

# Journal Pre-proof

Dopamine modulates social behaviour in cooperatively breeding fish

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

PII: S0303-7207(22)00097-1

DOI: <https://doi.org/10.1016/j.mce.2022.111649>

Reference: MCE 111649

To appear in: *Molecular and Cellular Endocrinology*

Received Date: 7 March 2021

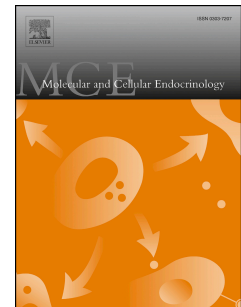
Revised Date: 24 March 2022

Accepted Date: 11 April 2022

Please cite this article as: Antunes, D.F., Soares, M.C., Taborsky, M., Dopamine modulates social behaviour in cooperatively breeding fish, *Molecular and Cellular Endocrinology* (2022), doi: <https://doi.org/10.1016/j.mce.2022.111649>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier B.V.



# Dopamine modulates social behaviour in cooperatively breeding fish

Diogo F. Antunes<sup>1,2</sup>; Marta C. Soares<sup>3,4</sup>; Michael Taborsky<sup>1</sup>

<sup>1</sup>Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, CH-3032

Hinterkappelen, Switzerland

<sup>2</sup>Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande,

1749-016 Lisboa, Portugal

<sup>3</sup>CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório

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

<sup>4</sup> BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-

661 Vairão, Portugal

Corresponding author:

Diogo F. Antunes

Institute of Ecology and Evolution

University of Bern

[diogo.antunes@iee.unibe.ch](mailto:diogo.antunes@iee.unibe.ch)

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

## Introduction

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

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

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

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

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

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

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

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

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

## Methods

### *Study species*

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

### *Subjects and housing conditions*

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

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 Tanganyika (Arnold and Taborsky, 2010). All the fish were fed with commercial flake food (5days/week) and defrosted fresh food (1day/week).

### *Pharmacological manipulation*

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



## Behavioural Analysis

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

## Experimental Design

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

To control for individual variation, we conducted a within-subject design and collected repeated behavioural measures for each individual. In total, eight *N. pulcher* groups were used (N= 16 helpers, eight small and eight large helpers); four groups were tested for the D1-like receptors and injected with three different dosages of the D1-like receptor agonist (SKF-38393: 0.0075, 0.04, 0.075 µg/gbw), the D1-like receptor antagonist (SCH-23390: 0.0015, 0.0075, 0.022 µg/gbw), and the control solution (0.9%NaCl; N=8 helpers from four different families), making a total of seven injections per individual with three to four days intervals between injections. The remaining four groups, were tested for the D2-like receptor activity and injected with three different dosages of the D2 agonist (Quinpirole: 0.0075, 0.03, 0.05 µg/gbw), the D2 antagonist (Metoclopramide: 0.0075, 0.04, 0.075 µg/gbw), and the control solution (0.9%NaCl; N=8 helpers from four different families). Making a total of seven injections per individuals with three to four days interval between injections. Intramuscular injection into the caudal muscle was performed for only one of the group’s helpers at a time. Each focal fish’s behaviour was recorded: its social interactions and with whom they occurred. Observations were done at four different time points: 15 min before the injection, and at 15 min, 30 min and 60 min after the injection. At the beginning of the experiment the shelters were filled with sand to stimulate digging behaviour. In case one of the helpers was evicted from the group it was replaced by another fish with the same size and sex (eleven replacements over the whole experiment). After the new helper was accepted and the family had stabilized again, we proceeded with the experiment.

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

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

*Statistical Analyses*

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

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

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

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

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

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

## **Results**

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

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

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

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

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

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

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

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

## Discussion

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

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

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

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

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

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

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



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

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

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



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

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

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

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

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

## Acknowledgements

We thank Jon Andreja Nuotclà and Mukta Watve for their constructive criticism on an earlier version of this manuscript; Evi Zwygart, Corinna von Kuerthy and Valentina Balzarini for logistical help during

the experiment. This study was supported by SNF-grants 31003A\_156152 and 31003A\_176174 to MT. M.C.S. is currently supported by National Funds through Fundação para a Ciência e a Tecnologia (DL57/2016/CP1440/CT0019).

## References

- Antunes, D.F., Teles, M.C., Zuelling, M., Friesen, C.N., Oliveira, R.F., Aubin-Horth, N., Taborsky, B., 2021. Early social deprivation shapes neuronal programming of the social decision-making network in a cooperatively breeding fish. *Mol. Ecol. mec.* 16019. <https://doi.org/10.1111/mec.16019>
- Aragona, B.J., 2009. Dopamine regulation of social choice in a monogamous rodent species. *Front. Behav. Neurosci.* 3, 1–11. <https://doi.org/10.3389/neuro.08.015.2009>
- Arnold, C., Taborsky, B., 2010. Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids. *Anim. Behav.* 79, 621–630. <https://doi.org/10.1016/j.anbehav.2009.12.008>
- Balshine, S., Leach, B., Neat, F., Reid, H., Taborsky, M., Werner, N., 2001. Correlates of group size in a cooperatively breeding cichlid fish ( *Neolamprologus pulcher* ). *Behav. Ecol. Sociobiol.* 50, 134–140. <https://doi.org/10.1007/s002650100343>
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67. <https://doi.org/10.18637/jss.v067.i01>
- Bello, E.P., Mateo, Y., Gelman, D.M., Noaín, D., Shin, J.H., Low, M.J., Alvarez, V.A., Lovinger, D.M., Rubinstein, M., 2011. Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nat. Neurosci.* 14, 1033–8. <https://doi.org/10.1038/nn.2862>
- Bender, N., Heg, D., Hamilton, I.M., Bachar, Z., Taborsky, M., Oliveira, R.F., 2006. The relationship between social status, behaviour, growth and steroids in male helpers and breeders of a cooperatively breeding cichlid. *Horm. Behav.* 50, 173–182. <https://doi.org/10.1016/j.yhbeh.2006.02.009>
- Bergmüller, R., Heg, D., Taborsky, M., 2005. Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. *Proc. Biol. Sci.* 272, 325–31. <https://doi.org/10.1098/rspb.2004.2960>
- Bergmüller, R., Taborsky, M., 2005. Experimental manipulation of helping in a cooperative breeder: helpers ‘pay to stay’ by pre-emptive appeasement. *Anim. Behav.* 69, 19–28. <https://doi.org/10.1016/j.anbehav.2004.05.009>
- Blumstein, D.T., Ebensperger, L.A., Hayes, L.D., Vásquez, R.A., Ahern, T.H., Burger, R., Dolezal, A.G.,

- Dosmann, A., González-mariscal, G., Harris, B.N., Emilio, A., 2010. Toward an integrative understanding of social behavior : new models and new opportunities. *Front. Behav. Neurosci.* 4, 1–9. <https://doi.org/10.3389/fnbeh.2010.00034>
- Bondar, N.P., Kudryavtseva, N.N., 2005. The effects of the D1 receptor antagonist SCH-23390 on individual and aggressive behavior in male mice with different experience of aggression. *Neurosci Behav Physiol* 35, 221–227.
- Bruintjes, R., Taborsky, M., 2011. Size-dependent task specialization in a cooperative cichlid in response to experimental variation of demand. *Anim. Behav.* 81, 387–394. <https://doi.org/10.1016/j.anbehav.2010.10.004>
- Bruintjes, R., Taborsky, M., 2008. Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Anim. Behav.* 75, 1843–1850. <https://doi.org/10.1016/j.anbehav.2007.12.004>
- Bshary, R., Würth, M., 2001. Cleaner fish *Labroides dimidiatus* manipulate client reef fish by providing tactile stimulation. *Proc. R. Soc. London. Ser. B Biol. Sci.* 268, 1495–1501. <https://doi.org/10.1098/rspb.2001.1495>
- Bukhari, S.A., Saul, M.C., Seward, C.H., Zhang, H., Bensky, M., James, N., Zhao, S.D., Chandrasekaran, S., Stubbs, L., Bell, A.M., 2017. Temporal dynamics of neurogenomic plasticity in response to social interactions in male threespined sticklebacks. *PLOS Genet.* 13, e1006840. <https://doi.org/10.1371/journal.pgen.1006840>
- Cooper, S.J., Al-Naser, H. a, 2006. Dopaminergic control of food choice: contrasting effects of SKF 38393 and quinpirole on high-palatability food preference in the rat. *Neuropharmacology* 50, 953–63. <https://doi.org/10.1016/j.neuropharm.2006.01.006>
- Courtin, J., Bitterman, Y., Müller, S., Hinz, J., Hagihara, K.M., Müller, C., Lüthi, A., 2022. A neuronal mechanism for motivational control of behavior. *Science (80-. ).* 375. <https://doi.org/10.1126/science.abg7277>
- Dall, S.R.X., Giraldeau, L.A., Olsson, O., McNamara, J.M., Stephens, D.W., 2005. Information and its use by animals in evolutionary ecology. *Trends Ecol. Evol.* 20, 187–193. <https://doi.org/10.1016/j.tree.2005.01.010>
- de Abreu, M.S., Maximino, C., Cardoso, S.C., Marques, C.I., Pimentel, A.F.N., Mece, E., Winberg, S., Barcellos, L.J.G., Soares, M.C., 2020. Dopamine and serotonin mediate the impact of stress on cleaner fish cooperative behavior. *Horm. Behav.* 125, 104813. <https://doi.org/10.1016/j.yhbeh.2020.104813>
- de Lima, M.N.M., Presti-Torres, J., Dornelles, A., Siciliani Scalco, F., Roesler, R., Garcia, V.A., Schröder, N., 2011. Modulatory influence of dopamine receptors on consolidation of object recognition

- memory. *Neurobiol. Learn. Mem.* 95, 305–310. <https://doi.org/10.1016/j.nlm.2010.12.007>
- Delville, Y., De Vries, G.J., Ferris, C.F., 2000. Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. *Brain. Behav. Evol.* 55, 53–76. <https://doi.org/10.1159/000006642>
- Dong, C.J., McReynolds, J.S., 1991. The relationship between light, dopamine release and horizontal cell coupling in the mudpuppy retina. *J. Physiol.* 440, 291–309. <https://doi.org/10.1113/jphysiol.1991.sp018709>
- Duftner, N., Sefc, K.M., Koblmüller, S., Salzburger, W., Taborsky, M., Sturmbauer, C., 2007. Parallel evolution of facial stripe patterns in the *Neolamprologus brichardi/pulcher* species complex endemic to Lake Tanganyika. *Mol. Phylogenet. Evol.* 45, 706–715. <https://doi.org/10.1016/j.ympev.2007.08.001>
- Fischer, S., Bohn, L., Oberhummer, E., Nyman, C., Taborsky, B., 2017. Divergence of developmental trajectories is triggered interactively by early social and ecological experience in a cooperative breeder. *Proc. Natl. Acad. Sci.* 114, E9300–E9307. <https://doi.org/10.1073/pnas.1705934114>
- Fischer, S., Zöttl, M., Groenewoud, F., Taborsky, B., 2014. Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proc. R. Soc. B Biol. Sci.* 281, 20140184. <https://doi.org/10.1098/rspb.2014.0184>
- Floresco, S.B., Phillips, A.G., 2001. Delay-dependent modulation of memory retrieval by infusion of a dopamine D1 agonist into the rat medial prefrontal cortex. *Behav. Neurosci.* 115, 934–939. <https://doi.org/10.1037/0735-7044.115.4.934>
- Freudiger, A., Josi, D., Thünken, T., Herder, F., Flury, J.M., Marques, D.A., Taborsky, M., Frommen, J.G., 2021. Ecological variation drives morphological differentiation in a highly social vertebrate. *Funct. Ecol.* 1365–2435.13857. <https://doi.org/10.1111/1365-2435.13857>
- Goodson, J.L., 2005. The vertebrate social behavior network: evolutionary themes and variations. *Horm. Behav.* 48, 11–22. <https://doi.org/10.1016/j.yhbeh.2005.02.003>
- Groenewoud, F., Frommen, J.G., Josi, D., Tanaka, H., Jungwirth, A., Taborsky, M., 2016. Predation risk drives social complexity in cooperative breeders. *Proc. Natl. Acad. Sci.* 113, 4104–4109. <https://doi.org/10.1073/pnas.1524178113>
- Gutter, A.S., 2004. Cleaner Fish Use Tactile Dancing Behavior as a Preconflict Management Strategy. *Curr. Biol.* 14, 1080–1083. <https://doi.org/10.1016/j.cub.2004.05.048>
- Hamilton, I., Bender, N., Heg, D., 2005. Size differences within a dominance hierarchy influence conflict and help in a cooperatively breeding cichlid. *Behaviour* 142, 1591–1613. <https://doi.org/10.1163/156853905774831846>
- Heg, D., Brouwer, L., Bachar, Z., Taborsky, M., 2005. Large group size yields group stability in the

- cooperatively breeding cichlid *Neolamprologus pulcher*. *Behaviour* 142, 1615–1641.  
<https://doi.org/10.1163/156853905774831891>
- Heg, D., Taborsky, M., 2010. Helper response to experimentally manipulated predation risk in the cooperatively breeding cichlid *Neolamprologus pulcher*. *PLoS One* 5, e10784.  
<https://doi.org/10.1371/journal.pone.0010784>
- Heimovics, S. a., Cornil, C. a., Ball, G.F., Ritters, L.V., 2009. D1-like dopamine receptor density in nuclei involved in social behavior correlates with song in a context-dependent fashion in male European starlings. *Neuroscience* 159, 962–973.  
<https://doi.org/10.1016/j.neuroscience.2009.01.042>
- Heimovics, S. a, Ritters, L. V, 2008. Evidence that dopamine within motivation and song control brain regions regulates birdsong context-dependently. *Physiol. Behav.* 95, 258–66.  
<https://doi.org/10.1016/j.physbeh.2008.06.009>
- Hert, E., 1985. Individual Recognition of Helpers by the Breeders in the Cichlid Fish *Lamprologus brichardi* (Poll, 1974). *Z. Tierpsychol.* 68, 313–325. <https://doi.org/10.1111/j.1439-0310.1985.tb00132.x>
- Hofmann, H.A., Beery, A.K., Blumstein, D.T., Couzin, I.D., Earley, R.L., Hayes, L.D., Hurd, P.L., Lacey, E.A., Phelps, S.M., Solomon, N.G., Taborsky, M., Young, L.J., Rubenstein, D.R., 2014. An evolutionary framework for studying mechanisms of social behavior. *Trends Ecol. Evol.* 29, 581–589. <https://doi.org/10.1016/j.tree.2014.07.008>
- Joëls, M., Baram, T.Z., 2009. The neuro-symphony of stress. *Nat. Rev. Neurosci.* 10, 459–66.  
<https://doi.org/10.1038/nrn2632>
- Jungwirth, A., Josi, D., Walker, J., Taborsky, M., 2015. Benefits of coloniality: Communal defence saves anti-predator effort in cooperative breeders. *Funct. Ecol.* 29, 1218–1224.  
<https://doi.org/10.1111/1365-2435.12430>
- Kasper, C., Schreier, T., Taborsky, B., 2019. Heritabilities, social environment effects and genetic correlations of social behaviours in a cooperatively breeding vertebrate. *J. Evol. Biol.* jeb.13494.  
<https://doi.org/10.1111/jeb.13494>
- Keeler, J.F., Pretsell, D.O., Robbins, T.W., 2014. Functional implications of dopamine D1 vs. D2 receptors: A “prepare and select” model of the striatal direct vs. indirect pathways. *Neuroscience* 282, 156–175. <https://doi.org/10.1016/j.neuroscience.2014.07.021>
- Lerena, D.A.M., Antunes, D.F., Taborsky, B., 2021. The interplay between winner–loser effects and social rank in cooperatively breeding vertebrates. *Anim. Behav.* 177, 19–29.  
<https://doi.org/10.1016/j.anbehav.2021.04.011>
- Loos, M., Pattij, T., Janssen, M.C.W., Counotte, D.S., Schoffemeer, A.N.M., Smit, A.B., Spijker, S., van

- Gaalen, M.M., 2010. Dopamine Receptor D1/D5 Gene Expression in the Medial Prefrontal Cortex Predicts Impulsive Choice in Rats. *Cereb. Cortex* 20, 1064–1070.  
<https://doi.org/10.1093/cercor/bhp167>
- Maruska, K.P., Anselmo, C.M., King, T., Mobley, R.B., Ray, E.J., Wayne, R., 2022. Endocrine and neuroendocrine regulation of social status in cichlid fishes. *Horm. Behav.* 139, 105110.  
<https://doi.org/10.1016/j.yhbeh.2022.105110>
- McIntyre, D.C., Healy, L.M., Saari, M., 1979. Intraspecies aggression and monoamine levels in rainbow trout (*Salmo gairdneri*) fingerlings. *Behav. Neural Biol.* 25, 90–98.  
[https://doi.org/10.1016/S0163-1047\(79\)90807-0](https://doi.org/10.1016/S0163-1047(79)90807-0)
- Melis, A.P., Warneken, F., Jensen, K., Schneider, A.-C., Call, J., Tomasello, M., 2011. Chimpanzees help conspecifics obtain food and non-food items. *Proc. R. Soc. B Biol. Sci.* 278, 1405–1413.  
<https://doi.org/10.1098/rspb.2010.1735>
- Messias, J.P.M., Paula, J.R., Grutter, A.S., Bshary, R., Soares, M.C., 2016a. Dopamine disruption increases negotiation for cooperative interactions in a fish. *Sci. Rep.* 6, 20817.  
<https://doi.org/10.1038/srep20817>
- Messias, J.P.M., Santos, T.P., Pinto, M., Soares, M.C., 2016b. Stimulation of dopamine D 1 receptor improves learning capacity in cooperating cleaner fish. *Proc. R. Soc. B Biol. Sci.* 283, 20152272.  
<https://doi.org/10.1098/rspb.2015.2272>
- Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors: from structure to function. *Physiol. Rev.* 78, 189–225. <https://doi.org/10.1186/1471-2296-12-32>
- Monte-Silva, K., Kuo, M.F., Thirugnanasambandam, N., Liebetanz, D., Paulus, W., Nitsche, M.A., 2009. Dose-dependent inverted U-shaped effect of dopamine (D2-like) receptor activation on focal and nonfocal plasticity in humans. *J. Neurosci.* 29, 6124–6131.  
<https://doi.org/10.1523/JNEUROSCI.0728-09.2009>
- Munro, A.D., 1986. The effects of apomorphine, d-amphetamine and chlorpromazine on the aggressiveness of isolated *Aequidens pulcher* (Teleostei, Cichlidae). *Psychopharmacology (Berl.)* 88, 124–128. <https://doi.org/10.1007/BF00310527>
- Naef, J., Taborsky, M., 2020a. Punishment controls helper defence against egg predators but not fish predators in cooperatively breeding cichlids. *Anim. Behav.* 168, 137–147.  
<https://doi.org/10.1016/j.anbehav.2020.08.006>
- Naef, J., Taborsky, M., 2020b. Commodity-specific punishment for experimentally induced defection in cooperatively breeding fish. *R. Soc. Open Sci.* 7. <https://doi.org/10.1098/rsos.191808>
- Nelson, R.J., Trainor, B.C., 2007. Neural mechanisms of aggression. *Nat. Rev. Neurosci.* 8, 536–546.  
<https://doi.org/10.1038/nrn2174>



- O'Connell, L. a., Hofmann, H. a., 2012. Evolution of a Vertebrate Social Decision-Making Network. *Science* (80-. ). 336, 1154–1157. <https://doi.org/10.1126/science.1218889>
- O'Connell, L. a., Hofmann, H.A., 2011. Genes, hormones, and circuits: An integrative approach to study the evolution of social behavior. *Front. Neuroendocrinol.* 32, 320–335. <https://doi.org/10.1016/j.yfrne.2010.12.004>
- O'Connell, L.A., Hofmann, H.A., 2011. The Vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *J. Comp. Neurol.* 519, 3599–3639. <https://doi.org/10.1002/cne.22735>
- Oliveira, R.F., 2009. Social behavior in context: Hormonal modulation of behavioral plasticity and social competence. *Integr. Comp. Biol.* 49, 423–440. <https://doi.org/10.1093/icb/icp055>
- Overli, O., Harris, C.A., Winberg, S., 1999. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain. Behav. Evol.* 54, 263–275. <https://doi.org/10.1159/000006627>
- Paula, J.R., Messias, J.P., Grutter, A.S., Bshary, R., Soares, M.C., 2015. The role of serotonin in the modulation of cooperative behavior. *Behav. Ecol.* 26, 1005–1012. <https://doi.org/10.1093/beheco/arv039>
- R Core Team, 2018. R: A Language and Environment for Statistical Computing.
- Robinson, G.E., Fernald, R.D., Clayton, D.F., 2008. Genes and Social Behavior. *Science* (80-. ). 322, 896–900. <https://doi.org/10.1126/science.1159277>
- Schultz, W., 2006. Behavioral theories and the neurophysiology of reward. *Annu. Rev. Psychol.* 57, 87–115. <https://doi.org/10.1146/annurev.psych.56.091103.070229>
- Schultz, W., 2002. Getting formal with dopamine and reward. *Neuron* 36, 241–263. [https://doi.org/10.1016/S0896-6273\(02\)00967-4](https://doi.org/10.1016/S0896-6273(02)00967-4)
- Seamans, J.K., Yang, C.R., 2004. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog. Neurobiol.* 74, 1–58. <https://doi.org/10.1016/j.pneurobio.2004.05.006>
- Silva, P.A., Trigo, S., Marques, C.I., Cardoso, G.C., Soares, M.C., 2020. Experimental evidence for a role of dopamine in avian personality traits. *J. Exp. Biol.* 223. <https://doi.org/10.1242/jeb.216499>
- Soares, M.C., 2017. The Neurobiology of Mutualistic Behavior: The Cleanerfish Swims into the Spotlight. *Front. Behav. Neurosci.* 11, 1–12. <https://doi.org/10.3389/fnbeh.2017.00191>
- Soares, M.C., Bshary, R., Fusani, L., Goymann, W., Hau, M., Hirschenhauser, K., Oliveira, R.F., 2010. Hormonal mechanisms of cooperative behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* 365, 2737–2750. <https://doi.org/10.1098/rstb.2010.0151>



- 752 Soares, Marta C., Cardoso, S.C., Malato, J.T., Messias, J.P.M., 2017. Can cleanerfish overcome  
 753 temptation? A selective role for dopamine influence on cooperative-based decision making.  
 754 *Physiol. Behav.* 169, 124–129. <https://doi.org/10.1016/j.physbeh.2016.11.028>
- 755 Soares, Marta C, Santos, T.P., Messias, J.P.M., 2017. Dopamine disruption increases cleanerfish  
 756 cooperative investment in novel client partners. *R. Soc. Open Sci.* 4, 160609.  
 757 <https://doi.org/10.1098/rsos.160609>
- 758 St. Onge, J.R., Abhari, H., Floresco, S.B., 2011. Dissociable Contributions by Prefrontal D1 and D2  
 759 Receptors to Risk-Based Decision Making. *J. Neurosci.* 31, 8625–8633.  
 760 <https://doi.org/10.1523/JNEUROSCI.1020-11.2011>
- 761 Stettler, P.R., F. Antunes, D., Taborsky, B., 2021. The serotonin 1A receptor modulates the social  
 762 behaviour within groups of a cooperatively-breeding cichlid. *Horm. Behav.* 129, 104918.  
 763 <https://doi.org/10.1016/j.yhbeh.2020.104918>
- 764 Taborsky, B., 2021. A positive feedback loop between sociality and social competence. *Ethology* 127,  
 765 774–789. <https://doi.org/10.1111/eth.13201>
- 766 Taborsky, B., 2016. Opening the Black Box of Developmental Experiments: Behavioural Mechanisms  
 767 Underlying Long-Term Effects of Early Social Experience. *Ethology* 122, 267–283.  
 768 <https://doi.org/10.1111/eth.12473>
- 769 Taborsky, B., Arnold, C., Junker, J., Tschopp, A., 2012. The early social environment affects social  
 770 competence in a cooperative breeder. *Anim. Behav.* 83, 1067–1074.  
 771 <https://doi.org/10.1016/j.anbehav.2012.01.037>
- 772 Taborsky, B., Oliveira, R.F., 2012. Social competence: an evolutionary approach. *Trends Ecol. Evol.*  
 773 27, 679–688. <https://doi.org/10.1016/j.tree.2012.09.003>
- 774 Taborsky, M., 2016. Cichlid fishes: A model for the integrative study of social behavior, in: Koenig ,  
 775 Walter D. and Dickinson, J.L. (Ed.), *Cooperative Breeding in Vertebrates: Studies of Ecology,*  
 776 *Evolution, and Behavior.* Cambridge University Press, pp. 272–293.
- 777 Taborsky, M., 1984a. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and  
 778 benefits. *Anim. Behav.* 32, 1236–1252. [https://doi.org/10.1016/S0003-3472\(84\)80241-9](https://doi.org/10.1016/S0003-3472(84)80241-9)
- 779 Taborsky, M., 1984b. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and  
 780 benefits. *Anim. Behav.* 32, 1236–1252. [https://doi.org/10.1016/S0003-3472\(84\)80241-9](https://doi.org/10.1016/S0003-3472(84)80241-9)
- 781 Taborsky, M., 1984c. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: Their costs and  
 782 benefits. *Anim. Behav.* 32, 1236–1252. [https://doi.org/10.1016/S0003-3472\(84\)80241-9](https://doi.org/10.1016/S0003-3472(84)80241-9)
- 783 Taborsky, M., Cant, M.A., Komdeur, J., 2021. The Evolution of Social Behaviour, The Evolution of  
 784 Social Behaviour. Cambridge University Press. <https://doi.org/10.1017/9780511894794>
- 785 Taborsky, M., Limberger, D., 1981. Helpers in fish. *Behav. Ecol. Sociobiol.* 8, 143–145.

<https://doi.org/10.1007/BF00300826>

Weitekamp, C.A., Nguyen, J., Hofmann, H.A., 2017. Social context affects behavior, preoptic area gene expression, and response to D2 receptor manipulation during territorial defense in a cichlid fish. *Genes, Brain Behav.* 16, 601–611. <https://doi.org/10.1111/gbb.12389>

Winberg, S., Nilsson, G.E., Olsén, H.K., 1991. Social rank and brain levels of monoamines and monoamine metabolites in Arctic charr, *Salvelinus alpinus* (L.). *J. Comp. Physiol. A* 168, 241–246. <https://doi.org/10.1007/BF00218416>

Winberg, S., Nilsson, G.E., Olsén, K.H., 1992. Changes in brain serotonergic activity during hierarchic behavior in Arctic charr (*Salvelinus alpinus* L.) are socially induced. *J. Comp. Physiol. A* 170, 93–99. <https://doi.org/10.1007/BF00190404>

Wong, M., Balshine, S., 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. *Biol. Rev. Camb. Philos. Soc.* 86, 511–30. <https://doi.org/10.1111/j.1469-185X.2010.00158.x>

Zöttl, M., Frommen, J.G., Taborsky, M., 2013a. Group size adjustment to ecological demand in a cooperative breeder. *Proc. Biol. Sci.* 280, 20122772. <https://doi.org/10.1098/rspb.2012.2772>

Zöttl, M., Heg, D., Chervet, N., Taborsky, M., 2013b. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nat. Commun.* 4, 1341. <https://doi.org/10.1038/ncomms2344>

Zupanc, G.K.H., Lamprecht, J., 2000. Towards a Cellular Understanding of Motivation: Structural Reorganization and Biochemical Switching as Key Mechanisms of Behavioral Plasticity. *Ethology* 106, 467–477. <https://doi.org/10.1046/j.1439-0310.2000.00546.x>

## Tables

Table 1. . Results of LMMs for the effects of the D1-like agonist (SKF-38393) from *Experiment 1* the dosage-dependence experiment (N= 8 helpers) on: a) aggression, b) submission (standardized for received aggression), c) affiliative behaviour; 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
<b>a) Aggression</b>	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	<b>0.036</b>
	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>b) Submission</b>	15	0.0075	0.295 $\pm$ 0.239	28	1.234	0.227
		0.04	0.572 $\pm$ 0.239	28	2.397	<b>0.023</b>
		0.075	0.535 $\pm$ 0.239	28	2.243	<b>0.033</b>
	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
<b>c) Affiliative</b>	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>

Table 2. Results of LMMs for the effects of the D1-like antagonist (SCH-23390) from *Experiment 1* the dosage-dependence experiment (N= 8 helpers) on: a) aggression, b) submission (standardized for received aggression), c) affiliative behaviour; 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
<b>a) Aggression</b>	15	0.0015	-0.227 $\pm$ 0.254	21	-0.894	0.381
		0.0075	-0.608 $\pm$ 0.250	21	-2.428	<b>0.024</b>
		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>b) Submission</b>	15	0.0015	0.575 $\pm$ 0.238	28	2.417	<b>0.022</b>
		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	<b>0.028</b>
		0.0075	0.658 $\pm$ 0.278	21	2.366	<b>0.028</b>
		0.022	0.796 $\pm$ 0.278	21	2.861	<b>0.009</b>
	60	0.0015	0.513 $\pm$ 0.240	22	2.139	<b>0.044</b>
		0.0075	0.525 $\pm$ 0.237	21	2.210	<b>0.038</b>
		0.022	0.342 $\pm$ 0.237	21	1.441	0.164
<b>c) Affiliative</b>	15	0.0015	0.558 $\pm$ 0.250	22	2.226	<b>0.037</b>
		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

Table 3. D2-like agonist (Quinpirole) from *Experiment 1*; 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
<b>a) Aggression</b>	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>b) Submission</b>	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
<b>c) Affiliative</b>	15	0.0075	0.508 $\pm$ 0.221	21	2.299	<b>0.032</b>
		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

Table 4. Results of LMMs for the effects of D2-like antagonist (Metoclopramide) from *Experiment 1*; 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
<b>a) Aggression</b>	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>b) Submission</b>	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
<b>c) Affiliative</b>	15	0.0075	0.556 $\pm$ 0.221	21	2.512	<b>0.020</b>
		0.04	0.493 $\pm$ 0.221	21	2.229	<b>0.037</b>
		0.075	0.431 $\pm$ 0.221	21	1.946	0.065
	30	0.0075	0.748 $\pm$ 0.255	28	2.936	<b>0.006</b>
		0.04	0.764 $\pm$ 0.255	28	2.998	<b>0.005</b>
		0.075	0.589 $\pm$ 0.255	28	2.312	<b>0.028</b>
	60	0.0075	0.530 $\pm$ 0.255	21	2.078	0.050
		0.04	0.577 $\pm$ 0.255	21	2.263	<b>0.034</b>
		0.075	0.344 $\pm$ 0.255	21	1.350	0.191

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

Drug	Behaviour	Task	Zero-Inflation	Estimate $\pm$ SE	z	p-value
<b>D1-like agonist</b> (SKF-38393: 0.075 $\mu$ g/gbw)	Aggression	Control	0.032	0.420 $\pm$ 0.228	1.84	0.065
		Digging	0.052	0.445 $\pm$ 0.264	1.69	0.092
		Egg predator	0.038	0.269 $\pm$ 0.213	1.26	0.208
	Submission	Control	1e <sup>-06</sup>	0.198 $\pm$ 0.206	0.96	0.336
		Digging	0.056	0.146 $\pm$ 0.208	0.70	0.484
		Egg predator	0.015	0.370 $\pm$ 0.196	1.89	0.059
	Affiliative	Control	0.306	-0.106 $\pm$ 0.511	-0.21	0.835
		Digging	0.264	0.479 $\pm$ 0.495	0.97	0.33
		Egg predator	1.0003e <sup>-06</sup>	0.773 $\pm$ 0.610	1.27	0.206
	<b>D1-like antagonist</b> (SCH-23390: 0.0075 $\mu$ g/gbw)	Control	0.032	0.115 $\pm$ 0.231	0.50	0.620
		Digging	0.052	0.164 $\pm$ 0.259	0.63	0.528
		Egg predator	0.038	0.138 $\pm$ 0.219	0.63	0.529
<b>D2-like agonist</b> (Quinpirole: 0.0075 $\mu$ g/gbw)	Aggression	Control	0.032	0.554 $\pm$ 0.230	2.41	<b>0.016</b>
		Digging	0.052	0.389 $\pm$ 0.256	1.52	0.129
		Egg predator	0.038	0.470 $\pm$ 0.218	2.16	<b>0.031</b>
	Submission	Control	1e <sup>-06</sup>	0.180 $\pm$ 0.206	0.87	0.383
		Digging	0.056	0.222 $\pm$ 0.216	1.03	0.302
		Egg predator	0.015	0.395 $\pm$ 0.210	1.88	0.060
	Affiliative	Control	0.306	0.203 $\pm$ 0.439	0.46	0.645
		Digging	0.264	0.224 $\pm$ 0.451	0.50	0.62
		Egg predator	1.0003e <sup>-06</sup>	0.947 $\pm$ 0.569	1.66	0.096

<b>D2-like antagonist</b> (Metoclopramide: 0.0075µg/gbw)	Submission	Control	1e <sup>-06</sup>	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 <sup>-06</sup>	0.051±0.634	0.08	0.936
	predator					
	Aggression	Control	0.032	0.418±0.237	1.76	0.078
		Digging	0.052	-0.127±0.256	-0.50	0.620
		Egg	0.038	0.077±0.221	0.35	0.727
	predator					
	Submission	Control	1e <sup>-06</sup>	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	<b>0.004</b>
	predator					
	Affiliative	Control	0.306	1.098±0.550	1.99	<b>0.046</b>
		Digging	0.264	0.134±0.449	0.30	0.77
		Egg	1.0003e <sup>-06</sup>	0.736±0.579	1.27	0.204
	predator					

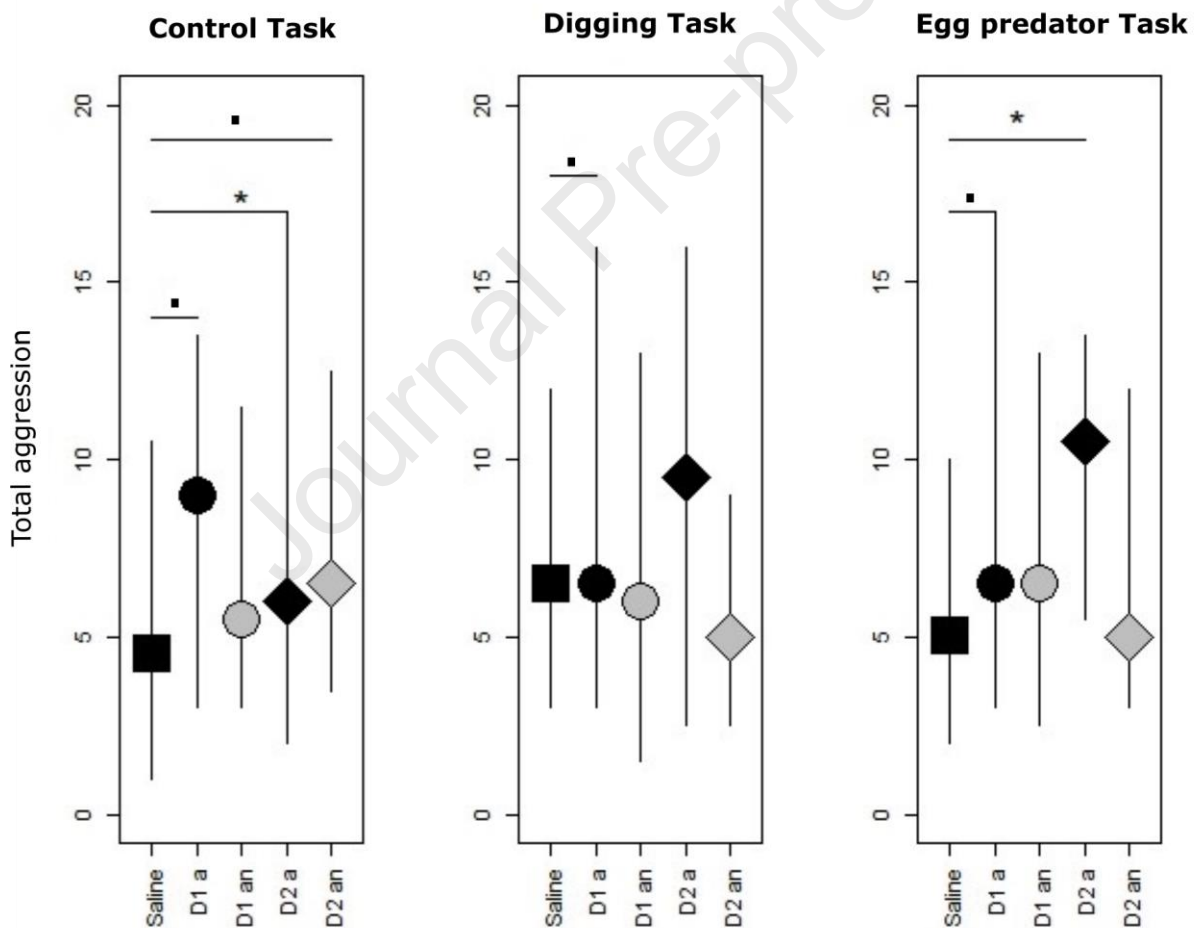
845

846



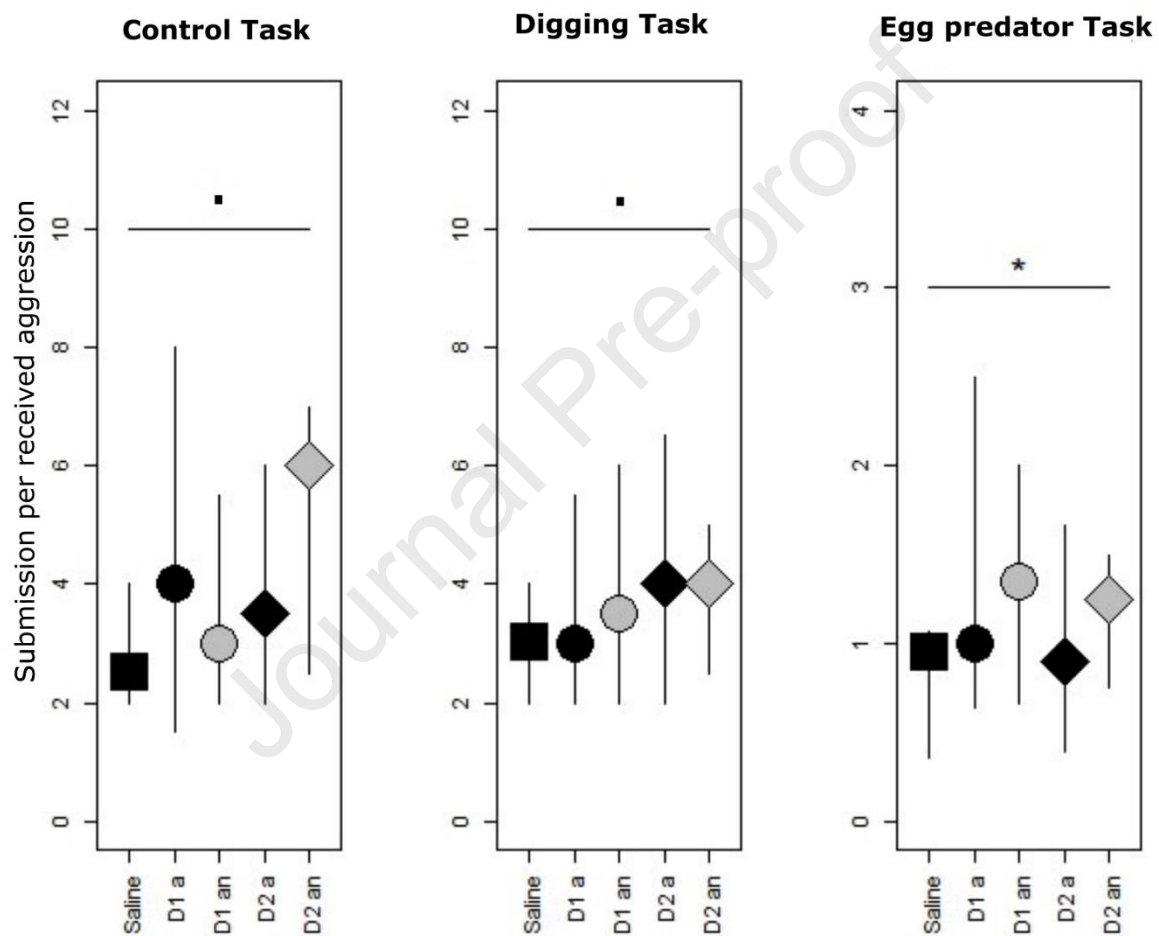
## Figures

Figure 1. Total aggression shown within 15min observations in the context-dependent experiment (experiment 2) during: a) control task, b) digging task and c) intruder task in the different treatments: saline represented as black squares, D1 agonist (SKF-38393) represented as black circles, D1 antagonist (SCH-23390) represented as grey circles, D2 agonist (Quinpirole) represented as black diamonds, D2 antagonist (Metoclopramide) represented as grey diamonds. Medians and interquartile ranges are shown. Significant differences are indicated with an asterisk and trends ( $0.1 > p\text{-value} > 0.05$ ) with a dot. The control task was always presented first (15min after injection), while digging and egg predator tasks were balanced to control for potential sequence effects (see *Methods*). See Table 5 for statistical details.



863

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.



871

## 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.