

Impaired approach to novelty and striatal alterations in the oxytocin receptor deficient mouse model of autism

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ABSTRACT

Long-standing studies established a role for the oxytocin system in social behavior, social reward, pair bonding and affiliation. Oxytocin receptors, implicated in pathological conditions affecting the social sphere such as autism spectrum disorders, can also modulate cognitive processes, an aspect generally overlooked.

Here we examined the effect of acute (pharmacological) or genetic (*Oxtr*^{-/-}) inactivation of oxytocin receptor-mediated signaling, in male mice, in several cognitive tests. In the novel object recognition test, both oxytocin receptor antagonist treated wild type animals and *Oxtr*^{-/-} mice lacked the typical preference for novelty. *Oxtr*^{-/-} mice even preferred the familiar object; moreover, their performance in the Morris water maze did not differ from wild types, suggesting that oxytocin receptor inactivation did not disrupt learning. Because the preference for novel objects could be rescued in *Oxtr*^{-/-} mice with longer habituation periods, we propose that the loss of novelty preferences following *Oxtr* inactivation is due to altered processing of novel contextual information.

Finally, we observed an increased expression of excitatory synaptic markers in the striatum of *Oxtr*^{-/-} mice and a greater arborization and higher number of spines/neuron in the dorsolateral area of this structure, which drives habit formation. Our data also indicate a specific reshaping of dorsolateral striatal spines in *Oxtr*^{-/-} mice after exposure to a novel environment, which might subtend their altered approach to novelty, and support previous work pointing at this structure as an important substrate for autistic behaviors.

1. Introduction

In the last two decades, the oxytocin (OXT) system has emerged as a possible new target for neuropsychiatric and neurodevelopmental disorders characterized by deficits in the social domain, including Autism Spectrum Disorder (ASD) (Kirsch, 2015). However, despite an intense clinical and preclinical investigation, the contribution of OXT to the pathogenesis of autism remains undefined and its efficacy to treat the main symptoms largely unproven (Guastella and Hickie, 2016). This is due not only to the complexity and heterogeneity of ASD at the genetic, epigenetic and environmental level but also to the still unclear mechanisms of action and targets of OXT in the brain.

Neuroimaging studies in humans and preclinical studies in rodents have classically focused on OXT amygdala, lateral septum and accumbens-related effects on social cognition and social reward (Ebner et al., 2000; Engelmann et al., 2000; Guzman et al., 2013; Lukas et al.,

2013; Ma et al., 2016; Menon et al., 2018; Ortiz et al., 2018; Zoicas et al., 2014). However, a more complex picture recently emerged, favoring the hypothesis that OXT promotes social adaption through multiple neuropsychological mechanisms: OXT increases salience of social cues by enhancing the attentional shift towards social stimuli (Wang et al., 2017), it is necessary in the ventral tegmental areas to elicit social reward (Hung et al., 2017), and modulates sensory experience in neocortical areas (Zheng et al., 2014) and spinal cord (Eliava et al., 2016). Accordingly, fMRI analyses of mouse (Galbusera et al., 2017) and rat (Dumais et al., 2017) brains show that the effects of OXT on the brain are complex. Furthermore, animal studies indicate an OXT-mediated tuning of neurotransmission (Owen et al., 2013). OXT determines a suppression in spontaneous pyramidal cell firing (i.e. noise) and an improvement in the fidelity and in the temporal precision of spike transmission (i.e. signal), improving signal/noise ratio of hippocampal and cortical circuits, suggesting a widespread role of this

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neuropeptide in the modulation of signal/noise ratio (Owen et al., 2013). This could represent a generalized mechanism by which endogenous OXT enhances information processing and cognitive performances. A general role of OXT on neuronal networks and connectivity is consistent with a recent human study showing that, in women, the neuropeptide is capable to enhance corticostriatal connectivity (Bethlehem et al., 2017). The striatum is a key node in the networks that subserve the ability to represent cognitive and affective mental states to both self and others. In particular, the ventral striatum (i.e. the nucleus accumbens, NAc) participates in the processing of affective functions, such as the representation of emotions, while the dorsal striatum is involved in motor and cognitive functions, including habit formation (Gasbarri et al., 2014; Malvaez and Wassum, 2018). While the role played by OXT in the ventral striatum has been deeply investigated (King et al., 2016), its role in the dorsal striatum has received far less attention. Interestingly, it has recently been proposed that OXT could facilitate a ventral to dorsal shift in activation of the corticostriatal loops (Tops et al., 2014).

The mouse genetically lacking the oxytocin receptor ($Oxtr^{-/-}$) displays a strong autistic-like phenotype (Sala et al., 2013; Sala et al., 2011). These mice have reduced sociability and social memory, as well as increased aggression; they have increased susceptibility to seizures accompanied by an altered ratio between glutamatergic and GABAergic synapses in hippocampal neurons. Finally, these mice show cognitive rigidity in the reversal phase of a T-maze learning test, suggesting a role of this neuropeptide in cognitive processes. One possibility is that an impairment in cognitive performance is present in $Oxtr^{-/-}$ mice, in line with previous evidence of the role of OXT in learning and memory (Chini et al., 2014). To test this hypothesis, we conducted cognitive learning tests after genetic and pharmacological inactivation of OXT receptors (OXTRs). The inactivation of OXTR eliminated preferences for novel objects in a novel object recognition test (NOR), a test that evaluates episodic memory. However, performances in the Morris water maze (MWM), a paradigm commonly used to assess spatial learning abilities, were unaffected, suggesting preserved learning and memory functions. We hypothesized that the lack of preference observed in the NOR test could be due to an altered approach to novelty, and not just to a learning and memory impairment. Indeed, our data indicate that longer habituation restores the learning and memory performances of $Oxtr^{-/-}$ mice, as assessed by a single object interaction test and the NOR test. To investigate the possible neural substrates that subvert the $Oxtr^{-/-}$'s altered approach to novelty, we considered the CA1 region of the hippocampus, which is highly reactive to novel environmental stimuli (Rinaldi et al., 2010), and the dorsal striatum, a region involved in habit formation (Lingawi and Balleine, 2012). We found alterations in excitation/inhibition markers (including the scaffolding proteins PSD95 and gephyrin and both GABAergic and glutamatergic receptors) and in dendritic spines in the dorsolateral striatum (DLS), but not in the hippocampus. Based on these findings we hypothesized that $Oxtr^{+/+}$ and $Oxtr^{-/-}$ respond differently to changes in environment. Our data showed that habituation for 7 days to a new external environment strongly modulated dendritic spines in the DLS in $Oxtr^{-/-}$, while $Oxtr^{+/+}$ modulated hippocampal spines. Our data are consistent with our idea that impairments of $Oxtr$ impact the mice's approach to novelty, interfering with the processing of novel stimuli.

2. Materials and methods

2.1. Animals

C57Bl/6J mice were purchased from Jackson laboratories. $Oxtr^{+/+}$ and $Oxtr^{-/-}$, originally generated by Takayanagi et al. (2005) have been obtained by Larry Young (Emory University) and maintained by heterozygous mating. Litters were genotyped by PCR. Male mice at the age of 2–3 months were housed in groups of four or five individuals of mixed genotypes. Even though specific hierarchical studies were not

carried out, in our conditions no signs of dominance were seen (i.e. barbering and/or skin lesions). $Oxtr^{+/+}$ and $Oxtr^{-/-}$ mice were then individually housed starting from one week before behavioral testing. Mice were maintained under a 12: 12 h light/dark cycle (lights on 08.00 am) at 20–22 °C and 40–60% relative humidity with ad libitum access to food and water.

All of the tests were conducted during the light portion of the cycle. All procedures involving wild type C57Bl/6J were approved by the Institutional Animal Care and Use Committee and conformed to National Institutes of Health guidelines (USA). For the other studies in $Oxtr^{+/+}$ and $Oxtr^{-/-}$, all experimental procedures followed the guidelines established by the Italian Council on Animal Care and were approved by Italian Government Decree No. 17/2013. Every effort was made to minimize the number of mice used and their suffering.

2.2. Experiment 1: behavioral effects caused by the pharmacological inhibition of OXTR

2.2.1. OTA administration

Wild type C57Bl/6J mice were given intraperitoneal injections of either vehicle (sterile PBS) or the nonpeptide L-368,899 (OTA) (L2540, Sigma-Aldrich, St. Louis, MO) which is a high affinity antagonist of the OXTR (Williams et al., 1994) and readily passes through the blood-brain barrier (Boccia et al., 2007). Unlike other OXTR antagonists, L-368,899 is a full antagonist inhibiting OXTR signal transduction via Gq, Gi, and Go signaling cascades (Duque-Wilckens et al., 2018). Based on previous behavioral studies and on pharmacokinetic data (Lee et al., 2015; Olszewski et al., 2013; Thompson et al., 1997; Wei et al., 2015), OTA 5 mg/kg was administered 30 min before the T1 phase (familiarization with objects) of the novel object recognition task.

2.2.2. Novel object recognition analysis

Object recognition was conducted over two days in an open plastic arena (45.7 × 45.7 × 34.3 cm). On day 1, animals were habituated to the test apparatus for 10 min. On day 2, animals were subjected to a familiarization trial (T1) and a novel object recognition trial (T2) with an inter-trial interval of 120 min. Each trial was 10 min long. 30 min before T1, mice were injected again with either vehicle or OTA. The objects consisted of orange plastic cones, green plastic tubes, and colored plastic Lego stacks of different shapes. During T1, the mouse was placed in the center of the arena between two objects and allowed to explore. During T2, one of the objects was changed for a new object. To reduce innate object preference, the nature of the stimuli (familiar or novel) was counterbalanced, as previously suggested (Ennaceur and Delacour, 1988). Time exploring the object was defined as the animal directing the nose towards the object within < 0.5 cm of the object. Sitting on, or leaning to, an object was not considered as exploratory behavior. A video camera mounted above the arena recorded the behavior and Anymaze (Stoelting) was used to automatically record behavior during each trial. The discrimination index [(time spent exploring novel object – time exploring familiar object)/(time spent exploring novel object + time exploring familiar object)] was calculated as described elsewhere (Pitsikas et al., 2001).

2.3. Experiment 2: behavioral effects caused by the genetic ablation of the $Oxtr$ gene

2.3.1. Behavioral analysis

$Oxtr^{+/+}$ and $Oxtr^{-/-}$ mice were separated in six cohorts and were exposed to the following behavioral tests: cohort 1: novel object recognition, 1-Day habituation; cohort 2: Morris water maze; cohort 3: object interaction test; cohort 4: marble burying and Y-maze; cohort 5: novel object recognition after 5-days habituation; cohort 6: spatial object recognition 1 day habituation; cohort 7: spatial object recognition, 5 days habituation.

2.3.2. Novel object recognition (NOR)

Object recognition was conducted over two days in an open plastic arena (38 cm × 30 cm × 18 cm). Animals were habituated to the test apparatus for 10 min for 1 (cohort 1) or 5 consecutive days (cohort 5), and subjected to a familiarization trial (T1) and a novel object recognition trial (T2) with an inter-trial interval of 120 min. To reduce innate object preference, the nature of the stimuli (familiar or novel) was counterbalanced, as previously suggested (Pitsikas et al., 2001). The objects consisted of white plastic cylinders and colored plastic Lego stacks of different shapes. During testing, each mouse was placed in the center of the arena between the two objects and allowed to explore the two objects for maximum 10 min. An experimenter blind to genotype measured the time spent close to each object. In the case they reached a total exploration time of the two objects of 30 s the analysis was stopped. Object recognition was defined as the animal being within < 0.5 cm from an object, with its nose pointing at it. Exploration was defined as directing the nose towards the object or touching the object with the nose. Sitting on, or leaning to, an object was not considered as exploratory behavior. The discrimination index [(time spent exploring novel object – time exploring familiar object)/(time spent exploring novel object + time exploring familiar object)] was calculated as described elsewhere (Pitsikas et al., 2001).

2.3.3. Morris water maze

The maze consisted of a circular pool (120 cm in diameter × 50 cm in height) filled with water maintained at $25 \pm 0.5^\circ\text{C}$. An escape circular platform, 10 cm in diameter, was immersed 0.5 cm under the surface of water in the center of the North West (NW) quadrant and remained in the same position throughout the learning trials. Floating plastic particles hid the platform (Zhang et al., 2013). For the familiarization trial, mice were placed in a random area inside the maze for 60 s. For four consecutive days mice were assigned to one learning session consisting of four trials separated by 60 min intervals in which each mouse was released to the pool from different starting points and trained to a constant platform position. The trial was terminated as soon as the mouse climbed on the escape platform or when 120 s elapsed, whichever occurred first. If an animal failed to find a submerged platform within 120 s, it was gently placed on the platform for the time of 30 s and it was given a latency score of 120 s. Twenty-four hours after the last trial, a probe test was performed during which the platform was removed. An experimenter blind to genotype evaluated the time spent in the target area. After each trial, mice were placed on a paper towel for additional drying and then put back into their home cages.

2.3.4. Object interaction test

Animals were divided into 3 experimental groups: no habituation, habituation to an automated activity cage for 10 min/day for 5 days prior to testing, habituation to the object in the home cage for 10 min/day for 5 days prior to testing. The experiment was carried out on Day 6 in the same activity cage entirely made of Perspex and fitted with two parallel horizontal infrared beams located 2 cm from the floor (41 cm × 41 cm × 32 cm; Basile, Varese, Italy). The test procedures were adapted from several protocols (Ennaceur et al., 2009; Green et al., 2013) (Wozniak et al., 2013) to ascertain the habituation factor. Each animal was put in the activity cage for the first 30 min and the number of horizontal counts recorded. An object (a metal pen holder 10.5 cm × 6 cm) was then placed in the center of the arena for the remaining 30 min for measurement of motor activity and interaction with the object. A video camera mounted above the chamber automatically recorded the entire experiment, and an experimenter blind to genotype measured offline the latency (sec) to the first investigation and the total number of investigations towards the object over the last 30-min period. Investigation of the object was quantified according to (Wozniak et al., 2013), evaluating the latency to the first object investigation and the number of rearings towards the object with the nose

approximately 5 mm or less from the object.

2.4. Experiment 3: neuronal morphological and biochemical characterization

2.4.1. Golgi staining

A separate cohort of 15 *Oxtr*^{+/+} and 12 *Oxtr*^{-/-} naïve mice at PN90 were used to evaluate the involvement of external environment change on dendritic spines and pre/post synaptic markers. Animals were moved in their cages from the housing facility to the behavioral unit for 7 days. The home cages had metal grid lids, so that the mice could see that the external environment changed, while avoiding physical contact between the animals and the experimenter, which could have introduced a confounding factor. At the end of the 7th day, mice were deeply anesthetized and intracardially perfused with 0.9% saline solution. Brains were impregnated for 6 days in Golgi-Cox solution and for 2 additional days in a 30% (w/v) sucrose solution. Coronal sections (100-μm-thick) were cut with a vibratome, mounted on gelatinized slides and stained according to the reference protocol of Gibb and Kolb (Gibb and Kolb, 1998). Regions of interest were identified with the help of the Franklin and Paxinos mouse brain atlas (“The mouse brain in stereotaxis coordinates” 3rd ed., 2008). Hippocampus subregion CA1 was evaluated between bregma -1.46 and -3.52 mm, and the dorsolateral striatum (DLS) between bregma 1.18 and -0.58 mm. Bright field Z-stack images were acquired with an Axiovert microscope (Zeiss) equipped with a Digital CCD Camera ORCA-AG (Hamamatsu). 63× and 20× magnification objectives were used for the analysis of spine density and of the dendritic arbors, respectively. Spines were counted on dendrites (II-IV order) from at least 3 neurons per animal. Protrusions longer than 2 μm or with no evident connection with the dendritic shaft were excluded from the count. Spine count and Sholl analysis to determine dendritic morphology were done with the software NeuronStudio (<http://research.mssm.edu/cnic/tools-ns.html>, Mount Sinai School of Medicine).

2.4.2. Western blotting

Hippocampi and striata were dissected from *Oxtr*^{+/+} and *Oxtr*^{-/-} mice at PN90 (3 animals per genotype) and lysed in ice-cold lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% Triton X-100) containing Protease Inhibitors (Sigma) and Phosphatase Inhibitors (Roche). Protein concentration was determined with the DC protein assay kit (Bio-Rad) and samples were diluted in 3× sample buffer (375 mM Tris-HCl, pH 6.8, 20% glycerol, 9% SDS, 10% β-mercaptoethanol, 0.05% bromophenol blue). Protein samples were separated by SDS-PAGE using 9% (w/v) acrylamide gels and transferred onto a 0.2 μm nitrocellulose membrane (GE Healthcare). Unspecific binding sites were saturated with a 30 min-incubation in BSA (5% w/v) or milk (5% w/v) in TBS (20 mM Tris-HCl, pH 7.4, 150 mM NaCl) at 37 °C. Proteins of interest were probed with the proper primary antibody and revealed by secondary antibodies conjugated with infrared-emitting-fluorophores. Signals were detected and quantified using an Odyssey scanner (Li-Cor) controlled by the ImageStudio software (Li-Cor). All the antibodies used and the respective suppliers and working dilutions are listed in Supplementary Table 1.

2.5. Statistical analysis

All results are presented as the means ± SEM. Where data distribution was normal and variance comparable (novel and spatial object recognition, motor activity), unpaired Student's *t*-test or two-way ANOVAs (with repeated measures when appropriate), followed by Tukey's or Bonferroni's post hoc tests were used. Non-parametric data were analyzed by Mann-Whitney test. For ANOVAs and Mann-Whitney test, effect sizes for each factor were calculated as eta-squared (η^2) which represents the proportion of variance that is accounted for by the factor being tested. An η^2 of 0.14 or greater was considered large

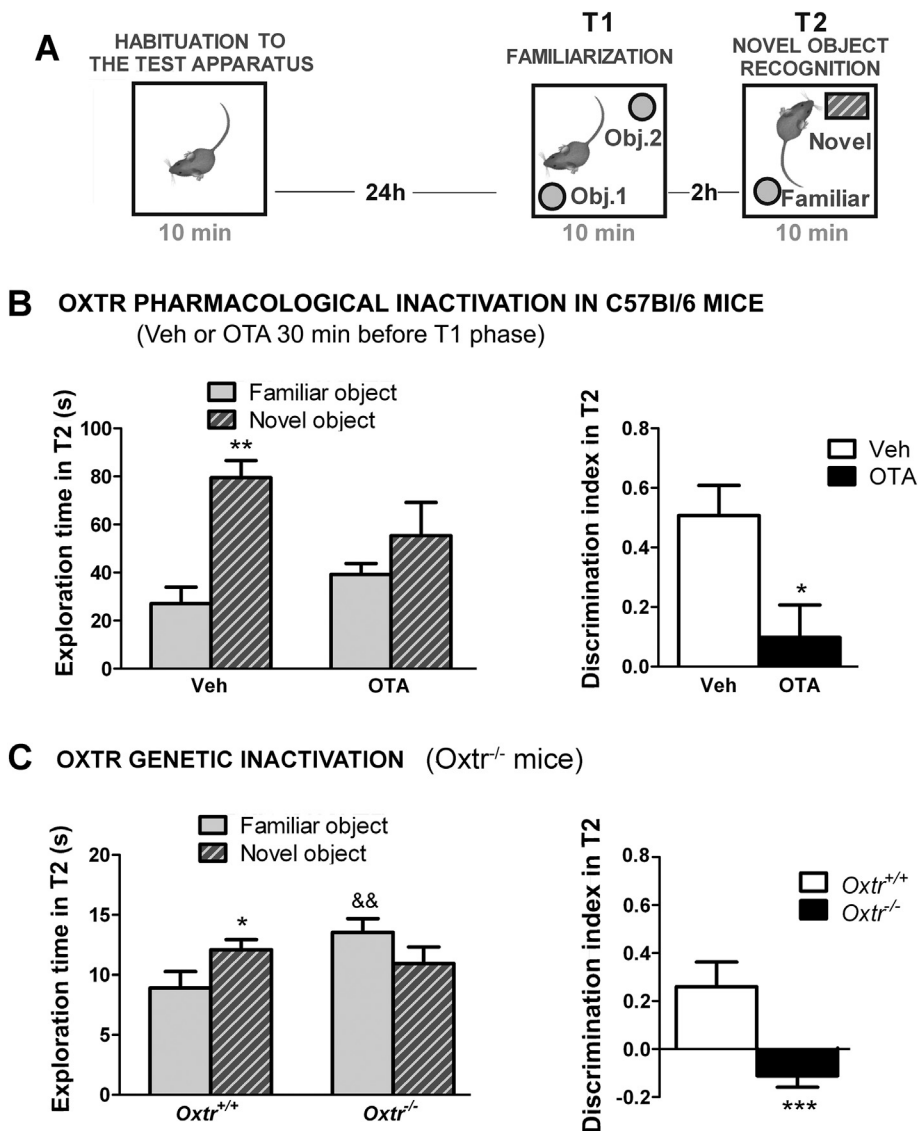


Fig. 1. OXTR inactivation impairs mice's performance in the NOR. **A.** Schematic illustration of the NOR procedure: habituation to the test apparatus was performed once 24 h before the test and lasted 10 min. Please note that objects 1 and 2 in T1 were different for the NOR in B and identical for the NOR in C. **B.** (left): C57Bl/6J mice treated with vehicle 30 min before familiarization (T1 phase) displayed a significantly higher mean exploration time of the novel object versus the familiar one in the T2 phase, while no difference was shown in C57Bl/6J mice treated with OTA ($p = 0.29$). ** $p < 0.01$ vs corresponding familiar object ($N = 6$ Veh and 7 OTA); **B.** (right): OTA administration (5 mg/kg i.p.) drastically reduced the discrimination index in the test. * $p < 0.05$ vs C57Bl/6J saline ($N = 6$ Veh and 7 OTA). **C.** (left): *Oxtr*^{+/+} mice displayed a significantly higher mean exploration time of the novel object versus the familiar one, while no difference was shown in *Oxtr*^{-/-} mice ($p = 0.24$). *Oxtr*^{-/-} also spent more time close to the familiar object in comparison to *Oxtr*^{+/+} mice. * $p < 0.05$ vs corresponding familiar object; && $p < 0.01$ vs corresponding *Oxtr*^{+/+} ($N = 11$ *Oxtr*^{+/+} and 15 *Oxtr*^{-/-}); **C.** (right): A difference in discrimination index was found between the two genotypes. *** $p < 0.001$ vs corresponding *Oxtr*^{+/+} ($N = 11$ *Oxtr*^{+/+} and 15 *Oxtr*^{-/-}). Data are presented as mean $\pm$ SEM.

whereas an η^2 between 0.06 and 0.13 was considered medium. For pairwise comparisons, effect sizes were calculated with Cohen's d analysis which evaluates the difference between means divided by standard deviation. In this measure, effect sizes were interpreted as small ($0.2 < d < 0.5$), moderate ($0.5 < d < 0.8$) and large ($d > 0.8$). Differences were considered statistically significant when $p < 0.05$. All statistical analyses were carried out by using Prism 6 software (GraphPad Software, Inc., USA). Results were deemed statistically significant when $p < 0.05$.

3. Results

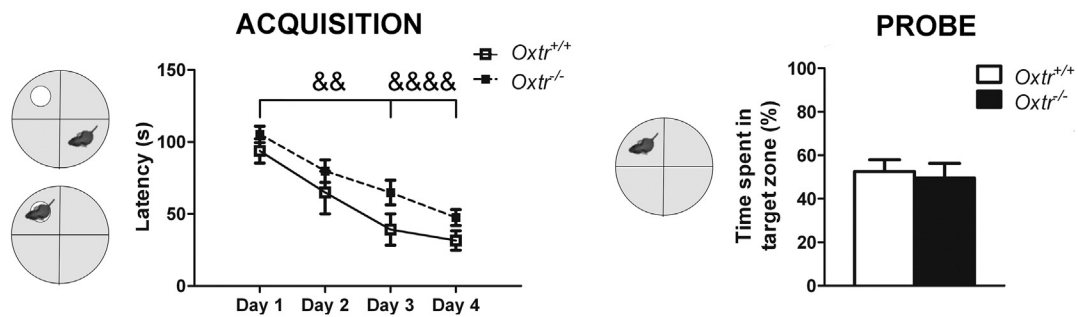
3.1. Blockade of OXTR causes impairments in the novel object recognition

In the attempt to better understand the overall contribution of the OXTR system to cognitive processes, we tested the performance of naïve C57Bl/6J mice in the NOR task, commonly used to evaluate episodic memory, when OXTRs had been blocked by OTA administration 30 min before the T1 phase of the test (familiarization with the objects, Fig. 1A). Fig. 1B shows that a single injection of the drug 30 min prior to the first exposure to the objects disrupts preference for the novel object in the NOR test (left: Object effect: $F_{(1,22)} = 0.43$, $p = 0.52$, $\eta^2 = 0.01$; Treatment effect: $F_{(1,22)} = 13.97$, $p = 0.001$, $\eta^2 = 0.35$; Object $\times$

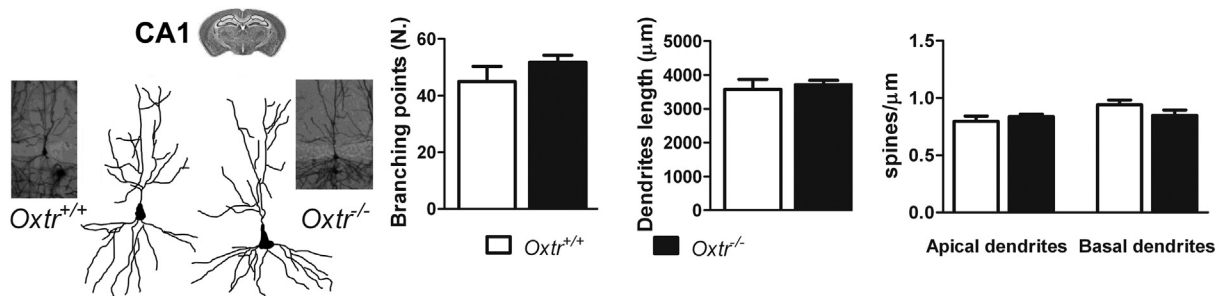
Treatment interaction: $F_{(1,22)} = 3.92$, $p = 0.06$, $\eta^2 = 0.10$; right: $t_{(11)} = 2.71$; $p = 0.02$, $d = 1.51$). As shown in Fig. S1, alterations in object explorations were not linked to increased fear/anxiety (recorded as freezing time, Fig. S1A) or to impaired locomotion (measured as distance travelled during the test, Fig. S1B); moreover, both groups of mice investigated the objects to the same extent during the acquisition phase of the test (Fig. S1C).

Deletion of the *Oxtr* gene in mice (*Oxtr*^{-/-} mice) results in very similar impairments in the NOR test (Fig. 1C). Two-way ANOVA revealed no significant effect among genotypes or objects, but a significant genotype $\times$ object interaction (Genotype effect: $F_{(1,48)} = 2.87$, $p = 0.096$, $\eta^2 = 0.495$; Object condition: $F_{(1,48)} = 0.31$, $p = 0.58$, $\eta^2 = 0.53$; Genotype $\times$ object condition interaction: $F_{(1,48)} = 6.83$, $p = 0.012$, $\eta^2 = 0.1178$). *Oxtr*^{-/-} mice did not display preference for any of the two objects in the test, indeed they spent an equal amount of time investigating the novel and familiar objects (Fig. 1C, left), leading to a much lower discrimination index than *Oxtr*^{+/+} mice (Fig. 1C, right; $t_{(24)} = 3.56$, $p = 0.001$, $d = 1.36$). Such results are usually interpreted as the inability to recognize the two objects, as rodents have a natural preference for novelty (see higher investigation time on the novel object for *Oxtr*^{+/+} mice, Fig. 1C left). Notably, the time spent to explore the familiar object was higher in *Oxtr*^{-/-} mice than in *Oxtr*^{+/+} mice, indicating an increased attention for the familiar object.

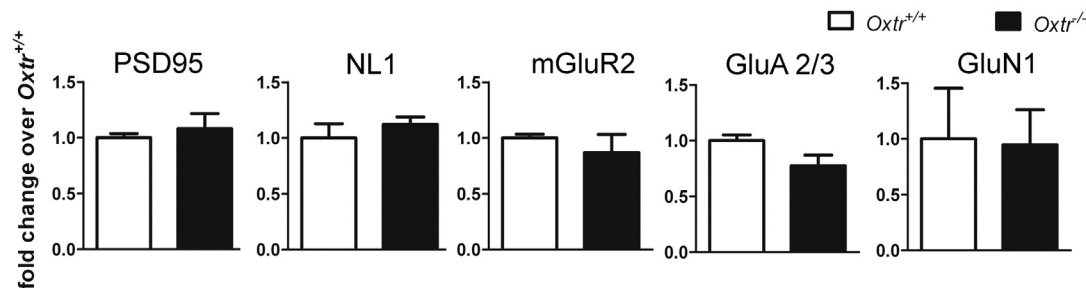
A MORRIS WATER MAZE



B NEURONAL MORPHOLOGY AND DENDRITIC SPINES



C HIPPOCAMPAL EXCITATORY POST-SYNAPTIC MARKERS



D HIPPOCAMPAL INHIBITORY POST-SYNAPTIC MARKERS

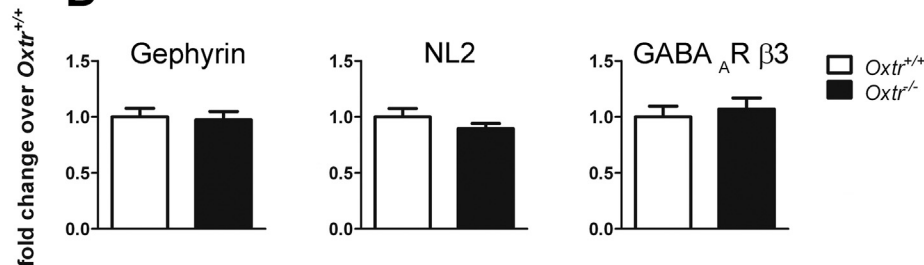


Fig. 2. $Oxtr^{-/-}$ mice perform normally in the Morris water maze, and do not display overt morphological and biochemical alterations in the CA1 of the hippocampus. A. (left): Schematic illustration of acquisition in the MWM procedure, and performance over time of $Oxtr^{+/+}$ and $Oxtr^{-/-}$ mice during this phase. $\&\&$ $p < 0.01$, $\&\&\&$ $p < 0.0001$ vs corresponding latency time during Day 1 ($N = 6$ $Oxtr^{+/+}$ and 12 $Oxtr^{-/-}$); A. (right): Schematic illustration of the probe test, where both genotypes spent an equal amount of time in the target zone ($N = 6$ $Oxtr^{+/+}$ and 12 $Oxtr^{-/-}$). B. (left): Representative pictures of Golgi-stained hippocampal pyramidal neurons in $Oxtr^{+/+}$ and $Oxtr^{-/-}$ mice; B. (right): Number of branching points ($N = 3$ $Oxtr^{+/+}$ and 6 $Oxtr^{-/-}$), dendrites' total length ($N = 3$ $Oxtr^{+/+}$ and 3 $Oxtr^{-/-}$) and spines' densities at apical and basal dendrites ($N = 9$ $Oxtr^{+/+}$ and 6 $Oxtr^{-/-}$) do not differ between the two genotypes. C, D. Quantification of adult hippocampal excitatory and inhibitory post-synaptic markers. None of the excitatory markers (top row, PSD95, NL1, mGluR2, GluA 2/3 and GluN1) nor of the inhibitory markers (bottom row: gephyrin, NL2 and GABA_A $\beta 3$) analyzed is altered in $Oxtr^{-/-}$ mice in comparison with $Oxtr^{+/+}$ mice ($N = 3$ $Oxtr^{+/+}$ and 3 $Oxtr^{-/-}$). All data are presented as mean $\pm$ SEM.

3.2. $Oxtr^{-/-}$ mice exhibit normal acquisition in the Morris water maze

The MWM is a test of spatial learning based on hippocampal function (Morris, 1984). If the poor outcome of the $Oxtr^{-/-}$ mice in the

NOR were due to hippocampal memory deficiencies, we would expect these animals to perform badly also in the MWM. However, our results suggest that $Oxtr^{-/-}$ mice have no deficits in hippocampal-based memory in comparison with $Oxtr^{+/+}$ mice, either during the

acquisition phase (Fig. 2A, left), or during the probe test (Fig. 2A, right). Two-way ANOVA revealed differences in genotype (as between subject factor: $F_{(1,64)} = 7.47$, $p = 0.008$, $\eta^2 = 5.96$), in time (as within factor: $F_{(3,64)} = 17.72$, $p < 0.0001$, $\eta^2 = 42.41$), but not in genotype $\times$ time interaction ($F_{(3,64)} = 0.23$, $p = 0.86$, $\eta^2 = 0.57$). Post hoc analysis revealed a significant decrease in the latency to find the platform during the third and fourth trials, compared to the corresponding first trial. Moreover, during the probe test both genotypes spent the same amount of time in each quadrant (Fig. 2A, right; $U_{(16)} = 33$, $p = 0.80$, $\eta^2 = 0.0001$). This is in agreement with previously reported results of unaffected spatial learning in these mice (Sala et al., 2011).

To corroborate the fact that $Oxtr^{-/-}$ mice's deficits in the NOR do not depend on impaired hippocampal function, we characterized by Golgi staining the properties of adult pyramidal neurons in the CA1 area of hippocampus in naïve animals (Fig. 2B). Spine density, dendritic length and complexity are similar in $Oxtr^{+/+}$ and $Oxtr^{-/-}$, indicating no major impact of the genetic deletion on the morphology of CA1 pyramidal neurons in young adults. Biochemical analysis of excitatory and inhibitory post-synaptic markers in hippocampi also revealed no differences between genotypes. Levels of the excitatory post-synaptic scaffolding proteins PSD95 and NL1, and of the glutamatergic markers mGluR2, GluA2/3 and GluN1, as well as the inhibitory post-synaptic markers gephyrin, NL2 and GABA_A receptor $\beta 3$ subunit, are not altered in the hippocampi of adult $Oxtr^{-/-}$ mice as compared to $Oxtr^{+/+}$ animals (Fig. 2C and D, respectively). Altogether, these data indicate no obvious impairments at the functional, morphological and biochemical levels in the hippocampus of adult $Oxtr^{-/-}$ mice.

3.3. The interest in a new object is rescued by habituation in $Oxtr^{-/-}$ mice

Impairments in the approach to novelty can affect cognitive abilities in novel environments. As we excluded memory impairments in $Oxtr^{-/-}$ mice, we tested whether the impaired behavior showed by $Oxtr^{-/-}$ mice in the NOR could be due to an altered approach to novelty. We evaluated the interaction of these mice with a novel object in a novel environment (the automated activity cage) (Fig. 3A). As expected, $Oxtr^{-/-}$ mice explored the unknown object less than $Oxtr^{+/+}$ mice (Fig. 3Ai, middle), and also displayed a longer latency to approach it for the first time (Fig. 3Ai, bottom). We ruled out that this was caused by motor impairment, as horizontal movements in the activity cage did not differ between $Oxtr^{-/-}$ and $Oxtr^{+/+}$ mice (Fig. 3Ai, top). Interestingly in two other tasks, the marble burying test and the spontaneous alternations on the Y-maze, $Oxtr^{-/-}$ mice also displayed longer latencies to approach the new objects (the marbles) and to entry the first arm in the Y maze (Fig. S2A and B, right). In these cases, however, their performance in the main tasks were similar to $Oxtr^{+/+}$ in terms of amount of buried marbles (Fig. S2A, left) and arms alternations (Fig. S2B, left), indicating no major defects in working memory and compulsive behavior in $Oxtr^{-/-}$ animals, and further supporting our hypothesis that novelty is an issue for these animals.

We next verified whether the approach to novel objects could be rescued by reducing the novel components presented in the test, namely the environment and the object, by habituating the animals to either of them. To do this, we analyzed motor activity and interaction with the object in mice previously habituated or not to the context or the object for 5 days. Analysis of the horizontal movements for 30 min in the activity cage indicated that 5 days of repeated interaction with the context, but not with the object, was enough to induce a significant decrease in motor activity in both genotypes (Fig. S3iii) (Habituation: $F_{(2,30)} = 9.13$, $p = 0.0008$, $\eta^2 = 17.22$; Genotype: $F_{(1,30)} = 1.06$, $p = 0.31$, $\eta^2 = 0.11$; Habituation $\times$ Genotype interaction: $F_{(2,30)} = 1.47$, $p = 0.24$, $\eta^2 = 1.08$) This was expected, since habituation is considered as a decline in behavioral response to a repeated stimulus (Rankin et al., 2009). The effects of the habituation to the object on motor activity became evident in the second 30 min of the test, when the object was introduced in the activity cage (Fig. 3Aii, top)

(Habituation: $F_{(2,30)} = 3.16$, $p = 0.05$, $\eta^2 = 17.22$; Genotype: $F_{(1,30)} = 0.04$, $p = 0.83$, $\eta^2 = 0.11$; Habituation $\times$ Genotype interaction: $F_{(2,30)} = 0.20$, $p = 0.81$, $\eta^2 = 1.08$). Habituation had a significant effect also on investigative behavior in terms of number of object investigations (Habituation: $F_{(2,30)} = 14.15$, $p < 0.0001$, $\eta^2 = 10.86$; Genotype: $F_{(1,30)} = 45.28$, $p < 0.0001$, $\eta^2 = 17.37$; Habituation $\times$ Genotype interaction: $F_{(2,30)} = 78.54$, $p < 0.0001$, $\eta^2 = 60.26$) and latency to the first investigation (Habituation: $F_{(2,30)} = 0.17$, $p = 0.84$, $\eta^2 = 0.64$; Genotype: $F_{(1,30)} = 1.03$, $p = 0.31$, $\eta^2 = 1.95$; Habituation $\times$ genotype interaction $F_{(2,30)} = 8.86$, $p = 0.0008$, $\eta^2 = 33.37$). The 5-day habituation reduced $Oxtr^{+/+}$ mice's interest towards the object, decreasing the number of object investigations and raising the latency to the first approach (Fig. 3Aii, middle and bottom), a normal response to repeated exposure to stimuli in healthy animals. It is of interest to note that habituation to the context was enough to damp down the efficacy of the novel object to attract the interest of the $Oxtr^{+/+}$ animals (Fig. 3Aiii, middle). Opposite outcomes were observed in $Oxtr^{-/-}$ mice, where habituation rescued the interest for the object irrespectively from the type of habituation (Fig. 3Aii and Aiii, middle). However, the latency to the first exploration was affected differently: context habituation normalized, whereas object habituation reduced, the latency time to the first object approach (Fig. 3Aii and Aiii, bottom), suggesting that $Oxtr^{-/-}$ could either prefer a known object or "escape" faster from the novel environment.

From this set of experiments, we can conclude that habituation to the environment has a profound impact on mice's exploratory behavior towards a novel object, although divergently between $Oxtr^{+/+}$ and $Oxtr^{-/-}$ mice. In fact, while $Oxtr^{+/+}$ mice lose interest in the object itself upon repeated exposure to it, $Oxtr^{-/-}$ mice seem to need habituation (to the environment) to be driven to explore an unfamiliar object.

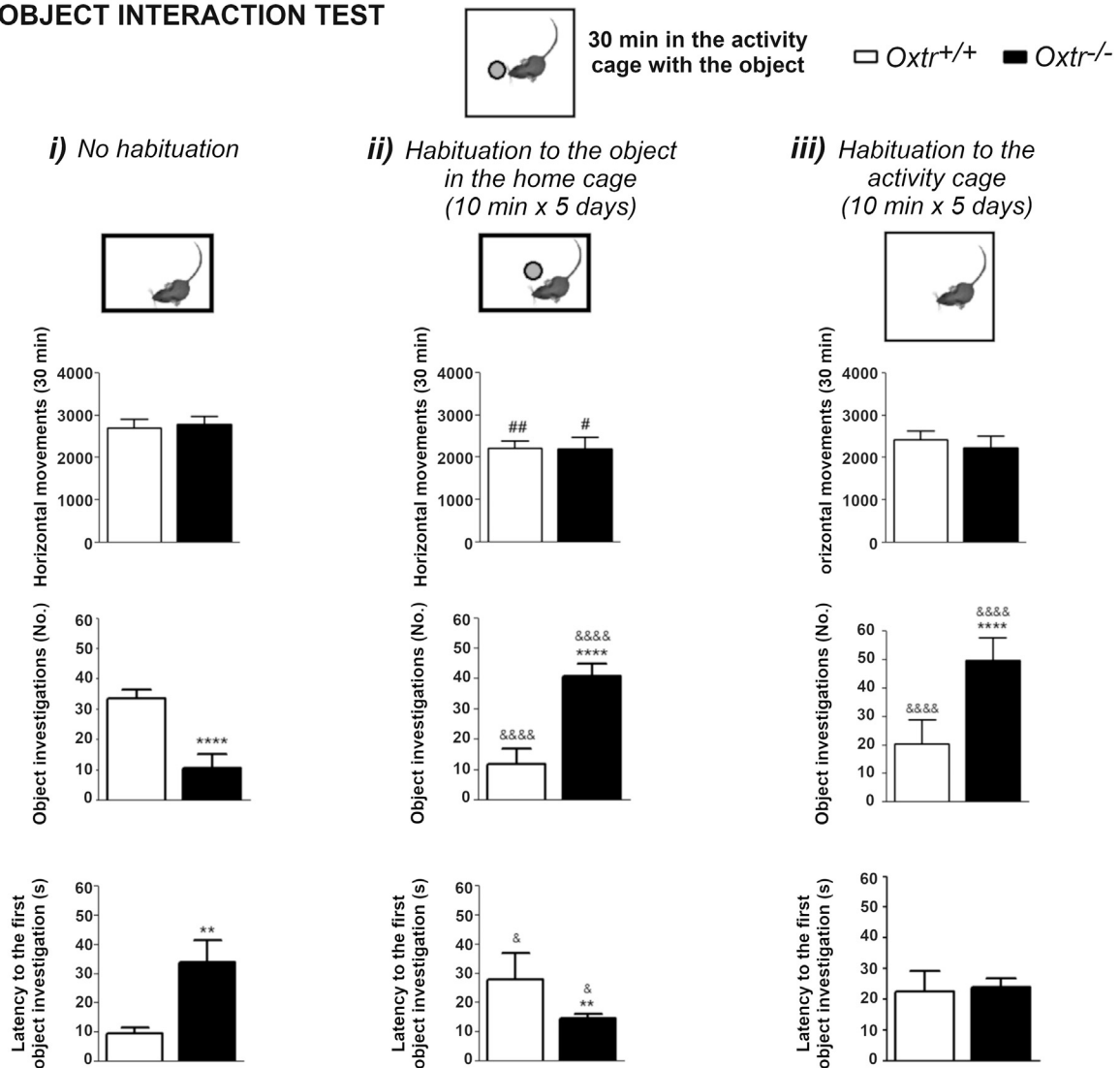
3.4. Habituation to the context rescued NOR deficits in $Oxtr^{-/-}$ mice

Since in the object interaction task the approach to novelty appeared altered in $Oxtr^{-/-}$ mice, but was rescued after 5-day habituation, we wondered whether habituation to the test cage (5 days vs the usual 1 day) could improve the performance of $Oxtr^{-/-}$ animals in the NOR (Fig. 3B). In line with our expectations, long habituation led to a change in the exploration times (Fig. 3Bi; Habituation: $F_{(1,30)} = 11.46$, $\eta^2 = 27.62$; $p = 0.03$; Genotype: $F_{(1,30)} = 0.041$, $p = 0.84$, $\eta^2 = 0.09$; Habituation $\times$ Genotype interaction: $F_{(1,30)} = 0.001$, $p = 0.97$, $\eta^2 = 0.003$) and to a normalization of the discrimination index in $Oxtr^{-/-}$ mice (Fig. 3Bii; $t_{(15)} = 0.01$, $p = 0.99$, $d = 0.01$). Interestingly, an analogous behavior was observed when $Oxtr^{-/-}$ mice were subjected to the spatial version (SOR) of the NOR task (Fig. S4). When a single habituation step to the home cage was provided (the standard NOR/SOR protocol), $Oxtr^{-/-}$ mice did not show any preference for the displaced object (Fig. S4B). However, 5 days of habituation decreased the exploration of the stationary object (Fig. S4B; Habituation: $F_{(3,48)} = 7.57$, $\eta^2 = 14.13$, $p = 0.0003$; Genotype: $F_{(1,48)} = 1.37$, $p = 0.885$, $\eta^2 = 0.86$; Habituation $\times$ Genotype interaction: $F_{(3,48)} = 27.65$, $p < 0.0001$, $\eta^2 = 55.06$), with a consequent reversion in the discrimination index (Fig. S4C; Habituation: $F_{(1,24)} = 9.52$, $p = 0.005$, $\eta^2 = 14.13$; Genotype: $F_{(1,25)} = 0.58$, $p = 0.45$, $\eta^2 = 23$; Habituation $\times$ Genotype interaction: $F_{(1,24)} = 7.28$, $p = 0.01$, $\eta^2 = 17.60$). In both situations, the discrimination index of $Oxtr^{-/-}$ mice indicates a recovered preference for the novel (or displaced) object and a loss of interest in the familiar one. This suggests that novel environments affect $Oxtr^{-/-}$ mice's cognitive performances, but a longer habituation is sufficient to rescue such defects.

3.5. Increased excitatory synapses in the striatum as a possible substrate for the impaired approach to novelty in $Oxtr^{-/-}$ mice

Striatal networks, in particular in the dorsal (Malvaez and Wassum,

A OBJECT INTERACTION TEST



B NOVEL OBJECT RECOGNITION

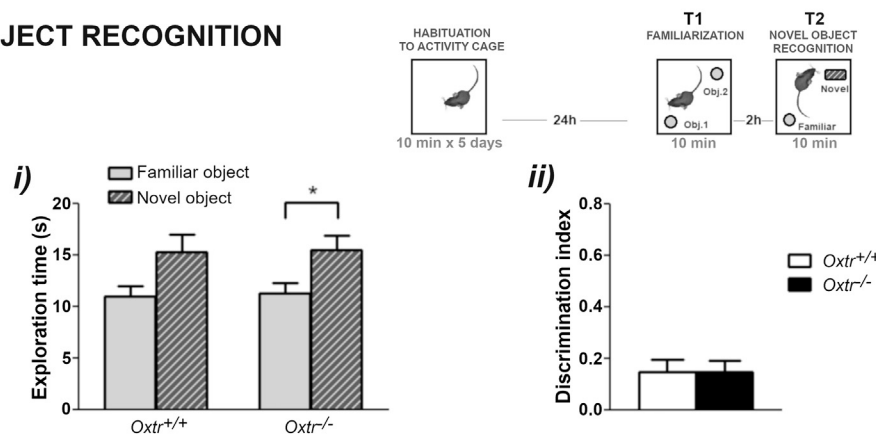
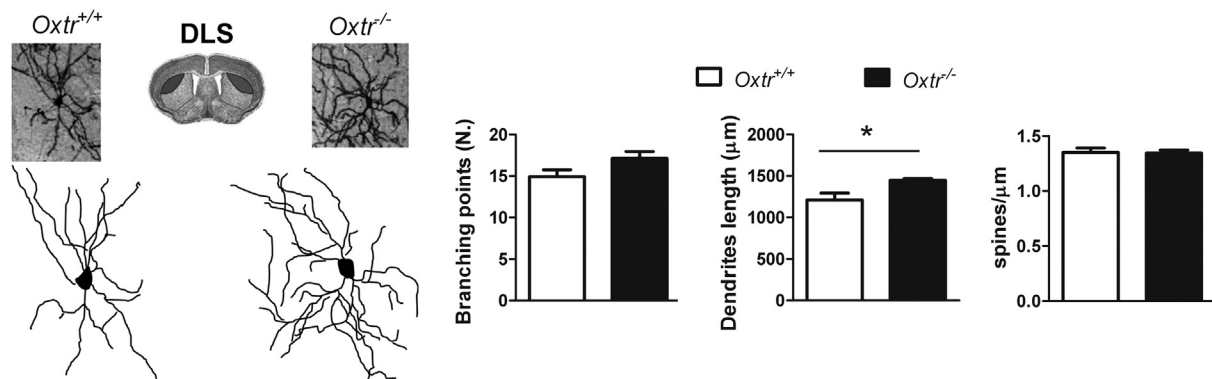
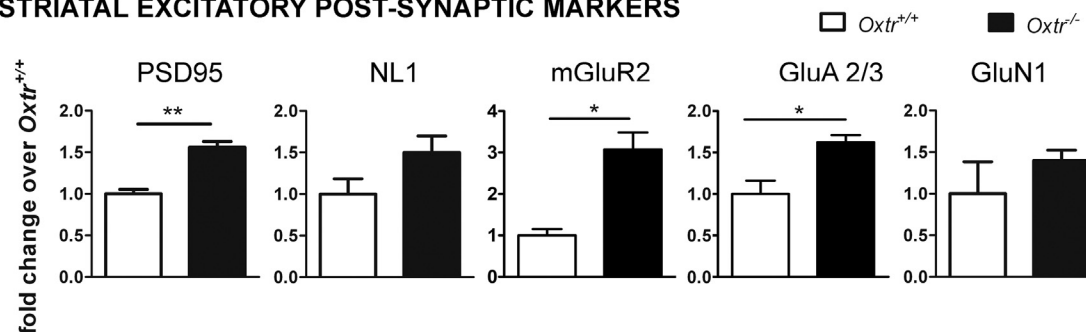


Fig. 3. Habituation rescues the exploratory behavior of *Oxttr*^{-/-} mice towards the object and restores preference for the novel object in the NOR. **A.** Object interaction test. **i).** When no habituation is provided, *Oxttr*^{-/-} mice display normal horizontal movements (top), but a reduced number of object investigations (middle) and an increased latency to the first object investigation (bottom). ***p* < 0.01, *****p* < 0.0001 vs corresponding *Oxttr*^{+/+} mice. **ii).** 5-Day habituation with the object decreased horizontal movements in both genotypes when compared to the first 30 min where no object was inserted. Moreover, it increased the number of object investigations (middle) and reduced the mean latency time to the first object exploration (bottom) in *Oxttr*^{-/-} mice. Opposite effects were seen in *Oxttr*^{+/+} mice. **iii).** 5-Day habituation to the activity cage had similar effects on the number of object investigations (middle), however the latency to the first exploration was similar in both genotypes (bottom). #*p* < 0.05, ##*p* < 0.01 vs corresponding first 30 min in the empty activity cage (see Fig. S2); ***p* < 0.01, *****p* < 0.0001 vs corresponding *Oxttr*^{+/+} mice; &*p* < 0.05, &&&&*p* < 0.0001 vs corresponding same genotype no habituation condition (N = 6 mice per group). **B.** Novel object recognition. **i).** Mean exploration time and **ii).** mean discrimination index in *Oxttr*^{-/-} mice are normalized by 5-Day habituation to the test apparatus. **p* < 0.05 vs corresponding familiar object (N = 7 *Oxttr*^{+/+} and 10 *Oxttr*^{-/-}). All data are presented as the mean ± SEM.

A NEURONAL MORPHOLOGY AND DENDRITIC SPINES



B STRIATAL EXCITATORY POST-SYNAPTIC MARKERS



C STRIATAL INHIBITORY POST-SYNAPTIC MARKERS

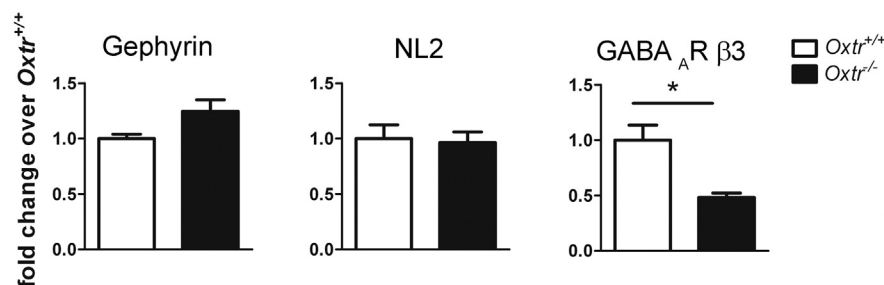


Fig. 4. Morphological and biochemical alterations in the Dorsolateral Striatum (DLS) in adult *Oxttr*^{-/-} mice. A. (left): Representative pictures of Golgi-stained DLS neurons in *Oxttr*^{+/+} and *Oxttr*^{-/-} mice. A. (right): Number of branching points (N = 3 *Oxttr*^{+/+} and 6 *Oxttr*^{-/-}) and dendrites' spines' density (N = 12 *Oxttr*^{+/+} and 9 *Oxttr*^{-/-}) do not differ between *Oxttr*^{+/+} and *Oxttr*^{-/-} mice; however, *Oxttr*^{-/-} mice have a significantly increased dendrites' length. *p < 0.05 vs corresponding *Oxttr*^{+/+} mice (N = 3 *Oxttr*^{+/+} and 3 *Oxttr*^{-/-}). B,C. Quantification of adult striatal Excitatory and Inhibitory post-synaptic markers. B. The excitatory markers PSD95, mGluR2 and GluA 2/3 are significantly increased, whereas NL1 and GluN1 are normal, in *Oxttr*^{-/-} mice when compared to *Oxttr*^{+/+} mice. C. The inhibitory marker GABA_A β3 is significantly reduced in *Oxttr*^{-/-} in comparison with *Oxttr*^{+/+} mice, whereas no alterations in gephyrin and NL2 were found. *p < 0.05, **p < 0.01 vs corresponding *Oxttr*^{+/+} mice (N = 3 *Oxttr*^{+/+} and 3 *Oxttr*^{-/-}). All data are presented as mean ± SEM.

2018) and dorsolateral striatum (Thorn et al., 2010), are known to strongly modulate habits, behavioral flexibility, routine formation and neophobia/familiarization.

Golgi staining of medium spiny neurons in this region at basal conditions (Fig. 4A) did not highlight significant differences in spine density (number of spines/μm) and in the number of branching points between *Oxttr*^{+/+} and *Oxttr*^{-/-} mice, but in *Oxttr*^{-/-} mice a bigger total length of dendrites was found (Fig. 4A, middle; $t_{(4)} = 2.78$, $p = 0.05$, $d = -2.27$). An enlarged dendritic arbor, in the presence of an unchanged spine density, results in a higher number of total spines per neuron. In line with the Golgi-staining data, in *Oxttr*^{-/-} striatal tissue, both metabotropic (mGluR2) and ionotropic (GluA2/3) glutamate receptors, and the scaffolding protein PSD95 were upregulated (Fig. 4B; PSD95: $t_{(4)} = 6.35$, $p = 0.003$, $d = -5.19$; mGluR2: $t_{(3)} = 3.83$, $p = 0.031$, $d = -3.49$; GluA2/3: $t_{(3)} = 3.86$, $p = 0.031$, $d = -3.52$).

Moreover, the ionotropic GABAergic receptor (subunit β3) was reduced in *Oxttr*^{-/-} mice (Fig. 4C; $t_{(4)} = 3.66$, $p = 0.02$, $d = 2.99$), indicating a defect in the inhibitory compartment, apparently not accompanied by a reduced number of inhibitory synapses, as the scaffolding protein Gephyrin was not reduced (Fig. 4C; $t_{(4)} = 2.26$, $p = 0.09$, $d = -1.84$). The other molecular markers observed (NL1, NL3 and GLUN1) did not show any alteration.

3.6. *Oxttr*^{-/-} mice shape dendritic spines in the DLS, but not in the hippocampus, when exposed to a novel environment

Our behavioral data strongly suggested that *Oxttr*^{-/-} mice's cognitive defects in the NOR were brought about by environmental novelty and rescued by habituation to the environment.

To evaluate if alterations in the DLS could be associated with the

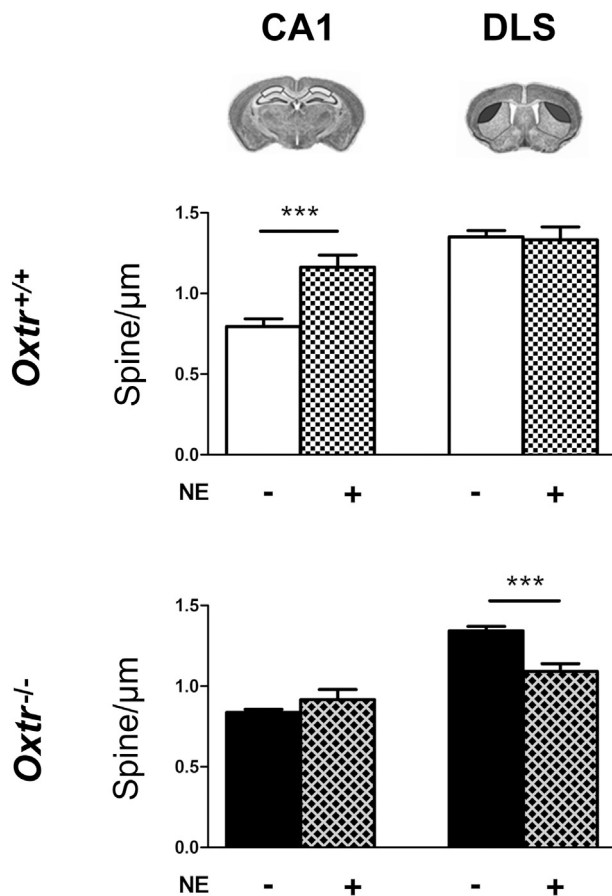


Fig. 5. *Oxttr*^{-/-} reshape DLS spines after exposure to a new environment. Effects on spine density of transferring mice in their home cages from their housing room to a new environment (behavioral facility room) for 7 days (NE condition). *Oxttr*^{+/+} mice increased spine density on apical dendrites of CA1 hippocampal pyramidal neurons (top row). *Oxttr*^{-/-} mice, instead, responded to such a change decreasing spine density in the DLS (bottom row). ****p* < 0.0001 vs corresponding non-ES condition (*N* = 6–12 mice per group). Data are presented as mean ± SEM.

defects in processing of environmental novelty and habituation in *Oxttr*^{-/-} mice, we exposed the animals to a novel environment (NE) external to their home cage. We moved mice in their cages from their housing room to the behavioral facility, left them to adapt for 7 days and then analyzed by Golgi staining adult neurons both in hippocampus and in DLS. Our results show that this environmental stimulus induces differential neurobiological responses in *Oxttr*^{+/+} and *Oxttr*^{-/-} mice: *Oxttr*^{+/+} animals display a hippocampal response, indicated by an increased density of dendritic spines on apical dendrites (Fig. 5, top; CA1 apical dendrites: *t*₍₁₃₎ = 4.48, *p* = 0.0006, *d* = -2.36), whereas hippocampi from *Oxttr*^{-/-} mice did not highlight any change in spine density (Fig. 5, bottom). However, *Oxttr*^{-/-} mice decreased spine density in the DLS after room change (Fig. 5, bottom; *t*₍₁₃₎ = 4.87, *p* = 0.0003, *d* = 2.56), a response not found in *Oxttr*^{+/+} mice (Fig. 5, top).

These data identify alterations of striatal neurons in adult *Oxttr*^{-/-} mice and suggests that an abnormal function of striatal circuits might be a likely neurobiological substrate for improper approach to novelty and impaired learning in this animal model of autism.

4. Discussion

Here we show that either genetic or pharmacological inactivation of *Oxttr* in male mice reduces preferences for novelty in an unfamiliar environment, and that this preference can be restored with repeated

exposure to the unfamiliar environment. In particular, our results indicate that male *Oxttr*^{-/-} mice lack the typical rodent innate interest for novelty, because when they are faced with a single novel object (object interaction tasks), they show an increased latency to the first object investigation and a reduced number of object investigations. A handful of studies have previously reported the involvement of the OXTerigic system in the interest towards novel objects, with variable results (Gard et al., 2012; Havranek et al., 2015; Madularu et al., 2014a; Madularu et al., 2014b). Consistent with our data, pharmacological blockade of OXTR in male mice impaired, while chronic OXT administration to male rats increased, the preference for the novel object in the NOR (Gard et al., 2012; Havranek et al., 2015). In contrast, OXT administration eliminated the preference for the novel object in male prairie voles and even favored the preference for the familiar object in ovariectomized female rats (Madularu et al., 2014a; Madularu et al., 2014b). Whether this variability is due to species or sex differences, or to different experimental protocols that overlooked habituation as a potential confounding factor, is unclear.

The lack of preferences for novel objects in *Oxttr*^{-/-} mice is unlikely due to memory impairments, as these mice performed well in spatial learning tasks not relying on novelty preference, such as the Morris water maze (this study) and in a T-maze (Lee et al., 2008; Sala et al., 2011). Interestingly, these animals display an increased latency to the first marble burying and to the first arm entry in the Y-maze, that could uncover a fear/anxiety-like state responsible for the impaired approach to new environment and objects. Indeed, because oxytocin is known to possess anxiolytic effects in humans and rodents (Neumann and Slattery, 2016; Bredewold and Veenema, 2018), increased anxiety could underlie neophobic responses in OXTR mutant mice. However, the genetic inactivation of OXTR was shown not to increase anxiety-like responses in mice (Lee et al., 2008; Sala et al., 2011) and, more recently, in voles (Horie et al., 2019), suggesting that the OXTR deficit does not impact general (non social) anxiety. Therefore, we favor the interpretation that the altered behavioral responses described here (i.e. the impaired approach to novel objects and environment in NOR and single object interaction tests, the increased latency in the marble burying test and in the Y-maze test) are consistent with an impaired approach to novelty and not with an increase in anxiety.

We showed that the interest in novel objects could be restored in *Oxttr*^{-/-} mice by prolonging the familiarization to the environment before the task. Previous studies showed that *Oxttr* knockout mice maintain high levels of interest in a conspecific even after repeated exposure (Ferguson et al., 2000). Our results suggest that this phenotype may extend to non-social contexts, as *Oxttr*^{-/-} mice maintain high levels of interest in objects, even with repeated exposure (Fig. 3Aii). As OXT plays a pivotal role in social behaviors, future studies should test whether interest in novel conspecifics is restored following repeated exposure to the context, as we observed in the NOR test. In relation to this point, it is important to mention that inactivation of OXTR in the lateral septum impaired social novelty without any effects in a standard NOR, suggesting that responses to social and nonsocial novelty contexts are independent (Mesic et al., 2015).

A possible explanation is that OXTRs contribute to process contextual information, a hypothesis consistent with previous reports showing that some behavioral effects of OXT are context-dependent (Johnson and Young, 2017). For example, through its interaction with the dopaminergic system, OXT exerts a modulatory role on reorienting attention to salient stimuli (Shamay-Tsoory and Abu-Akel, 2016). We speculate that in *Oxttr*^{-/-} mice contextual information is inefficiently processed, which leads to a failure in the NOR by altering the animals' approach to novelty. Our results also argue for a critical (re)interpretation of defective performances in behavioral tasks based on novelty, including the NOR test. Interestingly, impaired NOR performances were recently found also in the 16p11.2 heterozygous (Yang et al., 2015) and the AC5-KO mice (Kim et al., 2017), suggesting that an altered processing of novelty could be a shared feature of mice

presenting autistic-like phenotypes. In humans, alterations in context processing may contribute to the strong resistance to change observed in autism spectrum disorders, which lead to stereotyped routines, rigid thinking and perseverative interests (Gomot and Wicker, 2012).

Recently, it has been proposed that OXT is involved in general avoidance motivational processes that modulate the approach to social and non-social events and that a peculiar function of OXT is to shift the interest from novelty seeking towards the preference for familiarity (Harari-Dahan and Bernstein, 2014). Interestingly, the neural substrate proposed for such phenomenon is the striatum, where the shift from novelty to familiarity corresponds to shifts from ventral to dorsal areas (Tops et al., 2014). The molecular alterations we found in the dorsolateral striatum of *Oxtr*^{-/-} mice and the responsiveness of this region during habituation to a new environment are in line with this view. Such alterations, which suggest synaptic transmission variations in this area (increased number of spines and of excitatory synaptic markers), could thus subtend, at least partially, at the responsiveness of this region to the change of environment. Accordingly, a high level of basal extracellular striatal glutamate has been found in the dorsolateral striatum of mice exhibiting a low response to novel environments (Shakil et al., 2005). On the other hand, lack of hippocampal recruitment in *Oxtr*^{-/-} mice upon such environmental change is presumably not due to gross hippocampal impairments, at least based on our molecular and behavioral analyses. We rather hypothesize that the striatal response overrun, or at least prevailed over, the normal hippocampal response to novelty (Kim et al., 2014; Rinaldi et al., 2010). As OXT plays a pivotal role in hippocampal development by participating in the timing of the GABA switch (Leonzino et al., 2016), the absence of OXT signaling in the hippocampus of *Oxtr*^{-/-} mice might initiate, during early development, a rewiring process leading to enhanced responsiveness of striatal networks. Similarly, in C57Bl/6J mice treated with the OXTR antagonist, an acute block of OXTR activation could enhance responsiveness of striatal pathways. Importantly, OXT has been reported to enhance corticostriatal connectivity in women (Bethlehem et al., 2017), and to modulate synaptic activity and excitatory markers in the striatum (Dolen et al., 2013; Zhang et al., 2015). Finally, striatal abnormalities have been described in several genetic mouse models of autism spectrum disorders (Jaramillo et al., 2017; Peca et al., 2011; Rothwell, 2016; Welch et al., 2007) and several indications of striatal alterations in human patients have been gathered along the years (Fuccillo, 2016), pointing at this structure as a critical substrate for autistic conditions. This work paves the way for further studies aimed at a selective pharmacological and genetic blockage of OXTR expression and/or signaling in the DLS at different ages (around parturition, at weaning, during adolescence and in adult life), accompanied by a full characterization of synaptic and extrasynaptic signaling proteins and markers. These studies will allow identifying DLS substrates involved in OXT-regulated approach to novelty in non-social contexts at crucial periods of neurodevelopment and circuitry remodeling.

5. Conclusions

Our present results further support the knowledge that *Oxtr*^{-/-} mice express a complex behavioral phenotype that recapitulates several aspect of autism spectrum disorders. In addition to social behavior deficits (Sala et al., 2011), these mice display an impaired interest in novelty and their behavior towards novelty is strongly affected by the environmental context. The lack of an innate preference for novelty, accompanied by a strong preference for familiar objects and environments, fits in with the previously reported cognitive inflexibility of these mice (Sala et al., 2011). Most importantly, longer habituation to the testing environment was sufficient to rescue the poor performance of *Oxtr*^{-/-} in the standard NOR, indicating that this test could be used to assay novelty-based impairments in rodent models of autism. Finally, our data point to the dorsolateral striatum as a neurobiological substrate of the impaired approach to novelty and familiarization processes

to objects and surrounding environments, further underlining the importance of striatal networks in autism-relevant behaviors.

Author's contributions

M.L. designed, performed and interpreted biochemical and Golgi experiments and contributed to the design and interpretation of behavioral experiments. L.P., B.D., N.D.W., B.T. and M.S. designed, performed and interpreted behavioral experiments. I.C. performed Golgi staining. K.N. provided the *Oxtr*^{-/-} mice. M.B. contributed to animal breeding and maintenance, to Golgi experiments and to interpretation of biochemical and behavioral experiments. B.C. supervised the entire work and wrote the manuscript with V.G. and with the input of all co-authors.

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yhbeh.2019.06.007>.

References

- Bethlehem, R.A.I., Lombardo, M.V., Lai, M.C., Auyeung, B., Crockford, S.K., Deakin, J., Soubbranmanian, S., Sule, A., Kundu, P., Voon, V., Baron-Cohen, S., 2017. Intranasal oxytocin enhances intrinsic corticostriatal functional connectivity in women. *Transl. Psychiatry* 7, e1099. <https://doi.org/10.1038/tp.2017.72>.
- Boccia, M.L., Goursaud, A.P., Bachevalier, J., Anderson, K.D., Pedersen, C.A., 2007. Peripherally administered non-peptide oxytocin antagonist, L368,899, accumulates in limbic brain areas: a new pharmacological tool for the study of social motivation in non-human primates. *Horm. Behav.* 52, 344–351. <https://doi.org/10.1016/j.yhbeh.2007.05.009>.
- Bredewold, R., Veenema, A.H., 2018. Sex differences in the regulation of social and anxiety-related behaviors: insights from vasopressin and oxytocin brain systems. *Curr. Opin. Neurobiol.* 49, 132–140. <https://doi.org/10.1016/j.conb.2018.02.011>.
- Chini, B., Leonzino, M., Braidia, D., Sala, M., 2014. Learning about oxytocin: pharmacologic and behavioral issues. *Biol. Psychiatry* 76, 360–366. <https://doi.org/10.1016/j.biopsych.2013.08.029>.
- Dolen, G., Darvishzadeh, A., Huang, K.W., Malenka, R.C., 2013. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature* 501, 179–184. <https://doi.org/10.1038/nature12518>.
- Dumais, K.M., Kulkarni, P.P., Ferris, C.F., Veenema, A.H., 2017. Sex differences in neural activation following different routes of oxytocin administration in awake adult rats. *Psychoneuroendocrinology* 81, 52–62. <https://doi.org/10.1016/j.psyneuen.2017.04.003>.
- Duque-Wilckens, N., Steinman, M.Q., Busnelli, M., Chini, B., Yokoyama, S., Pham, M., Laredo, S.A., Hao, R., Perkeybile, A.M., Minie, V.A., Tan, P.B., Bales, K.L., Trainor, B.C., 2018. Oxytocin receptors in the anteromedial bed nucleus of the stria terminalis promote stress-induced social avoidance in female California mice. *Biol. Psychiatry* 83, 203–213. <https://doi.org/10.1016/j.biopsych.2017.08.024>.
- Ebner, K., Wotjak, C.T., Landgraf, R., Engelmann, M., 2000. A single social defeat experience selectively stimulates the release of oxytocin, but not vasopressin, within the septal brain area of male rats. *Brain Res.* 872, 87–92.
- Eliava, M., Melchior, M., Knobloch-Bollmann, H.S., Wahis, J., da Silva Gouveia, M., Tang, Y., Ciobanu, A.C., Triana Del Rio, R., Roth, L.C., Althammer, F., Chavant, V., Goumon, Y., Gruber, T., Petit-Demouliere, N., Busnelli, M., Chini, B., Tan, L.L., Mitre, M., Froemke, R.C., Chao, M.V., Giese, G., Sprengel, R., Kuner, R., Poisbeau, P., Seeburg, P.H., Stoop, R., Charlet, A., Grinevich, V., 2016. A new population of

- parvocellular oxytocin neurons controlling magnocellular neuron activity and inflammatory pain processing. *Neuron* 89, 1291–1304. <https://doi.org/10.1016/j.neuron.2016.01.041>.
- Engelmann, M., Wotjak, C.T., Ebner, K., Landgraf, R., 2000. Behavioural impact of intraseptally released vasopressin and oxytocin in rats. *Exp. Physiol.* 85, 125S–130S Spec No.
- Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. *Behav. Brain Res.* 31, 47–59.
- Ennaceur, A., Michalikova, S., Chazot, P.L., 2009. Do rats really express neophobia towards novel objects? Experimental evidence from exposure to novelty and to an object recognition task in an open space and an enclosed space. *Behav. Brain Res.* 197, 417–434. <https://doi.org/10.1016/j.bbr.2008.10.007>.
- 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. *Nat. Genet.* 25, 284–288.
- Fuccillo, M.V., 2016. Striatal circuits as a common node for autism pathophysiology. *Front. Neurosci.* 10, 27. <https://doi.org/10.3389/fnins.2016.00027>.
- Galbusera, A., De Felice, A., Girardi, S., Bassetto, G., Maschietto, M., Nishimori, K., Chini, B., Papaleo, F., Vassanelli, S., Gozzi, A., 2017. Intranasal oxytocin and vasopressin modulate divergent brainwide functional substrates. *Neuropsychopharmacology* 42, 1420–1434. <https://doi.org/10.1038/npp.2016.283>.
- Gard, P.R., Naylor, C., Ali, S., Partington, C., 2012. Blockade of pro-cognitive effects of angiotensin IV and physostigmine in mice by oxytocin antagonism. *Eur. J. Pharmacol.* 683, 155–160. <https://doi.org/10.1016/j.ejphar.2012.02.048>.
- Gasbarri, A., Pompili, A., Packard, M.G., Tomaz, C., 2014. Habit learning and memory in mammals: behavioral and neural characteristics. *Neurobiol. Learn. Mem.* 114, 198–208. <https://doi.org/10.1016/j.nlm.2014.06.010>.
- Gibb, R., Kolb, B., 1998. A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *J. Neurosci. Methods* 79, 1–4.
- Gomot, M., Wicker, B., 2012. A challenging, unpredictable world for people with autism spectrum disorder. *Int. J. Psychophysiol.* 83, 240–247. <https://doi.org/10.1016/j.ijpsycho.2011.09.017>.
- Green, M.R., Barnes, B., McCormick, C.M., 2013. Social instability stress in adolescence increases anxiety and reduces social interactions in adulthood in male Long-Evans rats. *Dev. Psychobiol.* 55, 849–859. <https://doi.org/10.1002/dev.21077>.
- Guastella, A.J., Hickie, I.B., 2016. Oxytocin treatment, circuitry, and autism: a critical review of the literature placing oxytocin into the autism context. *Biol. Psychiatry* 79, 234–242. <https://doi.org/10.1016/j.biopsych.2015.06.028>.
- Guzman, Y.F., Tronson, N.C., Jovasevic, V., Sato, K., Guedea, A.L., Mizukami, H., Nishimori, K., Radulovic, J., 2013. Fear-enhancing effects of septal oxytocin receptors. *Nat. Neurosci.* 16, 1185–1187. <https://doi.org/10.1038/nn.3465>.
- Harari-Dahan, O., Bernstein, A., 2014. A general approach-avoidance hypothesis of oxytocin: accounting for social and non-social effects of oxytocin. *Neurosci. Biobehav. Rev.* 47, 506–519. <https://doi.org/10.1016/j.neubiorev.2014.10.007>.
- Havranek, T., Zatkova, M., Lestanova, Z., Bacova, Z., Mravec, B., Hodosy, J., Strbak, V., Bakos, J., 2015. Intracerebroventricular oxytocin administration in rats enhances object recognition and increases expression of neurotrophins, microtubule-associated protein 2, and synapsin I. *J. Neurosci.* 35, 893–901. <https://doi.org/10.1002/jnr.23559>.
- Horie, K., Inoue, K., Suzuki, S., Adachi, S., Yada, S., Hirayama, T., Hidema, S., Young, L.J., Nishimori, K., 2019. Oxytocin receptor knockout prairie voles generated by CRISPR/Cas9 editing show reduced preference for social novelty and exaggerated repetitive behaviors. *Horm. Behav.* 111, 60–69. <https://doi.org/10.1016/j.yhbeh.2018.10.011>.
- Hung, L.W., Neuner, S., Polepalli, J.S., Beier, K.T., Wright, M., Walsh, J.J., Lewis, E.M., Luo, L., Deisseroth, K., Dolen, G., Malenka, R.C., 2017. Gating of social reward by oxytocin in the ventral tegmental area. *Science* 357, 1406–1411. <https://doi.org/10.1126/science.aan4994>.
- Jaramillo, T.C., Speed, H.E., Xuan, Z., Reimers, J.M., Escamilla, C.O., Weaver, T.P., Liu, S., Filonova, I., Powell, C.M., 2017. Novel Shank3 mutant exhibits behaviors with face validity for autism and altered striatal and hippocampal function. *Autism Res.* 10, 42–65. <https://doi.org/10.1002/aur.1664>.
- Johnson, Z.V., Young, L.J., 2017. Oxytocin and vasopressin neural networks: implications for social behavioral diversity and translational neuroscience. *Neurosci. Biobehav. Rev.* 76, 87–98. <https://doi.org/10.1016/j.neubiorev.2017.01.034>.
- Kim, J.M., Kim, D.H., Lee, Y., Park, S.J., Ryu, J.H., 2014. Distinct roles of the hippocampus and perirhinal cortex in GABA receptor blockade-induced enhancement of object recognition memory. *Brain Res.* 1552, 17–25. <https://doi.org/10.1016/j.brainres.2014.01.024>.
- Kim, H., Lee, Y., Park, J.Y., Kim, J.E., Kim, T.K., Choi, J., Lee, J.E., Lee, E.H., Kim, D., Kim, K.S., Han, P.L., 2017. Loss of adenylyl cyclase type-5 in the dorsal striatum produces autistic-like behaviors. *Mol. Neurobiol.* 54, 7994–8008. <https://doi.org/10.1007/s12035-016-0256-x>.
- King, L.B., Walum, H., Inoue, K., Eyrich, N.W., Young, L.J., 2016. Variation in the oxytocin receptor gene predicts brain region-specific expression and social attachment. *Biol. Psychiatry* 80, 160–169. <https://doi.org/10.1016/j.biopsych.2015.12.008>.
- Kirsch, P., 2015. Oxytocin in the socioemotional brain: implications for psychiatric disorders. *Dialogues Clin. Neurosci.* 17, 463–476.
- Lee, H.J., Caldwell, H.K., Macbeth, A.H., Tolu, S.G., Young III, W.S., 2008. A conditional knockout mouse line of the oxytocin receptor. *Endocrinology* 149, 3256–3263. <https://doi.org/10.1210/en.2007-1710>.
- Lee, S.Y., Park, S.H., Chung, C., Kim, J.J., Choi, S.Y., Han, J.S., 2015. Oxytocin protects hippocampal memory and plasticity from uncontrollable stress. *Sci. Rep.* 5, 18540. <https://doi.org/10.1038/srep18540>.
- Leonzino, M., Busnelli, M., Antonucci, F., Verderio, C., Mazzanti, M., Chini, B., 2016. The timing of the excitatory-to-inhibitory GABA switch is regulated by the oxytocin receptor via KCC2. *Cell Rep.* 15, 96–103. <https://doi.org/10.1016/j.celrep.2016.03.013>.
- Lingawi, N.W., Balleine, B.W., 2012. Amygdala central nucleus interacts with dorsolateral striatum to regulate the acquisition of habits. *J. Neurosci.* 32, 1073–1081. <https://doi.org/10.1523/JNEUROSCI.4806-11.2012>.
- Lukas, M., Toth, I., Veenema, A.H., Neumann, I.D., 2013. Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: male juvenile versus female adult conspecifics. *Psychoneuroendocrinology* 38, 916–926. <https://doi.org/10.1016/j.psyneuen.2012.09.018>.
- Ma, Y., Shamay-Tsoory, S., Han, S., Zink, C.F., 2016. Oxytocin and social adaptation: insights from neuroimaging studies of healthy and clinical populations. *Trends Cogn. Sci.* 20, 133–145. <https://doi.org/10.1016/j.tics.2015.10.009>.
- Madularu, D., Athanassiou, M., Yee, J.R., Kenkel, W.M., Carter, C.S., Mumby, D.G., 2014a. Oxytocin and object preferences in the male prairie vole. *Peptides* 61, 88–92. <https://doi.org/10.1016/j.peptides.2014.08.015>.
- Madularu, D., Athanassiou, M., Yee, J.R., Mumby, D.G., 2014b. Centrally-administered oxytocin promotes preference for familiar objects at a short delay in ovariectomized female rats. *Behav. Brain Res.* 274, 164–167. <https://doi.org/10.1016/j.bbr.2014.08.015>.
- Malvaez, M., Wassum, K.M., 2018. Regulation of habit formation in the dorsal striatum. *Curr. Opin. Behav. Sci.* 20, 67–74. <https://doi.org/10.1016/j.cobeha.2017.11.005>.
- Menon, R., Grund, T., Zoicas, I., Althammer, F., Fiedler, D., Biermeier, V., Bosch, O.J., Hiraoka, Y., Nishimori, K., Eliava, M., Grinevich, V., Neumann, I.D., 2018. Oxytocin signaling in the lateral septum prevents social fear during lactation. *Curr. Biol.* 28 (1066–1078), e1066. <https://doi.org/10.1016/j.cub.2018.02.044>.
- Mesic, I., Guzman, Y.F., Guedea, A.L., Jovasevic, V., Corcoran, K.A., Leaderbrand, K., Nishimori, K., Contractor, A., Radulovic, J., 2015. Double dissociation of the roles of metabotropic glutamate receptor 5 and oxytocin receptor in discrete social behaviors. *Neuropsychopharmacology* 40, 2337–2346. <https://doi.org/10.1038/npp.2015.81>.
- Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11, 47–60.
- Neumann, I.D., Slattery, D.A., 2016. Oxytocin in general anxiety and social fear: a translational approach. *Biol. Psychiatry* 79 (3), 213–221. <https://doi.org/10.1016/j.biopsych.2015.06.004>.
- Olszewski, P.K., Waas, J.R., Brooks, L.L., Herisson, F., Levine, A.S., 2013. Oxytocin receptor blockade reduces acquisition but not retrieval of taste aversion and blunts responsiveness of amygdala neurons to an aversive stimulus. *Peptides* 50, 36–41. <https://doi.org/10.1016/j.peptides.2013.09.008>.
- Ortiz, J.J., Portillo, W., Paredes, R.G., Young, L.J., Alcauter, S., 2018. Resting state brain networks in the prairie vole. *Sci. Rep.* 8, 1231. <https://doi.org/10.1038/s41598-017-17610-9>.
- Owen, S.F., Tuncdemir, S.N., Bader, P.L., Tirko, N.N., Fishell, G., Tsien, R.W., 2013. Oxytocin enhances hippocampal spike transmission by modulating fast-spiking interneurons. *Nature* 500, 458–462. <https://doi.org/10.1038/nature12330>.
- Peca, J., Feliciano, C., Ting, J.T., Wang, W., Wells, M.F., Venkatraman, T.N., Lascola, C.D., Fu, Z., Feng, G., 2011. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* 472, 437–442. <https://doi.org/10.1038/nature09965>.
- Pitsikas, N., Rigamonti, A.E., Cella, S.G., Locatelli, V., Sala, M., Muller, E.E., 2001. Effects of molsidomine on scopolamine-induced amnesia and hypermotility in the rat. *Eur. J. Pharmacol.* 426, 193–200.
- Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., McSweeney, F.K., Wilson, D.A., Wu, C.F., Thompson, R.F., 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol. Learn. Mem.* 92, 135–138. <https://doi.org/10.1016/j.nlm.2008.09.012>.
- Rinaldi, A., Romeo, S., Agustin-Pavon, C., Oliverio, A., Mele, A., 2010. Distinct patterns of Fos immunoreactivity in striatum and hippocampus induced by different kinds of novelty in mice. *Neurobiol. Learn. Mem.* 94, 373–381. <https://doi.org/10.1016/j.nlm.2010.08.004>.
- Rothwell, P.E., 2016. Autism spectrum disorders and drug addiction: common pathways, common molecules, distinct disorders? *Front. Neurosci.* 10, 20. <https://doi.org/10.3389/fnins.2016.00020>.
- Sala, M., Braidà, 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., Braidà, 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>.
- Shakil, S.S., Holmer, H.K., Moore, C., Abernathy, A.T., Jakowec, M.W., Petzinger, G.M., Meshul, C.K., 2005. High and low responders to novelty show differential effects in striatal glutamate. *Synapse* 58, 200–207. <https://doi.org/10.1002/syn.20198>.
- Shamay-Tsoory, S.G., Abu-Akel, A., 2016. The social salience hypothesis of oxytocin. *Biol. Psychiatry* 79, 194–202. <https://doi.org/10.1016/j.biopsych.2015.07.020>.
- 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. U. S. A.* 102, 16096–16101. <https://doi.org/10.1073/pnas.0505312102>.
- Thompson, K.L., Vincent, S.H., Miller, R.R., Colletti, A.E., Alvaro, R.F., Wallace, M.A., Feeney, W.P., Chiu, S.H., 1997. Pharmacokinetics and disposition of the oxytocin receptor antagonist L-368,899 in rats and dogs. *Drug Metab. Dispos.* 25, 1113–1118.

- Thorn, C.A., Atallah, H., Howe, M., Graybiel, A.M., 2010. Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. *Neuron* 66, 781–795. <https://doi.org/10.1016/j.neuron.2010.04.036>.
- Tops, M., Koole, S.L., H, I.J., Buisman-Pijlman, F.T., 2014. Why social attachment and oxytocin protect against addiction and stress: insights from the dynamics between ventral and dorsal corticostriatal systems. *Pharmacol. Biochem. Behav.* 119, 39–48. <https://doi.org/10.1016/j.pbb.2013.07.015>.
- Wang, D., Yan, X., Li, M., Ma, Y., 2017. Neural substrates underlying the effects of oxytocin: a quantitative meta-analysis of pharmaco-imaging studies. *Soc. Cogn. Affect. Neurosci.* 12, 1565–1573. <https://doi.org/10.1093/scan/nsx085>.
- Wei, D., Lee, D., Cox, C.D., Karsten, C.A., Penagarikano, O., Geschwind, D.H., Gall, C.M., Piomelli, D., 2015. Endocannabinoid signaling mediates oxytocin-driven social reward. *Proc. Natl. Acad. Sci. U. S. A.* 112, 14084–14089. <https://doi.org/10.1073/pnas.1509795112>.
- Welch, J.M., Lu, J., Rodriguez, R.M., Trotta, N.C., Peca, J., Ding, J.D., Feliciano, C., Chen, M., Adams, J.P., Luo, J., Dudek, S.M., Weinberg, R.J., Calakos, N., Wetsel, W.C., Feng, G., 2007. Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature* 448, 894–900. <https://doi.org/10.1038/nature06104>.
- Williams, P.D., Anderson, P.S., Ball, R.G., Bock, M.G., Carroll, L., Chiu, S.H., Clineschmidt, B.V., Culbertson, J.C., Erb, J.M., Evans, B.E., et al., 1994. 1-((7,7-Dimethyl-2(S)-(2(S)-amino-4-(methylsulfonyl)butylamido)bicyclo [2.2.1]heptan-1(S)-yl)methylsulfonyl)-4-(2-methylphenyl)piperazine (L-368,899): an orally bioavailable, non-peptide oxytocin antagonist with potential utility for managing preterm labor. *J. Med. Chem.* 37, 565–571.
- Wozniak, D.F., Diggs-Andrews, K.A., Conyers, S., Yuede, C.M., Dearborn, J.T., Brown, J.A., Tokuda, K., Izumi, Y., Zorumski, C.F., Gutmann, D.H., 2013. Motivational disturbances and effects of L-dopa administration in neurofibromatosis-1 model mice. *PLoS One* 8, e66024. <https://doi.org/10.1371/journal.pone.0066024>.
- Yang, M., Lewis, F.C., Sarvi, M.S., Foley, G.M., Crawley, J.N., 2015. 16p11.2 deletion mice display cognitive deficits in touchscreen learning and novelty recognition tasks. *Learn. Mem.* 22, 622–632. <https://doi.org/10.1101/lm.039602.115>.
- Zhang, Q., Du, X., Xu, Y., Dang, L., Xiang, L., Zhang, J., 2013. The effects of Gouqi extracts on Morris maze learning in the APP/PS1 double transgenic mouse model of Alzheimer's disease. *Exp. Ther. Med.* 5, 1528–1530. <https://doi.org/10.3892/etm.2013.1006>.
- Zhang, X., Li, Q., Zhang, M., Lam, S., Sham, P.C., Bu, B., Chua, S.E., Wang, W., McAlonan, G.M., 2015. The effect of oxytocin on social and non-social behaviour and striatal protein expression in C57BL/6N mice. *PLoS One* 10, e0145638. <https://doi.org/10.1371/journal.pone.0145638>.
- Zheng, J.J., Li, S.J., Zhang, X.D., Miao, W.Y., Zhang, D., Yao, H., Yu, X., 2014. Oxytocin mediates early experience-dependent cross-modal plasticity in the sensory cortices. *Nat. Neurosci.* 17, 391–399. <https://doi.org/10.1038/nn.3634>.
- Zoicas, I., Slattey, D.A., Neumann, I.D., 2014. Brain oxytocin in social fear conditioning and its extinction: involvement of the lateral septum. *Neuropsychopharmacology* 39, 3027–3035. <https://doi.org/10.1038/npp.2014.156>.