Dopamine modulates social behaviour in cooperatively breeding fish

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ABSTRACT

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

1. 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., 2010; Melis et al., 2011; Courin 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

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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 Artic char (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 waxwails (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 underlyling social behaviour (Wong and Balshine, 2011; B. Taborsky, 2016; M. 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; Bruinjes and Taborsky, 2011; Heg and Taborsky, 2010; M. 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 Asotutopia 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.

2. Methods

2.1. Study species

N. pulcher is a cooperatively breeding cichlid endemic to lake Tanganyika (Dufner 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; Bruinjes and Taborsky 2011; Naef and Taborsky, 2020b). Through alloparental care, territory defence and maintenance behaviours, helps pay-to-stay in the dominants’ territory (Bergmüller et al., 2005; Bruinjes and Taborsky, 2008; Zöttl et al., 2013b; Fischer et al.,
2014; M. 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 and Taborsky 2020a, b).

2.2. 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 Ethologi sche 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 near Mpulungu, Zambia, were used for our experiments. In total, 10 groups with two helpers (one large and one small) and a breeder pair were experimentally established. All group members had a minimum size difference of 5–10 mm 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 h and at a temperature of 27 ± 1 °C, simulating the conditions in Lake Tanganyika (Arnold and Taborsky, 2010). All the fish were fed with commercial flake food (5 day/week) and defrosted fresh food (1 day/week).

2.3. Pharmacological manipulation

To manipulate the dopaminergic system we performed intramuscular injections aiming at 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 DI-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 μg/mL); D1-like antagonist (SCH-23390: 0.1; 0.5; 1.5 μg/mL); D2-like receptor agonist (Quinpirole: 0.5; 2; 3 μg/mL); D2-like receptor antagonist (Metoclopramide: 0.5; 2.5; 5 μg/mL). Directly after preparation and at the beginning of the experiment the shelters were stored at –20 °C. As control we injected a saline solution (0.9% NaCl). The injected volume was 15 μL per gram of body weight (g bw); Paula et al., 2015; Messias et al., 2016a, 2016b; Stettler et al., 2021). To reduce stress, all tested fish were measured, weighed and anaesthetised with KoMed Sleep (Schönbach Pharmacy, Germany; 0.15 ml for a 300 ml water volume) before the injection. Injections were done using 0.5 ml insulin syringes (0.5 ml 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 min 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.

2.4. 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 Nederlands, 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.

2.5. Experimental design

a) Experiment 1: effects of dopaminergic 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-23930: 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 dopaminergic 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 intramuscular injections into the caudal muscle. Injected solutions contained either a D1-like receptors agonist (SKF-38393: 0.075 μg/gbw) or antagonist (SCH-23930: 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 15 min 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 vitatus*, which is a natural predator of *N. pulcher* eggs (Bruintjes 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).

2.6. 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 dopaminergic 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 fulfill 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 (standardized 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 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).

3. Results

a) Experiment 1: effects of dopaminergic 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 15 min, and also 30 min following treatment, and decreased slightly after 60 min (Table 1; SI Table 1). Submissive behaviour was enhanced 15 min after treatment with the intermediate and higher dosages of the D1 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 15 min after the injection, and this effect declined 30 and 60 min 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 30 min after injection. Affiliative behaviour was significantly enhanced 15 min after treatment with 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 dopaminergic effects on social responses

The D1-like receptor agonist tended to increase aggressive behaviour in the control situation of the 2nd 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, treatment with the D2-like receptor agonist significantly increased aggressive behaviour in the egg predator and control situations (Table 5, Fig. 3).
The D2-like receptors revealed no significant effects on aggression and control situation. In contrast, treatment increased submissive tendencies.

**Table 1**

Results of LMMs for the effects of the D1-like agonist (SKF-38393) from Experiment 1 (dosage-dependence; 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-value > 0.05).

| Behaviour | Time (min) | Dosage (µg/gbw) | Estimate ± SE | df | t   | p-value |
|-----------|------------|----------------|--------------|----|-----|--------|
| a) Aggression | 15 | 0.0075 | 0.755 ± 0.365 | 21 | 2.068 | 0.051 |
| | | 0.04 | 0.676 ± 0.365 | 21 | 1.851 | 0.078 |
| | | 0.075 | 0.706 ± 0.365 | 21 | 1.933 | 0.066 |
| | 30 | 0.0075 | 0.722 ± 0.349 | 21 | 2.066 | 0.051 |
| | | 0.04 | 0.679 ± 0.349 | 21 | 1.944 | 0.065 |
| | | 0.075 | 0.784 ± 0.349 | 21 | 2.242 | 0.036 |
| | 60 | 0.0075 | 0.641 ± 0.371 | 28 | 1.728 | 0.095 |
| | | 0.04 | 0.349 ± 0.371 | 28 | 0.941 | 0.354 |
| | | 0.075 | 0.718 ± 0.371 | 28 | 1.934 | 0.063 |
| b) Submission | 15 | 0.0075 | 0.295 ± 0.239 | 28 | 1.234 | 0.227 |
| | | 0.04 | 0.572 ± 0.239 | 28 | 2.397 | 0.023 |
| | | 0.075 | 0.535 ± 0.239 | 28 | 2.243 | 0.033 |
| | 30 | 0.0075 | 0.313 ± 0.213 | 28 | 1.471 | 0.153 |
| | | 0.04 | 0.227 ± 0.213 | 28 | 1.067 | 0.295 |
| | | 0.075 | 0.42 ± 0.213 | 28 | 0.665 | 0.511 |
| | 60 | 0.0075 | 0.312 ± 0.209 | 28 | 1.494 | 0.146 |
| | | 0.04 | 0.265 ± 0.209 | 28 | 1.270 | 0.215 |
| | | 0.075 | 0.171 ± 0.209 | 28 | 0.819 | 0.420 |
| c) Affiliation | 15 | 0.0075 | 0.434 ± 0.265 | 28 | 1.638 | 0.113 |
| | | 0.04 | 0.433 ± 0.265 | 28 | 1.635 | 0.113 |
| | | 0.075 | 0.290 ± 0.265 | 28 | 1.095 | 0.283 |
| | 30 | 0.0075 | 0.304 ± 0.247 | 21 | 1.230 | 0.232 |
| | | 0.04 | 0.353 ± 0.247 | 21 | 1.432 | 0.167 |
| | | 0.075 | 0.355 ± 0.247 | 21 | 1.436 | 0.166 |
| | 60 | 0.0075 | 0.397 ± 0.236 | 21 | 1.683 | 0.107 |
| | | 0.04 | 0.43 ± 0.236 | 21 | 1.828 | 0.082 |
| | | 0.075 | 0.432 ± 0.236 | 21 | 1.830 | 0.081 |

**Table 2**

Results of LMMs for the effects of the D1-like antagonist (SCH-23390) from Experiment 1 (dosage-dependence; 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-value > 0.05).

| Behaviour | Time (min) | Dosage (µg/gbw) | Estimate ± SE | df | t   | p-value |
|-----------|------------|----------------|--------------|----|-----|--------|
| a) Aggression | 15 | 0.0015 | -0.227 ± 0.254 | 21 | -0.894 | 0.381 |
| | | 0.0075 | -0.608 ± 0.250 | 21 | -2.428 | 0.024 |
| | | 0.022 | -0.269 ± 0.250 | 21 | -1.073 | 0.295 |
| | 30 | 0.0015 | -0.161 ± 0.280 | 21 | -0.577 | 0.570 |
| | | 0.0075 | -0.537 ± 0.276 | 21 | -2.016 | 0.057 |
| | | 0.022 | -0.23 ± 0.276 | 21 | -1.171 | 0.254 |
| | | 0.075 | -0.383 ± 0.302 | 21 | -1.267 | 0.219 |
| | 60 | 0.0015 | -0.543 ± 0.298 | 21 | -1.821 | 0.083 |
| | | 0.022 | -0.328 ± 0.298 | 21 | -1.101 | 0.283 |
| b) Submission | 15 | 0.0015 | 0.575 ± 0.238 | 28 | 2.417 | 0.022 |
| | | 0.0075 | 0.209 ± 0.238 | 28 | 0.879 | 0.387 |
| | | 0.022 | 0.356 ± 0.238 | 28 | 1.497 | 0.146 |
| | 30 | 0.0015 | 0.659 ± 0.281 | 22 | 2.350 | 0.028 |
| | | 0.0075 | 0.658 ± 0.278 | 21 | 2.366 | 0.028 |
| | | 0.022 | 0.796 ± 0.278 | 21 | 2.861 | 0.007 |
| | | 0.075 | 0.513 ± 0.240 | 22 | 2.139 | 0.044 |
| | 60 | 0.0015 | 0.525 ± 0.237 | 21 | 2.210 | 0.038 |
| | | 0.022 | 0.342 ± 0.237 | 21 | 1.441 | 0.164 |
| c) Affiliation | 15 | 0.0015 | 0.558 ± 0.250 | 22 | 2.226 | 0.037 |
| | | 0.0075 | 0.331 ± 0.249 | 21 | 1.249 | 0.197 |
| | | 0.022 | 0.179 ± 0.249 | 21 | 0.720 | 0.480 |
| | 30 | 0.0015 | 0.412 ± 0.362 | 28 | 1.133 | 0.267 |
| | | 0.0075 | 0.182 ± 0.362 | 28 | 0.502 | 0.620 |
| | | 0.022 | -0.104 ± 0.362 | 28 | -0.287 | 0.776 |
| | 60 | 0.0015 | 0.273 ± 0.334 | 28 | 0.818 | 0.420 |
| | | 0.0075 | -0.069 ± 0.334 | 28 | -0.208 | 0.837 |
| | | 0.022 | -0.104 ± 0.334 | 28 | -0.313 | 0.757 |

Fig. 1), while submissive and affiliative behaviours were not influenced. 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, treatment increased submissive tendencies in all three experimental situations, more so when environmental subjects were challenged by an egg predator (Table 5, Figs. 1, 2). This differed from the situation in experiment 1, 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.

4. Discussion

Our results demonstrate that the two classes of dopamine receptors have very distinct roles in behavioural regulation of subordinate helpers.
Table 3
D2-like agonist (Quinpirole) from Experiment 1 (dosage-dependence; 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-value > 0.05).

| Behaviour | Time (min) | Dosage (μg/gbw) | Estimate ± SE | df | t  | p-value |
|-----------|------------|-----------------|---------------|----|----|---------|
| a) Aggression | 15 | 0.0075 | 0.579 ± 0.366 | 21 | 1.582 | 0.128 |
| | | 0.03 | 0.666 ± 0.366 | 21 | 1.819 | 0.083 |
| | | 0.05 | 0.392 ± 0.366 | 21 | 1.071 | 0.296 |
| | 30 | 0.0075 | 0.377 ± 0.377 | 21 | 1.094 | 0.286 |
| | | 0.03 | 0.412 ± 0.377 | 21 | 1.094 | 0.286 |
| | | 0.05 | −0.055 ± 0.377 | 21 | −0.147 | 0.885 |
| | 60 | 0.0075 | 0.607 ± 0.371 | 21 | 1.636 | 0.117 |
| | | 0.03 | 0.585 ± 0.371 | 21 | 1.577 | 0.130 |
| | | 0.05 | 0.212 ± 0.371 | 21 | 0.573 | 0.573 |
| b) Submission | 15 | 0.0075 | 0.184 ± 0.234 | 21 | 0.787 | 0.443 |
| | | 0.03 | 0.029 ± 0.234 | 21 | 0.126 | 0.901 |
| | | 0.05 | −0.293 ± 0.234 | 21 | −1.251 | 0.225 |
| | 30 | 0.0075 | −0.225 ± 0.270 | 21 | −0.832 | 0.415 |
| | | 0.03 | −0.206 ± 0.270 | 21 | −0.762 | 0.455 |
| | | 0.05 | −0.229 ± 0.270 | 21 | −1.033 | 0.313 |
| | 60 | 0.0075 | 0.017 ± 0.193 | 21 | 0.087 | 0.931 |
| | | 0.03 | 0.052 ± 0.193 | 21 | 0.271 | 0.789 |
| | | 0.05 | −0.217 ± 0.193 | 21 | −1.125 | 0.273 |
| c) Affiliation | 15 | 0.0075 | 0.508 ± 0.221 | 21 | 2.299 | 0.032 |
| | | 0.03 | 0.397 ± 0.221 | 21 | 1.795 | 0.087 |
| | | 0.05 | 0.397 ± 0.221 | 21 | 1.795 | 0.087 |
| | 30 | 0.0075 | 0.334 ± 0.254 | 21 | 1.318 | 0.202 |
| | | 0.03 | −0.078 ± 0.254 | 21 | −0.306 | 0.762 |
| | | 0.05 | 0.241 ± 0.254 | 21 | 0.949 | 0.353 |
| | 60 | 0.0075 | 0.265 ± 0.252 | 21 | 1.051 | 0.355 |
| | | 0.03 | −0.154 ± 0.252 | 21 | −0.609 | 0.549 |
| | | 0.05 | 0.167 ± 0.252 | 21 | 0.662 | 0.515 |

Table 4
Results of LMMs for the effects of D2-like antagonist (Metoclopramide) from Experiment 1 (dosage-dependence; 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-value > 0.05).

| Behaviour | Time (min) | Dosage (μg/gbw) | Estimate ± SE | df | t  | p-value |
|-----------|------------|-----------------|---------------|----|----|---------|
| a) Aggression | 15 | 0.0075 | 0.191 ± 0.370 | 21 | 0.515 | 0.612 |
| | | 0.04 | 0.266 ± 0.370 | 21 | 0.718 | 0.480 |
| | 30 | 0.0075 | −0.430 ± 0.370 | 21 | −1.161 | 0.259 |
| | | 0.075 | 0.237 ± 0.371 | 21 | 0.637 | 0.531 |
| | | 0.04 | 0.250 ± 0.371 | 21 | 0.672 | 0.509 |
| | | 0.075 | −0.468 ± 0.371 | 21 | −1.259 | 0.222 |
| | 60 | 0.0075 | 0.564 ± 0.420 | 21 | 1.345 | 0.193 |
| | | 0.04 | 0.710 ± 0.420 | 21 | 1.691 | 0.106 |
| | | 0.075 | 0.219 ± 0.420 | 21 | 0.523 | 0.606 |
| b) Submission | 15 | 0.0075 | −0.006 ± 0.211 | 21 | −0.028 | 0.978 |
| | | 0.04 | −0.053 ± 0.211 | 21 | −0.249 | 0.805 |
| | | 0.075 | 0.173 ± 0.211 | 21 | 0.819 | 0.422 |
| | 30 | 0.0075 | −0.323 ± 0.255 | 28 | −1.265 | 0.216 |
| | | 0.04 | −0.220 ± 0.255 | 28 | −0.900 | 0.376 |
| | | 0.075 | 0.090 ± 0.255 | 28 | 0.352 | 0.728 |
| | 60 | 0.0075 | −0.233 ± 0.147 | 21 | −1.593 | 0.126 |
| | | 0.04 | 0.031 ± 0.147 | 21 | 0.212 | 0.834 |
| | | 0.075 | 0.169 ± 0.147 | 21 | 1.155 | 0.261 |
| c) Affiliation | 15 | 0.0075 | 0.556 ± 0.221 | 21 | 2.512 | 0.020 |
| | | 0.04 | 0.493 ± 0.221 | 21 | 2.229 | 0.037 |
| | | 0.075 | 0.431 ± 0.221 | 21 | 1.946 | 0.065 |
| | 30 | 0.0075 | 0.748 ± 0.255 | 28 | 2.936 | 0.006 |
| | | 0.04 | 0.764 ± 0.255 | 28 | 2.998 | 0.005 |
| | | 0.075 | 0.589 ± 0.255 | 28 | 2.312 | 0.028 |
| | 60 | 0.0075 | 0.530 ± 0.255 | 21 | 2.078 | 0.050 |
| | | 0.04 | 0.577 ± 0.255 | 21 | 2.263 | 0.034 |
| | | 0.075 | 0.344 ± 0.255 | 21 | 1.350 | 0.191 |

of 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 changes 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*. 
that both systems are relevant for the regulation of submissive behaviour. With results from previous serotonin manipulation experiments suggest that similarly to the results from the D1-like receptors manipulation (Stettler et al., 2021). Hence, our data together highlight the importance of testing the behavioral effects of exogenous neuropeptides, monoamines; Jo 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 (including e.g. serotonin; Stettler et al., 2021). For instance, in a similar study involving N. pulcher, the administration of a serotonin 1a receptor agonist decreased 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 for instance 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., 2017a).

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; Joels and Baram, 2009), which in combination with our pharmacological manipulations may have caused a similar behavioral 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 (including 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

| Drug                          | Behaviour | Task         | Zero-Inflation | Estimate ± SE | z      | p-value |
|-------------------------------|-----------|--------------|----------------|---------------|-------|---------|
| D1-like agonist (SKF-38393: 0.075µg/gbw) | Aggression | Control     | 0.032          | 0.420 ± 0.228 | 1.84  | 0.065   |
|                               |           | D 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   |
|                               |           | D Digging    | 0.056          | 0.146 ± 0.208 | 0.70  | 0.484   |
|                               |           | Egg predator | 0.015          | 0.370 ± 0.196 | 1.89  | 0.059   |
|                               | Affiliation| Control     | 3.06           | -0.106 ± 0.511 | -0.21 | 0.835   |
|                               |           | D Digging    | 0.264          | 0.479 ± 0.495  | 0.97  | 0.33    |
| D1-like antagonist (SCH-23390: 0.075µg/gbw) | Aggression | Control     | 0.032          | 0.115 ± 0.231 | 0.50  | 0.620   |
|                               |           | D 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   |
|                               |           | D Digging    | 0.056          | 0.222 ± 0.216  | 1.03  | 0.302   |
|                               |           | Egg predator | 0.015          | 0.395 ± 0.310  | 1.88  | 0.060   |
|                               | Affiliation| Control     | 3.06           | 0.203 ± 0.439  | 0.46  | 0.645   |
|                               |           | D Digging    | 0.264          | 0.224 ± 0.451  | 0.50  | 0.62    |
|                               |           | Egg predator | 1.000e-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   |
|                               |           | D Digging    | 0.052          | 0.389 ± 0.256  | 1.52  | 0.129   |
|                               |           | Egg predator | 0.038          | 0.470 ± 0.218  | 2.16  | 0.031   |
|                               | Submission| Control     | 1e-06          | 0.061 ± 0.210  | 0.29  | 0.773   |
|                               |           | D Digging    | 0.056          | 0.255 ± 0.204  | 1.25  | 0.213   |
|                               |           | Egg predator | 0.015          | 0.171 ± 0.210  | 0.81  | 0.416   |
|                               | Affiliation| Control     | 3.06           | 0.352 ± 0.590  | 0.60  | 0.551   |
|                               |           | D Digging    | 0.264          | 0.380 ± 0.440  | 0.86  | 0.39    |
|                               |           | Egg predator | 1.000e-06      | 0.051 ± 0.634  | 0.08  | 0.936   |
| D2-like antagonist (Metoclopramide: 0.0075µg/gbw) | Aggression | Control     | 0.032          | 0.418 ± 0.237  | 1.76  | 0.078   |
|                               |           | D Digging    | 0.052          | -0.127 ± 0.256 | -0.50 | 0.602   |
|                               |           | Egg predator | 0.038          | 0.077 ± 0.221  | 0.35  | 0.727   |
|                               | Submission| Control     | 1e-06          | 0.347 ± 0.200  | 1.73  | 0.083   |
|                               |           | D Digging    | 0.056          | 0.386 ± 0.203  | 1.90  | 0.057   |
|                               |           | Egg predator | 0.015          | 0.571 ± 0.197  | 2.91  | 0.004   |
|                               | Affiliation| Control     | 3.06           | 1.094 ± 0.550  | 1.99  | 0.046   |
|                               |           | D Digging    | 0.264          | 0.134 ± 0.449  | 0.30  | 0.77    |
in the nucleus accumbens lead to an increase of time spent in contact with a familiar mate (Aragonà, 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. dimissus) 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 interspecific 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 provision (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 and 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 Trinaor, 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., 1991, 1992). 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
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Fig. 2. Submission shown per received aggression within a 15 min observation 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-value > 0.05) with a dot. See Table 5 for statistical details.
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 breeders’ “payment” to the breeders to be allowed to stay in the territory, which enhances their survival chances (Taborsky and 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; Joels and Baram, 2009). Cortisol levels of subordinates are reduced with increasing levels of submission, showing the dominance (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 DA 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 Ritters, 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 Astotatilapia burtoni, D2 receptor activation lead to a decrease of aggression towards an intruder when reproductive opportunities existed, whereas blockade 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 neuro-endocrinological states, which are regulated through context- or state-dependent gene expression patterns in the brain (‘neurogenomic states’; Robinson et al., 2008). In threesprined sticklebacks, Gasterosteus aculeatus, a short territorial intrusion induced a change in their neurogenomic state, which included genes involved in hormone signalling and neurotransmitter transport (Bakhari 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 Astotatilapia 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2022.111649.

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