

RESEARCH ARTICLE

Experimental evidence for a role of dopamine in avian personality traits

Paulo A. Silva¹, Sandra Trigo¹, Cristiana I. Marques¹, Gonçalo C. Cardoso^{1,2} and Marta C. Soares^{1,*}

ABSTRACT

There is increasing interest in the genetic and physiological bases of behavioural differences among individuals, namely animal personality. One particular dopamine (DA) receptor gene (the dopamine receptor D4 gene) has been used as candidate gene to explain personality differences, but with mixed results. Here, we used an alternative approach, exogenously manipulating the dopaminergic system and testing for effects on personality assays in a social bird species, the common waxbill (*Estrilda astrild*). We treated birds with agonists and antagonists for DA receptors of both D1 and D2 receptor pathways (the latter includes the D4 receptor) and found that short-term manipulation of DA signalling had an immediate effect on personality-related behaviours. In an assay of social responses (mirror test), manipulation of D2 receptor pathways reduced time spent looking at the social stimulus (mirror image). Blocking D2 receptors reduced motor activity in this social assay, while treatment with a D2 receptor agonist augmented activity in this social assay but reduced activity in a non-social behavioural assay. Also, in the non-social assay, treatment with the D1 receptor antagonist markedly increased time spent at the feeder. These results show distinct and context-specific effects of the dopaminergic pathways on waxbill personality traits. Our results also suggest that experimental manipulation of DA signalling can disrupt a behavioural correlation (more active individuals being less attentive to mirror image) that is habitually observed as part of a behavioural syndrome in waxbills. We discuss our results in the context of animal personality, and the role of the DA system in reward and social behaviour.

KEY WORDS: Animal personality, Common waxbill, Dopamine D1-like receptors, Dopamine D2-like receptors, Motivation, Social responses

INTRODUCTION

There is much interest in the role of the dopaminergic (DAergic) system on animal personality, particularly avian personality, but research results have been inconsistent across species. The dopamine receptor D4 gene (*DRD4*) has been an important candidate gene in studies of avian personality because, in other taxa, *DRD4* is commonly associated with fear, novelty seeking and exploratory behaviour (Bailey et al., 2007; Hejjas et al., 2007; Momozawa et al., 2005; Munafò et al., 2008). Following an earlier


finding that polymorphisms in this candidate gene correlate with personality differences in great tits (*Parus major*; Fidler et al., 2007), several studies have searched for a relationship between personality type and *DRD4* in other avian species, especially in Passeriformes, where *DRD4* evolution is faster (Abe et al., 2011). In some species, this association was confirmed (Garamszegi et al., 2014; Holtmann et al., 2016; Mueller et al., 2014; van Dongen et al., 2015), in others, it was not (Atwell et al., 2012; Carvalho et al., 2013), and in the great tit it holds for some populations but not for others (Korsten et al., 2010; Mueller et al., 2013; Riyahi et al., 2015, 2017).

The ambivalence of these findings could be because *DRD4* codes for just one subtype of DA receptor, but the DAergic system involves several other receptors and neural pathways (Kubikova and Košťál, 2010). As for other vertebrates, the DAergic system of birds comprises several receptor types distributed in two main families: the D1-like receptor family includes the D1A, D1B and D1D receptors, and the D2-like receptor family includes the D2, D3 and D4 receptors (Callier et al., 2003; Kubikova and Košťál, 2010; Kubikova et al., 2010), the last of which is coded by the *DRD4* gene. This diversity in DA receptor types may be the cause of the uncertainty in correlations between *DRD4* polymorphism and personality types. Because D1-like receptors are solely found post-synaptically and D2-like receptors are autoreceptors (found both pre- and post-synaptically), D1-like receptors are acknowledged to increase neuronal signalling, while activation of D2-like receptors can decrease it (Ding and Perkel, 2002). This happens because the D2-like receptors can function as a control mechanism, being able to also inhibit DA neuron firing (Bello et al., 2011). Hence, depending on their relative abundance and distribution in different brain areas, D1-like and D2-like receptor pathways can modulate animal behaviour either through opposing effects or occasionally in a complementary way (Kleitz et al., 2009; St. Onge et al., 2011).

The DAergic system is known to affect multiple behavioural functions such as motivation, risk mediation, reward assessment and other roles in cognitive and motor function (e.g. Alcaro et al., 2007; Berridge and Robinson, 1998; Salamone and Correa, 2002), including long-lasting learning and reinforcement of actions to achieve desirable outcomes (Reynolds et al., 2001). DA is implicated in the estimation of future rewards, used for making decisions and calculating how much investment should be given to an action (de Boer et al., 2019). This is because DA is implicated in reinforcing neural pathways associated with positive experiences, such that DA signalling motivates behaviour in anticipation of good outcomes, and blockage of the DAergic pathways discourages actions associated with negative experiences (Frank et al., 2004; Salamone and Correa, 2002; Schultz, 1998). For example, repeated exposure to a food reward gradually transfers DA signalling onto the stimuli associated with the reward (e.g. the smell or other cue of the food), anticipating the reward (Schultz, 2010). Thus, if anticipation is crucial in decision making, the omission of an expected reward

¹CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, Vairão 4485-661, Portugal. ²Behavioural Ecology Group, Department of Biology, University of Copenhagen, 2100 Copenhagen Ø, Denmark.

*Author for correspondence (marta.soares@cibio.up.pt)

 P.A.S., 0000-0001-9747-7021; S.T., 0000-0002-9938-6606; C.I.M., 0000-0002-3406-9597; M.C.S., 0000-0002-5213-2377

may lead to emotional distress in humans (Abler et al., 2005), while in other mammals, birds and teleost fish it may induce aggressive and other more extreme behaviours (de Almeida and Miczek, 2002; Messias et al., 2016; Soares et al., 2017; Vindas et al., 2014; Zimmerman and Koene, 1998). More generally, the DAergic system helps to provide a motivational value to environmental cues or stimuli (either social or non-social), by influencing neural responses (Gobert et al., 1998; Schultz, 1998; Schultz et al., 1997). In humans, variations in DAergic function appear to be involved with differences in behavioural flexibility and personality, as it mediates motivational responses to uncertainty, novelty seeking and activity levels, learning and evaluation, impulsivity and aggression (Bromberg-Martin et al., 2010; DeYoung, 2013). In rodents, differences in DAergic signalling also appear to underlie the cognitive differences between more proactive and more reactive individuals (Caramaschi et al., 2013; Coppens et al., 2010; but see also Koolhaas et al., 2010). In avian species, the relationships between personality type and the DAergic system are as yet poorly understood and, as noted above, research has tended to concentrate on correlations with polymorphisms in the *DRD4* gene alone.

Here, we expand past research on avian models by, for the first time, manipulating pharmacologically both the D1-like and D2-like receptor pathways of the DAergic system in a bird species, the common waxbill [*Estrilda astrild* (Linnaeus 1758)], and testing for effects on stable behavioural differences that are related to personality in this species. Personality differences in common waxbills can be described along a reactive–proactive axis, similar to other species of birds (see Carere et al., 2010; Koolhaas et al., 1999), whereby more proactive individuals explore more a novel environment, and are more active but less attentive towards a social stimulus (a mirror image: Carvalho et al., 2013; Funghi et al., 2015). This personality axis of waxbills, however, does not appear to predict aggressiveness or position in social hierarchies (Funghi et al., 2015). Previous work found polymorphisms in the *DRD4* gene of common waxbills, but those polymorphisms did not predict personality differences in the common waxbill (Carvalho et al., 2013). After manipulation of the DAergic pathways, namely the D1-like and D2-like receptor families, we tested waxbills with a mirror test, a social assay that robustly assesses personality type in waxbills. Individual differences in these assays are consistent over extended periods of time (Funghi et al., 2015; Gomes et al., 2020), and they also predict individual differences in exploration (Carvalho et al., 2013). We also tested waxbills with a general activity assay after food deprivation, in an environment with a food source and a novel object, which is also often used as a personality assay in other species (Amy et al., 2017; Boogert et al., 2006; Mettke-Hofmann, 2012; Réale et al., 2007), as the DAergic system is involved in the reward and motivational aspects of feeding (Bello and Hajnal, 2010). Because the DAergic system involves several different receptors, and thus other genes in addition to *DRD4*, our neuropharmacological approach allowed a comprehensive test of whether DA is involved in determining behaviour that is related to avian personality.

MATERIALS AND METHODS

Experimental subjects

We obtained 24 common waxbills (12 males and 12 females) from certified breeders on 5 March 2018, and housed them at a research aviary at CIBIO (Vairão, Portugal). One male died before the end of the experiments, and therefore our sample size was 23. The birds were ringed and distributed into five metal cages (88.5 cm×30 cm×40 cm) in mixed-sex groups of $N=4$ or 5 birds per

cage. Common waxbills are highly gregarious and, thus, we kept them in groups to avoid the stress of being isolated. During their period in captivity, the birds were given *ad libitum* seeds (mixed seeds for exotic birds), water and sand with crushed oyster shell. The aviary had natural ventilation and light complemented with artificial light in a cycle that was adjusted to the natural photoperiod. Animal procedures used in this study were approved by the ORBEA – CIBIO-InBIO (reference no. 2017-01).

Manipulation of the DAergic system and experimental design

Experiments took place between 09:00 h and 13:00 h, from 12 March to 22 May 2018. Each bird received five treatments, one in each of five experimental rounds; different treatments separated in time by 9–14 days. Each treatment consisted of an intramuscular injection of a compound (saline, D1 or D2 receptor agonist, D1 or D2 receptor antagonist) on the right side of the pectoral muscle, followed by behavioural tests. In the first round of experiments, treatments were distributed in a balanced manner across all 23 birds and, in the following four rounds, each bird received a different treatment following a consistent order. This way, the different treatments were distributed evenly over time.

The control treatment consisted of the injection of 20 μ l saline solution, and the other four treatments consisted of the injection of a DA receptor agonist or antagonist diluted in 20 μ l saline solution. The dosage of each compound was based on Balthazart et al. (1997), using as reference the mean (\pm s.d.) body mass of our birds (8.7 \pm 0.6 g, measured before the experiments): treatment with the D1 receptor (D1R) agonist (SKF 38393, D047 Sigma-Aldrich, Darmstadt, Germany), D1R antagonist (SCH 23390, D054 Sigma-Aldrich) and D2 receptor (D2R) agonist (quinpirole, Q102 Sigma-Aldrich) used 1 mg kg⁻¹ body mass, and treatment with the D2R antagonist (spiperone, S7395 Sigma-Aldrich) used 10 mg kg⁻¹ body mass.

Each experimental bird was placed alone in the left side of the test cage and deprived of food for 1 h prior to the test. The test cage was identical to the home cages, and was located side-by-side with them, so that the experimental bird experienced the same acoustic environment as before. The experimental bird was injected with the corresponding treatment 45 min into the food deprivation period, and returned to the test cage for the remaining 15 min.

Afterwards, we performed two behavioural tests widely used in birds (Apfelbeck and Raess, 2008; Carvalho et al., 2013; Funghi et al., 2015; Webster and Lefebvre, 2001). The first test was an assay of activity, feeding and reaction to a novel object, which lasted for 10 min. The bird was then taken from the test cage and transported inside a paper bag to a smaller test cage (see below) for the second test – a mirror test – which took place after an interval of 5 min, and also lasted for 10 min (see time line in Fig. 1). Behavioural quantification of the videos was always performed by the same observer, who was blind to the experimental treatment. These behavioural assays are described below.

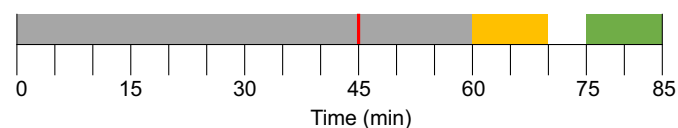


Fig. 1. Time line of experimental procedures and behavioural tests. Grey indicates the food deprivation period; yellow indicates the activity, feeding and novel object test period; white indicates the interval; and green indicates the mirror test period. The red line indicates experimental injection.

Activity, feeding and novel object test

This test started with removal of an opaque partition that separated the left side of the test cage, where the bird was located, from the right side, where an unfamiliar object had been placed next to a feeder, and the behaviour of the bird was filmed for 10 min. The test cage contained four perches equidistant from each other, similar to the home cages, and the feeder and novel object were located next to the rightmost perch (Fig. S1A). A different object was used in each of the five rounds of this test, so that individual birds were not tested twice with the same object: a blue ping-pong ball in the first round; fluorescent green Lego pieces in the second; a hedgehog-shaped toy in the third; a miniature sneaker in the fourth; and four clothes pegs of different colours in the fifth (Fig. S1B).

We quantified four behavioural variables from the videos. (1) Mean position relative to the feeder and novel object, computed as an index of position relative to the novel object and feeder based on the amount of time the individual spent on each perch. The perches were coded from 1 (furthest from novel object and feeder) to 4 (closest to novel object and feeder). The index of proximity to the novel object and feeder was calculated as $(1 \times \text{time in perch 1} + 2 \times \text{time in perch 2, etc.}) / (\text{time in perches 1 to 4})$. This proximity index can vary between 1 (always on the opposite side from the novel object and feeder) and 4 (always on the feeder or on the perch near the novel object). (2) Latency to the feeder, calculated as the amount of time, in seconds, that the bird took to go to the feeder for the first time. (3) Time at the feeder, i.e. the total time, in seconds, that the bird spent on all its visits to the feeder. (4) Movement, recorded as the number of changes between the four different zones of the cage (as described above). Movement to an adjacent zone was counted as 1, movement to a non-adjacent zone was counted as the number of zones traversed, and movements within the same zone were not counted.

As these four behavioural variables are not independent, and in order to reduce the number of response variables in this test, we ran a principal component analysis (PCA) on these four behaviours. The first principal component (PC) of this PCA explained 53.95% of variance, and was characterized by strong positive loadings of position relative to feeder and novel object (0.790) and time at the feeder (0.841), and negative loadings of latency to the feeder (−0.900) and movement (−0.130). High scores on this PC (hereafter ‘feeding PC’) indicate more time spent near or at the feeder, and a shorter latency to visit the feeder for the first time. The second PC (hereafter ‘activity PC’) explained 24.88% of variance, and was characterized by a strong loading of movement (0.989) and weak loadings of the other behaviours (latency to the feeder: −0.061; time at the feeder: −0.020; and mean position relative to the feeder and novel object: 0.114).

Mirror test

We conducted mirror tests as they are a good proxy for behavioural differences among individuals, are consistent over extended periods of time in common waxbills (Funghi et al., 2015; Gomes et al., 2020), and also predict differences in exploration behaviour (Carvalho et al., 2013). The mirror test was conducted in a small cage (24.5 cm × 17.5 cm × 16 cm) with three equally distanced perches. One end of the cage was entirely occupied by a mirror (17.5 cm × 16 cm), initially covered with a removable cardboard, and the other end was covered by a (fixed) piece of cardboard (Fig. S2). The cage was placed in the centre of an empty room (2 m × 2 m), about 75 cm above the floor. The behaviour of the bird was filmed for 10 min: the first 5 min with the mirror covered and the following 5 min with the mirror exposed, which was done by pulling the

cardboard cover off using a transparent wire from outside the room. As in previous studies with this species, waxbills did not react aggressively towards the mirror image (Carvalho et al., 2013; Funghi et al., 2015; Gomes et al., 2020) and, therefore, this test assays more general social responses. This makes sense for a non-territorial and highly gregarious species such as the waxbill (Clement et al., 1993).

Following Carvalho et al. (2013), we quantified seven behaviours that comprehensively characterize the birds’ activity in the cage, and then tested which behaviours increased from the period with the mirror covered to the period with the mirror exposed, suggesting a response to the mirror image. The following seven behaviours were quantified separately for the 5 min period with the mirror covered and with the mirror uncovered. (1) The mean position relative to the mirror, computed by first dividing the cage into 5 areas (1: closest to mirror, 2: close to first perch, 3: close to second perch, 4: close to third perch, and 5: closest to side opposite mirror) and then calculating the weighted average time (sum of area codes multiplied by time spent there, divided by total duration). This gives an index of proximity to the mirror that can vary from 1 (always near the mirror) to 5 (always near the opposite side). (2) The time facing the mirror, calculated as the length of time the head of the individual was oriented toward the mirror (i.e. within a 90 deg angle centred on the direction of the mirror). The time spent (3) grooming or (4) resting, calculated as the length of time the bird was, respectively, cleaning the bill or feathers, or in typical resting position with the plumage bulked. (5) The number of vocalizations. (6) The number of movements, counting transitions between adjacent positions as in the activity, feeding and novel object test above, but now in the three dimensions of the cage (vertical: floor, perch and hanging on top of cage; depth: hanging on near wall, not on wall, and on distal wall; horizontal: same five areas described for point 1), and then summing counts for the three dimensions. (7) The time spent in fast movements because, in addition to movements to a new location, quantified in point 6, waxbills would also fly or hop continuously, without stopping, for periods of time; we summed the length of time the bird was making these continuous, fast movements. A more detailed description of these behaviours can be found in Carvalho et al. (2013).

Four of these behaviours augmented significantly from before to after exposing the mirror: resting (means before and after exposing the mirror: 0.01 s and 0.02 s; Wilcoxon paired-sample test, $V=2$, $N=115$ tests, $P=0.004$), movement (means: 2.57 and 2.87; $V=659$, $P=0.025$), fast movements (means: 0.00004 s and 0.00091 s; $V=5.5$, $P=0.004$), and facing the mirror (means: 0.40 and 0.47; $V=2393$, $P=0.012$). The remaining three behavioural variables decreased or did not change significantly from before to after exposing the mirror, and were not analysed further: grooming (means: 0.0026 s and 0.0001 s; $V=141$, $P=0.002$), mean position relative to mirror (means: 3.30 and 3.27; $V=894$, $P=0.595$) and number of vocalizations (means: 0.31 and 0.43; $V=69.5$, $P=0.959$).

All previous studies subjecting common waxbills to a mirror test found that, amongst behaviours that differed from before to after exposing the mirror, all activity-related behaviours and time looking at the mirror weighed heavily in a first PC, but with opposing signal (Carvalho et al., 2013; Funghi et al., 2015; Gomes et al., 2020). We thus expected to obtain a first PC that discriminated proactive responses (birds reacting actively to the mirror image but not looking for long periods in its direction) from reactive responses (birds that were less active but more attentive to the mirror image).

However, unlike in previous studies, the first PC of a PCA on these four behaviours was little influenced by the time facing the mirror (trait loading: −0.330; in all previous work using mirror tests

on waxbill, the absolute value of the trait loading for time facing the mirror on PC1 has been >0.70 ; Carvalho et al., 2013; Funghi et al., 2015; Gomes et al., 2020). Instead, time facing the mirror weighed heavily on the second PC (trait loading: -0.847). Therefore, we decided to analyse time facing the mirror separately, and we summarized the other three behaviours in a PCA (full details are given in Table S1), whose first PC we refer to as ‘mirror activity PC’: this explained 39.75% of the variance and was characterized by strong positive loadings of movement (0.673) and fast movements (0.666), and strong negative loadings of resting (-0.544). High scores in the activity PC indicate more active responses to the mirror image (more movement and less resting). As before, we only used data from the period with the mirror uncovered for time facing the mirror.

Statistical analyses

Inspection of histograms showed right-skewed distributions for the feeding PC and activity PC. We transformed each of them to approach normality by first subtracting the minimum value from each PC score and then using a $\log(x+0.05)$ transformation. The distribution of time facing the mirror was approximately normal, but the distribution of the mirror activity PC was strongly leptokurtic. Kurtosis is difficult to correct using simple data transformations. We therefore used heavy tail Lambert W distributions (Goerg, 2011, 2015) to describe and transform this leptokurtic variable. Using the R package ‘LambertW’ (version 0.6.4; <https://cran.r-project.org/web/packages/LambertW/>), we estimated the moments of the distribution with the function IGMM (option ‘h’ for heavy tails), and then normalized the distribution based on those moments (function `get_input`).

To test whether some of the DAergic treatments were responsible for the breakdown of the expected negative association between time facing the mirror and the amount of activity in the mirror test, we ran a general linear model (GLM) with time facing the mirror as dependent variable, the mirror activity PC as covariate, treatment (control, D1R agonist, D1R antagonist, D2R agonist and D2R antagonist) as fixed factor, and their interaction. The effect of interest is the interaction term, which tests whether the relationship between time facing the mirror and the mirror activity PC changes depending on the type of DAergic treatment.

We used generalized linear mixed models (GLMMs) to test whether the control treatment differed from any of the DAergic treatments, separately for each behavioural variable (feeding PC, activity PC, time facing the mirror, and mirror activity PC) using the R package ‘glmmADMB’ (version 0.8.3.3; <http://glmmadmb.r-forge.r-project.org/>). In each GLMM, the behaviour was the dependent variable, treatment (control, D1R agonist, D1R antagonist, D2R agonist and D2R antagonist) was a fixed factor, and individual identity was a random factor, and we chose normal distribution with identity link function. By using individual identity as random factor, we controlled for among-individual differences in behaviour and tested for intra-individual effects of the different treatments. We examined residual $Q-Q$ plots from these models, and in every case residuals were approximately normally distributed. We report the GLMM contrasts (i.e. the simple coefficients, without having run an ANOVA on the GLMM), which test for differences between one level of the fixed factor (the control treatment) and each of the remaining levels (each DAergic treatment). As male and female waxbills do not differ along their reactive–proactive personality axis (Carvalho et al., 2013), and they had very similar responses in our experiments (Fig. S3), we report analyses for the two sexes altogether. All statistical analyses were performed in R (version 3.4.4; <http://www.R-project.org/>).

RESULTS

Covariation of behaviours

Overall, time facing the mirror was not significantly related to the amount of activity in the mirror test (GLM, effect of mirror activity PC: $F_{1,104}=0.100$, $P=0.752$; effect of treatment: $F_{4,104}=1.810$, $P=0.132$). Fig. 2 shows that in most treatments, including the control, time looking at the mirror tended to be negatively related to the mirror activity PC, similar to previous work with this species (Carvalho et al., 2013; Funghi et al., 2015; Gomes et al., 2020), but the opposite was true for the treatment with the D1R antagonist (Fig. 2). This suggests that the D1R antagonist might have been responsible for the breakdown of the expected negative relationship among these behaviours. However, the interaction term between treatment and mirror activity PC was not significant because of a large dispersion in the data ($F_{4,104}=0.552$, $P=0.698$; Fig. 2).

Activity, feeding and novel object test

In the activity, feeding and novel object test, treatment with the D1R antagonist caused birds to take less time to first approach the feeder and increased time spent at or near the feeder (feeding PC) compared with the control treatment ($z=2.59$, $P=0.010$; Table 1, Fig. 3A). Treatment with the D2R agonist caused a decrease in movement (activity PC) relative to the control ($z=-5.63$, $P<0.001$; Table 1, Fig. 3B).

Mirror test

In the mirror test, treatment with either the D2R agonist or the D2R antagonist caused a decrease in the time facing the mirror relative to the control treatment ($z=-1.97$, $P=0.049$, and $z=-2.52$, $P=0.012$, respectively; Table 1, Fig. 3C). Activity in the mirror test (mirror activity PC) decreased in relation to the control for treatment with the D1R antagonist and the D2R antagonist ($z=-2.47$, $P=0.014$, and $z=-4.11$, $P<0.001$, respectively; Table 1, Fig. 3D) and increased in relation to the control for treatment with the D2R agonist ($z=2.05$, $P=0.041$; Table 1, Fig. 3D).

DISCUSSION

In common waxbills, polymorphisms in the *DRD4* gene were previously reported not to correlate with personality differences

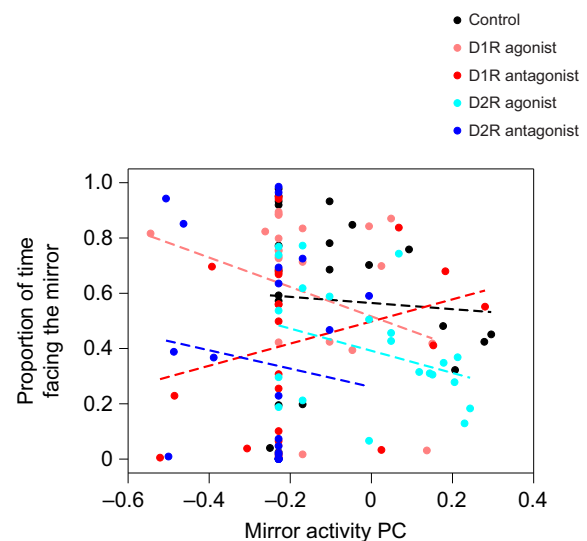


Fig. 2. Scatterplot of time looking at the mirror and scores in the mirror activity principal component (PC). Regression lines are included for each treatment. D1R, dopamine D1 receptor; D2R, dopamine D2 receptor.

Table 1. GLMM contrasts between behaviour in control and following each of the dopaminergic manipulations

	D1R agonist	D1R antagonist	D2R agonist	D2R antagonist
Activity, feeding and novel object test				
Feeding PC	$z=1.04$ ($P=0.297$)	$z=2.59$ ($P=0.010$)	$z=-0.87$ ($P=0.386$)	$z=0.93$ ($P=0.353$)
Activity PC	$z=-0.28$ ($P=0.782$)	$z=-1.08$ ($P=0.282$)	$z=-5.63$ ($P<0.001$)	$z=-1.76$ ($P=0.079$)
Mirror test				
Time facing the mirror	$z=0.27$ ($P=0.786$)	$z=-1.67$ ($P=0.094$)	$z=-1.97$ ($P=0.049$)	$z=-2.52$ ($P=0.012$)
Mirror activity PC	$z=-1.49$ ($P=0.137$)	$z=-2.11$ ($P=0.035$)	$z=2.05$ ($P=0.041$)	$z=-4.11$ ($P<0.001$)

D1R, dopamine D1 receptor; D2R, dopamine D2 receptor. Positive z-values indicate an increase over the control; negative z-values indicate a decrease. In all cases, the sample size is 23 individuals tested across five treatments. Significant results are in bold.

(Carvalho et al., 2013). However, our experimental manipulation of the DAergic D1R and D2R pathways had immediate effects on behaviours that, in waxbills, show stable differences among individuals and are related to personality. Namely, manipulation of the D2R affected behaviour in a social response task, the mirror test, which diagnoses personality type in waxbills (Carvalho et al., 2013). D2R facilitation increased activity levels when birds were exposed to a mirror image, and blockage of D2R transmission decreased this activity. Both these agonist and antagonist treatments decreased the time spent facing the mirror image. Importantly, the effect of D2R manipulation appears to be context dependent, as in the non-social assay (activity, feeding and novel object test), D2R facilitation had the opposite effect of decreasing activity. We also found that blockage of D1R signalling increased feeding in a non-social assay while decreasing the activity in a social context.

Overall relationship between DA and personality traits

DRD4 is an important candidate gene for demonstrating effects of the DAergic system on personality traits in other bird species (Abe et al., 2011; Garamszegi et al., 2014; Holtmann et al., 2016; Mueller et al., 2013, 2014; van Dongen et al., 2015) and other taxa (Bailey et al., 2007; Hejjas et al., 2007; Momozawa et al., 2005; Munafò et al., 2008). But the genetic basis of variation in complex phenotypes, such as behavioural differences among individuals, is probably mediated by the combined small effects of many genes (Gudbjartsson et al., 2008; Lettre et al., 2008; Weedon et al., 2008), and it has been argued that different genes could underlie variation

in identical avian personality traits across populations (Korsten et al., 2010). Therefore, and given the *DRD4* gene codes for a single receptor subtype among various others in the D2-like receptor family, it is not surprising that our manipulation of the DAergic system was able to demonstrate an effect on personality traits of the common waxbill, while a previous correlational study using *DRD4* polymorphisms could not (Carvalho et al., 2013). As noted earlier, the DAergic system includes several receptor types grouped in two main families, D1-like and D2-like receptor families, which can modulate behaviour in distinct manners (St. Onge et al., 2011). This is consistent with our experimental results, which differed depending on whether we manipulated the waxbill DAergic D1R and D2R pathways.

Our results suggest that in addition to affecting personality-related behaviours, manipulation of the DAergic system changed the normal among-individual correlation of behaviours. When consistent behavioural differences among individuals are observed for different behaviours and in a correlated manner, this is known as a behavioural syndrome (Roche et al., 2016; Sih et al., 2004). It was previously shown that waxbills behaving more actively when exposed to a mirror image also explore more in an open-field test (Carvalho et al., 2013), and look in the direction of the mirror image for less time (Carvalho et al., 2013; Funghi et al., 2015; Gomes et al., 2020). Individuals with this behavioural syndrome can be said to have more proactive, as opposed to reactive, personality types (Groothuis and Carere, 2005; Koolhaas et al., 1999; Réale et al., 2010; Sih et al., 2004). Unlike in all past work in

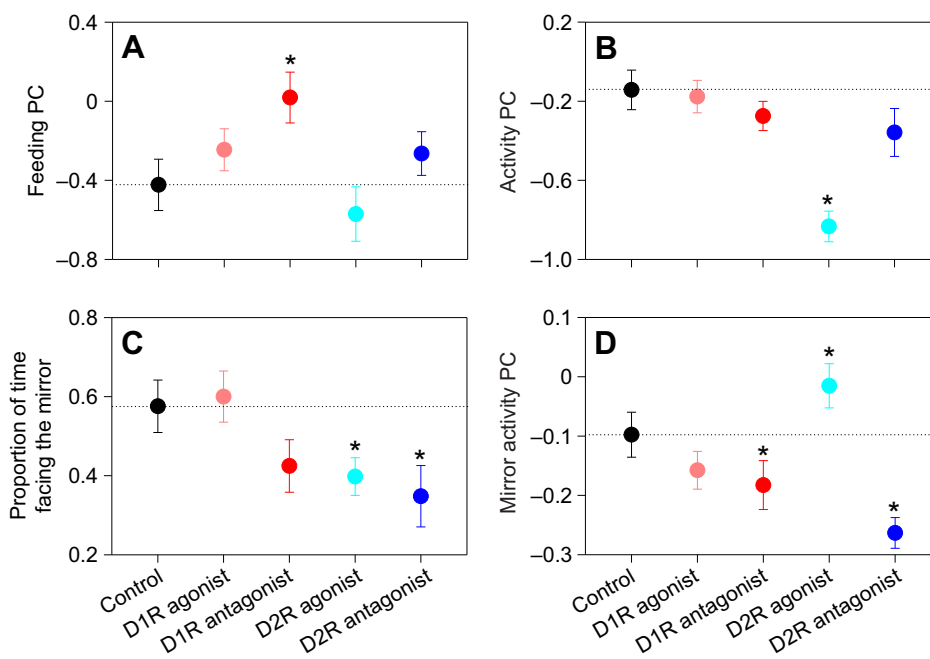


Fig. 3. PCs for the activity, feeding and novel object test and the mirror test. (A,B) Data for the activity, feeding and novel object test indicate a shorter latency to approach and more time at or near the feeder with the D1R antagonist (A) and a decrease in movement with the D2R agonist (B) relative to control. (C,D) Data for the mirror test indicate a decrease in the proportion of time looking at the mirror with the D2R agonist and antagonist (C), and less movement with the D1R and D2R antagonists and more movement with the D2R agonist (D) versus control. Means \pm s.e.m. for each experimental treatment. Asterisks mark significant differences in relation to the control.

which waxbills were studied with mirror tests (Carvalho et al., 2013; Funghi et al., 2015; Gomes et al., 2020), we found that activity and time spent looking at the mirror image were largely dissociated. Inspection of the data (see Fig. 2) suggests that treatment with the D1R antagonist may have been mainly responsible for this dissociation of behaviours. Thus, the DAergic system may be involved not only in determining personality-related behaviour in waxbills but also in maintaining the normal correlation of behaviours, or behavioural syndrome, observed across waxbill personality types. Our results, however, are only suggestive of an effect on behavioural correlations, and this hypothesis needs further investigation.

Effects of D1-like receptor manipulation

Inhibition of the D1-like receptors in the non-social assay caused birds to feed much more than in the control treatment, but did not change activity levels. DA is highly implicated in reward assessment and reinforcement behaviours (Berridge and Robinson, 1998; Heimovics et al., 2009), with the D1-like receptor pathways being particularly involved in reward-incentive learning (e.g. classic paradigms in which animals' operant behavioural response is tested in association with food, water, brain stimulation reward or pharmacological administration; Beninger and Miller, 1998). By inhibiting D1R transmission, and hence signalling for an outcome worse than predicted (i.e. a reward omission; Schultz, 1998), animals may enter a state of some distress which explains the great increase in feeding activity that we observed in waxbills. For example, blockage D1Rs in the cleaner fish (*Labroides dimidiatus*) induced them to initiate more interactions with clients and provide almost exclusively physical stimulation (Messias et al., 2016), especially to unfamiliar clients (Soares et al., 2017). In other words, reduction of D1R signalling seems to lead both waxbills and cleaner fish to a state of uncertainty regarding the future that causes waxbills to increase their feeding activity and cleaner fish to increase client stimulation.

Interestingly, the waxbills' response to D1R antagonism appeared to differ between contexts, as in the social assay (mirror test), D1R antagonism had the effect of reducing, rather than increasing, overall activity. Context-specific effects of DA signalling are not unexpected in birds, as it was previously shown that DA affects the motivation to sing (Schroeder and Ritters, 2006) but in a manner that is specific to certain social contexts (Heimovics and Ritters, 2008). In humans too, DA signalling is known to modulate positive (pleasure) and negative emotions in a context-specific manner, with disruption of DA signalling resulting in the absence of a response or, at the other extreme, the display of excessive reactions (Berridge and Kringelbach, 2015).

Effects of D2-like receptor manipulation

Experimental manipulations of the D2R pathways also revealed context-dependent effects: D2R facilitation in the social context increased activity, whereas in the non-social context it decreased activity. D2R pathways are known to regulate motivation related to social context (Choleris et al., 2011; Young and Wang, 2004). For instance, in male zebra finches (*Taeniopygia guttata*), D2R signalling inhibited aggressive mate competition without influencing courtship song (Kabelik et al., 2010) and, in unpaired female zebra finches, female song preferences were enhanced by D2R facilitation and disrupted by blocking D2R (Day et al., 2019). Our results in the social test corroborate this pattern but results in the non-social context were opposite to it, with D2R activation diminishing overall activity. Considering these context-dependent

results, further studies in which manipulation of D2R is coupled with more realistic social and non-social contexts would significantly advance our understanding of the proximal mechanisms by which birds fine-tune their behaviour in changing contexts.

While D2-like receptors were clearly implicated in the level of motor activity in the social assay (D2R agonist increased activity, and D2R antagonist decreased it), their effects on social attentiveness are less clear, as both D2R agonism and antagonism decreased how much birds looked at the social stimulus (mirror image). These results show that the D2-like receptor pathway is implicated in modulating attention to a social stimulus, but the functioning and direction of this modulation need further research. One possible explanation as to why D2R agonism and antagonism affected social attentiveness in the same direction is that the social stimulus lacked sexual valence (same-sex mirror images, rather than an individual of the opposite sex). In other species, elevated region-specific D2R brain signalling mediates partner preference (D2R signalling at the nucleus accumbens of female prairie voles, *Microtus ochrogaster*, and at the striatum of female zebra finches; Aragona et al., 2003; Liu and Wang, 2003; Tokarev et al., 2017). If the D2-like receptor pathway is specifically attuned to modulate attentiveness to sexual partners, then perhaps the lack of sexual valence of the mirror image would explain the absence of behavioural response with both agonist and antagonist D2R treatment. Moreover, the distinct effects of D2-like receptors on activity and on attentiveness to the social stimulus may indicate action at distinct brain sites; for example, affecting motor response and social recognition or memory. Brain regions differ in the density and/or distribution of DA receptors, which can be critical to dose-related effects of drugs (Balthazart et al., 1997). Depending on receptor density, and knowing that D2-like receptors can mediate auto-inhibition (because of their presynaptic and postsynaptic localization; De Mei et al., 2009), high DA availability could result in inhibition rather than enhancement of the social attentiveness to the mirror image.

Concluding remarks

DA is known to crucially influence various aspects of brain function, and thus modulate multiple behavioural functions (O'Connell and Hofmann, 2011, 2012; Soares, 2017). Here, we found that blockage of D1-like receptors affected feeding behaviour, with waxbills increasing their focus on food intake but switching to a rather immobile state during a social test. In contrast, manipulation of D2-like receptors affected activity when birds were exposed to a social stimulus (mirror image) in the opposite manner to which it affected activity in a non-social context. While previous work based on a candidate dopamine receptor gene (*DRD4*) did not find evidence for an association with personality in common waxbills (Carvalho et al., 2013), we documented distinct context-specific effects of the DAergic system, via either D1R or D2R pathways, on behaviours related to waxbill personality. We conclude that the DAergic system has a stronger effect on avian personality than is apparent from candidate-gene studies. Future research on animal personality should benefit from considering effects from distinct pathways within the DAergic system.

Acknowledgements

We thank Ana Cristina Gomes for help with the script of stats analyses and the two anonymous referees for constructive comments on previous versions of the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

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

Funding

This study was partially supported by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e a Tecnologia) project: PTDC/BIA-EVF/4852/2014. M.C.S. and G.C.C. are currently supported by National Funds through Fundação para a Ciência e a Tecnologia (DL57/2019/CP1440/CT0019 and DL57/2016/CP1440/CT0011, respectively), and P.A.S. and S.T. by Fundação para a Ciência e a Tecnologia project PTDC/BIA-ECO/32210/2017.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.216499.supplemental>

References

- Abe, H., Ito, S. and Inoue-Murayama, M. (2011). Polymorphisms in the extracellular region of dopamine receptor D4 within and among avian orders. *J. Mol. Evol.* **72**, 253-264. doi:10.1007/s00239-011-9432-9
- Abler, B., Walter, H. and Erk, S. (2005). Neural correlates of frustration. *Neuroreport* **16**, 669-672. doi:10.1097/00001756-200505120-00003
- Alcaro, A., Huber, R. and Panksepp, J. (2007). Behavioral functions of the mesolimbic dopaminergic system: An affective neuroethological perspective. *Brain Res. Rev.* **56**, 283-321. doi:10.1016/j.brainresrev.2007.07.014
- Amy, M., Ung, D., Béguin, N. and LeBoucher, G. (2017). Personality traits and behavioural profiles in the domestic canary are affected by sex and photoperiod. *Ethology* **123**, 885-893. doi:10.1111/eth.12662
- Apfelbeck, B. and Raess, M. (2008). Behavioural and hormonal effects of social isolation and neophobia in a gregarious bird species, the European starling (*Sturnus vulgaris*). *Horm. Behav.* **54**, 435-441. doi:10.1016/j.yhbeh.2008.04.003
- Aragona, B. J., Liu, Y., Curtis, J. T., Stephan, F. K. and Wang, Z. (2003). A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. *J. Neurosci.* **23**, 3483-3490. doi:10.1523/JNEUROSCI.23-08-03483.2003
- Atwell, J. W., Cardoso, G. C., Whittaker, D. J., Campbell-Nelson, S., Robertson, K. W. and Ketterson, E. D. (2012). Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. *Behav. Ecol.* **23**, 960-969. doi:10.1093/beheco/ars059
- Bailey, J. N., Breidenthal, S. E., Jorgensen, M. J., McCracken, J. T. and Fairbanks, L. A. (2007). The association of DRD4 and novelty seeking is found in a nonhuman primate model. *Psychiatr. Genet.* **17**, 23-27. doi:10.1097/YPG.0b013e32801140f2
- Balthazart, J., Castagna, C. and Ball, G. F. (1997). Differential effects of D1 and D2 dopamine-receptor agonists and antagonists on appetitive and consummatory aspects of male sexual behavior in Japanese quail. *Physiol. Behav.* **62**, 571-580. doi:10.1016/S0031-9384(97)00163-7
- Bello, N. T. and Hajnal, A. (2010). Dopamine and binge eating behaviors. *Pharmacol. Biochem. Behav.* **97**, 25-33. doi:10.1016/j.pbb.2010.04.016
- Bello, E. P., Mateo, Y., Gelman, D. M., Noain, D., Shin, J. H., Low, M. J., Alvarez, V. A., Lovinger, D. M. and Rubinstein, M. (2011). Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nat. Neurosci.* **14**, 1033. doi:10.1038/nn.2862
- Beninger, R. J. and Miller, R. (1998). Dopamine D1-like receptors and reward-related incentive learning. *Neurosci. Biobehav. Rev.* **22**, 335-345. doi:10.1016/S0149-7634(97)00019-5
- Berridge, K. C. and Kringelbach, M. L. (2015). Pleasure systems in the brain. *Neuron* **86**, 646-664. doi:10.1016/j.neuron.2015.02.018
- Berridge, K. C. and Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Brain Res. Rev.* **28**, 309-369. doi:10.1016/S0165-0173(98)00019-8
- Boogert, N. J., Reader, S. M. and Laland, K. N. (2006). The relation between social rank, neophobia and individual learning in starlings. *Anim. Behav.* **72**, 1229-1239. doi:10.1016/j.anbehav.2006.02.021
- Bromberg-Martin, E. S., Matsumoto, M. and Hikosaka, O. (2010). Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron* **68**, 815-834. doi:10.1016/j.neuron.2010.11.022
- Callier, S., Snapyan, M., Le Crom, S., Prou, D., Vincent, J.-D. and Vernier, P. (2003). Evolution and cell biology of dopamine receptors in vertebrates. *Biol. Cell* **95**, 489-502. doi:10.1016/S0248-4900(03)00089-3
- Caramaschi, D., Carere, C., Sgoifo, A. and Koolhaas, J. M. (2013). Neuroendocrine and autonomic correlates of animal personalities. In *Animal Personalities: Behavior, Physiology, and Evolution* (ed. C. Carere and D. Maestri), pp. 353-377. Chicago, London: The University of Chicago Press.
- Carere, C., Fawcett, T. W. and Caramaschi, D. (2010). Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses. *Curr. Zool.* **56**, 728-740. doi:10.1093/czoolo/56.6.728
- Carvalho, C. F., Leitão, A. V., Funghi, C., Batalha, H. R., Reis, S., Mota, P. G., Lopes, R. J. and Cardoso, G. C. (2013). Personality traits are related to ecology across a biological invasion. *Behav. Ecol.* **24**, 1081-1091. doi:10.1093/behecol/art034
- Choleris, E., Clipperton-Allen, A. E., Gray, D. G., Diaz-Gonzalez, S. and Welsman, R. G. (2011). Differential effects of dopamine receptor D1-type and D2-type antagonists and phase of the estrous cycle on social learning of food preferences, feeding, and social interactions in mice. *Neuropsychopharmacology* **36**, 1689-1702. doi:10.1038/npp.2011.50
- Clement, P., Harris, A. and Davies, J. (1993). *Finches and Sparrows: an Identification Guide*. Princeton University Press.
- Coppens, C. M., De Boer, S. F. and Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: Towards underlying mechanisms. *Philos. Trans. R. Soc. B Biol. Sci.* **365**, 4021-4028. doi:10.1098/rstb.2010.0217
- Day, N. F., Saxon, D., Robbins, A., Harris, L., Nee, E., Shroff-Mehta, N., Stout, K., Sun, J., Lillie, N., Burns, M. et al. (2019). D2 dopamine receptor activation induces female preference for male song in the monogamous zebra finch. *J. Exp. Biol.* **222**, jeb191510. doi:10.1242/jeb.191510
- de Almeida, R. M. M. and Miczek, K. A. (2002). Aggression escalated by social instigation or by discontinuation of reinforcement ("Frustration") in mice: inhibition by apirtoline: a 5-HT1B receptor agonist. *Neuropsychopharmacology* **27**, 171-181. doi:10.1016/S0893-133X(02)00291-9
- de Boer, L., Axelsson, J., Chowdhury, R., Riklund, K., Dolan, R. J., Nyberg, L., Bäckman, L., Guitart-Masip, M. (2019). Dorsal striatal dopamine D1 receptor availability predicts an instrumental bias in action learning. *Proc. Natl. Acad. Sci. USA* **116**, 261-270. doi:10.1073/pnas.1816704116
- De Mei, C., Ramos, M., Itaka, C. and Borrelli, E. (2009). Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. *Curr. Opin. Pharmacol.* **9**, 53-58. doi:10.1016/j.coph.2008.12.002
- DeYoung, C. G. (2013). The neuromodulator of exploration: a unifying theory of the role of dopamine in personality. *Front. Hum. Neurosci.* **7**, 1-26. doi:10.3389/fnhum.2013.00762
- Ding, L. and Perkel, D. J. (2002). Dopamine modulates excitability of spiny neurons in the avian basal ganglia. *J. Neurosci.* **22**, 5210-5218. doi:10.1523/JNEUROSCI.22-12-05210.2002
- Fidler, A. E., van Oers, K., Drent, P. J., Kuhn, S., Mueller, J. C. and Kempenaers, B. (2007). Drd4 gene polymorphisms are associated with personality variation in a passerine bird. *Proc. R. Soc. B Biol. Sci.* **274**, 1685-1691. doi:10.1098/rspb.2007.0337
- Frank, M. J., Seeberger, L. C. and O'Reilly, R. C. (2004). By carrot or by stick: cognitive reinforcement learning in Parkinsonism. *Science* **306**, 1940-1943. doi:10.1126/science.1102941
- Funghi, C., Leitão, A. V., Ferreira, A. C., Mota, P. G. and Cardoso, G. C. (2015). Social dominance in a gregarious bird is related to body size but not to standard personality assays. *Ethology* **121**, 84-93. doi:10.1111/eth.12318
- Garamszegi, L. Z., Mueller, J. C., Markó, G., Szász, E., Zsebok, S., Herczeg, G., Eens, M. and Török, J. (2014). The relationship between DRD4 polymorphisms and phenotypic correlations of behaviors in the collared flycatcher. *Ecol. Evol.* **4**, 1466-1479. doi:10.1002/ece3.1041
- Gobert, A., Rivet, J.-M., Audinot, V., Newman-Tancredi, A., Cistarelli, L. and Millan, M. J. (1998). Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto- and heteroreceptor-mediated control of release. *Neuroscience* **84**, 413-429. doi:10.1016/S0306-4522(97)00565-4
- Goerg, G. M. (2011). Lambert W random variables - A new family of generalized skewed distributions with applications to risk estimation. *Ann. Appl. Stat.* **5**, 2197-2230. doi:10.1214/11-AOAS457
- Goerg, G. M. (2015). The Lambert way to gaussianize heavy-tailed data with the inverse of Tukey's h transformation as a special case. *Sci. World J.* **25**, 909231. doi:10.1155/2015/909231
- Gomes, A. C. R., Guerra, S., Silva, P. A., Marques, C. I. J., Trigo, S., Boogert, N. and Cardoso, G. C. (2020). Proactive common waxbills make fewer mistakes in a cognitive assay, the detour-reaching task. *Behav. Ecol. Sociobiol.* doi:10.1007/s00265-020-2809-2
- Groothuis, T. G. G. and Carere, C. (2005). Avian personalities: characterization and epigenesis. *Neurosci. Biobehav. Rev.* **29**, 137-150. doi:10.1016/j.neubiorev.2004.06.010
- Gudbjartsson, D. F., Walters, G. B., Thorleifsson, G., Stefansson, H., Halldorsson, B. V., Zusmanovich, P., Sulem, P., Thorlacius, S., Gylfason, A., Steinberg, S. et al. (2008). Many sequence variants affecting diversity of adult human height. *Nat. Genet.* **40**, 609-615. doi:10.1038/ng.122
- Heimovics, S. A. and Ritters, L. V. (2008). Evidence that dopamine within motivation and song control brain regions regulates birdsong context-dependently. *Physiol. Behav.* **95**, 258-266. doi:10.1016/j.physbeh.2008.06.009
- Heimovics, S. A., Cornil, C. A., Ball, G. F. and 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. doi:10.1016/j.neuroscience.2009.01.042
- Hejjas, K., Vas, J., Topal, J., Szantai, E., Ronai, Z., Szekely, A., Kubinyi, E., Horvath, Z., Sasvari-Szekely, M. and Miklosi, A. (2007). Association of polymorphisms in the dopamine D4 receptor gene and the activity-impulsivity endophenotype in dogs. *Anim. Genet.* **38**, 629-633. doi:10.1111/j.1365-2052.2007.01657.x
- Holtmann, B., Grosser, S., Lagisz, M., Johnson, S. L., Santos, E. S. A., Lara, C. E., Robertson, B. C. and Nakagawa, S. (2016). Population differentiation and behavioural association of the two 'personality' genes DRD4 and SERT in dunlocks (*Prunella modularis*). *Mol. Ecol.* **25**, 706-722. doi:10.1111/mec.13514
- Kabelik, D., Kelly, A. M. and Goodson, J. L. (2010). Dopaminergic regulation of mate competition aggression and aromatase-Fos colocalization in vasotocin neurons. *Neuropharmacology* **58**, 117-125. doi:10.1016/j.neuropharm.2009.06.009
- Kleitz, H. K., Cornil, C. A., Balthazart, J. and Ball, G. F. (2009). Species differences in the relative densities of D1- and D2-like dopamine receptor subtypes in the Japanese quail and rats: an in vitro quantitative receptor autoradiography study. *Brain. Behav. Evol.* **73**, 81-90. doi:10.1159/000209864
- Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J. and Van Reenen, C. G. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neurosci. Biobehav. Rev.* **23**, 925-935. doi:10.1016/S0149-7634(99)00026-3
- Koolhaas, J. M., de Boer, S. F., Coppens, C. M. and Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Front. Neuroendocrinol.* **31**, 307-321. doi:10.1016/j.yfrne.2010.04.001
- Korsten, P., Mueller, J. C., Hermannstädter, C., Bouwman, K. M., Dingemans, N. J., Drent, P. J., Liedvogel, M., Matthysen, E., Van Oers, K., Van Overveld, T. et al. (2010). Association between DRD4 gene polymorphism and personality variation in great tits: a test across four wild populations. *Mol. Ecol.* **19**, 832-843. doi:10.1111/j.1365-294X.2009.04518.x
- Kubikova, L. and Košťál, L. (2010). Dopaminergic system in birdsong learning and maintenance. *J. Chem. Neuroanat.* **39**, 112-123. doi:10.1016/j.jchemneu.2009.10.004
- Kubikova, L., Wada, K. and Jarvis, E. D. (2010). Dopamine receptors in a songbird brain. *J. Comp. Neurol.* **518**, 741-769. doi:10.1002/cne.22255
- Lette, G., Jackson, A. U., Gieger, C., Schumacher, F. R., Berndt, S. I., Sanna, S., Eyheramendy, S., Voight, B. F., Butler, J. L., Guiducci, C. et al. (2008). Identification of ten loci associated with height highlights new biological pathways in human growth. *Nat. Genet.* **40**, 584-591. doi:10.1038/ng.125
- Liu, Y. and Wang, Z. X. (2003). Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience* **121**, 537-544. doi:10.1016/S0306-4522(03)00555-4
- Messias, J. P. M., Paula, J. R., Grutter, A. S., Bshary, R. and Soares, M. C. (2016). Dopamine disruption increases negotiation for cooperative interactions in a fish. *Sci. Rep.* **6**, 2-9. doi:10.1038/s41598-016-0005-4
- Metke-Hofmann, C. (2012). Head colour and age relate to personality traits in gouldian finches. *Ethology* **118**, 906-916. doi:10.1111/j.1439-0310.2012.02079.x
- Momozawa, Y., Takeuchi, Y., Kusunose, R., Kikusui, T. and Mori, Y. (2005). Association between equine temperament and polymorphisms in dopamine D4 receptor gene. *Mamm. Genome* **16**, 538-544. doi:10.1007/s00335-005-0021-3
- Mueller, J. C., Korsten, P., Hermannstaedter, C., Feulner, T., Dingemans, N. J., Matthysen, E., van Oers, K., van Overveld, T., Patrick, S. C., Quinn, J. L. et al. (2013). Haplotype structure, adaptive history and associations with exploratory behaviour of the DRD4 gene region in four great tit (*Parus major*) populations. *Mol. Ecol.* **22**, 2797-2809. doi:10.1111/mec.12282
- Mueller, J. C., Edelaar, P., Carrete, M., Serrano, D., Potti, J., Blas, J., Dingemans, N. J., Kempenaers, B. and Tella, J. L. (2014). Behaviour-related DRD4 polymorphisms in invasive bird populations. *Mol. Ecol.* **23**, 2876-2885. doi:10.1111/mec.12763
- Munafò, M. R., Yalcin, B., Willis-Owen, S. A. and Flint, J. (2008). Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. *Biol. Psychiatry* **63**, 197-206. doi:10.1016/j.biopsych.2007.04.006
- O'Connell, L. A. and Hofmann, H. A. (2011). The Vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *J. Comp. Neurol.* **519**, 3599-3639. doi:10.1002/cne.22735
- O'Connell, L. A. and Hofmann, H. A. (2012). Evolution of a vertebrate social decision-making network. *Science* **336**, 1154-1157. doi:10.1126/science.1218889
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T. and Dingemans, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biol. Rev.* **82**, 291-318. doi:10.1111/j.1469-185X.2007.00010.x
- Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V. and Montiglio, P.-O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos. Trans. R. Soc. B Biol. Sci.* **365**, 4051-4063. doi:10.1098/rstb.2010.0208
- Reynolds, J. N. J., Hyland, B. I. and Wickens, J. R. (2001). A cellular mechanism of reward-related learning. *Nature* **413**, 67-70. doi:10.1038/35092560
- Riyahi, S., Sánchez-Delgado, M., Calafell, F., Monk, D. and Senar, J. C. (2015). Combined epigenetic and intraspecific variation of the DRD4 and SERT genes influence novelty seeking behavior in great tit *Parus major*. *Epigenetics* **10**, 516-525. doi:10.1080/15592294.2015.1046027
- Riyahi, S., Björklund, M., Mateos-Gonzalez, F. and Senar, J. C. (2017). Personality and urbanization: behavioural traits and DRD4 SNP830 polymorphisms in great tits in Barcelona city. *J. Ethol.* **35**, 101-108. doi:10.1007/s10164-016-0496-2
- Roche, D. G., Careau, V. and Binning, S. A. (2016). Demystifying animal "personality" (or not): why individual variation matters to experimental biologists. *J. Exp. Biol.* **219**, 3832-3843. doi:10.1242/jeb.146712
- Salamone, J. D. and Correa, M. (2002). Motivational views of reinforcement: Implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav. Brain Res.* **137**, 3-25. doi:10.1016/S0166-4328(02)00282-6
- Schroeder, M. and Ritters, L. V. (2006). Pharmacological manipulations of dopamine and opioids have differential effects on sexually motivated song in male European starlings. *Physiol. Behav.* **88**, 575-584. doi:10.1016/j.physbeh.2006.05.011
- Schultz, W. (1998). Predictive reward signal of dopamine neurons. *J. Neurophysiol.* **80**, 1-27. doi:10.1152/jn.1998.80.1.1
- Schultz, W. (2010). Dopamine signals for reward value and risk: basic and recent data. *Behav. Brain Funct.* **6**, 24. doi:10.1186/1744-9081-6-24
- Schultz, W., Dayan, P. and Montague, P. R. (1997). A neural substrate of prediction and reward. *Science* **275**, 1593-1599. doi:10.1126/science.275.5306.1593
- Sih, A., Bell, A. M., Johnson, J. C. and Ziemba, R. E. (2004). Behavioral syndromes: an integrative overview. *Q. Rev. Biol.* **79**, 241-277. doi:10.1086/422893
- Soares, M. C. (2017). The Neurobiology of Mutualistic Behavior: The Cleanerfish Swims into the Spotlight. *Front. Behav. Neurosci.* **11**, 1-12. doi:10.3389/fnbeh.2017.00191
- Soares, M. C., Santos, T. P. and Messias, J. P. M. (2017). Dopamine disruption increases cleanerfish cooperative investment in novel client partners. *R. Soc. Open Sci.* **4**, 1-7. doi:10.1098/rsos.160609
- St. Onge, J. R., Abhari, H. and Floresco, S. B. (2011). Dissociable contributions by prefrontal D1 and D2 receptors to risk-based decision making. *J. Neurosci.* **31**, 8625-8633. doi:10.1523/JNEUROSCI.1020-11.2011
- Tokarev, K., Bruno, J. H., Ljubičić, I., Kothari, P. J., Helekar, S. A., Tchernichovski, O. and Voss, H. U. (2017). Sexual dimorphism in striatal dopaminergic responses promotes monogamy in social songbirds. *Elife* **6**, 1-20. doi:10.7554/eLife.25819
- van Dongen, W. F. D., Robinson, R. W., Weston, M. A., Mulder, R. A. and Guay, P.-J. (2015). Variation at the DRD4 locus is associated with wariness and local site selection in urban black swans. *BMC Evol. Biol.* **15**, 253. doi:10.1186/s12862-015-0533-8
- Vindas, M. A., Johansen, I. B., Vela-Avitua, S., Nørstrud, K. S., Aalgaard, M., Braastad, B. O., Höglund, E. and Øverli, Ø. (2014). Frustrative reward omission increases aggressive behaviour of inferior fighters. *Proc. R. Soc. B Biol. Sci.* **281**, 20140300. doi:10.1098/rspb.2014.0300
- Webster, S. J. and Lefebvre, L. (2001). Problem solving and neophobia in a columbiform-passeriform assemblage in Barbados. *Anim. Behav.* **62**, 23-32. doi:10.1006/anbe.2000.1725
- Weedon, M. N., Lango, H., Lindgren, C. M., Wallace, C., Evans, D. M., Mangino, M., Freathy, R. M., Perry, J. R. B., Stevens, S., Hall, A. S. et al. (2008). Genome-wide association analysis identifies 20 loci that influence adult height. *Nat. Genet.* **40**, 575-583. doi:10.1038/ng.121
- Young, L. J. and Wang, Z. (2004). The neurobiology of pair bonding. *Nat. Neurosci.* **7**, 1048-1054. doi:10.1038/nn1327
- Zimmerman, P. H. and Koene, P. (1998). The effect of frustrative nonreward on vocalisations and behaviour in the laying hen, *Gallus gallus domesticus*. *Behav. Processes* **44**, 73-79. doi:10.1016/S0376-6357(98)00035-7