



Research report

Functional interactions of dopamine cell groups reflect personality, sex, and social context in highly social finches



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HIGHLIGHTS

- Dopamine cell groups respond differentially to novel and familiar social stimuli.
- Fos responses of dopamine neurons are predicted by sex and personality.
- Sex and personality predict functional interactions of dopamine cell groups.

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ABSTRACT

Dopamine (DA) is well known for its involvement in novelty-seeking, learning, and goal-oriented behaviors such as social behavior. However, little is known about how DA modulates social processes differentially in relation to sex and behavioral phenotype (e.g., personality). Importantly, the major DA cell groups (A8–A15) are conserved across all amniote vertebrates, and thus broadly relevant insights may be obtained through investigations of avian species such as zebra finches (*Taeniopygia guttata*), which express a human-like social organization based on biparental nuclear families that are embedded within larger social groups. We here build upon a previous study that quantified multidimensional personality structures in male and female zebra finches using principal components analysis (PCA) of extensive behavioral measures in social and nonsocial contexts. These complex dimensions of behavioral phenotype can be characterized as Social competence/dominance, Gregariousness, and Anxiety. Here we analyze Fos protein expression in DA neuronal populations in response to social novelty and demonstrate that the Fos content of multiple dopamine cell groups is significantly predicted by sex, personality, social context, and their interactions. In order to further investigate coordinated neuromodulation of behavior across multiple DA cell groups, we also conducted a PCA of neural variables (DA cell numbers and their phasic Fos responses) and show that behavioral PCs are associated with unique suites of neural PCs. These findings demonstrate that personality and sex are reflected in DA neuron activity and coordinated patterns of neuromodulation arising from multiple DA cell groups.

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1. Introduction

With the advent of noninvasive neuroimaging techniques, exploring the mechanisms that are associated with human personality has become more feasible, so much so that personality neuroscience is emerging as a subdiscipline within psychology [1–3]. However, in order to examine neural systems that relate to aspects of personality at the cellular level, we must turn to non-human animal studies. We recently provided an extensive description of complex phenotype structure in a tractable, popular

species for genomic and behavioral studies—the highly social zebra finch (*Taeniopygia guttata*) [4], a socially monogamous, biparental species that lives in groups year-round. Because personality has been shown to profoundly impact the neural processing of social stimuli in humans [5], we explored the ways in which vasopressin-oxytocin (VP-OT) cell groups respond to novel and familiar social stimuli in relation to sex and multidimensional behavioral phenotypes (personality) in zebra finches. Using an alternate tissue series from the 80 animals phenotyped in this earlier study, we here conduct a similar analysis focused on dopamine (DA). DA is thought to be a strong generator of phenotypic diversity because of its involvement in motivation [6], affiliation [7,8], aggression, and sexual behavior [9,10], and thus we hypothesized that, similar to what we found with VP-OT neurons, the responses of DA neurons to social novelty vary in relation to sex and dimensions of personality.

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DA signaling is implicated in various aspects of phenotypic variation, such as individual differences in social impulsivity and novelty-seeking behavior [11,12]. However, although eight populations of DA neurons, A8–A15, are recognized in the forebrain and midbrain of amniote vertebrates [13,14], the vast majority of experimental data relate to only three—the A9 (substantia nigra), A10 (ventral tegmental area; VTA), and A12 (tuberoinfundibular hypothalamus). These cell groups have been studied primarily in relation to motor function, incentive motivation, and prolactin secretion, respectively, although other DA cell groups in the brain are known to be relevant to social behavior [15–18], hormonal regulation [19], and responses to stress [19–21]. In songbirds, the various DA cell groups exhibit distinct patterns of Fos response (a proxy marker of neural activity) to various social stimuli [18], and both individual and species differences are reflected in DA anatomy and/or socially induced Fos response, as shown in relation to affiliation (for the A10 cell group and the A11 cell group of midbrain central gray, CG) [7] and aggression (for the A10 cell group, A12 cell group; and A14 cell group of the medial hypothalamus and preoptic area, POA) [22].

We here present the personality profiles of male and female zebra finches as previously quantified [4], examine Fos expression in DA neurons following exposure to novel and familiar social partners, and explore the possibility that personality and sex relate to functional interactions across multiple DA cell groups.

2. Materials and methods

2.1. Animals

Forty male and 40 female zebra finches obtained as adults from a commercial supplier were used for these experiments. Subjects were housed in same-sex groups of 6–10 except for 3 days of testing in colonies, which contained 4 males and 4 females. Subjects were kept on a 14L:10D photoperiod with full spectrum lighting and were provided finch seed mix, cuttlebone, grit, and water ad libitum. The subjects and behavioral data used for the present experiments are the same as those presented in Ref. [4]. Tests were conducted in a humane manner and were approved by the Institutional Animal Care and Use Committee of Indiana University.

2.2. Behavioral phenotyping

Behavioral assays were conducted in the same order for all subjects and were completed within a 2-week period, with the exception of colony tests. Most of the assays were fairly short and were likely no more salient than a regular cage change, and therefore should not have produced carry-over effects that impacted subsequent measurements. The only assay that may have produced carry-over effects was the colony test, which involved intense courtship, aggression, and other social behaviors over multiple days. Therefore, colony observations were conducted last and were completed over a 6-week period.

2.2.1. Novelty-suppressed feeding

Food was removed from subjects' cages prior to lights-on in the morning. After lights-on, subjects were placed in a novel cage (31 cm W × 20 cm H × 36 cm D) that contained a novel purple nitrile glove hanging above a food dish. Subjects were then video recorded for 30 min and the latencies to move and feed were quantified.

2.2.2. Exploration of a novel environment

Subjects were placed in a flight cage (1.3 m W × 1.8 m H × 1.8 m D) with a tree branch cluster in each of the four corners. The latency to move and number of branch clusters explored was recorded during a 4 min trial.

2.2.3. Group size preference

Subjects were placed into a 1 m wide (0.43 m H × 0.36 m D) cage that was divided into seven zones by perches [23]. The perches at each end of the cage were approximately 4 cm from the cage wall, which adjoined a 0.5 m wide (0.43 m H × 0.36 m D) cage containing 2 novel same-sex stimulus birds at one end and 10 novel same-sex stimulus birds at the other (sides counterbalanced across subjects). The location of the subject was recorded every 15 s for 5 min. The amount of time spent with the large and small groups was defined as time spent on the perch closest to the stimulus cage.

2.2.4. Novel vs. familiar social preference

Using the same cage arrangement as just described for tests of group size preference, subjects were exposed to cages containing five novel same-sex conspecifics and five familiar same-sex cage-mates. The location of the subject was recorded every 15 s for 5 min, with sides counterbalanced across subjects. The amount of time spent with the novel and familiar groups was defined as time spent on the perch closest to the stimulus cage.

2.2.5. Colony observations

Behavioral observations in colonies were conducted as previously described [4,6,24]. Four subjects of each sex (all novel to each other) were moved into colony cages (1.3 m W × 0.43 m H × 0.36 m D) that contained 4 nest cups and shredded burlap nesting material. Focal observations were conducted 6 times over 3 days (AM/PM). Session 1 observations were 5 min per subject and began 10 min after the establishment of colonies, and Sessions 2–6 were 10 min each. The shorter observation period for Session 1 allowed for the quantification of behavior in all subjects during the initial burst of courtship and competitive aggression. The following behaviors were quantified: allopreen, follow, dance, directed song, undirected song, pick up nest item, carry nest item to nest, time spent on nest, copulation, latency to pair bond, and aggressive behaviors (displacements, threats, beak fences, and pecks). We also quantified displacements received from other birds. As in previous studies, all data except pairing were converted to units of behavior per minute not spent on the nest [4,6,24].

2.3. Principal components analysis

Principal components analysis (PCA) allows three or more variables to be reduced to a smaller number of factors that account for most of the variance observed in a full set of measured variables [25]. Because we obtained a large number of behavioral variables in the present dataset, we employed PCA to group statistically related variables, thereby reducing the number of variables. It is important to note that variables that load onto a principal component (PC) may not necessarily have a linear relationship. A loading of ≤ -0.4 or ≥ 0.4 is generally considered strong [25], and we therefore took the conservative approach of eliminating variables that did not load ≤ -0.300 or ≥ 0.300 , which removed several low-frequency behaviors (e.g., allopreen and copulation) from the analysis. PCA was conducted separately for males and females; however, the first three PCs that exhibited strong eigenvalues, ranging from 1.868 to 3.832, were very similar for both sexes (see Tables S1 and S2 from [4]). Given that both males and females had similar PC structures and because we wanted to directly compare the sexes, we conducted a final PCA using data from all subjects. Male-specific behaviors were excluded from the final analysis; no female-specific behaviors were quantified. Table 1 from [4] shows the final PCA structure, with variables that load most strongly (≤ -0.5 or ≥ 0.5) in bold font.

2.4. Behavioral manipulations for Fos analyses

Upon completion of the behavioral testing described above, all subjects were returned to their home cages. The Fos study was conducted one week later in order to avoid any carry-over effects from the last behavioral test. After PCA was run for all subjects ($n=80$), subjects were rank ordered by PC1, and every other bird was selected for the Fos study, with alternating, counterbalanced assignments to treatments (novel or familiar condition). We rank ordered subjects by PC1 in order to have a wide range of personalities for the IEG study; PC1 accounted for the most variance of the PCs and also loaded a diversity of behaviors, including novel-familiar social preferences. This yielded 20 birds of each sex ($n=10$ per condition) that covered the full range of PC1 scores [4]. Given that zebra finches are highly gregarious and often encounter novel conspecifics in the wild, we were interested in how variation in personality affects processing of social novelty. To test this, all subjects were transferred to a novel cage that contained either 4 familiar, same-sex cagemates or 4 novel, same-sex individuals. Subjects were sacrificed 90 min later. Familiar stimulus birds had been cagemates with subjects for approximately 6 months. We quantified expression of the immediate early gene protein Fos in DA neurons of male and female finches following interactions with familiar or novel birds.

2.5. Histology and immunocytochemistry

Subjects were sacrificed by isoflurane overdose and perfused with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Brains were removed, post-fixed overnight, and cryoprotected in 30% sucrose in PBS for 48 h prior to sectioning on a cryostat. Tissue was sectioned into three 40 μ m series. One series was immunofluorescently labeled for tyrosine hydroxylase (TH; to label catecholamine neurons in cell groups that are known to be dopaminergic) and Fos. Tissue was rinsed 5× for 10 min in 0.1 M PBS (pH 7.4), incubated for 1 h in block (PBS + 5% normal donkey serum + 0.3% Triton-X-100), and then incubated for approximately 40 h at 4 °C in primary antibodies diluted in PBS containing 2.5% normal donkey serum + 0.3% Triton-X-100. Primary antibodies used were sheep anti-TH (1:1000; Novus Biologicals, Littleton, CO) and rabbit anti-Fos (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA). The primary incubation was followed by two 30 min rinses in PBS. Tissue was then incubated in a donkey anti-rabbit secondary conjugated to Alexa Fluor 594 (5:1000; Invitrogen, Carlsbad, CA) and a donkey anti-sheep secondary conjugated to Alexa Fluor 488 (3:1000; Invitrogen). Secondaries were diluted in PBS containing 2.5% normal donkey serum + 0.3% Triton-X-100. Following two 30 min rinses in PBS, sections were mounted on subbed slides coverslipped with ProLong Gold antifade reagent containing DAPI nuclear stain (Invitrogen).

2.6. Quantification

Images were acquired at 5×, 10×, or 20× (depending on the brain region) using a Zeiss AxioImager microscope outfitted with an AxioCam HRm, z-drive, and an Apotome optical dissector (Carl Zeiss Inc., Göttingen, Germany). Cell counts were conducted from flattened z-stacks by an observer blind to condition using Photoshop CS3 (Adobe Systems, San Jose, CA) and Image J (National Institutes of Health, Bethesda, MD), as previously described [4,7,26]. Representative immunolabeling is shown in Fig. 1.

TH-Fos colocalization was quantified in eight catecholaminergic cell groups that are known to produce only DA. These cell groups are: A8, caudal tegmentum ("retrotrubral" in mammals); A9, substantia nigra; A10, VTA; A11, caudal periventricular hypothalamus and midbrain CG; A12, tuberoinfundibular hypothalamus; A13,

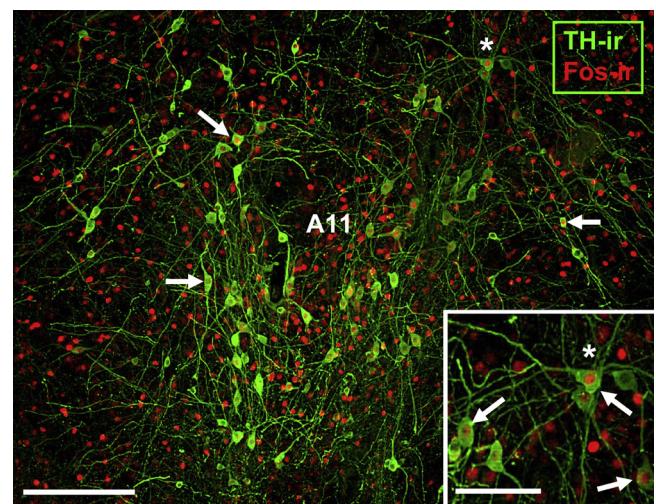


Fig. 1. Representative immunocytochemical colocalization of Fos and TH. Fos-ir neurons (red; Alexa Fluor 594) and TH-immunoreactive neurons (green; Alexa Fluor 488) in the A11 cell group of the caudal hypothalamus in a female zebra finch. Arrows highlight representative neurons showing TH-Fos colocalization. Scale bar = 50 μ m. Asterisk represents inset; scale bar = 100 μ m.

zona incerta; A14, medial hypothalamus and POA; and A15, lateral regions of the hypothalamus. TH-Fos colocalization was quantified in 1–3 sections per cell group, as follows: for A8, A9, A12, A13, and A15, 1 section, center of each cell group; A10, 2 sections, rostral and caudal as previously described [7]; A11, 2 sections, caudal hypothalamus and rostral CG; and A14, 3 sections distributed throughout the POA and hypothalamus. See [18] for an illustration of the distribution of A8–A15 TH-ir neurons in the avian brain.

2.7. Statistical analysis

In our first set of analyses, which were focused on phasic Fos responses (i.e., the rapid induction of c-Fos within a cell in response to an external stimuli; see [64]), data are expressed as the percent of TH neurons colocalizing Fos (henceforth termed TH-Fos colocalization). These data were analyzed using Sex × Context ANCOVAs with one of the three behavioral PCs in each model as a covariate. This approach was taken because inclusion of Sex, Context, and all three PCs in a single analysis would produce models that are overfit and not reliable [4,27]. We replicated this set of analyses using the raw number of double-labeled neurons (henceforth termed TH-Fos double-labeled neurons) as the dependent variable, providing a complementary view of phasic activity that is effectively weighted based on the numbers of TH-expressing neurons.

Finally, we also conducted a neural PCA that included 18 variables—TH cell numbers for each of the eight cell groups, including two sub-areas of the A11 cell group (caudal hypothalamus and CG), and the numbers of TH-Fos double-labeled neurons in each area. We then conducted a second set of Sex × Context ANCOVAs with one of the three behavioral PCs in each model as a covariate, and one of the six neural PCs as a dependent variable. It should be noted that these analyses are intended to explore the possible interactions of personality and DA systems, and because we do not have sufficient power to conduct corrections for multiple comparisons, some positive findings may represent type 1 errors.

Furthermore, while our data is appropriately analyzed using ANCOVAs (the results are presented as such), in order to best visually represent our findings in an intuitive manner, figures were made via correlations using JMP Statistical Software. Thus, the figures represent our results, but are not a direct result of ANCOVA modeling.

3. Results

3.1. Principal components of behavior

As previously described in [4], behavioral phenotypes were characterized for 40 male and 40 female zebra finches using assays of social preferences (group size and novel-familiar choice tests), anxiety-like behavior (novelty suppression of feeding and exploration), and observations of numerous behaviors (aggression, maintenance behaviors, sexual and non-sexual affiliative behavior, nesting, and pair bonding) in a colony environment. The combined PCA for males and females (Table 1) yields a significant model ($p < 0.0001$) containing three PCs of behavior, which we have previously characterized as: Social competence/dominance (PC1; a component that may reflect individual differences in the mechanisms of social cognition), which strongly loads variables such as dominance behaviors, latency to pair bond, and preferences for familiar social partners; Gregariousness (PC2), which strongly loads measures of group-size preference; and Anxiety (PC3), which primarily loads measures of novelty-suppressed feeding and exploration [4]. These PCs explain 20.3%, 13.7%, and 13% of the variance, respectively, and have eigenvalues of 3.040, 2.061, and 1.948. The analysis also yields four additional PCs that collectively explain 29.7% of the variance, however, here we focus only on the first three PCs because the sex-specific PCAs (see Tables S1 and S2 from [4]) show strong similarities only for the first three, more robust PCs. Importantly, the majority of the remaining variance (33.3%) is likely not intrinsic to the focal subjects, but rather reflects the fact that colony interactions involved many individuals. PC2 loadings and scores were multiplied by -1 in order to make the results more intuitive for interpretation; thus, higher scores are indicative of a higher level of gregariousness. Aggression data are entered separately for colony session 1 and sessions 2–6, given that aggression is initially focused on mate competition, but quickly shifts towards defense of a potential nest site. We have previously found that aggression is modulated differentially across these contexts [28] (also see [29]).

Previous characterizations of avian phenotypes have found a positive correlation between aggression and boldness [30–32]. Interestingly, however, as previously observed in [4], the PC structure here and additional regression analyses of aggression and anxiety do not support the findings that aggression and anxiety are positively correlated [4].

Table 1

Principal components matrix of zebra finch behavior^{a,b}

Behavioral measures	PC1 "Social competence/dominance"	PC2 "Gregariousness"	PC3 "Anxiety"
<i>Colony behaviors</i>			
Displacements, session 1	0.113	-0.330	0.384
Displacements, sessions 2–6	0.769	-0.235	0.062
Displaced by others, session 1	-0.379	-0.208	-0.256
Displaced by others, sessions 2–6	-0.595	-0.233	-0.199
Threats	0.685	-0.145	-0.204
Latency to pair bond	-0.560	0.255	0.289
Time on nest	0.740	-0.146	-0.263
<i>Choice tests</i>			
Time with novel birds	-0.543	-0.437	0.076
Time with familiar birds	0.501	0.168	0.053
Time with small group	0.001	-0.769	-0.091
Time with large group	0.094	0.759	0.003
<i>Anxiety tests</i>			
Latency to move (feed)	-0.052	0.290	0.461
Latency to feed	0.160	0.140	0.706
Latency to move (explore)	0.155	-0.244	0.641
Branches explored	-0.028	0.376	-0.598

^a PC structures were very similar in males and females, and sexes are shown combined. Loadings of ≥ 0.500 or ≤ -0.500 are bolded.

^b PC structure as previously reported in [4].

3.2. Fos responses of TH neurons vary according to sex, social context, and personality

3.2.1. Overview

In the first set of analyses, we focused on the contributions of sex, social context, and the three behavioral PCs to TH-Fos colocalization across the 8 TH cell groups (i.e., percent of TH neurons colocalizing Fos), and in a second set of analyses we use TH-Fos double-labeled neurons as the dependent variable. No significant effects are observed for the A9, A10, or hypothalamic A11 cell groups. However, numerous significant effects for TH-Fos colocalization and/or the number of double-labeled neurons are observed for other cell groups (A8, midbrain A11, A12, A13, A14, and A15), as summarized below and in Table 2. Small discrepancies in the degrees of freedom reflect the finding that a few subjects exhibited no TH-Fos colocalization in some brain regions.

3.2.2. A8 cell group (caudal tegmentum)

We find several significant effects for A8 neurons, involving Sex, Context, and all three behavioral PCs. In analyses of TH-Fos colocalization, we observe a trend for a main effect of Behavioral PC1 (Social competence/dominance) ($F_{(1,30)} = 3.625$, $p = 0.07$), with more socially competent/dominant birds tending to have higher levels of TH-Fos colocalization. In addition, we find that gregariousness has a negative relationship to TH-Fos colocalization in animals exposed to familiar same-sex conspecifics (Context \times Behavioral PC2; $F_{(1,30)} = 4.856$, $p = 0.04$). Furthermore, we observe a main effect of Behavioral PC3 (Anxiety) ($F_{(1,30)} = 5.098$, $p = 0.03$; Fig. 2), and in a Sex \times Context \times Behavioral PC2 analysis for TH-Fos colocalization, we also find a significant interaction of Sex and Context ($F_{(1,30)} = 6.183$, $p = 0.02$; Fig. 3). The ANCOVA including Behavioral PC3 also yields main effects of Sex ($F_{(1,30)} = 6.811$, $p = 0.01$) and Context ($F_{(1,30)} = 7.127$, $p = 0.01$), with males and all subjects exposed to novel conspecifics having greater levels of TH-Fos colocalization.

Using the number of TH-Fos double-labeled neurons as the dependent variable, we observe a Sex \times Context \times Behavioral PC2 interaction ($F_{(1,32)} = 5.361$, $p = 0.03$), reflecting a negative relationship between TH-Fos doubled-labeled neurons and gregariousness in females exposed to novel conspecifics and males exposed to familiar conspecifics. This analysis also yields a main effect of Context ($F_{(1,32)} = 4.749$, $p = 0.04$), with subjects exposed to novel conspecifics having higher numbers of TH-Fos double-labeled neurons. Lastly, the ANCOVA model including Behavioral PC3 yields

Table 2

Summary of ANCOVA results for TH-Fos colocalization and TH-Fos double-labeling.

Cell group ^a	Covariate included ^b	TH-Fos colocalization	TH-Fos double-labeling
A8	Behavioral PC1	Main effect of PC1 (trend)	Main effect of Context Sex × Context × PC2 interaction main effect of Sex
	Behavioral PC2	Context × PC2 interaction Sex × Context interaction	
	Behavioral PC3	Sex × Context	
A11	Behavioral PC1	Main effect of Sex	Sex × PC1 interaction Main effect of Sex Main effect of Context
	Behavioral PC2	Sex × Context × PC2 interaction	
A12	Behavioral PC2	Main effect of Context Main effect of PC2	
A13	Behavioral PC2	Context × PC2 interaction	Context × PC2 interaction main effect of PC2 main effect of PC3
	Behavioral PC3		
A14	Behavioral PC2		main effect of Sex Context × PC2 interaction
A15	Behavioral PC3	Main effect of Context Sex × PC3 interaction	Context × PC3 interaction

A12, tuberoinfundibular hypothalamus; A13, zona incerta; A14, medial hypothalamus and POA; A15, hypothalamus.

^a Brain regions associated with each cell group: A8, caudal tegmentum; A11, midbrain CG.^b Behavioral PC1—Social competence/dominance; Behavioral PC2—Gregariousness; Behavioral PC3—Anxiety.

a main effect of Sex ($F_{(1,32)} = 7.725, p = 0.01$), with males having significantly more TH-Fos double-labeled neurons than females.

3.2.3. A11 cell group (midbrain CG)

Although we did not find any significant effects or interactions for the A11 sub-population of the caudal hypothalamus, there are several significant interactions for the A11 sub-population of the CG. In a Sex × Context × Behavioral PC2 analysis for TH-Fos colocalization, we find a significant interaction of Sex, Context, and Behavioral PC2 ($F_{(1,32)} = 3.964, p = 0.05$; Fig. 4A). In the parallel analysis for TH-Fos double-labeled neurons, we observe a main effect of Context ($F_{(1,32)} = 5.507, p = 0.03$), which shows that animals exposed to novel same-sex conspecifics have greater numbers of TH-Fos double-labeled neurons than those exposed to familiar birds. In addition, analyses of Behavioral PC1 (Social

competence/dominance) for the number of TH-Fos double-labeled neurons reveal a significant interaction for Sex and Behavioral PC1 ($F_{(1,32)} = 7.048, p = 0.01$; Fig. 4B), and a main effect of Sex ($F_{(1,32)} = 9.952, p < 0.01$), with males having significantly more TH-Fos double-labeled neurons than females.

3.2.4. A12 cell group (tuberoinfundibular hypothalamus)

In a Sex × Context × Behavioral PC2 analysis for TH-Fos colocalization, we observe a main effect of Context ($F_{(1,32)} = 4.710, p = 0.04$), with birds exposed to novel same-sex conspecifics having higher levels of TH-Fos colocalization than birds exposed to familiar individuals. In addition, we find a main effect of Behavioral PC2 ($F_{(1,32)} = 4.225, p = 0.05$), reflecting a negative relationship between TH-Fos colocalization and gregariousness. We obtain no significant effects in analyses of TH-Fos double-labeled neurons.

3.2.5. A13 cell group (zona incerta)

In a Sex × Context × Behavioral PC2 analysis for TH-Fos colocalization, we obtain a significant interaction for Context and

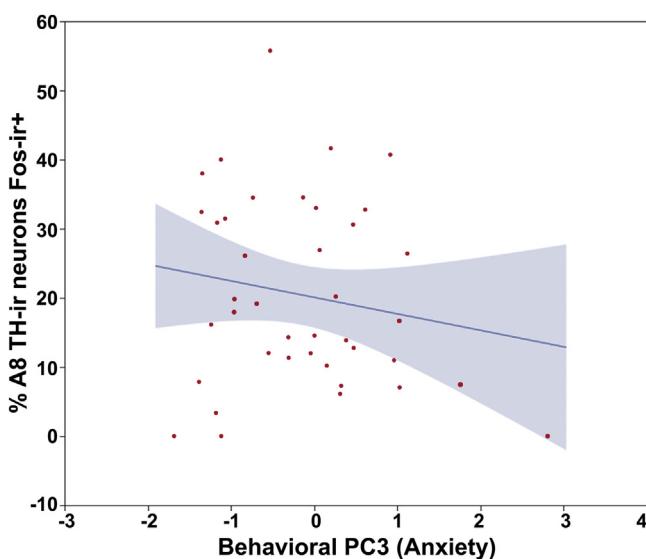


Fig. 2. TH-Fos colocalization in the A8 cell group (caudal tegmentum) correlates with Behavioral PC3 (Anxiety). Anxiety correlates negatively with A8 TH-Fos in male and female zebra finches. $F_{(1,30)} = 5.098, p = 0.03$. The 95% confidence interval is indicated by shading.

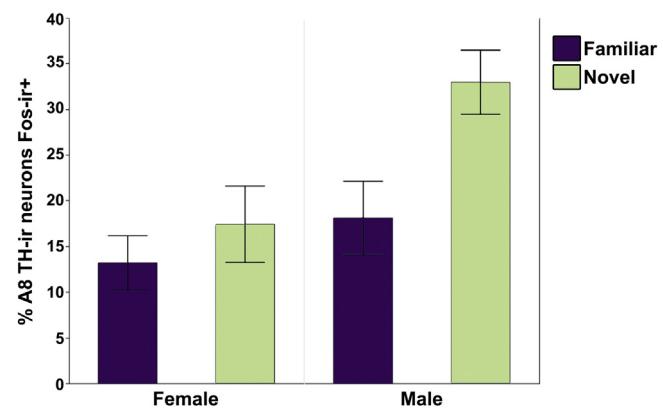


Fig. 3. TH-Fos colocalization in the A8 cell group (caudal tegmentum) varies as a function of an interaction between Sex and Context. A strong Sex × Context interaction is observed in an ANCOVA model for A8 TH-Fos colocalization with Behavioral PC2 (Gregariousness) as a covariate. $F_{(1,30)} = 6.183, p = 0.02$. Data are shown as mean + SEM.

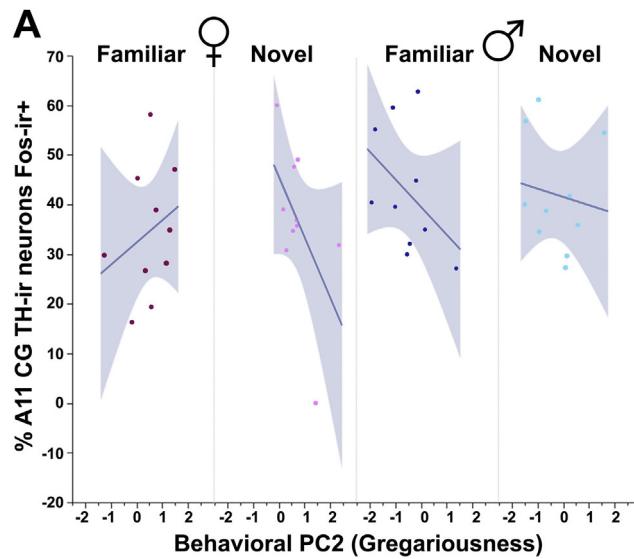


Fig. 4. TH-Fos colocalization and numbers of TH-Fos double-labeled neurons in the A11 cell group (midbrain CG) vary based on interactions between Sex, Context, Behavioral PC2 (Gregariousness), and Behavioral PC1 (Social competence/dominance). (A) Gregariousness correlates positively in females exposed to familiar same-sex conspecifics, but negatively in both sexes exposed to novel conspecifics and males exposed to familiar conspecifics. Sex \times Context \times Behavioral PC2 $F_{(1,32)} = 3.964, p = 0.05$. (B) Social competence/dominance correlates negatively with the number of A11 TH neurons expressing Fos in females, but positively in males. Sex \times Behavioral PC1 $F_{(1,32)} = 7.048, p = 0.01$. The 95% confidence interval is indicated by shading.

Behavioral PC2 ($F_{(1,32)} = 7.957, p < 0.01$; Fig. 5). Furthermore, in the parallel analysis of TH-Fos double-labeled neurons, we find a significant interaction for Context and Behavioral PC2 ($F_{(1,32)} = 8.463, p = 0.01$), as well as a main effect of Behavioral PC 2 ($F_{(1,32)} = 7.941, p = 0.01$). These effects reflect a positive relationship between the number of TH-Fos double-labeled neurons and gregariousness in subjects exposed to novel conspecifics and a positive relationship between the number of double-labeled neurons and gregariousness. Although Behavioral PC3 (Anxiety) makes no significant contributions to the model for TH-Fos colocalization in the A13 cell group, we nonetheless observe a main effect of Behavioral PC3 for TH-Fos double-labeling ($F_{(1,32)} = 6.482, p = 0.02$). Here we find that anxiety relates negatively to the number of TH-Fos double-labeled neurons.

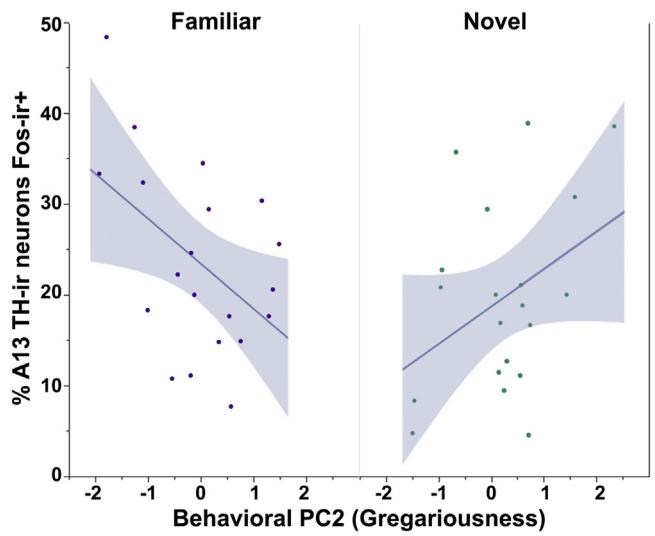


Fig. 5. TH-Fos colocalization in the A13 cell group (zona incerta) as a function of an interaction between Context and Behavioral PC2 (Gregariousness). Gregariousness correlates negatively with TH-Fos colocalization in subjects exposed to familiar same-sex conspecifics, but not in subjects exposed to novel same-sex conspecifics. Context \times Behavioral PC2 interaction, $F_{(1,32)} = 7.957, p < 0.01$. The 95% confidence interval is indicated by shading.

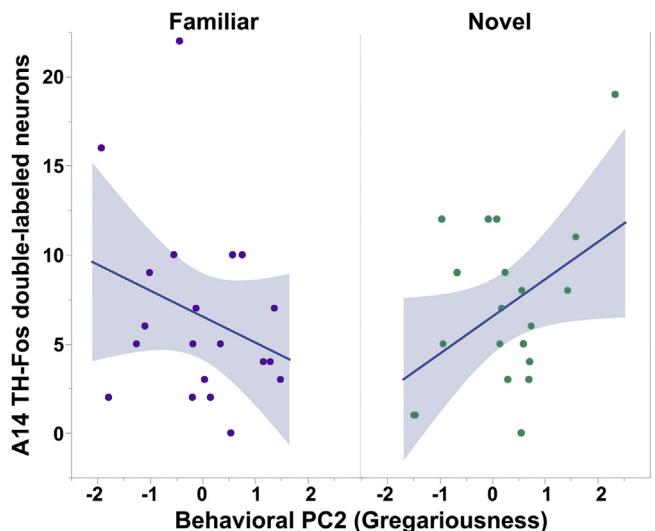


Fig. 6. Numbers of TH-Fos double-labeled neurons in the A14 cell group (medial hypothalamus and POA) as a function of an interaction between Context and Behavioral PC2 (Gregariousness). Gregariousness correlates negatively with A14 TH-Fos double-labeled neurons in subjects exposed to familiar same-sex conspecifics, but positively in subjects exposed to novel same-sex conspecifics. Context \times Behavioral PC2 interaction, $F_{(1,32)} = 4.926, p = 0.03$. The 95% confidence interval is indicated by shading.

3.2.6. A14 cell group (medial hypothalamus and POA)

Although we do not observe any significant effects for TH-Fos colocalization for this TH cell group, we do find significant effects for the number of TH-Fos double-labeled neurons. A Sex \times Context \times Behavioral PC2 analysis yields a main effect of Sex ($F_{(1,32)} = 3.898, p = 0.05$), with males having significantly more TH-Fos double-labeled neurons than females, and a significant interaction for Context and Behavioral PC2 ($F_{(1,32)} = 4.926, p = 0.03$; Fig. 6).

3.2.7. A15 cell group (hypothalamus)

In a Sex \times Context \times Behavioral PC3 analysis for TH-Fos colocalization, we observe a main effect of Context ($F_{(1,31)} = 7.467, p = 0.01$),

Table 3Principal components matrix of zebra finch TH cell numbers and Fos co-expression ("Neural PCs")^a

	PC1	PC2	PC3	PC4	PC5	PC6
A8 TH neurons	0.861	-0.028	-0.054	-0.040	-0.168	-0.087
A8 TH double-labeled neurons	0.829	0.045	0.079	-0.071	-0.277	-0.187
A9 TH neurons	0.687	0.062	-0.183	-0.119	0.112	0.410
A9 TH double-labeled neurons	0.773	0.047	-0.353	0.021	-0.326	-0.154
A10 TH neurons	0.543	0.307	0.102	0.066	0.278	0.580
A10 TH double-labeled neurons	0.508	0.095	-0.063	0.547	0.238	0.236
A11 CG TH neurons	0.449	-0.141	0.538	-0.428	0.178	-0.153
A11 CG TH double-labeled neurons	0.509	-0.148	0.570	-0.382	0.005	-0.217
A11 HYP TH neurons	-0.268	0.247	0.602	-0.302	-0.005	0.011
A11 HYP TH double-labeled neurons	-0.071	0.394	0.610	0.092	-0.338	0.047
A12 TH neurons	-0.014	-0.426	0.608	0.407	0.252	-0.136
A12 TH double-labeled neurons	0.208	-0.443	0.591	0.526	0.256	0.001
A13 TH neurons	0.272	0.417	-0.300	-0.099	0.586	-0.394
A13 TH double-labeled neurons	0.102	0.640	-0.089	0.229	0.369	-0.475
A14 TH neurons	-0.158	0.789	0.234	0.110	-0.040	0.094
A14 TH double-labeled neurons	0.161	0.622	0.359	0.277	-0.384	0.002
A15 TH neurons	-0.080	0.322	0.187	-0.545	0.359	0.297
A15 TH double-labeled neurons	-0.152	0.775	0.005	0.096	-0.046	-0.061

^a Loadings of ≥ 0.500 or ≤ -0.500 are bolded.

with subjects exposed to familiar conspecifics having greater levels of TH-Fos colocalization than those exposed to novel individuals. We also find a significant interaction of Sex and Behavioral PC3 ($F_{(1,31)} = 7.428, p = 0.01$), with TH-Fos colocalization relating positively to anxiety in females, but negatively in males. A single experimental female was removed from this analysis because the subject was 2.8 standard deviations from the mean. In addition, the analysis including Behavioral PC3 (Anxiety) for TH-Fos double-labeled neuron numbers reveals a significant interaction of Context and Behavioral PC3 ($F_{(1,32)} = 7.941, p = 0.01$), with the number of TH-Fos double-labeled neurons relating positively to anxiety in females and negatively in males. The female that was an outlier for TH-Fos colocalization was not a significant outlier for the number of TH-Fos double-labeled neurons and is included in this analysis.

3.3. Neural principal components are extensively related to sex, social context, and personality

Because individual DA cell groups act in the context of co-modulation by other DA cell groups, we conducted a PCA that includes both TH-immunoreactive cell numbers and the numbers of those neurons that were double-labeled for Fos. In order to take into account individual differences in DA anatomy, we included the number of TH neurons, which represent a constitutive (relatively constant) factor, in addition to the number of TH-Fos double-labeled neurons, which represents a phasic factor. This yields a highly significant model ($p < 0.0001$) and six PCs that collectively explain 75.9% of the variance (Table 3). The eigenvalues for these PCs are 3.797, 3.045, 2.567, 1.653, 1.391, and 1.199. Neural PC1 loads A8, A9, A10, and A11 variables in a positive manner. In contrast, Neural PC2 loads A13, A14, and A15 variables positively. Neural PC3 loads A11 midbrain and hypothalamic and A12 variables positively, whereas Neural PC4 loads A10 and A12 double-labeled TH neurons positively and A15 TH cells negatively. Finally Neural PC5 loads only A13 TH cell numbers positively, and Neural PC6 loads only A10 TH cell numbers positively.

In order to determine how the three behavioral PCs, Sex and Context relate to these neural PCs, we conducted Sex \times Context ANCOVAs, each of which included a single behavioral PC as a covariate and one of the six neural PCs as a dependent variable. These analyses reveal strong contributions of Sex, Context and all three behavioral PCs, and each behavioral PC tends to relate to a distinct subset of neural PCs. As described below, Behavioral PC1 (Social competence/dominance) relates primarily to Neural PC3 and Neural PC4; Behavioral PC2 (Gregariousness) relates primarily to Neural

Table 4
Summary of ANCOVA results for Neural PCs.

Neural PC ^a	Covariate included ^b	Effect/interaction
Neural PC2	Behavioral PC2	Context \times Behavioral PC2 interaction Sex \times Behavioral PC2 interaction
Neural PC3	Behavioral PC1 Behavioral PC3	Sex \times Context interaction Sex \times Context interaction
Neural PC4	Behavioral PC1	Main effect of Sex Main effect of Behavioral PC1 Sex \times Behavioral PC1 interaction
	Behavioral PC2	Sex \times Behavioral PC2 interaction
Neural PC5	Behavioral PC2 Behavioral PC3	Main effect of Behavioral PC2 Main effect of Behavioral PC3

^a Loading for Neural PCs: Neural PC2 loads A13, A14, and A15 variables positively. Neural PC3 loads A11 midbrain and hypothalamic and A12 variables positively. Neural PC4 loads A10 and A12 double-labeled TH neurons positively and A15 TH cells negatively. Neural PC5 loads only A13 TH cell numbers positively.

^b Behavioral PC1—Social competence/dominance; Behavioral PC2—Gregariousness; Behavioral PC3—Anxiety.

PC2 and Neural PC5; and Behavioral PC3 (Anxiety) relates primarily to Neural PC3 and Neural PC5. These results are summarized in Table 4.

The ANCOVA model for Neural PC3 with Behavioral PC1 (Social competence/dominance) as a covariate reveals a significant interaction of Sex and Context ($F_{(1,32)} = 4.408, p = 0.04$), with females exposed to novel same-sex conspecifics having higher Neural PC3 scores than females exposed to familiar birds, whereas males exposed to novel conspecifics exhibit lower Neural PC3 scores compared to males exposed to familiar individuals. In addition, the comparable model for Neural PC4 yields a main effect of Sex ($F_{(1,32)} = 5.597, p = 0.02$), with males having significantly higher Neural PC4 scores than females, and a main effect of Behavioral PC1 ($F_{(1,32)} = 6.139, p = 0.02$), with Neural PC4 relating negatively to Social competence/dominance. This analysis also yields a significant interaction of Sex and Behavioral PC1 ($F_{(1,32)} = 8.724, p < 0.01$; Fig. 7).

Using Behavioral PC2 (Gregariousness) as a covariate in a model for Neural PC2, we observe a significant interaction of Context and Behavioral PC2 ($F_{(1,32)} = 7.704, p = 0.01$; Fig. 8A), and a significant Sex \times Behavioral PC2 interaction ($F_{(1,32)} = 4.442, p = 0.04$; Fig. 8B). In the comparable model for Neural PC4, we also find a significant Sex \times Behavioral PC2 interaction ($F_{(1,32)} = 4.460, p = 0.04$), reflecting a negative relationship between Neural PC4 and gregariousness in females, but not males. For the model for Neural PC5 we observe a main effect of Behavioral PC2 ($F_{(1,32)} = 10.516, p < 0.01$), reflecting a positive relationship between Neural PC5 and gregariousness.

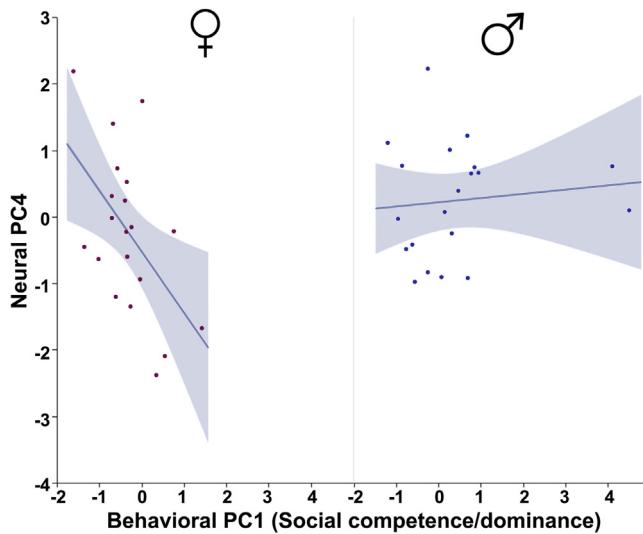


Fig. 7. Behavioral PC1 (Social competence/dominance) interacts with Sex to predict Neural PC4. The correlation between Behavioral PC1 (Social competence/dominance) and Neural PC4 is strongly negative in females, but not in males. Sex \times Behavioral PC1, $F_{(1,32)} = 8.724, p < 0.01$. The 95% confidence interval is indicated by shading.

Finally, using Behavioral PC3 (Anxiety) as a covariate in a model for Neural PC3, we observe a significant Sex \times Context interaction ($F_{(1,32)} = 4.228, p = 0.05$), with females exposed to novel conspecifics having higher Neural PC3 scores than females exposed to familiar birds, whereas males exposed to novel conspecifics have lower Neural PC3 scores than males exposed to familiar birds. The comparable model for Neural PC5 yields a main effect for Behavioral PC3 ($F_{(1,32)} = 6.873, p = 0.01$), reflecting a negative relationship between Neural PC5 and anxiety.

4. Discussion

We recently described the structure of complex behavioral phenotypes ("personalities") in male and female zebra finches and demonstrated that VP-OT cell groups function in complex ways that reflect sex, social context, and three axes of personality [4].

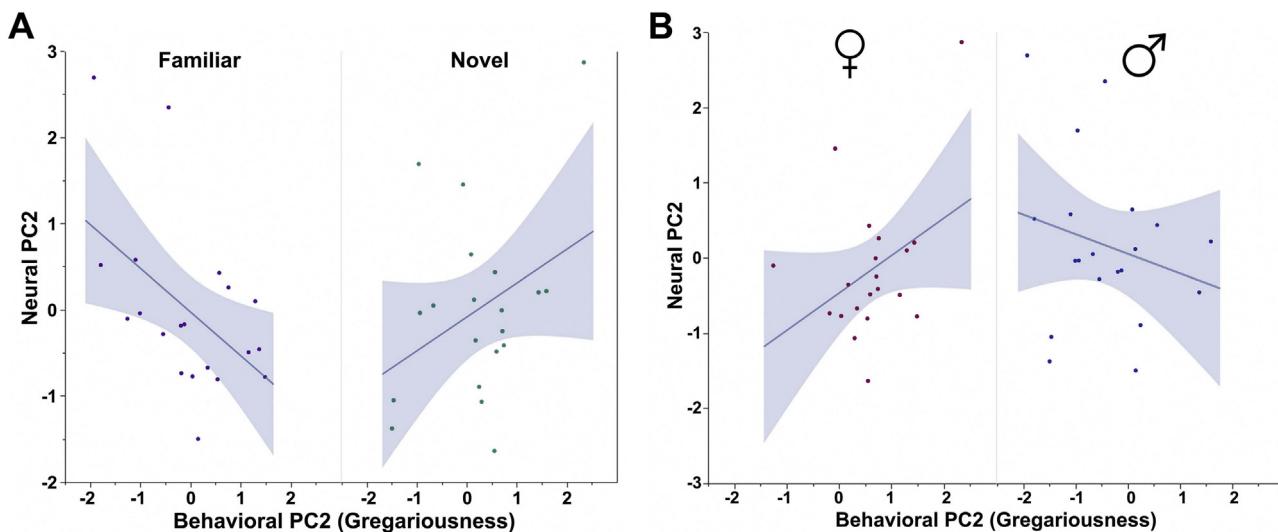


Fig. 8. Behavioral PC2 (Gregariousness) interacts with Context to predict Neural PC2. (A) The correlation between Behavioral PC2 (Gregariousness) and Neural PC2 is strongly negative in subjects exposed to familiar same-sex conspecifics, but positive in subjects exposed to novel conspecifics. Context \times Behavioral PC2 $F_{(1,32)} = 7.704, p = 0.01$. (B) The correlation between Behavioral PC2 (Gregariousness) and Neural PC2 is positive in females, but negative in males. Sex \times Behavioral PC2 interaction, $F_{(1,32)} = 4.442, p = 0.04$. The 95% confidence interval is indicated by shading.

Because DA is an important modulator of appetitive and consummatory behaviors and social behavior [7,33–35], we now expand upon those findings and show that responses of numerous DA cell groups also function in relation to personality, social context, and sex. Perhaps the greatest surprise in the present experiment was the observation of 11 significant effects and interactions for the DA A8 cell group of the caudal tegmentum. This population has garnered virtually no interest from behavioral neuroscientists, yet the present results suggest that these cells are profoundly relevant to sex-, phenotype- and context-specific patterns of behavioral modulation.

4.1. Personality in zebra finches

Although trait dimensions of human personalities are typically determined via self-reports and written questionnaires, personality structures in nonhuman primates and other species (e.g., cats and dogs) are quantified by observing overt behaviors. However, complex personality profiles have not been generated for common, laboratory animals that are tractable for neurobiological experiments, such as rodents, birds, or fish. Generally, studies of personality across mammals (e.g., humans, macaques, dogs) have revealed similar trait dimensions with some species-specific variation. Components of personality include Neuroticism, Agreeableness, Extraversion, Openness, and Conscientiousness in humans [36], and Anxiety, Sociability, Connectedness, Dominance, Activity, and Aggressiveness in nonhuman mammals [37,38]. We have used methods similar to those used for mammals to define dimensions of personality in zebra finches [4] and find that trait dimensions for zebra finches are somewhat similar to mammals. We quantified a total of 23 social and nonsocial behaviors and analyzed them using PCA, which resulted in three robust PCs of behavior. We have characterized these components of zebra finch personality as Social competence/dominance, Gregariousness, and Anxiety [4].

4.2. Functional profile of dopamine cell groups

DA is well known for its involvement in learning, reward-seeking, and motivated behaviors, including social behavior [39,40]. However, as with the VP-OT cell groups [41–43], DA cell

groups tend to show extensive overlap in their projections (e.g., [44]), making it difficult to draw conclusions about the functions of specific cell groups from pharmacological data. Hence, of the 8 DA cell groups, extensive data are available for only 4—the A9 cell group of the substantia nigra, the A10 cell group of the VTA, the A12 cell group of the tuberoinfundibular hypothalamus, and the A14 cell group of the medial hypothalamus and POA. Surprisingly, we observe no significant effects for the A9 and A10 populations, but do observe significant effects for the A12 and A14 cell groups, plus the A8 cell group of the caudal tegmentum, A11 cell group of the midbrain CG, A13 cell group of the zona incerta, and the A15 cell group of the lateral hypothalamic region.

4.2.1. A8 cell group

A clear standout among the DA cell groups is the A8 population, where we obtained 11 significant effects. The majority of the significant interactions include Sex and Context for individual analyses that include each of the PCs, suggesting that this cell group is highly sensitive to social context and in a manner that is tied to sex. To our knowledge, only two studies have examined the behavioral functions of the A8 neurons. Kabelik et al. [34] found that TH-Fos colocalization of these cells correlates positively with performance of appetitive and consummatory sexual behaviors and consummatory aggressive behavior in lizards. In addition, in zebra finches, TH-Fos colocalization increases in response to aggression, courtship, and copulation [18]. Given that we observe numerous significant interactions from analyses with all three PCs, the present findings provide further support that the A8 cell group is a potent modulator of various aspects of social behavior and also demonstrate that these neurons function in a sex-specific manner. Here we show that males have higher levels of TH-Fos colocalization and greater numbers of TH-Fos double-labeled neurons than females and also find that A8 TH neurons respond differently to social novelty based on sex as well as phenotypic variation in gregariousness. For example, more gregarious females exposed to novel same-sex conspecifics have fewer TH-Fos double-labeled neurons, whereas the opposite is true for males exposed to novel conspecifics. However, more gregarious males exposed to familiar conspecifics have fewer TH-Fos double-labeled neurons than less gregarious males exposed to familiar individuals.

4.2.2. A10 cell group

The A10 cell group of the VTA has been extensively studied in relation to its effects on incentive motivation, aversion, and reward [45]. Consistent with these functions, A10 neurons exhibit immediate early gene responses to sexual interactions in both birds and rodents [18,46–48]. Numerous studies in birds also demonstrate an involvement in sexually motivated song [7,39,49]. In both birds and mammals, functional variation is observed along the rostrocaudal axis of the VTA [7,50], and we find that in zebra finches, male courtship singing correlates with TH-Fos colocalization only in the caudal VTA [7]. Interestingly, gregarious finch species exhibit relatively more DA neurons in the caudal VTA than do territorial finch species, and relatively more neurons are observed in male zebra finches that reliably court females than those that do not, suggesting that these cells play an important role in establishing affiliation phenotypes [7]. Given that this DA cell group is involved in various aspects of social and sexual behavior, we were surprised to observe no significant effects or interactions for the A10 neurons in the present study.

4.2.3. A11 cell group

As with the A10 neurons, A11 neurons of the midbrain CG exhibit immediate early gene responses to sociosexual interactions [7,47,51], and directed singing in male zebra finches correlates

positively with TH-Fos colocalization [7]. Absolute numbers of TH-positive cells in the CG also correlate positively with phenotypic variation in rates of courtship singing, and males that reliably fail to court females fall at the lower end of both correlations (i.e., for TH-Fos colocalization and TH-ir cell numbers). However, following exposure to a same-sex conspecific, there are no differences between gregarious and territorial finch species in either the Fos response of TH-ir neurons in the CG or in the absolute numbers of those neurons [7]. Thus, rather than playing a role in general affiliation, as hypothesized for the caudal A10 population, these cells may contribute to phenotypic variation in sexual motivation and/or social competence. In support of this hypothesis, we here find that male zebra finches with higher Behavioral PC1 (Social competence/dominance) scores have greater numbers of TH-Fos double-labeled neurons in the A11 cell group. In contrast, this is not observed with females; instead we find that female zebra finches with higher Behavioral PC1 (Social competence/dominance) scores have fewer numbers of TH-Fos double-labeled neurons. Interestingly, males have greater numbers of A11 TH-Fos double-labeled neurons in the CG than females. Additional sex differences are also observed in the Fos responses of A11 TH neurons in relation to social context and Behavioral PC2 (Gregariousness), suggesting that these cells not only contribute to phenotypic variation in sexual motivation and social competence, but also to aspects of affiliation.

4.2.4. A12 cell group

The A12 DA neurons are important regulators of reproduction, particularly via their inhibitory effects on prolactin secretion [19,52,53], although to our knowledge, their direct contributions to behavior are not known. However, TH-Fos colocalization increases in these cells in response to physiological and immobilization stressors [20] and following an immune challenge in mice [19]. Consistent with these findings, male zebra finches show high levels of TH-Fos colocalization in the A12 neurons following social isolation; this colocalization is significantly reduced following 90 min with another male and further decreased following sexual interactions with a female [18]. The present data further demonstrate a main effect of Behavioral PC2 (Gregariousness), with birds that are less gregarious tending to have higher levels of TH-Fos colocalization. Hence, A12 TH neurons may play a particularly important role in the modulation of social anxiety.

4.2.5. A13 cell group

Few experiments have examined the functions of A13 DA neurons of the zona incerta. This cell group has been implicated in the control of paradoxical sleep in rats [54] and may play a role in migraine attacks [55]; however studies that have investigated the social behavioral functions of A13 neurons have not generated any significant findings. For instance, two studies that examined the Fos response of A13 DA neurons to social, sexual, and aggressive behavior found no significant increases in TH-Fos colocalization [18,34]. Nonetheless, we here observe significant interactions that include Context, Behavioral PC2 (Gregariousness), and Behavioral PC3 (Anxiety). The present data show that both A13 TH-Fos colocalization and the number of TH-Fos double-labeled neurons relate positively to Behavioral PC2 (Gregariousness) in subjects exposed to novel conspecifics, suggesting that the A13 cell group is important in the processing of social novelty. In addition, the positive relationship between the number of TH-Fos double-labeled neurons and Behavioral PC2 also supports the notion that these cells are important modulators of social behavior. Our results also suggest that A13 TH neurons may have anxiolytic properties, given the negative relationship between the number of TH-Fos double-labeled neurons and Behavioral PC3 (Anxiety). Interestingly, of the

cell groups that we obtained significant effects for, the A13 cell group is the only DA population that shows no effects of Sex.

4.2.6. A14 cell group

DA neurons of the A14 cell group are present in the medial hypothalamus and POA and are thought to be involved in sexual behavior [44,56]. Interestingly, *in vivo* microdialysis revealed that DA release in the medial POA of quail is specifically linked to sexual motivation rather than to copulatory behavior per se [57]. Only one study to date has confirmed that A14 DA neurons of the POA are activated in response to copulation and showed that, in zebra finches, male sexual behavior is accompanied by increases in TH-Fos colocalization [18]. However, studies in songbirds have demonstrated that A14 neurons in the medial preoptic nucleus (POM) respond to singing behavior in a context-dependent manner [58], and examination of TH labeling reveals a negative relationship between breeding context song and indices of TH immunoreactivity [59]. Furthermore, optical density of TH in the A14 cell group negatively correlates with clumping behavior (non-sexual affiliation) in zebra finches [48]. Such negative correlations are difficult to interpret given that other findings suggest an involvement of A14 DA in sexual and social behaviors, but the decreased TH-immunolabeling may be the result of end-product inhibition of TH by consistent high DA concentrations [48,59].

In the present study, we observe a main effect of Sex and a significant interaction of Context and Behavioral PC2 (Gregariousness) for the number of A14 double-labeled neurons. These analyses show that males exhibit significantly more TH-Fos double-labeled neurons than females, suggesting that the A14 cell group may play a stronger role in the modulation of social behavior in males. Furthermore, the positive relationship between the number of TH-Fos double-labeled cells and Behavioral PC2 in birds exposed to novel same-sex conspecifics and the negative relationship for birds exposed to familiar conspecifics suggests that A14 TH neurons are particularly important for the processing of social novelty.

4.2.7. A15 cell group

To our knowledge, the only direct functional data available for the A15 DA neurons demonstrate that they regulate seasonal breeding and the transition to anestrus in sheep via inhibition of gonadotropin-releasing hormone secretion [60]. Interestingly, a previous experiment failed to show significant activation of these neurons by a variety of sociosexual stimuli in male zebra finches [18], although based on the present data, this may reflect the fact that the earlier experiment did not control for variation in the personality of subjects. We here obtain significant effects and interactions that include Context, Sex, and Behavioral PC3 (Anxiety) for both TH-Fos colocalization and the number of TH-Fos double-labeled neurons, suggesting that A15 neurons modulate context- and sex-specific anxiety responses. Both TH-Fos colocalization and the number of TH-Fos double-labeled neurons tended to relate positively to Behavioral PC3 in females, but negatively in males, suggesting that A15 TH neurons may have anxiogenic properties in female zebra finches, yet anxiolytic properties in males.

4.3. Neuromodulatory patterning

Although numerous studies suggest that individual DA cell groups exert unique effects on behavior, behavioral output and behavioral phenotype likely reflect patterns of coordinated neuromodulation arising from multiple DA cell groups. This is strongly suggested by the fact that mesencephalic DA populations develop in parallel from common embryonic tissues and that several cell groups (e.g., A8, A9, and A10) exhibit partial overlap in their projection fields [33,61,62]. Midbrain cell groups also exhibit overlapping projections with forebrain cell groups [44].

In order to examine how DA cell groups function in concert to produce unique effects on behavior in a manner that relates to personality, we conducted a Neural PCA that included TH cell numbers and the numbers of TH neurons that colocalized Fos. This analysis yielded six PCs that collectively account for 75.9% of the variance. Each Neural PC loads a unique combination of variables and thus these Neural PCs may reflect collective modulation by specific DA cell groups on target brain areas. It is not surprising that Neural PC1 loads variables from A10 and A11 positively given that numerous studies have found that these cell groups exhibit similar functional profiles in birds and rodents [7,18,46,47,51]. Notably, the A8 cell group also loads positively onto Neural PC1, and the present data suggest that this population may play an even more important role in phenotypic variation than do the A10 and A11 cell groups. Of additional interest is Neural PC4, which loads variables from the A10 and A12 cell groups positively and variables from the A15 cell group negatively. The A10 and A12 cell groups have been implicated in sexual behavior [18,46,47] and reproduction [19,52,53], respectively, and our data here suggest that the A15 cell group functions primarily in relation to anxiety. Thus, the loadings for Neural PC4 suggest that DA cell groups that modulate reproductive behaviors may function in opposition of those that modulate anxiety-like behavior.

Overall, we find that all Neural PCs have significant interactions with at least one of the Behavioral PCs and Sex or Context. Furthermore, each Behavioral PC relates to a unique suite of Neural PCs, suggesting that personality is reflected by unique patterns of simultaneous activity across numerous DA cell populations.

5. Conclusions

Although DA is known to influence a wide variety of social behaviors, to our knowledge, no studies have examined how the activity of individual cell groups relates to personality structure. The majority of functional data for DA neuronal populations exists for the A9 (substantia nigra) and A10 (VTA) cell groups, which are involved in motor function and incentive motivation, respectively. Interestingly, in the present study, we observe significant interactions of sex, social context, and personality for all but the A9 and A10 DA cell groups. However, the findings here provide novel insights into how numerous other DA cell groups function in context-, sex- and phenotypic-specific ways. For example, to our knowledge, only two prior studies have examined the Fos responses of A8 DA neurons of the caudal tegmentum and revealed that these cells respond to sexual and aggressive behaviors. Here, we provide support for these findings and find that A8 neuronal activity relates not only to aggression and sexual behavior (reflected in Behavioral PC1), but also to gregariousness and anxiety-like behavior. Finally, from the analyses of Neural PCs, we demonstrate that DA cell groups interact in complex ways to predict dimensions of personality, suggesting that complex behavioral phenotype is the result of coordinated DA modulation arising from multiple neuronal populations.

As demonstrated here (and in [4]), dopamine and nonapeptides modulate social processes differentially in relation to sex and behavioral phenotype. Other studies have shown that these systems also modulate social behavior differentially in relation to species and aspects of personality [7,11,12]. The findings here, in conjunction with those from [4] on nonapeptides, provide novel insights into the mechanisms underlying sex- and phenotype-specific modulation of behavior. Given that dopamine-modulated reward systems likely interact with nonapeptides to mediate social behavior [63], an interesting future direction would be to examine the influence that these two systems have on each other to produce distinct behavioral outputs.

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