

RESEARCH ARTICLE

A test of context- and sex-dependent dopaminergic effects on the behavior of a gregarious bird, the common waxbill, *Estrilda astrild*

Sandra Trigo*, Paulo A. Silva, Gonçalo C. Cardoso and Marta C. Soares

ABSTRACT

The dopaminergic (DAergic) system has well-known influences on behavioral and cognitive functions. Previous work with common waxbills (*Estrilda astrild*) reported context-specific DAergic effects that could have been due to social environment. Manipulating the dopamine D2-like receptor family (D2R) pathways had opposing effects on behavior depending on whether waxbills were tested alone or in a small cage with a mirror as a social stimulus. As waxbills are highly gregarious, it was hypothesized that being alone or perceiving that they have a companion might explain this context dependence. To test context-dependent DAergic effects, we compared behavioral effects of D2R manipulation in waxbills in the same familiar environment, but either alone or with a familiar, same-sex companion. We found that D2R agonism decreased movement and feeding, similar to previous results when testing waxbills alone. However, contrary to the hypothesis of dependence on social context, we found that the behavioral effects of the D2R agonist were unchanged when waxbills were tested with a companion. The context dependence reported earlier might thus be due to other factors, such as the stress of being in a novel environment (small cage) or with an unfamiliar social stimulus (mirror image). In tests with a companion, we also found a sex-specific social effect of D2R manipulation: D2R blocking tended to decrease aggression in males but to increase it in females. Together with past work, our results suggest that DAergic effects on behavior involve different types of context or sex dependence.

KEY WORDS: Dopamine system, Dopamine D2-like receptors, Social context, Social behavior, Common waxbill

INTRODUCTION

The dopaminergic (DAergic) system has well-established behavioral and cognitive functions that are known to be conserved across vertebrates, with the involvement of dopamine (DA) in the avian brain fundamentally comparable to its role in the mammalian brain (reviewed in O'Connell and Hofmann, 2011). The DAergic system is known to mediate novelty-seeking, learning and goal-oriented behaviors (Rose et al., 2013), including motivation, reinforcement and reward assessment, memory consolidation (Wise, 2004) and, in distinct pathways, other cognitive and motor functions (Ayano, 2016). Regarding social decision making, DA is implicated in reinforcing neural pathways associated with

positive experiences, such that DA signaling motivates behavior in anticipation of good outcomes, and blockage of DAergic pathways discourages actions associated with negative experiences (reviewed in Puig et al., 2014). Indeed, DA plays a crucial role in evaluating rewards and adjusting the motivational state of the individual (Lucas et al., 2004; Young et al., 2011; Ritters, 2012), having multiple influences on behavioral responses, including food intake (Khodadadi et al., 2017), food reward (Moe et al., 2014), song learning and its control (Fee and Goldberg, 2011; Simonyan et al., 2012; Budzillo et al., 2017), egg production and broodiness (Xu et al., 2010), maternal care (Chokchaloemwong et al., 2015) and aggressiveness (Komiyama et al., 2014; Kops et al., 2017). Considering the crucial importance of DA signaling in modulating individual decision making and how it relates to the value of social experiences, the DAergic system seems a good candidate to further study the role of social context in behavioral modulation.

In birds, as in other vertebrates, the DA system operates via two classes of receptor: the D1-like and D2-like receptor families (D1R and D2R; Smeets et al., 2000; Kubikova and Kostál, 2010; Haug-Baltzell et al., 2015), with activation of D1R generally increasing neuronal firing, and activation of D2R decreasing it (Kai et al., 2015; but see Hopf et al., 2003). In addition to putatively having opposing functions and potentially differing in neuronal distribution, D2R also have higher affinity for DA than do D1R (Missale et al., 1998), and evidence has accumulated with respect to D2R influences in animal socio-sexual behavioral responses. For example, in male Syrian hamsters (*Mesocricetus auratus*), D2R antagonism decreases the appeal of a sexual cue (Bell and Sisk, 2013). Also, in male zebra finches (*Taeniopygia guttata*), D2R agonism blocks aggressive displays when competing for mates, but not courtship singing (Kabelik et al., 2011), and in the African cichlid fish *Astatotilapia burtoni*, treatment with a D2R antagonist decreases intruder-directed aggression in a mating setting, but increases aggression in the neutral context (Weitekamp et al., 2017). Both these results with the zebra finch and the cichlid fish point to context-dependent effects of DA on social behavior. It was reported that quails have a higher ratio of D2R to D1R compared with rats (Kleitz et al., 2009), suggesting that the D2R system may have a particularly important influence on avian social behavior. As D2R are autoreceptors (i.e. found both pre- and post-synaptically), they may have different functions, and may be able to inhibit DA neuron firing, synthesis and release, thus functioning as a negative feedback mechanism (Bello et al., 2012).

In a recent study with common waxbills (*Estrilda astrild*), manipulation of D2R pathways affected behavior in a context-dependent manner (Silva et al., 2020). When waxbills were tested alone in a large cage, similar to their home cages, D2R agonism decreased activity and feeding. But, when waxbills were tested in a smaller cage, facing a mirror image, D2R agonism instead increased activity, rather than decreasing it, and it was D2R antagonism that decreased activity (Silva et al., 2020). As waxbills are very

CIBIO/InBIO—Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.

*Author for correspondence (strigo@cibio.up.pt)

© S.T., 0000-0002-9938-6606; P.A.S., 0000-0001-9747-7021; G.C.C., 0000-0001-6258-1881; M.C.S., 0000-0002-5213-2377

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gregarious, living in flocks year-round (Payne, 2010), it was thus suggested that this context dependence could be due to birds perceiving they were alone or, when facing their mirror image, with a companion (Silva et al., 2020). Nonetheless, alternative interpretations for this context dependence persist, because the study (Silva et al., 2020) used different physical settings (cage size and location) for tests alone or with the mirror image, and further work is necessary to test whether social setting is indeed the cause of this context-specific effect of DA.

Here, we expand on previous research, using the common waxbill as a model, manipulating pharmacologically the D2R pathways of the DAergic system and testing for behavioral effects in two different social contexts. After manipulation of the DAergic pathways with either an agonist or an antagonist for D2R, we quantified waxbill behavior in a familiar environment, in either the presence or absence of a same-sex familiar companion bird. The two social contexts were tested in an otherwise identical setting, including the same location and experimental cage. This allowed us to disambiguate whether social context mediates the context-specific behavioral effects of the DAergic system that were reported earlier by Silva and colleagues (2020), in which case we would expect social context to modulate the effects of DR2 on waxbill motor activity and feeding. In addition, as these experiments used same-sex companions as social stimuli for both male and female experimental birds, we controlled for possible sex differences in social context, and therefore were also able to study sex-dependent DAergic effects, and DAergic effects on social interactions and aggression.

MATERIALS AND METHODS

Experimental animals

We obtained 24 common waxbills, *Estrilda astrild* (Linnaeus 1758) (12 males and 12 females), from certified breeders in March 2018, and housed them at a research aviary in CIBIO-InBIO (Vairão, Portugal). The birds had numbered and colored leg rings for individual identification and were distributed across six metal cages (88.5 cm×30 cm×40 cm) in mixed-sex groups of four birds per cage (2 males and 2 females). As common waxbills are highly gregarious, we kept them in groups to avoid the stress of isolation. Cages were visually but not acoustically isolated from each other. The birds received *ad libitum* mixed seeds (Tropical Finches Prestige, Versele-Laga), water and mixed grit with crushed oyster shell (Grit with Coral Prestige, Versele-Laga). The aviary had natural ventilation and light, complemented with artificial light in a cycle adjusted to the natural photoperiod. Procedures used in this study were evaluated and approved by the ORBEA (Organism for Animal Welfare) of CIBIO-InBIO (ethics assessment no. ORBEA_2019_Estrilda).

Experimental design

Experiments took place during mornings, between 09:00 h and 12:30 h, from 22 July to 5 September 2019. This time of year is within the very long breeding season of waxbills in this region, after the peak of breeding (May) and before its end (September/October; Sanz-Aguilar et al., 2015; Beltrão et al., 2021a), although these birds do not reproduce when living in this type of cage. Before the start of the experiments, birds were randomly divided into two groups: one male and one female per cage were used as experimental birds, and one male and one female per cage were used as companion birds. There were six rounds of experiments, three rounds with the experimental birds tested alone, and three rounds tested with a familiar, same-sex companion bird

(i.e. experimental males had their male cage-mate as a companion, and experimental females had their female cage-mate as a companion).

Each experimental bird received three treatments – control, D2R agonist and D2R antagonist – and was tested alone or with a social companion. Different tests on the same bird were separated in time by 7 days. The birds did not all receive the same sequence of treatments, but instead received different sequences following a balanced design. In the first round of experiments, a third of birds received the control treatment, a third received the D2R agonist and a third received the D2R antagonist; additionally, in each treatment, half the birds were tested alone and half were tested with a companion. In subsequent rounds, the treatment and social context that each bird received changed, as shown in Table S1, so that treatments and social contexts were evenly distributed over time. We used this balanced design so that date or order effects would not confound experimental results.

Each treatment consisted of an intramuscular injection of a compound, on the right side of the pectoral muscle, followed by a behavioral test. We chose to use an intramuscular injection, following previously published protocols in this (Silva et al., 2020) and other species (chukar *Alectoris chukar*: Dickens et al., 2009; house sparrow *Passer domesticus*: Gao and Deviche, 2018, 2019), because it does not threaten vital organs and because, given the small size of waxbills, subcutaneous injections are difficult to perform. One female died before the start of the experiments and another died before the end.

The control treatment consisted of injection of 20 µl saline solution, and the other two treatments consisted of injection of a DA receptor agonist or antagonist diluted in 20 µl saline solution. Dosages of each compound were identical to those in Silva et al. (2020), based on Balthazart et al. (1997), and were scaled for the mean body mass of our birds (8.7 g, measured before the experiments, no sex differences in mass): treatment with the D2R agonist (quinpirole, Q102 Sigma-Aldrich, Darmstadt, Germany) used 1 mg kg⁻¹ body mass, and treatment with the D2R antagonist (spiperone, S7395 Sigma-Aldrich) used 10 mg kg⁻¹ body mass.

One hour before each behavioral test, the home cage of the experimental bird was divided in half with a partition, and the experimental bird was placed with its companion in the right side and deprived of food until the behavioral test. Forty-five minutes into the food deprivation period, the experimental bird was briefly taken from the cage, injected with the corresponding treatment, and then returned to the right side of its home cage for the remaining 15 min of food deprivation (Fig. S1). If tested alone, the experimental bird was then transferred to the test cage or, if tested with its companion, both birds were transferred to the test cage (Fig. S2). The test cage was identical to the home cage, and was located next to it, thus sharing the same acoustic and visual environment. We then performed a behavioral assay to quantify activity, feeding and aggressiveness. The assay consisted of filming (with a Canon LEGRIA HF M306 camera, fixed on a wall across the aviary room) the behavior of the birds during 15 min in the test cage. The test cage had four perches in symmetrical positions, similar to the home cages, and had two feeders, one placed next to the rightmost perch, and the other next to the leftmost perch (Fig. S2). After each behavioral assay, the birds were returned to their home cage.

Quantification of behavior

Quantification of behavior of the experimental bird, from the video recordings, was performed by the same observer (P.A.S.), who was

blind to the experimental treatment, using the software BORIS (Behavioural Observation Research Interactive Software, version 7.9.4; Friard and Gamba, 2016). Four behavioral variables were quantified. (1) Time at the feeder, i.e. the total time, in seconds, that the experimental bird spent on all its visits to the feeder (i.e. perched at the feeder or next to the feeder but with the head reaching it for feeding; we did not observe waxbills resting at the feeder rather than eating). (2) Latency to visit the feeder, in seconds, calculated as the amount of time that the experimental bird took to go to the feeder for the first time. (3) Movement, assessed as the number of changes made by the focal bird between different locations in the cage. These locations were: each of the four perches, each of the two feeders, and each of the four zones on the cage floor (these four zones were defined by their proximity to each of the four perches; Fig. S2). Movements within the same location were not counted. (4) Aggressive behaviors (only in tests with a companion bird), counted as the number of aggressive displays or attacks: stretching the body towards the companion (which could be accompanied by opening the beak or flicking the wings), pecking at the companion, or displacing the companion by flying towards it.

Time at the feeder and latency to the feeder are strongly correlated, and we summarized them with a principal component analysis (PCA) whose first principal component (PC) explained 77.99% of variance and had a strong positive loading of time at the feeder (0.883) and a strong negative loading of latency to visit the feeder (−0.883). High scores on this PC (hereafter ‘feeding PC’) indicate more time spent at the feeder, and a shorter latency to visit the feeder for the first time.

Statistical analyses

We used generalized linear mixed models (GLMMs) to test whether the control treatment differed from the D2R agonist or D2R antagonist treatments, separately for each of the three behavioral traits (activity, feeding PC and aggressiveness) using the R package ‘lme4’ (version 1.1-23; Bates et al., 2014) and ‘lmerTest’ (version 3.1-2; Kuznetsova et al., 2017). In each GLMM, one of these three behavioral traits was the dependent variable, the fixed factors were treatment (control, D2R agonist and D2R antagonist), sex (female or male) and social context (alone or with companion), and we used individual identity as a random factor to test for within-individual effects of the different treatments while controlling for among-individual differences in behavior. We also included the interaction

between treatment and sex, and between treatment and social context, to test whether DAergic effects differ between the sexes or social contexts, respectively. When an interaction between treatment and sex was statistically significant, we performed a *post hoc* analysis, using similar GLMMs separately for females and for males (without the factor sex, or interactions with sex). We checked model assumptions using the R package ‘performance’ (version 0.4.7; Lüdtke et al., 2020) to compare residuals from models with different distributions and link functions. Accordingly, we chose a Gaussian distribution with log link function in the GLMM for feeding PC, a Poisson distribution in the GLMM for aggressiveness and a negative binomial distribution in the GLMM for activity. We were interested in comparing behavior between the control treatment and either of the DAergic treatments, but not in comparing behavior between the two DAergic treatments because, in the absence of a difference to the control, the latter comparison is not interpretable. Therefore, we report GLMM contrasts (i.e. simple coefficients, without having run an ANOVA after the GLMM), which allowed us to test differences between one level of the fixed factor (the control treatment) and each of the remaining levels (D2R agonist or D2R antagonist). All statistical analyses were performed in R version 4.0.0 (<http://www.R-project.org/>).

RESULTS

Treatment with the D2R agonist caused a decrease in activity (contrast of D2R agonist: $Z=-2.193$, $P=0.0283$; Table 1, Fig. 1). Females were on average more active than males ($Z=-2.133$, $P=0.0329$; Table 1, Fig. 1), and the interactions with sex were significant for the effects of both the D2R agonist ($Z=2.470$, $P=0.0135$; Table 1, Fig. 1) and the D2R antagonist ($Z=2.204$, $P=0.0275$; Table 1, Fig. 1). *Post hoc* analyses showed that these interactions between DAergic treatment and sex were due to females decreasing activity in the D2R agonist treatment relative to the control, but not males (Table S2). There were no significant effects of, or interactions with, social context (i.e. tested alone or with a companion bird; Table 1, Fig. 1).

Feeding PC scores also decreased in the D2R agonist treatment compared with the control (contrast of D2R agonist: $Z=-3.073$, $P=0.0021$; Table 1, Fig. 2), indicating that birds took more time to reach the feeder and spent less time at the feeder. There were no significant effects of, or interactions with, sex or social context (Table 1, Fig. 2).

Table 1. GLMM contrasts between behavior [activity, feeding principal component (PC) or aggressiveness] in control conditions and following each of the dopaminergic (DAergic) manipulations

	Activity		Feeding PC		Aggressiveness	
	Estimate	Z (P)	Estimate	Z (P)	Estimate	Z (P)
Contrast with control treatment						
D2R agonist	−1.05	−2.19 (0.03)	−0.54	−3.07 (0.002)	−0.98	−1.45 (0.15)
D2R antagonist	−0.42	−0.91 (0.36)	−0.26	−1.77 (0.08)	0.49	1.08 (0.28)
Contrast with alone treatment						
With companion	0.04	0.10 (0.92)	0.20	1.44 (0.15)		
Contrast with female						
Male	−0.82	−2.13 (0.03)	−0.53	−1.82 (0.07)	−0.16	−0.20 (0.84)
2-Way interaction with DAergic treatment						
D2R agonist×With companion	−0.18	−0.34 (0.74)	−0.28	−1.21 (0.23)		
D2R antagonist×With companion	0.003	0.01 (0.99)	−0.03	−0.16 (0.87)		
D2R agonist×Male	1.35	2.47 (0.01)	0.28	1.19 (0.24)	−0.41	−0.39 (0.70)
D2R antagonist×Male	1.21	2.20 (0.03)	0.24	1.04 (0.30)	−1.87	−2.06 (0.04)

Sample size is 11 birds (5 females and 6 males). The reference level for the factor ‘treatment’ is the control and comparison levels are D2R agonist and D2R antagonist; for the factor ‘social context’ the reference level is alone and the comparison level is with companion; and for the factor ‘sex’ the reference level is female and the comparison level is male; the tested effects and interactions are named after their comparison levels. Bold indicates significance.

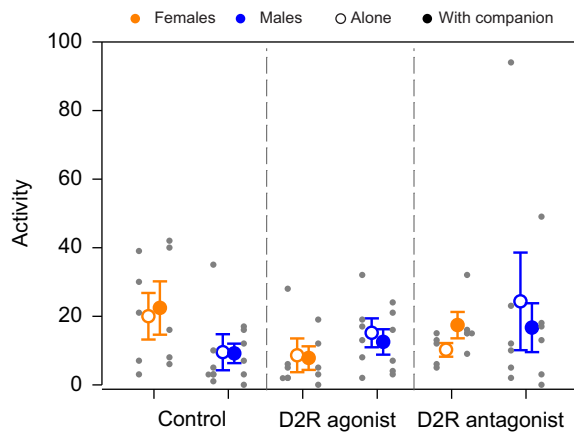


Fig. 1. Activity of common waxbills, measured as counts of movements in the cage, according to sex, dopaminergic (DAergic) treatment and social context. Data are means \pm s.e.m. ($N=11$, 5 females and 6 males; gray circles are data for individual birds). Analysis of activity indicates a decrease in movement with the dopamine D2-like receptor family (D2R) agonist relative to control, sex differences in activity, significant interactions of sex and DAergic treatment, and no effect of social context. See Table 1 for statistical results.

Finally, analyses of social aggressiveness in tests with a companion bird showed a significant interaction between sex and the D2R antagonist treatment ($Z=-2.058$, $P=0.0395$; Table 1, Fig. 3). Inspection of Fig. 3 suggests that this interaction was due to treatment with the D2R antagonist having the opposed effects of increasing female aggressiveness and reducing male aggressiveness relative to the control, although *post hoc* tests analyzing each sex separately did not show significant effects of the D2R antagonist on aggressiveness (Table S2), possibly because these *post hoc* tests used a smaller sample size and have lower statistical power.

DISCUSSION

Our short-term experimental manipulation of the DAergic D2R pathways had immediate effects on the activity, feeding and aggressive behavior of common waxbills. Similar to previous research, when testing waxbills alone (Silva et al., 2020), D2R agonism decreased activity levels and reduced feeding. Contrary to

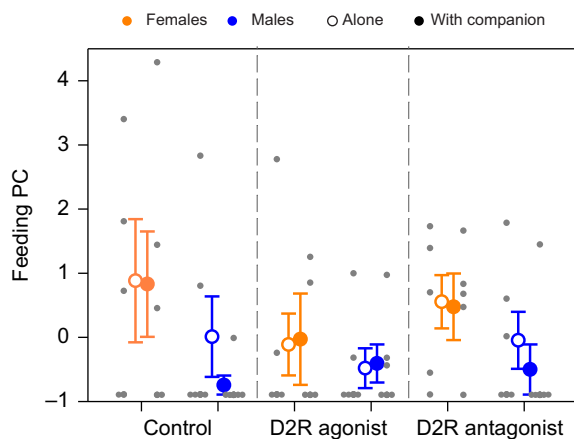


Fig. 2. Feeding principal component (PC) scores according to sex, DAergic treatment and social context. Data are means \pm s.e.m. ($N=11$, 5 females and 6 males; gray circles are data for individual birds). Analysis of feeding PC scores indicates decreased feeding with the D2R agonist, relative to the control, and no effects of social context or sex. See Table 1 for statistical results.

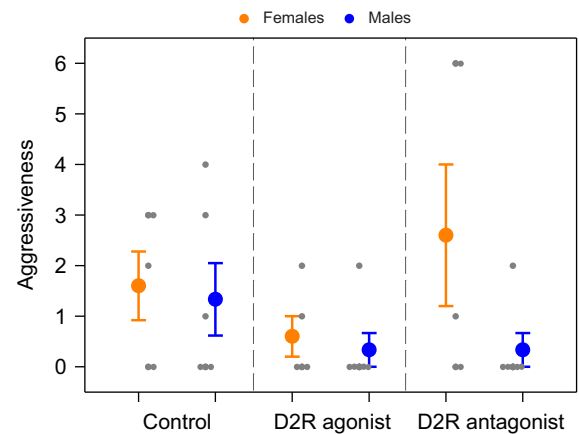


Fig. 3. Aggressiveness of common waxbills, measured as the number of aggressive behaviors, according to sex and DAergic treatment. Data are means \pm s.e.m. ($N=11$, 5 females and 6 males; gray circles are data for individual birds). Analysis of aggressiveness indicates a significant interaction of D2R antagonist treatment and sex. See Table 1 for statistical results.

the hypothesis that these effects could be dependent on social isolation, we found that social context (being alone or with a same-sex companion) did not interact with the effects of D2R on activity or feeding. Interestingly, we also found that aggressive behavior, when tested with a companion, showed a sex-specific effect of the D2R antagonist, with D2R blockage tending to decrease aggression in males but to increase it in females. Below we discuss these results in turn.

D2R effects on waxbill activity levels

In previous work with common waxbills, experimental manipulation of the D2R pathways pointed to a context-dependent effect: D2R facilitation decreased activity in a non-social context but increased activity in tests with a mirror image (Silva et al., 2020). Our new results show that D2R agonism decreased activity levels overall, whether waxbills were tested in isolation or with a companion bird. These new results confirm the findings of Silva et al. (2020) when testing waxbills in a non-social context, but oppose the suggestion that behavioral effects could be different in a social context. Instead, in light of our results, the context-dependent change reported by Silva et al. (2020) may not have been due to the presence or absence of a mirror image functioning as a social stimulus. Here, we used a well-controlled experimental approach, where each bird was tested either alone or with a familiar, same-sex companion, and we found that this did not change the behavioral effect of D2R manipulation. While all our experimental treatments were conducted in the same environment, similar to that of the waxbills' home cages, Silva et al. (2020) conducted their mirror tests in a different cage (smaller than the home cages and the non-social treatment) and in a different aviary room. We thus hypothesize that the context-dependent results of Silva et al. (2020) could have been due to that novel, unfamiliar environment where mirror tests were conducted, which could have raised stress, a known mediator of the mesolimbic DAergic system (Sim et al., 2013; Trainor, 2011). Indeed, previous evidence shows that the DAergic system is highly susceptible to stress, with moderate stressors increasing DAergic function, and intense stressors potentially inhibiting it (Marinelli, 2007).

Here, we observed that females had overall higher activity levels than males, irrespective of DAergic treatment or social context, which could have led to differential effects of DAergic treatment

between the sexes: females had high activity levels in control conditions and decreased activity following treatment with the D2R agonist, while male activity levels were already low in control conditions and remained unchanged upon D2R manipulation. These statistically significant interactions need to be viewed cautiously, because they resulted largely from a sex difference in the control treatment, and previous work in the wild (Carvalho et al., 2013) and in the laboratory (Funghi et al., 2015; Silva et al., 2020) did not find that male and female waxbills differ in their level of activity. In the case of females, a putative explanation is that the activation of D2 auto-receptors (that exist both pre- and post-synaptically) may decrease both the excitability of DA neurons and the release of DA, thus leading to a reduction in locomotion (Jackson and Westlind-Danielsson, 1994; Missale et al., 1998). The differences between sexes, however, are not easily interpretable, and more research is needed to confirm whether a sex-dependent DAergic effect on activity does indeed exist.

D2R effects on waxbill feeding behavior

In addition to reducing overall activity, we also observed that exogenous D2R agonism decreased feeding behavior, with birds feeding less and taking longer to access the feeder compared with those in the control treatment. Feeding behavior and activity may be related behavioral effects, which are difficult to dissociate (as accessing the feeder implies changing location). Despite not having applied a formal statistical correction for conducting two tests on these related hypotheses (feeding and activity), the tests showed a similar and statistically significant main effect of D2R agonism, indicating that the result was not fortuitous as a result of multiple testing (this could be the case if only one of these results were statistically significant). Previous work with common waxbills had shown a similar, albeit statistically non-significant, decrease in feeding behavior upon treatment with the D2R agonist (Silva et al., 2020). Similar to the results with activity levels, discussed above, this DAergic effect on feeding was also independent of social context, again indicating that context-dependent DAergic effects reported earlier (Silva et al., 2020) were not due to the birds perceiving to be alone or not, but to other factors.

While it is well known that DA has effects on feeding behavior in several species (e.g. *Drosophila melanogaster*: Marella et al., 2012; fish: Leal et al., 2013; rats: Zigmond and Stricker, 1972; Ferrari and Giuliani, 1994; mice: Zhou and Palmiter, 1995; non-human primates: Schultz et al., 1993; and humans: Small et al., 2003), work on birds is still scarce and, among the few published studies, results have been contradictory. In neonatal chicks, intracerebroventricular DA injections caused no difference in food intake compared with controls (Denbow et al., 1983; Bungo et al., 2010), whereas in another study, intracerebroventricular DA injections caused a significant decrease in food intake in chickens (Zendehdel et al., 2014). Moreover, in pigeons, the D2R antagonist sulpiride increased food intake in females and water intake in males (24 h following injection; Abbasnezhad and Joneydi, 2007). In previous work with common waxbills, we found that blockage of D1R pathways increased feeding (Silva et al., 2020). Here, studying the D2R pathways instead, we found that D2R activation inhibited feeding behavior. These different results with the D1R and D2R pathways may be explained because D1R and D2R are found in different cell groups (Wei et al., 2018), and they may exert opposing actions (Balthazart et al., 1997; Kleitz-Nelson et al., 2010; Beaulieu and Gainetdinov, 2011) as, unlike D1R, D2R are auto-receptors and can regulate the DA system by feedback inhibition (Ford, 2014). Therefore, it is possible that above certain levels of D2R activation,

there is an overall inhibition of the DAergic system, and thus shifting towards an overall inhibition of the feeding and activity response.

D2R effects on waxbill aggressiveness

Common waxbills are very gregarious, and it is common to observe aggressive behaviors (e.g. aggressive open-beak posture, pecking or displacing others) when they are competing for food either towards familiar birds in birdcages (especially after a period of food deprivation; Funghi et al., 2015, 2018) or when living in open spaces (Beltrão et al., 2021b). Our treatment with D2R agonist did not significantly affect waxbill aggressiveness, but D2R antagonism produced sex-specific effects, which appeared to result from a tendency to decrease aggressive behavior in males and to increase it in females (see Fig. 3). In other model systems, impulsive aggression has often been associated with higher activity in the DAergic system (Harrison et al., 1997; for a review, see Netter and Rammsayer, 1991), e.g. elevated levels of DA were responsible for aggressive behavior in rodents (Hadfield, 1983; Miczek et al., 1994; Tidey and Miczek, 1996). In humans, the DAergic hyperfunction can be associated with impulsivity and emotional dysregulation (Chotai et al., 1998; for a review, see Friedel, 2004), and the effects of the D2R antagonist sulpiride can impair the ability to recognize angry facial expressions (Lawrence et al., 2002). These results with rodents and humans are in agreement with the trend for decreased aggressiveness that we found in male waxbills after treatment with a D2R antagonist. Opposite to these results, previous work with zebra finches found that D2R agonism inhibited male–male competitive aggression to access a female mate (Kabelik et al., 2010). The apparently different results in zebra finches and waxbills could perhaps be due to the different experimental designs used: competitive aggression to access a female mate in zebra finches, and here aggression in a non-sexual context.

To interpret the sex-specific effect of D2R antagonism on waxbill aggressiveness, it is useful to consider differences in how the DAergic system may influence aggression depending on social dominance hierarchies. Previous evidence with humans and non-human primates has shown that individuals of higher social rank have greater D2R expression in the striatum (Morgan et al., 2002; Martinez et al., 2010; Yamaguchi et al., 2017), and D2R manipulations may modulate behavioral traits that are required to gain higher social rank, such as aggressive displays. For example, Yamaguchi and colleagues (2017) found that D2R antagonist administration to groups of macaques and mice with high social rank attenuated social dominance hierarchies, whereas the same drug treatment did not alter social dominance hierarchies among individuals of lower social rank. Similarly, it was shown that D2R agonism increases aggression in low-aggression strains of laying hens (Dennis and Cheng, 2011), while antagonism of D2R reduced the frequency of aggressive behavior in both low-aggression and high-aggression hen strains (Dennis and Cheng, 2011). Thus, the baseline aggressiveness of individuals can be important to determine which type of effect DA has on aggressive behavior. If male waxbills were socially dominant over females, our finding of a sex-specific effect of D2R antagonism (D2R antagonism tended to decrease aggressive behavior in males but to increase it in females) would agree with the above results from primates, mice and hens. However, male and female common waxbills do not differ strongly in overall aggressiveness or in social dominance, either when housed in bird cages (Funghi et al., 2015, 2018) or when moving in an open space (Beltrão et al., 2021b). Female waxbills, instead, have been reported to be less aggressive towards males than towards other

females (Funghi et al., 2015). Albeit minor, this sex difference in waxbill aggressiveness and social dominance may provide a hint as to why DAergic effects were sex dependent. Similar to the suggestion based on work with mammals (Morgan et al., 2002; Martinez et al., 2010; Yamaguchi et al., 2017), D2R antagonism might have reduced aggressiveness only in the putatively more socially dominant males, perhaps as a result from them having higher expression of D2R and thus responding strongly to a putative reduction of regular basal levels of DA (see Morgan et al., 2002).

In conclusion, our results suggest the involvement of the DAergic system in several different behaviors – activity, food intake and aggressiveness – and, together with previous research with the common waxbill (Silva et al., 2020), suggest that these DAergic effects involve different types of context or sex dependence.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.T., G.C.C., M.C.S.; Methodology: S.T., P.A.S., G.C.C.; Formal analysis: S.T., P.A.S.; Resources: G.C.C.; Writing - original draft: S.T.; Writing - review & editing: S.T., P.A.S., G.C.C., M.C.S.; Project administration: G.C.C.

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Data availability

The dataset from this experiment is available from figshare: <https://doi.org/10.6084/m9.figshare.19242714>.

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