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Oxytocin receptor signalling modulates novelty recognition but not social preference in zebrafish

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Abstract

Sociality is a complex phenomenon that involves the individual´s motivation to approach their conspecifics, along with social cognitive functions that enable individuals to interact and survive. The nonapeptide oxytocin (OXT) is known to regulate sociality in many species. However, the role of OXT in specific aspects of sociality is still not well understood. In the present study, we investigated the contribution of the OXT receptor (OXTR) signalling in two different aspects of zebrafish social behaviour: social preference, by measuring their motivation to approach a shoal of conspecifics, and social recognition, by measuring their ability to discriminate between a novel and familiar fish, using a mutant zebrafish lacking a functional OXTR. Although *oxtr* mutant zebrafish displayed normal attraction to a shoal of conspecifics, they exhibited reduced social recognition. We further investigated whether this effect would be social-domain specific by replacing conspecific fish by objects. Although no differences were observed in object approach, *oxtr* mutant fish also exhibited impaired object recognition. Our findings suggest that OXTR signalling regulates a more general memory recognition of familiar vs novel entities, not only in social but also in a non-social domain, in zebrafish.

KEYWORDS
autism spectrum disorders, oxytocin, social preference, social recognition, zebrafish

1 | INTRODUCTION

Nonapeptides are known to be important players in the regulation of social behaviour across taxa.[1] They evolved from a common ancestral molecule, the arginine vasotocin, after a gene duplication event in early-jawed fish.[2] One copy originated the vasopressin-like peptides, which retained the most primitive functions of the ancestral molecule, namely the regulation of osmotic balance. The other originated the oxytocin (OXT)-like peptides, which have greatly diverged across species, being recruited for diverse homeostatic processes, including parturition and lactation in mammals. Throughout evolution, these nonapeptides have also been involved in the regulation of social behaviours, with vasopressin being more involved in aggression and agonistic behaviours and OXT in affiliative behaviours and sociality.[3]

Ribeiro and Nunes contributed equally to this work.

The increasing reports supporting a role of OXT in the regulation of sociality across vertebrates have linked this nonapeptide to diseases associated with social affiliative deficits, such as autism-spectrum disorders.[4] Thus, OXT has been the focus of a significant research effort in translational social neuroscience, and the interest in understanding the mechanisms through which it regulates sociality is growing at a fast pace.

Both pharmacological and genetic approaches have been employed to investigate the role of the OXT system in sociality. However, sociality has been often seen as a unitary variable, studied mainly as the preference for an individual to approach conspecifics. Sociality, however, is a multidimensional phenomenon, including not only a motivational domain reflected by the willingness of an individual to approach and engage with others, but also a cognitive domain, which includes different cognitive functions that enable individuals to interact.[5] In particular, the ability for individuals to recognise others (also known as social memory recognition), such that they can use their previous experience to modulate subsequent interactions with specific conspecifics, plays a key role in the evolution of individuality in social interactions. Whether OXT plays a role in these different domains of sociality, motivation vs cognitive functions, is not entirely clear. There are some studies showing that different aspects of sociality may be differently affected by OXT; however, these results are not consistent throughout the literature. For example, although prairie voles exhibiting a dysfunctional OXT receptor (OXTR) display normal approach to conspecifics, they show an impairment in social recognition.[6] The same results have been observed using a conditional lateral septum-specific OXTR-deficient mice, showing deficits in social recognition, although not in social preference.[7] These two studies support a differential role of OXT in distinct domains of sociality, with the OXTR in the lateral septum being a target area for social recognition memory. However, two other independent studies, using a different mice *oxtr* knockout, revealed impairments not only in social recognition memory, but also in social approach.[8,9] Thus, although available data are consistent for the effects of OXT on social recognition,[10] it remains unclear whether OXT has an effect on social approach or not.

The role of OXT-like peptides in sociality of other taxa, such as fish, has been less characterised and the findings are controversial. The majority of these studies employed pharmacological approaches using varied concentrations of exogenous OXT-like peptides and/or receptor agonists and antagonists. Although, in some studies, the administration of OXT-like peptides or its receptor agonist has shown to decrease or not to change social preference in cooperatively breeding cichlids, *Neolamprologus pulcher* and in zebrafish,[11,12] other studies, using lower concentrations of these ligands, showed an effect in social preference that followed an inverted-U dose-response curve.[13] Interestingly, the same highest OXT concentration that did not induce changes in sociality as reported in a study by Braida et al[13] was also found to recover the deficits of social preference mediated by MK-801 in an independent study without affecting the preference for the conspecifics when administered alone.[14] Furthermore, although OXT-like peptides do not increase zebrafish preference for the shoal, vasopressin-like peptides do[12] or exhibit the same dose-dependent effect observed for OXT-like peptides.[13]

Despite the abovementioned controversial findings on the effects of OXT on social approach in fish, to the best of our knowledge, there are no reports on the OXT effects in social memory recognition among teleosts. Furthermore, as a result of recent advances in genetic-editing techniques, we have now genetic tools available that allow us to dissect, in a more controllable manner, the role of the oxytocinergic system in fish. Thus, the present study aimed to investigate the contribution of the oxytocinergic signalling in two different aspects of social behaviour in a well-established social animal model, the zebrafish. For this purpose, we used a zebrafish mutant line exhibiting a dysfunctional OXTR to assess the OXT signalling contribution with respect to both social and non-social preference and memory recognition.

2 | MATERIALS AND METHODS

2.1 | Animal models

Oxytocin receptor mutant zebrafish, *Danio rerio*, line (*oxtr*$^{-/-}$), raised in a TL-mixed background, were generated in the laboratory of Dr Gil Levkowitz (Weizmann Institute of Science) using a transcription activator-like effector nuclease (TALEN) genome-editing tool. Our previous data showed that mutant *oxtr*$^{(-/-)}$ fish exhibit an impaired oxytocinergic signalling (validation of the line in Nunes et al[15]). Zebrafish genotyping was performed according with the procedure described by Blechamnn et al[16]. The region of interest was amplified by a polymerase chain reaction (PCR) and sequenced using the primers: sense 5′-TGCGCGAGGAAAACTAGTT-3′ and antisense 5′ AGCAGACACTCAGAATGGTCA-3′.

Zebrafish were raised and bred under standard conditions. They were kept in mixed sex groups (10 adults L^{-1}) in a recirculation life support system (14:10 hour light/dark photocycle at 28°C, pH 7.0, conductivity 1000 μS cm^{-1}) and fed live food and commercial processed dry food, twice a day. From 2016 onward, the colony has remained free of pathogens. Pre-filter sentinels were tested negative for mycobacteria and *Pseudoloma neurophilia*. All experiments were conducted in accordance with standard operating procedures of the Instituto Gulbenkian de Ciência and Direcção Geral de Alimentação e Veterinária (DGAV), Portugal and by the Weizmann Institute´s Institutional Animal Care and Use Committee (IACUC).

2.2 | Behavioural experiments

Behavioural tests were conducted in adult zebrafish (3-6 months old) to characterise the OXTR signalling role on social and object preference, as well as social and object memory recognition. We also performed an open field test to assess anxiety-like behaviours. All tests were conducted in the light phase of the photoperiod. Both female and male zebrafish were used in the experiments. In all tests, the

behavioural set-up was placed on top of an infrared light box and illuminated from below. Fish were video-recorded from above. Video acquisition was performed using PINNACLE STUDIO, version 14 (Corel Corporation, Ottawa, ON, Canada). All behaviour analyses were performed with EthoVision video tracking system (Noldus Information Technologies, Wageningen, The Netherlands) and data were extracted to excel files (Microsoft Corp., Redmond, WA, USA) for further analysis. Fish with a mean speed of ± 2 SD were excluded from the analysis.

2.3 | Social (shoal) preference

The social preference test followed the protocol described by Wircer et al[17]. The test tank measured 20 × 19 × 5 cm (length × width × height). An individual zebrafish was placed in a starting box at one side of the arena. At the other side, there were two side-by-side compartments: one containing a shoal of four zebrafish, two males and two females, whereas the other was kept empty (Figure 1A). The stimulus shoal used matched the genotype of the focal fish. To avoid side biases, the shoal stimuli were randomly assigned to one of these two compartments. All compartments were completely sealed to block transmission of chemical and vibrational stimuli and thus, only visual cues were accessible.

The focal fish was first allowed to acclimate to the set-up, spending 5 minutes in the starting box. Then, the starting box was lifted and the fish allowed to explore the whole arena for 10 minutes. The percentage of cumulative time fish spent closer (one zebrafish body length) to each compartment ($\%T_{shoal}$ and $\%T_{empty}$) was used to calculate the social preference score [$\%T_{shoal}/(\%T_{shoal} + \%T_{empty})$] and the stimuli exploration score [$(T_{shoal} + T_{empty})/T_{total}$]. Mean velocity was also extracted from Ethovision. A social preference score of 0.5 indicates no preference, whereas above 0.5 indicates preference towards the shoal.

2.4 | Object preference

An object preference test was adapted from the social preference test, with stimuli fish being replaced by four red or four green 0.5-mL microcentrifuge tubes. The set-up used was 30 × 15 × 10 cm. Fish was first placed in the arena for a 10 minutes acclimatisation phase without visual access to the stimuli. Afterwards, an opaque partition between the fish and the stimuli was removed and the two side-by-side compartments became visually accessible: one containing the microcentrifuge tubes and the other kept empty (Figure 1G). The colours of the microcentrifuge tubes were chosen based on previous work that showed an innate zebrafish preference towards red and green objects.[18] The percentage cumulative time fish spent near each compartment (objects vs empty) was used to calculate the object preference score [$\%T_{objects}/(\%T_{objects} + \%T_{empty})$] and stimuli exploration score [$(T_{objects} + T_{empty})/T_{total}$]. An object preference score of 0.5 indicates no preference, whereas a score above 0.5 indicates a preference for objects.

2.5 | Social memory recognition

The behavioural set-up for the social recognition test was similar to the object preference set-up described above. The focal fish was placed for 10 minutes in the experimental arena for an acclimatisation phase. Then, zebrafish was allowed to interact visually with two novel conspecifics, each placed in one of the side-by-side stimuli compartments (phase I: novel-novel test phase) (Figure 2A). After 10 minutes, an opaque partition blocked the visual access to the stimuli compartments. Focal fish that did not approach both stimuli in phase I were excluded and not considered for further tests. Both stimuli fish were removed from the side-by-side compartments with one of the conspecifics being placed back to the original compartment, becoming the familiar stimulus, whereas a novel conspecific fish (ie, unfamiliar to the focal fish), was placed in the other compartment. In this way, both stimuli fish, familiar and novel, were handled to control for any effects of handling stress in the stimuli fish. After 5 minutes, the opaque partition was lifted, and the focal fish was allowed to visually interact with both conspecifics for 10 minutes (phase II: novel- familiar test phase) (Figure 2G). Focal fish and stimuli fish were from different holding tanks, so that all stimuli fish were unfamiliar to the focal fish at the beginning of the experiment. Focal and stimuli fish were matched by sex, size, age and genotype. The time fish spent near each compartment was used to calculate a preference score for phase I [$\%T_{Novel1}/(\%T_{Novel1} + \%T_{Novel2})$] and a social novelty preference score for phase II [$(\%T_{Novel}/(\%T_{Novel} + \%T_{Familiar})$]. A novelty preference score above 0.5 indicates a preference towards novel conspecifics. A stimuli exploration score was also calculated in both phases of the experiment [$(T_{novel1} + T_{novel\ 2(or\ familiar\ fish)})/T_{total}$].

2.6 | Object memory recognition

The object recognition test was performed in a similar manner to the social recognition test described above, with stimuli fish being replaced by a 0.5-mL microcentrifuge tubes. In the novel-novel test phase, two microcentrifuge tubes of the same colour were shown (Figure 3A). Fish that did not approach both objects were excluded. In the novel-familiar test phase, one red (familiar) and one green (novel) microcentrifuge tubes were shown (Figure 3G). Colours were switched between replicates to avoid a specific preference towards the colour. We used colour-cue objects because it has been reported that zebrafish are better in discriminating colour changes than changes in object shape or size.[18]

2.7 | Statistical analysis

Normality of the data was tested with both the Shapiro-Wilk and D´Agostino & Pearson omnibus normality tests. To assess for differences between control $oxtr^{(+/+)}$ and $oxtr^{(-/-)}$ mutant groups, we performed a Mann-Whitney test or unpaired *t* test, in case of

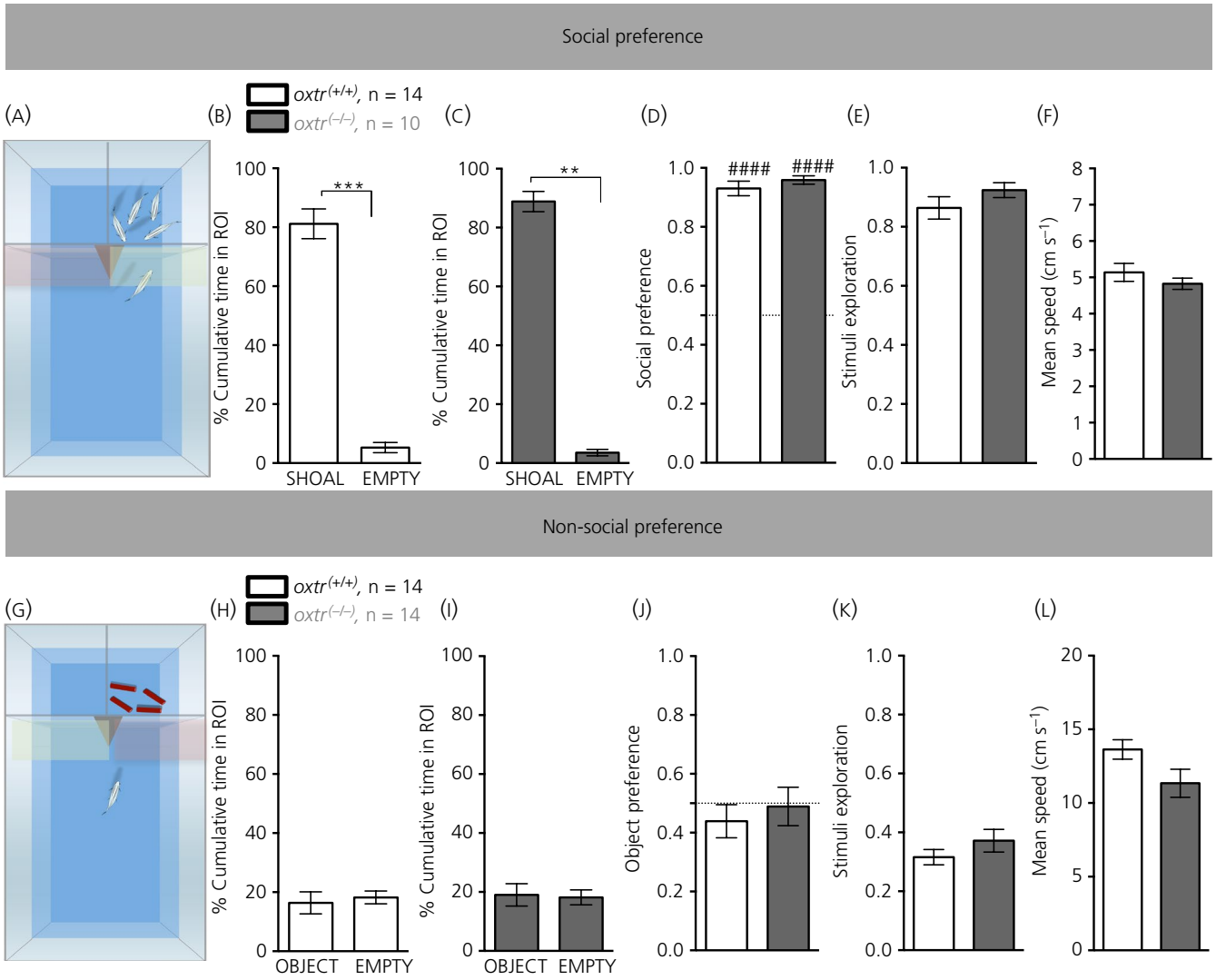

FIGURE 1 Mutant *oxtr*$^{(-/-)}$ fish show normal social and non-social preference. A, Schematic of the social preference behavioural set-up: focal fish are allowed to chose between a shoal of conspecifics vs an empty tank. B, Control *oxtr*$^{(+/+)}$ spent significantly more time near the shoal vs empty compartments ($P = 0.0001$, n = 14, Wilcoxon matched-pairs signed rank test). C, *Oxtr*$^{(-/-)}$ spent significantly more time near the shoal vs empty compartments ($P = 0.002$, Wilcoxon matched-pairs signed rank test). D, Both genotypes exhibit preference towards a shoal ($P < 0.0001$, one sample *t* test). Social preference score measured as percentage cumulative time fish spent near the shoal compared to the total time spent in both stimuli. E, Both genotypes explore the stimuli similarly. ($P = 0.52$, Mann-Whitney test). Stimuli exploration score measured as cumulative time fish spent near the shoal and empty compartments compared to the total time of the trial. F, *oxtr*$^{(+/+)}$ and *oxtr*$^{(-/-)}$ mutants exhibited similar mean speed ($P = 0.34$, unpaired *t* test). G, Schematic of the object preference behavioural set-up where the shoal of fish was replaced by four 0.5-mL microcentrifuge tubes. H, *Oxtr*$^{(+/+)}$ fish spent equal time near the objects vs empty compartments ($P = 0.39$, Wilcoxon matched-pairs signed rank test). I, *Oxtr*$^{(-/-)}$ mutants spent equal time near the objects vs empty compartment ($P = 0.87$, paired *t* test). J, No preference towards the object is observed (*oxtr*$^{(+/+)}$: $P = 0.30$, *oxtr*$^{(-/-)}$: $P = 0.86$, one sample *t* test). K, Both control *oxtr*$^{(+/+)}$ and their mutant siblings, *oxtr*$^{(-/-)}$, exhibit similar stimuli exploration scores ($P = 0.63$, unpaired *t* test) and (L) mean speed values ($P = 0.06$, unpaired *t* test). *Oxtr*$^{(+/+)}$ represented by white bars and mutant *oxtr*$^{(-/-)}$ fish by grey bars. Values are the mean ± SEM. ROI, region of interest

non-parametric or parametric data, respectively. The % cumulative time fish spent in each region of interest, within the same genotype, was compared using a Wilcoxon matched-paired signed rank test or paired *t* test, in case of non-parametric or parametric data, respectively.

Statistical analyses were performed using PRISM, version 6.0c (GraphPad Software Inc., San Diego, CA, USA). For all tests, $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Mutants *oxtr*$^{(-/-)}$ exhibit normal social approach

To investigate the oxytocinergic signalling role in social approach, we tested mutant *oxtr*$^{(-/-)}$ fish and their control *oxtr*$^{(+/+)}$ siblings for their preference/motivation to approach a shoal of conspecifics (Figure 1A). When given a choice between a shoal of fish vs an

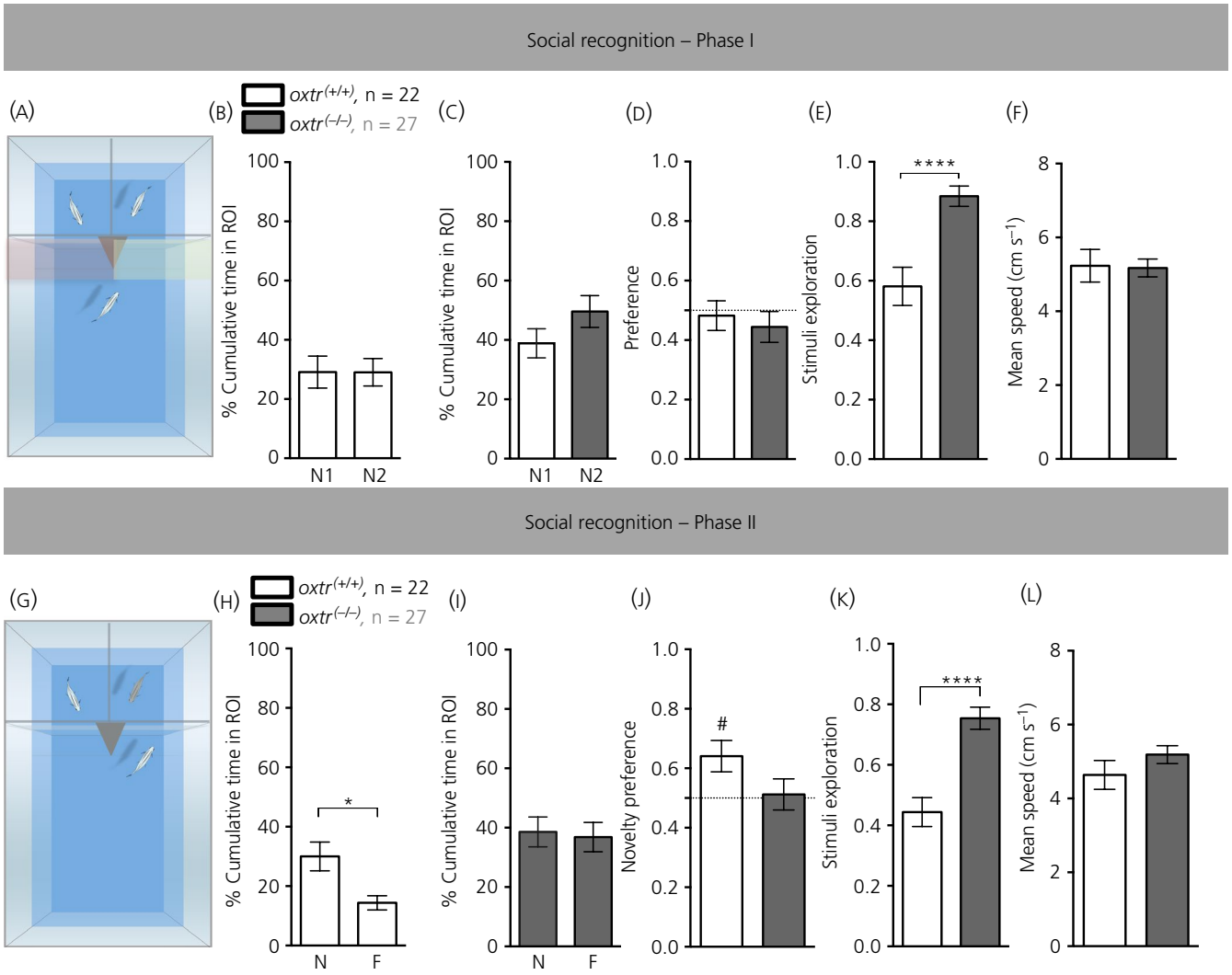

FIGURE 2 Mutant *oxtr*$^{(-/-)}$ fish exhibit impaired social memory recognition. A, Schematic of phase I of the behavioural test: focal fish are given a choice between two novel conspecifics. B, *Oxtr*$^{(+/+)}$ fish spent equal time in both novel stimuli, N1 and N2 ($P = 0.82$, Wilcoxon matched-pairs signed rank test). C, *Oxtr*$^{(-/-)}$ mutants spent equal time in both stimuli ($P = 0.34$, Wilcoxon matched-pairs signed rank test). D, Preference score indicates no preference towards one conspecific vs the other (*oxtr*$^{(+/+)}$: $P = 0.73$, *oxtr*$^{(-/-)}$: $P = 0.29$, one sample *t* test). Preference for one conspecific measured as percentage of time fish spent near conspecific N1 compared to the total time fish spent near both conspecifics (N1 + N2). E, Mutant *oxtr*$^{(-/-)}$ fish explore the two conspecifics significantly more than the *oxtr*$^{(+/+)}$ group ($P < 0.0001$, Mann-Whitney test) with (F) no differences observed in mean speed ($P = 0.54$, Mann- Whitney test). G, Schematic of phase II of the behavioural test: focal fish are given a choice between a novel vs familiar fish. H, *Oxtr*$^{(+/+)}$ fish spend significantly more time near novel than familiar fish ($P = 0.01$, Wilcoxon matched-pairs signed rank test). I, *Oxtr*$^{(-/-)}$ mutant fish spent equal time in both stimuli ($P = 0.93$, Wilcoxon matched-pairs signed rank test). (J) *Oxtr*$^{(+/+)}$ *fish*, but not *oxtr*$^{(-/-)}$, exhibit social novelty preference (*oxtr*$^{(+/+)}$:$P = 0.01$, *oxtr*$^{(-/-)}$: $P = 0.82$, one sample *t* test). (K) Mutant *oxtr*$^{(-/-)}$ fish explored more the novel vs familiar stimuli than *oxtr*$^{(+/+)}$ fish ($P < 0.0001$, Mann-Whitney test) and (L) exhibited similar mean speed to their controls ($P = 0.22$, unpaired *t* test). *Oxtr*$^{(+/+)}$ represented by white bars and mutant *oxtr*$^{(-/-)}$, fish by grey bars. Values are the mean ± SEM. ROI, region of interest

empty tank, *oxtr*$^{(+/+)}$ approached and spent a significantly higher percentage of time closer to their conspecifics than to the empty compartment (81.16 ± 5.04% in shoal vs 5.22 ± 1.74% in empty, $P = 0.0001$, Wilcoxon matched-paired signed rank test, n = 14) (Figure 1B). Mutant *oxtr*$^{(-/-)}$ siblings displayed similar levels of social preference to those of *oxtr*$^{(+/+)}$ (88.85 ± 3.44% close to shoal vs 3.54 ± 1.10% close to empty, $P = 0.002$, n = 10) (Figure 1C). Both genotypes exhibited a social preference score significantly higher than 0.5 ($P < 0.0001$ for *oxtr*$^{(+/+)}$, $P < 0.0001$ for *oxtr*$^{(-/-)}$, one sample *t* test) (Figure 1D) and similar between *oxtr*$^{(+/+)}$ and *oxtr*$^{(-/-)}$ mutants ($P = 0.71$, Mann-Whitney test) (Figure 1D). Both genotypes explored the stimuli equally ($P = 0.52$, Mann-Whitney test) (Figure 1E) and exhibited a similar mean velocity ($P = 0.34$, unpaired *t* test) (Figure 1F).

We also quantified the zebrafish motivation to approach objects, by replacing the shoal of conspecifics by four red or green 0.5-mL microcentrifuge tubes (Figure 1G). Because *oxtr*$^{(+/+)}$ responded similarly to red and green ($P = 0.90$, preference for red, n = 5 vs green,

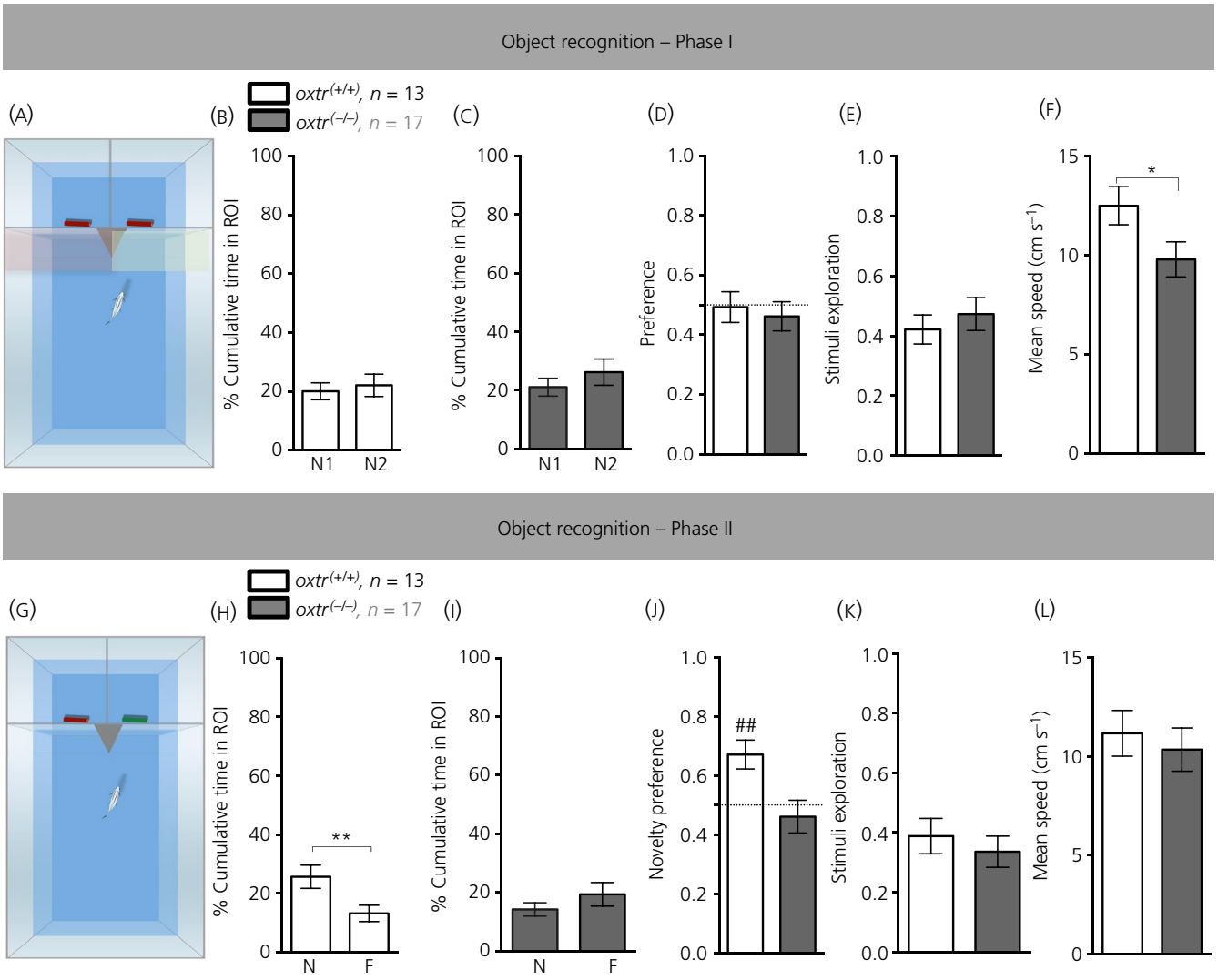

FIGURE 3 Mutant *oxtr*$^{(-/-)}$ fish show impaired non-social recognition memory. A, Schematic of phase I of the behavioural test: focal fish are given a choice between two novel objects. B, *Oxtr*$^{(+/+)}$ fish spent equal time in both novel stimuli, N1 and N2 ($P = 0.67$, paired *t* test). C, Mutant *oxtr*$^{(-/-)}$ fish spent equal time in both stimuli ($P = 0.43$, Wilcoxon matched-pairs signed rank test). D, No preference observed towards one vs the other object (*oxtr*$^{(+/+)}$: $P = 0.89$, *oxtr*$^{(-/-)}$: $P = 0.45$, one sample *t* test). E, No differences observed in stimuli exploration ($P = 0.50$, unpaired *t* test); however, (F) *oxtr*$^{(+/+)}$ fish exhibit a significantly higher mean speed than their *oxtr*$^{(-/-)}$ mutant siblings ($P = 0.05$, unpaired *t* test). G, Schematic of phase II of the behavioural test: focal fish are given a choice between a novel vs familiar object (different colours). H, *Oxtr*$^{(+/+)}$ fish spend significantly more time near a novel than a familiar object ($P = 0.003$, paired *t* test). I, Mutant *oxtr*$^{(-/-)}$ fish spent equal time in both novel and familiar stimuli ($P = 0.38$, Wilcoxon matched-pairs signed rank test. J, Novelty preference was observed for *oxtr*$^{(+/+)}$ fish ($P = 0.004$, one sample *t* test). K, No differences in stimuli exploration ($P = 0.51$, unpaired *t* test) and (L) mean speed of fish during test trial ($P = 0.74$, Mann-Whitney test) between *oxtr*$^{(+/+)}$ and *oxtr*$^{(-/-)}$ mutant fish. *oxtr*$^{(+/+)}$ represented by white bars and mutant *oxtr*$^{(-/-)}$ fish by grey bars. Values are the mean ± SEM. ROI, region of interest

n = 9, Mann-Whitney test), the results with the two colours were combined. Control *oxtr*$^{(+/+)}$ approached both the objects and empty compartments equally, showing no preference for any of the stimuli (object vs empty, $P = 0.39$, n = 14, Wilcoxon matched-paired signed rank test) (Figure 1H). Similarly, *oxtr*$^{(-/-)}$ fish spent equal time in both stimuli (object vs empty, $P = 0.87$, n = 14, paired *t* test) (Figure 1I). For both genotypes, the object preference score was not different from 0.5 ($P = 0.30$ for *oxtr*$^{(+/+)}$ and $P = 0.86$ for *oxtr*$^{(-/-)}$, one sample *t* test) (Figure 1J). Furthermore, no differences on object preference score were observed between genotypes ($P = 0.57$, unpaired *t* test) (Figure 1J). Finally, *oxtr*$^{(+/+)}$ and their *oxtr*$^{(-/-)}$ mutant siblings explored the object stimuli equally ($P = 0.63$, unpaired *t* test) (Figure 1K) and significantly less than when live shoal was present (for shoal vs object in *oxtr*$^{(+/+)}$: $P < 0.0001$, Mann-Whitney test; and *oxtr*$^{(-/-)}$: $P < 0.0001$, unpaired *t* test). However, *oxtr*$^{(-/-)}$ mutants exhibited a nearly significant decrease in mean speed, when compared to their *oxtr*$^{(+/+)}$ siblings ($P = 0.06$, unpaired *t* test) (Figure 1L).

These results show that zebrafish are highly attracted to their conspecifics and exhibit a higher motivation to interact with conspecifics than to engage with motionless objects. Furthermore, our data

suggest that, in our experimental system, motivation to approach conspecifics is not affected by OXTR deficiency.

3.2 | Oxytocin receptor modulates social recognition

To investigate the role of OXT signalling in social recognition, we first exposed a focal fish to two novel conspecifics (phase I: novel-novel) (Figure 2A). Focal *oxtr*$^{(+/+)}$ fish spent an equal amount of time near the two novel fish (novel 1 vs novel 2, $P = 0.82$, n = 22, Wilcoxon matched-paired signed rank test) (Figure 2B). Similarly, *oxtr*$^{(-/-)}$ mutants did not discriminate between the two ($P = 0.34$, n = 27, Wilcoxon matched-paired signed rank test) (Figure 2C). Both genotypes exhibited a preference score close to 0.5 (for *oxtr*$^{(+/+)}$, $P = 0.73$; for *oxtr*$^{(-/-)}$ mutants, $P = 0.29$, one sample *t* test) (Figure 2D), which is indicative of no preference towards any of the stimuli. However, *oxtr*$^{(-/-)}$ mutants explored significantly more both stimuli than the controls ($P < 0.0001$, Mann-Whitney test) (Figure 2E). No differences in mean speed were observed between the two genotypes ($P = 0.54$, Mann-Whitney test) (Figure 2F).

In phase II, when the focal fish had to discriminate between a novel and a familiar conspecific (Figure 2G), *oxtr*$^{(+/+)}$ fish spent significantly more time near the novel than the familiar fish (30.01 ± 4.84% near novel vs 14.38 ± 2.38% near familiar, n = 22, $P = 0.01$, Wilcoxon matched-paired signed rank test) (Figure 2H), whereas *oxtr*$^{(-/-)}$ did not seem to discriminate between the two, since no differences were observed between % cumulative of time *oxtr*$^{(-/-)}$ fish spent near novel vs familiar stimuli ($P = 0.93$, n = 27, Wilcoxon matched-paired signed rank test) (Figure 2I). In agreement with these findings, we observed that the novelty score was significantly higher from 0.5 in *oxtr*$^{(+/+)}$ ($P = 0.01$, n = 22, one-sample *t* test) (Figure 2J) but not in *oxtr*$^{(-/-)}$ ($P = 0.82$, n = 27, one sample *t* test) (Figure 2J). Furthermore, no correlation was observed between the preference score for the side in phase I where the novel conspecific was placed in phase II, and the preference score for the novel fish in phase II (for oxtr$^{(+/+)}$: $r = -0.13$, $P = 0.56$, n = 22, Pearson correlation; for oxtr$^{(-/-)}$: $r = 0.1289$, $P = 0.52$, n = 27, Pearson correlation). Together these results indicate that *oxtr*$^{(+/+)}$ fish are able to discriminate between familiar and novel conspecifics and exhibit a preference towards the novel ones, which is not observed in *oxtr*$^{(-/-)}$ fish, supporting a role of OXTR signalling in social memory recognition. As observed in phase I of the test, *oxtr*$^{(-/-)}$ also explored significantly more the stimuli than their control siblings ($P < 0.0001$, Mann-Whitney test) (Figure 2K). No differences were found in mean swimming speed between the two genotypes ($P = 0.22$, unpaired *t* test) (Figure 2L).

3.3 | Mutants *oxtr*$^{(-/-)}$ display deficits in object recognition

To assess whether the impairment in social recognition in *oxtr*$^{(-/-)}$ were specific of the social domain, we replaced the fish stimuli by 0.5 mL- microcentrifuge tubes of the same colour (either both red or both green, phase I of test) (Figure 3A). Control *oxtr*$^{(+/+)}$ explored both objects equally ($P = 0.67$, n = 13, paired *t* test) (Figure 3B). Also, *oxtr*$^{(-/-)}$ mutants spent the same amount of time near each of the objects ($P = 0.43$, n = 17, Wilcoxon matched-paired signed rank test) (Figure 3C). Thus, for both genotypes, the mean preference score was not significantly different from 0.5 ($P = 0.89$ for *oxtr*$^{(+/+)}$, and $P = 0.45$ for *oxtr*$^{(-/-)}$, one sample *t* test) (Figure 3D). Furthermore, no differences were observed in stimuli exploration ($P = 0.50$, unpaired *t* test) (Figure 3E). However, *oxtr*$^{(-/-)}$ swam at lower speeds than their control siblings ($P = 0.05$, unpaired *t* test) (Figure 2F).

In phase II of the test (familiar vs novel object) (Figure 2G), *oxtr*$^{(+/+)}$ fish spent significantly more time closer to the novel than the familiar object ($P = 0.003$, n = 13, paired *t* test) (Figure 3H), whereas *oxtr*$^{(-/-)}$ mutant fish did not discriminate between the two ($P = 0.38$, n = 17, Wilcoxon matched-paired signed rank test) (Figure 3I). Thus, in contrast to the *oxtr*$^{(-/-)}$ fish, control *oxtr*$^{(+/+)}$ exhibited a novelty preference score significantly higher than 0.5 (for *oxtr*$^{(+/+)}$: $P = 0.004$, for *oxtr*$^{(-/-)}$: $P = 0.49$, one sample *t* test) (Figure 3J). No differences between genotypes were observed in stimuli exploration ($P = 0.51$, unpaired *t* test) (Figure 3H) and mean swimming speed ($P = 0.74$, Mann-Whitney test) (Figure 3K).

Overall, these results show that zebrafish OXTR is not involved in a social domain-specific memory recognition but, instead, in a general memory recognition process.

3.4 | Mutant *oxtr*$^{(-/-)}$ exhibit normal anxiety-like behaviours

We next performed the open field test to assess for anxiety-related behaviours (see Supporting information, Figure S1A). Mutant *oxtr*$^{(-/-)}$ fish and their control siblings, *oxtr*$^{(+/+)}$, spent similar % cumulative of time in center of the arena($P = 0.64$, n = 18, Mann-Whitney test) (see Supporting information, Figure S1B) and exhibited similar mean speed ($P = 0.33$, n = 18, unpaired *t* test) (see Supporting information, Figure S1C). These results suggest that *oxtr*$^{(-/-)}$ mutants exhibit normal levels of anxiety and locomotion.

Overall, our findings indicate that, although *oxtr*$^{(-/-)}$ mutants displayed similar levels of social preference and anxiety-related behaviours to those of their controls, they do exhibit a significant impairment in both social and non-social recognition memory.

4 | DISCUSSION

In the present study, we disrupted OXT signalling using TALEN-mediate genome editing to generate specific genomic deficiency of one of the two zebrafish OXTR. We found that the OXTR differentially regulates distinct aspects of social behaviour. Mutant *oxtr*$^{(-/-)}$ fish showed no impairment of social preference, although they did exhibit a significant impairment in both social and non-social memory recognition. These findings suggest that, in zebrafish, OXT-like

peptides act on particular aspects of social behaviour, more specifically on cognitive rather than on motivational functions of sociality, which has also been observed in other species.[6,7] Moreover, our findings also show that these effects of OXT on memory recognition are not specific to the social domain but, instead, they are of the general domain, also affecting object recognition.

The lack of effect of OXT signalling disruption on social preference reported in the present study is in contrast with previous findings showing an effect of OXT on social preference in different species, including zebrafish. However, these previous reports mainly relied on the effects of the administration of exogenous OXT or its receptor antagonists/agonists on an individual´s approach to conspecifics,[19-22] and pharmacological approaches are not always conclusive given dose-dependent effects, as well as the common cross-reactivity of nonapeptides antagonists and agonists at their respective receptors. Moreover, genetic knockout of either *oxt* or *oxtr* genes have yield variable social preference phenotypes.[9,23] Although OXT/OXTR-deficient animals were shown to exhibit impaired approach to their conspecifics,[9] they did not exhibit altered social preference in other independent reports.[7,23] In agreement with the latter reports, we show here that zebrafish lacking functional OXTR exhibits normal social preference behaviour. Our results suggest that, at least in zebrafish, OXTR is not directly involved in the motivational domain of sociality, specifically, the drive to approach conspecifics. However, it should be noted that, in the present study, we knocked-out one of the two OXT receptors present in zebrafish, namely the OXTR and not the OXTR-like (OXTRL) receptor. It is therefore possible that, in zebrafish, both OXTR and OXTRL have redundant roles in the regulation of social motivation. Another possibility for the lack of motivational effect on OXTR deficiency zebrafish, as reported in the present study, could be a result of the presence of a functional OXTRL in our *oxtr*$^{(-/-)}$ mutants that would rescue their social preference phenotype. Future studies with *oxtrl*$^{(-/-)}$ mutants and with *oxtr*$^{(-/-)}$/*oxtrl*$^{(-/-)}$ double mutants are needed to disentangle these two scenarios. Alternatively, this effect can also be attributed to the use of a non-conditional mutant, allowing time for compensatory mechanisms such as up-regulation of alternative pathways (eg, those mediated by OXTRL or vasopressin receptors).

Although we have observed a normal social preference in our *oxtr*$^{(-/-)}$ mutants, we did observe that an impaired oxytocinergic signalling affected social recognition, comprising a cognitive function that has been shown consistently to be affected by OXT in other species.[6-10] It has already been reported that the zebrafish is able to discriminate between novel and familiar conspecifics, exhibiting social recognition memory,[24,25] and, in the present study, for the first time, we have shown that this mechanism is regulated by OXT signalling, suggesting a conserved mechanism across species.[6,7] Interestingly, a conditional deletion of OXTR in lateral septum of mice impairs social memory but not social approach.[7] Moreover, knockout of mGLUR5 in this brain region abolishes social preference at the same time as leaving social recognition intact, suggesting that different neuronal types contribute to social preference vs social memory. In the present study, we have used a non-conditional *oxtr*$^{(-/-)}$ that abolishes the function of the receptor in all organism. OXT receptors are widely expressed throughout the forebrain and midbrain of teleost fish (African cichlid), including the ventral telencephalon Vv area, which is homologous to the lateral septum in mammals.[26] Thus, it is possible that Vv is also implicated in social memory in zebrafish. Interestingly, a cholinergic neuronal population in the Vv region has recently been implicated in social preference in zebrafish.[27] Given the lack of social preference impairment in our *oxtr*$^{(-/-)}$, we hypothesise that OXTR receptors are not expressed in this cholinergic neuronal population of Vv, and that another OXTR sensitive neuronal population that regulates social recognition may also be present in the Vv. Moreover, similarly to what happens in autism spectrum disorders, oxtr$^{(-/-)}$ mutant fish displayed an intense focus attention on the stimuli, spending more time near the stimuli, which was reflected by a higher stimuli exploratory score in oxtr$^{(-/-)}$ than in the wild-type siblings. These observations open doors for future experiments aiming to explore the role of oxytocin signalling on attention.

Finally, although, in some studies, the effect of social recognition appears to be specific of the social domain,[7] we showed that zebrafish *oxtr*$^{(-/-)}$ mutants exhibited impaired social and object recognition. In accordance with the present study, there are also reports on OXT effects on non-social recognition in both rats and humans. Intracerebroventricular administration of OXT in rats enhances object recognition behaviour along with changes in neuronal growth factors and cytoskeletal proteins in hippocampus.[28] Also, administration of OXT in humans enhances both social and non-social memory.[29] Taken together, these results suggest that OXT is important for memory recognition in general, which is an argument in favour of social recognition being a general domain cognitive ability rather than an evolutionary specialisation for the social domain.

In conclusion, in the present study, OXTR signalling is suggested to regulate a general memory recognition ability that plays a key role in both social and non-social cognition in zebrafish.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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