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Targeting selectively oxytocin receptor signalling efficiently improves social interaction in Fmr1 KO mice

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► To cite this version:

Caroline Gora, Nicolas Azzopardi, Lucile Drobecq, Emmanuel Pecnard, Patrick Schnider, et al.. Targeting selectively oxytocin receptor signalling efficiently improves social interaction in Fmr1 KO mice. 2024. hal-04740514

HAL Id: hal-04740514

<https://hal.science/hal-04740514v1>

Preprint submitted on 16 Oct 2024

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1 Title: **Targeting selectively oxytocin receptor signalling efficiently improves social**
2 **interaction in *Fmr1* KO mice**

3

4 Running title: **Selective OTR agonists restore sociability in *Fmr1* KO mice**

5

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18

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24 **Word count:** 3570/4000 (excluding abstract, methods, references and figure legends)

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26

27 **ACKNOWLEDGEMENTS**

28 Mouse breeding and care was performed at INRAE Animal Physiology Facility
29 (<https://doi.org/10.15454/1.5573896321728955E12>). This project has received funding from
30 the European Research Council (ERC) under the European Union's Horizon 2020 research and
31 innovation programme (grant agreement No. 851231). We thank Dr Pascale Crépieux and Dr
32 Eric Reiter for their advice on the manuscript. LPP and CG acknowledge the LabEx
33 MabImprove (grant ANR-10-LABX-53-01) for the financial support in co-financing CG's PhD
34 fellowship. We used ChatGPT, developed by OpenAI, for assistance with English editing. The
35 conducted research was not preregistered with an analysis plan in an independent,
36 institutional registry.

37

38 **CONFLICT OF INTEREST STATEMENT**

39 PDP, PS and ChG are or were employed by F. Hoffmann-La Roche AG, Basel, Switzerland.
40 The other authors declare no conflict of interest.

41

42 **BULLET POINT SUMMARY**

43 **What is already known**

- 44 • Drugs, including OT and AVP, are inefficient to improve social impairments in
45 individuals with autism
- 46 • Targeting OTR and V_{1A} receptors remains the most relevant option for the
47 management of autism.

48 **What does this study add**

- 49 • Selective OTR agonists biased towards Gαq signalling improve sociability in *Fmr1* KO
50 mice.
- 51 • The V_{1A} antagonist RO6893074 is not sufficient to improve sociability in *Fmr1* KO mice.

52 **What is the clinical significance**

- 53 • Selective Gαq-biased OTR agonists represent a promising strategy for the
54 management of autism.

55

56 **ABSTRACT**

57 **Background and Purpose:** No drugs targeting the core social features of autism spectrum
58 disorder (ASD) have been approved. Although clinical trials with oxytocin (OT) and vasopressin
59 (AVP) have yielded mixed results, targeting their receptors remains the most promising
60 pharmacological strategy for addressing social impairments in ASD. This study aims to identify
61 which receptors and signalling pathways within this family can sustainably improve social
62 impairments.

63 **Experimental Approach:** We used dose-response and kinetic analyses, along with
64 mathematical modelling, to evaluate OT, AVP, their homologs, and novel synthetic ligands on
65 G protein coupling, β -arrestins recruitment, and internalisation of mouse oxytocin (OTR) and
66 vasopressin (V_{1A} , V_{1B} , V_2) receptors in Neuro-2a cells. We tested acute and subchronic
67 administration of OTR agonists and the novel V_{1A} receptor antagonist, alongside OT and AVP,
68 for their effects on social interaction in *Fmr1* KO mice, a model exhibiting ASD-like features.

69 **Key Results:** While OT, AVP and most compounds were non-selective across the four
70 receptors, the OTR agonists TGOT or RO6958375 and the V_{1A} antagonist RO6893074 were
71 selective. TGOT or RO6958375, favouring $G\alpha_q$ signalling, enhanced social interactions in *Fmr1*
72 KO mice while showing minimal effects in wild-type mice. In contrast, OT, AVP or RO6893074
73 exhibited limited efficacy in *Fmr1* KO mice.

74 **Conclusion and Implications:** Selective OTR agonists, unlike OT and AVP, effectively improved
75 social impairments in *Fmr1* KO mice after acute and subchronic treatment. These findings

76 highlight the necessity for developing highly selective OTR $G\alpha_q$ -biased agonists to achieve
77 clinical outcomes in ASD.

78

79 Keywords (3-7 words): **oxytocin receptors, vasopressin receptors, G protein-coupled**
80 **receptors, signalling pathways, autism spectrum disorder, Fragile X syndrome, social**
81 **interaction**

82 INTRODUCTION

83 Autism spectrum disorder (ASD) is a neurodevelopmental condition characterised by
84 impairments in social communication and interaction, along with stereotyped or repetitive
85 behaviours (American Psychiatric Association, 2013). It affects approximately 1% of the global
86 population (Zeidan et al., 2022). Recent genome-wide association studies have identified
87 hundreds of candidate genes ([SFARI Gene](#)), implicating convergent neurobiological
88 mechanisms such as gene expression regulation and neuronal communication, signalling or
89 plasticity (De Rubeis et al., 2014; Satterstrom et al., 2020; Pintacuda et al., 2023). Fragile X
90 syndrome, a monogenic disorder frequently associated with ASD and intellectual disability,
91 results from the loss of FMRP expression (Pieretti et al., 1991; Verheij et al., 1993). To date,
92 no effective pharmacological options targeting core social features have been approved for
93 individuals with ASD. Placebo effects, lack of efficacy, and the heterogeneity of patients within
94 the spectrum have prevented success in phase 3 clinical trials.

95 G protein-coupled receptors (GPCRs), are targeted by around 30% of approved drugs (Hauser
96 et al., 2017; Alexander et al., 2021) and are key regulators of downstream signalling that are
97 dysregulated in ASD (De Rubeis et al., 2014; Satterstrom et al., 2020; Pintacuda et al., 2023).
98 Over 200 pathogenic variants in 26 GPCR genes have been associated with ASD ([SFARI Gene](#),
99 Annamneedi et al., 2023). Thus, GPCRs have emerged as robust and specific therapeutic
100 targets for ASD. Among them, oxytocin (OT) or vasopressin (AVP) bind to oxytocin (OTR) and
101 vasopressin (V_{1A} and V_{1B}) receptors in the brain, modulating social behaviours (Busnelli et al.,
102 2013; Quintana et al., 2019; Rigney et al., 2022; Theofanopoulou et al., 2022). These receptors
103 are primarily coupled to $G\alpha_{q/11}$ proteins, leading to the activation of the IP_3 - Ca^{2+} signalling
104 pathway and recruit β -arrestins upon ligand binding (Busnelli et al., 2013). Although results
105 have been inconsistent, some clinical trials have reported improvements in social scales and

106 repetitive behaviours following intranasal administration of OT (Bernaerts et al., 2020;
107 Annamneedi et al., 2023; Daniels et al., 2023; Guastella et al., 2023). Notably, while plasma
108 and brain concentrations of OT are not correlated (Valstad et al., 2017; Gora et al., 2024),
109 individuals with lower plasma OT levels tend to respond better to exogenous OT
110 administration (Parker et al., 2017). Furthermore, recent studies suggest that combining OT
111 administration with behavioural interventions enhances sociability more than OT alone (Ford
112 and Young, 2022; Daniels et al., 2023; Pantouli et al., 2024). Similarly intranasal AVP
113 administration was well tolerated in early trials and improved social responsiveness,
114 particularly in children with ASD and low plasma AVP concentrations (Parker et al., 2019).
115 While administration of the V_{1A} antagonists balovaptan or RG7713 enhanced social skills in
116 adults with ASD in phase 2 or exploratory clinical studies (Umbricht et al., 2017; Bolognani et
117 al., 2019), balovaptan did not show improvement over placebo in phase 3 trials (Hollander et
118 al., 2022; Jacob et al., 2022). Despite this, OTR and V_{1A} receptors remain the most promising
119 targets to improve sociability in individuals with ASD (Annamneedi et al., 2023). Further
120 research is needed to identify the most suitable ligands, targets and downstream signalling
121 pathways.

122 In this study, we aim to enhance our understanding of why compounds targeting OTR and V_{1A}
123 receptors have underperformed in clinical trials. We used a systematic pharmacological
124 approach to assess both existing and novel ligands, determining which ligand-receptor
125 pairings are most promising *in vitro* and in *Fragile X Mental Retardation 1* knockout (*Fmr1* KO)
126 mice.

127 RESULTS

128 **Oxytocin or vasopressin act as partial agonists on mouse vasopressin or oxytocin receptors** 129 **in murine Neuro-2a cells**

130 To address the inconsistent results from OT and AVP clinical trials, it is essential to investigate
131 their distinct pharmacological profiles on OTR, V_{1A}, V_{1B} and V₂ receptors. We assessed their
132 kinetics and concentration-response relationships across the four murine receptors,
133 evaluating ten signalling outputs in the murine Neuro-2a neuroblastoma cell line, using BRET1
134 assays over a 30-min period and luminescence assay for Ca²⁺ release (Figure 1A). Spider plots
135 illustrated that all receptors recruited miniGq, leading to intracellular Ca²⁺ mobilisation, and
136 both β -arrestin-1 and -2 and internalised (using CAAX and FYVE sensors) following OT or AVP
137 stimulation (Figures 1C, S1, Table S1). As expected, V₂ also recruited miniGs and induced
138 cAMP production. None of the receptors recruited miniGi or reduced forskolin-induced cAMP
139 production. Overall, AVP acted as a partial agonist on OTR compared to OT, while OT was a
140 partial agonist on V_{1A} receptors across these assays (except for Ca²⁺ mobilisation). OT acted as
141 a full agonist with lower potency than AVP on V_{1B} and V₂ receptors, although it was less
142 efficient at recruiting β -arrestin on V₂ receptors (Figures 1C, S1). The pharmacological profiles
143 of OT and AVP for the four human receptors in human HEK293A cells were comparable to
144 those observed for the murine receptors (Figure S2, Table S2).

145 To estimate the potential role of receptor internalisation in the lack of efficacy resulting from
146 repeated OT and AVP administration during clinical trials, we quantified the β -arrestin
147 recruitment and receptor internalisation kinetics for the four receptors using mathematical
148 modelling (Figure 1B). The model (1), which incorporates receptor internalisation, β -arrestin
149 recruitment, and subsequent receptor recycling, provided the best fit for the BRET1 data
150 (Material and Methods, Figure S3, Table S3). The parameter identifiability procedure yielded

151 robust normalised parameter estimation with small confidence intervals for predicted affinity
152 ($1/K_D$) and for kinetic parameters, β -arrestin recruitment (k_τ), internalisation (k_{int}) and
153 recycling (k_{rec} ; [Table S3](#)), except for OT on the V_{1A} receptor and AVP on OTR where the E_{max}
154 were not reached. We found that receptor recycling was statistically significant except for
155 AVP on OTR, and was slower than internalisation within the 30-minute period ($k_{rec} \approx 0.1 \text{ min}^{-1}$
156 ¹ and slightly higher for AVP on V_2 receptor; [Figure 1D](#), [Table S3](#)). Radar plots highlighted key
157 differences in kinetic parameters, showing that OT on OTR, OT and AVP on V_{1B} and AVP on V_{1A}
158 and V_2 receptors shared similar parameter landscapes, and to a lesser extent, OT on V_2 ([Figure](#)
159 [1D](#), [Table S3](#)). These ligand-receptor pairings exhibited comparatively higher affinity ($1/K_D$),
160 recycling (k_{rec}) and internalisation (k_{int}) rates, along with lower β -arrestin recruitment (k_τ).
161 Conversely, despite large confidence intervals, AVP on OTR and OT on V_{1A} receptors showed
162 lower affinity ($1/K_D$) and internalisation (k_{int}), and higher β -arrestin recruitment (k_τ , [Table S3](#)).
163 Using GPCR ligand bias analyses (Kolb et al., 2022), we found that AVP was significantly biased
164 towards miniGq on the OTR compared to OT ([Figure 2A](#), [Table 1](#)). OT and AVP did not induce
165 any other significant biased signalling on the OTR, V_{1A} and V_{1B} receptors, as both G protein

166 and β -arrestin recruitment profiles were similar (Figure 2A). Overall, miniGq and β -arrestin-2
167 recruitment served as discriminant BRET1 assays to identify ligand bias (Table 1).

168 In conclusion, while OT and AVP activated all four receptors, with distinct potency and
169 efficacy, they induced similar β -arrestin recruitment and receptor internalisation profiles.
170 Only AVP displayed a partial biased agonist profile at the OTR.

171

172 **Revisiting existing and novel ligands targeting oxytocin and vasopressin receptors in Neuro-** 173 **2a cells**

174 To identify the most specific and potentially biased ligands, we compared various agonists
175 and antagonists targeting oxytocin and vasopressin receptors, focusing on miniGq and β -
176 arrestin-2 recruitment in Neuro-2a cells (Figures 2, S4, Tables 1, S1).

177 The bird and fish OT homologues mesotocin and isotocin recruited both miniGq and β -
178 arrestin-2 at all the four receptors, except for isotocin on the V_{1A} receptor (Figures 2A, S4A,
179 Tables 1, S1). Notably, mesotocin and isotocin were biased towards miniGq on OTR, V_{1A} and
180 V_2 receptors and on OTR, V_{1B} and V_2 receptors respectively, compared to OT and AVP. The
181 bird AVP homologue vasotocin was more efficient than AVP on OTR, V_{1A} and V_{1B} and displayed
182 also a significant bias only for OTR (Figures 2A, S4A, Tables 1, S1).

183 Among synthetic peptide OTR agonists, RO6958375 (Janz et al., 2023) and TGOT (Elands et
184 al., 1988) activated OTR, and to a lesser extent V_2 for RO6958375, but neither activated V_{1A}
185 and V_{1B} , in contrast to OT (Figures 2B-C, Table S1). Both synthetic ligands were identified as
186 miniGq-biased agonists on OTR, and V_2 receptors for RO6958375, being less potent and
187 efficient in β -arrestin-2 recruitment (Figure 2B-C, Tables 1, S1). However, kB7-OT (Koehbach
188 et al., 2013) and carbetocin previously described as a $G\alpha_q$ -biased OTR agonist (Passoni et al.,

189 2016), did not activate the receptors in our experiments, except for V₂ with carbetocin
190 showing a miniGq-biased profile (Figures 2B, S4B, Table S1).

191 The OTR antagonists atosiban (Goodwin et al., 1994) and L-371,257 (Williams et al., 1995)
192 displayed additional antagonistic effects on V_{1A} and V₂, respectively (Figures 2D). In contrast,
193 the novel V_{1A} antagonist RO6893074, related to balovaptan (Schnider et al., 2020), displayed
194 high selectivity and did not block OTR, V_{1B} and V₂ receptors (Figures 2D). Furthermore,
195 RO6893074 did not inhibit a selection of 47 human receptors, channels, transporters and
196 enzymes when tested at 3 μM (Table S4). Notably, none of the antagonists exhibited partial
197 agonist effects or any bias on the four oxytocin or vasopressin receptors when tested alone
198 (Figure S4C).

199 In conclusion, TGOT and RO6958375 are selective, partial miniGq-biased agonists for OTR
200 while RO6893074 is a selective V_{1A} antagonist. Therefore, these ligands, alongside the
201 reference peptides OT and AVP, are of interest for assessing their effects on social interaction
202 outcomes.

203

204 **Oxytocin or vasopressin administration did not enhance social interactions in *Fmr1* KO mice**

205 To better understand the mixed outcomes of previous clinical trials, we compared the effects
206 of acute (Day 0) and subchronic (Day 7) intranasal administration of OT and AVP at doses of
207 0, 20 and 40 μg.kg⁻¹ in WT and *Fmr1* KO mice (Figures 3, S5-7, Table S5). The *Fmr1* KO mouse
208 model is well-established for studying Fragile X syndrome and ASD (Kat et al., 2022), affecting
209 oxytocin neurons (Lewis et al., 2020; Gora et al., 2024). Using the Live Mouse Tracker (de
210 Chaumont et al., 2019), which automatically assesses multiple parameters of social
211 interaction among two unfamiliar sex-, treatment- and genotype-matched mice, we

212 confirmed that *Fmr1* KO mice displayed consistent social interaction impairments compared
213 to WT mice, in both sexes (Figure S5).

214 Neither acute nor subchronic intranasal administration of OT, regardless of dose, improved
215 nose contacts and huddling behaviours — two parameters of social exploration, in *Fmr1* KO
216 mice (Figures 3, S6), while acute administration of OT at 40 $\mu\text{g.kg}^{-1}$ reduced their time spent
217 in 'move in contact' (Figure S6A). In contrast, acute administration of OT at 20 $\mu\text{g.kg}^{-1}$
218 enhanced nose contacts and reduced the time spent 'stop isolated' in WT animals,
219 irrespective of treatment duration, while the highest dose reduced stretch-attend postures
220 (SAP) — an index of anxious-like behaviours (Figures 3, S6). Acute intranasal administration
221 of AVP decreased the time spent in nose contacts and huddling behaviours in *Fmr1* KO mice
222 at 40 $\mu\text{g.kg}^{-1}$, while subchronic intranasal administration of AVP at 20 $\mu\text{g.kg}^{-1}$ increased these
223 social parameters in this group (Figures 3B, S6, Table S5).

224 In conclusion, neither OT nor AVP efficiently improved social impairments in *Fmr1* KO mice,
225 while AVP affected social interaction in WT mice.

226

227 **Targeting selectively oxytocin receptors improves social interactions in *Fmr1* KO mice**

228 To determine whether selective targeting of OTR signalling, in contrast to non-selective OT,
229 could effectively enhance social interactions in *Fmr1* KO mice, we administered selective OTR
230 peptide agonists TGOT (intranasally, at 0, 20 and 40 $\mu\text{g.kg}^{-1}$) and RO6958375 (subcutaneously,
231 at 0, 0.03, 0.06 and 0.12 mg.kg^{-1}) in both WT and *Fmr1* KO mice (Figures 4, S7, Table S5). Acute
232 intranasal administration of TGOT at 20 $\mu\text{g.kg}^{-1}$ increased social exploration in *Fmr1* KO mice,
233 as evidenced by enhanced nose contacts and huddling behaviours. Subchronic administration
234 of TGOT at 40 $\mu\text{g.kg}^{-1}$ also increased nose contacts but did not affect other parameters in
235 *Fmr1* KO mice (Figure 4). In contrast, both acute and subchronic administration of TGOT at

236 the highest dose (40 $\mu\text{g.kg}^{-1}$) reduced all social parameters in WT mice and increased the time
237 spent isolated and in SAP (Figures 4, S7). Both acute or subchronic administration of the OTR
238 agonist RO6958375 at low (0.03 mg.kg^{-1}) and moderate (0.06 mg.kg^{-1}) doses increased social
239 interaction in *Fmr1* KO mice, as well as social motivation ('social approach' and 'move in
240 contact') at the moderate dose (Figures 4). Acute administration of the moderate dose also
241 increased nose contacts and huddling behaviours in WT mice. However, subchronic exposure
242 to the highest dose (0.12 mg.kg^{-1}) reduced social motivation and increased anxious-like
243 behaviours in WT mice (Figures 4, S7).

244 We also tested the small compound V_{1A} antagonist RO6893074 (intraperitoneally at 0, 25, 50
245 and 100 mg.kg^{-1}) in both WT and *Fmr1* KO mice, aiming to redirect endogenous oxytocin
246 release on OTR by blocking V_{1A} receptors (Figures 4, S7, Table S5). Acute administration of
247 RO6893074 at 50 mg.kg^{-1} increased social interaction in *Fmr1* KO mice (Figure 4). However,
248 administration at 100 mg.kg^{-1} decreased locomotion, as evidenced by reduced 'move in
249 contact' and increased 'stop isolated' time, diminishing social interaction in both WT and
250 *Fmr1* KO mice (Figures 4, S7). Acute administration of the highest dose had sedative effects,
251 while subchronic administration showed variable enhancement of nose contacts and
252 huddling behaviours in WT mice (Figure 4).

253 The pharmacokinetics of RO6893074 following a single intraperitoneal dose of 5 mg.kg^{-1} and
254 50 mg.kg^{-1} in mice, indicated an average maximum plasma concentration (C_{max}) of
255 approximately 220 ng.mL^{-1} and 7000 ng.mL^{-1} , with a time to reach C_{max} (T_{max}) of 0.5 hours
256 and 0.84 hours, respectively. The area under the plasma concentration-time curve from time
257 0 to infinity ($\text{AUC}_{0-\infty}$) was 581 and 16400 ng.h.mL^{-1} , respectively. The elimination clearance
258 ($\text{CL} = \text{Dose}/\text{AUC}$) was dose-dependent. Based on free plasma exposure of 6.3% (considering
259 the compound is not a substrate for mouse P-glycoprotein and has good permeability) and *in*

260 *in vitro* V_{1A} binding, the corresponding brain V_{1A} receptor occupancy was estimated to be
261 approximately 49% and 96% at doses of 5 mg.kg⁻¹ and 50 mg.kg⁻¹, respectively.
262 Administration at doses of 10 mg.kg⁻¹ and 100 mg.kg⁻¹ is expected to yield approximately 65%
263 and 98% occupancy, respectively. Biological effects of V_{1A} blockade are expected to be
264 detectable between 70 to 90% receptor occupancy (Grimwood and Hartig, 2009). Higher
265 exposure may lead to inhibition of off-target receptors, inducing non-specific side effects, as
266 observed in WT mice ([Figure 4A](#)).

267 In conclusion, selective OTR agonists TGOT at 20 µg.kg⁻¹ or RO6958375 at 0.03 and 0.06
268 mg.kg⁻¹ restored social impairments in *Fmr1* KO mice, while TGOT at 40 µg.kg⁻¹ reduced
269 sociability in WT mice. RO6958375 displayed the most favourable dose effect with fewer side
270 effects. The V_{1A} antagonist RO6893074 showed some beneficial effects in *Fmr1* KO mice at 50
271 mg.kg⁻¹, resulting in 96% V_{1A} receptor occupancy, although these effects were not sustained
272 with subchronic administration.

273 DISCUSSION

274 In our study, we evaluated the efficacy, potency and selectivity of various ligands targeting
275 oxytocin and vasopressin receptors in neuronal heterologous cells. Despite similar binding
276 affinities (Busnelli et al., 2013), OT and AVP exhibited distinct efficacy and potency across their
277 receptors. They primarily recruit miniGq and β -arrestins at OTR, V_{1A} , and V_{1B} receptors, while
278 the V_2 receptor also activates miniGs. These findings are in line with previous studies
279 (Birnbaumer, 2000; Busnelli et al., 2012, 2013; Heydenreich et al., 2022). None of the
280 receptors coupled with miniGi proteins or mediated cAMP inhibition in our assays, which
281 contrasts with previous reports for OTR or V_2 receptors (Strakova and Soloff, 1997; Busnelli
282 et al., 2012; Heydenreich et al., 2022). The lower affinity of the chimeric miniGi protein may
283 explain these differences and may also account for the variations observed in the calcium
284 assay. Previous studies identified an association between $G\alpha_{i3}$ and OTR in rat myometrium
285 and CHO cells (Strakova and Soloff, 1997; Strakova et al., 1998) and described conformational
286 changes between $G\alpha_{i/o}$ and $G_{\beta\gamma}$ subunits or OTR fused to YFP and G_{γ} upon OT stimulation in
287 HEK293 cells (Busnelli et al., 2012). These findings imply that OTR could signal through $G_{\beta\gamma}$
288 release, as previously shown (Camps et al., 1992), although this was not detected in our
289 assays. While β -arrestin-1 and -2 recruitment was comparable across receptors and ligands,
290 we cannot exclude the possibility of distinct conformational changes between the two β -
291 arrestins (Haider et al., 2022). Isotocin, mesotocin or vasotocin displayed pharmacological
292 profiles similar to OT or AVP, respectively, and did not act as biased agonists on the four
293 receptors. Surprisingly, kB7-OT and carbetocin failed to activate OTR in our assays, except for
294 carbetocin on V_2 receptors, which contrasts with previous studies (Koehbach et al., 2013;
295 Passoni et al., 2016). This difference may be attributed to lower receptor expression in
296 HEK293A cells and distinct signalling assays (miniGq vs. $G\alpha$ - G_{γ} BRET assays). We identified

297 TGOT and RO6958375 peptides as miniGq-biased agonists for OTR and V₂ receptors — a
298 profile previously reported only for TGOT on OTR (Busnelli et al., 2013). Given that V₂ is not
299 expressed in neurons or glia and does not participate in social behaviours (Ostrowski et al.,
300 1992; Kato et al., 1995), the administration of these agonists *in vivo* primarily targets social
301 inputs regulated by OTR in the brain. Intranasal administration of OT and AVP showed limited
302 efficacy in improving social impairments in *Fmr1* KO mice. Our results align with previous
303 studies (Lindenmaier et al., 2022; Pan et al., 2022) and reflect the outcomes of clinical trials
304 where intranasal administration did not consistently yield therapeutic effects in individuals
305 with ASD (Guastella et al., 2010; Bernaerts et al., 2020; Annamneedi et al., 2023; Daniels et
306 al., 2023). The therapeutic potential of OT may be diminished by the activation of both
307 oxytocin and vasopressin receptors, despite OT activating V_{1A} receptors to a lesser extent *in*
308 *vitro*. Potential hetero-oligomerization between OTR and V_{1A} (Terrillon et al., 2003) or V_{1B}
309 receptors could also impact *in vivo* outcomes. In addition, OT has poor blood brain barrier
310 penetration even following intranasal administration, resulting in low brain exposure and high
311 interindividual variability (Leng et al., 2022).

312 Conversely, selective OTR agonists TGOT or RO6958375 improved social interactions in WT
313 and *Fmr1* KO mice at low doses. This supports the hypothesis that unselective activation of
314 vasopressin receptors by endogenous OT may counteract the pro-social effects of OTR
315 activation. The combination of OT administration with behavioural interventions appears
316 more effective in reducing social deficits (Ford and Young, 2022; Daniels et al., 2023; Pantouli
317 et al., 2024). Our experimental approach, which exposed mice to repeated social interactions
318 with unfamiliar mice, may have enhanced the effectiveness of TGOT and RO6958375.
319 Longitudinal studies could provide more comprehensive insights into the persistence of
320 therapeutic effects, including post-treatment outcomes. TGOT and RO6958375 showed

321 distinct effects in *Fmr1* KO and WT mice. RO6958375 administration consistently improved
322 social interactions in *Fmr1* KO mice without affecting WT sociability, suggesting a more
323 favourable profile than intranasal TGOT. The different administration routes (intranasal vs.
324 subcutaneous) may also influence brain exposure and reduce interindividual exposure
325 variability.

326 The two OTR agonists, characterised by lower β -arrestin recruitment efficiency and potency
327 *in vitro* compared to OT, may provide sustained OTR signalling with limited desensitisation
328 and internalisation, offering a crucial therapeutic advantage for chronic treatment.
329 Differences between these OTR agonists may be attributed to their distinct kinetic profiles or
330 efficacy *in vitro*. Ligands that are selectively biased towards G protein or β -arrestin pathways
331 have been shown to optimise therapeutic effects for other neurological disorders while
332 minimising potential side effects of the other signalling pathways (Park et al., 2016; Lee et al.,
333 2021; Kolb et al., 2022).

334 In contrast, the acute selective blockade of the V_{1A} receptor with RO6893074 was less
335 beneficial for social approach in *Fmr1* KO mice than OTR agonists, but did acutely increase
336 nose contact and huddling. The suprathreshold intraperitoneal dose of 100 mg.kg⁻¹ affected
337 locomotion, likely due to off-target effects. This suggests that blocking V_{1A} receptors may elicit
338 different behavioural outcomes than OTR activation. Therefore, combining RO6893074 with
339 an OTR agonist could potentially enhance therapeutic outcomes for social features.

340 Targeting the OTR with low doses of selective agonists improves social interactions in *Fmr1*
341 KO mice, while eliciting only limited side effects in WT mice. These findings open new avenues
342 for developing OTR-targeted therapies for ASD. Although challenging, the development of
343 OTR positive allosteric modulators enhancing OTR signalling in brain regions where OT is
344 endogenously released holds promise. Additionally, $G_{\alpha_{q/11}}$ - or β -arrestin-biased agonists that

345 induce signalling bias towards specific pathways and limit side effects may be effective
346 therapies for ASD. Understanding the precise contributions of each receptor to social
347 interaction, as well as their expression levels and the role of peptides remains crucial. Our
348 study offers new insights into OTR activation and V_{1A} inhibition on sociability. Future research
349 should investigate the effects of selective V_{1A} agonists (Andrés et al., 2002; Marir et al., 2013)
350 or the OTR antagonist atosiban, as well as the role of V_{1B} receptors, which have more
351 restricted expression in the brain (Young et al., 2006; Stevenson and Caldwell, 2012), to
352 elucidate their contributions. A limitation of this study is the lack of direct measurement of
353 mouse brain exposure for the tested compounds. This data would provide more accurate
354 estimates of brain receptor occupancy and the relative contributions of G-protein signalling
355 versus β -arrestin recruitment.

356 While our study focused on social features, assessing effects on stereotyped behaviours,
357 cognitive flexibility or anxiety would provide a more comprehensive evaluation of treatment
358 efficacy. Testing OTR agonists in mouse models using a molecular stratification approach
359 based on specific molecular markers across models mimicking the autism spectrum (Gora et
360 al., 2024), may enhance translatability and facilitate the development of personalised
361 medicines for individuals with ASD.

362 **MATERIALS AND METHODS**

363 **Compounds**

364 For *in vitro* assays, drug powders of oxytocin (OT; 1910, Tocris Bioscience™, UK), (Arg⁸)-
365 vasopressin (AVP; 2935, Tocris Bioscience™, UK), (Butyryl¹,Tyr(Me)²)-1-Carbaoxytocin
366 (carbetocin; 4040269, Bachem, Switzerland), (Ser⁴,Ile⁸)-oxytocin (isotocin; 4030890, Bachem,
367 Switzerland), (Ile⁸)-oxytocin (mesotocin; 4030888, Bachem, Switzerland), (Arg⁸)-vasotocin
368 (vasotocin; 4100576, Bachem, Switzerland), (Thr⁴,Gly⁷)-oxytocin (TGOT; 4013837, Bachem,
369 Switzerland), RWJ22164 (atosiban; 4065438, Bachem, Switzerland), (Gly⁵,Thr⁷,Ser⁹)-oxytocin
370 (kB7-OT; 4144391, Bachem, Switzerland), L-371,257 (2410, Tocris Bioscience™, UK),
371 RO6958375 OTR peptide agonist (c[Gly-Tyr-Ile-Gln-Asn-Glu]-*trans*-4-fluoro-Pro-Leu-Gly-NH₂,
372 Roche pharmaceutical, Switzerland) (Janz et al., 2023) and RO6893074 V_{1A} chemical
373 antagonist (*trans*-2-(4-(4-(4-chlorophenyl)-5-methyl-4*H*-1,2,4-triazol-3-
374 yl)cyclohexyloxy)pyridine, Roche pharmaceutical, Switzerland) were dissolved at 10⁻² M in
375 100% DMSO (20-139, Sigma-Aldrich, USA), aliquoted and stored at -20°C until further use. On
376 the testing day, all compounds were diluted in PBS 1X + 10 mM HEPES (CSTHEP00-OP, Eurobio,
377 France) at 4°C and tested alone. In addition for their antagonist effects, atosiban, L-371,257
378 and RO6893074 V_{1A} antagonists were diluted at EC₈₀ (concentration reaching 80% of maximal
379 efficacy) in 10⁻⁶ M of OT for OTR or in 10⁻⁶ M of AVP for V_{1A} and V_{1B} or 10⁻⁸ M for V₂.
380 Coelenterazine luciferase substrate (R3078C, Interchim, France) was protected from light
381 exposure and stored at 1 mM in 100% ethanol (64-17-5, Carlo Erba Reagents, France) and
382 then diluted to 5 μM final in PBS 1X.

383 For *in vivo* assays, OT, AVP and TGOT were diluted at 20 and 40 μg.kg⁻¹ in NaCl 0.9%
384 (190/12604022/1021, Braun, Germany), corresponding to low and moderate doses (0.03 and
385 0.06 IU) at 4°C and stored in aliquot for daily use at -20°C (vehicle: NaCl 0.9%). RO6958375

386 and RO6893074 compounds were formulated at Roche Pharmaceutical at 0.03, 0.06 and 0.12
387 mg.kg⁻¹ in PBS 1X for subcutaneous administration of RO6958375 OTR peptide agonist
388 (vehicle: PBS 1X), and at 25, 50 and 100 mg.kg⁻¹ in 0.1% polysorbate Tween-80, 0.21% citric
389 acid monohydrate, 0.8% sodium chloride, sodium hydroxide 1N at pH 7 diluted in water for
390 intraperitoneal injection of RO6893074 V_{1A} small molecule antagonist (vehicle: 0.1%
391 polysorbate Tween-80, 0.21% citric acid monohydrate, 0.8% sodium chloride, sodium
392 hydroxide 1N at pH 7) and stored in aliquot for daily use at 4°C until further use.

393

394 ***Cell culture***

395 Neuro-2a mouse neuroblastoma (Neuro-2a; CCL-131™, RRID:CVCL_0470, ATCC, USA) cells
396 were regularly tested for negative mycoplasma contamination were cultured in Eagle's
397 Minimal Essential Medium (EMEM; CM1MEM40-01, Eurobio, France) supplemented with
398 10% (v/v) foetal bovine serum (CVFSVF00-01, Eurobio, France), 1% penicillin/streptomycin
399 (100 U.mL⁻¹, 100 µg.mL⁻¹, respectively; 15140-122, Eurobio, France) and 1% L-glutamine (2
400 mM; CSTGLU00-0U, Eurobio, France), and maintained at 37°C with 5% CO₂. When reaching
401 90% confluency, cells were washed with PBS 1X (CS1PBS01K-BP, Eurobio, France) followed by
402 trypsin (0.5 g.L⁻¹, L-EDTA 0.2 g.L⁻¹, without phenol red; CEZTDA00-0U, Eurobio, France)
403 treatment. After cell counting, cells were transiently transfected in suspension using the

404 Metafectene Pro transfection reagent (T040-5.0, BioNTex Laboratories, Germany) according
405 to the manufacturer's protocol.

406

407 ***G protein, β -arrestin recruitment and internalisation using Bioluminescence Resonance***
408 ***Energy Transfer (BRET) assays***

409 In 96-well plates (30196, SPL Life Sciences, Korea), around 40,000 Neuro-2a cells per well were
410 transiently co-transfected with the mouse oxytocin receptor cDNA *Oxtr* or mouse vasopressin
411 *Avpr1a*, *Avpr1b* subcloned using HindIII and XhoI in pcDNA3 plasmid or with the *Avpr2*
412 receptors in pcDNA3.1(+) plasmid, fused in their C-terminus to the BRET donor *Renilla*
413 *reniformis* luciferase (RLuc8) at 50 ng/well, along with BRET acceptor sensors containing a
414 yellow fluorescent proteins (YFP, Venus or YPET) at 50 ng/well each. For the miniG protein
415 recruitment, we used the pNluc-C1 vector encoding the miniG proteins fused in their N-
416 terminus to the Nuclear Export Signal (NES) to avoid their transport to the nucleus; NES-
417 Venus-mG_s, NES-Venus-mG_i or NES-Venus-mG_q (provided by Pr Nevin A. Lambert, Augusta,
418 GA, USA) (PMID: 29523687), namely miniGs, miniGi and miniGq, respectively. MiniGs proteins
419 are engineered G α subunits with modifications such as the exclusion of the α -helical domain,
420 a shortened N-terminus which removes both membrane anchors and the G $\beta\gamma$ -binding surface,
421 specific mutations to enhance *in vitro* protein stability and a mutation in the C-terminal 5-
422 helix to stabilise receptor-miniG complexes even in the presence of guanine nucleotides. For
423 miniGq and miniGi, the five C-terminal amino acids of miniGs, which form the main receptor
424 binding site and are key determinants of coupling specificity, were replaced with those of G α_q ,
425 necessitating three mutations. For miniGi, 9 mutations were done into the G α_5 helix to
426 change the coupling specificity from G α_s to G α_i (Nehmé et al., 2017; Wan et al., 2018). For β -
427 arrestin recruitment, we used YPET- β -arrestin-1 or YPET- β -arrestin-2 (provided by Dr M.G.

428 Scott, Paris, France) (Kamal et al., 2009) encoded in pcDNA3.1 plasmid. For internalisation
429 experiments, the yellow fluorescent protein YPET was fused to the CAAX box of KRas protein
430 and the FYVE domain of endofin, according to (Lee et al., 2005; Namkung et al., 2016), namely
431 YPET-CAAX addressed at the plasma membrane or YPET-FYVE addressed at the endosome
432 membrane, respectively. Their sequences were synthesised at Twist Bioscience and
433 subcloned in pcDNA3.1 plasmid by recombination between HindIII and XhoI restriction sites.
434 For cAMP assay, pcDNA3.1(+) encoding untagged murine receptors and the CAMYEL
435 (provided by Dr Lily Jiang, Texas, USA) (Jiang et al., 2007), a cAMP EPAC sensor (YFP-Epac-
436 RLuc8) were co-transfected at 50 ng/well.

437 48 hours after transfection, Neuro-2a cells were starved for 4 hours in phenol red-free DMEM
438 (21063-029, Gibco, France) at 37°C in 5% CO₂. Cells incubated with coelenterazine substrate
439 (5 µM) were first measured for 5 minutes at 480 ± 20 nm and 530 ± 25 nm (Mithras2 LB 943
440 with the Mikrowin 2010 software, Berthold Technologies GmbH & Co., Germany). Then, cells
441 were rapidly stimulated with the different agonists at 3.10⁻⁵ M, 10⁻⁵ M, 10⁻⁶ M, 10⁻⁷ M, 10⁻⁸ M,
442 10⁻⁹ M, 10⁻¹⁰ M diluted in PBS 1X and HEPES 10 mM final, or PBS 1X and HEPES 10 mM alone
443 for the baseline, then 96-well plates were recorded for 30 minutes. When testing the effect
444 of the antagonists, they were tested at the same concentration as agonists and in presence
445 of OT at EC₈₀ for OTR or AVP at EC₈₀ for the V_{1A}, V_{1B} or V₂ receptors. For cAMP production and
446 inhibition BRET assays, compounds were tested in absence or presence of forskolin at 5.10⁻⁶
447 M (F6886, Sigma-Aldrich, USA), respectively. Each condition was performed in triplicate

448 within the same 96-well plate, and all experiments were independently repeated at least
449 three times.

450

451 ***Calcium mobilisation assays using luminescence***

452 In 6-well plates (353046, Corning, USA), around 400,000 Neuro-2a cells per well were plated
453 24 hours at 37°C with 5% CO₂ before transfection. Then, cells were transiently transfected
454 with the untagged mouse oxytocin (*Oxtr*) or vasopressin (*Avpr1a*, *Avpr1b* or *Avpr2*) receptor
455 cDNAs subcloned using HindIII and XhoI in the pcDNA3.1(+) plasmid at 1 µg/well and pPD16
456 vector encoding the calcium-sensitive bioluminescent protein aequorin from the jellyfish
457 *Aequorea victoria* jellyfish (GFP-aequorin provided by Dr Bertrand Lambolez, Paris, France) at
458 2 µg per well (Drobac et al., 2010). 48 hours after transfection, Neuro-2a cells were Ca²⁺-
459 deprived for 4 hours at 37°C with 5% CO₂ using the Hank's Balanced Salt Solution (CS1SSH22-
460 0U HBSS; Eurobio, France) with no calcium or phenol red and containing 5 µM coelenterazine
461 in the dark. Subsequently, cells were harvested and centrifuged at 500g for 5 minutes. Cell
462 pellets were resuspended in around 2.10⁶ cells.mL⁻¹ of HBSS with 1.26 mM of calcium
463 (CS1SSH23-0U; Eurobio, France) and 5 µM coelenterazine, and incubated for one hour at 37°C
464 in the dark. OT and AVP ligands were diluted in HBSS with 1.26 mM of calcium across at 10⁻
465 ⁵M, 10⁻⁶M, 10⁻⁷M, 10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M, 10⁻¹¹M, 10⁻¹²M final concentrations on ice, and HBSS
466 with 1.26 mM of calcium alone for baseline. Ten µL per well of each concentration of ligand
467 (5X) was loaded in duplicate into a 384-well plate and 40 µL of cells (around 40,000 cells per

468 well) were injected into each well. Luminescence of calcium-bound aequorin signals was
469 continuously measured for 25 seconds.

470

471 ***In vitro data modelling and analysis***

472 *BRET and calcium assays*

473 The 530 nm/480 nm BRET ratios were normalised to the initial unstimulated BRET ratio and
474 then computed into “Induced BRET” values by subtracting the control (PBS) ratio at each
475 measurement time point. Then, the area under curves (AUC) were estimated over the 30-
476 minute induced BRET measurements or directly for calcium assays over the 25-second
477 measurements for each receptor, ligand and concentration combination to study the
478 concentration–response relationship. Concentration-response curves were modelled with a
479 four-parameters log-logistic equation, with E_{\min} (minimal response), E_{\max} (maximal response),
480 EC_{50} (concentration to reach $E_{\max}/2$) and h (sigmoidicity coefficient). Concentration-response

481 curve values were then normalised to the E_{\max} value of the reference ligand for each receptor
482 (i.e. OT for OTR and AVP for V_{1A} , V_{1B} and V_2 receptors).

483 Ligand bias calculation has been done according to GPCR ligand bias guidelines (Kolb et al.,
484 2022). For each normalised dose-response curve, we computed a transduction coefficient
485 $\log(\tau/K_A)$ defined by the following equation:

$$\text{response} = \frac{E_m}{1 + \exp\left\{n \ln\left[1 + \frac{\exp(\beta_1)}{[A]}\right] - n\beta_2\right\}}$$

486
487 Where $\beta_1 = \ln(K_A)$ and $\beta_2 = \ln(\tau)$ and $\log(\tau/K_A) = (\beta_2 - \beta_1)/\ln(10)$.

488 This composite parameter reflects the affinity and efficacy of the ligand-receptor interaction
489 and the induction of downstream signalling pathways measured by each assay (Kenakin et al.,
490 2012).

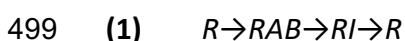
491

492 Mathematical modelling of β -arrestin-2 recruitment and Internalisation assays

493 In this section, we detail the kinetic analysis on β -arrestin-2, FYVE and CAAX induced BRET
494 signals. This methodology was independently applied for each murine receptor OTR, V_{1A} , V_{1B}
495 and V_2 , and each endogenous ligand OT and AVP.

496 *1) Kinetic model*

497 We adapted and extended the methodology presented in (Hoare et al., 2020). More precisely,
498 we used the following kinetic reaction network model :



500 where R denotes the unbound membrane receptor, RAB denotes the receptor-agonist- β -
501 arrestin-2 complex, and RI denotes the internalised receptor. We assume fast binding kinetics
502 between the agonist and the receptor and that the agonist is in excess compared to the
503 receptor (Hoare et al., 2020). We further assume that the free β -arrestin-2 is in excess

504 compared to the receptor-agonist complex, thus its depletion can be neglected. As a result,
505 the model **(1)** is a first-order kinetic reaction network whose dynamics is represented by the
506 linear three-dimensional ordinary differential equation (ODE):

$$\begin{aligned} \frac{d}{dt}R &= -k_{\tau} \frac{A}{A + K_D} R + k_{rec} RI \\ \frac{d}{dt}RAB &= k_{\tau} \frac{A}{A + K_D} R - k_{int} RAB \\ \frac{d}{dt}RI &= k_{int} RAB - k_{rec} RI \end{aligned}$$

507 **(2)**

508 where A (mol) is the agonist concentration, K_D (mol) is the binding affinity of the agonist and
509 the receptor, k_{τ} (min^{-1}) is β -arrestin-2 recruitment rate, k_{int} (min^{-1}) is the internalisation rate
510 and k_{rec} (min^{-1}) is the recycling rate. The initial condition associated with system **(2)** is:

$$511 R(0)=R_{tot}, RAB(0)=0, RI(0)=0,$$

512 where R_{tot} (mol) is the total quantity of receptors.

513 *II) Model fitting and parameter estimation*

514 The CAAX induced BRET ratio was taken as proportional to the loss of receptors at plasma
515 membrane, $R-R_{tot}$, the β -arrestin-2 induced BRET ratio was taken as proportional to the
516 quantity of receptor-agonist- β -arrestin-2 complex RAB , and the FYVE induced BRET ratio is

517 taken proportional to the quantity of internalised receptor RI , with standard gaussian
 518 measurement errors with unknown variance, as follows :

519 **(3)** $CAAXinducedBRETratio = y_1 = k_{bret,1} \cdot (R - R_{tot}) + \varepsilon_{0,\sigma_1}$

520 $Barr_2inducedBRETratio = y_2 = k_{bret,2} \cdot RAB + \varepsilon_{0,\sigma_2}$

521 $FyveinducedBRETratio = y_3 = k_{bret,3} \cdot RI + \varepsilon_{0,\sigma_3}$

522 where $k_{bret,1}$, $k_{bret,2}$ and $k_{bret,3}$ are proportional constant, and ε_{0,σ_1} , ε_{0,σ_2} and ε_{0,σ_3} are standard
 523 centred standard gaussian distribution of variances σ_1 , σ_2 and σ_3 , respectively.

524 As detailed in (Raue et al., 2013), model adimensionalisation and reparameterisation is a
 525 prerequisite for a successful and meaningful estimation. To that, we divided each variable by
 526 R_{tot} and transformed the system **(2)** into the following equivalent system:

$$\begin{aligned} \frac{d}{dt} x_1 &= k_{int} \left(-\overline{k_\tau} \frac{A}{A + K_D} x_1 + \overline{k_{rec}} x_3 \right) \\ \frac{d}{dt} x_2 &= k_{int} \left(\overline{k_\tau} \frac{A}{A + K_D} x_1 - x_2 \right) \\ \frac{d}{dt} x_3 &= k_{int} \left(x_2 - \overline{k_{rec}} x_3 \right) \\ x_1(0) &= 1, \quad x_2(0) = 0, \quad x_3(0) = 0 \\ y_1 &= \overline{k_{bret,1}} (x_1 - 1) + \varepsilon_{0,\sigma_1} \\ y_2 &= \overline{k_{bret,2}} x_2 + \varepsilon_{0,\sigma_2} \\ 527 \quad \mathbf{(4)} \quad y_3 &= \overline{k_{bret,3}} x_3 + \varepsilon_{0,\sigma_3} \end{aligned}$$

528 where, $\overline{k_\tau} = \frac{k_\tau}{k_{int}}$, $\overline{k_{rec}} = \frac{k_{rec}}{k_{int}}$, $\overline{k_{bret,1}} = \frac{k_{bret,1}}{R_{tot}}$, $\overline{k_{bret,2}} = \frac{k_{bret,2}}{R_{tot}}$, $\overline{k_{bret,3}} = \frac{k_{bret,3}}{R_{tot}}$,

529 In the ODE system **(4)**, k_{int} has (min^{-1}) unit, A is the (known) agonist concentration, K_D has molar
 530 unit, and all other constants are unitless. This model has a total of 10 unknown parameters
 531 whose search intervals are given in [Table S3](#).

532 We solved the system of ODE **(4)** using the *AMICI* package (Fröhlich et al., 2021) and
 533 performed parameter estimation following the maximum likelihood approach. We specified
 534 the parameter estimation problem following the Petab (Schmiester et al., 2021) format and
 535 solved it using the pyPESTO toolbox (Schälte et al., 2023), in Python. Parameter confidence

536 intervals were determined using the profile likelihood approach, implemented in pyPESTO. In
537 addition, we verified that the parameter estimation problem is theoretically well-posed using
538 the *StructuralIdentifiability* package in Julia (Dong et al., 2023), which shows that all
539 parameters of model **(4)** are structurally identifiable given data on y_1, y_2, y_3 for at least two
540 agonist doses. We report in [Table S3](#) maximum likelihood estimates with their profile-based
541 confidence intervals.

542 *III) Model selection*

543 The model fitting procedure reveals over-fitting issues for model **(4)** and for some of the
544 agonist-receptor combinations, resulting in practical parameter unidentifiability and large
545 confidence parameter intervals. Further analyses revealed large uncertainties in the $\overline{k_{rec}}$
546 parameter, suggesting that a model without recycling could equally fit the data ($k_{rec} = 0$).

547 Therefore, we asked whether the following submodel could be more suitable I to describe the
548 data:



550 Using the same methodology as in section I and II, we obtained the following reduced model:

$$\begin{aligned}\frac{d}{dt}x_1 &= k_{int} \left(-\overline{k_\tau} \frac{A}{A + K_D} x_1 \right) \\ \frac{d}{dt}x_2 &= k_{int} \left(\overline{k_\tau} \frac{A}{A + K_D} x_1 - x_2 \right) \\ \frac{d}{dt}x_3 &= \overline{k_{bret,3}} x_2 \\ x_1(0) &= 1, \quad x_2(0) = 0, \quad x_3(0) = 0 \\ y_1 &= \overline{k_{bret,1}}(x_1 - 1) + \varepsilon_{0,\sigma_1} \\ y_2 &= \overline{k_{bret,2}}x_2 + \varepsilon_{0,\sigma_2} \\ \text{551 } \mathbf{(6)} \quad y_3 &= x_3 + \varepsilon_{0,\sigma_3}\end{aligned}$$

552 where, $\overline{k_\tau} = \frac{k_\tau}{k_{int}}$, $\overline{k_{bret,1}} = \frac{k_{bret,1}}{R_{tot}}$, $\overline{k_{bret,2}} = \frac{k_{bret,2}}{R_{tot}}$, $\overline{k_{bret,3}} = \frac{k_{bret,3}}{R_{tot}}$

553 In the ODE system **(6)**, k_{int} has (min^{-1}) unit, A is the (known) agonist concentration, K_D has
554 molar unit, and all other constants are unitless. This reduced model has a total of 9 unknown
555 parameters whose search intervals are given in [Table S3](#).

556 We performed parameter estimation of model **(6)** using the same methodology for model **(4)**.
557 We report in [Table S3](#) maximum likelihood estimates with their profile-based confidence
558 intervals. For each receptor-agonist combination we performed a model selection procedure
559 to select either model **(4)** or model **(6)**. We report in [Table S3](#) standard likelihood ratio p-
560 values ($\alpha=0.05$) and AIC and BIC criteria.

561

562 **Animals**

563 All mouse breeding, care and experimental procedures were in accordance with the European
564 and French Directives and approved by the local ethical committee CEEA Val de Loire N°19
565 and the French ministry of teaching, research and innovation (APAFIS #18035-

2018121213436249). Following heterozygous breeding scheme, independent cohorts of *Fmr1* KO (provided by Rob Willensem (Mientjes et al., 2006)) and WT mice were generated from a minimum of 3 different homozygous non-inbred couples maintained on a mixed 50%-50% C57BL/6J;129S2 background in the same breeding room of the animal facility. Mice were outcrossed with fresh mixed backgrounds every 5-10 generations, and between them, with WT mice from other lines, to prevent inbreeding and ensure consistency across independent batches. This *in vivo* experimental model in mice replicates aspects of the human Fragile X syndrome (Kat et al., 2022). Two months old naive males and females from *Fmr1* KO (n = 235, 107 males and 128 females) and WT (n = 188, 84 males and 104 females) animals were raised in groups of 2-4 animals on a 12-hour light/dark regular cycle, with food and water *ad libitum* and controlled temperature (21°C) and humidity (50%) in conventional health housing status, exempt from any monitored viral, bacterial, mycoplasma, fungi, parasites or pathological lesions, except detected mouse norovirus and *helicobacter spp.* Cages of WT and *Fmr1* KO mice were randomly allocated by an experienced experimenter to a treatment to obtain at least 8 animals (4 males and 4 females) per group.

Mouse intraperitoneal single dose pharmacokinetic with RO6893074 and V_{1A} Receptor occupancy calculation

An exploratory single-dose pharmacokinetic study in three C57BL/6J male mice (Charles River) was conducted with RO6893074 V_{1A} small molecule antagonist following intraperitoneal administration at the doses of 5 mg.kg⁻¹ and 50 mg.kg⁻¹. The compound was formulated as a microsuspension in HPMC / DOSS (1.25% / 0.1%) / Parabens, with a pH of 6. Blood samples were collected at 6 time points: 0.25, 0.5, 1, 3, 5, 7 hours after administration and the

588 compound was detected using mass spectrometry. Pharmacokinetic parameters were
589 derived from the plasma concentration versus time profile.

590 Using *in vitro* plasma free fraction ($f_{up} = 6.3\%$) and *in vitro* V_{1A} binding data (mouse V_{1A} $K_i =$
591 39 nM , $n = 3$), the following E_{max} equation was used to calculate receptor occupancy at C_{max}
592 (assuming competitive inhibition):

$$\text{Receptor occupancy} = B_{\max} \times \frac{C_p \times f_{up}}{K_i + C_p \times f_{up}} = B_{\max} \times \frac{\frac{C_p \times f_{up}}{K_i}}{1 + \frac{C_p \times f_{up}}{K_i}}$$

593

594 where B_{\max} is the maximum binding, assumed to be 100%, C_p is the plasma concentration of the
595 compound, f_{up} is the free fraction in plasma, and K_i represents *in vitro* binding to the receptor.

596

597 **Drug administration**

598 WT and *Fmr1* KO mice were administered every mornings for 8 consecutive days from day 0
599 (acute) to day 7 (subchronic) with OT, AVP or TGOT (20 or $40 \mu\text{g.kg}^{-1}$, 0.2 mL.kg^{-1} , intranasally,
600 15 minutes prior to behavioural tests), RO6958375 OTR agonist (0.03 , 0.06 or 0.12 mg.kg^{-1} ,
601 10 mL.kg^{-1} , subcutaneously, 30 minutes prior to behavioural tests) or RO6893074 V_{1A}
602 antagonist (25 , 50 or 100 mg.kg^{-1} , 10 mL.kg^{-1} , intraperitoneally, 30 minutes prior to
603 behavioural tests) based on previous studies (Peñagarikano et al., 2015; Janz et al., 2023) and
604 Roche recommendations, along with their respective vehicles (for OT, AVP or TGOT: NaCl
605 0.9% , intranasally, 0.2 mL.kg^{-1} ; for RO6958375: PBS $1\times$, intraperitoneally, 10 mL.kg^{-1} ; for
606 RO6893074: 0.1% polysorbate Tween-80, 0.21% citric acid monohydrate, 0.8% sodium
607 chloride, sodium hydroxide 1N at pH 7 , subcutaneously, 10 mL.kg^{-1}). For intranasal
608 administration, each drop was placed alternately to each nostril until the animal aspirated the
609 drop into its nasal cavity. As both TGOT and RO6958375 OTR agonists can activate the V_2

610 receptor, we carefully monitored the mouse weight, which remained stable, for potential
611 diuretic effects of V₂ receptor activation.

612

613 ***Social interactions using the Live Mouse Tracker***

614 One week before the behavioural experiments, RFID chips (APT12 PIT Tag, Biomark, USA)
615 were inserted under the skin on the lateral and ventral side of the abdomen in *Fmr1* KO and
616 WT mice under Isoflurane gas anaesthesia (FR/V/4397332 3/2021, Isoflurin®; Axience SAS,
617 France) and a Procaine local anaesthetic (FR/V/6012689 8/2013, Procamidor®; Axience SAS,
618 France) at 40 µg/10g⁻¹ subcutaneously 10 min at the insertion site. All behavioural tests were
619 carried out, when possible, in the morning, to avoid any circadian cycle effect, in a dedicated
620 quiet room and a dim light intensity of 15 lux. Males were always tested before females to
621 prevent any sexual olfactory bias. The Live Mouse Tracker (LMT) (LMT_SOURIS, Rodent
622 Phenotyping Toolkit, France (de Chaumont et al., 2019)) is a reproducible and automatic
623 tracking device that identifies in real time over 30 behavioural parameters of reciprocal social
624 interaction using constant RFID detection and an infrared–depth sensing camera. Two mice
625 matched by sex, age, genotype and treatment that had never met before were placed in the
626 LMT for 10 min at 15 lux in a transparent red Plexiglass open field (50 x 25 x 30 cm). Floors
627 were covered with a thin layer of fresh wood litter to favour normal locomotion, well-being
628 and reduced anxious-like behaviours. Social interaction tests were performed 3 days before
629 the treatment (D-3), the first day (D0) and the last day (D7) of treatment (acute vs. subchronic
630 effects). Multi-behavioural parameters were analysed, such as individual events (moving
631 isolated, stopped isolated, rearing), dyadic state events (moving in contact, following,
632 stopped in contact, contact side by side, nose-to-nose contact, nose-anogenital contact),
633 locomotion (% of time moving) and anxious-like behaviours (stretch-attend posture). SQLite

634 databases containing the coordinates of each mouse and movies were generated for each run
635 of interaction. All individual behavioural parameters were extracted using Python scripts
636 (github.com/fdechaumont/lmt-analysis version v1.0.6). “Nose contacts” or “huddling” were
637 the sum of nose-to-nose and nose-to-anogenital region contacts, or contact side by side in
638 same or in opposite directions, respectively. For each mouse line, mice injected with vehicles
639 were used as control of those injected with the different drug compounds at D0 and D7. While
640 experimenters were not blinded to the conditions during the tests to prevent any exchange
641 of mice, scoring was conducted automatically for reciprocal social interaction by the Live
642 Mouse Tracker. All animal criteria have been reported in agreement with the ARRIVE
643 guidelines (Kilkenny et al., 2010). The SAL, PBS and VEH WT group consisted of independent
644 batches of WT mice, serving as the controls for each mouse line or treatment condition and
645 batch.

646

647 **Statistical analysis**

648 Computation and subsequent statistical analysis were performed using R software (version
649 4.4.0). Parameter values of the concentration-effect curves were estimated by a nonlinear
650 mixed-effect model (population approach) using Monolix Suite 2024R1 (Lixoft, Antony,
651 France). Concentration-Effect curve parameters E_{max} , $\log(EC_{50})$ and $\Delta\log(\tau/Ka)$ were compared
652 to the parameters of the receptor referent-ligand with a Student t-test, taking into account
653 the mean and standard deviation estimated by the population approach and the number of
654 experimental replicates.

655 No animal outliers were removed from the analysis. For the Live Mouse Tracker data analysis,
656 Kruskal-Wallis tests were conducted using the rstatix package (Alboukadel Kassambara,

657 2023)(R package version 0.7.2) followed by Dunn's *post hoc* tests. Sex differences were
658 analysed only before treatment allocation, as sample sizes were too small for comparison.

659

660 **Data availability statement**

661 All *in vitro* and *in vivo* raw data, means and statistics are available in [supplementary Tables](#).

662 Codes and configuration files are available on the research.data.gouv.fr. Movies of
663 behavioural experiments are available upon request.

664

665 **AUTHOR CONTRIBUTIONS**

666 PS and ChG provided materials and an unpublished tool compound; CG, ChG and LPP designed
667 the experiments; CG, LD, EP and LPP performed the experiments and contributed to the data
668 collection; CG, LD, ChG, RY, NA and LPP contributed to the interpretation of data; RY and NA
669 performed all data integration, modelling and statistical analysis; PDP performed the brain
670 receptor occupancy modelling; CG and LPP wrote the original drafts; CG, LD, EP, PS, PDP, ChG,
671 RY, NA and LPP reviewed and edited the manuscript; LPP contributed to the funding
672 acquisition, project conceptualization and supervision.

673

- 674 **ABBREVIATIONS**
- 675 **ASD** autism spectrum disorders
- 676 **AVP** Arginine vasopressin
- 677 ***Avpr1A*** mouse vasopressin V_{1A} receptor gene
- 678 ***Avpr1B*** mouse vasopressin V_{1B} receptor gene
- 679 ***Avpr2*** mouse vasopressin V₂ receptor gene
- 680 ***AVPR1A*** human vasopressin V_{1A} receptor gene
- 681 ***AVPR1B*** human vasopressin V_{1B} receptor gene
- 682 ***AVPR2*** human vasopressin V₂ receptor gene
- 683 **BORIS** behavioural observation research interactive software
- 684 **BRET** bioluminescence resonance energy transfer
- 685 **DMEM** dulbecco's modified eagle medium
- 686 **EMEM** eagle's minimal essential medium
- 687 **FMR1** fragility mental retardation 1
- 688 **HBSS** hank's balanced salt solution
- 689 **HEK293A** human embryonic kidney 293A
- 690 **kB7-OT** [Gly⁵,Thr⁷,Ser⁹]-Oxytocin
- 691 **KO** knockout
- 692 **LMT** live mouse tracker
- 693 **Neuro-2a** mouse neuroblastoma Neuro-2a cell line
- 694 **OT** oxytocin
- 695 **OTR** oxytocin receptor
- 696 ***Oxtr*** mouse oxytocin receptor gene
- 697 ***OXTR*** human oxytocin receptor gene

- 698 **RLuc8** sea pansy renilla reniformis luciferase
- 699 **SAP** stretch-attend postures
- 700 **TGOT** [Thr4,Gly7]OT
- 701 **WT** wild-type

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922

923 **FIGURE LEGENDS**

924 **Figure 1 Pharmacological systemic analysis of OT and AVP effects on murine oxytocin and**
925 **vasopressin receptors in Neuro-2a cells**

926 (A) schematic representation on miniGq, miniGi, miniGs, β -arrestin-1, β -arrestin-2
927 recruitments, intracellular Ca^{2+} mobilisation, cAMP production or forskolin-induced
928 inhibition, and membrane CAAX and endosome FYVE internalisation of mouse OTR, V_{1A} , V_{1B} ,
929 V_2 receptors following OT (red) and AVP (blue) stimulation in the murine Neuro-2a cell line.
930 (B) Mathematical model equation fitting with β -arrestin-2 recruitment, internalisation and
931 recycling profiles of the four receptors. (C) Spider plots illustrate the relative effect of OT and
932 AVP on the ten intracellular inputs of these four receptors. (D) Radar plot summarise the
933 robust predicted affinity (K_D) and kinetic parameters of β -arrestin-2 recruitment (k_τ),
934 internalisation (k_{int}) and recycling (k_{rec}) for each receptor and endogenous ligand, except for
935 OT on the V_{1A} receptor and AVP on OTR. A, agonist; AVP, arginine vasopressin; ext,
936 extracellular space; int, intracellular compartment; OT, oxytocin; R, receptor; RAB, receptor-
937 agonist- β -arrestin-2 complex; RI, internalised receptor.

938

939 **Figure 2 Effect of orthologs and synthetic ligands on murine oxytocin and vasopressin**
940 **receptors in Neuro-2a cells**

941 (A) Bias plot of an equimolar comparison of the orthologs isotocin (slate), mesotocin (khaki),
942 and vasotocin (mauve) in miniGq and β -arrestin-2 recruitment at mouse OTR, V_{1A} , V_{1B} , V_2
943 receptors in the murine Neuro-2a cell line, with OT (red) and AVP (blue) as reference ligands
944 for OTR and vasopressin receptors, respectively. (B) Bias plot of the synthetic ligands TGOT
945 (mustard), RO6958375 (purple), carbetocin (light blue) and kB7 (light brown) in miniGq and
946 β -arrestin-2 recruitment at the four receptors. (C) Dose-response curves of the OTR agonists

947 TGOT and RO6958375 in miniGq and β -arrestin-2 recruitment at the four receptors. (D) Dose-
948 response curves of the antagonists atosiban (yellow), RO6893074 (green) and L-371,257 (dark
949 brown) in the presence of OT and AVP at their EC₈₀ concentrations on OTR and vasopressin
950 receptor miniGq and β -arrestin-2 recruitment. AVP, arginine vasopressin; OT, oxytocin.

951

952 **Figure 3 Acute and subchronic effect of OT and AVP on social motivation and exploration in**
953 ***Fmr1* KO and WT mice**

954 In the Live Mouse Tracker, the cumulative time in nose contact, social approach and huddling
955 behaviours over a 10 min period were compared following 15 minutes of acute (A) or
956 subchronic (B) intranasal administration of OT or AVP at low and moderate dose in WT (NaCl
957 0.9% (SAL; grey): n = 34, 10 males and 24 females; OT 20 $\mu\text{g}\cdot\text{kg}^{-1}$ (pink): n = 14, 4 males and
958 10 females; OT 40 $\mu\text{g}\cdot\text{kg}^{-1}$ (red): n = 8, 4 males and 4 females; AVP 20 $\mu\text{g}\cdot\text{kg}^{-1}$ (light blue): n =
959 9, 5 males and 4 females; AVP 40 $\mu\text{g}\cdot\text{kg}^{-1}$ (dark blue): n = 8, 4 males and 4 females) and *Fmr1*
960 KO mice (SAL (grey): n = 46, 22 males and 24 females; OT 20 $\mu\text{g}\cdot\text{kg}^{-1}$ (pink): n = 8, 4 males and
961 4 females; OT 40 $\mu\text{g}\cdot\text{kg}^{-1}$ (red): n = 8, 4 males and 4 females; AVP 20 $\mu\text{g}\cdot\text{kg}^{-1}$ (light blue): n =
962 16, 5 males and 11 females; AVP 40 $\mu\text{g}\cdot\text{kg}^{-1}$ (dark blue): n = 12, 8 males and 4 females). Data
963 are presented as mean \pm sd (raw values, mean and statistics in [Table S5](#)). Statistical analysis
964 was conducted using Kruskal-Wallis tests followed by Dunn post hoc tests with asterisks

965 indicating treatment effect ($p = P$ adjusted): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

966 AVP, arginine vasopressin; KO, *Fmr1* KO mice; OT, oxytocin; SAL, saline; WT, wild-type.

967

968 **Figure 4 Acute and subchronic effect of TGOT, RO6958375 and RO6893074 on social**
969 **motivation and exploration in *Fmr1* KO and WT mice**

970 In the Live Mouse Tracker, the cumulative time in nose contact, social approach and huddling

971 behaviours over a 10 min period were compared following 15 minutes of acute (A) or

972 subchronic (B) administration of TGOT (intranasal) and RO6958375 (subcutaneous) OTR

973 agonists and RO6893074 V_{1A} antagonist (intraperitoneal) at low, moderate and high dose in

974 WT (SAL for TGOT (grey): $n = 34$, 10 males and 24 females; TGOT 20 $\mu\text{g} \cdot \text{kg}^{-1}$ (light brown): $n =$

975 10, 4 males and 6 females; TGOT 40 $\mu\text{g} \cdot \text{kg}^{-1}$ (brown): $n = 10$, 6 males and 4 females; PBS 1X

976 for RO6958375 (PBS; grey): $n = 19$, 10 males and 9 females; RO6958375 0.03 $\text{mg} \cdot \text{kg}^{-1}$ (light

977 violet): $n = 9$, 5 males and 4 females; RO6958375 0.06 $\text{mg} \cdot \text{kg}^{-1}$ (violet): $n = 8$, 4 males and 4

978 females; RO6958375 0.12 $\text{mg} \cdot \text{kg}^{-1}$ (dark violet): $n = 10$, 5 males and 5 females; 0.1% Tween-

979 80 for RO6893074 (VEH; grey): $n = 20$, 10 males and 10 females; RO6893074 25 $\text{mg} \cdot \text{kg}^{-1}$ (light

980 green): $n = 11$, 5 males and 6 females; RO6893074 50 $\text{mg} \cdot \text{kg}^{-1}$ (green): $n = 8$, 4 males and 4

981 females; RO6893074 100 $\text{mg} \cdot \text{kg}^{-1}$ (dark green): $n = 10$, 4 males and 6 females) and *Fmr1* KO

982 mice (0 = NaCl 0.9% for TGOT (grey): $n = 46$, 22 males and 24 females; TGOT 20 $\mu\text{g} \cdot \text{kg}^{-1}$ (light

983 brown): $n = 12$, 6 males and 6 females; TGOT 40 $\mu\text{g} \cdot \text{kg}^{-1}$ (brown): $n = 13$, 7 males and 6 females;

984 0 = PBS 1X for RO6958375 (grey): $n = 27$, 11 males and 16 females; RO6958375 0.03 $\text{mg} \cdot \text{kg}^{-1}$

985 (light violet): $n = 13$, 4 males and 9 females; RO6958375 0.06 $\text{mg} \cdot \text{kg}^{-1}$ (violet): $n = 14$, 5 males

986 and 9 females; RO6958375 0.12 $\text{mg} \cdot \text{kg}^{-1}$ (dark violet): $n = 12$, 6 males and 6 females; 0 = 0.1%

987 Tween-80 for RO6893074 (grey): $n = 21$, 11 males and 10 females; RO6893074 25 $\text{mg} \cdot \text{kg}^{-1}$

988 (light green): $n = 9$, 4 males and 5 females; RO6893074 50 $\text{mg} \cdot \text{kg}^{-1}$ (green): $n = 14$, 6 males

989 and 8 females; RO6893074 100 mg.kg⁻¹ (dark green): n = 10, 4 males and 6 females). Data are
990 presented as mean ± sd (raw values, mean and statistics in [Table S5](#)). Statistical analysis was
991 conducted using Kruskal-Wallis tests followed by Dunn post hoc tests with asterisks indicating
992 treatment effect ($p = P$ adjusted): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. KO, *Fmr1*
993 KO mice; OTR, oxytocin receptor; PBS, Phosphate-buffered saline; SAL, saline; TGOT,
994 (Thr⁴,Gly⁷)-oxytocin; VEH; vehicle; WT, wild-type.

995

Figure 1 Pharmacological systemic analysis of OT and AVP effects on murine oxytocin and vasopressin receptors in Neuro-2a cells

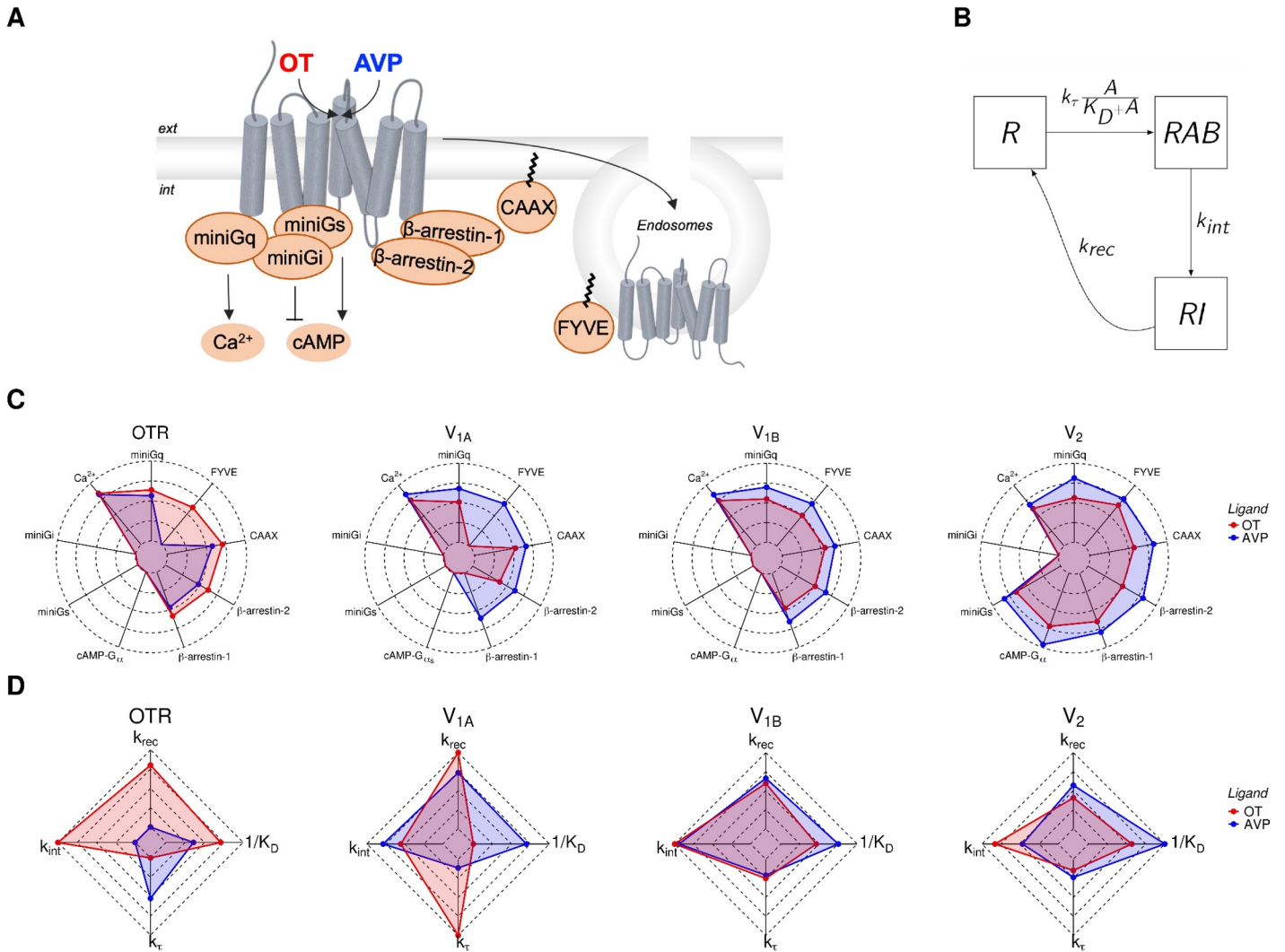


Figure 2 Effect of orthologs and synthetic ligands on murine oxytocin and vasopressin receptors in Neuro-2a cells

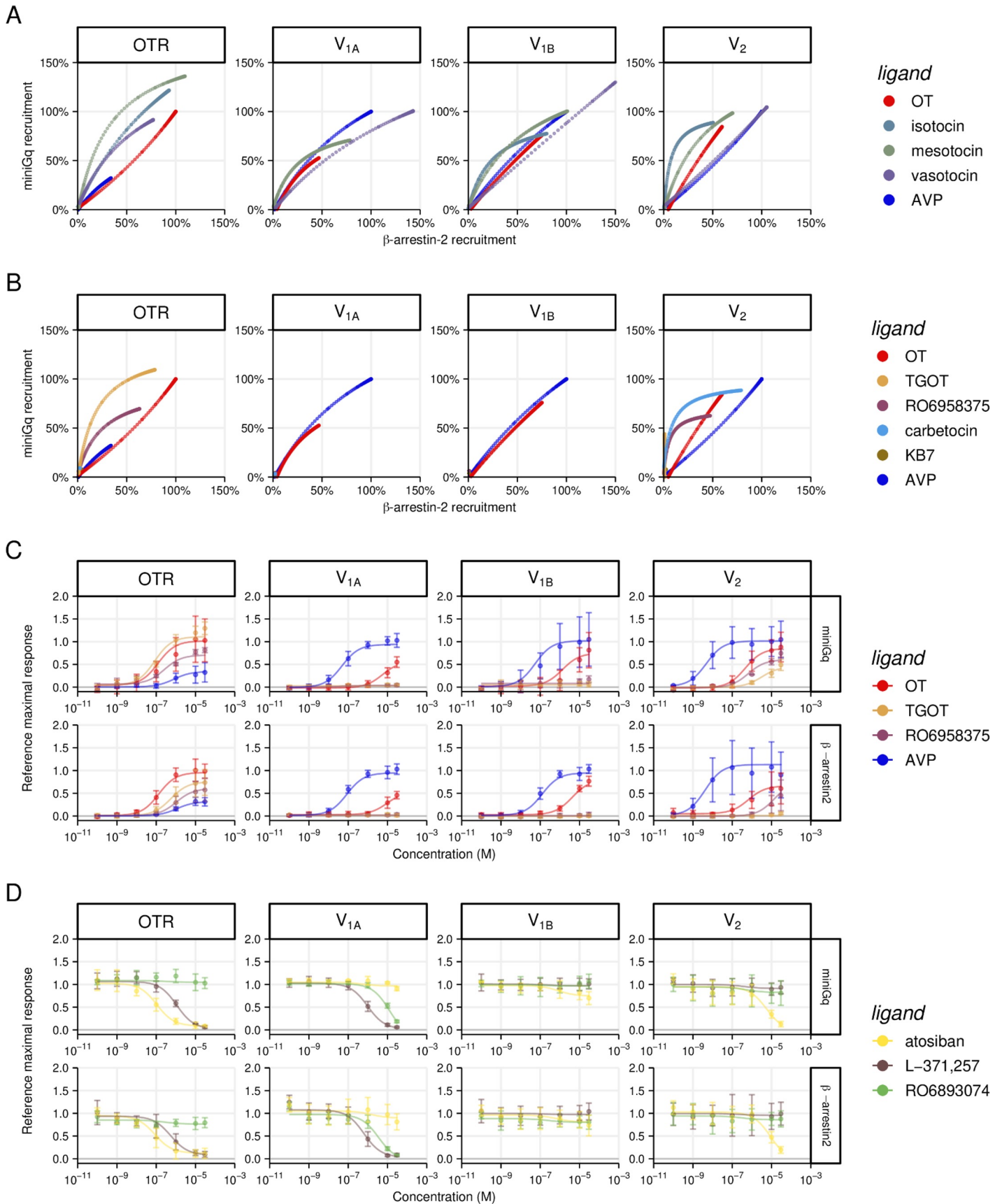


Figure 3 Acute and subchronic effect of OT and AVP on social motivation and exploration in *Fmr1* KO and WT mice

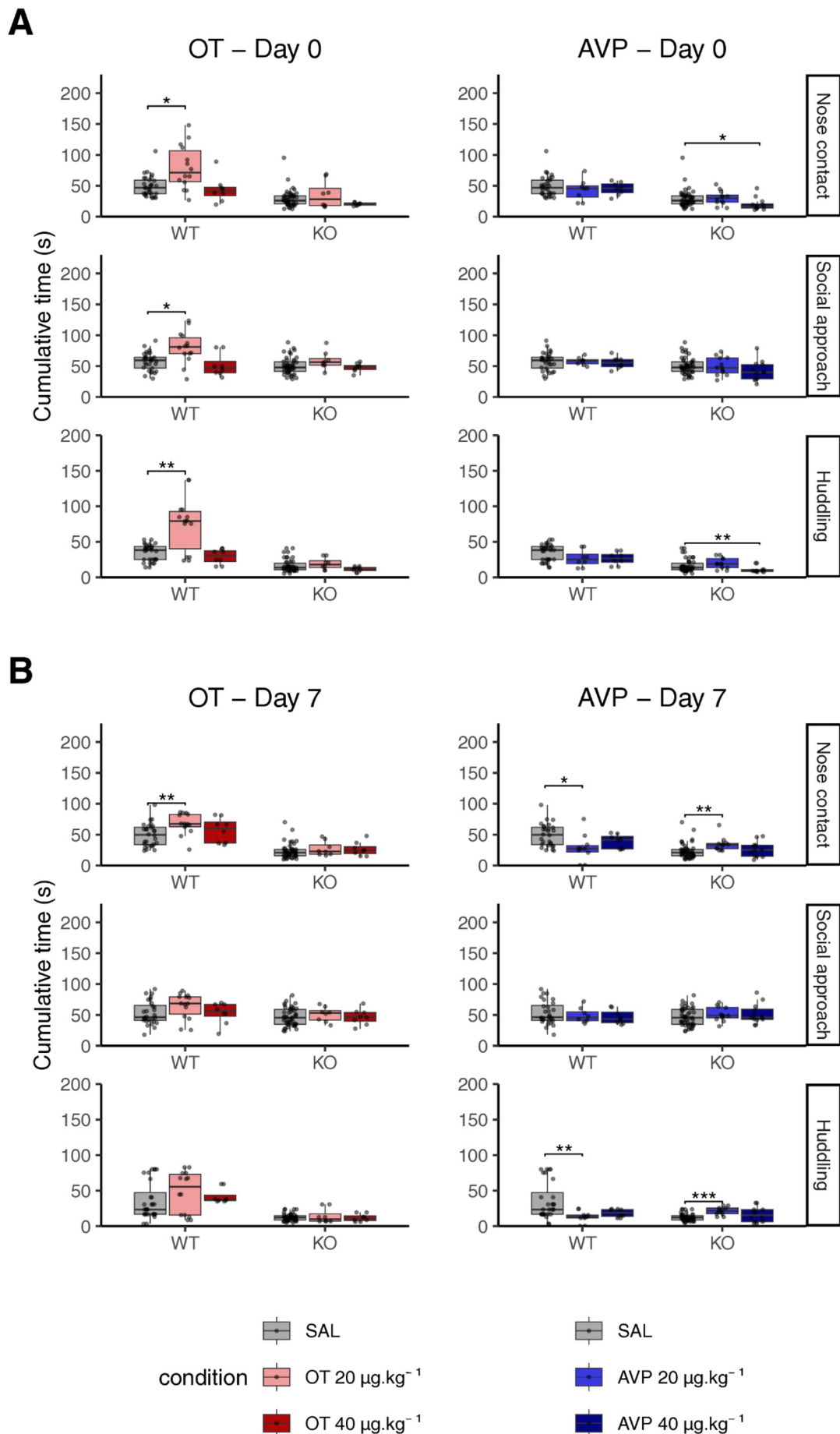
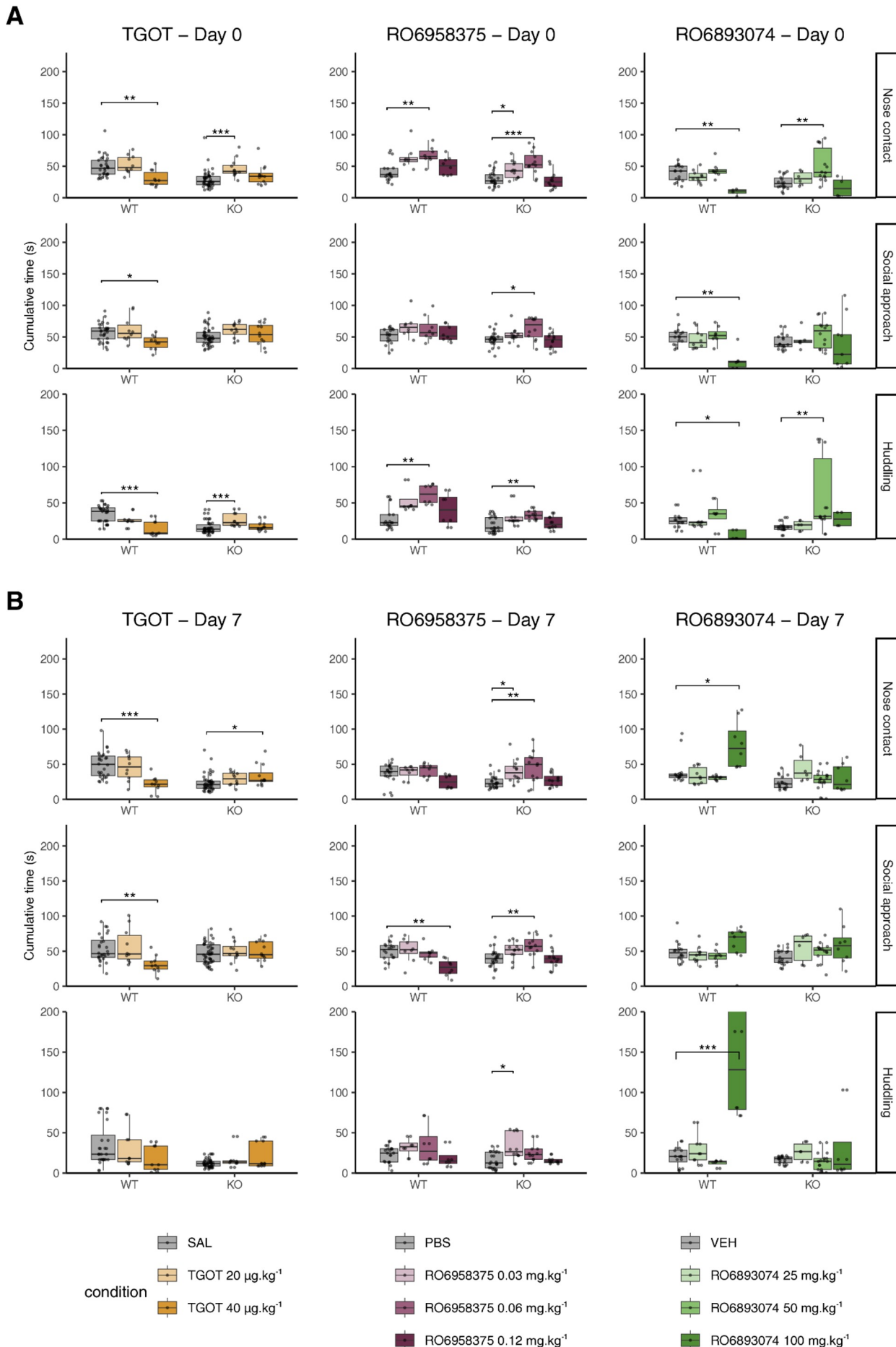


Figure 4 Acute and subchronic effect of TGOT, RO6958375 and RO6893074 on social motivation and exploration in *Fmr1* KO and WT mice



996 **TABLE LEGENDS**

997

998 **Table 1: Bias factors of the ligands on murine oxytocin and vasopressin receptors in Neuro-**

999 **2a**

1000 Statistical analysis was conducted using Student t-test with asterisks indicating bias effect (p

1001 = P value): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. RE, relative efficiency compared

1002 to the reference ligand $10\Delta\log(\tau/Ka)$; bias factor, $RE\beta_{arr2}/RE_{Gq}$; §, ligand of reference.

Receptor	Ligand	$\log(\tau/Ka)$		$\Delta\log(\tau/Ka)$		$\Delta\Delta\log(\tau/Ka)$	RE		Bias factor
		Gq	β_{arr2}	Gq	β_{arr2}		Gq	β_{arr2}	
OTR	OT§	6.68 ± 0.06	6.82 ± 0.07	0.00 ± 0.08	0.00 ± 0.09	0.00 ± 0.13	1.00	1.00	1
OTR	TGOT	7.00 ± 0.08	6.07 ± 0.04	0.32 ± 0.10	-0.75 ± 0.08	*** -1.13 ± 0.14	02.09	0.18	0.08
OTR	RO6958375	6.39 ± 0.07	5.70 ± 0.05	-0.30 ± 0.09	-1.12 ± 0.08	*** -0.89 ± 0.13	0.51	0.08	0.15
OTR	isotocin	6.31 ± 0.03	5.96 ± 0.05	-0.37 ± 0.07	-0.86 ± 0.08	*** -0.50 ± 0.12	0.43	0.14	0.32
OTR	mesotocin	7.35 ± 0.05	6.75 ± 0.04	0.67 ± 0.07	-0.07 ± 0.08	**** -0.76 ± 0.12	4.69	0.85	0.18
OTR	vasotocin	7.18 ± 0.10	6.66 ± 0.04	0.49 ± 0.11	-0.16 ± 0.08	* -0.70 ± 0.18	3.11	0.69	0.22
OTR	carbetocin	ND	ND	ND	ND	-0.13 ± 0.85	ND	ND	ND
OTR	KB7	ND	ND	ND	ND	-0.12 ± 1.18	ND	ND	ND
OTR	AVP	5.63 ± 0.11	5.44 ± 0.09	-1.05 ± 0.12	-1.38 ± 0.12	* -0.31 ± 0.15	0.09	0.04	0.47
V1A	OT	4.80 ± 0.11	4.63 ± 0.10	-2.42 ± 0.12	-2.43 ± 0.11	0.02 ± 0.17	0.00	0.00	0.98
V1A	TGOT	ND	ND	ND	ND	0.15 ± 0.90	ND	ND	ND
V1A	RO6958375	5.74 ± 0.49	ND	-1.49 ± 0.50	ND	0.47 ± 0.85	0.03	ND	ND
V1A	isotocin	ND	ND	ND	ND	0.15 ± 1.03	ND	ND	ND
V1A	mesotocin	5.28 ± 0.06	4.89 ± 0.05	-1.95 ± 0.08	-2.17 ± 0.06	* -0.27 ± 0.14	0.01	0.01	0.6
V1A	vasotocin	7.24 ± 0.08	7.07 ± 0.04	0.01 ± 0.10	0.01 ± 0.06	0.00 ± 0.12	1.02	1.02	1
V1A	carbetocin	ND	ND	ND	ND	0.14 ± 1.16	ND	ND	ND
V1A	KB7	ND	ND	ND	ND	0.20 ± 0.82	ND	ND	ND
V1A	AVP§	7.23 ± 0.06	7.06 ± 0.04	0.00 ± 0.08	0.00 ± 0.06	0.00 ± 0.11	1.00	1.00	1
V1B	OT	5.25 ± 0.08	5.24 ± 0.04	-1.72 ± 0.10	-1.67 ± 0.06	0.11 ± 0.18	0.02	0.02	1.14
V1B	TGOT	ND	ND	ND	ND	0.12 ± 1.14	ND	ND	ND
V1B	RO6958375	ND	ND	ND	ND	0.10 ± 1.18	ND	ND	ND
V1B	isotocin	5.71 ± 0.08	5.03 ± 0.05	-1.26 ± 0.11	-1.88 ± 0.06	* -0.59 ± 0.17	0.05	0.01	0.24
V1B	mesotocin	5.88 ± 0.10	5.42 ± 0.07	-1.09 ± 0.12	-1.49 ± 0.08	-0.41 ± 0.18	0.08	0.03	0.4
V1B	vasotocin	7.44 ± 0.07	7.39 ± 0.04	0.47 ± 0.10	0.49 ± 0.06	0.02 ± 0.15	2.95	03.07	01.04
V1B	carbetocin	ND	ND	ND	ND	0.21 ± 1.07	ND	ND	ND
V1B	KB7	ND	ND	ND	ND	0.10 ± 1.16	ND	ND	ND
V1B	AVP§	6.97 ± 0.07	6.91 ± 0.04	0.00 ± 0.10	0.00 ± 0.06	0.00 ± 0.18	1.00	1.00	1
V2	OT	6.18 ± 0.05	5.86 ± 0.06	-2.11 ± 0.14	-2.52 ± 0.15	* -0.29 ± 0.19	0.01	0.00	0.39
V2	TGOT	5.06 ± 0.12	ND	-3.23 ± 0.18	ND	1.12 ± 0.72	0.00	ND	ND
V2	RO6958375	5.78 ± 0.14	4.60 ± 0.13	-2.51 ± 0.19	-3.78 ± 0.19	* -1.24 ± 0.21	0.00	0.00	0.05
V2	isotocin	6.08 ± 0.06	4.99 ± 0.12	-2.21 ± 0.14	-3.39 ± 0.18	*** -1.19 ± 0.19	0.01	0.00	0.07
V2	mesotocin	6.70 ± 0.07	6.14 ± 0.03	-1.58 ± 0.15	-2.24 ± 0.14	** -0.63 ± 0.17	0.03	0.01	0.22
V2	vasotocin	8.19 ± 0.16	8.29 ± 0.06	-0.10 ± 0.20	-0.09 ± 0.15	-0.02 ± 0.21	0.80	0.82	01.03
V2	carbetocin	6.00 ± 0.05	4.55 ± 0.09	-2.29 ± 0.14	-3.83 ± 0.16	** -1.48 ± 0.19	0.01	0.00	0.03
V2	KB7	ND	ND	ND	ND	-0.07 ± 0.77	ND	ND	ND
V2	AVP§	8.29 ± 0.13	8.38 ± 0.14	0.00 ± 0.18	0.00 ± 0.19	0.00 ± 0.22	1.00	1.00	1

1003

1004

SUPPLEMENTARY INFORMATION

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Supplementary Materials and Methods

Cell culture, G protein, beta-arrestin and internalisation assays using Bioluminescence Resonance Energy Transfer (BRET)

Human embryonic kidney 293A (HEK293A; ATCC, Manassas, VA, USA, CRL-1573™, RRID:CVCL_6910) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; CM1DME68-01, Eurobio, France) supplemented with 10% (v/v) foetal bovine serum (CVFVSF00-01, Eurobio, France) and 1% penicillin/streptomycin (100 U.mL⁻¹, 100 µg.mL⁻¹ respectively; 15140-122, Eurobio, France) and maintained at 37°C with 5% CO₂. When reaching 90% confluency, cells were washed with PBS 1X (CS1PBS01K-BP, Eurobio, France) followed by trypsin (0.5 g.L⁻¹, L-EDTA 0.2 g.L⁻¹, without phenol red; CEZTDA00-0U, Eurobio, France) treatment. After cell counting, cells were transiently transfected in suspension using the Metafectene Pro transfection reagent (T040-5.0, BioNTex Laboratories, Germany) according to the manufacturer's protocol.

In 96-well plates (30196, SPL Life Sciences, Korea), HEK293A cells (70, 000 cells/well) were transiently co-transfected with the human oxytocin receptor *OXR* or human vasopressin receptors *AVPR1a*, *AVPR1b* subcloned with restriction enzymes HindIII and XhoI in pcDNA3 plasmid or with the *AVPR2* receptors in pcDNA3.1(+) plasmid, fused in their C-terminus to the BRET donor *Renilla reniformis* luciferase (RLuc8) at 25 ng per well, along with BRET acceptor sensors containing a yellow fluorescent proteins (YFP, Venus or YPET) at 50 ng per well each, miniGq or or YPET- β -arrestin-2. 48 hours after transfection, HEK293A cells were starved for 4 hours in phenol red-free DMEM (21063-029, Gibco, France) at 37°C in 5% CO₂. Cells incubated with cœlenterazine substrate (5 µM) were first measured for 5 minutes at 480 ± 20 nm and 530 ± 25 nm measurements (Mithras2 LB 943 with the Mikrowin 2010 software, Berthold Technologies GmbH & Co., Germany). Then, cells were rapidly stimulated with the different agonists at 3.10⁻⁵ M, 10⁻⁵ M, 10⁻⁶ M, 10⁻⁷ M, 10⁻⁸ M, 10⁻⁹ M, 10⁻¹⁰ M diluted in PBS 1X and HEPES 10 mM final, or PBS 1X and HEPES 10 mM alone for the baseline, then 96-well plates were recorded for 30 minutes.

Supplementary Figures

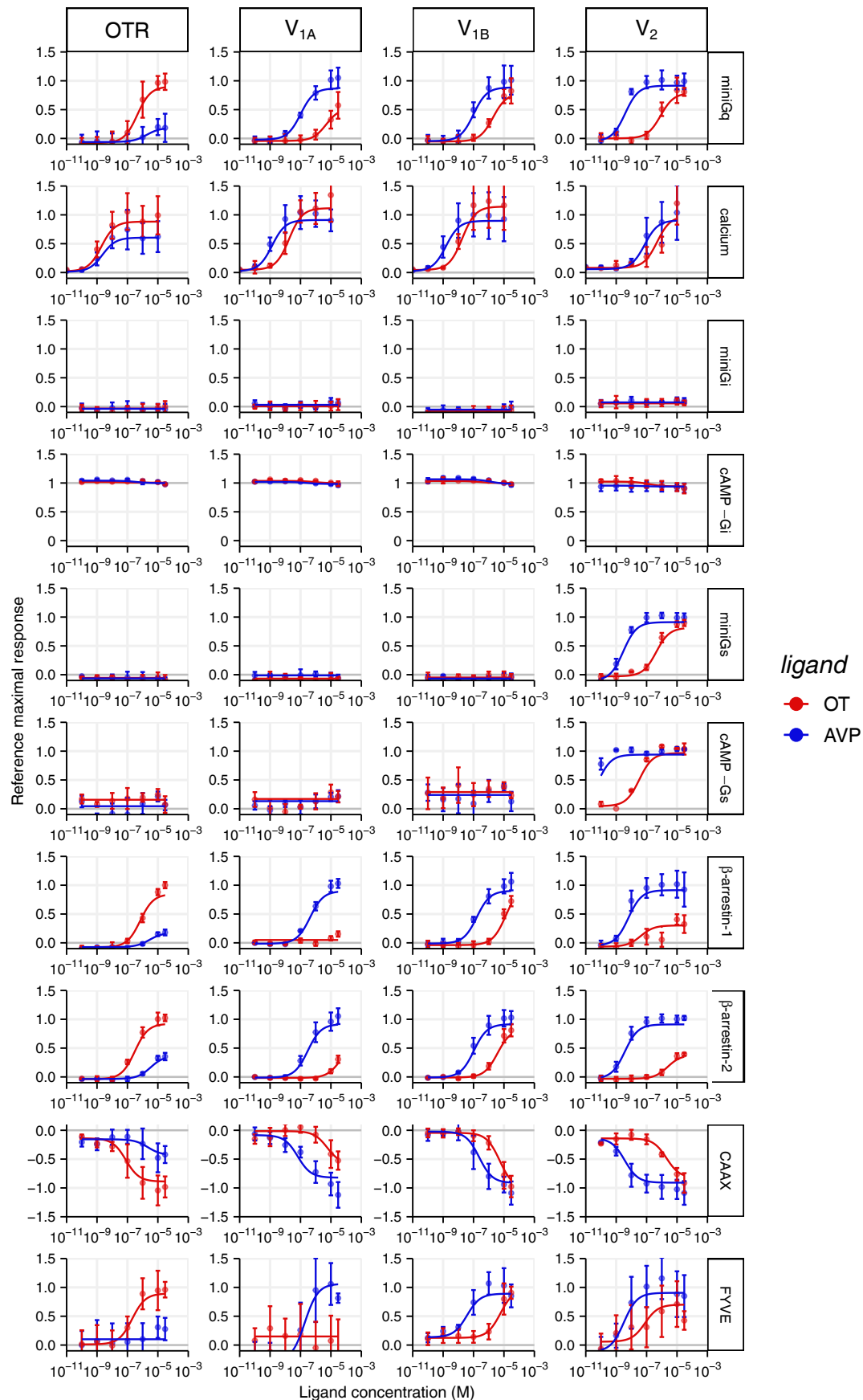


Figure S1: Relative efficacy and potency of OT and AVP on murine oxytocin and vasopressin receptors in Neuro-2a cells

Relative dose-response of OT (red) and AVP (blue) on miniGq, miniGi, miniGs, β -arrestin-1, β -arrestin-2 recruitment, intracellular Ca^{2+} mobilisation, cAMP production (cAMP - Gs) or forskoline-induced inhibition (cAMP - Gi), and CAAX and FYVE internalisation profiles of mouse OTR, V_{1A} , V_{1B} , V_2 receptors in murine Neuro-2A cell lines. The dose-response curve represents the mean of the biological replicates ($n = 3-5$ per sensor; Table S1).

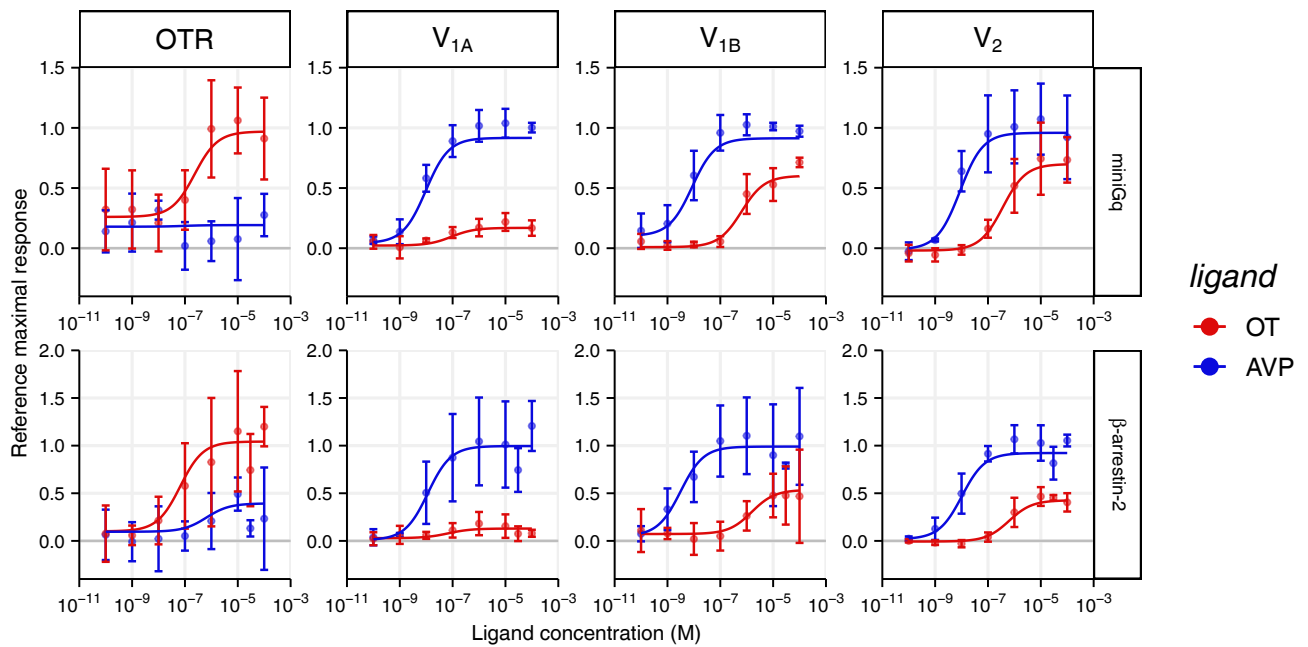


Figure S2: Relative efficacy and potency of OT and AVP on human oxytocin and vasopressin receptors in HEK293A cells

Relative dose-response of OT (red) and AVP (blue) on miniGq, and β -arrestin-2 recruitment profiles of human OTR, V_{1A} , V_{1B} , V_2 receptors in human HEK293A cell lines. The dose-response curve represents the mean of the biological replicates ($n = 3-5$ per sensor; Table S2).

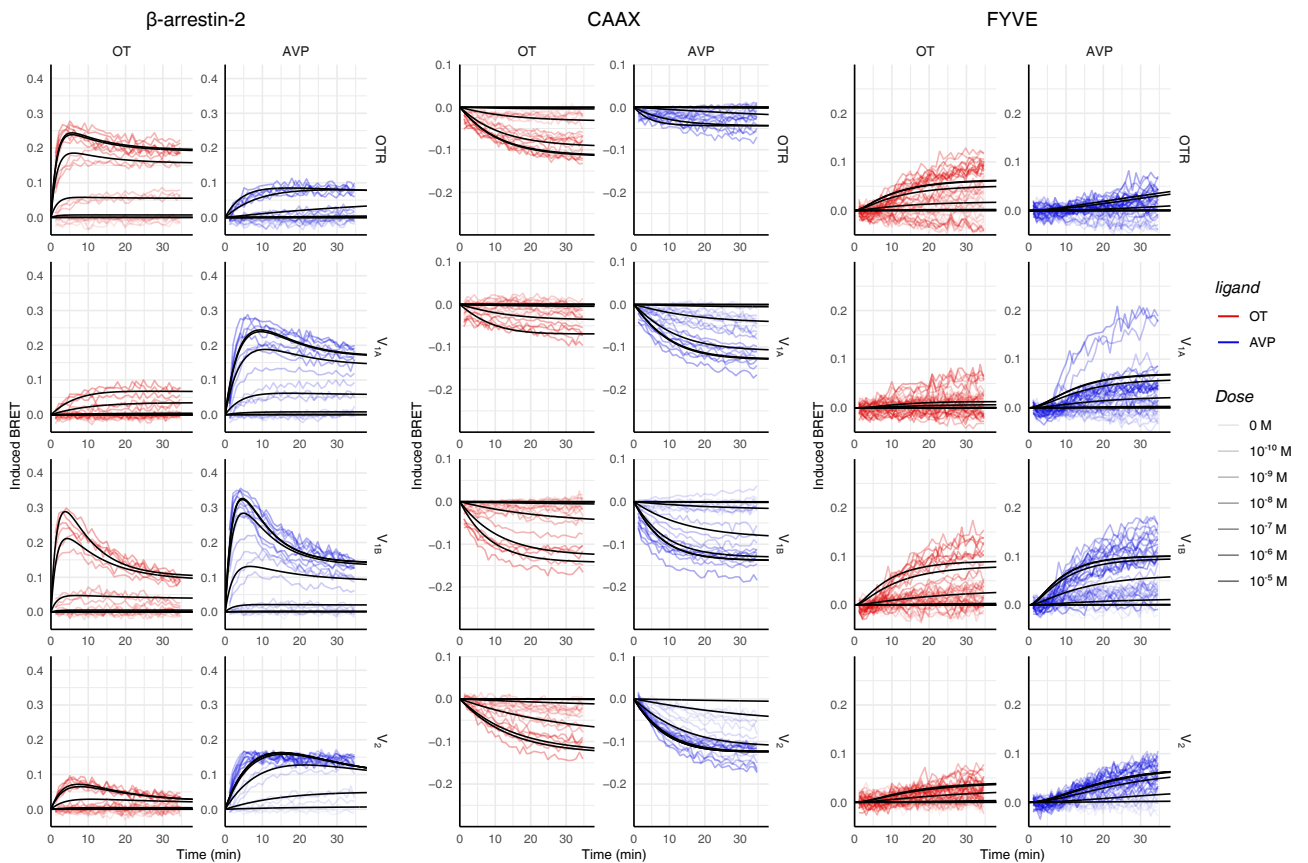
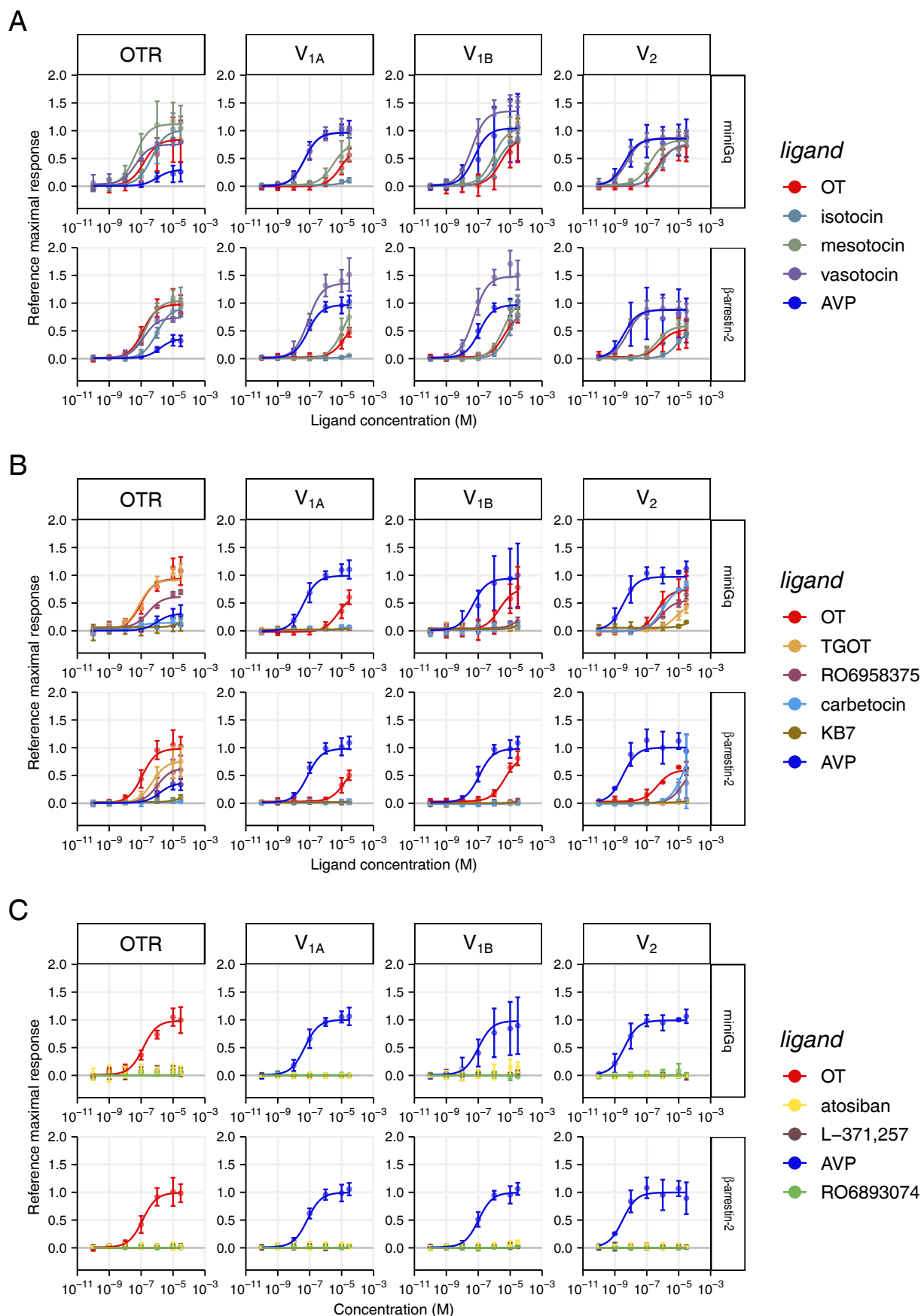


Figure S3: Kinetic modelling of OT and AVP on murine oxytocin and vasopressin receptors in Neuro-2a cells

Kinetics and model fitting of OT (red) and AVP (blue) as reference ligands on β -arrestin-2 recruitment, and CAAX and FYVE internalisation profiles of mouse OTR, V_{1A} , V_{1B} , V_2 receptors in murine Neuro-2A cell lines ($n = 3-5$ per sensor; Tables S1 and S3).



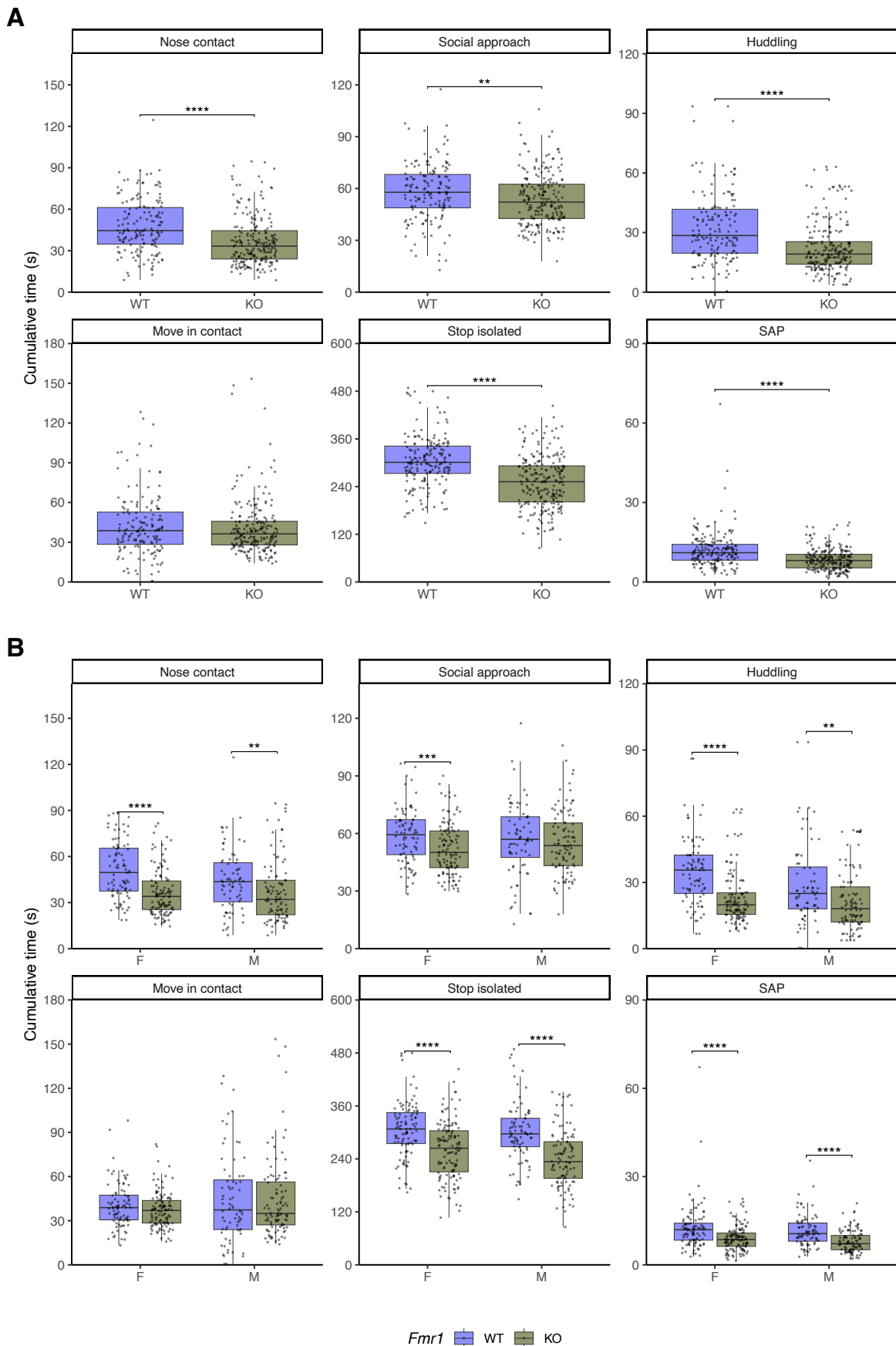


Figure S5: *Fmr1* KO males and females display social impairments compared to WT mice in the Live Mouse Tracker

In the Live Mouse Tracker, the cumulative time in nose contact, social approach, huddling, 'move in contact', 'stop isolated' and SAP over a 10 min period were compared three days before the first administration between WT (purple; $n = 188$, 84 males and 104 females) and *Fmr1* KO (kaki; $n = 235$, 107 males and 128 females) mice (**A**) and between WT and *Fmr1* KO males and females (**B**). *Fmr1* KO mice showed reduced time spent in nose contact, social approach, huddling behaviour, isolation and stretch-attend posture compared to WT mice, independently of sex, with the exception of social approach in males. Data are presented as mean \pm sd (raw values, mean and statistics in Table S2). Statistical analysis was conducted using Kruskal-Wallis tests followed by Dunn post hoc tests with asterisks indicating genotype or sex effect ($p = P$ adjusted): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. F, females; M, males; KO, *Fmr1* KO mice; SAP, Stretch-attend posture; WT, wild-type.

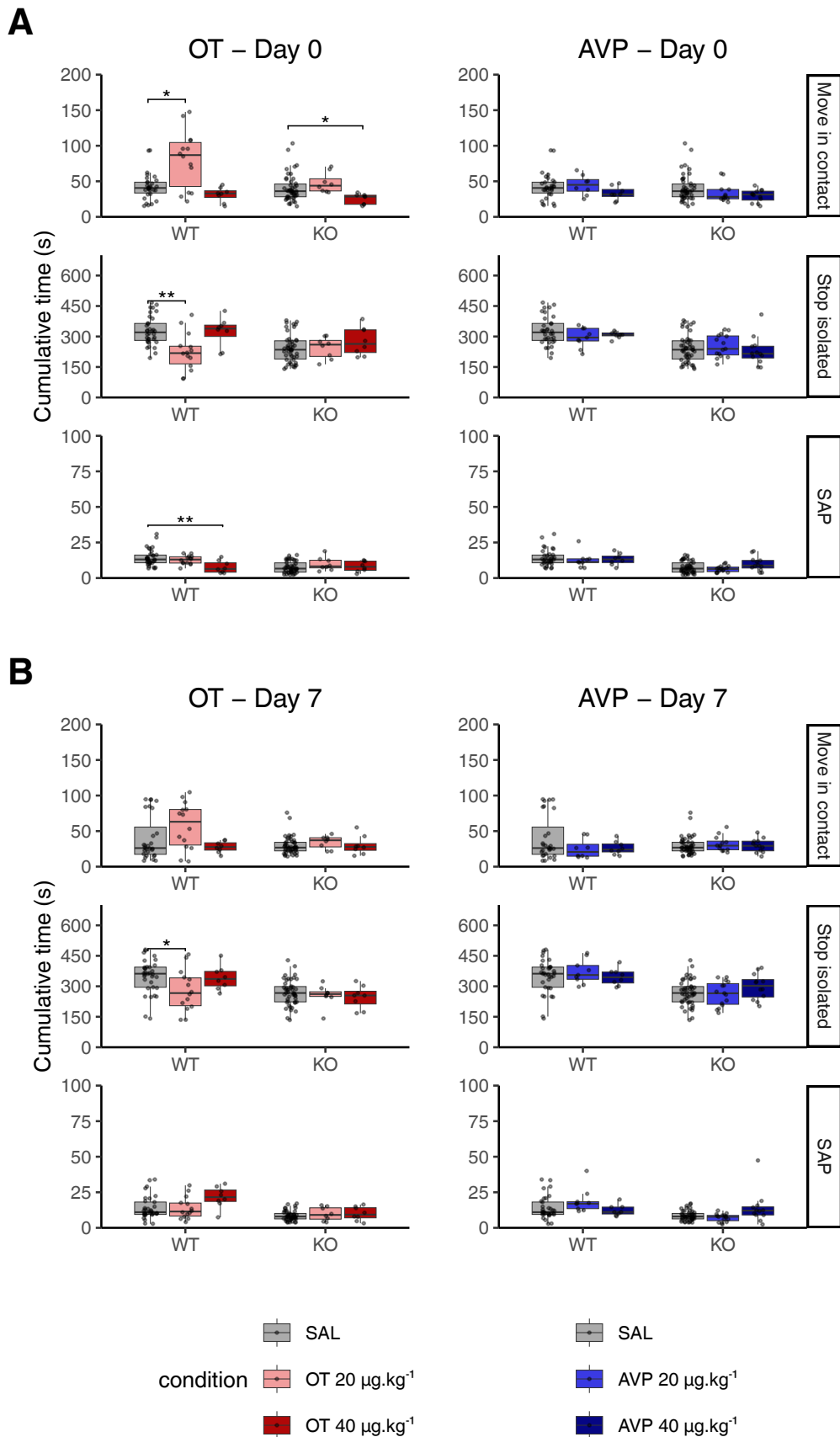


Figure S6: Effect of OT and AVP on social motivation, isolation and anxious-like behaviours in *Fmr1* KO and WT mice

In the Live Mouse Tracker, the cumulative time in 'move in contact', 'stop isolated' and SAP over a 10 min period were compared following 15 minutes of acute (A) or subchronic (B) intranasal administration of OT or AVP at low and moderate dose in WT (0 or NaCl 0.9% (grey): n = 34, 10 males and 24 females; OT 20 µg.kg⁻¹ (pink): n = 14, 4 males and 10 females; OT 40 µg.kg⁻¹ (red): n = 8, 4 males and 4 females; AVP 20 µg.kg⁻¹ (light blue): n = 9, 5 males and 4 females; AVP 40 µg.kg⁻¹ (dark blue): n = 8, 4 males and 4 females) and *Fmr1* KO mice (0 (grey): n = 46, 22 males and 24 females; OT 20 µg.kg⁻¹ (pink): n = 8, 4 males and 4 females; OT 40 µg.kg⁻¹ (red): n = 8, 4 males and 4 females; AVP 20 µg.kg⁻¹ (light blue): n = 16, 5 males and 11 females; AVP 40 µg.kg⁻¹ (dark blue): n = 12, 8 males and 4 females). Data are presented as mean ± sd (raw values, mean and statistics in Table S2). Statistical analysis was conducted using Kruskal-Wallis tests followed by Dunn post-hoc tests with asterisks indicating treatment effect ($p = P$ adjusted): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. AVP, vasopressin; OT, oxytocin; KO, *Fmr1* KO mice; SAL, saline; SAP, Stretch-attend posture; WT, wild-type.

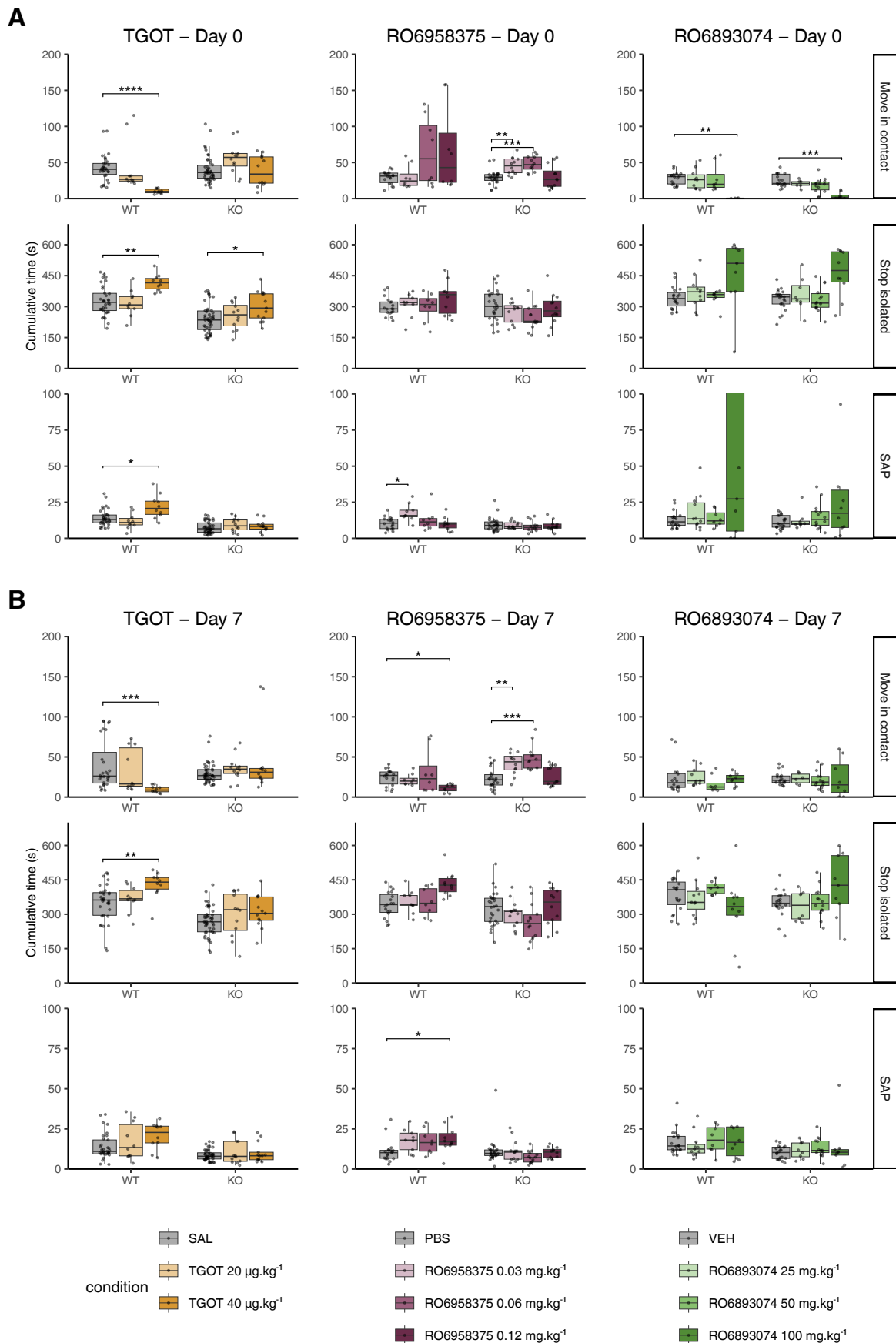


Figure S7: Effect of TGOT, RO6958375 and RO6893074 on social motivation, isolation and anxious-like behaviours in *Fmr1* KO and WT mice

In the Live Mouse Tracker, the cumulative time in 'move in contact', 'stop isolated' and SAP over a 10 min period were compared following 15 minutes of acute (A) or subchronic (B) administration of TGOT (intranasal) and RO6958375 (subcutaneous) OTR agonists and RO6893074 V_{1A} antagonist (intraperitoneal) at low, moderate and high dose in WT (0 = NaCl 0.9% for TGOT (grey): n = 34, 10 males and 24 females; TGOT 20 $\mu\text{g.kg}^{-1}$ (light brown): n = 10, 4 males and 6 females; TGOT 40 $\mu\text{g.kg}^{-1}$ (brown): n = 10, 6 males and 4 females; 0 = PBS 1X for RO6958375 (grey): n = 19, 10 males and 9 females; RO6958375 0.03 mg.kg^{-1} (light violet): n = 9, 5 males and 4 females; RO6958375 0.06 mg.kg^{-1} (violet): n = 8, 4 males and 4 females; RO6958375 0.12 mg.kg^{-1} (dark violet): n = 10, 5 males and 5 females; 0 = 0.1% Tween-80 for RO6893074 (grey): n = 20, 10 males and 10 females; RO6893074 25 mg.kg^{-1} (light green): n = 11, 5 males and 6 females;

RO6893074 50 mg.kg⁻¹ (green): n = 8, 4 males and 4 females; RO6893074 100 mg.kg⁻¹ (dark green): n = 10, 4 males and 6 females) and *Fmr1* KO mice (0 = NaCl 0.9% for TGOT (grey): n = 46, 22 males and 24 females; TGOT 20 µg.kg⁻¹ (light brown): n = 12, 6 males and 6 females; TGOT 40 µg.kg⁻¹ (brown): n = 13, 7 males and 6 females; 0 = PBS 1X for RO6958375 (grey): n = 27, 11 males and 16 females; RO6958375 0.03 mg.kg⁻¹ (light violet): n = 13, 4 males and 9 females; RO6958375 0.06 mg.kg⁻¹ (violet): n = 14, 5 males and 9 females; RO6958375 0.12 mg.kg⁻¹ (dark violet): n = 12, 6 males and 6 females; 0 = 0.1% Tween-80 for RO6893074 (grey): n = 21, 11 males and 10 females; RO6893074 25 mg.kg⁻¹ (light green): n = 9, 4 males and 5 females; RO6893074 50 mg.kg⁻¹ (green): n = 14, 6 males and 8 females; RO6893074 100 mg.kg⁻¹ (dark green): n = 10, 4 males and 6 females). Data are presented as mean ± sd (raw values, mean and statistics in Table S2). Statistical analysis was conducted using Kruskal-Wallis tests followed by Dunn post hoc tests with asterisks indicating treatment effect ($p = P$ adjusted): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. KO, *Fmr1* KO mice; OTR, oxytocin receptor; PBS, Phosphate-buffered saline; SAL, saline; SAP, stretch-attend postures; TGOT, (Thr α ,Gly α)-oxytocin; VEH; vehicle; WT, wild-type.

SUPPLEMENTARY TABLES

Table S1: Pharmacological characteristics of ligands on murine oxytocin and vasopressin receptors in Neuro-2a cells

Receptor	Ligand	Emax		log(EC50)	
		Gq	barr2	Gq	barr2
OTR	AVP	0.93 ± 0.16 **	1.71 ± 0.35 ***	-6.06 ± 0.28 *	-5.88 ± 0.25 **
OTR	KB7	ND	ND	ND	ND
OTR	OT§	2.82 ± 0.38	4.84 ± 0.34	-6.75 ± 0.25	-6.89 ± 0.12
OTR	RO6958375	1.97 ± 0.26 *	3.15 ± 0.39 ***	-6.49 ± 0.27	-5.96 ± 0.15 ****
OTR	TGOT	3.08 ± 0.34	3.86 ± 0.37 **	-6.96 ± 0.25	-6.26 ± 0.14 ***
OTR	carbetocin	ND	ND	ND	ND
OTR	isotocin	3.47 ± 0.39	4.63 ± 0.37	-6.28 ± 0.19	-6.04 ± 0.10 ****
OTR	mesotocin	3.82 ± 0.35 *	5.30 ± 0.30	-7.34 ± 0.14 *	-6.72 ± 0.11
OTR	vasotocin	2.57 ± 0.33	3.73 ± 0.29 **	-7.18 ± 0.27	-6.86 ± 0.12
V1A	AVP§	5.99 ± 0.42	5.01 ± 0.37	-7.37 ± 0.09	-7.09 ± 0.13
V1A	KB7	ND	ND	ND	ND
V1A	OT	3.76 ± 0.50 ****	3.34 ± 0.83 *	-5.26 ± 0.13 ****	-4.87 ± 0.22 ****
V1A	RO6958375	0.26 ± 1.24 *	ND	-7.12 ± 1.65	ND
V1A	TGOT	ND	ND	ND	ND
V1A	carbetocin	ND	ND	ND	ND
V1A	isotocin	ND	ND	ND	ND
V1A	mesotocin	4.56 ± 0.56 *	5.54 ± 0.95	-5.61 ± 0.20 **	-4.91 ± 0.14 ****
V1A	vasotocin	6.02 ± 0.42	7.16 ± 0.42 ****	-7.33 ± 0.12	-7.09 ± 0.12
V1B	AVP§	2.92 ± 0.43	4.81 ± 0.44	-7.08 ± 0.23	-6.93 ± 0.10
V1B	KB7	ND	ND	ND	ND
V1B	OT	2.48 ± 0.71	4.09 ± 0.49	-5.44 ± 0.33 *	-5.36 ± 0.15 ****
V1B	RO6958375	ND	ND	ND	ND
V1B	TGOT	ND	ND	ND	ND
V1B	carbetocin	ND	ND	ND	ND
V1B	isotocin	2.38 ± 0.61	4.94 ± 0.76	-5.78 ± 0.30 *	-5.06 ± 0.15 ****
V1B	mesotocin	3.05 ± 0.54	5.42 ± 0.50	-5.86 ± 0.24 *	-5.43 ± 0.11 ****
V1B	vasotocin	3.92 ± 0.48	7.46 ± 0.37 ***	-7.27 ± 0.19	-7.26 ± 0.07 **
V2	AVP§	3.53 ± 0.24	5.59 ± 0.43	-8.33 ± 0.18	-8.43 ± 0.12
V2	KB7	ND	ND	ND	ND
V2	OT	3.04 ± 0.29	3.39 ± 0.35 **	-6.27 ± 0.15 ***	-6.20 ± 0.18 ***
V2	RO6958375	2.29 ± 0.37	4.06 ± 0.85 *	-5.97 ± 0.32 *	-4.80 ± 0.20 ****
V2	TGOT	1.83 ± 1.01	ND	-5.27 ± 0.37 *	ND
V2	carbetocin	3.24 ± 0.46	7.05 ± 0.93 *	-5.98 ± 0.17 **	-4.75 ± 0.15 ****
V2	isotocin	3.21 ± 0.39	3.49 ± 0.96 **	-6.14 ± 0.17 ***	-5.14 ± 0.22 ****
V2	mesotocin	3.50 ± 0.31	3.98 ± 0.32 **	-6.74 ± 0.18 ***	-6.35 ± 0.11 ****
V2	vasotocin	3.69 ± 0.29	5.87 ± 0.31	-8.16 ± 0.14	-8.20 ± 0.08

Table S2: Pharmacological characteristics of OT and AVP on human oxytocin and vasopressin receptors in HEK293A cells

Receptor	Ligand	Emax		log(EC50)	
		Gq	barr2	Gq	barr2
OTR	AVP	ND	0.44 ± 0.17 ***	ND	-6.47 ± 0.74
OTR	OT§	0.83 ± 0.16	1.44 ± 0.18	-6.69 ± 0.48	-7.12 ± 0.30
V _{1A}	AVP§	5.29 ± 0.42	3.32 ± 0.25	-8.12 ± 0.14	-7.93 ± 0.13
V _{1A}	OT	1.01 ± 0.17 ***	0.43 ± 0.10 ****	-7.18 ± 0.42 *	-7.30 ± 0.69
V _{1B}	AVP§	3.25 ± 0.28	3.01 ± 0.20	-8.19 ± 0.25	-8.55 ± 0.19
V _{1B}	OT	2.13 ± 0.35 *	1.60 ± 0.30 ****	-6.19 ± 0.24 ***	-5.84 ± 0.28 ****
V ₂	AVP§	5.46 ± 0.42	8.71 ± 0.48	-8.06 ± 0.13	-8.20 ± 0.10
V ₂	OT	3.93 ± 0.45 *	4.27 ± 0.43 ****	-6.49 ± 0.16 ***	-6.23 ± 0.13 ****

Table S3: List of parameters for each model equation and model selection

The table reports the parameter search intervals (in log10 scale) for the first model (equation (4) in the material and method), the maximum likelihood estimate and their profile-based confidence intervals for the first model (equation (4) in the material and method), the parameter search intervals (in log10 scale) for the second model (equation (6) in the material and method), the maximum likelihood estimate and their profile-based confidence intervals for the second model (equation (6) in the material and method), and the model selection calculations.

Parameters_model_1

Parameter Name	parameterScale	lowerBound	upperBound
k_rec	log10	0.000001	100
k_tau	log10	0.0001	100
k_int	log10	0.001	10
KD	log10	1,00E-12	0.1
kbret,1	log10	0.001	1000
kbret,2	log10	0.001	1000
kbret,3	log10	0.001	1000
sd_1	log10	0.001	10
sd_2	log10	0.001	10
sd_3	log10	0.001	10

MLE_CI_model_1

Normalised parameter name	parameter value	OTR		V1A		V1B		V2	
		OT	AVP	OT	AVP	OT	AVP	OT	AVP
k_rec	MLE	-0,98	-6,00	0,29	-0,64	-1,31	-1,08	-1,27	-0,24
	left CI	-1,04	-6,00	0,08	-0,70	-1,37	-1,13	-1,41	-0,31
	right CI	-0,91	0,08	0,57	-0,57	-1,25	-1,03	-1,16	-0,18
k_tau	MLE	-1,38	2,00	2,00	-0,65	-0,75	-0,73	-0,66	0,32
	left CI	-1,47	1,63	1,03	-0,74	-0,82	-0,79	-0,77	0,11
	right CI	-1,29	2,00	2,00	-0,55	-0,69	-0,67	-0,55	0,49
k_int	MLE	-0,21	-2,30	-1,14	-0,65	-0,21	-0,33	-0,56	-1,32
	left CI	-0,24	-2,38	-1,23	-0,70	-0,25	-0,37	-0,63	-1,42
	right CI	-0,17	-2,06	-1,06	-0,61	-0,18	-0,30	-0,49	-1,19
KD	MLE	-6,44	-4,43	-2,41	-6,36	-5,04	-6,69	-5,63	-8,09
	left CI	-6,46	-4,58	-3,32	-6,40	-5,07	-6,71	-5,68	-8,16
	right CI	-6,41	-4,33	-2,26	-6,33	-5,01	-6,66	-5,58	-8,02
sd_1	MLE	-1,62	-1,69	-1,71	-1,65	-1,71	-1,62	-1,73	-1,68
	left CI	-1,64	-1,71	-1,72	-1,67	-1,73	-1,63	-1,74	-1,69
	right CI	-1,61	-1,68	-1,69	-1,64	-1,70	-1,60	-1,71	-1,66
sd_2	MLE	-1,70	-1,82	-1,89	-1,60	-1,75	-1,61	-1,78	-1,66
	left CI	-1,71	-1,83	-1,91	-1,61	-1,76	-1,62	-1,79	-1,67
	right CI	-1,69	-1,81	-1,88	-1,59	-1,73	-1,60	-1,77	-1,64
sd_3	MLE	-1,52	-1,69	-1,62	-1,47	-1,55	-1,47	-1,71	-1,70
	left CI	-1,53	-1,70	-1,63	-1,48	-1,56	-1,48	-1,72	-1,71
	right CI	-1,51	-1,68	-1,61	-1,47	-1,54	-1,46	-1,70	-1,69
kbret_1	MLE	-0,42	-1,36	-0,89	-0,62	-0,72	-0,71	-0,79	-0,85
	left CI	-0,46	-1,38	-0,95	-0,66	-0,73	-0,72	-0,82	-0,88
	right CI	-0,37	-1,34	-0,82	-0,58	-0,70	-0,69	-0,76	-0,80
kbret_2	MLE	0,83	-1,03	-0,72	0,22	0,46	0,41	-0,27	-0,50
	left CI	0,75	-1,04	-0,81	0,15	0,41	0,37	-0,35	-0,56
	right CI	0,90	-1,00	-0,63	0,29	0,51	0,46	-0,19	-0,41
kbret_3	MLE	-0,63	-0,60	-1,16	-0,80	-0,89	-0,81	-1,26	-0,89
	left CI	-0,67	-0,81	-1,29	-0,83	-0,91	-0,82	-1,29	-0,91
	right CI	-0,59	-0,51	-0,98	-0,77	-0,88	-0,79	-1,23	-0,86

Parameters_model_2

Parameter Name	parameterScale	lowerBound	upperBound
k_tau	log10	0.0001	100
k_int	log10	0.001	10
KD	log10	1,00E-12	0.1
sd_1	log10	0.001	10
sd_2	log10	0.001	10
sd_3	log10	0.001	10
kbret_1	log10	0.001	1000
kbret_2	log10	0.001	1000
kbret_3	log10	0.001	1000

MLE_CI_model_2

Normalised parameter name	parameter value	OTR		V1A		V1B		V2	
		OT	AVP	OT	AVP	OT	AVP	OT	AVP
k_tau	MLE	-1,96	2,00	2,00	1,01	-1,26	-1,32	-0,59	0,78
	left CI	-2,03	1,63	1,56	0,92	-1,30	-1,36	-0,72	0,70
	right CI	-1,90	2,00	2,00	1,09	-1,21	-1,29	-0,45	0,87
k_int	MLE	-0,11	-2,30	-1,40	-1,59	-0,03	-0,13	-0,64	-1,63
	left CI	-0,14	-2,38	-1,50	-1,64	-0,06	-0,16	-0,72	-1,69
	right CI	-0,08	-2,06	-1,30	-1,55	0,00	-0,10	-0,56	-1,58
KD	MLE	-6,47	-4,43	-2,64	-5,81	-5,16	-6,84	-5,62	-7,95
	left CI	-6,49	-4,58	-3,05	-5,86	-5,19	-6,86	-5,67	-7,99
	right CI	-6,45	-4,33	-2,47	-5,76	-5,13	-6,81	-5,57	-7,91
sd_1	MLE	-1,56	-1,69	-1,70	-1,64	-1,64	-1,52	-1,72	-1,67
	left CI	-1,58	-1,71	-1,72	-1,65	-1,65	-1,54	-1,74	-1,68
	right CI	-1,55	-1,68	-1,69	-1,62	-1,63	-1,51	-1,71	-1,65
sd_2	MLE	-1,70	-1,82	-1,89	-1,56	-1,75	-1,59	-1,77	-1,66
	left CI	-1,71	-1,83	-1,90	-1,58	-1,76	-1,60	-1,79	-1,67
	right CI	-1,68	-1,81	-1,87	-1,55	-1,73	-1,57	-1,76	-1,64
sd_3	MLE	-1,53	-1,69	-1,62	-1,48	-1,56	-1,48	-1,71	-1,70
	left CI	-1,54	-1,70	-1,63	-1,49	-1,57	-1,49	-1,72	-1,71
	right CI	-1,52	-1,68	-1,61	-1,47	-1,55	-1,48	-1,70	-1,69
kbret_1	MLE	-0,26	-1,36	-1,02	-0,96	-0,67	-0,63	-0,84	-0,91
	left CI	-0,30	-1,38	-1,06	-0,97	-0,68	-0,64	-0,86	-0,92
	right CI	-0,22	-1,34	-0,97	-0,95	-0,66	-0,62	-0,81	-0,90
kbret_2	MLE	1,36	-1,03	-0,78	-0,50	0,85	0,88	-0,32	-0,64
	left CI	1,30	-1,04	-0,86	-0,52	0,81	0,85	-0,42	-0,66
	right CI	1,42	-1,00	-0,70	-0,48	0,89	0,91	-0,23	-0,61
kbret_3	MLE	-0,49	-0,60	-1,40	-0,81	-0,85	-0,75	-1,30	-0,84
	left CI	-0,53	-0,81	-1,49	-0,84	-0,86	-0,76	-1,33	-0,87
	right CI	-0,44	-0,51	-1,32	-0,78	-0,84	-0,73	-1,28	-0,81

Model_selection

receptor	ligand	best_obj	ndata	model	npar	lrt_stat	lrt_pvalue	aic	bic
OTR	OT	-19 552,44	8820	model_2	9	523,28	8,20E-116	525,28	532,36
		-19 814,07	8820	model_1	10	0	1	0	0
V1A		-22 145,73	8820	model_2	9	69,26	8,64E-17	71,26	78,34
		-22 180,36	8820	model_1	10	0	1	0	0
V1B		-20 557,43	8820	model_2	9	542,40	5,66E-120	544,40	551,49
		-20 828,63	8820	model_1	10	0	1	0	0
V2	-22 512,59	8785	model_2	9	59,57	1,18E-14	61,57	68,65	
	-22 542,37	8785	model_1	10	0	1	0	0	
OTR	AVP	-22 426,93	8785	model_2	9	0	1	0	0
		-22 426,93	8785	model_1	10	1,29E-05	NA	-2,00	-9,08
V1A		-18 724,91	8820	model_2	9	529,27	4,07E-117	531,27	538,35
		-18 989,54	8820	model_1	10	0	1	0	0
V1B		-18 358,01	8820	model_2	9	885,58	1,34E-194	887,58	894,67
		-18 800,81	8820	model_1	10	0	1	0	0
V2	-21 611,89	8820	model_2	9	69,91	6,19E-17	71,91	79,00	
	-21 646,85	8820	model_1	10	0	1	0	0	

Table S4: Results for RO6893074 from the CEREP selectivity screen. The table reports the percentage of inhibition at 3 μ M of the RO6893074 on 35 human receptors, channels or transporters, as well 12 human enzymes tested in functional assays and reported as percentage enzyme inhibition over basal activity. Enzyme assays are noted with an asterisk*. Negative numbers are due to assay noise.

Assay	% inhibition at 3µM	Assay	% inhibition at 3µM
A1 (h) (agonist radioligand)	-1	5-HT2B (h) (agonist radioligand)	51
A3 (h) (agonist radioligand)	-10	5-HT3 (h) (antagonist radioligand)	5
alpha 1A (h) (antagonist radioligand)	5	sigma (non-selective) (h) (agonist radioligand)	-2
alpha 2A (h) (antagonist radioligand)	6	sst4 (h) (agonist radioligand)	32
beta 1 (h) (agonist radioligand)	-2	GR (h) (agonist radioligand)	-9
AT1 (h) (antagonist radioligand)	-1	ERalpha (h) (agonist radioligand)	2
BZD (central) (agonist radioligand)	2	AR (h) (agonist radioligand)	-17
D1 (h) (antagonist radioligand)	-12	Ca2+ channel (L, diltiazem site) (benzothiazepines) (antagonist radioligand)	5
D2S (h) (agonist radioligand)	2	Na+ channel (site 2) (antagonist radioligand)	1
glycine (strychnine-insensitive) (antagonist radioligand)	-2	norepinephrine transporter (h) (antagonist radioligand)	5
H1 (h) (antagonist radioligand)	0	5-HT transporter (h) (antagonist radioligand)	-11
H2 (h) (antagonist radioligand)	-11	*COX2 (h)	9
H3 (h) (agonist radioligand)	-13	*PDE5 (h) (non-selective)	-15
MT3 (ML2) (agonist radioligand)	45	*ACE (h)	-22
M2 (h) (antagonist radioligand)	2	*HIV-1 protease (h)	2
M4 (h) (antagonist radioligand)	10	*CDK2 (h) (cycA)	-3
N muscle-type (h) (antagonist radioligand)	-3	*GSK3alpha (h)	-1
kappa (KOP) (agonist radioligand)	29	*GSK3beta (h)	-1
mu (MOP) (h) (agonist radioligand)	6	*ZAP70 kinase (h)	-17
PPARGamma (h) (agonist radioligand)	14	*acetylcholinesterase (h)	7
FP (h) (agonist radioligand)	10	*MAO-A (h)	11
5-HT1A (h) (agonist radioligand)	-12	*MAO-B (h) recombinant enzyme	0
5-HT2A (h) (agonist radioligand)	-4	*xanthine oxidase/ superoxide O2- scavenging	-6

Table S5: raw, mean, standard deviation and statistics of social interaction in the Live Mouse

Tracker