive days. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

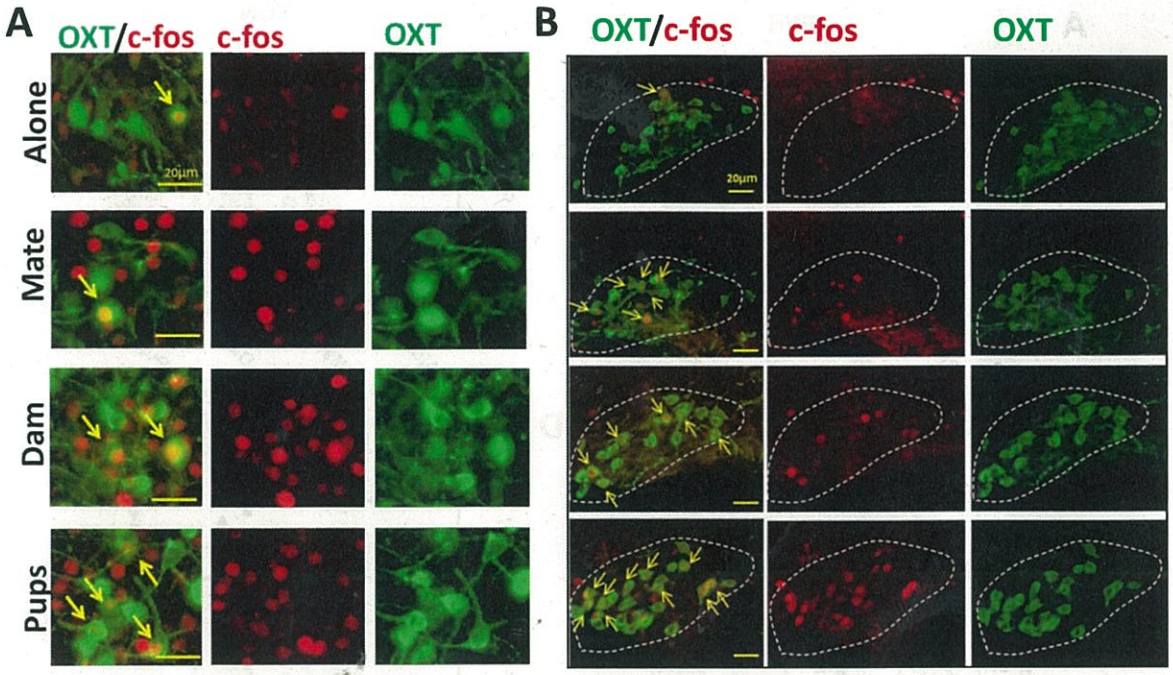

Fig. 3. Representative photomicrographs of c-Fos (red) and oxytocin (green) double immunostaining. The images shown are sections from the PVN (A) and SON (B) of WT sires after the resident-intruder test, which was preceded by different types of family-cue stimulation, as in Fig. 2. PVN, paraventricular nucleus; SON, supraoptic nucleus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. c-Fos immunoreactivity in the hypothalamus following exposure to family cues

It has been reported that downregulation of OT or lesion of the hypothalamic PVN reduces maternal aggression in rats (Consiglio, 1996; Giovenardi et al., 1998). This finding suggests that one of the brain regions important in maternal aggression is an area of the hypothalamus termed the '*hypothalamic aggression area*' (Numan, 2015). Therefore, we investigated neuronal activation in the PVN and SON of the animals in the four different sire groups using c-Fos staining 1 h after the resident-intruder test. As shown in Fig. 3, the number of c-Fos-positive cells (red) increased dramatically in the PVN (Fig. 3A) and SON (Fig. 3B) of sires, stimulated by their mates, dams with pups, or directly by pups. However, quantitative analysis of the individual mouse with the increased number of c-Fos-positive cells showed only tendency, but no significant difference between four stimuli in the PVN (Fig. 4A; one-way ANOVA, $F_{3,15} = 3.03$, $P = 0.062$, $\eta^2 = 0.37$). In the SON, significant difference was observed between males alone and sires exposed to dams. However, the number of c-Fos-positive cells was equal in the three sire groups stimulated by family cues, with no difference among these groups (Fig. 4B, one-way ANOVA, $F_{3,15} = 4.8$, $P = 0.015$, $\eta^2 = 0.49$; with Bonferroni's *post hoc* analysis, $^*P < 0.05$).

The above data indicate that cells in the hypothalamus were activated after the exposure of sires to family cues, without discrimination between nonlactating mates and lactating dams. To elucidate whether OT is involved in this neuronal activation, we compared the levels of c-Fos in oxytocinergic neurons. As shown in the merged images, the co-localization of OT (green) and c-Fos (red) immunoreactivity in the PVN (Fig. 3A) and SON (Fig. 3B) increased in sires exposed to family cues. However, in the PVN, OT and c-Fos colocalization was not increased in sires exposed to nonlactating mates, with similar levels as in sires without family cues. Colocalization was the highest in sires exposed to pups compared with the other sire groups (Fig. 4C, one-way ANOVA, $F_{3,15} = 9.88$, $P = 0.0008$, $\eta^2 = 0.66$; with Bonferroni's *post hoc* analysis, $^{**}P < 0.01$).

In contrast, c-Fos staining of oxytocinergic neurons in the SON in sires exposed to any of the three types of family cues was equally higher than that in sires without family cues (alone), with only significant difference between sires alone and dams (Fig. 4D; one-way ANOVA, $F_{3,15} = 5.1$, $P = 0.012$, $\eta^2 = 0.5$; with Bonferroni's *post hoc* analysis, $^*P < 0.05$). Importantly, no significant difference was detected between sires exposed to nonlactating or lactating dams. These results show that the activation of oxytocinergic neurons by direct pup stimuli occurred only in the PVN, though only tendency was observed for indirect stimulation by pups with dams. Because a significant increase by the pup direct stimulation was not observed in the SON, we conclude that the PVN is more critical than the SON for discriminating external pup cues from other stimuli. Moreover, the observed differences in OT and c-Fos immunoreactivity in OT neurons in the PVN can in part explain the observed differences in the pattern of aggressive behavior shown in Fig. 2.

3.4. Resident-intruder testing of CD38-knockout mice

Paternal aggression in WT ICR males was associated with the activation of oxytocinergic neurons in the PVN in the hypothalamic aggression area. Therefore, we explored whether paternal aggression induced by the extrinsic factor, pups, is modified by the intrinsic factor of OT release. To this end, we used CD38 KO mice (Kato et al., 1999), in which OT release into the brain is impaired (Higashida et al., 2012, 2018; Jin et al., 2007). The genetic background of CD38 KO mice is the ICR strain; therefore, we used mice of the ICR strain as controls and compared the aggressive behavior of mice of the two genotypes.

We tested paternal aggression in virgin male mice and sires of the CD38 KO strain using the resident-intruder test under the conditions described in Fig. 1. Surprisingly, the latency to begin attacking was very long in both strains of virgin mice (415 ± 60s, $n = 10$) and sires (498 ± 44 s, n = 10; Fig. 5A). However, the latency to begin attacking was longer in the KO sires than in the WT sires (Fig. 1B; 90 ± 16 s, n = 10, $P < 0.001$).

We also measured three parameters of aggression (latency, number and duration of attacks) in the four types of KO males as described in

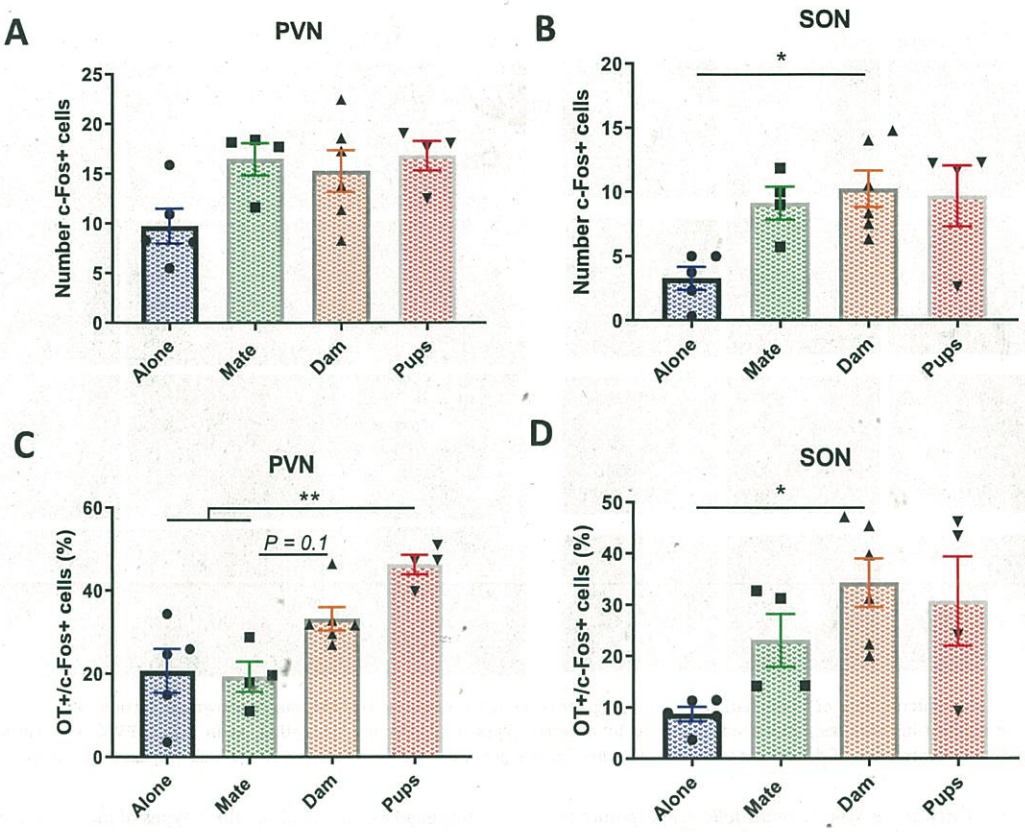

Fig. 4. Activation of neurons in the PVN and SON of the hypothalamus of WT sires exposed to different family-cue stimuli. Number of individual mice showing c-Fos-positive cells in 4–6 brains in the PVN (A) and in the SON (B) of WT sires 1 h after the resident-intruder test. Number of individual mice showing percentage of c-Fos-positive oxytocinergic neurons in the PVN (C) and SON (D). The social stimuli indicated are the same as in Fig. 2. The data are presented as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$. PVN, paraventricular nucleus; SON, supraoptic nucleus.

Fig. 2. As measured by latency to attack (one-way ANOVA, $F_{3,37} = 0.47$, $P = 0.71$, $\eta^2 = 0.036$), number of attacks (one-way ANOVA $F_{3,37} = 0.44$, $P = 0.72$, $\eta^2 = 0.034$) and duration of attacks (one-way ANOVA, $F_{3,37} = 0.39$, $P = 0.76$, $\eta^2 = 0.031$), we observed no significant differences in aggression in KO sires primed by different family cues (Fig. 5B–D). Interestingly, this decreased aggression phenotype differs markedly from the increased aggression observed in OT receptor KO female mice (Sala et al., 2013).

In the PVN of CD38 KO mice, the number of c-Fos-positive cells and the c-Fos immunoreactivity in oxytocinergic neurons were detected (Fig. 6A). Quantitative analysis of individual mice with four different stimuli showed no difference in c-Fos-positive cells (Fig. 6B, one-way ANOVA, $F_{3,14} = 0.67$, $P = 0.58$, $\eta^2 = 0.12$) and the c-Fos immunoreactivity in oxytocinergic neurons (Fig. 6C; one-way ANOVA, $F_{3,14} = 0.06$, $P = 0.98$, $\eta^2 = 0.013$). The data suggest that cells in the PVN cannot be activated by family cues in KO sires.

3.5. Recovery of paternal aggression by oxytocin in CD38-knockout mice

We then asked whether the phenotype of aggression can be modified by increasing the levels of an endogenous brain factor, OT, by external administration. OT can pass the blood-brain barrier through a transporter, the receptor for advanced glycation end-products (Higashida et al., 2017; Yamamoto et al., 2019)

To address this question, the aggressive behavior of CD38 KO sires was tested following intraperitoneal injection of OT (Fig. 7A). Injection of OT significantly decreased the latency of attack (Fig. 7B; two-tailed Student's *t*-test, $n = 7$, $P < 0.001$, Cohen's d = 1.69), and the number of attacks increased significantly to the WT level (Fig. 7C; two-tailed Student's *t*-test, n = 7, $P < 0.05$, Cohen's d = 1.22) compared with the animals that received PBS injection. There was no difference between the OT and PBS-injected CD38 KO sires in the duration of aggression (Fig. 7D; two-tailed Student's *t*-test, n = 7, $P = 0.18$, Cohen's d = 0.76).

3.6. Cross-genetic research in the resident-intruder test

We found that WT sires displayed more intense aggression against intruders than KO sires when the same genotype combinations, such as WT virgin male and sire residents against WT intruders and KO residents against KO intruders, were used. To examine the effect of genotype on the resident and intruder relationship, we performed cross-genotype experiments. The different genotype combinations used are illustrated in Supplementary Figs. 1 and 2. WT sires were not more aggressive against KO intruders and were not more aggressive against WT intruders than the virgin males (Supplementary Fig. 1). CD38 KO sires displayed lower levels of aggression to both WT and CD38 KO intruders (Supplementary Figs. 2). No differences were observed in any genotype combinations.

3.7. Paternal aggression and anxiety

We tested anxiety-related behaviors using the open field and elevated plus maze tests using three types of sires (sires maintained alone and sires exposed to dams or pups). The results revealed no significant differences in the animals' anxiety levels (Supplementary Fig. 3).

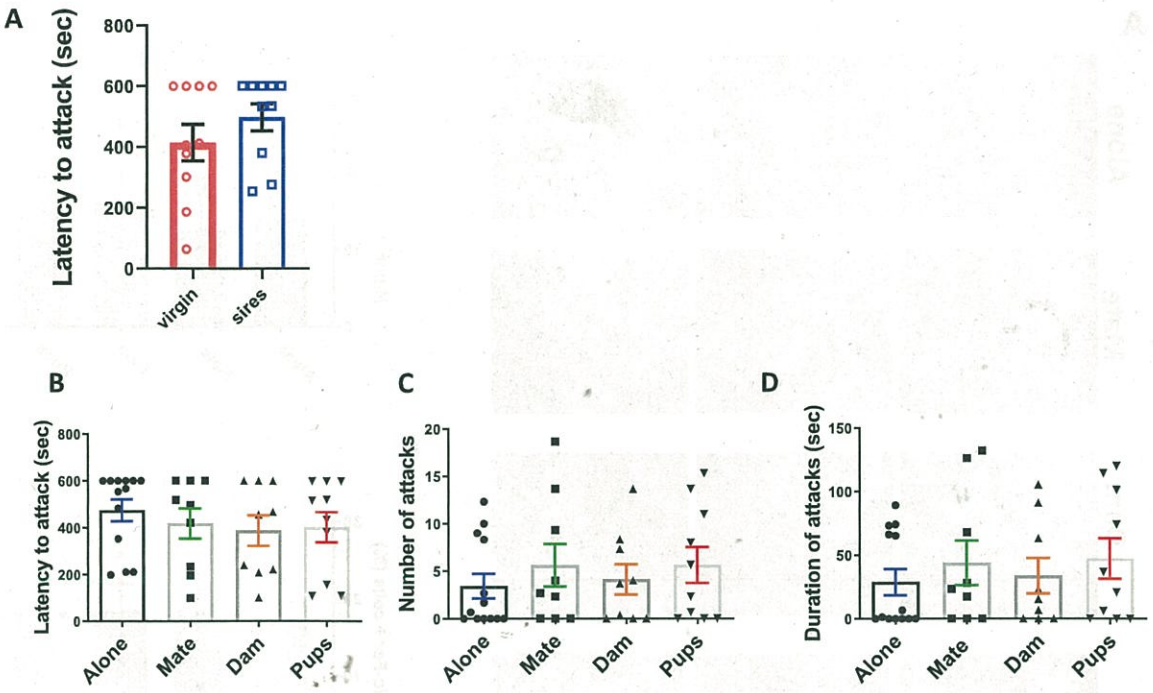

Fig. 5. Aggressive behavior of CD38 KO sires exposed to different family-cue stimuli. (A) Latency to attack (s) in the resident-intruder test in virgin males (n = 10) and sires (n = 10). The data are presented as the mean ± SEM. Latency to attack (B), number of attacks (C) and duration of attacks (D) by CD38 KO sires that received different family-cue stimuli as shown in Fig. 2. Alone, n = 13; Mate, n = 9; Dam, n = 9; Pups, n = 10. The data are presented as the mean ± SEM. Note that the results shown in A–D display no statistically significant differences.

4. Discussion

In this study, we demonstrated the aggressive behavior of WT and CD38 KO mouse sires of the nonmonogamous ICR strain. First, we found that new fathers displayed more aggression toward male intruders than that displayed by virgin male mice. This result is consistent with previously reported paternal behavior in monogamous mice (Lei et al., 2017; Trainor et al., 2008), in which reproductive experience was shown to increase aggressive behavior. Next, we compared the aggression induced in sires that were exposed to different family cues. The sires whose contact with their partners and biological pups was withdrawn for 7 days soon after delivery of the pups (pup information-deprived) did not attack immediately and showed the same latency to attack as the virgin males. This result shows that to form and maintain parenthood, sires require the continuous presence of pup cues (Dulac et al., 2014). Previously, we have shown that isolation of sires in new cages away from the family (discontinuity) for only 3–5 min was sufficient to reduce paternal retrieval (Liu et al., 2013).

The sires exposed repeatedly to mate dams for 7 days that were completely isolated from their biological pups (that is, the dams had no experience with lactation) did not display a quick attack and showed no significant differences from isolated sires in attack latency. In contrast, sires exposed to lactating dams were quick to attack, similar to sires directly exposed to pups. In both cases, latency to attack was significantly decreased, and the number and duration of attacks significantly increased. Lactating dams may transmit pup information, including pup-associated chemosignals (Isogai et al., 2018) that provide olfactory cues; such cues are important, as they seem to be critical for aggression (Numan and Young, 2016).

Probably, the differences in sires' attack behavior after exposure to the lactating and non-lactating dams may be explained by pups' chemosignals transmitted through the lactating dams. Taken together, these results suggest that paternal aggressive behavior in the ICR mouse strain depends largely on the animals' reproductive experience, consistent with observations in rats (Giovenardi et al., 2000).

It has been reported that after parturition, the pups' odor, together with visual and auditory cues, becomes the relevant regulator of maternal behavior (Lonstein et al., 2015; Lonstein and Gammie, 2002; Rogers and Bales, 2019). Interestingly, it was recently reported that pups carry pup-associated chemicals (hemoglobins, submandibular gland protein c and vasopressin V1 receptor ligands from dams and nest materials) (Isogai et al., 2018). Although these chemicals were originally identified as inducing aggression in virgin males, it is highly possible that they were used by lactating dams in our experiments.

A preliminary time course experiment showed that aggression continued during 3 successive postpartum days and that it clearly increased in every test, suggesting that paternal behavior is progressively established over 3 days (Supplementary Fig. 4). It has been shown that California male mice (*Peromyscus californicus*) are very aggressive toward unfamiliar females after the pups are born (Kowalczyk et al., 2018). In rats, pup development results in a natural decline in maternal aggressive behavior (Giovenardi et al., 2000). Taken together, the findings suggest that direct physical contact with pups (nurturing), which increases somatosensory input to the brain, is the most effective means of transmission of pup information to sires (Abraham and Feldman, 2018; Feldman et al., 2019; Rogers and Bales, 2019).

Furthermore, we found that CD38 KO sires were less aggressive against intruders than WT mice, with longer attack latency, shorter duration of attacks or fewer attacks. In addition, the attacks by the CD38 KO sires were much weaker than those of WT virgin male mice. Based on our data, the CD38 KO sires displayed a lower level of aggression toward both WT and CD38 KO intruders (Supplementary Figs. 1 and 2). The same was true for the WT mice, in which the combination of genotypes between resident and intruder mice was not essential, as no difference was observed with any genotype combination. This finding is consistent with a report that showed reduced aggressive behavior in mice with targeted disruption of the *Oxt* gene (DeVries et al., 1997). In contrast, Ragnauth et al. (2005) reported that

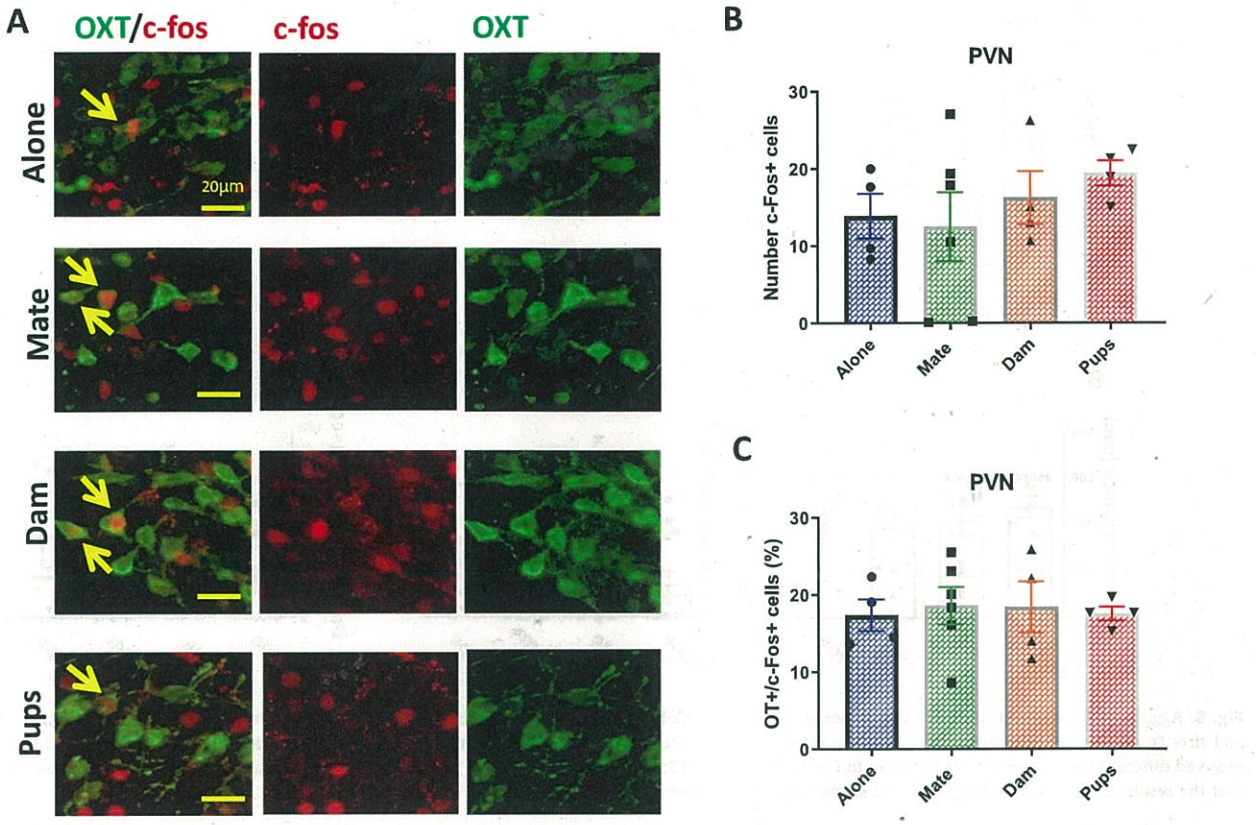

Fig. 6. Representative photomicrographs of c-Fos (red) and oxytocin (green) double immunostaining in CD38KO sires. Images are sections from the PVN (A) of animals sacrificed after the resident-intruder test, which was administered after the animals had received different family-cue stimuli as shown in Fig. 2. (B) Number of individual mice showing Fos-positive cells and (C) percentage of OT-positive neurons among the c-Fos-positive cells in the PVN. The data are presented as the mean ± SEM. Note that there is no significant difference in B and C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

female *Oxt* KO adults, but not mothers, kept in a seminatural environment display exaggerated aggressive behavior. Sala et al. (2011) reported pharmacologic rescue of aggression in male adult OT receptor-null mice that were not fathers.

Importantly, exogenous OT supplementation increased aggressiveness in CD38 KO sires to the level observed in WT fathers. We previously reported the restoration of paternal care behavior by OT supplementation in CD38 KO mice (Akther et al., 2013). The main effects

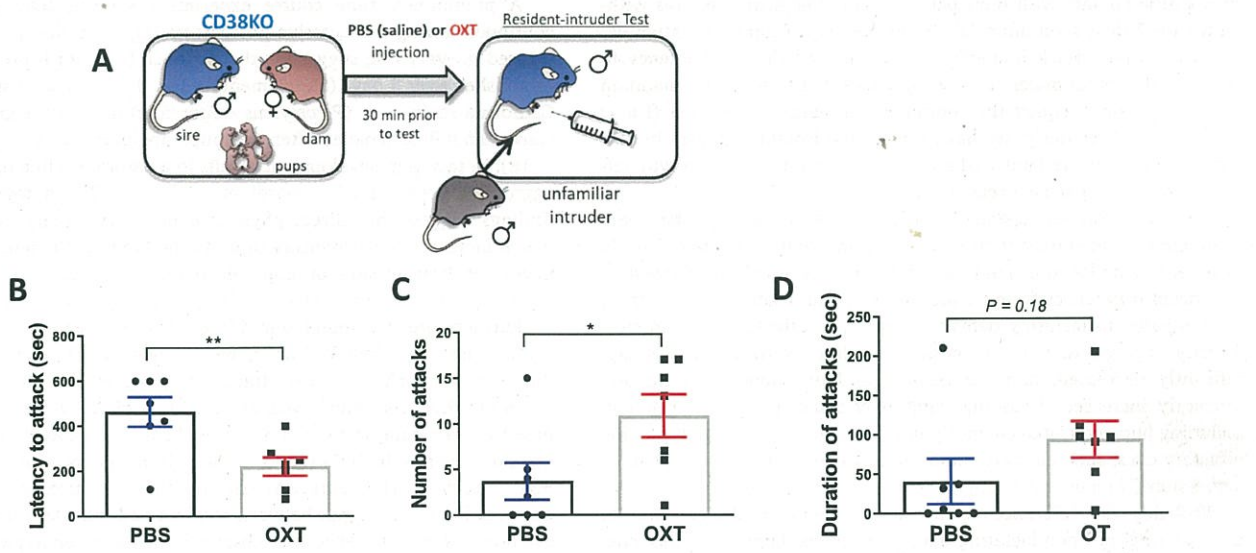

Fig. 7. Recovery effects of oxytocin on paternal aggression in CD38 KO sires. (A) Scheme of the experiment. CD38 KO sires were intraperitoneally injected with oxytocin or saline and subjected to the resident-intruder test. (B) Latency to attack (*s*). (C) Number of attacks. (D) Duration of attacks (*s*) (PBS, $n = 7$; Oxytocin (OT), $n = 7$). The results shown are the means of the results obtained on 3 successive days. The data are presented as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

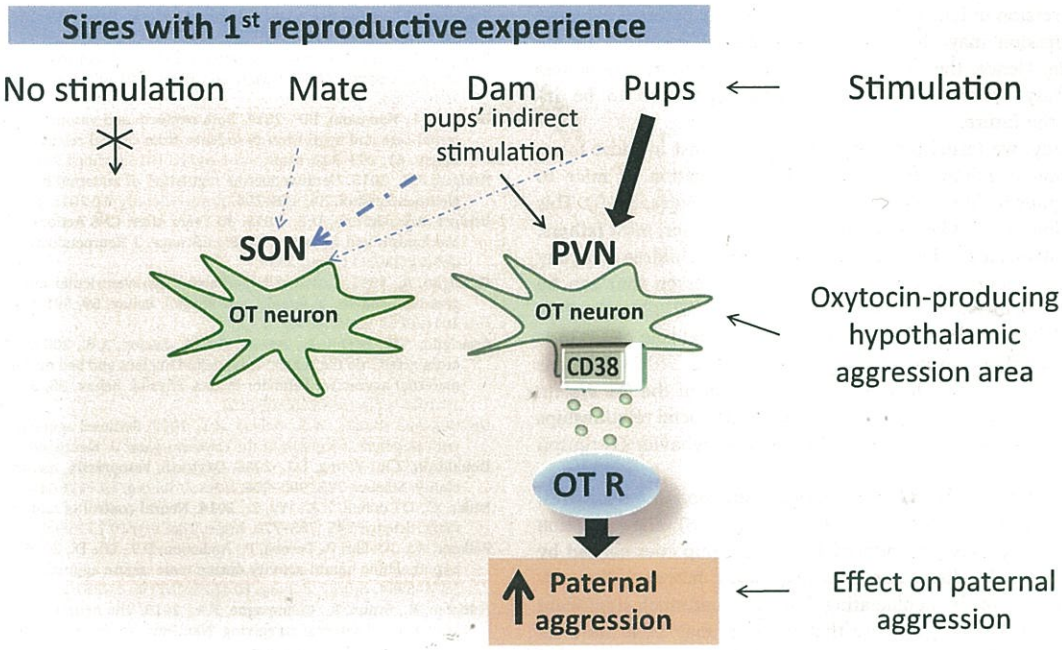

Fig. 8. Scheme showing the involvement of CD38 in the PVN in pup-induced paternal aggression. Among social (family) cues, direct pup cues activate OT neurons (thick lines) more than indirect pup cues (from lactating dams) in the PVN. In the SON, activation by dams may be only meaningful signals (dashed lines). In CD38 KO mice, no such activation of PVN neurons and paternal aggression was found; thus, CD38 and/or a CD38-dependent OT release signal are critical for paternal aggression. Peripheral administration of OT rescued impaired paternal aggression in CD38 KO sires, suggesting that OT receptor stimulation in PVN neurons triggers paternal aggression.

of peripheral application of OT are controversial; however, we have recently shown that RAGE (the receptor for advanced glycation end products) is an OT-binding protein in human serum and that RAGE is the transporter through which OT crosses the blood-brain barrier in mice (Higashida et al., 2017; Yamamoto et al., 2019). Therefore, we are confident that peripherally applied OT reaches the brain and can function in the PVN, as previously reported (Smith et al., 2019).

Supplementation with OT may mimic CD38-mediated OT release in the brain (Higashida et al., 2012, 2018; Jin et al., 2007), and this is probably the reason for the rescue of impaired pup retrieval by CD38 KO sires. As reviewed by de Jong and Neumann (2017) and Higashida et al. (2019), exogenous OT administration can be an anxiolytic treatment for psychiatric disorders in humans. This conclusion is reasonable because it is well established that brain OT is a key regulator of emotional behaviors in mice and humans (Donaldson and Young, 2008; Kohl and Dulac, 2018). In contrast, OT had an inhibitory effect on aggressive behavior (frequency of frontal attack) in lactating rats after it was injected into the central amygdaloid nucleus and the nucleus stria terminalis (Consiglio et al., 2005).

Maternal aggression that is associated with anxiety during the lactation period of female mice has been well documented (Bosch, 2005, 2011, 2013). In contrast, the effect of anxiety or stress on paternal aggression is poorly understood (Kowalczyk et al., 2018) and differs from intermale aggression originating from the ventromedial hypothalamus (Falkner et al., 2014; Hashikawa et al., 2017). Our results obtained using sires maintained alone and sires exposed to dams or pups revealed no significant differences in the anxiety levels of the animals (Supplementary Fig. 3). Thus, we concluded that aggression induced by pup or dam cues and anxiety are not interrelated. This conclusion suggests that pup protection is associated with paternal behavior and not with anxiety in ICR mice.

Numan (2015) reported that several brain areas are involved in aggression. Kruk (2014) reported that the hypothalamus, specifically the hypothalamic aggression area, which includes the ventromedial hypothalamus, the dorsomedial hypothalamic nucleus, the anterior hypothalamic nucleus, PVN parvocellular cells and PVN magnocellular cells, is one of these areas (Dulac et al., 2014; Feldman et al., 2019). Therefore, it was reasonable to examine the PVN in the current experiments. Pup-derived signals travel from the accessory olfactory bulb to the above hypothalamic areas, including the PVN, which discriminates pup signals from other signals (Fig. 8). This idea is consistent with the results of a previous study (Dulac et al., 2014). However, it has been noted that the ventromedial hypothalamic nucleus is important in intermale aggression (Falkner et al., 2014; Hashikawa et al., 2017).

Neural activation marked by c-Fos immunoreactivity in the PVN and SON was observed in sires that exhibited aggression (Lonstein and Gammie, 2002). In a study that examined maternal aggression, increased c-Fos was detected in the PVN but not in the SON (Gammie and Nelson, 2001). A novel point in our study is the observed increase in the number of OT-positive cells with c-Fos immunoreactivity during paternal aggression (Fig. 8).

> 10 brain regions, including the central amygdaloid nucleus and the bed nucleus of the stria terminalis, are functioning in maternal brain (Consiglio et al., 2005; Numan and Young, 2016; Rogers and Bales, 2019). As discussed recently, the mPOA and the VP are important brain regions that impair mate-dependent paternal behaviors when electrically lesioned and govern parental behaviors by galanin location in the mPOA (Kohl et al., 2018). Moreover, mPOA (Kohl et al., 2018) and NAcc (Akther et al., 2013) neurons are activated during paternal behaviors in response to various stimuli, including chemosensory cues, as evidenced by c-Fos expression. c-Fos expression was significantly increased in the medial amygdala and the mPOA of biparental male mice brain after aggression (Kohl et al., 2018; Numan, 2015; Trainor et al., 2008). Several other areas are involved in eliciting paternal behavior together with increased aromatase activity (Akther et al., 2015). Considering the previous observation that lesions of the PVN reduced

maternal aggression in female rats (Consiglio, 1996), paternal care and paternal aggression may share the same neuronal pathways in the parental brain. Hence, the brain areas relevant to paternal responses and the sensory system related to pup protection need to be investigated in the future.

In this study, we tested paternal aggression against intruder mice; such aggression is related to the instinctive motivation of mice to protect their pups (Feldman et al., 2019; Rogers and Bales, 2019). This type of situation can also be observed in human behavior; most fathers, if they have established fatherhood, will protect their children from any kind of danger (Nelson and Trainor, 2007). The lesson that can be learned from the current result is the involvement of CD38 in the PVN in this process in mice (Jin et al., 2007). A functional role of CD38 is expected in fatherhood in humans (Modi and Young, 2012). Humans with CD38 deficiency, which results in dysfunction of the OT system (Jin et al., 2007; Higashida et al., 2012), may forget social relationships (amnesia) and do not display bounded protective behavior (Ferguson et al., 2000 and 2002).

In conclusion, pup cues induced paternal aggression in mouse sires. This aggression was regulated by CD38 and OT (Fig. 8). The activation of oxytocinergic neurons was induced by indirect pup cues carried by lactating dams and by direct pup cues (exposure); however, the activation of such neurons by nonlactating dams was not much significant in the PVN. Therefore, we propose that the PVN may be a more responsive hypothalamic aggression area than the SON. These findings indicate a possible mechanism of offspring protection by male subjects.

Acknowledgements

We thank Michal Bell, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Funding

We acknowledge financial supports from Kanazawa University SAKIGAKE project 2018 and the Program for Fostering Globally Talented Researchers from Japan Society for the Promotion of Science.

Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2020.104695.

References

Abraham, E., Feldman, R., 2018. The neurobiology of human allomaternal care; implications for fathering, coparenting, and children's social development. Physiol. Behav. 193, 25–34. https://doi.org/10.1016/j.physbeh.2017.12.034.

Abraham, E., Hendler, T., Shapira-Lichter, I., Kanat-Maymon, Y., Zagoory-Sharon, O., Feldman, R., 2014. Father's brain is sensitive to childcare experiences. Proc. Natl. Acad. Sci. U. S. A. 111, 9792–9797. https://doi.org/10.1073/pnas.1402569111.

Akther, S., Korshnova, N., Zhong, J., Liang, M., Cherepanov, S.M., Lopatina, O., Komleva, Y.K., Salmina, A.B., Nishimura, T., Fakhrul, A.A., Hirai, H., Kato, I., Yamamoto, Y., Takasawa, S., Okamoto, H., Higashida, H., 2013. CD38 in the nucleus accumbens and oxytocin are related to paternal behavior in mice. Mol Brain 6, 41. https://doi.org/10.1186/1756-6606-6-41.

Akther, S., Fakhrul, A.A.K.M., Higashida, H., 2014. Effects of electrical lesions of the medial preoptic area and the ventral pallidum on mate-dependent paternal behavior in mice. Neurosci. Lett. 570, 21–25. https://doi.org/10.1016/j.neulet.2014.03.078.

Akther, S., Huang, Z., Liang, M., Zhong, J., Fakhrul, A.A.K.M., Yuhi, T., Lopatina, O., Salmina, A.B., Yokoyama, S., Higashida, C., Tsuji, T., Matsuo, M., Higashida, H., 2015. Paternal retrieval behavior regulated by brain estrogen synthetase (aromatase) in mouse sires that engage in communicative interactions with pairmates. Front. Neurosci. 9. https://doi.org/10.3389/fnins.2015.00450.

Bosch, O.J., 2005. Brain oxytocin correlates with maternal aggression: link to anxiety. J. Neurosci. 25, 6807–6815. https://doi.org/10.1523/JNEUROSCI.1342-05.2005.

Bosch, O.J., 2011. Maternal nurturing is dependent on her innate anxiety: the behavioral roles of brain oxytocin and vasopressin. Horm. Behav. 59, 202–212. https://doi.org/10.1016/j.yhbeh.2010.11.012.

Bosch, O.J., 2013. Maternal aggression in rodents: brain oxytocin and vasopressin mediate pup defence. Phil. Trans. R. Soc. B 368, 20130085. https://doi.org/10.1098/rstb.2013.0085.

Bosch, O.J., Neumann, I.D., 2012. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: from central release to sites of action. Horm. Behav. 61, 293–303. https://doi.org/10.1016/j.yhbeh.2011.11.002.

Bridges, R.S., 2015. Neuroendocrine regulation of maternal behavior. Front. Neuroendocrinol. 36, 178–196. https://doi.org/10.1016/j.yfrne.2014.11.007.

Bridges, R.S., Grattan, D.R., 2018. 30 Years After: CNS Actions of Prolactin: Source, Mechanism and Physiological Significance. J Neuroendocrinol e12669. doi:https://doi.org/10.1111/jne.12669.

Consiglio, A., 1996. Lasion of hypothalamic paraventricular nucleus and maternal aggressive behavior in female rats. Physiol. Behav. 59, 591–596. https://doi.org/10.1016/0031-9384(95)02117-5.

Consiglio, A.R., Borsoi, A., Pereira, G.A.M., Lucion, A.B., 2005. Effects of oxytocin microinjected into the central amygdaloid nucleus and bed nucleus of stria terminalis on maternal aggressive behavior in rats. Physiol. Behav. 85, 354–362. https://doi.org/10.1016/j.physbeh.2005.05.002.

DeVries, A.C., Young, W.S., Nelson, R.J., 1997. Reduced aggressive behaviour in mice with targeted disruption of the oxytocin gene. J. Neuroendocrinol. 9, 363–368.

Donaldson, Z.R., Young, L.J., 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. Science 322, 900–904. https://doi.org/10.1126/science.1158668.

Dulac, C., O'Connell, L.A., Wu, Z., 2014. Neural control of maternal and paternal behaviors. Science 345, 765–770. https://doi.org/10.1126/science.1253291.

Falkner, A.L., Dollar, P., Perona, P., Anderson, D.J., Lin, D., 2014. Decoding ventromedial hypothalamic neural activity during male mouse aggression. J. Neurosci. 34, 5971–5984. https://doi.org/10.1523/JNEUROSCI.5109-13.2014.

Feldman, R., Braun, K., Champagne, F.A., 2019. The neural mechanisms and consequences of paternal caregiving. Nat. Rev. Neurosci. 20, 205–224. https://doi.org/10.1038/s41583-019-0124-6.

Ferguson, J.N., Young, L.J., Hearn, E.F., Matzuk, M.M., Insel, T.R., Winslow, J.T., 2000. Social Amnesia in Mice Lacking the Oxytocin Gene. Natural Genetics 25 (3), 284–288. https://doi.org/10.1038/77040.

Ferguson, J.N., Young L.J., Insel T.R., 2002. The Neuroendocrine Basis of Social Recognition. Frontiers in Neuroendocrinology 23 (2), 200–224. https://doi.org/10.1006/frne.2002.0229.

Finkenwirth, C., Burkart, J.M., 2018. Why help? Relationship quality, not strategic grooming predicts infant-care in group-living marmosets. Physiol. Behav. 193, 108–116. https://doi.org/10.1016/j.physbeh.2018.02.050.

Finkenwirth, C., Martins, E., Deschner, T., Burkart, J.M., 2016. Oxytocin is associated with infant-care behavior and motivation in cooperatively breeding marmoset monkeys. Horm. Behav. 80, 10–18. https://doi.org/10.1016/j.yhbeh.2016.01.008.

Gammie, S.C., Nelson, R.J., 2001. cFOS and pCREB activation and maternal aggression in mice. Brain Res. 898, 232–241. https://doi.org/10.1016/S0006-8993(01)02189-8.

Giovenardi, M., Padoin, M.J., Cadore, L.P., Lucion, A.B., 1998. Hypothalamic paraventricular nucleus modulates maternal aggression in rats: effects of ibotenic acid lesion and oxytocin antisense. Physiol. Behav. 63, 351–359. https://doi.org/10.1016/S0031-9384(97)00434-4.

Giovenardi, M., Consiglio, A.R., Barros, H.M.T., Lucion, A.B., 2000. Pup age and aggressive behavior in lactating rats. Braz. J. Med. Biol. Res. 33, 1083–1088. https://doi.org/10.1590/S0100-879X2000000900015.

Hashikawa, Y., Hashikawa, K., Falkner, A.L., Lin, D., 2017. Ventromedial hypothalamus and the generation of aggression. Front. Syst. Neurosci. 11, 94. https://doi.org/10.3389/fnsys.2017.00094.

Heiming, R.S., Mnning, A., Jansen, F., Kloke, V., Lesch, K.-P., Sachser, N., 2013. To attack, or not to attack? The role of serotonin transporter genotype in the display of maternal aggression. Behav. Brain Res. 242, 135–141. https://doi.org/10.1016/j.bbr.2012.12.045.

Higashida, H., Yokoyama, S., Kikuchi, M., Munesue, T., 2012. CD38 and its role in oxytocin secretion and social behavior. Horm. Behav. 61, 351–358. https://doi.org/10.1016/j.yhbeh.2011.12.011.

Higashida, H., Furuhara, K., Yamauchi, A.-M., Deguchi, K., Harashima, A., Munesue, S., Lopatina, O., Gerasimenko, M., Salmina, A.B., Zhang, J.-S., Kodama, H., Kuroda, H., Tsuji, C., Suto, S., Yamamoto, H., Yamamoto, Y., 2017. Intestinal transepithelial permeability of oxytocin into the blood is dependent on the receptor for advanced glycation end products in mice. Sci. Rep. 7, 7883. https://doi.org/10.1038/s41598-017-07949-4.

Higashida, H., Yuhi, T., Akther, S., Amina, S., Zhong, J., Liang, M., Nishimura, T., Liu, H.-X., Lopatina, O., 2018. Oxytocin release via activation of TRPM2 and CD38 in the hypothalamus during hyperthermia in mice: implication for autism spectrum disorder. Neurochem. Int. 119, 42–48. https://doi.org/10.1016/j.neuint.2017.07.009.

Higashida, H., Munesue, T., Kosaka, H., Yamasue, H., Yokoyama, S., Kikuchi, M., 2019. Social interaction improved by oxytocin in the subclass of autism with comorbid intellectual disabilities. Diseases 7, 24. https://doi.org/10.3390/diseases7010024.

Isogai, Y., Wu, Z., Love, M.I., Ahn, M.H.-Y., Bambah-Mukku, D., Hua, V., Farrell, K., Dulac, C., 2018. Multisensory logic of infant-directed aggression by males. Cell 175 e17, 1827–1841. https://doi.org/10.1016/j.cell.2018.11.032.

Jin, D., Liu, H.-X., Hirai, H., Torashima, T., Nagai, T., Lopatina, O., Shnayder, N.A., Yamada, K., Noda, M., Seike, T., Fujita, K., Takasawa, S., Yokoyama, S., Koizumi, K., Shiraishi, Y., Tanaka, S., Hashii, M., Yoshihara, T., Higashida, K., Islam, M.S., Yamada, N., Hayashi, K., Noguchi, N., Kato, I., Okamoto, H., Matsushima, A., Salmina, A., Munesue, T., Shimizu, N., Mochida, S., Asano, M., Higashida, H., 2007. CD38 is critical for social behaviour by regulating oxytocin secretion. Nature 446, 41–45. https://doi.org/10.1038/nature05526.

de Jong, T.R., Neumann, I.D., 2017. Oxytocin and aggression, in: Hurlemann, R., Grinevich, V. (Eds.), Behavioral Pharmacology of Neuropeptides: Oxytocin. Springer International Publishing, Cham, pp. 175–192. https://doi.org/10.1007/7854_2017_13.

de Jong, T.R., Beiderbeck, D.I., Neumann, I.D., 2014. Measuring virgin female aggression in the female intruder test (FIT): effects of oxytocin, estrous cycle. and anxiety. PLoS ONE 9, e91701. https://doi.org/10.1371/journal.pone.0091701.

Kato, I., Yamamoto, Y., Fujimura, M., Noguchi, N., Takasawa, S., Okamoto, H., 1999. CD38 disruption impairs glucose-induced increases in cyclic ADP-ribose, $[Ca^{2+}]_i$, and insulin secretion. J. Biol. Chem. 274, 1869–1872. https://doi.org/10.1074/jbc.274.4.1869.

Kohl, J., Dulac, C., 2018. Neural control of parental behaviors. Curr. Opin. Neurobiol. 49, 116–122. https://doi.org/10.1016/j.conb.2018.02.002.

Kohl, J., Babayan, B.M., Rubinstein, N.D., Autry, A.E., Marin-Rodriguez, B., Kapoor, V., Miyamishi, K., Zweifel, L.S., Luo, L., Uchida, N., Dulac, C., 2018. Functional circuit architecture underlying parental behaviour. Nature 556, 326–331. https://doi.org/10.1038/s41586-018-0027-0.

Koolhaas, J.M., Coppens, C.M., de Boer, S.F., Buwalda, B., Meerlo, P., Timmermans, P.J. A., 2013. The Resident-intruder Paradigm: A Standardized Test for Aggression, Violence and Social Stress. JoVE 4367. doi:https://doi.org/10.3791/4367.

Kowalczyk, A.S., Davila, R.F., Trainor, B.C., 2018. Effects of social defeat on paternal behavior and pair bonding behavior in male California mice (Peromyscus californicus). Horm. Behav. 98, 88–95. https://doi.org/10.1016/j.yhbeh.2017.12.010.

Kruk, M.R., 1991. Ethology and pharmacology of hypothalamic aggression in the rat. Neurosci. Biobehav. Rev. 15, 527–538.

Kruk, M.R., 2014. Hypothalamic attack: a wonderful artifact or a useful perspective on escalation and pathology in aggression? a viewpoint. In: Miczek, K.A., Meyer-Lindenberg, A. (Eds.), Neuroscience of Aggression. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 143–188. https://doi.org/10.1007/7854_2014_313.

Lei, K., Liu, Y., Smith, A.S., Lonstein, J.S., Wang, Z., 2017. Effects of pair bonding on parental behavior and dopamine activity in the nucleus accumbens in male prairie voles. Eur. J. Neurosci. 46, 2276–2284. https://doi.org/10.1111/ejn.13673.

Liang, M., Zhong, J., Liu, H.-X., Lopatina, O., Nakada, R., Yamauchi, A.-M., Higashida, H., 2014. Pairmate-dependent pup retrieval as parental behavior in male mice. Front. Neurosci. 8. https://doi.org/10.3389/fnins.2014.00186.

Liu, H.-X., Lopatina, O., Higashida, C., Fujimoto, H., Akther, S., Inzhutova, A., Liang, M., Zhong, J., Tsuji, T., Yoshihara, T., Sumi, K., Ishiyama, M., Ma, W.-J., Ozaki, M., Yagitani, S., Yokoyama, S., Mukaida, N., Sakurai, T., Hori, O., Yoshioka, K., Hirao, A., Kato, Y., Ishihara, K., Kato, I., Okamoto, H., Cherepanov, S.M., Salmina, A.B., Hirai, H., Asano, M., Brown, D.A., Nagano, I., Higashida, H., 2013. Displays of paternal mouse pup retrieval following communicative interaction with maternal mates. Nat. Commun. 4, 1346. https://doi.org/10.1038/ncomms2336.

Lonstein, J.S., Fleming, A.S., 2002. Parental Behaviors in Rats and Mice, in: Crawley, J.N., Gerfen, C.R., Rogawski, M.A., Sibley, D.R., Skolnick, P., Wray, S. (Eds.), Current Protocols in Neuroscience. John Wiley & Sons, Inc., Hoboken, NJ, USA, p. ns0815s17. doi:https://doi.org/10.1002/0471142301.ns0815s17.

Lonstein, J.S., Gammie, S.C., 2002. Sensory, hormonal, and neural control of maternal aggression in laboratory rodents. Neurosci. Biobehav. Rev. 26, 869–888.

Lonstein, J.S., Lévy, F., Fleming, A.S., 2015. Common and divergent psychobiological mechanisms underlying maternal behaviors in non-human and human mammals. Horm. Behav. 73, 156–185. https://doi.org/10.1016/j.yhbeh.2015.06.011.

Lopatina, O., Yoshihara, T., Nishimura, T., Zhong, J., Akther, S., Fakhrul, A.A.K.M., Liang, M., Higashida, C., Sumi, K., Furuhara, K., Inahata, Y., Huang, J.-J., Koizumi, K., Yokoyama, S., Tsuji, T., Petugina, Y., Sumarokov, A., Salmina, A.B., Hashida, K., Kitao, Y., Hori, O., Asano, M., Kitamura, Y., Kozaka, T., Shiba, K., Zhong, F., Xie, M.-J., Sato, M., Ishihara, K., Higashida, H., 2014. Anxiety- and depression-like behavior in mice lacking the CD157/BST1 gene, a risk factor for Parkinson's disease. Front. Behav. Neurosci. 8. https://doi.org/10.3389/fnbeh.2014.00133.

Modi, M.E., Young, L.J., 2012. The oxytocin system in drug discovery for autism: animal models and novel therapeutic strategies. Horm. Behav. 61, 340–350. https://doi.org/10.1016/j.yhbeh.2011.12.010.

Nelson, R.J., Trainor, B.C., 2007. Neural mechanisms of aggression. Nat. Rev. Neurosci. 8, 536–546. https://doi.org/10.1038/nrn2174.

Numan, M., 2015. Neurobiology of social behavior: toward an understanding of the prosocial and antisocial brain. In: Elsevier/AP, Academic Press is an imprint of Elsevier, London, UK. MA, Waltham.

Numan, M., Stolzenberg, D.S., 2009. Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. Front. Neuroendocrinol. 30, 46–64. https://doi.org/10.1016/j.yfrne.2008.10.002.

Numan, M., Young, L.J., 2016. Neural mechanisms of mother–infant bonding and pair bonding: similarities, differences, and broader implications. Horm. Behav. 77, 98–112. https://doi.org/10.1016/j.yhbeh.2015.05.015.

Palanza, P., Parmigiani, S., 1994. Functional analysis of maternal aggression in the house mouse (mus musculus domesticus). Behav. Process. 32, 1–16. https://doi.org/10.1016/0376-6357(94)90023-X.

Paterson, A.T., Vickers, C., 1985. Stimulation of aggression in male mice by alpha-MSH and its relation to light phase and to saline intake effects. Behav. Brain Res. 15, 183–189.

Perry, A.N., Sue Carter, C., Cushing, B.S., 2015. Effects of postnatal estrogen manipulations on juvenile alloparental behavior. Horm. Behav. 75, 11–17. https://doi.org/10.1016/j.yhbeh.2015.07.019.

Ragnauth, A.K., Devidze, N., Moy, V., Finley, K., Goodwillie, A., Kow, L.-M., Muglia, L.J., Pfaff, D.W., 2005. Female oxytocin gene-knockout mice, in a semi-natural environment, display exaggerated aggressive behavior: female OTKO mice in a semi-natural environment. Genes Brain Behav. 4, 229–239. https://doi.org/10.1111/j.1601-183X.2005.00118.x.

Rogers, F.D., Bales, K.L., 2019. Mothers, fathers, and others: neural substrates of parental care. Trends Neurosci. 42, 552–562. https://doi.org/10.1016/j.tins.2019.05.008.

Sala, M., Braida, D., Lentini, D., Busnelli, M., Bulgheroni, E., Capurro, V., Finardi, A., Donzelli, A., Pattini, L., Rubino, T., Parolaro, D., Nishimori, K., Parenti, M., Chini, B., 2011. Pharmacologic rescue of impaired cognitive flexibility, social deficits, increased aggression, and seizure susceptibility in oxytocin receptor null mice: a neurobehavioral model of autism. Biol. Psychiatry 69, 875–882. https://doi.org/10.1016/j.biopsych.2010.12.022.

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. https://doi.org/10.1111/j.1365-2826.2012.02385.x.

Saltzman, W., Ziegler, T.E., 2014. Functional significance of hormonal changes in mammalian fathers. J. Neuroendocrinol. 26, 685–696. https://doi.org/10.1111/jne.12176.

Smith, A.S., Korgan, A.C., Young, W.S., 2019. Oxytocin delivered nasally or intraperitoneally reaches the brain and plasma of normal and oxytocin knockout mice. Pharmacol. Res. 146, 104324. https://doi.org/10.1016/j.phrs.2019.104324.

Takayanagi, Y., Yoshida, M., Bielsky, I.F., Ross, H.E., Kawamata, M., Onaka, T., Yanagisawa, T., Kimura, T., Matzuk, M.M., Young, L.J., Nishimori, K., 2005. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. Proc. Natl. Acad. Sci. 102, 16096–16101. https://doi.org/10.1073/pnas.0505312102.

Trainor, B.C., Finy, M.S., Nelson, R.J., 2008. Paternal aggression in a biparental mouse: parallels with maternal aggression. Horm. Behav. 53, 200–207. https://doi.org/10.1016/j.yhbeh.2007.09.017.

Veenema, A.H., Bredewold, R., Neumann, I.D., 2007. Opposite effects of maternal separation on intermale and maternal aggression in C57BL/6 mice: link to hypothalamic vasopressin and oxytocin immunoreactivity. Psychoneuroendocrinology 32, 437–450. https://doi.org/10.1016/j.psyneuen.2007.02.008.

Winslow, J.T., Hearn, E.F., Ferguson, J., Young, L.J., Matzuk, M.M., Insel, T.R., 2000. Infant vocalization, adult aggression, and fear behavior of an oxytocin null mutant mouse. Horm. Behav. 37, 145–155. https://doi.org/10.1006/hbeh.1999.1566.

Yamamoto, Y., Liang, M., Munesue, S., Deguchi, K., Harashima, A., Furuhara, K., Yuhi, T., Zhong, J., Akther, S., Goto, H., Eguchi, Y., Kitao, Y., Hori, O., Shiraishi, Y., Ozaki, N., Shimizu, Y., Kamide, T., Yoshikawa, A., Hayashi, Y., Nakada, M., Lopatina, O., Gerasimenko, M., Komleva, Y., Malinovskaya, N., Salmina, A.B., Asano, M., Nishimori, K., Shoelson, S.E., Yamamoto, H., Higashida, H., 2019. Vascular RAGE transports oxytocin into the brain to elicit its maternal bonding behaviour in mice. Commun Biol 2, 76. https://doi.org/10.1038/s42003-019-0325-6.

Yoshimura, H., Watanabe, K., Ogawa, N., 1988. Psychotropic effects of ginseng saponins on agonistic behavior between resident and intruder mice. Eur. J. Pharmacol. 146, 291–297. https://doi.org/10.1016/0014-2999(88)90305-6.

Zhong, J., Amina, S., Liang, M., Akther, S., Yuhi, T., Nishimura, T., Tsuji, C., Tsuji, T., Liu, H.-X., Hashii, M., Furuhara, K., Yokoyama, S., Yamamoto, Y., Okamoto, H., Zhao, Y.J., Lee, H.C., Tominaga, M., Lopatina, O., Higashida, H., 2016. Cyclic ADP-ribose and heat regulate oxytocin release via CD38 and TRPM2 in the hypothalamus during social or psychological stress in mice. Front. Neurosci. 10. https://doi.org/10.3389/fnins.2016.00304.