Social amnesia in mice lacking the oxytocin gene

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The development of social familiarity in rodents depends predominantly on olfactory cues and can critically influence reproductive success[1,2]. Researchers have operationally defined this memory by a reliable decrease in olfactory investigation in repeated or prolonged encounters with a conspecific[3–6]. Brain oxytocin (OT) and vasopressin (AVP) seem to modulate a range of social behaviour from parental care to mate guarding[7]. Pharmacological studies indicate that AVP administration may enhance social memory[8–10], whereas OT administration may either inhibit or facilitate social memory depending on dose, route or paradigm[1,11–13]. We found that male mice mutant for the oxytocin gene (*Oxt*$^{-/-}$) failed to develop social memory, whereas wild-type (*Oxt*$^{+/+}$) mice showed intact social memory. Measurement of both olfactory foraging and olfactory habituation tasks indicated that olfactory detection of non-social stimuli is intact in *Oxt*$^{-/-}$ mice. Spatial memory and behavioural inhibition measured in a Morris water-maze, Y-maze, or habituation of an acoustic startle also seemed intact. Treatment with OT but not AVP rescued social memory in *Oxt*$^{-/-}$ mice, and treatment with an OT antagonist produced a social amnesia-like effect in *Oxt*$^{+/+}$ mice. Our data indicate that OT is necessary for the normal development of social memory in mice and support the hypothesis that social memory has a neural basis distinct from other forms of memory.

We characterized social memory in male mice during repeated pairings with the same ovariectomized mouse. *Oxt*$^{+/+}$ mice showed a characteristic decline in the time spent investigating an ovariectomized female, with a full recovery following the introduction of a new female. *Oxt*$^{-/-}$ mice showed no decline (Fig. 1*a*,*c*), a pattern consistent with a memory deficit[5]. When we presented *Oxt*$^{+/+}$ males with a new female, they showed high levels of investigation at each encounter (Fig. 1*b*). Investigation of a stimulus female by *Oxt*$^{+/+}$ males remained significantly reduced when re-tested after 30 or 60 minutes, but not after 90 minutes (data not shown). The social behaviour of the stimulus females directed at *Oxt*$^{-/-}$ versus *Oxt*$^{+/+}$ male mice during these tests did not differ.

The social memory deficit of *Oxt*$^{-/-}$ males was not limited to ovariectomized females. We detected significant genotype-depen-

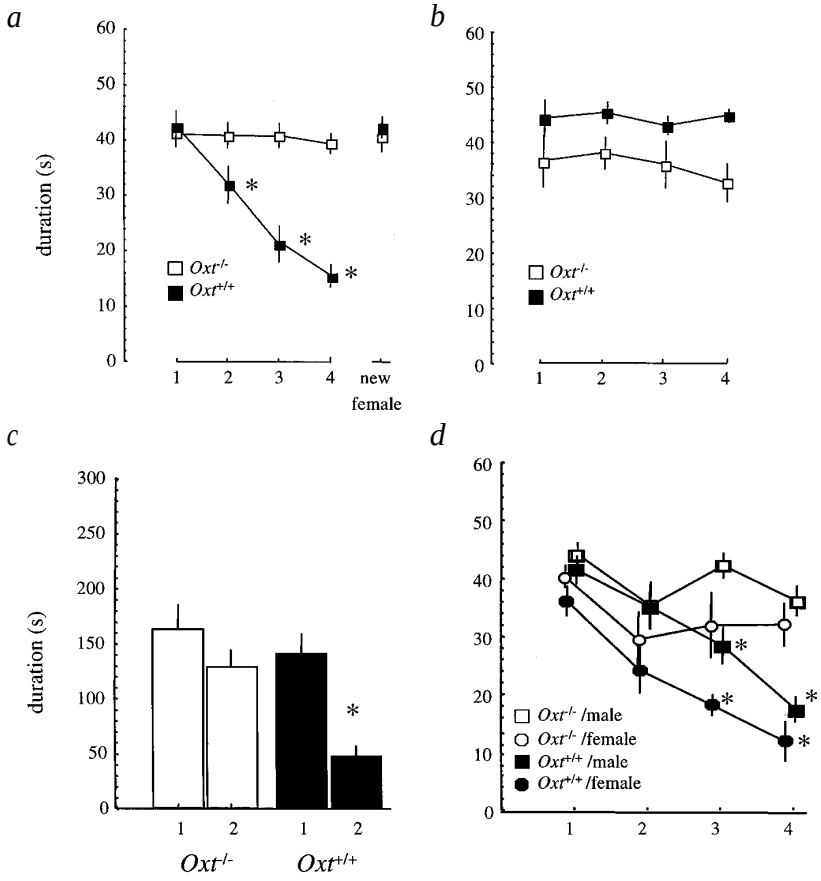

Fig. 1 Social memory in mice. ***a***, Social memory by *Oxt*$^{-/-}$ (open symbols) and *Oxt*$^{+/+}$ (filled symbols) male mice was measured as a difference in olfactory investigation. Data depict mean +/–1 s.e.m. for the amount of time (s) allocated to investigation of the same ovariectomized (OVX) female during each of four successive 1-min trials. A fifth 'dishabituation' trial depicts the response of males to the presentation of a new female in a 1-min pairing 10 min after the fourth trial. Asterisks represent a significant decrease between each trial compared with the first trial for *Oxt*$^{+/+}$ males (measured with Newman-Keuls (q) comparisons; second, q=4.11; third, q=8.15; fourth trial, q=10.4, $P<0.05$). ***b***, Depiction of olfactory investigation when a different OVX stimulus female is presented in each repeated trial. Stimulus females were rotated between successive males so that new males in each of four trials investigated each female. The persistence of social investigation by males in these tests indicates that changes in female behaviour associated with repeated encounters with males do not account for the decline in male interest when meeting the same female in repeated trials. ***c***, Depiction of olfactory investigation of the same OVX stimulus presented in each of two 5-min trials with an intertrial interval of 30 min. *Oxt*$^{-/-}$ mice showed no significant reduction of olfactory investigation in the second meeting with a stimulus female, whereas *Oxt*$^{+/+}$ mice showed an approximately 70% reduction (q=3.2, P <0.05). ***d***, Depiction of significant differences in olfactory investigation by *Oxt*$^{-/-}$ (open symbols) and *Oxt*$^{+/+}$ (filled symbols) mice of intact male (squares; effect of genotype: F(1,104)=17.9, $P<0.05$) or intact female (circles; effect of genotype: F(1,56)=16.9, $P< 0.05$) stimulus mice during each of four successive 1-min trials.

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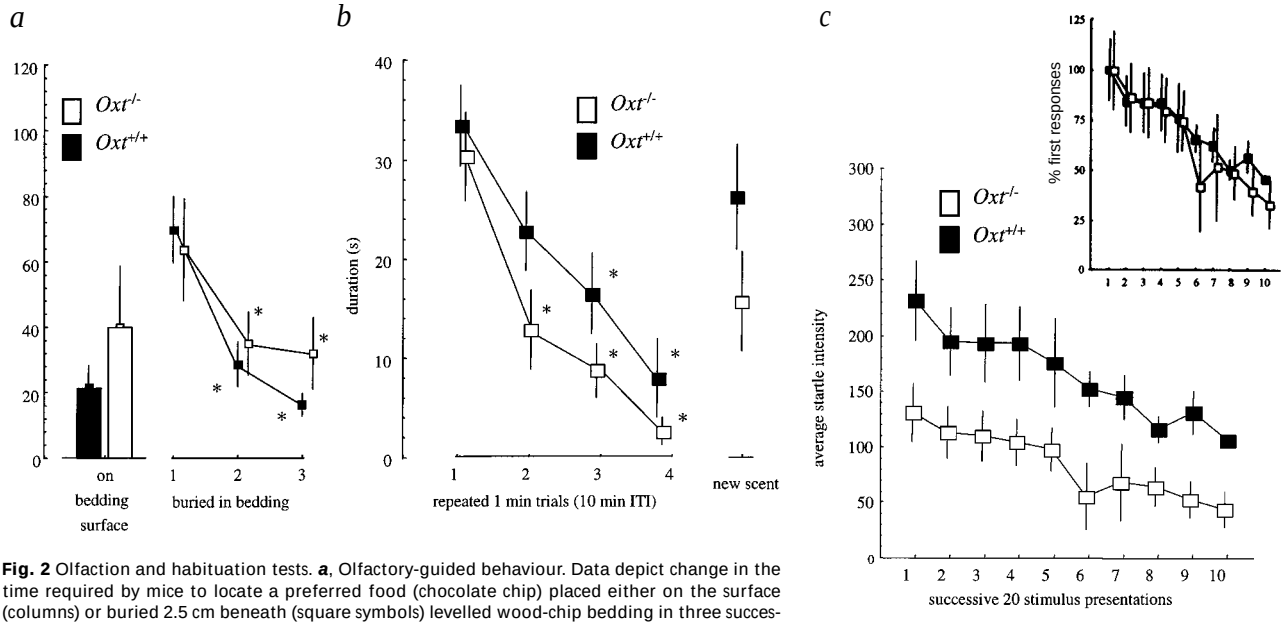


Fig. 2 Olfaction and habituation tests. ***a***, Olfactory-guided behaviour. Data depict change in the time required by mice to locate a preferred food (chocolate chip) placed either on the surface (columns) or buried 2.5 cm beneath (square symbols) levelled wood-chip bedding in three successive trials (ITI=10 min; effect of repeated testing, $F(3,42)=3.56$, $P<0.05$). We did not detect genotype-dependent differences. ***b***, Habituation to a novel olfactory cue. Although $Oxt^{+/+}$ mice (filled symbols) showed higher overall levels of investigation (effect of genotype, $(F(4,65)=8.35$, $P<0.05)$, both wild-type and mutant mice showed a change in the amount of time spent investigating a lemon-scented cylinder during repeated 1-min presentations (ITI=10 min; effect of repeated trial, $F(4,65)=12.09$, $P<0.05$). Also shown is a dishabituation response when we combined lemon and vanilla scents to form a new scent. ***c***, Habituation of an acoustic startle response. Data depict the mean +/– 1 s.e.m. startle response measured in successive blocks of 20 acoustic stimulus presentations for $Oxt^{-/-}$ (open symbols) and $Oxt^{+/+}$ (filled symbols) mice (effect of genotype, $F(1,100)=51.41$, $P<0.05$; effect of repeated testing, $F(9,100)=3.98$, $P<0.05$). Also depicted (inset) is the same response data expressed as percentage of response measured in the first block of 20 trials. These data demonstrate that although the intensity of response differs between genotypes, the rate of habituation does not.

dent differences for investigation of intact female or male mice. $Oxt^{+/+}$ males showed similar declines in olfactory investigation of both types of stimuli, whereas $Oxt^{-/-}$ males showed persistent interest during repeated presentations (Fig. 1*d*). To determine whether the deficits in social memory might be due to impairments in olfactory function or behavioural inhibition, we examined performance in an olfactory-guided foraging task and in olfactory and acoustic habituation. $Oxt^{-/-}$ and $Oxt^{+/+}$ mice, tested in repeated trials, both learned to locate buried food as rapidly as they located food placed on the surface of cage bedding (Fig. 2*a*). $Oxt^{+/+}$ mice spent more time investigating a lemon-scented object than $Oxt^{-/-}$ mice, but both genotypes rapidly habituated to the scent and dishabituated when the scent was changed (Fig. 2*b*). $Oxt^{+/+}$ mice had significantly higher startle responses during habituation to an acoustic stimulus than $Oxt^{-/-}$ mice over all trials (Fig. 2*c*), but when data were expressed and analysed as a percentage of the average for the first 20 trials, the rate of habituation was identical for $Oxt^{-/-}$ and $Oxt^{+/+}$ mice (Fig. 2*c*).

We also examined the spatial memory of $Oxt^{+/+}$ and $Oxt^{-/-}$ mice in a Morris water maze and a two-trial Y-maze task. Both genotypes showed a characteristic decline in the amount of time and distance travelled to locate a submerged platform (Fig. 3*a*,*b*), and comparable performance in a probe trial (Fig. 3*c*). $Oxt^{+/+}$ mice have a slower swim velocity and longer latencies compared with $Oxt^{-/-}$ males. Similarly, we found no genotype-dependent differences for spatial recognition during the test trial of the two-trial Y-maze (Fig. 3*d*).

Acute intraventricular administration of OT completely rescued social memory in $Oxt^{-/-}$ mice (Fig. 4*a*), measured both as a reduction in interest in the stimulus female and as the recovery of interest when a new female was presented. Injection of the OT receptor antagonist, OTA, had no measurable effect on olfactory investigation by $Oxt^{-/-}$ mice, but did delay the decline in olfactory investigation by $Oxt^{+/+}$ mice. Administration of AVP had no significant effect on either $Oxt^{-/-}$ or $Oxt^{+/+}$ mice (Fig. 4*a*) in this paradigm at the dose tested. To ensure that a compensatory upregulation or redistribution of OT receptors within the olfactory processing pathway did not facilitate the rescue of the $Oxt^{-/-}$ phenotype by OT, we performed receptor autoradiography using ^{125}OTA in both $Oxt^{+/+}$ and $Oxt^{-/-}$ mice (Fig. 4*b*). No difference in receptor distribution or density in $Oxt^{+/+}$ and $Oxt^{-/-}$ mice was apparent in any region examined.

The impairment of social memory does not depend on deficits in main olfactory bulb function because $Oxt^{-/-}$ mice did not have deficits in either olfactory-guided foraging or habituation to a non-social olfactory stimulus. Memory impairment did not seem to be global because spatial memory measured in both the Morris water-maze and the two-trial Y-maze memory tests was intact. Deficits in social memory also do not reflect global defects in behavioural inhibition because habituation to both a non-social olfactory and an acoustic stimulus were intact.

OT receptors are abundant in brain regions associated with mnemonic processing[14,15], such as the hippocampus, lateral septum and bed nucleus of the stria terminalis. Olfaction in rodents involves two sensory organs: the main olfactory bulb, which is involved in general olfaction, and the vomeronasal organ[16]. Individual recognition is thought to involve the detection of pheromones by the vomeronasal organ[16], which projects to the accessory olfactory bulb (AOB) and then to the medial (MeA) and cortical (CoA) nuclei of the amygdala. The AOB, MeA and CoA are particularly rich in OT receptors and are therefore candidate sites for OT action in the formation of social memory in mice. For example, OT in the olfactory bulbs facilitates rat maternal behaviour[18] and social recognition of a ewe for her lambs[17]. Although this work suggests that social memory may represent a special type of olfactory memory[16], the sensory basis for the deficit found in $Oxt^{-/-}$ mice, if any, remains to be determined.

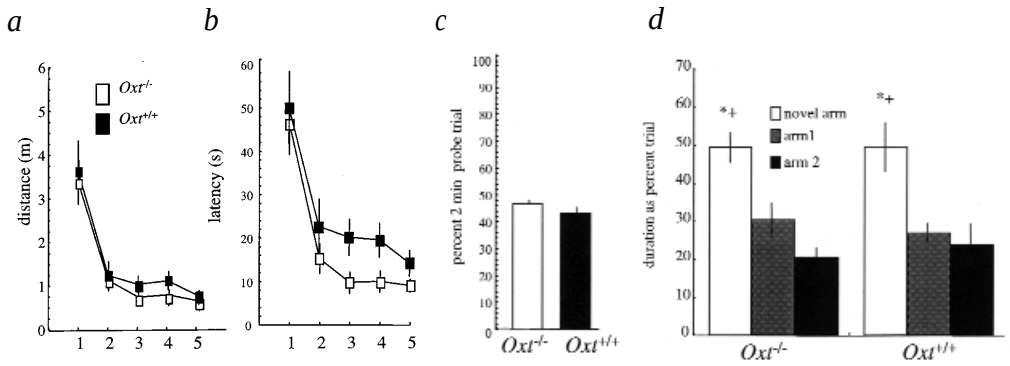
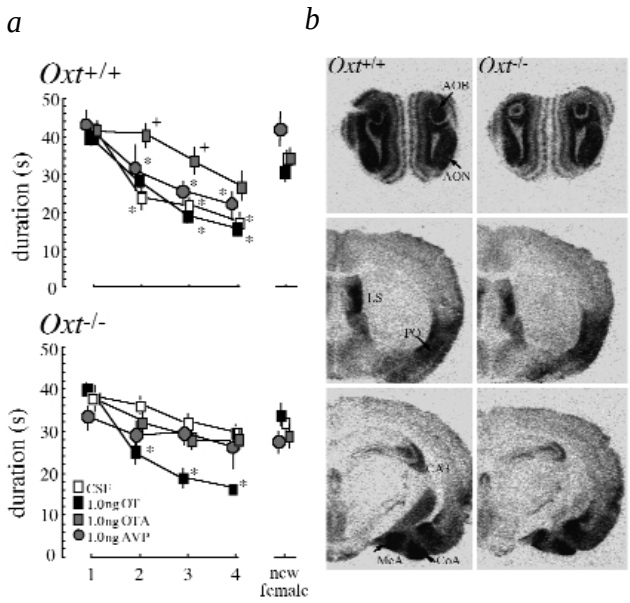


Fig. 3 Performance in spatial memory tests. Morris water maze data depict the decline in the mean (+/–1 s.e.m) distance (***a***; effect of repeated testing: F(4,290)=26.4, $P<0.05$) and latency (***b***; effect of repeated testing: F(4,290)=20.5, $P<0.05$) required for $Oxt^{-/-}$ (open symbols) and $Oxt^{+/+}$ (filled symbols) to locate a submerged platform averaged over four repeated trials within five successive daily sessions. We detected genotype-dependent differences for latency (F(1,290)=4.28, $P<0.05$) and swim speed (F(1,290)=9.04, $P<0.05$), but not for distance travelled. Analysis did not detect interaction effects on any measures of water-maze performance. Also portrayed is the amount of time spent in the platform quadrant during a 2-min probe trial on day 6 (***c***). We did not detect significant genotype-dependent differences. ***d***, Performance in the two-trial Y-maze test. We did not detect significant genotype-dependent differences in the behaviour of mice during the familiarization trial. During the test trial, subjects showed a preference for exploring the new arm expressed as amount of time (mean +/–1 s.e.m) and activity allocated to the new versus two familiar arms during the recall trial (effect of arm preference-duration, F(2,28)=6.62, $P<0.05$; effect of arm preference-distance traversed, F(2,28)=5.87, $P<0.05$). We did not detect significant genotype-dependent differences. *, Significant difference between the new arm and arm 1; +, significant difference between the new arm and arm 2.

Previous efforts to characterize a role for OT in the social memory of rodents indicated a complex relationship[19,20]. OT infused into the cerebral ventricles of male rats can enhance social memory, but only at low doses[21,22]. OT has no effect at moderate doses[21] and may interfere with memory at high doses[23]. Although the enhancement of social memory by OT has been postulated to involve the medial preoptic area[24] or olfactory bulbs[13], these effects are not blocked by local OT or AVP antagonists and thus may be occurring at a distant or unidentified receptor[24]. Engelmann *et al.* recently reported[12] that central administration of OT enhances social memory in female rats whereas infusion of an OT antagonist produces amnestic effects. We also examined the social investigation of conspecific mice by female mutant mice and found a similar pattern of deficit to that shown by males. The initial level of investigation by both $Oxt^{-/-}$ and $Oxt^{+/+}$ females was less vigorous than males and consequently it has been much more difficult to measure a robust deficit in this paradigm.



Methods

$Oxt^{-/-}$ and $Oxt^{+/+}$ mice (overall n=42 per genotype) used here were descended from the recombinant hybrid mouse originally constructed from 129S7/SvEvBrd-$Hprt^{b\text{-}m2}$ and C57BL/6J background strains[25]. We mated germline chimaeric males (generated from mutant 129S7/SvEvBrd-$Hprt^{b\text{-}m2}$ embryonic stem cells) to C57BL/6J females to generate $Oxt^{-/-}$ mice. We derived experimental animals from obligate litters by mating F_3, F_4 or F_5 $Oxt^{-/-}$ or $Oxt^{+/+}$ mice to yield litters composed entirely of either $Oxt^{-/-}$ or $Oxt^{+/+}$ pups. Not more than four mice from any one litter were used to ensure that pups came from at least six separate parental pairs. We cross-fostered pups of both genotypes to $Oxt^{+/+}$ mothers within 2 h of birth to provide comparable postnatal experience for both litter types ($Oxt^{-/-}$ mothers do not lactate, consequently pups must be cross-fostered to survive). Pups were weaned at approximately 21 d post-natal and housed in same-sex groups of siblings until adulthood (>40 d). The social memory phenotype described here was stable through three generations of mice (F_4–F_6) recruited for these studies.

All mice were housed under standard vivarium conditions (23.5±0.5 °C, 12 h light/dark cycle, food and water provided *ad libitum*). Adult (>50 d), group housed ovariectomized female CD-1 mice (Charles River Laboratories), and experimentally naive group-housed $Oxt^{+/+}$ male and female mice were used as stimulus animals for the social memory test. We have previously determined that ovariectomized females provide consistent stimulus value with minimal aggression in repeated testing[5].

Social memory. We transferred mice at age 40–50 d from group to individual housing for 7–10 d before testing to permit establishment of a home-cage territory. Testing began when a stimulus mouse was introduced into the home cage of each subject (n=18 per genotype) for a 1-min confrontation. At the end of the 1-min trial, we removed the stimulus animal and returned it to an individual holding cage. We repeated

Fig. 4 Pharmacological studies. ***a***, Social memory of $Oxt^{+/+}$ (top) and $Oxt^{-/-}$ (bottom) male mice was measured after administration of doses of CSF, OT, OTA and AVP (ICV). Depicted are peptide effects on mean +/–1 s.e.m. duration of olfactory investigation during each of 4 successive 1-min trials. Asterisks represent a significant decrease in olfactory investigation compared with the first trial. +, Significant effect of TA compared with vehicle within the associated trial (second, q=5.53; third, q=4.86, q=10.4; $P<0.05$) determined by Newman–Keuls (q) comparisons. A fifth 'dishabituation' trial depicts the response of males to the presentation of a new female in a 1-min pairing 10 min after the fourth trial. ***b***, OT receptor distribution in the brain of $Oxt^{+/+}$ (left) and $Oxt^{-/-}$ (right) mice. Oxytocin receptors are abundant in the main and accessory olfactory bulb (AOB), the lateral septum (LS), primary olfactory cortex (PO), CA3 layer of the hippocampus, and the medial (MeA) and cortical (CoA) nuclei of the amygdala. Analysis did not detect genotype-dependent difference in these structures.

this sequence for four trials with 10-min inter-trial intervals and introduced each stimulus to the same resident in all four trials unless otherwise indicated. In a fifth 'dishabituation' trial, we introduced each stimulus mouse to a different resident male mouse. A separate group (n=8 per genotype) was tested in a two-trial paradigm (5-min interaction with 30-min ITI). Behaviour was recorded on videotape and subsequently scored by trained raters (inter-rater reliability >90%) using a computer-assisted data acquisition system. We used described behavioural definitions, clustering and protocols[5,6].

Olfaction and habituation tests. We examined performance in two tests of olfactory behaviour. For the foraging task (adapted from ref. 26), food was withheld from individually housed mice (n=8 per genotype, randomly selected from the males used in the social memory test) for 16–24 h before testing. In the first phase of this test, we provided mice with small pieces of chocolate for 12 h. After mice consumed all pieces, we gave them chow. Two days later we again withheld food for 16–24 h. Testing began when we transferred each mouse from his home cage to a holding cage. After we placed a small piece of chocolate on the surface of the bedding of the cage, we returned each subject mouse to his home cage and recorded the latency to locate the chocolate chip. This procedure was repeated three more times with the chocolate chip buried in different positions of the cage beneath 2–3 cm of levelled bedding.

For olfactory habituation (adapted from refs 27,28), we packed a small perforated tube (5 cm long, 1.5 cm diameter) with cotton, placed a 10-µl drop of lemon extract in the cotton, and placed the scented tube in the home cage of each mouse for 1 min. We repeated this four times at 10-min intervals. In a fifth trial 10 min later, we added a 10-µl drop of vanilla extract to the lemon and again placed the tube in each subject's cage. We operationally defined olfactory investigation as nasal contact with the tube. We measured habituation to a startle stimulus using the automated SR-LAB system (San Diego Instruments). Adult male $Oxt^{-/-}$ (n= 6) and $Oxt^{+/+}$ mice (n=6) completed a test a session composed of 200 presentations of an acoustic startle stimulus (40 ms, 118 dB) regularly presented at intervals of 10 s. We computed mean startle amplitude data for successive 20-stimulus presentations for each subject to yield 10 startle response values per session.

Spatial memory tests. Ten $Oxt^{-/-}$ and $Oxt^{+/+}$ males (~80–120 d) used in the social memory task but not used in olfactory testing were tested in a Morris water maze[29]. We rendered water in a tank (120-cm diameter, 30-cm depth) opaque with white latex paint. In four trials each day, subjects were placed at all of four systematically varied positions equidistant along the edge of the tank with an inter-trial interval of 10 min. We measured latency and distance required to locate a submerged platform (2.5 cm square) located in the tank. We scheduled testing of maze performance during the last half of the light portion of the light-dark cycle. We repeated sessions on five successive days and conducted a 2-min probe trial with no platform on the sixth day.

A separate group of male mice (n=8 per genotype) was tested under red-light illumination in a two-trial Y-maze task[30]. The maze (arms: 34 cm long, 8 cm wide and 14.5 cm deep) was constructed of clear Plexiglass and equipped with guillotine doors isolating each arm. The experiment consisted of two trials separated by a 30-min inter-trial time interval. During the familiarization phase (trial 1), one arm of the Y-maze was closed with a guillotine door. We placed mice in one of the two remaining arms and allowed them to explore the maze for 5 min. During the retrieval phase (trial 2), we removed the door and animals had free access to all three arms. Other investigators have interpreted failure to show preference for the novel arm to reflect a deficit in spatial recognition[30].

We recorded activity in both mazes using the Noldus Ethovision system, which automatically quantifies distance traversed, velocity, latency and time spent by a mouse in defined sections of the maze.

Pharmacological studies. We implanted a separate group of mice with intraventricular cannuli. We anaesthetized mice with ketamine and xylazine, made a 1-mm midline incision across the top of the skull and placed the mouse in a stereotaxic apparatus. After cleaning the periosteum, we made a 1-mm hole 0.11-mm lateral to bregma and implanted a 26-gauge stainless-steel infusion cannula (Plastic One) 0.26 mm below the skull surface into the lateral ventricle. We secured the cannula to the skull with dental cement and inserted a stylus to maintain cannula patency. We made injections using a 33-gauge stainless-steel injector attached to PE-10 tubing fitted to a 10-µl Hamilton syringe. Injections began 3–4 d after mice recovered from surgery. One-nanogram doses of OT, OTA or AVP were dissolved in artificial cerebrospinal fluid (CSF) and delivered to awake, restrained mice in a 4-µl volume infused over 60–90 s. Subjects were tested beginning 2 min after the injection cannula was removed. Each subject received all doses with a minimum of 48–72 h between each administration. A CSF vehicle control trial preceded the first and third and followed the last peptide administration. Each mouse received OT and AVP as their first and second peptide dosing. For the third and fourth peptide dosing, we administered OT and OTA using a counterbalanced design within subject. The guide cannula remained patent throughout the experiment as measured by evidence of movement of an air bubble placed in the PE-10 line. At the completion of the dosing and testing series, we infused a 4-µl volume of 10% India ink in CSF, rapidly killed the mice, extracted brains and inspected them for evidence of ink in the ventricles. Only mice showing ink (n=7 per genotype) in the ventricles were included for analysis.

Oxytocin receptors. For the radioligand receptor binding, we processed slide-mounted sections for receptor autoradiography using ^{125}I-labelled d$(CH_2)_5$[Tyr(Me)$_2$,Tyr^{9}NH$_2$]ornithine vasotocin (NEN) as described previously[25]. Analysis of behaviour measured during the social memory test, Morris water maze, and olfactory and acoustic startle tests compared the influence of genotype ($Oxt^{-/-}$ or $Oxt^{+/+}$) during repeated trials using fixed-factor, repeated measure ANOVAs. For the two-trial Y-maze test, genotype-dependent differences in preference for new versus previously explored arms of the maze (expressed as percentage duration of second trial spent in each arm) were analysed using a two-factor ANOVA with repeated measures for different arms of the Y-maze. Analysis of Morris water maze data included a third factor representing five daily sessions composed of four trials per session. If we detected a significant main or interaction effect, we used a post-hoc Newman-Keuls Test comparison (q) to measure individual treatment group differences. In all tests, $P<0.05$ was accepted as significant.

Acknowledgements

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