Neurogenomic landscape associated with status-dependent cooperative behavior

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Abstract

The neurogenomic mechanisms mediating cooperative behaviors are understudied. We leveraged extensive transcriptomic and behavioral data on a neotropical bird (*Pipra filicauda*) that perform cooperative courtship displays to better understand the underlying mechanisms. Cooperative display is modulated by testosterone, it promotes cooperation in non-territorial birds, but suppresses cooperation in territory holders. We sought to understand the neurogenomic underpinnings of these three related traits: social status, cooperative behavior, and testosterone phenotype. To do this, we profiled gene expression in 10 brain nuclei spanning the social decision-making network (SDMN), and two key endocrine tissues that regulate social behavior. We associated gene expression with each bird's behavioral and endocrine profile derived from 3 years of repeated measures taken from free-living birds in the Ecuadorian Amazon. We found

distinct landscapes of constitutive gene expression were associated with social status, testosterone phenotype, and cooperation, reflecting the modular organization and engagement of neuroendocrine tissues. Sex-steroid and neuropeptide signaling appeared to be important in mediating status-specific relationships between testosterone and cooperation, suggesting shared regulatory mechanisms with male aggressive and sexual behaviors. We also identified differentially regulated genes involved in cellular activity and synaptic potentiation, suggesting multiple mechanisms underpin these genomic states. Finally, we identified SDMN-wide gene expression differences between territorial and floater males that could form the basis of "status-specific" neurophysiological phenotypes, potentially mediated by testosterone and growth hormone. Overall, our findings provide new, systems-level insights into the mechanisms of cooperative behavior, and suggest that differences in neurogenomic state are the basis for individual differences in social behavior.

Introduction

Cooperation is the synchronized behavior(s) of two or more individuals to produce a net fitness benefit for one or more individuals (Taborsky and Taborsky 2015). An individual's decisions about cooperation are based on the immediate social stimulus and individual condition, which is based on recent and distant past experiences, developmental effects, and underlying genetics. These effects can ultimately produce repeatable, among-individual variation in behaviors – a behavioral phenotype – where some individuals tend to be more cooperative than others. To understand the mechanisms of cooperation, one needs to understand the neurophysiological signal processing of the stimulus through to the behavioral response, as well as the genetic and neurophysiological components that underpin behavioral phenotypes. Indeed, understanding these mechanisms enables us to link how genetic and neurophysiological architecture can constrain and facilitate the evolution of cooperation (Soares et al. 2010; Hofmann et al. 2014; Taborsky and Taborsky 2015). Differences in cooperative behavioral phenotype are in part achieved by the pleiotropic yet modular effects of hormone signaling systems (Ketterson et al. 2009; Cox 2020). Hormonal pleiotropy mediates phenotypic integration, whereby multiple traits covary with a hormonal signal (Ketterson et al. 2009; Cox 2020), sometimes in opposite directions. For example, elevated testosterone typically promotes aggression but inhibits parental care in males (Wingfield et al. 2001). In contrast, through variation in hormone receptor expression, tissues across the body are often modular in their response to hormone signals, which enables diverse and flexible responses to hormonal and social signals and trait independence (Ketterson et al. 2009; Lipshutz et al. 2019).

Cooperation, like other social behaviors, appears to be mediated by differences in circulating hormones, such as sex steroids, and aspects of hormone-signaling pathways, including the distribution and abundance of hormone receptors in neural tissues (Soares et al. 2010; Kasper et al. 2017). The neural substrates modulating social behaviors are conserved across vertebrates (O'Connell and Hofmann 2011). Known as the social decision making network (SDMN), these reciprocally interconnected brain nuclei are rich in steroid receptors, steroidogenic enzymes, and steroid-modulated neuropeptide systems (Newman 1999; Goodson 2005; O'Connell and Hofmann 2011). The SDMN is both modular and dynamic, whereby different combinations of brain regions are activated in response to different social contexts (Newman 1999; Goodson 2005). This model means the common practice of measuring the

neurological response in a single nucleus is not reflective of all systems engaged in modulating a given social behavior. Variation among individuals in the expression of neuroendocrine genes across the SDMN likely underlies repeatable individual differences in social behavior and sets conditions for behavioral phenotypes that respond differently to circulating hormones and social stimuli. For example, the up or down-regulation of genes involved in sex-steroid signaling pathways across nuclei of the SDMN can alter the sensitivity of the male brain to testosterone and manifest as differential behavioral responses to a given hormone signal. Emerging research is showing that behavioral phenotypes are correlated with differences in constitutive gene expression or neurogenomic state among individuals characterized by suites of genes co-expressed across behaviorally relevant brain regions (Antunes et al. 2021; Kabelik et al. 2021; Lattin et al. 2021). However, given the diversity of behavioral components associated with cooperation (Díaz-Muñoz et al. 2014; Kasper et al. 2017), questions remain on how neuroendocrine mechanisms operate to produce the variety of social and cooperative behaviors among individuals. Indeed, our understanding of the neuroendocrine mechanisms underlying seemingly paradoxical male-male reproductive cooperative behaviors is only emerging (DuVal and Goymann 2011; Díaz-Muñoz et al. 2014; Ryder et al. 2020; Vernasco et al. 2020; Jones and DuVal 2021; Loveland et al. 2021). For the first time, we characterize gene expression across the neuroendocrine system in relation status-specific androgenic modulation of a male-male cooperative behavior.

The wire-tailed manakin (*Pipra filicauda*) is a neotropical lek breeding bird species in which unrelated males perform cooperative displays to attract females (Figure 1A). Long-term display partnerships are established between territorial and subordinate non-territorial (floater) males and form the basis of complex social networks (Figure 1A, B) (Ryder et al. 2008; Ryder, Blake, et al. 2011). In this bird, territoriality is a pre-requisite to siring offspring, and floater males follow an age-graded queue for territorial status (Ryder et al. 2008; Ryder et al. 2009). Cooperation is directly linked to fitness, whereby territory holders with more display partners sire more offspring, and floaters with more partners are more likely to ascend to territorial status (Ryder et al. 2009). Individuals have a repeatable 'testosterone phenotype' in which territorial males tend to have higher testosterone than floaters (Figure 1D) (Ryder, Horton, et al. 2011; Ryder et al. 2020). Remarkably, circulating testosterone has a status-specific effect on the cooperative display behavior: experimental and observational evidence show that higher testosterone levels

103 are antagonistic to cooperation in territorial males but promote cooperation in floater males
104 (Ryder et al. 2020; Vernasco et al. 2020) (Figure 1C).

105 To explore the neurogenomic basis of the status-specific androgenic modulation of
106 cooperative behavior in these birds, we quantify transcriptome-level gene expression in the
107 SDMN and pituitary and testes of free-living male wire-tailed manakins sampled in the
108 Ecuadorian Amazon. Captive studies can obscure ecologically and evolutionary relevant
109 variation in environmental and behavioral responses (Griffith et al. 2017; Kasper et al. 2017), so
110 we demonstrate the exciting applicability of neuro-transcriptional methods in the challenging
111 conditions of the rainforest. In this study, we aim to characterize the genes and neuroendocrine
112 pathways that modulate individual variation in cooperative behavior, as well as those related
113 social status and individual variation in testosterone phenotype as a modifier of both social status
114 and cooperative behavior. We characterize the constitutive gene expression for each of these
115 traits within and between nodes of the SDMN and the endocrine tissues. Given the androgenic
116 regulation of cooperative display (Ryder et al. 2020; Vernasco et al. 2020), we hypothesize that
117 differences in gene regulation will manifest in SDNM nuclei with high density of sex-steroid
118 receptors and pituitary and gonadal tissues (Newman 1999; Goodson 2005). We further reason
119 that androgenic regulation will relate to differential expression of genes relating to sex-steroid
120 signaling and metabolism, as well as three key neuropeptide systems involved in social behavior
121 (Goodson and Thompson 2010; Kingsbury and Wilson 2016). Given the modular nature of the
122 SDNM, we predict that many gene expression patterns will be brain region-specific (Figure 1G).
123 However, given the pleiotropic effects of testosterone (Ketterson et al. 2009; Cox 2020), we also
124 predict that there will be signatures of testosterone's systemic influence on gene expression
125 patterns across the brain. We discuss our results in the context of modes of phenotypic evolution
126 and similarities with mechanisms of other social behaviors.

127 **Materials and Methods**

128 All methods described here were approved by Smithsonian Animal Care and Use Committee
129 (protocols 12-12,14-25,17-11), as well as the Ecuadorian Ministry of the Environment (MAE-
130 DNB-CM-2015-0008), and samples were transported with export permit (006-016-EXP-1C-
131 FAU-DNB/MA) and import permit (USDA APHIS 126133). Detailed methods can be found in
132 the supplementary material, and full code from analyses in R v 4.0.2 are available on the GitHub

site (https://periperipatus.github.io/PIFI_brain_transcriptome/) and on FigShare (10.25573/data.22186516).

Field Methods

For RNA sequencing we sampled 16 wild territorial and floater wire-tailed manakin males from Tiputini Biodiversity Station in the lowland Amazon rainforest of Ecuador (Table S1.1). Males were categorized by social status as either territorial or floater. The territorial males sampled (n=9) were males in definitive plumage that held a consistent display territory over the study period, while floaters (n=7) had either definitive or pre-definitive plumage and did not occupy consistent territories within leks (Ryder et al. 2008). On 12 of these 16 males, we used repeated testosterone sampling (Figure 1D, Supplement 1) to characterize each male's testosterone phenotype (mean testosterone). Previous research has shown that territorial males have, on average, higher circulating testosterone than floaters (Ryder et al., 2020; Ryder, Horton, et al., 2011), and the males sampled for this study reflect that pattern (Figure 1D). A male's testosterone phenotype is represented by mean of the residuals of a linear regression from repeated sampling of testosterone concentration (Supplement 1 for more detail, see also (Ryder et al. 2020)). Mean testosterone as a measure of testosterone phenotype was shown to be repeatable within individuals (Ryder et al. 2020). In the broader population, the relationship between testosterone phenotype and social network measures used as a proxy for cooperation was status-specific, with testosterone suppressing cooperation in territorial males but increasing cooperation in floaters (Ryder et al. 2020) (Figure 1C).

Social network structure and individual variation in behavior were characterized using autonomous radio-telemetry and tag proximity detections within territories on a total population of 180 male manakins over multiple years (Ryder et al. 2012; Dakin and Ryder 2018; Ryder et al. 2020). We used this tagging data to calculate social network strength for the same 12 males with repeated testosterone measures. Social network strength is a proxy for cooperative tendency and is the time spent interacting with other males averaged over the study period (Figure 1E), and was the most repeatable within individuals (Ryder et al. 2020). This social network proxy for cooperation was most strongly status specific relation to an individual's testosterone phenotype (Ryder et al. 2020) (Figure 1C), but the overall frequency of cooperative interactions did not differ between territorial and floater males (Figure 1E).

163 ***Microdissection and RNAseq***
164 Methods for brain extraction, tissue preservation, cryosectioning, microdissection, and RNA
165 extraction followed those published previously (Horton, Ryder, et al. 2020). Briefly, birds were
166 euthanized by decapitation near the site of capture immediately after blood sampling for
167 testosterone. Whole brains were extracted within 4-6 minutes of euthanasia and immediately
168 frozen in powdered dry ice. To capture the full Hypothalamic-Pituitary-Gonadal axis critical to
169 sex-steroid regulation, we additionally sampled the pituitary gland and testes, which were
170 sampled immediately after the brain. Brains and testes were kept frozen on dry ice in the field (<
171 4 hours) until transferred into a 21-day liquid nitrogen-charged Arctic Express™ dry shipper
172 (Thermo Scientific) where they were stored at cryogenic temperatures until imported into the
173 United States. Pituitaries were preserved in RNAlater™ (Invitrogen) at ambient temperatures
174 until imported into the United States.

175 The brains were cryosectioned, and frozen sections were microdissected to excise tissue from 10
176 different nuclei involved in social behavior – nine of which are from the SDMN. The SDMN
177 reflects the interconnectivity of two networks involved in regulating social behavior (O'Connell
178 and Hofmann 2011), constituting nuclei from both the Social Behavior Network (SBN)
179 (Newman 1999; Goodson 2005) and the Mesolimbic Reward System (MRS) (Olds and Milner
180 1954; O'Connell and Hofmann 2011). Figure 1F indicates the nomenclature used and the
181 relationships of these nuclei to the SBN and MRS. To clarify bird specific nomenclature: the
182 arcopallium intermedium (AI) is a region that may be, in part, homologous the basolateral
183 amygdala of mammals, a node of the MRS (O'Connell and Hofmann 2011), which is
184 hypothesized to play a role in androgen-dependent manakin display behavior (Fusani et al.
185 2014); and the nucleus taenia (TnA) is homologous to the mammalian medial amygdala
186 (O'Connell and Hofmann 2011). Finally, we sampled the paraventricular nucleus (PVN) for a set
187 of 10 nuclei sampled. This is a region that is not yet formally recognized as part of the SDMN,
188 despite its neuropeptide projections to nodes of the SDMN and established role as a major
189 regulator of vertebrate social behavior (Goodson and Thompson 2010; Goodson and Kingsbury
190 2013). For a graphical representation of the tissues sampled see Figure 1F, with the full list of
191 individuals collected presented in Table S1.1 and tissues in Table S1.2.

192 Microdissected tissues from both hemispheres were combined for sequencing. RNA was
193 extracted and sequenced according to previously described methods (Horton, Ryder, et al. 2020).

194 RNA libraries for the various tissues and field seasons were randomized amongst sequencing
195 batches (Supplement 1, Table S1.1 & S1.2). All samples analyzed are associated with Bioproject
196 PRJNA437157, and SRR12660169-198, SRR19521260-271, SRR19521432-575.

197 ***Gene expression analyses***

198 Reads were mapped to the annotated *Pipra filicauda* genome (GCA_003945595.1) using
199 splice-aware mapper STAR v.2.7.5 (Dobin et al. 2013), and transcripts were counted against
200 exons using featureCounts v.2.0.1 (Liao et al. 2014). For subsequent analyses we used a total of
201 170 libraries, and quality filtering of individuals and gene-counts was performed before the
202 analysis of each tissue (Supplement 1 & 3). To further explore tissue and brain nucleus function
203 in this species (Horton, Ryder, et al. 2020), we characterized differences in gene expression
204 among tissues and brain nuclei using principal components analysis in PCAtool*s* and Weighted
205 Gene Co-Expression Analysis (WGCNA) (Langfelder and Horvath 2008; Blighe and Lun 2020)
206 (Supplement 2). The number of individuals used in each tissue varies owing to quality filtering
207 and variable sampling of individuals across all brain regions (Table S1.1 & S1.2).

208 To describe the role of tissues and brain nuclei in relation to these hormonal and
209 behavioral phenotypes we conducted filtering (supplement 1), differential expression
210 (Supplement 3) and co-expression (Supplement 5) analysis in each tissue and nucleus separately.
211 For a subset of tissues, we had an extra four individuals characterized for social status from a
212 previous study (Horton, Ryder, et al. 2020), for which behavioral and hormone sampling data
213 were unavailable. Owing to this, and to not overparameterize the models that account for batch
214 effects we identified differentially expressed genes (DEGs) associated with each of our traits of
215 interest (social status, testosterone phenotype and social network strength) separately. These
216 were run as separate negative binomial generalized linear models for each trait that accounted for
217 batch effects where necessary using DESeq2 (Love et al. 2014) (For detailed description see
218 Supplement 3) in R v 4.0.2 (R Core Team 2020). Mean testosterone and social network strength
219 were input to DESeq2 as continuous variables. In these cases, significant DEGs were interpreted
220 to be correlated with the predictor variable, and Log_2 Fold Change (LFC) value represents the
221 LFC of gene expression per unit of the predictor variable. In all cases, significant DEGs were
222 identified using the Wald Test and the default FDR corrected p-value of 0.1 ($q<0.1$). Further, for
223 each tissue and nucleus we identified gene co-expression networks using WGCNA. The identity
224 of genes of interest identified by WGCNA were similar to those in DESeq2. For brevity and

225 simplicity we focus mostly on the differential expression results, but the full results are presented
226 in Supplement 5 (WGCNA) and Supplement 3 (differential expression).

227 Due to the key role testosterone plays in modulating behavior in this species (Ryder et al.
228 2020), we conducted further detailed analysis on nine candidate genes involved in sex-hormone
229 metabolism and signaling (Figure S4.1), and a further nine steroid sensitive neuropeptides and
230 their receptors (Figure S4.2). Any candidate gene identified by differential gene expression
231 (DGE) analysis with an uncorrected $p < 0.05$ was considered "significant" and examined further.
232 In addition to analyzing models of status, mean testosterone and social network strength
233 separately, we also explored whether candidate genes were involved in mediating status specific
234 relationships between mean testosterone and social behavior (Ryder et al. 2020) by extracting
235 "significant" candidate genes from a final DGE interaction analysis (model form: expression ~
236 status x mean testosterone). Owing to the overfitting concerns discussed above, we primarily
237 considered this model as a method for identifying which of our candidate genes showed status-
238 specific expression with respect to mean testosterone, rather than identifying new DEGs. The list
239 of putative relationships between genes and traits were plotted (Supplement 4), and any
240 relationship with highly influential observations, or with $R^2 \leq 0.2$, was excluded from our final
241 set. For candidates derived from the interaction effect models, and for genes that were associated
242 with multiple traits, we decided the most convincing relationship based on a stepdown AIC
243 procedure (Supplement 4). Given the substantially smaller set of candidate genes we considered
244 uncorrected p-values to be significant ($p<0.05$), but report the FDR corrected value as well.

245 To explore whether neuroanatomy constrains the types of genes expressed among brain
246 regions, we characterized overlap in gene expression patterns among brain nuclei by comparison
247 of ranked gene-lists (Supplement 6). The gene-lists were ranked by $-\log_{10}$ transforming the p-
248 values and multiplying by the direction of expression. Then these gene lists were compared for
249 similarity between each pair of tissues by calculating a) Pearson correlation and b) rank-rank
250 hypergeometric overlap (RRHO) test (Cahill et al. 2018).

251 Testosterone is pleiotropic in that it can affect multiple traits by influencing gene
252 expression in similar ways in many different target tissues (Ketterson et al. 2009; Cox 2020), and
253 it is crucial to social status-specific regulation of behavior in the wire-tailed manakin (Ryder et
254 al. 2020). We reasoned that these effects would be reflected in shared signatures of differential

255 expression across brain nuclei. To explore systemic gene expression patterns (i.e., consistent
256 patterns across the entire brain), we used the transformed p-values from the aforementioned
257 analysis and calculated the median p-value across all brain nuclei. Genes that had a median p-
258 value < 0.05 (transformed value > |1.3|) were considered consistently differentially expressed
259 across the brain. Further, the eigenvalues for each principal component (PC) from the whole-
260 brain PCA analysis (Figure 1G inset) were Pearson correlated with our variables of interest using
261 pcatools::eigencorplot() (Blighe and Lun 2020) (Supplement 1). Correlations observed with
262 social status were further validated by a linear mixed model in package 'nlme' (Pinheiro et al.
263 2016). The model form included individual as a random effect and incorporated batch variables
264 that were also correlated with the same PC (Supplement 1).

265 We used *clusterProfiler* v3.16.1(Yu et al. 2012) to determine patterns of functionality in
266 DEGs and WGCNA modules with Gene Ontology enrichment analyses. If the total number of
267 FDR corrected DEGs was less than 40, we used gene set enrichment analysis instead which
268 leverages the rank order of p-values and direction of expression (Supplement 3).

269

270 Results

271 *Landscape of gene expression among neural and endocrine tissues*

272 Our analyses revealed substantial differences in gene expression among tissues and brain
273 regions (Figure 1G; Figure S2.7). As expected, the pituitary was associated with high expression
274 levels of genes key to hormonal regulation (e.g., PRL, GH1, TSHB, POMC; Figure S2.3), while
275 the testes showed high expression levels of genes associated with germ cells and flagellar
276 motility (e.g., PKDREJ, BOLL, DRC7; Figure S2.3). Within the brain, the majority of gene
277 expression variation was explained by differences between brain regions (Figure 1G inset) and
278 was reflected in brain region-specific gene co-expression networks (Figure S2.7). These
279 expression patterns were more similar between adjacent nuclei, forming major brain-region
280 groupings of the amygdala, hypothalamus, and midbrain (Figure 1G). Our 18 candidate
281 neuroendocrine genes (Table S1.3) also showed tissue and brain region-specific expression
282 (Table 2.2, Figures S4.1-4.2).

Social status and testosterone phenotype are associated with tissue and brain region-specific patterns of gene expression

We observed a landscape of gene expression in association with testosterone phenotype (Figure 2A) and social status (Figure 2B), that was characterized by a high number of DEGs (or co-expression modules) identified in some tissues, but not others. Similarly, across brain regions we identified relationships between many of our 18 candidate genes and status, testosterone phenotype or their interaction (Figure 2C). Mean testosterone was a continuous predictor in DESeq2, as such DEGs showed up-or downregulation with a higher testosterone phenotype (Figure 2A), while DEGs identified in social status indicated up or down regulation in territorial males (Figure 2B). The number of trait-associated co-expression modules were similar to our results from differential gene expression, so we primarily focus on the tissues with large numbers of DEGs and significant candidate genes for the main manuscript, but see Supplements 3 and 5 for all results. The number of DEGs in each tissue was more similar between testosterone phenotype and status than any other comparison of traits examined ($R = 0.5$, $p = 0.08$, Figure S3.7), and this pattern appeared to hold across co-expression modules (Supplement 5) and candidate gene analysis (Figure 2C & Figure 3B). This similarity was expected because testosterone phenotype and status themselves are correlated (Figure 1D) (Ryder, Horton, et al. 2011; Ryder et al. 2020).

The testes and TnA each had over 1,000 genes differentially expressed (at uncorrected p-value < 0.05) in association with both testosterone phenotype and status (144 and 252 genes after FDR correction $q<0.01$ in association with mean T; 2 and 13 after FDR correction in association with social status). DEGs identified in association with testosterone phenotype in the testes were enriched for genes involved with response to corticosterone (GO:0051412) and synaptic transmission and organization (e.g., GO:0007268, GO:0050808) among others (Figure S3.20). Notable DEGs included steroidogenic acute regulatory protein (STAR, LFC = -1.1, $p = 2x10^{-7}$, $q = 0.0003$, $R^2 = 0.6$) and candidate gene AVPR1A (LFC = 0.8, $p = 5x10^{-5}$, $q = 0.01$, $R^2 = 0.7$; Figure S4.4). Note that Log_2 Fold Change (LFC) represents change in gene expression per unit of the predictor variable. However, these transcriptional differences do not correspond to significant differences in testis size in relation to testosterone phenotype (Table S3.2), nor social status (Table S3.1 & S3.2).

313 Among the 252 DEGs associated with testosterone phenotype in TnA, there was non-
314 significant enrichment for genes involved in neural crest migration (GO:0001755), learning and
315 memory (GO:0007613, GO:0007612), as well as long-term synaptic potentiation (e.g.,
316 GO:0060291) (Figure S3.28). Among the DEGs associated with testosterone phenotype in TnA,
317 candidate gene estrogen receptor 2 (ESR2; aka Erβ) was upregulated in TnA of males with
318 higher testosterone (Figure 2C; LFC = 0.8, p = 0.001, q = 0.08, R^2 = 0.4). Other notable DEGs in
319 TnA included upregulation of immediate early gene JUN, also a 'coral1' hub gene (module
320 membership (MM) = 0.93; LFC = 0.61, p = 0.0004, q = 0.05) and corticotropin releasing
321 hormone receptor 2 (CRHR2, LFC = 1, q = 0.006, R^2 = 0.5).

322 Not all nuclei showed similar patterns between status and testosterone phenotype. For
323 example, ICo of the midbrain showed 161 DEGs associated with testosterone phenotype after
324 FDR correction, but did not stand out in association with social status (3 DEGs). Most of the
325 significantly enriched GO terms were biological processes relating to motor proteins and cilia
326 (e.g., GO:0003341, Figure S3.26). Intriguingly, although the POM did not reveal a high number
327 of DEGs in association with testosterone phenotype (45), our analysis revealed differential
328 expression for compelling gene ontology categories and candidate neuroendocrine genes (Figure
329 S3.25, Figure S4.10, Figure S5.7). Notably, males with higher testosterone exhibited
330 upregulation of candidate gene androgen receptor (AR) in POM (Figure 2D). Indeed, the
331 'floralwhite' module comprised many of the upregulated DEGs and was significantly associated
332 with both testosterone phenotype and to a lesser extent social status (Figure S5.6). This module
333 showed enrichment for biological processes relating to G protein-coupled receptor signaling
334 pathways (GO:0007186) and neuropeptide signaling pathway (GO:0007218), with statistically
335 non-significant enrichment for steroid receptor signaling (GO:0030518), learning and memory
336 (GO:0007611), and nervous system development (GO:0007399) (Figure S3.7). Indeed, many of
337 the module hub genes were also DEGs associated with testosterone phenotype, and interact with
338 candidate sex hormone pathways, prolactin releasing hormone (PRLH, MM = 0.91, LFC = 2.4, q
339 = $5x10^{-5}$, R^2 = 0.6, Figure S3.25A) and growth regulating estrogen receptor binding 1 (GREB1,
340 MM = 0.86, LFC = 0.6, q = $3x10^{-5}$, R^2 = 0.7, Figure S3.25A), gonadotropin releasing hormone 1
341 (GNRH1, MM = 0.76, LFC = 0.4, p = 0.006, q = 0.2, R^2 = 0.4). Other notable 'floralwhite' hub
342 genes include the candidate gene progesterone receptor (PGR, MM = 0.81) which was also
343 associated with social status (Figure 2C; LFC = 0.4 p = 0.006, q = 0.7) and serotonin receptor 5A

344 (HTR5A, MM = 0.87, LFC = 0.4, p = 0.04, q = 0.6). Candidate gene aromatase
345 (LOC113993669/CYP19A1) was also upregulated in males with higher testosterone phenotype
346 (Figure 2C, LFC = 0.6, p = 0.02, q = 0.4, R^2 = 0.5), but was found in the 'lightcoral' co-
347 expression module (MM= 0.86).

348 Despite overall similarity in gene expression associations with testosterone phenotype
349 and status, some brain regions were more strongly associated with status or status × testosterone
350 phenotype interaction (Figure 2B). Likewise, expression in many of the 18 candidate
351 neuroendocrine genes were associated with status or the interaction with testosterone in different
352 nuclei (Figure 2C). PVN of the hypothalamus showed the most DEGs associated with social
353 status after FDR correction (42). Within these, there was statisticaly significant enrichment for
354 cytoskeletal movement genes (Figure S3.12B) involved in intracellular transport and cilia (e.g.,
355 GO:0003341, GO:0061640), and non-significant enrichment for cholesterol biosynthesis
356 (GO:0006695, GO:0045540). Candidate gene expression also revealed status-specific
357 associations with testosterone phenotype in the PVN, that is a statistical interaction between
358 status and testosterone phenotype (Figure 2C). In particular, AR expression was positively
359 correlated with testosterone phenotype in floater males, but not in territorial males (Figure 2F;
360 LFC = -0.7, p = 0.008, q = 0.9). The PVN of territorial males also showed higher VIPR1 (Figure
361 S4.8; LFC = 0.3, p = 0.02, q = 0.4) and lower ESR2 expression (LFC = -0.6, p = 0.006, q = 0.2).
362 In BSTm, a region that bridges the SDMN and MRS, there were more than 1000 putative DEGs,
363 but only nine after FDR correction. Among these, AR expression was higher in territorial males
364 than in floater males (Figure 2E; LFC = 0.6, p = 0.0007, q =0.2). Further, testosterone
365 metabolism gene 5-alpha reductase (SRD5A2) was upregulated in the LS of territorial males
366 (Figure S4.20; LFC = 0.7, p = 0.0005, q = 0.4). The VIP system is also implicated in status-
367 specific responses to testosterone in the TnA, where VIPR1 was upregulated in association with
368 testosterone in territorial, but not floater males (Figure S4.17; LFC = 1, p = 0.04 q = 0.7).

369 GCt was a hotbed for gene expression associated with social status and status ×
370 testosterone phenotype interaction (Figure 1B, Figure S3.6). In association with social status,
371 gene set enrichment analysis revealed there was a significant tendency for downregulation of
372 genes relating to synapse assembly (GO:0051965), and response estradiol (GO:0032355), and
373 upregulation of genes relating to RNA processing (e.g., GO:0000184, GO:0006396) and

374 translation (e.g., GO:0006413, GO:0006412) (Figure S3.15). While, candidate neuroendocrine
375 genes showed status specific expression in association with testosterone phenotype in this region
376 including progesterone receptor (PGR, Figure S4.15; LFC = 1.9, p= 0.003, q = 0.1), estrogen
377 receptor 1 (ESR1; aka Erα; LFC = 1.1 p = 0.04, q = 0.4), prolactin receptor (PRLR; LFC = 0.7, p
378 = 0.01, q = 0.2), and vasoactive intestinal peptide (VIP; Figure S4.15; LFC = 1.6, q = 0.005, q =
379 0.1). Further, candidate gene aromatase was downregulated with higher testosterone phenotype
380 in the GCt (LFC = -1.3, p = 0.002, q = 0.3, R^2 = 0.4).

381 ***Cooperative behavior is associated with a distinct gene expression landscape***

382 Network strength (the number of cooperative interactions between individuals) varies
383 widely among territorial and floater males (Figure 1E), representing a key axis of variation in
384 social behavior in this species. We predicted that the associations between gene expression and
385 cooperative behavior would be different from those observed for social status and testosterone
386 phenotype. Indeed, we found a distinctive landscape of constitutive gene expression across
387 tissues relating to cooperative behavior (Figure 3A, Figure S3.5), as well as among candidate
388 genes (Figure 3B). These results are based on results from a continuous predictor and can be
389 interpreted as genes with significant up-or downregulation with increasing social network
390 strength. AH had the largest number of DEGs associated with a male's social network strength.
391 In this nucleus there were no significantly enriched GO terms, but there was non-significant
392 enrichment for genes involved in RNA metabolism (e.g., GO:0043928) and regulation of
393 translation (e.g., GO:0017148), development (e.g., GO:0061061, GO:0042472) and synaptic
394 potentiation (e.g., GO:0007274) (Figure S3.35). The pituitary also stood out in the abundance of
395 differentially expressed genes associated with male's social network strength (109 genes after
396 FDR correction q < 0.1). There were no significantly enriched GO categories after FDR
397 correction (Figure S3.33B), but among the non-significant terms were actin filament
398 organization (GO:0007015), neural differentiation (e.g., GO:0031103, GO:0045664) and the cell
399 cycle (e.g., GO:0043065, GO:0071158.) Among the DEGs in the pituitary, candidate genes
400 prolactin (PRL) (Figure 3C; LFC = -0.6, p=5x10^{-5} q = 0.03, R^2 = 0.7), ESR2 (Figure S4.5; LFC
401 = -0.7, p = 0.04, q = 0.5, R^2 = 0.5) and VIPR2 (Figure S4.5; LFC = -0.4, p = 0.008, q = 0.3, R^2 =
402 0.4) were downregulated in highly cooperative males. Intriguingly, the PVN showed a weak
403 correlation of one of our candidate genes: oxytocin (OXT) expression decreased with a male's
404 social network strength (Figure 3D; LFC = -0.3, p = 0.04, q = 0.6, R^2 = 0.2). Further, although

405 there was less representation of sex-steroid related pathways in the candidate gene analysis
406 (Figure 2B), PGR was downregulated in males with higher social network strength in BSTm
407 (Figure 2E; LFC = -0.3, $p = 5x10^{-4}$, q = 0.2, $R^2 = 0.5$).

408 Many genes in the LS were differentially expressed in association with an individual's
409 social network strength (98 genes after FDR correction $q < 0.1$). There were no GO categories
410 significantly enriched after FDR correction (Figure S3.42), but among the top non-significant
411 terms were those broadly involved in development (e.g., GO:0048538, GO:0048593), and neural
412 organization (e.g., GO:0099175, GO:0050771). However, the expression of our candidate genes
413 in the LS was not linked to social network strength. Although relatively few genes were
414 differentially expressed in BSTm with respect to social network strength, the LS and BSTm
415 shared many genes with similar patterns of expression in association with strength as shown by
416 RRHO analysis (Cahill et al. 2018) (Figure 3G, F). In contrast, the LS and BSTm shared few
417 genes with similar expression in association with testosterone phenotype (Figure 4H, I) or social
418 status (Figure S6.1A), suggesting that these regions are uniquely engaged in modulating
419 cooperative behavior. Indeed, pairwise correlations of gene expression associated with
420 testosterone phenotype broadly recapitulated the neuroanatomical similarity of tissues sampled
421 (Figure 3H) and perhaps reflects similarities in pathways related to sex-steroid signaling (Figure
422 2C).

423 ***Testosterone phenotype and social status are associated with systemic gene expression***
424 We reasoned that the pleiotropic effects of testosterone and its role in social status in the
425 manakins (Ryder et al. 2020) would also produce systemic effects on gene expression, or similar
426 patterns of expression across multiple regions. Social status and testosterone phenotype were
427 linked to the highest number of consistently differentially expressed genes (60 and 66 genes with
428 median uncorrected $p < 0.05$ respectively), while only 10 genes were consistently differentially
429 expressed in association with social network strength (Supplement 6). Further, PC5 of the whole
430 brain analysis was significantly associated with social status in a linear mixed model that
431 corrected for batch confounds and repeated sampling of individuals (Figure S2.5). There was
432 overlap between the genes and GO categories between the consistently differentially regulated
433 genes with social status and testosterone phenotype and genes identified as associated with PC5.
434 But hereon we focus on genes related to social status in the main manuscript for simplicity.

435 The top two most consistently differentially regulated genes were the upregulation in the
436 brains of territorial males of an immune system gene CLEC2B (Figure 4A) and down regulation
437 of development gene ROBO4 (Figure 4B). The functional categories of these genes were
438 reflected in the patterns of GO biological process enrichment, though none of these terms were
439 significant after FDR correction (Figure 4C). There was also evidence for global differential
440 regulation of telomere maintenance genes (Figure 4C), with downregulation of POLD-1-like
441 (LOC114000574) and upregulation of RAD51 in territorial males. Further, there was evidence
442 for global regulation of cholesterol biosynthesis (Figure 4C), and glutathione transferases (Figure
443 S2.6, Table S6.2), both of which are relevant in steroid hormone biosynthesis. These findings of
444 global expression differences among status classes could be partly caused by the upregulation of
445 growth hormone (GH1) in the pituitary of territorial males (Figure 4D; LFC = 0.8, $p = 3x10^{-5}$, q
446 = 0.01). This result holds in the model that accounts for the interaction between social status and
447 mean T (LFC = 0.9, $p = 6x10^{-5}$, q = 0.05).

448 **Discussion**

449 Using the most comprehensive transcriptome-level sampling of the SDMN to-date
450 (Lopes and König 2020; Antunes et al. 2021; Bentz, Niederhuth, et al. 2021; Bentz, George, et
451 al. 2021; Kabelik et al. 2021), our findings highlight both modular and integrative neurogenomic
452 organization underlying individual variation in cooperative behavior. The relative roles of each
453 are central to the development and expression of any complex trait (Ketterson et al. 2009;
454 Lipshutz et al. 2019; Cox 2020). We discuss our findings in relation to each theme in turn.

455 **Different landscapes of gene expression with each trait reflect modular organization of the**
456 **neuroendocrine system**

457 Modularity is reflected in how different brain nuclei, endocrine tissues, and genetic
458 pathways within individuals are associated with the expression of different traits. Such
459 neurogenomic landscapes, characterized by the upregulation of neuroendocrine genes in some
460 brain regions and downregulation in others, would allow differential sensitivity to testosterone
461 and other signaling molecules among SDMN nuclei. These neurogenomic states can be likened
462 to the model of immediate early gene (IEG) activation across the SDMN after a social stimulus
463 (Newman 1999; Goodson 2005). In that 'landscape' model, it is the pattern of IEG expression
464 across multiple brain nuclei that correlates with behavioral responses, not the up or
465 downregulation of particular genes within a single nucleus. This original model considered this

466 landscape of IEG expression (and later neuropeptides) in the context of proximate responses to
467 social stimuli. Our findings extend this model in two ways: by considering transcriptomic
468 variation and how long-term neurogenomic states underlie individual variation in social
469 behavior.

470 In the landscape of constitutive gene expression in male wire-tailed manakins, total
471 number of DEGs and candidate gene expression implicate constitutive differences in most brain
472 regions, but the brain regions with higher numbers of DEGs or candidate gene expression may be
473 more important for mediating certain traits. Some brain regions showed associations between
474 gene expression and both social status and testosterone phenotype (e.g., POM) or only
475 testosterone phenotype (e.g., TnA, testes). This may be because these centers are involved in
476 testosterone mediated traits irrespective of social status (O'Connell and Hofmann 2012).
477 Meanwhile, others appeared to be more related to social status and status specific relationships
478 with testosterone phenotype (e.g., PVN, GCt), or an individual's strength of cooperative
479 behavior (e.g., PIT, LS). Our results also suggest that the identity and function of these genes
480 vary across tissues and brain regions (Figure 3GH). However, most analyses revealed DEGs
481 relating to neural development, synaptic potentiation or organization and genes relating to
482 cellular metabolism, or transcription/translation regulation. Thus, individual variation is
483 mediated by neurogenomic states that encode synaptic modifications and connectivity
484 (mechanism of memory formation), larger scale shifts in cellular activity, and neural structure
485 (Clayton et al. 2019; George et al. 2020)). These mechanisms may be supported or feedback on
486 the variation in elements of sex-steroid signaling we investigated in detail (*sensu*
487 "neuroendocrine action potential" (Clayton et al. 2019)).

488 We found ample evidence for modularity in steroid related gene expression, particularly
489 in association with social status, testosterone phenotype or their interaction. Unsurprisingly, we
490 found evidence that status-specific regulation of behavior could be modulated directly by
491 testosterone through AR in multiple nuclei (e.g., POM, PVN, BSTm). Higher AR expression is
492 linked to display performance in golden-collared manakins (Schlinger et al. 2013). Further, the
493 conversion of testosterone to estradiol by the enzyme aromatase and subsequent binding to
494 estrogen receptors is a key pathway in the regulation of testosterone-dependent male behaviors
495 more broadly (Schlinger and Balthazart 2013), and we found evidence for the role of aromatase

496 and estrogen signalling role in multiple nuclei. Our results also highlight the importance of
497 neural VIP expression in modulating multiple aspects of social behavior (Kingsbury and Wilson
498 2016; Horton, Michael, et al. 2020), as it was the only neuropeptide system with widespread
499 patterns of differential expression with respect to all our traits of interest.

500 Notably, VIP and other steroid receptors may play a role in status-specific responses to
501 testosterone in GCt. Although tyrosine hydroxylase (TH) neurons in the GCt have been
502 associated with courtship behaviors (Goodson et al. 2009; Ben-Tov et al. 2021), our finding of
503 association with VIP and courtship behaviors broadly speaking in GCt is novel (Kingsbury and
504 Wilson 2016). GCt coordinates social stimuli and downstream motor processes that control
505 behavior. For example, in the songbird brain, GCt links POM to the song control system and
506 thereby mediates androgen-dependent singing behavior (Haakenson et al. 2020). Given that
507 cooperative display in wire-tailed manakins requires coordinated physical (and vocal displays in
508 cooperative *Chiroxiphia* manakins (Trainer et al. 2002)), the status-specific differential
509 expression of genes for steroid receptors and steroid-sensitive neuropeptides in GCt suggest that
510 this SDMN region may play a unique role in mediating status-specific relationships between
511 testosterone and cooperative behavior.

512 The pattern of co-upregulation of steroid related genes in the POM of males with high
513 testosterone phenotype (and territorial males) point to this region's role as a potent steroid
514 regulator. The POM is involved in regulating male aggression, sexual behavior and parental care
515 (O'Connell and Hofmann 2011), and stimulates gonadal testosterone production through
516 gonadotropin releasing hormone (*GnRH*). Indeed, the observed increase in GNRH1 transcription
517 may relate to transcriptional changes indicating enhanced steroid production in the testes. In our
518 results, the co-upregulation of AR and GNRH1 would seem contradictory due to the negative
519 regulatory effects of androgens and AR on *GnRH* (Brayman et al. 2012), though these may not
520 interact at the cell population level or may show different temporal patterns of translation.
521 Regardless, activation and steroid receptor concentration in POM is associated with social status
522 differences and social status ascension cichlid fish (Maruska et al. 2013), and mice (Lee et al.
523 2022). In the same cichlid species, different preoptic cell types, including homologs for PVN,
524 showed higher activation in one of the two partners in cooperative territorial defense
525 (Weitekamp and Hofmann 2017). Indeed, testosterone can link the POM to the Hypothalamic

526 Pituitary Adrenal (HPA) axis via the PVN (Williamson et al. 2010), which in our study also
527 showed evidence of status specific gene expression, including AR. Aforementioned previous
528 work, and the close linkages between the PVN and POM (Williamson et al. 2010), suggest these
529 nuclei in wire-tailed manakins could be involved in multiple elements of behavior depending on
530 cell types, social contexts and timescales investigated.

531 The number of differentially expressed genes observed in TnA implicates this region as a
532 hub for neurogenomic pathways involved in regulation of testosterone mediated traits. The avian
533 homolog of the medial amygdala (O'Connell and Hofmann 2011), TnA integrates sensory
534 information and has been associated with the regulation of diverse social behaviors (Raam and
535 Hong 2021). Indeed, our results may reflect persistent activation of this area, based on the
536 upregulation of IEGs such as JUN and SYT7 in males with high testosterone phenotype, and
537 differential regulation of other genes involved in long-term synaptic potentiation (Clayton 2000;
538 Marrone et al. 2008; O'Connell et al. 2012; Clayton et al. 2019). Further, our results implicate
539 potential estrogenic regulation in this region via increased ESR2 in males with higher
540 testosterone phenotype. Indeed, estrogenic regulation through ESR1 has been shown to regulate
541 status-specific aggression in birds (Horton et al. 2014; Merritt et al. 2020) and prosocial behavior
542 in prairie voles (Cushing et al. 2008). Although ESR2 is not well characterized in birds, it may
543 function in the medial amygdala of mammals to regulate social recognition (Lymer et al. 2018),
544 and sexual regulation (Nakata et al. 2016), and is associated with higher social rank in the
545 forebrains of cichlid fish (Burmeister et al. 2007). Further, the differential expression of stress
546 steroid receptor (CRHR2) hints again at the previously unexplored role of the HPA axis in
547 modulating display behavior and social status in this species. Indeed, social ranks often differ in
548 their glucocorticoid profiles (Goymann and Wingfield 2004) or regulatory responses to
549 glucocorticoids (Snyder-Mackler et al. 2019). The related lance-tailed manakins (*Chiroxiphia*
550 *lanceolata*), which also display cooperatively, vary in testosterone as well as corticosterone
551 profiles according to social status (DuVal and Goymann 2011; Jones and DuVal 2021). Finally,
552 the fish homolog of TnA appears mediate aggression and cooperation in cooperative territory
553 defense in an actor-specific manner (Weitekamp et al. 2017; Weitekamp and Hofmann 2017),
554 supporting a role for modulating cooperation in wire-tailed manakins as well.

555 The time spent interacting with other males (social network strength), our proxy for
556 cooperative behavior, is associated with a unique pattern of gene expression among tissues and
557 brain regions. Some of these signatures are difficult to parse, for example many genes are
558 differentially expressed in AH, yet lack clear GO enrichment. Prior to our study AH has
559 associated with regulation of aggression rather than cooperation in males, and acting via AVP
560 receptors and VIP (O'Connell and Hofmann 2011; Horton, Michael, et al. 2020). Although we
561 do not find any evidence for the role of these genes in the AH, our findings may suggest that
562 cooperative displays are derived from displays of territorial aggression, and/or gene expression is
563 inhibiting aggression in these displays. Indeed, cooperative displays may act as a way of
564 maintaining dominance hierarchies in wire-tailed manakins.

565 LS showed many differentially expressed genes associated with cooperation, and highly
566 cooperative individuals may show differences in neural structure based on the enriched GO
567 terms. Patterns of expression in BSTm were similar, but weaker based on fewer statistically
568 significant DEGs. BSTm has neural projections into LS, and vasopressin (AVP) and VIP
569 neurons in the BSTm and receptors for these neuropeptides in the LS are associated with
570 affiliative behavior and gregariousness in birds (Kelly et al. 2011; Kelly and Goodson 2013;
571 Kingsbury and Wilson 2016). Those prior studies suggest a role for these brain nuclei in
572 processing social information acutely, whereas our study suggests differences among individuals
573 in cooperative tendency is related to constitutive differences in gene expression and neural
574 structure, perhaps relating to excitability of these regions in response to social information. Thus,
575 cooperative display is regulated by some of the same conserved brain regions as other types of
576 affiliative behavior. That we only observed a large number of DEGs in the LS and not in other
577 nuclei may suggest that other unsampled nuclei in the mesolimbic reward system are important
578 for modulation of cooperative tendency (e.g., (Paula et al. 2015; Weitekamp et al. 2017; de
579 Abreu et al. 2020; Lopes and König 2020).

580 We did not find convincing evidence for any variation in sensitivity to nonapeptides
581 (vasopressin and oxytocin) in the brain (AVPR2 in hypothalamic nuclei showed transcript counts
582 <30). However, the localization of these receptors in particular brain regions may be more
583 important for modulating behavior than a change in their abundance (Goodson and Thompson
584 2010). We did find evidence for differential sensitivity to AVP in the pituitary, again implicating

585 stress response pathways in mediating social status and cooperation in this species. Further, we
586 did find a weak negative relationship between oxytocin expression and social network strength in
587 the PVN. Although in apparent contrast to the widely held belief that oxytocin promotes sociality
588 (as in humans (Feldman 2012)), this among our other observations may indicate that cooperative
589 displays are a derivative of aggressive displays. However, nonapeptides have complex and
590 sometimes species-specific relationships with affiliative behavior (Goodson and Thompson
591 2010; Maruska et al. 2022), and can also be associated with social status (Lee et al. 2022).
592 Further, the ratio of peptide to mRNA can be an important factor indicating higher turnover
593 (usage) in certain contexts (Maruska et al. 2022). Therefore, we do not have sufficient evidence
594 that oxytocin inhibits cooperation in this species, and further experimental work is needed.

595 Our results establish a variety of mechanisms that could underpin individual variation in
596 cooperative behavior, and its status specific modulation by testosterone. We find evidence for
597 multiple forms of neuroplasticity, through steroid receptor variation, synaptic potentiation and
598 neural development, as well as cellular activity (Clayton et al. 2019). Indeed, testosterone
599 manipulations are known to induce changes in neuroanatomy in behaviorally relevant brain
600 nuclei (Kabelik et al. 2008), suggesting a direct role for testosterone-mediated neural growth and
601 flexibility in modulating male-male cooperation (Soares et al. 2010). Our results provide
602 promising targets for further study on the mechanisms underlying cooperative behavior,
603 including examination of neuroanatomical features and the use of hormonal or other
604 pharmaceutical manipulations and RNAi technologies to examine causal relationships between
605 neural gene expression and behavior (e.g., (Merritt et al. 2020)).

606 **Similarities in gene expression reflect the role of testosterone as a phenotypic**
607 **integrator.**

608 Similarities in gene expression among tissues are predicted as a result of the pleiotropic
609 effects of testosterone and support the role of the hormone as a mediator of phenotypic
610 integration. The extent of gene expression similarities and differences among tissues may reflect
611 the degree of integration and independence (or modularity), respectively, that can either
612 constrain or facilitate adaptive evolution (Ketterson et al. 2009; Lipshutz et al. 2019). We
613 identified similarities in testosterone associated gene expression between brain regions that
614 broadly recapitulated tissue ontology. We also identified consistent, brain-wide (systemic) gene

615 expression differences associated predominantly with social status and testosterone phenotype.
616 However, systemic results did not explain as much variation in overall neural gene expression as
617 with some axes of personality (Kabelik et al. 2021; Lattin et al. 2021), suggesting that the brain
618 region-specific patterns may be more important in modulating male wire-tailed manakin
619 behavior. Moreover, the broader associations of social status with metabolic, immune, and
620 telomere gene ontology categories suggests differences in allostatic load among status classes in
621 male-male cooperative systems (Goymann and Wingfield 2004; Jones and DuVal 2021;
622 Vernasco et al. 2021). Further, differential global regulation of telomeres may explain the
623 relative stability of telomere length in territorial wire-tailed manakin males (Vernasco et al.
624 2021), and collectively these results may represent metabolic adaptations for territoriality.

625 Our results highlight two additional hormones that may have a system-wide role in the
626 status-specific modulation of cooperative display behavior. We found differences in the
627 expression of genes for important behavioral and metabolic hormones (prolactin and growth
628 hormone) in the pituitary, and may reflect organism wide metabolic adaptations. Indeed,
629 previous work on this species showed that more social males had shorter telomeres (Vernasco et
630 al. 2021), and social bond strength has been associated with signatures of energy metabolism and
631 stress (Anderson et al. 2022; Simons et al. 2022). The anterior pituitary is the primary production
632 site for both hormones, and thus gene expression patterns here are likely good predictors for
633 circulating levels of those hormones and thus signal strength. PRL transcript was downregulated
634 in more cooperative males. Prolactin is best known for its role in promoting parental care
635 behaviors, including in cooperative breeders (Schoech et al. 1996), but emerging studies suggest
636 it could play a role in inhibiting aggression in cooperative breeders (Medger et al. 2019; Gilbert
637 et al. 2022) and promotion of nest defense behavior (Mohamed et al. 2016). However, the
638 negative relationship observed appears contradictory to a general model of increased prolactin
639 promoting broadly affiliative social interactions, and again may reflect the origin of these
640 cooperative displays from aggressive or competitive interactions (Díaz-Muñoz et al. 2014). The
641 other compelling phenotypic integrator for status-dependent behaviors in the wire-tailed manakin
642 comes from the gene encoding growth hormone (GH1). Ascension in social rank in the
643 cooperatively displaying manakins is associated with age (McDonald 1989; DuVal 2007; Ryder
644 et al. 2008). It was therefore surprising that territorial males in our study showed increased
645 expression of GH1 in the pituitary as compared to floater males. However, in anolis lizards,

646 stimulation of the growth hormone axis is associated with increased testosterone that enables
647 phenotypic divergence between males and females (Cox et al. 2017). Without gross
648 morphological differences in wire-tailed manakin status-classes, growth-hormone could have
649 subtle effects on multiple systems enabling the status-specific regulation of behaviors. In
650 particular, status differences in GH1 expression may explain repeated signatures of neural
651 development and protein synthesis in our results (Sonksen 2006), and may reflect the increased
652 metabolic demands on displaying territorial males through muscle performance and regulation of
653 oxidative stress (Hu et al. 2019). The neuroethological role of growth hormone are not well
654 known, but its release may be linked to stress (Greenwood and Landon 1966; McCormick et al.
655 1998). Growth hormone administration promotes increased aggression in mice (Matte 1981), and
656 fish (Jönsson et al. 1998; Johansson et al. 2004). Indeed, growth hormone inhibitor somatostatin
657 and its receptors have also been implicated in regulating aggression and social status in cichlid
658 fish independent of androgens (Hofmann and Fernald 2000; Trainor and Hofmann 2006) whilst
659 natal androgen treatment increased aggression and somatostatin receptor expression in zebra
660 finches (Bentz, Niederhuth, et al. 2021). We did some evidence for negative relationship
661 between SSTR1 and SSTR2 with testosterone phenotype in the testes, and positive association
662 between SSTR1 and testosterone phenotype in the POM but these were not significant after FDR
663 correction. Thus, our results suggest that the growth hormone axis may play a role in the status-
664 specific regulation of wire-tailed manakin cooperative displays, and increased GH in territorial
665 males may be an important inhibitor of cooperation by promoting aggression. Future work on
666 social behavior should consider the role of growth hormone and somatostatin in conjunction with
667 sex steroids and glucocorticoids to gain novel insights into its function in behavioral regulation.

668 ***Conclusion***

669 Across the SDMN and HPG of the male wire-tailed manakin we observed correlations with
670 social status, cooperation and testosterone phenotype in patterns of gene regulation and a
671 plurality of gene functions, including sex-steroid and neuropeptide signaling. This landscape of
672 gene expression speaks to the modular nature of behavioral mechanisms (Newman 1999;
673 Goodson 2005; Lipshutz et al. 2019) and highlights the continuum between complete phenotypic
674 integration and modularity (Ketterson et al. 2009). Much of the previous work on the
675 mechanisms of social and cooperative behaviors focus on relatively few candidate genes in

676 broad-scale brain divisions (e.g., hypothalamus) or in one or a few particular nuclei. Our work
677 presented here is one of the most comprehensive examinations to date of region-specific gene
678 expression in all nuclei of the SDMN, albeit correlative only. Our correlative findings help us to
679 better understand cooperative behavior (Soares et al. 2010) by linking neurogenomics with
680 endocrine mechanisms, and generate further hypotheses to test in further experiments to
681 implicate causality. We further demonstrate and emerging idea that constitutive landscapes in
682 gene expression across the brain are associated with repeatable differences in behavioral
683 phenotype (Antunes et al. 2021; Kabelik et al. 2021; Lattin et al. 2021). We also provide
684 tantalizing glimpses into nuclei and genomic pathways involved in the status-specific regulation
685 of this cooperative display behavior. Although questions remain on the acute mediators of
686 cooperative display behavior in the wire-tailed manakin, we demonstrate the power and
687 flexibility of a system-wide approach. We propose that landscapes of constitutive gene
688 expression for multiple genes and across many tissues and brain regions, like those observed
689 here, form the foundation of consistent inter-individual differences in any behavior, upon which
690 flexible and acute responses to social stimuli are built.

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702 **Data and materials availability:** Raw read data are available associated with BioProject
703 PRJNA437157, and all code used to conduct analyses is available here:
704 https://github.com/periperipatus/PIFI_brain_transcriptome

References

de Abreu MS, Maximino C, Cardoso SC, Marques CI, Pimentel AFN, Mece E, Winberg S, Barcellos LJG, Soares MC. 2020. Dopamine and serotonin mediate the impact of stress on cleaner fish cooperative behavior. *Horm. Behav.* 125.

Anderson JA, Lea AJ, Voyles TN, Akinyi MY, Nyakundi R, Ochola L, Omondi M, Nyundo F, Zhang Y, Campos FA, et al. 2022. Distinct gene regulatory signatures of dominance rank and social bond strength in wild baboons. *Philos. Trans. R. Soc. B* [Internet] 377. Available from: https://royalsocietypublishing.org/doi/10.1098/rstb.2020.0441

Antunes DF, Teles MC, Zuelling M, Friesen CN, Oliveira RF, Aubin-Horth N, Taborsky B. 2021. Early social deprivation shapes neuronal programming of the social decision-making network in a cooperatively breeding fish. *Mol. Ecol.* [Internet] 30:4118–4132. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/mec.16019

Ben-Tov M, Duarte F, Mooney R. 2021. A neural hub that coordinates learned and innate courtship behaviors. *bioRxiv* [Internet]:2021.09.09.459618. Available from: https://www.biorxiv.org/content/10.1101/2021.09.09.459618v1

Bentz AB, George EM, Wolf SE, Rusch DB, Podicheti R. 2021. Experimental competition induces immediate and lasting effects on the neurogenome in free-living female birds. *Proc. Natl. Acad. Sci. USA* 118:e2016154118.

Bentz AB, Niederhuth CE, Carruth LL, Navara KJ. 2021. Prenatal testosterone triggers long-term behavioral changes in male zebra finches: unravelling the neurogenomic mechanisms. *BMC Genomics* 22:158.

Blighe K, Lun A. 2020. PCAtools: Everything Principal Components Analysis.

Brayman MJ, Pepa PA, Mellon PL. 2012. Androgen Receptor Repression of Gonadotropin-Releasing Hormone Gene Transcription via Enhancer 1. *Mol. Cell. Endocrinol.* [Internet] 363:92. Available from: /pmc/articles/PMC3447085/

Burmeister SS, Kailasanath V, Fernald RD. 2007. Social dominance regulates androen and estrogen receptor gene expression. *Bone* 51:164–170.

Cahill K, Huo Z, Tseng GC, Logan RW, Seney ML. 2018. Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach. *Sci. Rep.* 8:9588.

Clayton DF. 2000. The genomic action potential. *Neurobiol. Learn. Mem.* 74:185–216.

Clayton DF, Anreiter I, Aristizabal M, Frankland PW, Binder EB, Citri A. 2019. The role of the genome in experience-dependent plasticity: Extending the analogy of the genomic action potential. *Proc. Natl. Acad. Sci.* 117:201820837.

Cox RM. 2020. Sex steroids as mediators of phenotypic integration, genetic correlations, and evolutionary transitions. *Mol. Cell. Endocrinol.* [Internet] 502:110668. Available from: https://doi.org/10.1016/j.mce.2019.110668

Cox RM, Cox CL, McGlothlin JW, Card DC, Andrew AL, Castoe TA. 2017. Hormonally Mediated Increases in Sex-Biased Gene Expression Accompany the Breakdown of Between-Sex Genetic Correlations in a Sexually Dimorphic Lizard. *https://doi.org/10.1086/690105* [Internet] 189:315–332. Available from: https://www.journals.uchicago.edu/doi/10.1086/690105

Cushing BS, Perry A, Musatov S, Ogawa S, Papademetriou E. 2008. Estrogen Receptors in the Medial Amygdala Inhibit the Expression of Male Prosocial Behavior. *J. Neurosci.* [Internet] 28:10399–10403. Available from: https://www.jneurosci.org/content/28/41/10399

Dakin R, Ryder TB. 2018. Dynamic network partnerships and social contagion drive cooperation. *Proc. R. Soc. B Biol. Sci.* 285:20181973.

Díaz-Muñoz SL, DuVal EH, Krakauer AH, Lacey EA. 2014. Cooperating to compete: Altruism, sexual selection and causes of male reproductive cooperation. *Anim. Behav.* [Internet] 88:67–78. Available from: http://dx.doi.org/10.1016/j.anbehav.2013.11.008

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29:15–21.

DuVal EH. 2007. Adaptive advantages of cooperative courtship for subordinate male lance-tailed manakins. *Am. Nat.* 169:423–432.

DuVal EH, Goymann W. 2011. Hormonal correlates of social status and courtship display in the cooperatively lekking lance-tailed manakin. *Horm. Behav.* [Internet] 59:44–50. Available from: http://dx.doi.org/10.1016/j.yhbeh.2010.10.004

Feldman R. 2012. Oxytocin and social affiliation in humans. *Horm. Behav.* [Internet] 61:380–391. Available from: https://pubmed.ncbi.nlm.nih.gov/22285934/

Fusani L, Donaldson Z, London SE, Fuxjager MJ, Schlinger BA. 2014. Expression of androgen receptor in the brain of a sub-oscine bird with an elaborate courtship display. *Neurosci. Lett.* [Internet] 578:61–65. Available from: http://dx.doi.org/10.1016/j.neulet.2014.06.028

George JM, Bell ZW, Condliffe D, Dohrer K, Abaurrea T, Spencer K, Leitão A, Gahr M, Hurd PJ, Clayton DF. 2020. Acute social isolation alters neurogenomic state in songbird forebrain. *Proc. Natl. Acad. Sci.* 117:201820841.

Gilbert JD, Rossiter SJ, Bennett NC, Faulkes CG. 2022. The elusive role of prolactin in the sociality of the naked mole-rat. *Horm. Behav.* 143:105196.

Goodson JL. 2005. The vertebrate social behaviour network: evolutionary themes and variations. *Horm. Behav.* 48:11–22.

Goodson JL, Kabelik D, Kelly AM, Rinaldi J, Klatt JD. 2009. Midbrain dopamine neurons reflect affiliation phenotypes in finches and are tightly coupled to courtship. *Proc. Natl. Acad. Sci.* [Internet] 106:8737–8742. Available from: https://www.pnas.org/content/106/21/8737

Goodson JL, Kingsbury MA. 2013. What's in a name? Considerations of homologies and nomenclature for vertebrate social behavior networks. *Horm. Behav.* 64:103–112.

Goodson JL, Thompson RR. 2010. Nonapeptide mechanisms of social cognition, behavior and species-specific social systems. *Curr. Opin. Neurobiol.* 20:784–794.

Goymann W, Wingfield JC. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. *Anim. Behav.* 67:591–602.

Greenwood FC, Landon J. 1966. Growth hormone secretion in response to stress in man. *Nature* 210:540–541.

Griffith SC, Crino OL, Andrew SC, Nomano FY, Adkins-Regan E, Alonso-Alvarez C, Bailey IE, Bittner SS, Bolton PE, Boner W, et al. 2017. Variation in Reproductive Success Across Captive Populations: Methodological Differences, Potential Biases and Opportunities. *Ethology* [Internet] 123:1–29. Available from: http://doi.wiley.com/10.1111/eth.12576

Haakenson CM, Balthazart J, Ball GF. 2020. Cognition and Behavior Effects of Inactivation of the Periaqueductal Gray on Song Production in Testosterone-Treated Male Canaries (*Serinus canaria*). *eNeuro* [Internet] 7:ENEURO.0048-20.2020. Available from: https://doi.org/10.1523/ENEURO.0048-20.2020

Hofmann H a., Beery AK, Blumstein DT, Couzin ID, Earley RL, Hayes LD, Hurd PL, Lacey E a., Phelps SM, Solomon NG, et al. 2014. An evolutionary framework for studying mechanisms of social behavior. *Trends Ecol. Evol.* [Internet] 29:1–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0169534714001608

Hofmann HA, Fernald RD. 2000. Social Status Controls Somatostatin Neuron Size and Growth. *J. Neurosci.* [Internet] 20:4740–4744. Available from: https://www.jneurosci.org/content/20/12/4740

Horton BM, Hudson WH, Ortlund EA, Shirk S, Thomas JW, Young ER, Zinzow-Kramer WM, Maney DL. 2014. Estrogen receptor α polymorphism in a species with alternative behavioral phenotypes. *Proc. Natl. Acad. Sci. U. S. A.* [Internet] 111:1443–1448. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1317165111

Horton BM, Michael C, Mackenzie P, Maney DL. 2020. Vasoactive intestinal peptide as a mediator of the effects of a supergene on social behavior. *Proc. R. Soc. B Biol. Sci.* 287:20200196.

Horton BM, Ryder TB, Moore IT, Balakrishnan CN. 2020. Gene expression in the social behavior network of the wire-tailed manakin (*Pipra filicauda*) brain. *Genes, Brain Behav.*:e12560.

Hu B, Hu S, Yang M, Liao Z, Zhang D, Luo Q, Zhang X, Li H. 2019. Growth hormone receptor gene is essential for chicken mitochondrial function in vivo and in vitro. *Int. J. Mol. Sci.* 20:1–13.

Johansson V, Winberg S, Jönsson E, Hall D, Björnsson B. 2004. Peripherally administered growth hormone increases brain dopaminergic activity and swimming in rainbow trout. *Horm. Behav.* 46:436–443.

Jones B, DuVal EH. 2021. Glucocorticoids correlate with and predict social status in the cooperatively breeding lance-tailed manakin (*Chiroxiphia lanceolata*). In: Integrative and Comparative Biology. Vol. 61. p. E428-429.

Jönsson E, Johnsson J, Björnsson B. 1998. Growth hormone increases aggressive behavior in juvenile rainbow trout. *Horm. Behav.* 33:9–15.

Kabelik D, Julien AR, Ramirez D, O'Connell LA. 2021. Social boldness correlates with brain gene expression in male green anoles. *Horm. Behav.* 133:105007.

Kabelik D, Weiss SL, Moore MC. 2008. Steroid hormones alter neuroanatomy and aggression independently in the tree lizard. *Physiol. Behav.* [Internet] 93:492. Available from: /pmc/articles/PMC4286361/

Kasper C, Vierbuchen M, Ernst U, Fischer S, Radersma R, Raulo A, Cunha-Saraiva F, Wu M, Mobley KB, Taborsky B. 2017. Genetics and developmental biology of cooperation. *Mol. Ecol.* 26:4364–4377.

Kelly AM, Goodson JL. 2013. Behavioral relevance of species-specific vasotocin anatomy in gregarious finches. *Front. Neurosci.* [Internet] 7:242. Available from: /pmc/articles/PMC3865460/

Kelly AM, Kingsbury MA, Hoffbuhr K, Schrock SE, Waxman B, Kabelik D, Thompson RR, Goodson JL. 2011. Vasotocin neurons and septal V1a-like receptors potently modulate songbird flocking and responses to novelty. *Horm. Behav.* 60:12–21.

Ketterson ED, Atwell JW, McGlothlin JW. 2009. Phenotypic integration and independence: Hormones, performance, and response to environmental change. *Integr. Comp. Biol.* 49:365–379.

Kingsbury MA, Wilson LC. 2016. The Role of VIP in Social Behavior: Neural Hotspots for the Modulation of Affiliation, Aggression, and Parental Care. *Integr. Comp. Biol.* [Internet] 56:1238–1249. Available from: https://academic.oup.com/icb/article/56/6/1238/2647097

Langfelder P, Horvath S. 2008. WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559.

Lattin CR, Kelly TR, Kelly MW, Johnson KM. 2021. Constitutive gene expression differs in three brain regions important for cognition in neophobic and non-neophobic house sparrows (*Passer domesticus*). *bioRxiv* [Internet]:2021.07.06.451290. Available from: https://www.biorxiv.org/content/10.1101/2021.07.06.451290v1

Lee W, Dwortz MF, Milewski TM, Champagne FA, Curley JP. 2022. Social status mediated variation in hypothalamic transcriptional profiles of male mice. *Horm. Behav.* 142:105176.

Liao Y, Smyth GK, Shi W. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30:923–930.

Lipshutz SE, George EM, Bentz AB, Rosvall KA. 2019. Evaluating testosterone as a phenotypic integrator: From tissues to individuals to species. *Mol. Cell. Endocrinol.* [Internet] 496:110531. Available from: https://doi.org/10.1016/j.mce.2019.110531

Lopes PC, König B. 2020. Wild mice with different social network sizes vary in brain gene expression. *BMC Genomics* 21:1–14.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for

RNA-seq data with DESeq2. *Genome Biol.* 15:550.

Loveland JL, Giraldo-Deck LM, Lank DB, Goymann W, Gahr M, Küpper C. 2021. Functional differences in the hypothalamic-pituitary-gonadal axis are associated with alternative reproductive tactics based on an inversion polymorphism. *Horm. Behav.* [Internet] 127:104877. Available from: https://doi.org/10.1016/j.yhbeh.2020.104877

Lymer JM, Sheppard PAS, Kuun T, Blackman A, Jani N, Mahbub S, Choleris E. 2018. Estrogens and their receptors in the medial amygdala rapidly facilitate social recognition in female mice. *Psychoneuroendocrinology* 89:30–38.

Marrone DF, Schaner MJ, Mcnaughton BL, Worley PF, Barnes CA. 2008. Immediate-Early Gene expression at rest recapitulates recent experience. *J. Neurosci.* 28:1030–1033.

Maruska KP, Anselmo CM, King T, Mobley RB, Ray EJ, Wayne R. 2022. Endocrine and neuroendocrine regulation of social status in cichlid fishes. *Horm. Behav.* 139:105110.

Maruska KP, Zhang A, Neboori A, Fernald RD. 2013. Social Opportunity Causes Rapid Transcriptional Changes in the Social Behaviour Network of the Brain in an African Cichlid Fish. *J. Neuroendocrinol.* 25:145–157.

Matte AC. 1981. Growth hormone and isolation-induced aggression in wild male mice. *Pharmacol. Biochem. Behav.* [Internet] 14 Suppl 1:85–87. Available from: https://pubmed.ncbi.nlm.nih.gov/7195589/

McCormick SD, Shrimpton JM, Carey JB, O'Dea MF, Sloan KE, Moriyama S, Björnsson BT. 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. *Aquaculture* 168:221–235.

McDonald DB. 1989. Cooperation Under Sexual Selection : Age-Graded Changes in a Lekking Bird. *Am. Nat.* 134:709–730.

Medger K, Bennett NC, Ganswindt SB, Ganswindt A, Hart DW. 2019. Changes in prolactin, cortisol and testosterone concentrations during queen succession in a colony of naked mole-rats (Heterocephalus glaber): a case study. *Naturwissenschaften* [Internet] 106. Available from: https://pubmed.ncbi.nlm.nih.gov/31089819/

Merritt JR, Grogan KE, Zinzow-kramer WM, Sun D, Ortlund EA. 2020. A supergene-linked estrogen receptor drives alternative phenotypes in a polymorphic songbird. :1–8.

Mohamed RA, Shukry M, Mousa-balabel TM, Elbassiouny AA. 2016. Assessment of Plasma Prolactin and Nest Defense Behaviour During Breeding Cycle of Pigeon (Columba livia domestica). *J. Environ. Agric. Sci.* 7:19–22.

Nakata M, Sano K, Musatov S, Yamaguchi N, Sakamoto T, Ogawa S. 2016. Effects of Prepubertal or Adult Site-Specific Knockdown of Estrogen Receptor β in the Medial Preoptic Area and Medial Amygdala on Social Behaviors in Male Mice. *eNeuro* [Internet] 3:579–588. Available from: /pmc/articles/PMC4819287/

Newman SW. 1999. The medial extended amygdala in male reproductive behavior: A node in the mammalian social behavior network. *Ann. N. Y. Acad. Sci.* 877:242–257.

O'Connell LA, Hofmann HA. 2011. The Vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *J. Comp. Neurol.* 519:3599–3639.

O'Connell LA, Hofmann HA. 2012. Social Status Predicts How Sex Steroid Receptors Regulate Complex Behavior across Levels of Biological Organization. *Endocrinology* [Internet] 153:1341–1351. Available from: https://academic.oup.com/endo/article/153/3/1341/2424042

O'Connell LA, Matthews BJ, Hofmann HA. 2012. Isotocin regulates paternal care in a monogamous cichlid fish. *Horm. Behav.* 61:725–733.

Olds J, Milner P. 1954. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J. Comp. Physiol. Psychol.* [Internet] 47:419–427. Available from: /record/1955-06866-001

Paula JR, Messias JP, Grutter AS, Bshary R, Soares MC. 2015. The role of serotonin in the modulation of cooperative behavior. *Behav. Ecol.* [Internet] 26:1005–1012. Available from: https://academic.oup.com/beheco/article/26/4/1005/209523

Pinheiro J, Bates D, DebRoy S, Sarkar D. 2016. Linear and Nonlinear Mixed Effects Models. R. package v 3.1-128.

R Core Team. 2020. R: A language and environment for statistical computing. Available from: https://www.r-project.org/

Raam T, Hong W. 2021. Organization of neural circuits underlying social behavior: a consideration of the medial amygdala. *Curr. Opin. Neurobiol.* [Internet] 68:124. Available from: /pmc/articles/PMC8243811/

Ryder TB, Blake JG, Parker PG, Loiselle BA. 2011. The composition, stability, and kinship of reproductive coalitions in a lekking bird. *Behav. Ecol.* 22:282–290.

Ryder TB, Dakin R, Vernasco BJ, Evans BS, Horton BM, Moore IT. 2020. Testosterone modulates status-specific patterns of cooperation in a social network. *Am. Nat.* 195:82–94.

Ryder TB, Horton BM, Moore IT. 2011. Understanding testosterone variation in a tropical lek-breeding bird. *Biol. Lett.* 7:506–509.

Ryder TB, Horton BM, Van Den Tillaart M, De Dios Morales J, Moore IT. 2012. Proximity data-loggers increase the quantity and quality of social network data. *Biol. Lett.* 8:917–920.

Ryder TB, McDonald DB, Blake JG, Parker PG, Loiselle BA. 2008. Social networks in the lek-mating wire-tailed manakin (*Pipra filicauda*). *Proc. R. Soc. B Biol. Sci.* 275:1367–1374.

Ryder TB, Parker PG, Blake JG, Loiselle BA. 2009. It takes two to tango: Reproductive skew and social correlates of male mating success in a lek-breeding bird. *Proc. R. Soc. B Biol. Sci.* 276:2377–2384.

Schlinger B, Balthazart J. 2013. Aromatase and Behavior: Concepts Gained from Studies of Aromatase in the Avian Brain. In: Brain Aromatase, Estrogens, and Behavior. Available from: https://psycnet.apa.org/record/2012-25571-010

Schlinger BA, Barske J, Day L, Fusani L, Fuxjager MJ. 2013. Hormones and the neuromuscular

control of courtship in the golden-collared manakin (*Manacus vitellinus*). *Front. Neuroendocrinol.* [Internet] 34:143. Available from: /pmc/articles/PMC3995001/

Schoech SJ, Mumme L R, Wingfield.ast C J. 1996. Prolactin and helping behaviour in the cooperatively breeding Florida scrub-jay, *Aphelocoma c. coerulescens*. *Anim. Behav.* 52:445–456.

Simons ND, Michopoulos V, Wilson M, Barreiro LB, Tung J. 2022. Agonism and grooming behaviour explain social status effects on physiology and gene regulation in rhesus macaques. *Philos. Trans. R. Soc. B Biol. Sci.* 377.

Snyder-Mackler N, Sanz J, Kohn JN, Voyles T, Pique-Regi R, Wilson ME, Barreiro LB, Tung J. 2019. Social status alters chromatin accessibility and the gene regulatory response to glucocorticoid stimulation in rhesus macaques. *Proc. Natl. Acad. Sci. U. S. A.* [Internet] 116:1219–1228. Available from: https://www.pnas.org/doi/abs/10.1073/pnas.1811758115

Soares MC, Bshary R, Fusani L, Goymann W, Hau M, Hirschenhauser K, Oliveira RF. 2010. Hormonal mechanisms of cooperative behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* 365:2737–2750.

Sonksen P. 2006. Growth Hormone Replacement Therapy and Life Quality. Future Perspectives. In: Nyberg F, editor. The Somatotrophic Axis in Brain Function. Elsevier Inc. p. 327–333.

Taborsky M, Taborsky B. 2015. Evolution of genetic and physiological mechanisms of cooperative behaviour. *Curr. Opin. Behav. Sci.* 6:132–138.

Trainer JM, McDonald DB, Learn WA. 2002. The development of coordinated singing in cooperatively displaying long-tailed manakins. *Behav. Ecol.* 13:65–69.

Trainor BC, Hofmann HA. 2006. Somatostatin Regulates Aggressive Behavior in an African Cichlid Fish. *Endocrinology* [Internet] 147:5119–5125. Available from: https://academic.oup.com/endo/article/147/11/5119/2500141

Vernasco BJ, Dakin R, Majer AD, Haussmann MF, Ryder TB, Moore IT. 2021. Longitudinal dynamics and behavioural correlates of telomeres in male wire-tailed manakins. *Funct. Ecol.* [Internet] 35:450–462. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/1365-2435.13715

Vernasco BJ, Horton BM, Moore IT, Ryder TB. 2020. Reduced cooperative behavior as a cost of high testosterone in a lekking passerine bird. *Behav. Ecol.* [Internet] 31:401–410. Available from: https://academic.oup.com/beheco/advance-article/doi/10.1093/beheco/arz201/5673236

Weitekamp CA, Hofmann HA. 2017. Neuromolecular correlates of cooperation and conflict during territory defense in a cichlid fish. *Horm. Behav.* 89:145–156.

Weitekamp CA, Nguyen J, Hofmann HA. 2017. Neuromolecular regulation of aggression differs by social role during joint territory defense. *Integr. Comp. Biol.* 57:631–639.

Williamson M, Bingham B, Gray M, Innala L, Viau V. 2010. The Medial Preoptic Nucleus Integrates the Central Influences of Testosterone on the Paraventricular Nucleus of the Hypothalamus and Its Extended Circuitries. *J. Neurosci.* [Internet] 30:11762. Available

from: /pmc/articles/PMC6633408/

Wingfield JC, Lynn SE, Soma KK. 2001. Avoiding the 'Costs' of Testosterone: Ecological Bases of Hormone-Behavior Interactions. *Brain. Behav. Evol.* [Internet] 57:239–251. Available from: https://www.karger.com/Article/FullText/47243

Yu G, Wang L-G, Han Y, He Q-Y. 2012. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omi. A J. Integr. Biol.* 16:284–287.

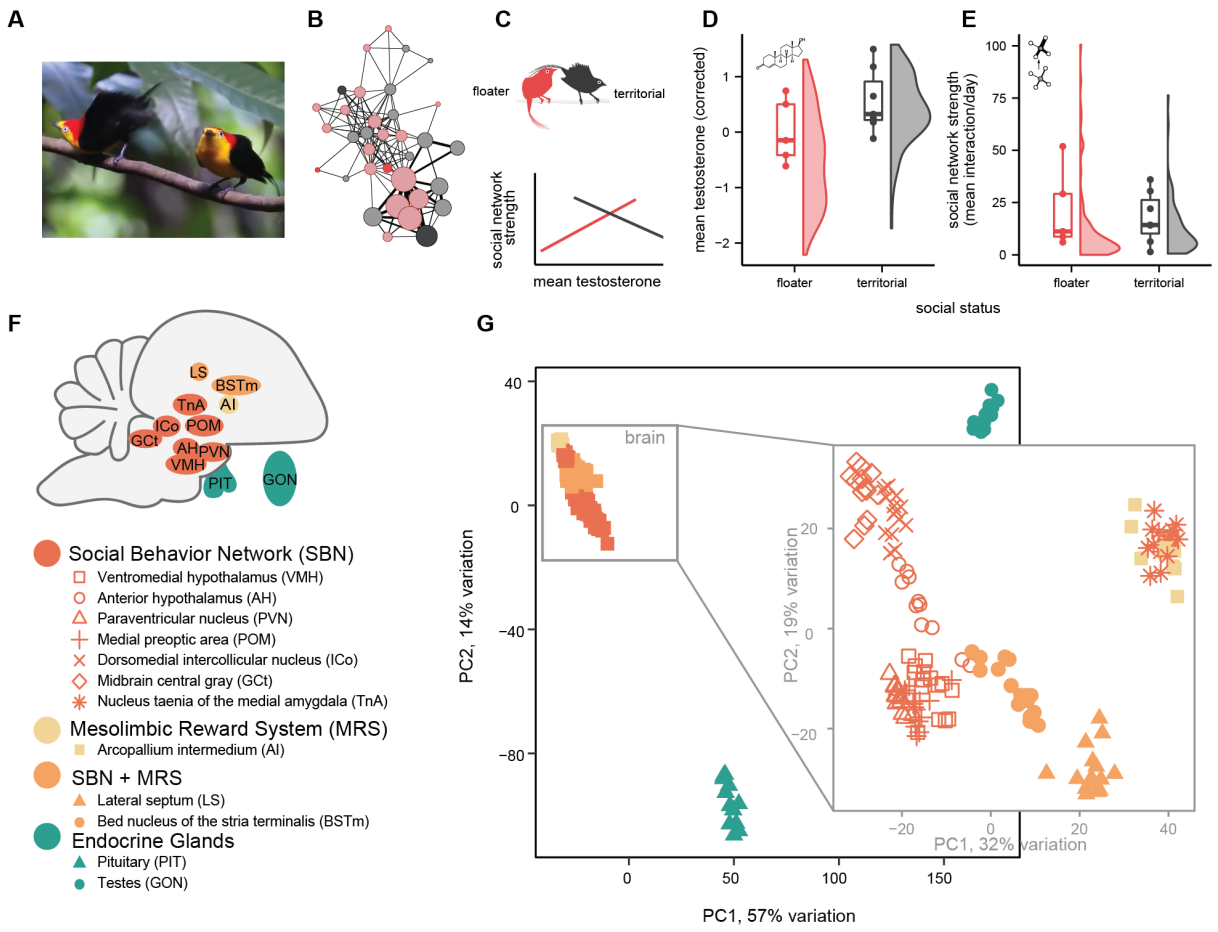

Fig. 1. Circulating testosterone and neuroendocrine tissues are involved in the cooperative behavior of wire-tailed manakins.
A) Males engage in cooperative displays that form the basis of B) complex social networks. The example shown is a single lek over one week, where node size indicates a male's average social network strength (measure of overall time spent interacting), and edge width represents the frequency of interactions between a pair of individuals. Node color denotes floater (red) and territorial (gray) male status, where darker colors were included in the study. C) A male's testosterone phenotype (mean testosterone (corrected)) influences his cooperative tendency differently in territorial and floater classes. D) Twelve individuals (points) were from the larger population with repeated measures of testosterone (density), and E) proximity-data logging measures of social network strength. Note the total sample size for individuals characterized for social status is N=16 is larger than for testosterone or sociality (N=12). F) RNAseq characterized gene expression from each individual in 10 testosterone-sensitive brain regions and two endocrine tissues. Structures shown are not to scale. Colors represent functional groups (O'Connell and Hofmann 2011). G & inset) principal component analysis of transcriptomic data reveals distinct gene expression profiles among brain regions and endocrine tissues (colors and shapes as F). (photo by Alice Boyle).

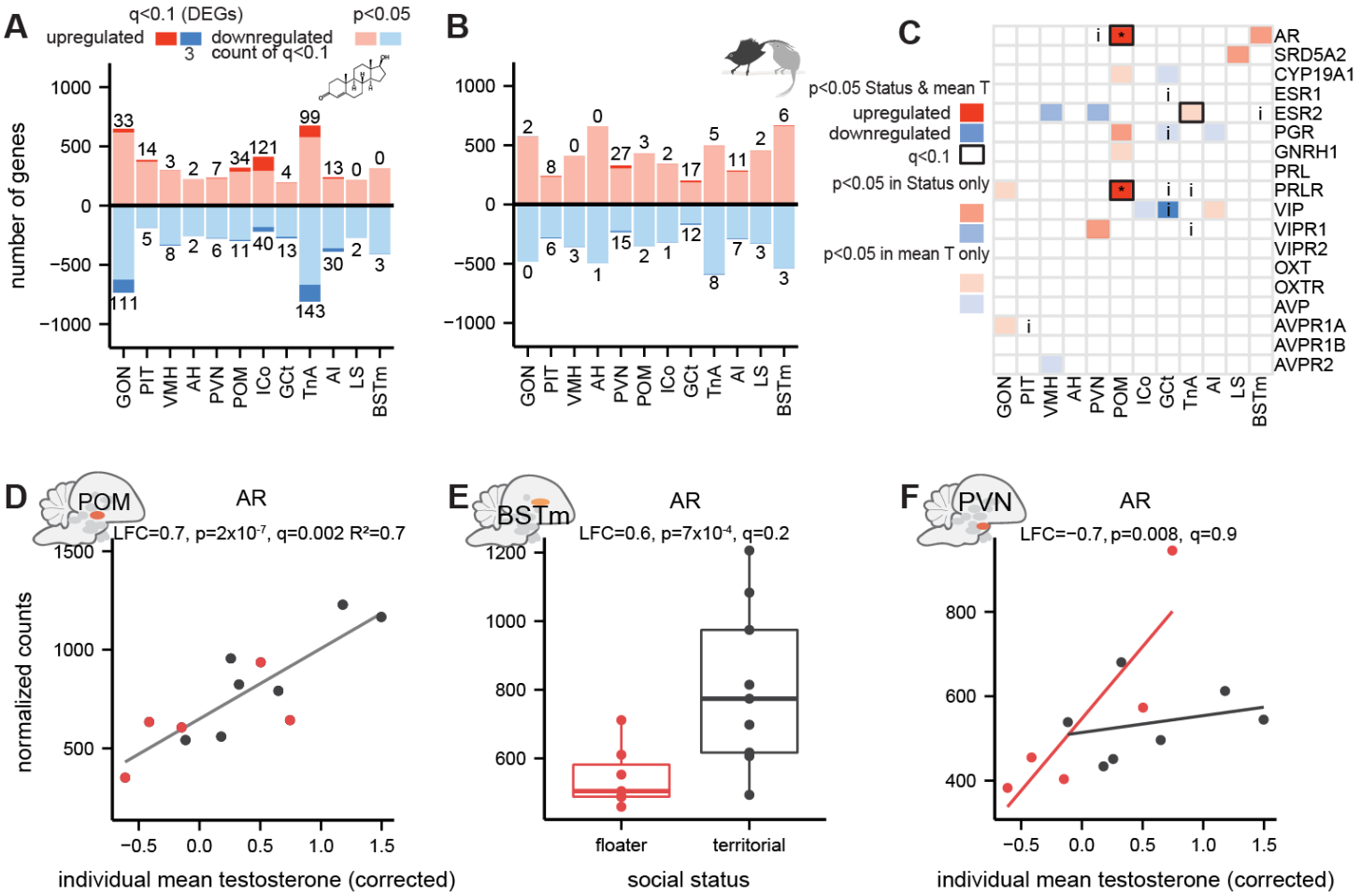

Fig. 2. Neurogenomic landscape of social status and hormone phenotype.
A) There is a landscape of gene expression in male wire-tailed manakins with high testosterone phenotype (mean testosterone (corrected)) relative to lower testosterone phenotype. Red bars indicate number of genes that are upregulated in males with higher testosterone, whereas blue indicates genes that are downregulated in males with higher testosterone. Darker colors and text indicate number of differentially expressed genes after FDR correction. B) The landscape of gene expression in relation to social status, where red indicates genes upregulated in territorial males. C) Summary of candidate gene analysis and filtering, where colors indicate direction of expression (as previous panels), but intensity reflects which of the models were significant, and "i" indicates there was an interaction effect, and a black box indicates result was significant after FDR correction for all genes in that region's transcriptome. D) AR was upregulated in POM of males with higher testosterone. where point colors indicate social status (red = floater; black = territorial. Fitted line from linear regression. Subheader indicates key results from DESeq2: Log_2 Fold Change (LFC), uncorrected p-value (p), and correction for multiple testing (q) and R^2 correlation coefficient calculated separately. E) AR was upregulated in the BSTm of territorial males. F) AR shows status-specific expression in relation to testosterone phenotype in the PVN. Grey area represents the 95% confidence interval as determined by linear regression of gene ~ Status×mean_T. Note that maximum sample size for analysis of social status was N=16, while

only 12 individuals were sampled for testosterone and behavior, but the actual sample size varies slightly in each tissue owing to quality filtering.

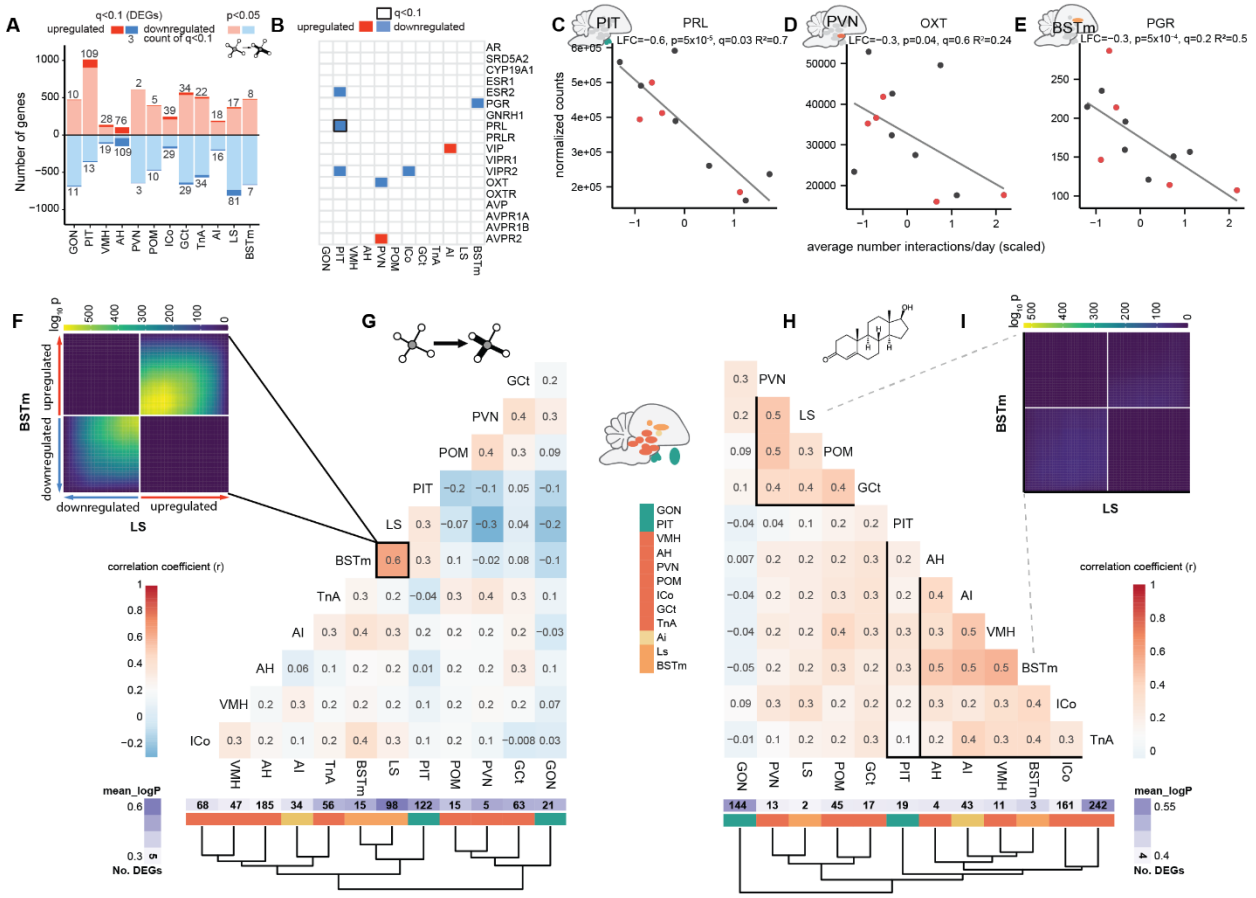

Fig. 3. A distinct landscape of gene expression in relation to cooperative behavior.
A) Landscape of gene expression in male more cooperative wire-tailed manakins (social network strength). Darker colors and text indicate number of genes after FDR correction. Red indicates genes that are upregulated in males with higher social network strength, whereas blue indicates genes that are downregulated in these males. B) Summary of candidate gene analysis and filtering, where colors indicate direction of expression (as previous panels) in relation to individuals with higher social network strength. A black box indicates the result remained significant after FDR correction for all genes in that region's transcriptome. C) Prolactin (PRL) in the pituitary shows decreased expression in males with higher social network strengths. D) Oxytocin (OXT) transcript expression decreases in males with higher social network strength. E) Progesterone Receptor (PGR) has lower transcript expression in males with higher social network strength. F) RRHO2 analysis reveals similar profiles of gene expression associated with cooperation in the LS and BSTm, G) but otherwise generally low similarity of gene expression among tissues in association with cooperation. H) Similarity of gene expression among tissues in relation to testosterone phenotype roughly recapitulates ontogeny of brain regions, I) and there is no correlation between testosterone related gene expression profiles in the LS and BSTm. G,H) Cell color and number are the Pearson correlation coefficients of gene expression between tissues, correlating $\log_{10}$ transformed differential expression p-values (times the direction of

differential expression). Black boxes outline significant tissue correlation clusters based on 1,000 bootstrap resamples of the correlation matrix without FDR correction.

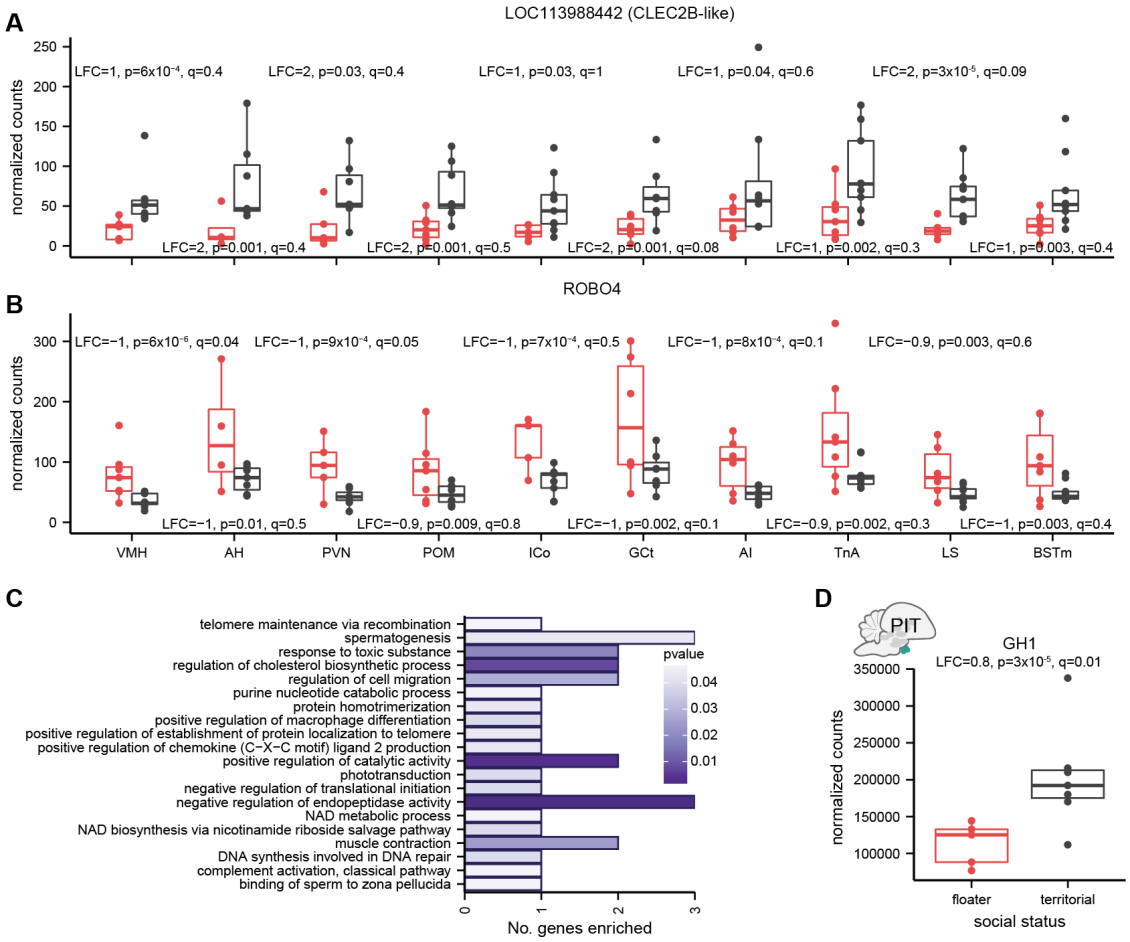

Fig. 4: Global neural gene expression differences between social classes
A) Immune gene CLEC2B-like was consistently upregulated in the brains of territorial individuals, and B) developmental gene ROBO4 was consistently downregulated in the brains of territorial males. C) Enriched GO categories among genes that were consistently up- or downregulated between territorial and floater males, where bar color indicates uncorrected p-value. D) These broader signatures of developmental differences could be explained by upregulation of growth hormone transcript in the pituitary of territorial males.