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Dopamine modulates social behaviour in cooperatively breeding fish

Diogo F. Antunes, Marta C. Soares, Michael Taborsky



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1 Dopamine modulates social behaviour in cooperatively breeding fish

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3 Diogo F. Antunes^{1,2}; Marta C. Soares^{3,4}; Michael Taborsky¹

4 ¹Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032

5 Hinterkappelen, Switzerland

6 ²Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande,

7 1749-016 Lisboa, Portugal

8 ³CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório

9 Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

10 ⁴ BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-

11 661 Vairão, Portugal

12

13 Corresponding author:

14 Diogo F. Antunes

15 Institute of Ecology and Evolution

16 University of Bern

17 diogo.antunes@iee.unibe.ch

18

19

20 **Abstract**

21 Dopamine is part of the reward system triggering the social decision-making network in the brain. It
22 has hence great potential importance in the regulation of social behaviour, but its significance in the
23 control of behaviour in highly social animals is currently limited. We studied the role of the
24 dopaminergic system in social decision-making in the cooperatively breeding cichlid fish,
25 *Neolamprologus pulcher*, by blocking or stimulating the dopaminergic D1-like and D2-like receptors.
26 We first tested the effects of different dosages and timing of administration on subordinate group
27 members' social behaviour within the group in an unchallenging environment. In a second
28 experiment we pharmacologically manipulated D1-like and D2-like receptors while experimentally
29 challenging *N. pulcher* groups by presenting an egg predator, and by increasing the need for territory
30 maintenance through digging out sand from the shelter. Our results show that the D1-like and D2-
31 like receptor pathways are differently involved in the modulation of aggressive, submissive and
32 affiliative behaviours. Interestingly, the environmental context seems particularly crucial regarding
33 the role of the D2-like receptors in behavioural regulation of social encounters among group
34 members, indicating a potential pathway in agonistic and cooperative interactions in a pay-to-stay
35 scenario. We discuss the importance of environmental information in mediating the role of
36 dopamine for the modulation of social behaviour.

37

38 Keywords: Cooperative breeding, dopamine, social behaviour, D1 receptors, D2 receptors,
39 *Neolamprologus pulcher*, aggression, submission, affiliation, pay-to-stay

40

41 Introduction

42 Group-living animals acquire social information, either through evolved signals or through
43 inadvertent social information (social cues), from their group members (Dall et al., 2005; Taborsky et
44 al. 2021). Such social information is then integrated in the central nervous system (Oliveira, 2009),
45 which coordinates the activity of all parts of the body and adjusts the resulting behaviour continually
46 to the dynamic changes of the social environment. An individual's ability to behave according to the
47 available social information determines its social competence (Taborsky and Oliveira, 2012), which
48 involves regulatory mechanisms allowing for rapid behavioural changes. These mechanisms induce
49 socially driven biochemical switching that act on existing neural networks (Zupanc and Lamprecht,
50 2000). During the last decade, research highlighted some basic regulatory mechanisms of social
51 behaviour in vertebrates, including the cognitive and neurophysiological processes underlying
52 decision-making (Soares et al., 2010a; Melis et al., 2011; Courtin et al., 2022; Maruska et al., 2022).
53 The vertebrate brain structures involved in social decision-making appear to be highly conserved and
54 are referred to as 'social decision-making network' (SDMN; O'Connell and Hofmann, 2011) consisting
55 of several interconnected brain nuclei from the forebrain and midbrain, including the mesolimbic
56 reward system (Goodson, 2005; O'Connell and Hofmann, 2011). The SDMN involves several
57 neurophysiological systems, including steroid hormones and monoaminergic action (e.g. serotonin,
58 dopamine and noradrenaline), and it is highly sensitive to dopaminergic mediation (O'Connell and
59 Hofmann, 2011, 2012). This makes dopamine a key candidate to study the neuroendocrine
60 mechanisms underlying social behaviour.

61 Dopamine (DA) is a neurotransmitter involved in several neurochemical and neurohormonal
62 processes modulating animal behaviour (Soares, 2017). It is involved in risk assessment and
63 anticipatory responses to reward-associated stimuli (Heimovics et al., 2009). Dopaminergic activity is
64 crucial for determining the salience of (social) stimuli, deeming them as positive/rewarding or as
65 negative/penalising (Schultz, 2006), which enables animals to learn to anticipate the outcomes of
66 social interactions, consequently resulting in appropriate decision-making (Schultz, 2002).

67 The dopaminergic system has two major classes of receptors, called 'D1-like' and 'D2-like'.
68 Their activity can lead to opposing effects depending on the level of stimulation, as both receptor
69 classes follow an inverted-U shaped dose-response curve (Seamans and Yang, 2004; Monte-Silva et
70 al., 2009). D1-like receptors elicit neuron firing, while the D2-like receptors inhibit neuron firing and
71 dopamine synthesis (Bello et al., 2011). For this reason these two receptors may have antagonistic
72 effects on behaviour (St. Onge et al., 2011). For instance, in rats the blockage of the D2-like
73 receptors was shown to increase aggression, while blockage of the D1-like receptors had the
74 opposite effect (Bondar and Kudryavtseva, 2005). Additionally, dopaminergic activity is an important

75 modulator of a wide variety of social behaviours. For instance, in Arctic charr (*Salvelinus alpinus*),
76 subordinate fish have lower dopaminergic activity, which coincides with reduced aggression
77 (Winberg et al., 1991). In cleaner wrasses (*Labroides dimidiatus*), D1-like receptors are responsible
78 for reward salience, the perception of cost and benefits in interactions with clients, and in learning
79 (Messias et al., 2016a,b; Soares et al., 2017a,b). In common waxbills (*Estrilda astrild*),
80 pharmacological facilitation of the D2-like receptors increased activity in a social context, whereas in
81 a non-social context it decreased activity (Silva et al., 2020). This apparent diversity of functions
82 suggests that the role of the two types of dopamine receptors should be scrutinized more deeply
83 and independently from each other to unravel their significance, particularly in highly social animals.

84 The neurophysiological mechanisms that mediate social interactions in highly social animals
85 are yet little understood. In complex social systems, group living individuals frequently engage in
86 social interactions in which individuals flexibly respond to the dynamic social environment
87 (Blumstein et al., 2010; Taborsky and Oliveira, 2012; Hofmann et al., 2014). In such animals social
88 information is permanently updated within the SDMN and biochemical switching of
89 neurophysiological systems is necessary to build their behavioural response (Zupanc and Lamprecht,
90 2000; O'Connell and Hofmann, 2011). To better understand the role of the dopaminergic system in
91 regulating social interactions in highly social animals, we used the cooperatively breeding cichlid
92 *Neolamprologus pulcher* that serves as a model system for the study of social evolution and the
93 neuroendocrine mechanisms underlying social behaviour (Wong and Balshine, 2011; B. Taborsky,
94 2016; Antunes et al., 2021; Taborsky, 2021). *N. pulcher* lives in size-structured social groups with a
95 linear hierarchy (Taborsky and Limberger, 1981; Taborsky, 1984, 2016; Balshine et al., 2001;
96 Hamilton et al., 2005). Within these groups, *N. pulcher* have individualized relationships, role
97 differentiation and division of labour (Taborsky and Limberger, 1981; Hert, 1985; Bruintjes and
98 Taborsky, 2011; Heg and Taborsky, 2010; Taborsky, 2016;). Group members are perpetually involved
99 in socio-positive and agonistic interactions, and making appropriate social decisions is an important
100 determinant of Darwinian fitness (Arnold and Taborsky, 2010; Taborsky et al., 2012; Taborsky and
101 Oliveira, 2012; Zöttl et al., 2013a; Lerena et al., 2021).

102 In this study we focused on how the dopaminergic system regulates social behaviour in
103 different contexts, and how this affects interactions among group members. We asked two
104 questions to further our understanding of the regulation of social behaviour by the dopaminergic
105 system: (1) How is social behaviour modulated by D1-like and D2-like receptors in dependence of the
106 agonists and antagonists dosage? (2) Does the regulatory function of D1-like and D2-like receptors
107 vary between different behaviours and social contexts? To answer these questions we
108 pharmacologically manipulated the activity of D1-like and D2-like receptors in the cooperatively

109 breeding cichlid *Neolamprologus pulcher*. For this purpose, we administered both a D1-like receptor
110 agonist and antagonist, and a D2-like receptor agonist and antagonist, and compared behavioural
111 responses to social challenges with a control situation in which a saline solution was given. Since our
112 aim was to understand how the dopaminergic system can modulate social and cooperative
113 interactions, we performed exogenous pharmacological manipulations on helpers from pre-
114 established families of *N. pulcher*. Taking into account insights from previous studies done in cleaner
115 wrasses (*Labroides dimidiatus*; Messias et al., 2016b, 2016a), in the first experiment we tested
116 compound time-dependent and dosage-dependent modulation of social behaviour by D1-like and
117 D2-like receptor pathways, as different quantities of agonists and antagonists might induce
118 divergent behavioural effects (Stettler et al., 2021). In the second experiment we tested effects of
119 dopaminergic compounds on *N. pulcher* social behaviour and group interactions when exposed to
120 different environmental contexts, this time only using a single dosage of agonist and antagonist for
121 each receptor type. The dosage used in the second experiment was determined from the
122 behavioural effects on social behaviour observed in the first experiment.

123 Previous research has shown that in *N. pulcher*, the behavioural regulation of cooperative
124 effort of unrelated helpers depends on specific functions and environmental contexts. Territory
125 maintenance of helpers, which mainly consists of digging out sand from the breeding chamber, is
126 controlled by breeders punishing idle subordinates through aggressive attacks. Helpers abstaining
127 from defending the territory against egg predators are either punished by breeders' attacks, or they
128 compensate for previous idleness by increased defence effort on subsequent occasions (Naef and
129 Taborsky, 2020a, 2020b). The role of dopamine in regulating behaviour is context-dependent. For
130 instance, in *Astatotilapia burtoni* the pharmacological blockage of the D2-like receptors reduced
131 aggression towards an intruder depending on the reproductive status of the female (Weitekamp et
132 al., 2017). Therefore, in our second experiment we investigated the role of D1-like and D2-like
133 receptors in regulating social behaviours on *N. pulcher* helpers that were experimentally exposed to
134 different contexts involving the need for help. We pharmacologically manipulated the activity of D1-
135 like and D2-like receptor pathways in *N. pulcher* helpers that were experimentally exposed to an
136 increased need for help in two distinct situations: (i) territory maintenance (digging sand out of the
137 shelter), and (ii) defence against an egg predator. Behavioural regulation of these tasks by the
138 interaction between dominant breeders and subordinate helpers was shown to differ in dependence
139 of the type of cooperative effort required (Naef and Taborsky, 2020a, 2020b). We hypothesised that
140 the dopaminergic system is involved in the mediation of the social encounters between group
141 members by affecting aggressive, submissive and affiliative behaviours in response to the
142 experimental manipulation of the need for help through the specific environmental challenges.

143 Based on information from other model systems we predicted that the D1-like and D2-like receptors
144 have complementary effects on *N. pulcher* behaviour; the activity of D1-like receptors was expected
145 to modulate aggressive and submissive behaviours, while the D2-like receptors were hypothesised
146 to instead modulate affiliative behaviour.

147

148 **Methods**

149 *Study species*

150 *N. pulcher* is a cooperatively breeding cichlid endemic to lake Tanganyika (Duftner et al.,
151 2007; Taborsky, 1984). Cooperative breeding in *N. pulcher* has evolved in response to exceptionally
152 high predation risk, leading to the formation of groups to successfully defend their offspring
153 (Taborsky, 1984; Groenewoud et al., 2016; Heg et al., 2005; Freudiger et al., 2021). Dominants and
154 subordinate group members (helpers) cooperatively defend the territory against fish and egg
155 predators (Taborsky and Limberger, 1981; Jungwirth et al., 2015; Naef and Taborsky, 2020a). Helpers
156 also maintain the breeding chamber by digging out sand and keeping the entrance clear (Taborsky
157 and Limberger, 1981; Bruintjes & Taborsky 2011; Naef and Taborsky, 2020b). Through alloparental
158 care, territory defence and maintenance behaviours, helpers pay-to-stay in the dominants' territory
159 (Bergmüller et al., 2005; Bruintjes and Taborsky, 2008; Zöttl et al., 2013b; Fischer et al., 2014;
160 Taborsky, 2016). Helpers appease dominants either by defending and maintaining the territory or by
161 enhancing their submissive display behaviours (Bergmüller and Taborsky, 2005; Taborsky et al.,
162 2012; Fischer et al., 2014, 2017; Naef & Taborsky 2020a, b).

163

164 *Subjects and housing conditions*

165 All experimental procedures were approved by the Veterinary Office of the Kanton Bern, Switzerland
166 (licence number BE74/15), and carried out in accordance with the standards of the National
167 Institutes of Health Guide for the Care and Use of Animal Experiments, USA, as well as the EU
168 directive 2010/63/EU for animal experiments. All cichlids used in the experiments were bred and
169 housed at the Ethologische Station Hasli, Institute of Ecology and Evolution of the University of Bern,
170 which is a licenced breeding facility for cichlid fish (licence number BE 4/11, Veterinary Office of the
171 Kanton Bern, Switzerland). Second to fourth generation offspring of wild caught *N. pulcher* from
172 Kasakalawe point near Mpulungu, Zambia, were used for our experiments. In total, 10 groups with
173 two helpers (one large and one small) and a breeder pair each were experimentally established. All
174 group members had a minimum size difference of 5-10mm standard length (SL) between them.
175 Groups were kept in 50L tanks with two flowerpot halves as shelters and one semi-transparent
176 plastic bottle mounted near the water surface as additional shelter. The fish were kept under a light:

177 dark cycle of 13:11 hrs and at a temperature of $27^{\circ}\text{C}\pm 1^{\circ}\text{C}$, simulating the conditions in Lake
178 Tanganyika (Arnold and Taborsky, 2010). All the fish were fed with commercial flake food
179 (5days/week) and defrosted fresh food (1day/week).

180

181 *Pharmacological manipulation*

182 To manipulate the dopaminergic system we performed intramuscular injections on the caudal
183 muscle. SKF-38393 (D047, Sigma Aldrich, Deisenhofen, Germany), a D1-like receptor agonist, and
184 SCH-23390 (D054, Sigma Aldrich, Deisenhofen, Germany), a D1-like receptor antagonist were used.
185 For the D2-like receptor activity manipulation, we used Quinpirole hydrochloride (Q102, Sigma
186 Aldrich, Deisenhofen, Germany), a D2-like receptors agonist, and Metoclopramide (M0763, Sigma
187 Aldrich, Deisenhofen, Germany), a D2-like receptor antagonist. The drugs were chosen based on
188 previous results in other fish model systems (Missale et al., 1998; Cooper and Al-Naser, 2006;
189 Messias et al., 2016b). Dosages for both D1-like and D2-like receptor manipulations were similar but
190 slightly lower than those previously used in other model systems (Cooper and Al-Naser, 2006; de
191 Lima et al., 2011; Dong and McReynolds, 1991; Loos et al., 2010; Messias et al., 2016a). Drugs order
192 was randomized, and the experimenter was blinded to the treatment to avoid sequence effects and
193 observer biases. The drugs were dissolved in saline solution (0.9% NaCl) to reach the desired
194 concentrations: D1-like receptor agonist (SKF-38393: 0.5, 2.5, 5 $\mu\text{g}/\text{mL}$); D1-like antagonist (SCH-
195 23390: 0.1; 0.5; 1.5 $\mu\text{g}/\text{mL}$); D2-like receptor agonist (Quinpirole: 0.5; 2; 3 $\mu\text{g}/\text{mL}$); D2-like receptor
196 antagonist (Metoclopramide: 0.5; 2.5; 5 $\mu\text{g}/\text{mL}$). Directly after preparation and whenever the drug
197 solutions were not used, they were stored at -20°C . As control we injected a saline solution (0.9%
198 NaCl). The injected volume was $15\mu\text{L}$ per gram of body weight (gbw; Paula et al., 2015; Messias et
199 al., 2016a, 2016b; Stettler et al., 2021). To reduce stress, all tested fish were measured, weighed,
200 sexed and anaesthetised with KoiMed Sleep (Schönbach Pharmacy, Germany; 0.15ml for a 300ml
201 water volume) before the injection. Injections were done using 0.5ml insulin syringes (0.5ml
202 MYJECTOR, Terumo Medical Corporation, Elkton, MD 21921, USA). After the injection, the fish were
203 placed in a recovery box with an air stone to recuperate, and when the fish was fully recovered from
204 the anaesthesia it was put back into its home tank but kept in isolation until the first behavioural
205 measurement started. The whole procedure was performed within 5 minutes from catching the
206 focal until the focal was back in the home tank. Injections for the same fish were performed after
207 three to four day intervals. Within the groups, the hierarchy of the focal individual for each
208 injection/trial was chosen in a balanced order to ensure that each fish was tested one after the other
209 with at least one day interval between injections. The experimenter (DFA) was blind to the drug and
210 dosage injected in the focal subject.

211

212 *Behavioural Analysis*

213 All behavioural recordings comprised 15 min live scoring of behaviours within the home tank of the
214 fish, using the software “Observer” version 5.0.25 (Noldus, The Netherlands, 2003). The experimenter
215 (DFA) was blind to the treatment while scoring the behaviours. The following behaviours were
216 scored: Overt aggression (bite, ram, mouth-fight), restrained aggression (fin-spread and opercular
217 spread), affiliative behaviour (bumping, i.e., a soft-touching of the body of another individual with
218 the mouth), submissive behaviour (tail quiver; for a detailed description of the behaviours see
219 Taborsky, 1984 and Taborsky and Limberger, 1981). The information regarding all the interactions in
220 which the focal fish were engaged was recorded, identifying the actor and the recipient of each
221 interaction.

222

223 *Experimental Design*224 *a) Experiment 1: effects of dosage and timing of application on social behaviours*

225 To control for individual variation, we conducted a within-subject design and collected repeated
226 behavioural measures for each individual. In total, eight *N. pulcher* groups were used (N= 16 helpers,
227 eight small and eight large helpers); four groups were tested for the D1-like receptors and injected
228 with three different dosages of the D1-like receptor agonist (SKF-38393: 0.0075, 0.04, 0.075 µg/gbw
229), the D1-like receptor antagonist (SCH-23390: 0.0015, 0.0075, 0.022 µg/gbw), and the control
230 solution (0.9%NaCl; N=8 helpers from four different families), making a total of seven injections per
231 individual with three to four days intervals between injections. The remaining four groups, were
232 tested for the D2-like receptor activity and injected with three different dosages of the D2 agonist
233 (Quinpirole: 0.0075, 0.03, 0.05 µg/gbw), the D2 antagonist (Metoclopramide: 0.0075, 0.04, 0.075
234 µg/gbw), and the control solution (0.9%NaCl; N=8 helpers from four different families). Making a
235 total of seven injections per individuals with three to four days interval between injections.

236 Intramuscular injection into the caudal muscle was performed for only one of the group’s helpers at
237 a time. Each focal fish’s behaviour was recorded: its social interactions and with whom they
238 occurred. Observations were done at four different time points: 15 min before the injection, and at
239 15 min, 30 min and 60 min after the injection. At the beginning of the experiment the shelters were
240 filled with sand to stimulate digging behaviour. In case one of the helpers was evicted from the
241 group it was replaced by another fish with the same size and sex (eleven replacements over the
242 whole experiment). After the new helper was accepted and the family had stabilized again, we
243 proceeded with the experiment.

244

245 *b) Experiment 2: context-dependence of dopamine receptor effects on social responses*

246 Similarly to experiment 1, we performed a within-subject design to control for individual variation.
247 For this experiment the eight groups utilised in experiment 1 and two additional groups were used
248 after an interval of 2 months past the end of experiment 1. In total, 20 focal helpers (ten small and
249 ten large helpers) were treated with intra-muscular injections into the caudal muscle. Injected
250 solutions contained either a D1-like receptors agonist (SKF-38393: 0.075 µg/gbw) or antagonist (SCH-
251 23390: 0.0075 µg/gbw), or a D2-like receptor agonist (Quinpirole: 0.0075 µg/gbw) or antagonist
252 (Metoclopramide: 0.0075 µg/gbw), or a saline solution as control (0.9%NaCl). We used a single
253 dosage for each of the test drugs. Behavioural observations started 15min after the injection, in
254 accordance with the results from experiment 1 on treatment effects on *N. pulcher* behaviour. Only
255 one helper was injected per trial. Every focal helper experienced a 3 days break between trials to
256 avoid potential stress from repeated capture and manipulation. The aim of this experiment was to
257 test the helpers' behavioural response to environmental challenges in dependence of our
258 manipulations of the dopaminergic system. Two distinct tasks were experimentally assigned to each
259 group: a) a digging task, where the helpers were challenged to perform shelter maintenance
260 behaviour and b) an egg predator intrusion into the territory. In the first task, the shelters were filled
261 with sand directly before the observation, during which we counted the frequency of digging (sand
262 removal from the shelter) performed by the focal helper, and all interactions with the group
263 members. For the intruder task, we used *Telmatochromis vittatus*, which is a natural predator of *N.*
264 *pulcher* eggs (Bruintjes and Taborsky, 2011)(Zöttl et al., 2013b). During this task, the numbers of
265 aggressive behaviours and displays of the focal helper towards the intruder were recorded, together
266 with all interactions occurring among group members. In the control situation, the fish did not face
267 any environmental challenges and we recorded every interaction within the group. The sequence of
268 the tasks was balanced to prevent sequence effects. In case one of the helpers was evicted from the
269 group, it was replaced by another fish with the same size and sex (in total two replacements
270 occurred).

271

272 *Statistical Analyses*

273 All tests and plots were done using the software R (R Core Team, 2018), version 4.0.3.

274

275 *a) Experiment 1: effects of dosage and timing of application on social behaviours*

276 The two treatments (D1 and D2 receptor treatments) were analysed separately, since each fish was
277 exposed only to one treatment. All occurrences of restrained and overt aggressive behaviours were
278 summed up and analysed as total aggression. Behavioural frequencies were corrected for the effect

279 of the injection by subtracting the baseline values obtained from each fish, i.e. all behaviours
280 performed during the recording period before the pharmacological treatment. We log-transformed
281 the behavioural data to fulfil the normality criterion. Corrected behavioural frequencies were
282 analysed by fitting linear mixed-effect models (LMM) using the package “lme4” (Bates et al., 2015).
283 Separate models were fitted for each observation time point. As the experiment was based on
284 repeated measurements, fish identity was included in the models as a random factor. LMMs were
285 fitted to analyse the frequencies of performed activities including aggressive, submissive
286 (standardised by received aggression), affiliative, and digging behaviours as dependent variable. All
287 initial models included dosage and helper rank as fixed factors. Models were simplified by backwards
288 selection (Bates et al., 2015), whereas dosage was always kept in the model. The assumptions of
289 normality of the error term were checked by Shapiro-Wilk tests and visual inspection of quantile-
290 quantile plots of model residuals to detect skew and kurtosis, as well as Tukey-Anscombe plots to
291 check for homogeneity of variance.

292

293 *b) Experiment 2: context-dependence of dopamine receptor effects on social responses*

294 Every focal fish was injected with all test drugs, therefore we included in the analysis fish identity as
295 random variable. The behavioural frequencies were analysed separately for each environmental
296 manipulation. A general linear mixed-effect model (GLMM) following a negative binomial
297 distribution was fitted to analyse the behavioural frequencies using the package “glmmadmb”
298 (<http://glmmadmb.r-forge.r-project.org>). A zero-inflation term was included in all models. Models
299 were fitted to analyse the frequencies of each behavioural class, aggression, submission
300 (standardized by received aggression), and affiliative behaviour as dependent variables. All initial
301 models included treatment (i.e. the drug injected) and helper rank as fixed factors. Model
302 simplification was performed by backwards selection (Bates et al., 2015), whereas treatment was
303 kept in the final model. Digging behaviour and defence against the intruder were excluded from
304 these analyses due to the low frequencies of these events (six digging events in total, including
305 between 1 and 30 digging actions: 1 with Metoclopramide, 3 with Quinpirole, 1 with SCH-23390 and
306 1 with SKF-38393; seven defence events against the presented egg predator in total, including
307 between 1 and 12 individual attacks: 1 with Metoclopramide, 4 with Quinpirole, 1 with SCH-23390
308 and 2 with SKF-38393).

309

310 **Results**

311 *a) Experiment 1: effects of dosage and timing of application on social behaviours*

312 In response to the D1-like receptor manipulation treatment, we found that enhancing this
313 receptor activity with the *agonist* induced an increase of aggressive behaviour with rising dosages
314 (Table 1). After injection with the highest dosage, aggressive behaviour tended to increase 15min
315 after the injection, and aggression was significantly higher 30min after the injection than before,
316 which decreased again slightly after 60min from injection (Table 1; SI Table 1). Submissive behaviour
317 was enhanced 15min after treatment with the middle and high dosages of the agonist, whereas no
318 effects were determined on affiliative behaviour (Table 1; SI Table 1).

319 When injected with the intermediate dosage of the D1-like *antagonist*, the aggressive
320 behaviour of treated fish decreased significantly already 15min after the injection, and this effect
321 declined 30 and 60min after the injection (Table 2; SI Table 1). The lower and higher dosages
322 rendered no significant effects. Overall, submissive behaviour of treated fish increased after
323 injecting the antagonist, and regardless of dosage this effect was strongest 30min after injection.
324 Affiliative behaviour was significantly enhanced 15min after injecting the lowest dosage of the
325 antagonist (Table 2; SI Table 1).

326 No significant effects were found on aggressive and submissive behaviours when D2-like
327 receptor *agonist* was applied (Table 3; SI Table 1). Affiliative behaviour increased 15 min after
328 injection of the lowest dosage of D2-like receptor *agonist* (Table 3; SI Table 1).
329 The D2 *antagonist* also caused no significant effects on aggression and submission (Table 4; SI Table
330 1) but generally, blocking the D2-like receptors raised affiliative behaviour significantly in
331 comparison to the pre-injection control (Table 4; SI Table 1).

332

333 *b) Experiment 2: context-dependence of dopamine receptor effects on social responses*

334 The D1-like receptor *agonist* tended to increase aggressive behaviour in the control situation
335 of the 2nd experiment, confirming results from experiment 1. However, the exposure to
336 environmental challenges seemed to mitigate this effect. In contrast, submissive behaviour exhibited
337 towards other group members only increased in the egg predator treatment in experiment 2, and
338 not in the digging challenge or the control situation (Table 5, Fig.1). Similar to experiment 1,
339 affiliative behaviour was not affected by D1-like receptor *agonist* injection (Table 5). The D1-like
340 receptor *antagonist* tended to increase submissive and affiliative behaviours similarly to experiment
341 1, but again only when the helpers were experimentally exposed to the egg predator (Table 5, Fig.2).
342 Aggressive behaviour was not affected at all by applying D1-like receptor *antagonist* in experiment
343 2.

344 In contrast to the responses determined in the first experiment, applying the D2-like
345 receptor *agonist* significantly increased aggressive behaviour in the egg predator and control

346 situations (Table 5, Fig.1), while submissive and affiliative behaviours were not influenced by this
347 treatment. The injection of D2-like receptor *antagonist* tended to increase the propensity of test
348 subjects to show aggressive behaviour but solely in the control situation. In contrast, it increased
349 submissive tendencies in all three experimental situations, particularly so when environmental
350 subjects were challenged by an egg predator (Table 5, Fig. 1, Fig. 2). This differed from the situation
351 in experiment one, in which manipulations of the D2-like receptors revealed no significant effects on
352 aggression and submission. The D2-like receptor *antagonist* treatment also enhanced affiliative
353 behaviours in the control treatment of experiment 2, which confirmed the result obtained in
354 experiment 1.

355

356 Discussion

357 Our results demonstrate that the two classes of dopamine receptors have very distinct roles
358 in behavioural regulation of subordinate helpers in a cooperatively breeding fish. Our first
359 experiment revealed the D1-like receptor pathways modulating aggression and submission, while
360 the D2-like receptor mediation strongly affected affiliative behaviour. In our second experiment
361 which included several distinct environmental challenges, we found that stimulating the activity of
362 the D2-like receptors increased aggression of helpers toward other group members during the egg
363 predator and control tasks, whereas the blockage of the D2-like receptors produced a significant
364 increase of performed submission and affiliation. Interestingly, our environmental challenges
365 seemed to reduce the effects of D1-like receptor manipulations on the aggression of test subjects
366 shown against other group members. These results suggest that the regulatory function of the D1-
367 like and D2-like receptors for the modulation of social behaviour depends on the environmental
368 challenges to which group members are exposed.

369 Experiment 1 revealed a significant role of D1-like receptors in the modulation of aggressive
370 and submissive behaviours of *N. pulcher* subordinate helpers, while the D2-like receptor pathways
371 mediated affiliative behaviour. Specifically, the higher dosage of the D1-like receptors *agonist* we
372 used significantly increased both aggression and submission, whereas lower dosages showed less
373 pronounced effects. The intermediate dosage of the D1-like receptor *antagonist* significantly
374 decreased aggression, whereas it increased submission. Our results highlight the importance of
375 testing the behavioural effects of exogenous pharmacological manipulations over time, not only to
376 identify the minimum interval between treatment and observation, but also to reveal the short-term
377 effectiveness of different dosages. Indeed, we show that 60min after the treatment, most of the
378 behavioural response effects were no longer significant.

379 Moreover, our results show that the administration of both D1-like receptor agonist and
380 antagonist lead to an increase in submissive behaviour, which seems contradictory. Despite our
381 efforts to reduce handling-stress, we cannot exclude that these results might have been influenced
382 by stress that could have activated other neurophysiological systems, including the monoaminergic
383 pathway (Joëls and Baram, 2009; de Abreu et al., 2020). Alternatively, we hypothesise that
384 complementary pathways regulating submissive behaviour may exist, either through direct D1
385 activity or through blocking D1, which may trigger other neuroendocrine pathways (involving e.g.
386 serotonin; Stettler et al., 2021). For instance, in a similar study in *N. pulcher*, the administration of a
387 serotonin 1a receptor agonist has decreased the helpers' submissive behaviour (Stettler et al., 2021).
388 Hence, our data together with results from previous serotonin manipulation experiments suggest
389 that both systems are relevant for the regulation of submissive behaviour, which may complicate the
390 interpretation of results when only one system is manipulated at a time. In other species, the
391 significance of D1-like receptors in the regulation of social behaviour has rarely been studied, but in
392 cleaner wrasses, *Labroides dimidiatus*, the D1-like receptors play an important role in the modulation
393 of both intraspecific cooperation and interspecific client familiarization. Pharmacological blockage of
394 the D1-like receptors increased tactile stimulation events to clients and the duration of the
395 interactions (Messias et al., 2016a), including unfamiliar ones (Soares et al., 2017).

396 The D2-like receptors seem to modulate affiliative behaviour, as the lowest dosage of the
397 agonist and the low and medium dosages of the antagonist significantly increased affiliative
398 behaviour. Our results suggest, that similarly to the results from the D1-like receptors manipulation,
399 two alternative mechanisms might explain these results. One possibility that we cannot exclude is
400 that potential handling-stress activated other neurophysiological systems (e.g. steroid hormones,
401 neuropeptides, monoamines; Joëls and Baram, 2009), which in combination with our
402 pharmacological manipulations may have caused a similar behavioural effect in both agonist and
403 antagonist administrations. Alternatively, complementary pathways may exist that regulate
404 affiliative behaviour, either through direct D2 activity or through blocking D2, which may trigger
405 other neuroendocrine pathways (involving e.g. serotonin; Stettler et al., 2021). In *N. pulcher*, the
406 serotonin receptor 1a modulates affiliative behaviour; the administration of the receptor agonist
407 increases affiliative behaviour, while application of the receptor antagonist decreases affiliative
408 behaviour (Stettler et al., 2021). Again, the involvement of different neuroendocrine regulatory
409 systems may impede the interpretation of responses to the manipulation of only one of these
410 systems at a time. While there are few data on the regulation of social behaviour involving the D2-
411 like receptor pathway in other animals, in male prairie voles, activation of the D2-like receptors in

412 the nucleus accumbens lead to an increase of time spent in contact with a familiar mate (Aragona,
413 2009).

414 Our results from experiment 1 suggest that both D1-like and D2-like receptors combined
415 may contribute relevantly to the modulation of social interactions, and these two pathways seem to
416 complement each other. While the D1-like pathway is involved in regulating aggressive and
417 submissive behaviour, the D2-like pathways seems to mainly affect affiliative behaviour. In cleaner
418 wrasses (*L. dimiatus*) the dopaminergic activity is involved in regulating cleaner/client interactions
419 and the blockage of D2 pathways caused an increased number of tactile stimulation (when cleaners
420 touch the body of clients by using their pectoral and pelvic fins), whereas it did not affect the
421 amount of time spent with providing it (Messias et al., 2016a). Cleaner wrasses use tactile
422 stimulation in their negotiation with clients, serving to prolong the interaction, or to appease clients
423 after cheating (Bshary and Würth, 2001; Grutter, 2004). The effects of pharmacological blockage of
424 the D2 pathways pointed towards its role in the regulation and maintenance of social interactions. In
425 contrast, D1 blockade impaired the cleaner wrasses' overall behaviour (Messias et al 2016a). The
426 provision of tactile stimulation has been argued to be a costly behaviour (Bshary and Würth, 2001),
427 and the relative contributions of each DA pathway (D1 and D2) revealed similar results but
428 complementary functions, with the D1 pathways regulating the overall interactions (duration of the
429 interaction, time spent performing tactile stimulation and the proportion of interactions with tactile
430 stimulation), and the D2 pathways mediating solely the frequency of tactile stimulation (Messias et
431 al., 2016a). Similarly, in *N. pulcher* we show that D1 and D2 pathways seem to complementarily
432 regulate social interactions, through the modulation of aggressive, submissive and affiliative
433 behaviour.

434 In highly social animals, the environmental context is typically very dynamic and individuals
435 are required to respond appropriately to all kinds of situations (Taborsky et al., 2012; Taborsky and
436 Oliveira, 2012). Group members constantly acquire information from the environment including
437 their social partners (e.g., whether they contest resources, demand or offer support, or are
438 reproductively receptive). In the central nervous system, social information is integrated in the
439 SDMN, where dopamine plays a key role (O'Connell & Hofmann, 2011). Our results from experiment
440 2 show that the stimulation of D2-like receptors caused an increase of aggressive behaviour in *N.*
441 *pulcher*, which corroborates results from other model systems. For instance, in rodents some of the
442 nuclei from the social decision-making network are involved in the modulation of aggression,
443 particularly under mediation of the activity of D2-like receptors (Delville et al., 2000; Nelson and
444 Trainor, 2007). In teleosts, the dopaminergic system is known to regulate aggressive behaviour,
445 which is related to social hierarchy (McIntyre et al., 1979; Weitekamp et al., 2017; Winberg et al.,

446 1992, 1991). Subordinate fish show higher dopaminergic activity in their hypothalamus (Overli et al.,
447 1999). In cichlid fish (*Aequidens pulcher*), administration of generalist dopamine D1-like and D2-like
448 receptor agonists (apomorphine) and antagonists (chlorpromazine) both reduced aggressive
449 behaviours (Munro, 1986). Through independently manipulating the D1 and D2 pathways in our
450 study we show that dopaminergic regulation of social behaviour is complex, with D1 and D2
451 pathways complementing each other in regulating social interactions within cooperatively breeding
452 groups.

453 The D1 and D2 receptor pathways are functionally different, having different selectivity to
454 DA, different distributions and brain densities. Importantly, because D2-like receptors are mostly
455 autoreceptors that are present both pre- and postsynaptically, the activation of D2 receptors can
456 induce a negative feedback inhibiting dopamine neuron firing, synthesis and release (Bello et al.,
457 2011). This contrasts the D1 more typically postsynaptic function. We hypothesise that the D2-like
458 receptor stimulation may be working to inhibit the system, this way reducing neuron firing and
459 dopamine release (Beaulieu and Gainetdinov, 2011; Tritsch and Sabatini, 2012) and ultimately
460 contributing to an increase in aggressive motivation due to a decrease in DA availability. Thereby,
461 the D2-like pathway may play an important role in the fine-tuning of aggressive behaviours.

462 Interestingly, the blockage of the D2 pathways induced submissive behaviour, particularly
463 when test subjects were exposed to an egg predator. This corroborates evidence found in other
464 teleost fish that submissive individuals, which constantly receive aggression from dominants,
465 showing lower brain DA baseline levels compared to dominant individuals (Winberg et al., 1991).
466 Fish that are frequently attacked by dominants apparently experience changes in the catecholamine
467 levels when compared to dominants (McIntyre et al., 1979), and these changes might be associated
468 with stress (de Abreu et al., 2020). Our results point towards effects of D2-like receptors on the
469 regulation of submissive behaviour in accordance with an individual's internal state, i.e. the
470 individual's state-dependent neurophysiological profile. Moreover, in other fish species subordinates
471 were shown to have lower levels of dopamine (McIntyre et al., 1979), with the activation of the D2-
472 like receptors inducing a negative feedback (Bello et al., 2011). By blocking the D2-like receptors
473 with a lower dosage of antagonist in comparison to previous studies (Messias et al., 2016a;
474 Weitekamp et al., 2017) we hypothesize that the antagonist may have targeted mainly D2
475 presynaptic receptors (Keeler et al., 2014), thereby apparently producing an overall inhibition of the
476 system. Our results from D1-like receptor stimulation suggest that these receptors may also be
477 involved in the regulation of submissive behaviours, which indicates that D1 and D2 pathways may
478 have complementary functions when it comes to responding submissively. Contrary to experiment 2,
479 in experiment 1 focal groups were not exposed to specific environmental challenges, but group

480 members could also engage in sand digging, which was possible throughout both experiments. In
481 experiment 2, the environmental manipulations created a change in the groups' needs, hence the
482 behavioural modulatory role of dopamine could have differed depending on the environmental
483 challenges. Our data suggest that the D1 pathway regulates the helper's submission during
484 unchallenging scenarios (experiment 1), while the D2 pathway regulates submission when the
485 environmental context demands help (experiment 2). This hypothesis needs further testing in future
486 studies in order to disentangle the relative importance of each receptor class in the regulation of
487 submissive behaviour. In *N. pulcher*, submission plays a crucial role in the helpers' "payment" to the
488 breeders to be allowed to stay in the territory, which enhances their survival chances (Taborsky &
489 Limberger, 1981; Taborsky, 1984). When there is need for help in the territory, helpers can appease
490 the breeders either by increasing their helping efforts or by showing submission (Bergmüller et al.,
491 2005; Naef and Taborsky, 2020a). When helpers are experimentally prevented from defending
492 against an egg predator, they increase their submissive displays towards the breeders (Naef and
493 Taborsky, 2020a). Our results suggest that such appeasement is regulated via the D2 pathways, as
494 D2 activity regulates helper's submissive behaviour when there is a need for help. In addition,
495 breeder aggression toward helpers seems to be influenced by the D1 pathways, which in turn may
496 raise stress of subordinates and release submissive behaviour (de Abreu et al., 2020; Joëls and
497 Baram, 2009). Cortisol levels of subordinates are reduced with increasing levels of submission shown
498 toward the dominants (Bender et al., 2006), apparently alleviating stressful situations for helpers in
499 breeder-helper conflicts (Bergmüller and Taborsky, 2005).

500 The expression of affiliative behaviour in *N. pulcher* helps to maintain group cohesion and to
501 stabilise the hierarchy among individuals (Hamilton et al., 2005). Our results show that during the
502 control situation in experiment 2, blocking the D2-receptors significantly increased affiliation, which
503 confirmed the results from the first experiment. This effect was absent during the digging and
504 intruder tasks, where the environmental challenges apparently demanded different behavioural
505 responses. The propensity to show affiliative behaviour was shown to be heritable in *N. pulcher*
506 (Kasper et al., 2019), suggesting that the D2 receptors might play a decisive role in the evolution of
507 group-living in this species, particularly with regard to affiliation and the consequent acceptance in
508 the group.

509 When environmental challenges were provided, we did not find a significant effect of our
510 D1-like receptor manipulations on the helpers' behaviour. The major difference between our two
511 experiments was that the experimentally induced environmental challenges created a demand for
512 specific behavioural responses of helpers, which inevitably altered the interactions between the
513 group members as shown in previous studies (Taborsky 1985; Zöttl et al. 2013a). A context-

514 dependent role of dopamine was also shown in previous studies. For instance in European starlings,
515 *Sturnus vulgaris*, dopaminergic regulation of song production in the brain differs depending on
516 contexts (breeding vs non-breeding; Heimovics and Riters, 2008). In common waxbills, *Estrilda*
517 *astrild*, the D1-like pathway regulates activity depending on context, reducing activity in a social
518 context while increasing it in a non-social context (Silva et al., 2020). In *Astatotilapia burtoni*, D2
519 receptor activation lead to a decrease of aggression towards an intruder when reproductive
520 opportunities existed, whereas blockage inhibited aggression towards an intruder in the same
521 context while increasing aggression in a neutral context (Weitekamp et al., 2017). These different
522 effects on aggressive behaviour from D2-like receptor manipulations were supposedly due to
523 context-dependent receptor occupancy (Weitekamp et al., 2017). In addition, different
524 environmental contexts might be linked to different neuro-endocrinological states, which are
525 regulated through context- or state-dependent gene expression patterns in the brain
526 (“neurogenomic states”; Robinson et al., 2008). In threespined sticklebacks, *Gasterosteus aculeatus*,
527 a short territory intrusion induced a change in the their neurogenomic state, which included genes
528 involved in hormone signalling and neurotransmitter transport (Bukhari et al., 2017). We
529 hypothesise that our experimentally induced environmental challenges altered the helpers’ neuro-
530 endocrine state in response, for example through changes in baseline D1-like and D2-like receptor
531 occupancy. In rats, the D1-like receptor agonist in the prefrontal cortex had opposite effects on
532 performance in a radial maze task in individuals with different memory traces, due to differences in
533 pre-existing dopamine levels (Floresco and Phillips, 2001).

534 Our results provide evidence for a decisive role of D1 and D2 receptors in the modulation of
535 social interactions. However, further research is needed to better understand their function within
536 specific brain regions, particularly within the SDMN. For instance, we performed intramuscular
537 injections, leading to a systemic exposure to the drugs instead of a localized manipulation. The
538 densities of dopaminergic neurons may differ between different brain regions. For instance, in
539 *Astatotilapia burtoni* the central part of the ventral telencephalon (Vc) and the preoptic area (POA)
540 have a higher density of dopaminergic cells than the dorsomedial telencephalon. Further research
541 should focus on region-specific manipulations of the dopaminergic system. Additionally, as some of
542 the behaviours of interest were shown at low frequencies, future studies should consider an
543 increase of observation time while taking into account the time-dependent effects we found.

544

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809 **Tables**

810 Table 1. . Results of LMMs for the effects of the D1-like agonist (SKF-38393) from *Experiment 1* the
 811 dosage-dependence experiment (N= 8 helpers) on: a) aggression, b) submission (standardized for
 812 received aggression), c) affiliative behaviour; significant p-values are indicated in bold; trends are
 813 indicated in italic ($0.1 > p\text{-value} > 0.05$).

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Behaviour	Time (min)	Dosage ($\mu\text{g}/\text{gbw}$)	Estimate \pm SE	df	t	p-value
a) Aggression	15	0.0075	0.755 \pm 0.365	21	2.068	<i>0.051</i>
		0.04	0.676 \pm 0.365	21	1.851	<i>0.078</i>
		0.075	0.706 \pm 0.365	21	1.933	<i>0.066</i>
	30	0.0075	0.722 \pm 0.349	21	2.066	<i>0.051</i>
		0.04	0.679 \pm 0.349	21	1.944	<i>0.065</i>
		0.075	0.784 \pm 0.349	21	2.242	0.036
	60	0.0075	0.641 \pm 0.371	28	1.728	<i>0.095</i>
		0.04	0.349 \pm 0.371	28	0.941	0.354
		0.075	0.718 \pm 0.371	28	1.934	<i>0.063</i>
b) Submission	15	0.0075	0.295 \pm 0.239	28	1.234	0.227
		0.04	0.572 \pm 0.239	28	2.397	0.023
		0.075	0.535 \pm 0.239	28	2.243	0.033
	30	0.0075	0.313 \pm 0.213	28	1.471	0.153
		0.04	0.227 \pm 0.213	28	1.067	0.295
		0.075	0.142 \pm 0.213	28	0.665	0.511
	60	0.0075	0.312 \pm 0.209	28	1.494	0.146
		0.04	0.265 \pm 0.209	28	1.270	0.215
		0.075	0.171 \pm 0.209	28	0.819	0.420
c) Affiliative	15	0.0075	0.434 \pm 0.265	28	1.638	0.113
		0.04	0.433 \pm 0.265	28	1.635	0.113
		0.075	0.290 \pm 0.265	28	1.095	0.283
	30	0.0075	0.304 \pm 0.247	21	1.230	0.232
		0.04	0.353 \pm 0.247	21	1.432	0.167
		0.075	0.355 \pm 0.247	21	1.436	0.166
	60	0.0075	0.397 \pm 0.236	21	1.683	0.107
		0.04	0.431 \pm 0.236	21	1.828	<i>0.082</i>
		0.075	0.432 \pm 0.236	21	1.830	<i>0.081</i>

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818 Table 2. Results of LMMs for the effects of the D1-like antagonist (SCH-23390) from *Experiment 1* the
 819 dosage-dependence experiment (N= 8 helpers) on: a) aggression, b) submission (standardized for
 820 received aggression), c) affiliative behaviour; significant p-values are indicated in bold; trends are
 821 indicated in italic ($0.1 > p\text{-value} > 0.05$).

Behaviour	Time (min)	Dosage ($\mu\text{g}/\text{gbw}$)	Estimate \pm SE	df	t	p-value
a) Aggression	15	0.0015	-0.227 \pm 0.254	21	-0.894	0.381
		0.0075	-0.608 \pm 0.250	21	-2.428	0.024
		0.022	-0.269 \pm 0.250	21	-1.073	0.295
	30	0.0015	-0.161 \pm 0.280	21	-0.577	0.570
		0.0075	-0.557 \pm 0.276	21	-2.016	<i>0.057</i>
		0.022	-0.323 \pm 0.276	21	-1.171	0.254
	60	0.0015	-0.383 \pm 0.302	21	-1.267	0.219
		0.0075	-0.543 \pm 0.298	21	-1.821	<i>0.083</i>
		0.022	-0.328 \pm 0.298	21	-1.101	0.283
b) Submission	15	0.0015	0.575 \pm 0.238	28	2.417	0.022
		0.0075	0.209 \pm 0.238	28	0.879	0.387
		0.022	0.356 \pm 0.238	28	1.497	0.146
	30	0.0015	0.659 \pm 0.281	22	2.350	0.028
		0.0075	0.658 \pm 0.278	21	2.366	0.028
		0.022	0.796 \pm 0.278	21	2.861	0.009
	60	0.0015	0.513 \pm 0.240	22	2.139	0.044
		0.0075	0.525 \pm 0.237	21	2.210	0.038
		0.022	0.342 \pm 0.237	21	1.441	0.164
c) Affiliative	15	0.0015	0.558 \pm 0.250	22	2.226	0.037
		0.0075	0.331 \pm 0.249	21	1.331	0.197
		0.022	0.179 \pm 0.249	21	0.720	0.480
	30	0.0015	0.412 \pm 0.362	28	1.133	0.267
		0.0075	0.182 \pm 0.362	28	0.502	0.620
		0.022	-0.104 \pm 0.362	28	-0.287	0.776
	60	0.0015	0.273 \pm 0.334	28	0.818	0.420
		0.0075	-0.069 \pm 0.334	28	-0.208	0.837
		0.022	-0.104 \pm 0.334	28	-0.313	0.757

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830 Table 3. D2-like agonist (Quinpirole) from *Experiment 1*; significant p-values are indicated in bold;
 831 trends are indicated in italic ($0.1 > p\text{-value} > 0.05$).
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Behaviour	Time (min)	Dosage ($\mu\text{g}/\text{gbw}$)	Estimate \pm SE	df	t	p-value
a) Aggression	15	0.0075	0.579 \pm 0.366	21	1.582	0.128
		0.03	0.666 \pm 0.366	21	1.819	<i>0.083</i>
		0.05	0.392 \pm 0.366	21	1.071	0.296
	30	0.0075	0.377 \pm 0.377	21	1.000	0.329
		0.03	0.412 \pm 0.377	21	1.094	0.286
		0.05	-0.055 \pm 0.377	21	-0.147	0.885
	60	0.0075	0.607 \pm 0.371	21	1.636	0.117
		0.03	0.585 \pm 0.371	21	1.577	0.130
		0.05	0.212 \pm 0.371	21	0.573	0.573
b) Submission	15	0.0075	0.184 \pm 0.234	21	0.787	0.440
		0.03	0.029 \pm 0.234	21	0.126	0.901
		0.05	-0.293 \pm 0.234	21	-1.251	0.225
	30	0.0075	-0.225 \pm 0.270	21	-0.832	0.415
		0.03	-0.206 \pm 0.270	21	-0.762	0.455
		0.05	-0.279 \pm 0.270	21	-1.033	0.313
	60	0.0075	0.017 \pm 0.193	21	0.087	0.931
		0.03	0.052 \pm 0.193	21	0.271	0.789
		0.05	-0.217 \pm 0.193	21	-1.125	0.273
c) Affiliative	15	0.0075	0.508 \pm 0.221	21	2.299	0.032
		0.03	0.397 \pm 0.221	21	1.795	<i>0.087</i>
		0.05	0.397 \pm 0.221	21	1.795	<i>0.087</i>
	30	0.0075	0.334 \pm 0.254	21	1.318	0.202
		0.03	-0.078 \pm 0.254	21	-0.306	0.762
		0.05	0.241 \pm 0.254	21	0.949	0.353
	60	0.0075	0.265 \pm 0.252	21	1.051	0.305
		0.03	-0.154 \pm 0.252	21	-0.609	0.549
		0.05	0.167 \pm 0.252	21	0.662	0.515

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835 Table 4. Results of LMMs for the effects of D2-like antagonist (Metoclopramide) from *Experiment 1*;
 836 significant p-values are indicated in bold; trends are indicated in italic ($0.1 > p\text{-value} > 0.05$).

Behaviour	Time (min)	Dosage ($\mu\text{g/gbw}$)	Estimate \pm SE	df	t	p-value
a) Aggression	15	0.0075	0.191 \pm 0.370	21	0.515	0.612
		0.04	0.266 \pm 0.370	21	0.718	0.480
		0.075	-0.430 \pm 0.370	21	-1.161	0.259
	30	0.0075	0.237 \pm 0.371	21	0.637	0.531
		0.04	0.250 \pm 0.371	21	0.672	0.509
		0.075	-0.468 \pm 0.371	21	-1.259	0.222
	60	0.0075	0.564 \pm 0.420	21	1.345	0.193
		0.04	0.710 \pm 0.420	21	1.691	0.106
		0.075	0.219 \pm 0.420	21	0.523	0.606
b) Submission	15	0.0075	-0.006 \pm 0.211	21	-0.028	0.978
		0.04	-0.053 \pm 0.211	21	-0.249	0.805
		0.075	0.173 \pm 0.211	21	0.819	0.422
	30	0.0075	-0.323 \pm 0.255	28	-1.265	0.216
		0.04	-0.230 \pm 0.255	28	-0.900	0.376
		0.075	0.090 \pm 0.255	28	0.352	0.728
	60	0.0075	-0.233 \pm 0.147	21	-1.593	0.126
		0.04	0.031 \pm 0.147	21	0.212	0.834
		0.075	0.169 \pm 0.147	21	1.155	0.261
c) Affiliative	15	0.0075	0.556 \pm 0.221	21	2.512	0.020
		0.04	0.493 \pm 0.221	21	2.229	0.037
		0.075	0.431 \pm 0.221	21	1.946	<i>0.065</i>
	30	0.0075	0.748 \pm 0.255	28	2.936	0.006
		0.04	0.764 \pm 0.255	28	2.998	0.005
		0.075	0.589 \pm 0.255	28	2.312	0.028
	60	0.0075	0.530 \pm 0.255	21	2.078	<i>0.050</i>
		0.04	0.577 \pm 0.255	21	2.263	0.034
		0.075	0.344 \pm 0.255	21	1.350	0.191

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840 Table 5. Effects of pharmacological manipulation during environmental challenges (*Experiment 2*).
 841 Results of negative binomial GLMMs with zero-inflation term for the effects of the pharmacological
 842 manipulation of the D1-like and D2-like receptor from the context-dependence experiment (N=100
 843 observations from 20 helpers); significant p-values are indicated in bold; trends are indicated in italic
 844 (0.1 > p-value > 0.05). Behavioural observations started 15min after the injection.

Drug	Behaviour	Task	Zero-Inflation	Estimate ± SE	z	p-value	
D1-like agonist (SKF-38393: 0.0075µg/gbw)	Aggression	Control	0.032	0.420±0.228	1.84	0.065	
		Digging	0.052	0.445±0.264	1.69	0.092	
		Egg predator	0.038	0.269±0.213	1.26	0.208	
	Submission	Control	1e ⁻⁰⁶	0.198±0.206	0.96	0.336	
		Digging	0.056	0.146±0.208	0.70	0.484	
		Egg predator	0.015	0.370±0.196	1.89	0.059	
	Affiliative	Control	0.306	-0.106±0.511	-0.21	0.835	
		Digging	0.264	0.479±0.495	0.97	0.33	
		Egg predator	1.0003e ⁻⁰⁶	0.773±0.610	1.27	0.206	
	D1-like antagonist (SCH-23390: 0.0075µg/gbw)	Aggression	Control	0.032	0.115±0.231	0.50	0.620
			Digging	0.052	0.164±0.259	0.63	0.528
			Egg predator	0.038	0.138±0.219	0.63	0.529
Submission		Control	1e ⁻⁰⁶	0.180±0.206	0.87	0.383	
		Digging	0.056	0.222±0.216	1.03	0.302	
		Egg predator	0.015	0.395±0.210	1.88	0.060	
Affiliative		Control	0.306	0.203±0.439	0.46	0.645	
		Digging	0.264	0.224±0.451	0.50	0.62	
		Egg predator	1.0003e ⁻⁰⁶	0.947±0.569	1.66	0.096	
D2-like agonist (Quinpirole: 0.0075µg/gbw)		Aggression	Control	0.032	0.554±0.230	2.41	0.016
			Digging	0.052	0.389±0.256	1.52	0.129
		Submission	Egg predator	0.038	0.470±0.218	2.16	0.031
	predator						

	Submission	Control	1e ⁻⁰⁶	0.061±0.210	0.29	0.773
		Digging	0.056	0.255±0.204	1.25	0.213
		Egg	0.015	0.171±0.210	0.81	0.416
		predator				
	Affiliative	Control	0.306	0.352±0.590	0.60	0.551
		Digging	0.264	0.380±0.440	0.86	0.39
		Egg	1.0003e ⁻⁰⁶	0.051±0.634	0.08	0.936
		predator				
D2-like	Aggression	Control	0.032	0.418±0.237	1.76	0.078
antagonist		Digging	0.052	-0.127±0.256	-0.50	0.620
<i>(Metoclopramide:</i>		Egg	0.038	0.077±0.221	0.35	0.727
<i>0.0075µg/gbw)</i>		predator				
	Submission	Control	1e ⁻⁰⁶	0.347±0.200	1.73	0.083
		Digging	0.056	0.386±0.203	1.90	0.057
		Egg	0.015	0.571±0.197	2.91	0.004
		predator				
	Affiliative	Control	0.306	1.098±0.550	1.99	0.046
		Digging	0.264	0.134±0.449	0.30	0.77
		Egg	1.0003e ⁻⁰⁶	0.736±0.579	1.27	0.204
		predator				

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847 **Figures**

848

849 Figure 1. Total aggression shown within 15min observations in the context-dependent experiment

850 (experiment 2) during: a) control task, b) digging task and c) intruder task in the different

851 treatments: saline represented as black squares, D1 agonist (SKF-38393) represented as black circles,

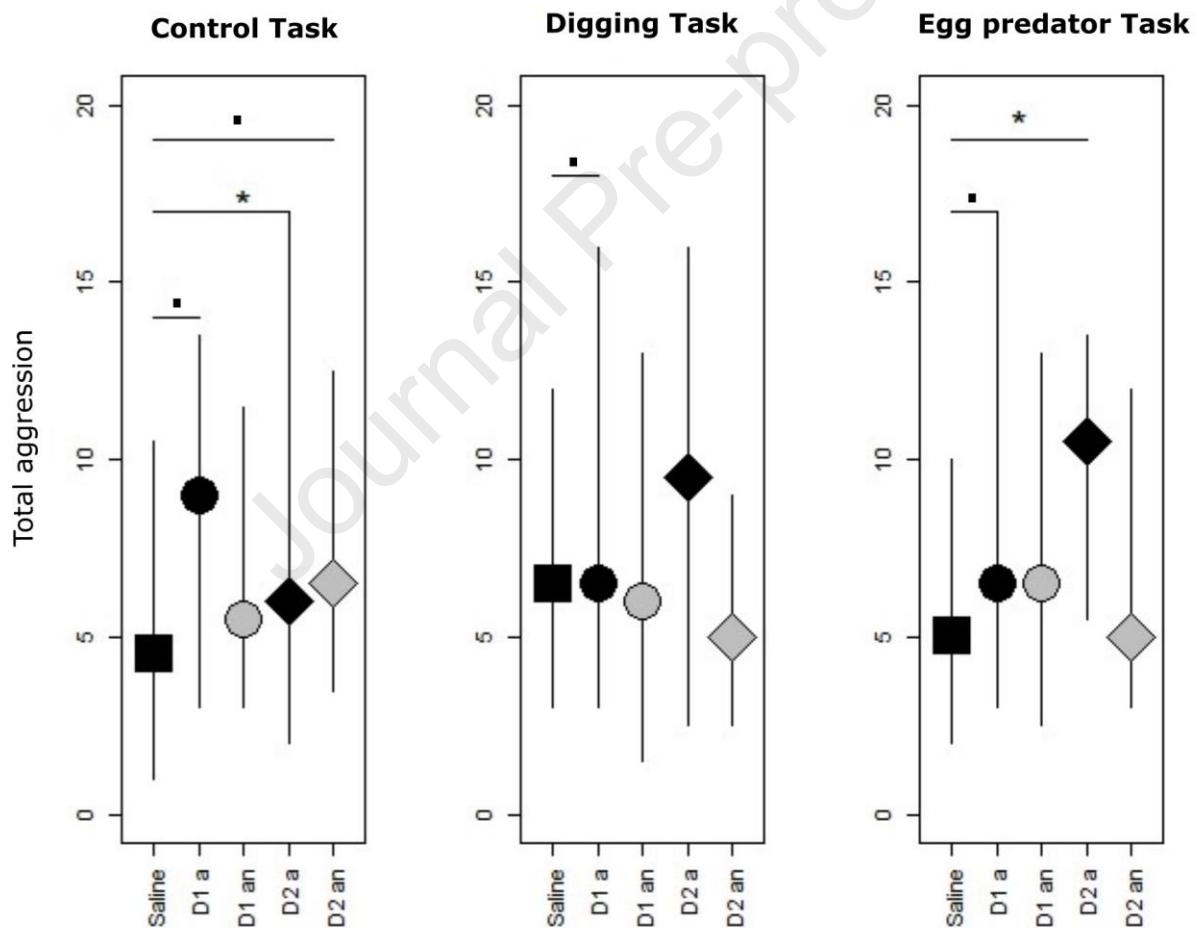
852 D1 antagonist (SCH-23390) represented as grey circles, D2 agonist (Quinpirole) represented as black

853 diamonds, D2 antagonist (Metoclopramide) represented as grey diamonds. Medians and

854 interquartile ranges are shown. Significant differences are indicated with an asterisk and trends (0.1 >

855 p -value > 0.05) with a dot. The control task was always presented first (15min after injection), while856 digging and egg predator tasks were balanced to control for potential sequence effects (*see*857 *Methods*). See Table 5 for statistical details.

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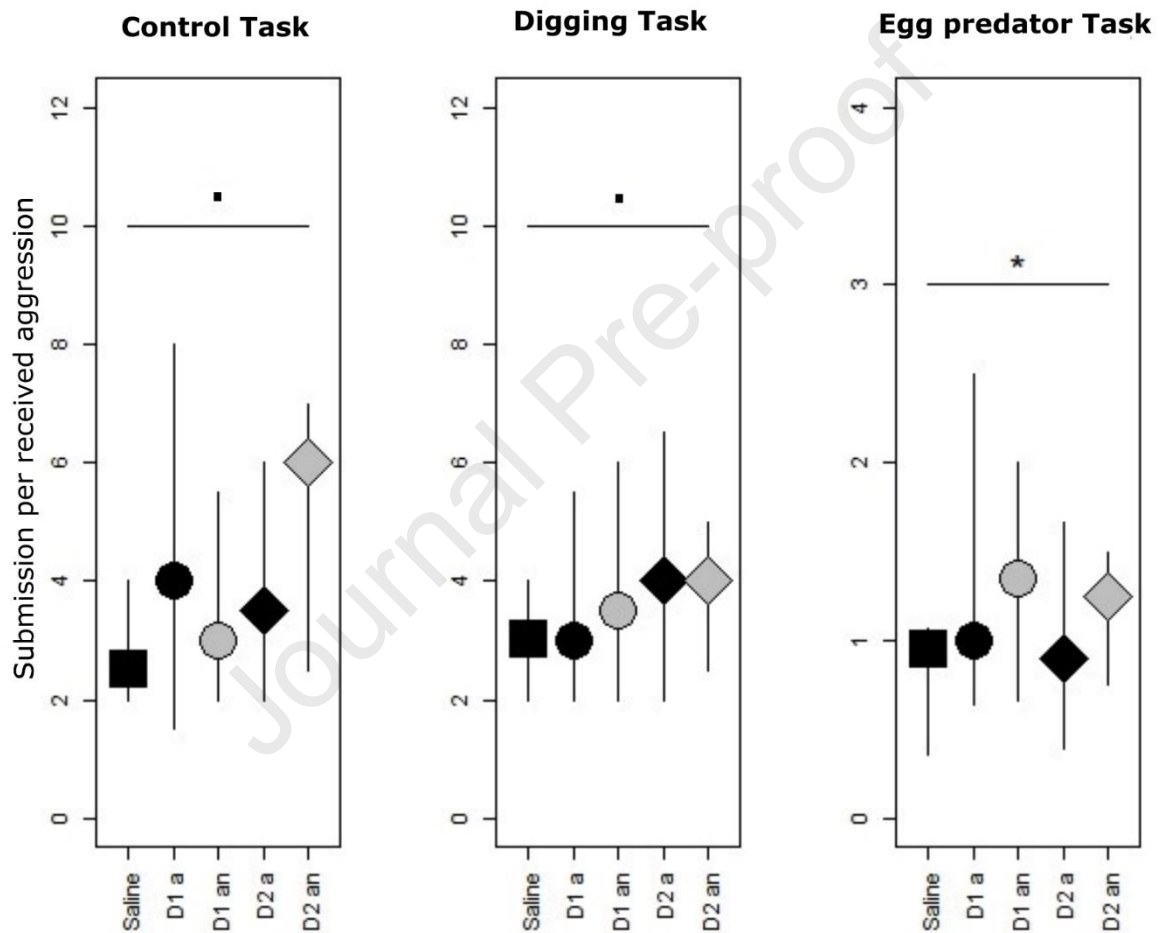
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864 Figure 2. Submission shown per received aggression within a 15 min observation in the context-
 865 dependent experiment (experiment 2) during: a) control task, b) digging task and c) intruder task in
 866 the different treatments: saline represented as black squares, D1 agonist (SKF-38393) represented as
 867 black circles, D1 antagonist (SCH-23390) represented as grey circles, D2 agonist (Quinpirole)
 868 represented as black diamonds, D2 antagonist (Metoclopramide) represented as grey diamonds.
 869 Medians and interquartile ranges are shown. Significant differences are indicated with an asterisk
 870 and trends ($0.1 > p\text{-value} > 0.05$) with a dot. See Table 5 for statistical details.



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Highlights

- The dopaminergic system plays an important role in determining the salience of social stimuli.
- Group-living animals continuously acquire information from social partners, and adjust their behaviour to the available information.
- We show in a cichlid fish that dopamine plays an important role in modulating social interactions.
- D1-like and D2-like receptors are differently involved in the modulation of aggressive, submissive and affiliative behaviours.
- Environmental context seems to be important for the D2-like receptor's behavioural regulation of social encounters.