

# Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice

Yuki Takayanagi<sup>\*†</sup>, Masahide Yoshida<sup>\*</sup>, Isadora F. Bielsky<sup>‡</sup>, Heather E. Ross<sup>‡</sup>, Masaki Kawamata<sup>\*</sup>, Tatsushi Onaka<sup>§</sup>, Teruyuki Yanagisawa<sup>¶</sup>, Tadashi Kimura<sup>¶</sup>, Martin M. Matzuk<sup>\*\*</sup>, Larry J. Young<sup>‡</sup>, and Katsuhiko Nishimori<sup>\*††</sup>

<sup>\*</sup>Laboratory of Molecular Biology, Department of Molecular and Cell Biology, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai, Miyagi 981-8555, Japan; <sup>‡</sup>Center for Behavioral Neuroscience, Department of Psychiatry and Behavioral Sciences and Yerkes National Primate Research Center, Emory University, 954 Gatewood Road NE, Atlanta, GA 30322; <sup>§</sup>Department of Physiology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan; <sup>¶</sup>Laboratory of Molecular Pharmacology, Departments of Physiology and Pharmacology, Graduate School of Medicine, Tohoku University, 2-1 Seiryomachi, Aoba-ku, Sendai, Miyagi 980-8575, Japan; <sup>¶</sup>Division of Obstetrics and Gynecology, Department of Specific Organ Regulation, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan; and <sup>\*\*</sup>Departments of Pathology, Molecular and Cellular Biology, and Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Edited by Donald W. Pfaff, The Rockefeller University, New York, NY, and approved September 9, 2005 (received for review June 24, 2005)

The oxytocin receptor (OXTR) and its ligand, oxytocin (OXT), regulate reproductive physiology (i.e., parturition and lactation) and sociosexual behaviors. To define the essential functions of OXTR, we generated mice with a null mutation in the *Oxtr* gene (*Oxtr*<sup>-/-</sup>) and compared them with OXT-deficient (*Oxt*<sup>-/-</sup>) mice. *Oxtr*<sup>-/-</sup> mice were viable and had no obvious deficits in fertility or reproductive behavior. *Oxtr*<sup>-/-</sup> dams exhibited normal parturition but demonstrated defects in lactation and maternal nurturing. Infant *Oxtr*<sup>-/-</sup> males emitted fewer ultrasonic vocalizations than wild-type littermates in response to social isolation. Adult *Oxtr*<sup>-/-</sup> males also showed deficits in social discrimination and elevated aggressive behavior. Ligand *Oxt*<sup>-/-</sup> males from *Oxt*<sup>-/-</sup> dams, but not from *Oxt*<sup>+/-</sup> dams, showed similar high levels of aggression. These data suggest a developmental role for the OXT/OXTR system in shaping adult aggressive behavior. Our studies demonstrate that OXTR plays a critical role in regulating several aspects of social behavior and may have important implications for developmental psychiatric disorders characterized by deficits in social behavior.

lactation | maternal behavior | ultrasonic vocalization | social discrimination | aggressive behavior

Oxytocin (OXT), a nonapeptide hormone, was the first peptide hormone whose structure was determined and the first to be chemically synthesized in a biologically active form (1, 2). OXT is produced primarily in the paraventricular and supraoptic nuclei of the hypothalamus (3) and secreted mainly from the posterior pituitary gland. In addition, OXT fibers project to various brain regions (4) where OXT functions as a neurotransmitter or neuromodulator. Besides its classical functions (i.e., induction of labor and milk ejection), OXT plays an important role in social behavior (i.e., sexual behavior, maternal behavior, affiliation, and social memory), the estrous cycle, penile erection, and ejaculation (4–7).

The actions of OXT are mediated via binding to the OXTR receptor (OXTR). OXTR contains seven transmembrane domains and belongs to the class 1 family of G protein-coupled receptors. In response to ligand binding, OXTR mainly leads to stimulation of phospholipase C by interacting with G<sub>αq/11</sub>. OXTR is widely expressed in the reproductive tract (i.e., uterus, mammary gland, ovary, testis, and prostate), brain, and kidney in mammals (4), suggesting that signaling through OXTR could elicit a wide variety of effects.

OXT-deficient (*Oxt*<sup>-/-</sup>) mice displayed impairments in milk ejection (8, 9) and social recognition (10) but no obvious defects in parturition (8, 9). Although OXT is a strong uterotonic and is considered to drive parturition, the previous observations unexpectedly and clearly prove that OXT is not essential for labor in mice. In contrast, *Oxtr* mRNA expression in the uterus is up-regulated dramatically at term (11), uterine sensitivity to

OXT increases just before parturition (12, 13), and OXTR antagonists delay parturition in mice (14), suggesting an indispensable role for OXTR in mouse parturition. We therefore suspected that a redundant signaling through OXTR during parturition might compensate for the absence of OXT in *Oxt*<sup>-/-</sup> mice. Furthermore, growing evidence suggests a role for OXTR in the modulation of social behaviors. To further study the functions of the OXT/OXTR system, we generated mice lacking OXTR (*Oxtr*<sup>-/-</sup>) and evaluated their reproductive functions and social behaviors. In addition, we further compared their maternal behavior and male aggressive behavior with those of ligand *Oxt*<sup>-/-</sup> mice.

## Materials and Methods

**Generation of *Oxtr*<sup>-/-</sup> Mutant Mice and Genotyping.** To construct the targeting vector, mouse 129/Sv strain-derived genomic clones (11) were used. The *Oxtr* gene targeting vector was designed to insert a phosphoglycerate kinase promoter-neomycin resistance cassette (PGK-Neo) into intron 3 and three *loxP* sites around exons 2 and 3 and the PGK-Neo (Fig. 1A). A 6.3-kb XhoI-BamHI fragment was used as the 5' homology region, a 2.2-kb BamHI-SphI fragment containing exons 2 and 3 was inserted between two *loxP* sites, and a 2.8-kb SphI-SphI fragment was used as the 3' homology region. An MC1 promoter-herpes simplex virus-thymidine kinase cassette (MC1TK) was used for negative selection. We linearized this construct with SalI and electroporated it into E14TG2a embryonic stem cells. G418 and FIAU (Moravak Biochemicals, Brea, CA) doubly resistant clones were screened by Southern blot analysis. We generated chimeric mice by microinjection of heterozygous embryonic stem cell clones into C57BL/6J blastocysts. We mated chimeric males to CAG-*cre* transgenic female mice (15) to recombine the *loxP* sites and yield mice with an *Oxtr* deleted allele (Fig. 1A). Offspring from intercrosses of heterozygous (*Oxtr*<sup>+/-</sup>) littermates were genotyped by Southern blot analysis (Fig. 1B). The care and use of mice in this study were approved by the Institutional Animal Care and Use Committee of Tohoku University.

**Maintenance of Mice.** In addition to *Oxtr*<sup>-/-</sup> mice generated in the present study, we also used *Oxt*<sup>-/-</sup> mice generated previously (8)

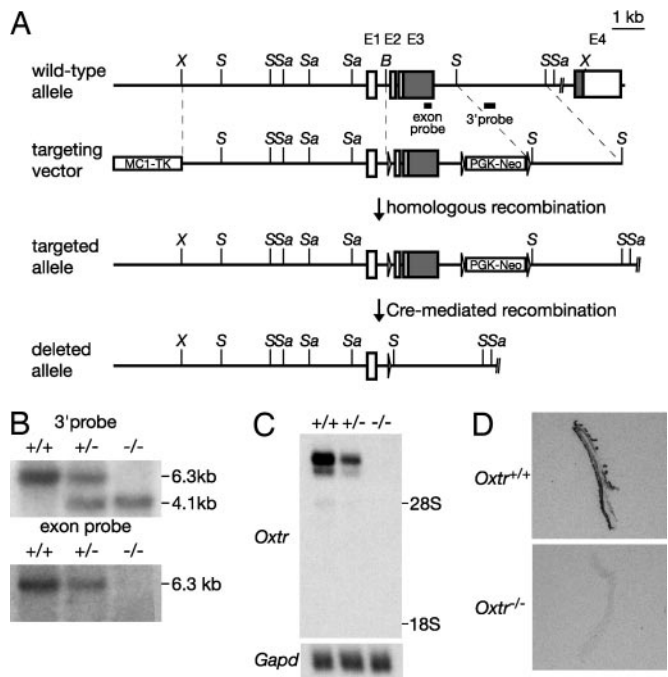
This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: OXT, oxytocin; OXTR, OXT receptor; AVP, arginine vasopressin; AVPR1a, AVP receptor 1A.

<sup>†</sup>Present address: Department of Physiology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan.

<sup>††</sup>To whom correspondence should be addressed. E-mail: knishimori@mail.tains.tohoku.ac.jp.

© 2005 by The National Academy of Sciences of the USA



**Fig. 1.** Generation of *Oxt*<sup>-/-</sup> mice. (A) The wild-type and mutant *Oxt* loci and gene targeting constructs. Exons (E) are indicated by boxes (white boxes, 5' and 3' UTRs; gray boxes, coding regions). Positions of restriction enzyme sites and the probes used for Southern blot analysis are shown (X, XhoI; S, SphI; Sa, SacI; B, BamHI). The *loxP* sites are represented by arrows (not to scale). (B) Southern blot analysis of genomic DNA from littermate progeny from *Oxt*<sup>+/-</sup> crosses. SacI-digested tail DNA was hybridized with the radiolabeled probes indicated in A. (C) Northern blot analysis of poly(A)<sup>+</sup> RNA (2  $\mu$ g per lane) from the pregnant uteri (day 19 of gestation) of *Oxt*<sup>+/+</sup>, *Oxt*<sup>+/-</sup>, and *Oxt*<sup>-/-</sup> mice. The blot was sequentially hybridized with *Oxt* and *Gapd* cDNA probes. (D) OXTR-binding autoradiograms of uteri from pregnant *Oxt*<sup>+/+</sup> and *Oxt*<sup>-/-</sup> mice (day 19 of gestation).

to distinguish between the role of the ligand, OXT, and that of the receptor, OXTR, in the regulation of maternal and aggressive behaviors. *Oxt*<sup>-/-</sup>, *Oxt*<sup>+/-</sup>, and *Oxt*<sup>+/+</sup> mice were maintained on a mixed 129  $\times$  C57BL/6J genetic background. *Oxt*<sup>-/-</sup> and *Oxt*<sup>+/+</sup> mice used in this study were maintained on a mixed 129  $\times$  C57BL/6J genetic background, as described (8). In the maternal behavior and aggressive behavior tests, we used *Oxt*<sup>-/-</sup> and *Oxt*<sup>+/+</sup> mice or *Oxt*<sup>-/-</sup> and *Oxt*<sup>+/+</sup> mice from heterozygous intercrosses. For analysis of the potential effects of maternal OXT, we also used intercrosses of homozygous *Oxt*<sup>-/-</sup> and *Oxt*<sup>+/+</sup> mice to generate *Oxt*<sup>-/-</sup> and *Oxt*<sup>+/+</sup> mice from homozygous parents followed by crossfostering with C57BL/6J females.

**Southern and Northern Blot Analyses.** For Southern blot analysis, 3  $\mu$ g of genomic DNA extracted from the tail was digested with SacI and loaded on 1% agarose gels. For Northern blot analysis, total RNA from tissues was isolated with TRIzol Reagent (Invitrogen), and poly(A)<sup>+</sup> RNA was purified by using oligotex-dT30/super (Takara Bio, Otsu, Japan) and loaded on formaldehyde-agarose gels. These DNA and RNA samples were subjected to electrophoresis and transferred to Byodyne B nylon membranes (Pall). The membranes were hybridized to <sup>32</sup>P-labeled probes. Probes for Southern blots were obtained by digestion with restriction enzyme, and probes for Northern blots were obtained by RT-PCR [*Oxt* probe, spanning from 170 to 429 nucleotides (259 base pairs) in the mouse *Oxt* mRNA (GenBank accession no. D86599) coding region; glyceraldehyde-3-phosphate dehydrogenase (*Gapd*) probe, spanning from 39 to 1,111 nucleotides (1,073 base pairs) of the mouse *Gapd* mRNA

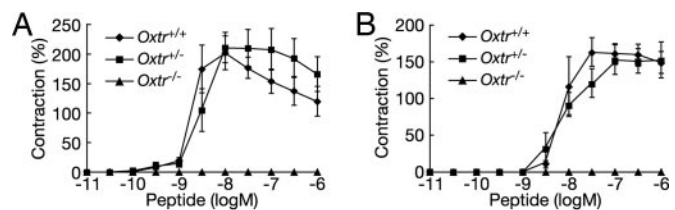
(GenBank accession no. NM008084)], and labeled with Megaprime DNA labeling systems (Amersham Biosciences) with [<sup>32</sup>P]-dCTP. Membranes were stripped and reprobed for *Gapd* to ensure equal loading.

**Maternal Behavior Test.** Maternal behavior was tested on both postpartum (13–18 weeks old) and virgin females (7–9 weeks old). Pregnant females were individually housed a few days before parturition and tested for maternal behavior on the morning of parturition. Nesting material was provided 1 day before testing. Each new mother was observed for 20 min with minimal disturbance, and the following data points were recorded: time crouching over pups and percentage of newborns scattered. One hour after the removal of her pups, each female was exposed to three 1- to 3-day-old foster pups from a wild-type dam, which were placed in each corner of the cage distant from her nest. During the next 30 min, each female was continuously observed, and the following data points were recorded: latency to sniff and retrieve each pup and latency and duration of crouching over all three pups in the nest. Different pups were used in each test. Virgin females were individually housed for 2 days before testing. For 2 consecutive days, each female was exposed to three 1- to 3-day-old foster pups for 30 min as described above. Only the second test was scored.

**Ultrasonic Vocalization Test and Measurement of Locomotor Activity in Pups.** Seven-day-old male pups from seven *Oxt*<sup>+/-</sup> breeding pairs were tested between 1 and 4 h before the dark phase. The parents were removed from the home cage 20 min before testing, and the cage was placed on a heated surface at 35°C until testing was completed. Each pup was placed into a Plexiglass recording chamber (40  $\times$  40 cm) for 3 min. Vocalizations were recorded by using an ultrasonic detector and analyzed as WAV files (16). The number of 4.5  $\times$  4.5-cm grids crossed during the test was also noted.

**Social Discrimination Test.** Before testing, males (4–7 months old) were individually housed and exposed to ovariectomized females for varying periods of time (15–40 min) for 2 days to reduce mating bouts during test sessions. The social discrimination test (17) consisted of placing an ovariectomized C57BL/6J stimulus female into the home cage of the experimental male for 5 min. After a 30-min interexposure interval, the female (SAME, Fig. 4) was then placed back into the cage along with another C57BL/6J ovariectomized stimulus female (NOVEL, Fig. 4) for 5 min. The amount of time spent investigating each female's anogenital or perioral area was then scored from the video-recorded session. Interactions including sexual behavior were excluded from the analysis. Females were exposed to only one male per day to reduce male odor contamination.

**Resident-Intruder Aggression Test.** Ten-week-old resident males were individually housed for  $\approx$ 4 weeks before testing. Ten-week-old C57BL/6J mice, housed in groups, were used as intruders.



**Fig. 2.** Reproductive functions in *Oxt*<sup>-/-</sup> mice. (A and B) The amplitude of OXT- (A) or AVP- (B) stimulated contractions of myometrial strips isolated from pregnant mice (day 19 of gestation) of each genotype. These investigations were performed as described (21).

**Table 1. Effect of genotype on reproductive functions**

Fertility and sexual behavior in male and female mice					Parturition and maternal behavior in female mice				
Pairing		No. of mating trials	Mating rate, %	Pregnancy rate, %	Average litter size	No. of pregnant females	Onset of labor, day	Duration of labor, hour	Postnatal survivors per total births, %
Male	Female								
<i>Oxtr</i> <sup>+/+</sup>	× <i>Oxtr</i> <sup>+/+</sup>	79	35	71	7.5 ± 0.7	11	19.1 ± 0.1	3.8 ± 0.4	96
<i>Oxtr</i> <sup>-/-</sup>	× <i>Oxtr</i> <sup>+/+</sup>	63	41	65	8.2 ± 0.9	10	19.2 ± 0.1	4.8 ± 0.9	92
<i>Oxtr</i> <sup>+/+</sup>	× <i>Oxtr</i> <sup>-/-</sup>	43	42	67	8.6 ± 0.5	10	19.2 ± 0.1	4.8 ± 0.9	0
<i>Oxtr</i> <sup>-/-</sup>	× <i>Oxtr</i> <sup>-/-</sup>	82	33	59	8.4 ± 0.8	13	19.3 ± 0.1	3.2 ± 0.2	0

The profile of reproductive functions in *Oxtr*<sup>+/+</sup> and *Oxtr*<sup>-/-</sup> mice (male, 10–25 weeks old; female, 10–15 weeks old). Each genotype was mated and females were selected without reference to ovulatory cycle. Mating rate denotes the ratio of plugged females to matings, and pregnancy rate denotes the ratio of pregnant females to plugged females. The morning when the copulation plug was observed is designated as day 0.5 of gestation. The data represent mean ± SEM.

Two tests of 5 min each with a 5-min interval were performed. New intruder mice were used in each test. The following data points were recorded: attack duration, frequency and latency, and latency to first attack.

**Additional Details.** For further details, see *Supporting Text*, which is published as supporting information on the PNAS web site.

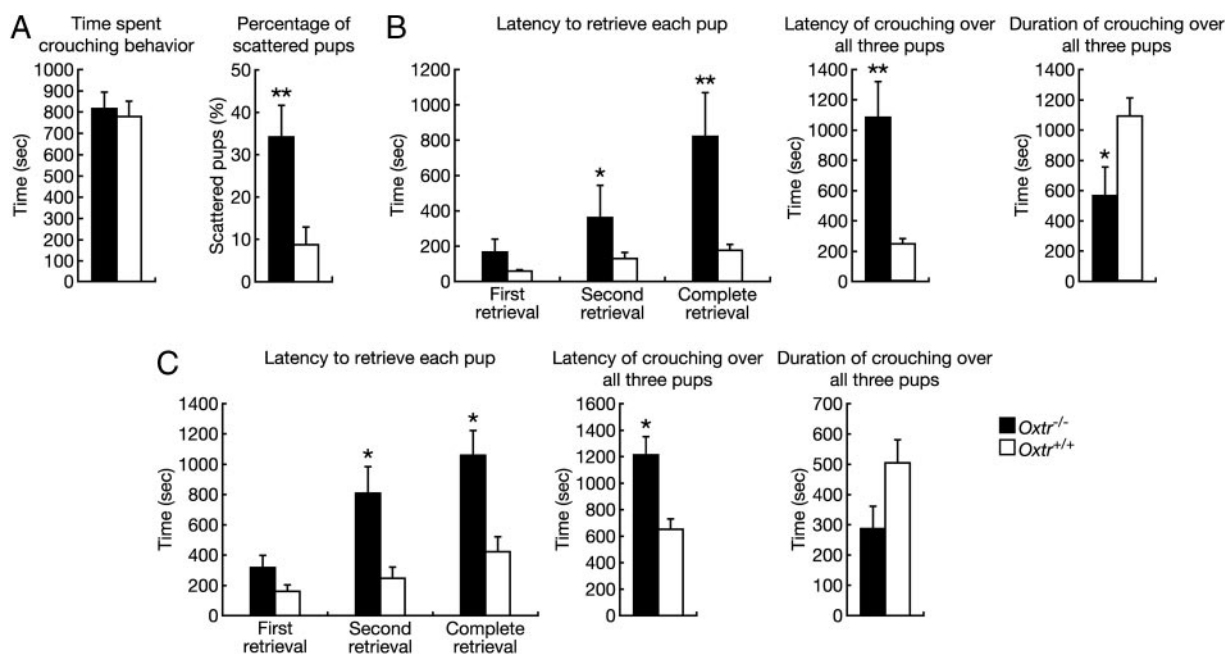
**Results**

**Generation of *Oxtr*<sup>-/-</sup> Mutant Mice.** To define the roles of OXTR in the reproductive and central nervous systems, we generated OXTR-deficient mice by gene targeting (Fig. 1*A*). The disruption of the *Oxtr* gene locus (Fig. 1*B*), the absence of *Oxtr* transcripts (Fig. 1*C*), and the absence of OXTR binding (Fig. 1*D*) in *Oxtr*<sup>-/-</sup> mice confirmed that the recombined allele is null. A 1:2:1 Mendelian distribution of the progeny from heterozygous intercrosses was observed [(*Oxtr*<sup>+/+</sup>:*Oxtr*<sup>+/-</sup>:*Oxtr*<sup>-/-</sup>), 65:133:69 (males); 79:133:78 (females)], indicating that OXTR is not required for embryonic development.

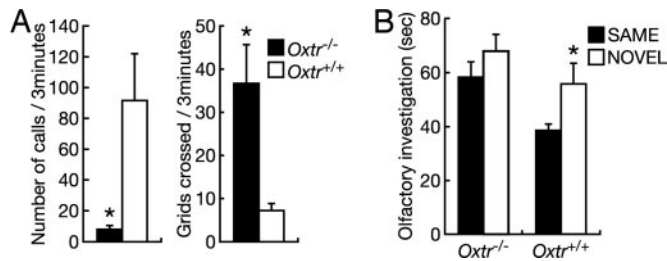
**Reproductive Functions in *Oxtr*<sup>-/-</sup> Mice.** In *Oxtr*<sup>-/-</sup> mice, OXT-induced contractions in pregnant myometrium were not evident

(Fig. 2*A*). Because arginine vasopressin (AVP), another non-peptide hormone synthesized in the paraventricular and supraoptic nuclei and secreted from the posterior pituitary, acts as a partial agonist of OXTR (18, 19) and also stimulates uterine contractions (20, 21), we also examined AVP-induced myometrium contractions in *Oxtr*<sup>-/-</sup> mice. The myometrium of pregnant *Oxtr*<sup>-/-</sup> mice did not respond to AVP (Fig. 2*B*). Receptor autoradiography confirmed the absence of OXTR binding in pregnant myometrium of *Oxtr*<sup>-/-</sup> mice (Fig. 1*D*). We previously reported the inability to detect AVP receptor 1A (*Avpr1a*) mRNA in the wild-type mouse uterus by using RT-PCR (21). Taken together, these findings prove that AVP-induced uterine contractions in pregnant wild-type mice are mediated solely by OXTR, consistent with our previous report (21).

To examine reproductive function, *Oxtr*<sup>-/-</sup> and *Oxtr*<sup>+/+</sup> mice were mated in all possible combinations. Contrary to our prediction, the onset and duration of labor were normal in *Oxtr*<sup>-/-</sup> females (Table 1). Furthermore, *Oxtr*<sup>-/-</sup> mice exhibited normal rates of mating and pregnancy and litter sizes, demonstrating that OXTR is not essential for either male or female reproductive function. However, all offspring from



**Fig. 3.** Maternal nurturing in female *Oxtr*<sup>-/-</sup> mice. (*A*) Observation of newly postpartum *Oxtr*<sup>-/-</sup> (*n* = 9) and *Oxtr*<sup>+/+</sup> (*n* = 10) females before tests for maternal behavior. (*B* and *C*) Tests for maternal behavior. Pup retrieval and crouching behaviors in *Oxtr*<sup>-/-</sup> (*n* = 9) and *Oxtr*<sup>+/+</sup> (*n* = 10) postpartum females (*B*), and *Oxtr*<sup>-/-</sup> (*n* = 15) and *Oxtr*<sup>+/+</sup> (*n* = 7) virgin females (*C*) from heterozygous intercrosses. Failure to retrieve or crouch was assigned as 30 min, the length of the observation period. \*, *P* < 0.05 and \*\*, *P* < 0.01 (Mann–Whitney *U* test). Error bars, standard error.



**Fig. 4.** Infant ultrasonic vocalization and adult social discrimination. (A) Measurements of social isolation-induced ultrasonic vocalizations (Left) and locomotor activity (Right) in *Oxt<sup>-/-</sup>* ( $n = 8$ ) and *Oxt<sup>+/+</sup>* ( $n = 10$ ) male pups from heterozygous intercrosses. (B) Test for social discrimination test. After the first exposure to a female and an interexposure interval, this female (SAME) was placed back along with another female (NOVEL). *Oxt<sup>-/-</sup>* ( $n = 14$ ) and *Oxt<sup>+/+</sup>* ( $n = 10$ ) males were examined for investigation times directed to the SAME or NOVEL females. \*,  $P < 0.05$  (Mann-Whitney  $U$  test). Error bars, standard error.

*Oxt<sup>-/-</sup>* dams died within 24 h after birth, regardless of the genotype of the offspring (Table 1). This mortality was likely due to defects in lactation, because milk was not observed in the digestive tracts of pups from *Oxt<sup>-/-</sup>* dams. All offspring from *Oxt<sup>-/-</sup>* mice were successfully fostered to *Oxt<sup>+/+</sup>* mice, and thus the survival defect in the pups lies entirely with the *Oxt<sup>-/-</sup>* dams. Histological analysis of mammary glands in *Oxt<sup>-/-</sup>* females indicated that the development of mammary tissues during gestation and milk production were normal except for the accumulation of milk in ducts of the postpartum mammary glands (data not shown). Thus, *Oxt<sup>-/-</sup>* females failed to lactate, similar to *Oxt<sup>-/-</sup>* females (8).

***Oxt<sup>-/-</sup>* Female Mice Display Defects in Maternal Nurturing.** Because OXT plays a role in maternal behavior (7), we examined maternal behavior in *Oxt<sup>-/-</sup>* mice. Initially, the behavior of postpartum females was observed for 20 min in their home cages. Both *Oxt<sup>-/-</sup>* and *Oxt<sup>+/+</sup>* females built nests and spent the majority of this period crouching over their pups ( $P > 0.05$ ), but pups of *Oxt<sup>-/-</sup>* females were often found scattered around the cage ( $P < 0.01$ ) (Fig. 3A). Following this initial observation, we monitored the dams' responses to three pups placed in different corners of the cage for 30 min. *Oxt<sup>-/-</sup>* dams displayed a significantly longer latency to retrieve the pups (first retrieval,  $P > 0.05$ ; second retrieval,  $P < 0.05$ ; and complete retrieval,  $P < 0.01$ ) or to crouch over the pups ( $P < 0.01$ ) and spent less time crouching over the pups ( $P < 0.05$ ) than *Oxt<sup>+/+</sup>* females (Fig. 3B). The impairment of retrieval was not due to the failure of the mothers to detect the pups, because latency to approach and sniff the pups was similar to wild-type mothers ( $11.7 \pm 5.0$  s compared with  $9.9 \pm 4.2$  s;  $P > 0.05$ ). Additionally, virgin *Oxt<sup>-/-</sup>* females displayed a similar phenotype (Fig. 3C), suggesting that OXT is required for nurturing responses to pups outside the physiological context of pregnancy and parturition. In contrast, both postpartum and virgin *Oxt<sup>-/-</sup>* females displayed normal maternal behavior (data not shown), consistent with an earlier

study (22). This could be explained by other ligands, such as AVP, activating this receptor.

The decrease in maternal behavior of postpartum *Oxt<sup>-/-</sup>* females could be explained as a reflection of the inability to lactate. However, our data showed that virgin *Oxt<sup>-/-</sup>* females that have not experienced lactation displayed an impairment of maternal behavior (Fig. 3C), and *Oxt<sup>-/-</sup>* females showed normal maternal behavior despite their inability to lactate (data not shown). These results strongly suggest that any deficits in maternal behavior in these mice would be a clear indication of a specific behavioral deficit.

#### Decreased Ultrasonic Vocalizations and Increased Locomotor Activity in Infant *Oxt<sup>-/-</sup>* Males.

Next, we examined isolation-induced ultrasonic vocalizations and locomotor activity of infant *Oxt<sup>-/-</sup>* males. *Oxt<sup>-/-</sup>* males, like *Oxt<sup>-/-</sup>* males (16), emitted significantly fewer calls than did wild-type littermates ( $P < 0.05$ ) (Fig. 4A), while displaying significantly higher levels of locomotor activity during the test ( $P < 0.05$ ) (Fig. 4A). These results suggest that perhaps *Oxt<sup>-/-</sup>* males are less distressed by social isolation and shift their behavior toward more exploratory activity than do wild-type littermates.

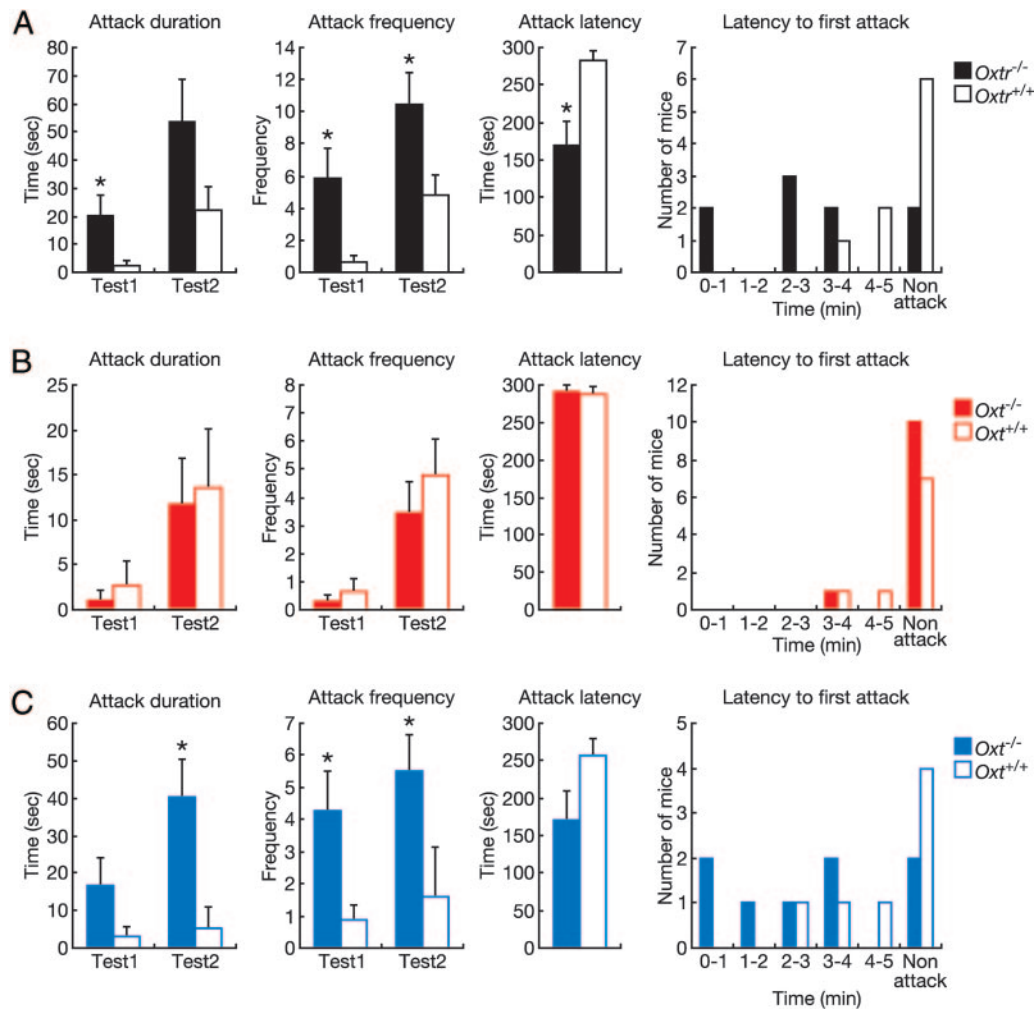
**Social Amnesia in *Oxt<sup>-/-</sup>* Mice.** Because *Oxt<sup>-/-</sup>* mice display social amnesia (10), we examined social discrimination in adult *Oxt<sup>-/-</sup>* males. Males were initially exposed to an ovariectomized C57BL/6J female. After a 30-min separation, the male was simultaneously exposed to this same female and a novel female of the same strain. As expected, *Oxt<sup>+/+</sup>* males spent significantly more time investigating the novel compared with the familiar female ( $P < 0.05$ ) (Fig. 4B) and therefore were able to discriminate between the two. However, *Oxt<sup>-/-</sup>* males spent a similar amount of time investigating both females ( $P > 0.05$ ) (Fig. 4B), suggesting an impairment of social discrimination. There was no difference between the two genotypes in the amount of time spent investigating the initial female ( $P > 0.05$ ), indicating that the *Oxt<sup>-/-</sup>* males' deficit was not due to a difference in exposure time or motivation to investigate a female (data not shown). However, *Oxt<sup>-/-</sup>* males were able to discriminate outbred CD-1 stimulus females (data not shown), suggesting that the deficit represents an impairment rather than a complete disruption in social recognition.

#### *Oxt<sup>-/-</sup>* Males Display High Levels of Aggression Due to the Lack of OXT Activation During Prenatal Development.

Because we observed more wounded mice in group-housed males from cages containing *Oxt<sup>-/-</sup>* mice than cages containing only *Oxt<sup>+/+</sup>* mice (Table 2), we assessed aggressive behavior using the resident-intruder test. *Oxt<sup>-/-</sup>* resident males attacked the intruder with shorter latency ( $P < 0.05$ ), for longer duration (test1,  $P < 0.05$ ; and test2,  $P > 0.05$ ), and with higher frequency (test1,  $P < 0.05$ ; and test2,  $P < 0.05$ ) compared with *Oxt<sup>+/+</sup>* residents (Fig. 5A). In contrast, in adjacent cages containing male *Oxt<sup>-/-</sup>* mice, the rate of wounded mice was similar to that in *Oxt<sup>+/+</sup>* mice (Table 2). Furthermore, aggressive behavior of *Oxt<sup>-/-</sup>* mice in the resident-intruder test was indistinguishable from *Oxt<sup>+/+</sup>* males (Fig. 5B).

**Table 2. The number of wounded mice (3–9 months old) in cages, including each genotype**

Genotypes of members in the same cage	No. of observed mice	Wounded mice	Unwounded mice	Wounded per total, %	Mice per cage
<i>Oxt<sup>+/+</sup></i>	37	1	36	2.7	4.1
<i>Oxt<sup>+/+</sup></i> , <i>Oxt<sup>+/-</sup></i> , <i>Oxt<sup>-/-</sup></i>	54	18	36	33.3	4.0
<i>Oxt<sup>+/+</sup></i>	21	0	21	0.0	3.5
<i>Oxt<sup>+/+</sup></i> , <i>Oxt<sup>+/-</sup></i> , <i>Oxt<sup>-/-</sup></i>	62	2	60	3.2	4.1



**Fig. 5.** Aggressive behavior as measured by the resident-intruder test. (A and B) Aggressive behavior in the resident-intruder test of *Oxt*<sup>-/-</sup> ( $n = 9$ ) and *Oxt*<sup>+/+</sup> ( $n = 9$ ) mice (A) and *Oxt*<sup>-/-</sup> ( $n = 11$ ) and *Oxt*<sup>+/+</sup> ( $n = 9$ ) mice (B) from heterozygous intercrosses. (C) Aggressive behavior in the resident-intruder test of *Oxt*<sup>-/-</sup> ( $n = 8$ ) and *Oxt*<sup>+/+</sup> ( $n = 7$ ) mice from intercrosses of homozygous *Oxt*<sup>-/-</sup> and *Oxt*<sup>+/+</sup> mice and fostering by C57BL/6J females. \*,  $P < 0.05$  (Mann-Whitney  $U$  test). Error bars, standard error.

To investigate this discrepancy in aggression phenotypes between *Oxt*<sup>-/-</sup> and *Oxt*<sup>-/-</sup> mice, we examined possible compensatory effects of the *Oxt* gene mutation. OXTR and AVPR1a autoradiography in the brain showed no OXTR binding in *Oxt*<sup>-/-</sup> mice and the density of AVPR1a binding was similar between *Oxt*<sup>+/+</sup> ( $5,826 \pm 602.3$  dpm/mg) and *Oxt*<sup>-/-</sup> ( $4,787 \pm 463$  dpm/mg) mice (Fig. 6, which is published as supporting information on the PNAS web site). Northern blot analysis failed to detect any RNA products with sequence similarity to *Oxt* mRNA in the brain (Fig. 7, which is published as supporting information on the PNAS web site). In addition, *Oxt*<sup>-/-</sup> mice showed no differences in *Oxt* and *Avp* mRNA expression in the hypothalamus (data not shown), pituitary OXT (*Oxt*<sup>+/+</sup>,  $202.6 \pm 10.7$  ng per pituitary,  $n = 9$ ; *Oxt*<sup>-/-</sup>,  $222.8 \pm 9.3$  ng per pituitary,  $n = 7$ ) and AVP (*Oxt*<sup>+/+</sup>,  $471.6 \pm 70.9$  ng per pituitary,  $n = 9$ ; *Oxt*<sup>-/-</sup>,  $453.3 \pm 28.3$  ng per pituitary,  $n = 7$ ), plasma OXT (*Oxt*<sup>+/+</sup>,  $36.3 \pm 5.2$  pg/ml,  $n = 5$ ; *Oxt*<sup>-/-</sup>,  $39.3 \pm 6.1$  pg/ml,  $n = 5$ ), or plasma testosterone levels (*Oxt*<sup>+/+</sup>,  $1,329.4 \pm 277.7$  pg/ml,  $n = 10$ ; *Oxt*<sup>-/-</sup>,  $1,148.7 \pm 225.5$  pg/ml;  $n = 10$ ). These results indicated that there were no apparent dysregulations of AVP, AVPR1a, or testosterone, each of which is known to influence aggression.

OXT in the dam can transfer through the placental barrier

(23–25), and *Oxt* mRNA is present in the mouse brain during embryonic development (Fig. 8, which is published as supporting information on the PNAS web site). Furthermore, perinatal injections of OXT in prairie voles have an impact on adult behavior (26). Therefore, we hypothesized that *in utero* exposure to OXT might have rescued the aggression phenotype in *Oxt*<sup>-/-</sup> mice derived from heterozygous matings. Therefore, we analyzed aggression in *Oxt*<sup>-/-</sup> mice generated from homozygous matings (Fig. 5C), creating an OXT-free developmental environment. Like *Oxt*<sup>-/-</sup> males, *Oxt*<sup>-/-</sup> males generated from homozygous matings exhibited highly aggressive behavior, as reported (16). Our findings indicate that embryonic exposure to OXT affects the development of aggression in adulthood, although other intrauterine factors in *Oxt*<sup>-/-</sup> dams could also influence aggression. However, because increased aggression is seen in *Oxt*<sup>-/-</sup> mice from *Oxt*<sup>+/+</sup> mothers, it suggests that the defect lies in the pup.

## Discussion

This study provides a comprehensive analysis of mice lacking the *Oxt* gene. Although OXT has been used to induce or augment labor in humans, and the OXTR antagonist delays labor in wild-type outbred mice (14), parturition is initiated and proceeds

