Social Status Predicts How Sex Steroid Receptors Regulate Complex Behavior across Levels of Biological Organization

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Social status strongly affects behavior and physiology, in part mediated by gonadal hormones, although how each sex steroid acts across levels of biological organization is not well understood. We examine the role of sex steroids in modulating social behavior in dominant (DOM) and subordinate (SUB) males of a highly social fish, *Astatotilapia burtoni*. We first used agonists and antagonists to each sex steroid receptor and found that androgens and progestins modulate courtship behavior only in DOM, whereas estrogens modulate aggressive behavior independent of social status. We then examined the hormonal and physiological responses to sex steroid receptor antagonist treatment and uncovered substantial changes in circulating steroid hormone levels and gonad size only in SUB, not in DOM. Consistent with status-based physiological sensitivities to drug manipulation, we found that neuropeptide and steroid receptor gene expression in the preoptic area was sensitive only in SUB. However, when we compared the transcriptomes of males that received either vehicle or an estrogen receptor antagonist, 8.25% of all genes examined changed expression in DOM in comparison with only 0.56% in SUB. Finally, we integrate behavior, physiology, and brain gene expression to infer functional modules that underlie steroid receptor regulation of behavior. Our work suggests that environmentally induced changes at one level of biological organization do not simply affect changes of similar magnitude at other levels, but that instead very few key pathways likely serve as conduits for executing plastic responses across multiple levels. **(*Endocrinology* 153: 1341–1351, 2012)**

Behavior and physiology are profoundly affected by an individual's social status. To function in a social group, every group member integrates information about its own internal physiological state with external social information into a behavioral output that ultimately promotes its fitness. Although behavioral and hormonal responses within social groups are relatively well understood in mammals and fish (1–5), researchers have only recently begun to examine the molecular events in the brain that mediate the behavioral responses of individuals in different social states (6, 7). Despite the importance of integrating external and internal signals within the brain into subsequent behavioral changes, studies investigating this integration are limited due to the general difficulty of incorporating multiple levels of analysis such as environment, physiology, and molecular responses in the nervous system. Thus, our present understanding of how physiology, brain gene expression, and social environment regulate behavior in social groups is still limited.

Sex steroid hormones are excellent candidates for mediating the integration of external and internal information into an adaptive behavioral response. These hormones vary across many physiological contexts such as sex, social status, breeding condition, and season (6–10). Such variability is important in regulating an animal's behavioral response to both reproductive and aggressive contexts (11). Circulating hormones robustly respond to social stimulation and have been extensively studied in the

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Abbreviations: AR, Androgen receptor; AVT, arginine vasotocin; CA, cyproterone acetate; DHT, dihydrotestosterone; DOM, dominant; ER, estrogen receptor; gbw, gram body weight; GEE, generalized estimating equations; GSI, gonadosomatic index; IST, isotocin; 11KT, 11-ketotestosterone; 17α-20β-P, 17α-20β-dihydroprogesterone; POA, preoptic area; PR, progesterone receptor; qPCR, quantitative PCR; SUB, subordinate.

context of the androgen challenge response to aggressive interactions, a physiological response conserved across vertebrates (11, 12). Sex steroids integrate these social and physiological cues into a molecular response by acting through either nuclear hormone receptors, which function as transcription factors and modulate gene expression (reviewed in Ref. 13), or more quickly through membrane-bound receptors that trigger a signal transduction cascade (14, 15).

To study the proximate mechanisms of male-typical behavior in a social hierarchy, we use the African cichlid fish, *Astatotilapia burtoni*, a model system in social neuroscience and behavioral genomics (16, 17). *A. burtoni* males have two plastic behavioral phenotypes. Brightly colored dominant (DOM) males aggressively defend territories where they court and spawn with females. Subordinate (SUB) males are dull in coloration, reproductively suppressed, and school with females. The brain gene expression profiles of these two phenotypes differ substantially (18). Given the opportunity, SUB will attain DOM status in an astonishing transition that is accompanied by a concerted change in gene expression in brain and testes as well as a rapid response in circulating steroid hormones (6). However, we know relatively little about how sex steroid hormone receptors mediate behavior in relation to social status and across levels of biological organization (including, *e.g.* behavior, physiology, and brain gene expression).

We examined the differential role of sex steroid hormones in the regulation of behavior in these two social phenotypes with the hypotheses that 1) steroid hormone receptors mediate distinct behavioral components and 2) the gene regulatory actions of sex steroid hormone receptors differ with social status. We first determined how androgen receptors (AR), estrogen receptors (ER), and the progesterone receptor (PR) modulate social behavior and circulating hormone levels in DOM and SUB within a community setting. Additionally, we examined steroid receptor regulation of gene expression in the preoptic area (POA), a brain region that regulates male aggressive and sexual behaviors in all vertebrates (19, 20).

Materials and Methods

Animals

A. burtoni from a wild-caught stock population were maintained in a naturalistic community as previously described (21) with eight males and eight females per 110-liter tank. A total of 34 DOM (length, 4.877 ± 0.1264 cm; weight, 3.341 ± 0.2851 g) and 32 SUB (length, 4.615 ± 0.0851 cm; weight, 2.486 ± 0.1289 g) were used in this study. All work was carried out in compliance with the Institutional Animal Care and Use Committee at The University of Texas at Austin.

Behavior and pharmacology

Male *A. burtoni* chosen for this study were stable in social status for 1 wk before injections. Only one animal per tank was manipulated at a given time. Animals were injected ip with 10 μl mineral oil per gram body weight (gbw) for 2 d (to allow for within-individual comparisons) and then with a sex steroid receptor agonist or antagonist for the next 2 d. Fish were immediately placed back into the home tank after injection. One group of males used as the control group for all end-point analyses were injected with vehicle for all 4 d. Five-minute focal observations were conducted between 0800 and 1100 h the following morning of each day after injections and for 2 d after injections ended (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org) by an observer blind to the treatment. This observation paradigm avoided nongenomic fast-acting effects of steroid hormones (14). Social behaviors recorded are described elsewhere (22) and include aggressive (chasing and biting), sexual (quivering and leading), territorial (border dispute, carousel, and threats), and subordinate (fleeing) displays.

We chose the following doses based on a dose-response experiment in DOM (Supplemental Fig. 2) where three doses of each drug were tested to see which had the largest effect on aggressive and/or courtship behavior: 0.4 μg/gbw 17β-estradiol (Steraloids, Newport, RI; n = 8 SUB and n = 10 DOM), 0.13 μg/gbw dihydrotestosterone (DHT; Sigma Chemical Co., St. Louis, MO; n = 8 per social status), 0.125 μg/gbw 17α-20β-dihydroprogesterone (17α-20β-P; Steraloids; n = 8 per social status), 1.6 μg/gbw ICI182780 (ER antagonist; Sigma; n = 8 per social status), 0.83 μg/gbw cyproterone acetate (AR antagonist; Sigma; n = 8 per social status), and 1.6 μg/gbw ZK0112993 (PR antagonist; Bayer Schering Pharma AG, Berlin, Germany; n = 8 per social status). Dose-response curves were not conducted in SUB, because social suppression by DOM males may mask any effect of a drug, and isolating SUB for testing will result in a transition to DOM status within hours, a period too short to conduct a dose-response curve manipulating nuclear hormone receptors. DHT was used, rather than the teleost-specific 11-ketotestosterone (11KT), because DHT binds AR with higher affinity than 11KT (23, 24). All antagonists have been shown to bind their respective teleost sex steroid receptors [ICI182780 binds all three ER (25); cyproterone acetate binds both AR (23); and ZK0112993 binds PR (26)].

On the last day of behavioral observations, we recorded weight and length of each individual that received an antagonist or only vehicle and drew blood from the dorsal aorta using heparinized 26-gauge butterfly infusion sets (Becton Dickinson, Mountain View, CA). Gonads were removed and weighed for calculation of the gonadosomatic index (GSI: testes mass divided by body mass times 100). Brains were rapidly dissected, embedded in Tissue-Tek OCT Compound (Sakura Finetek U.S.A., Torrance, CA), fresh frozen on dry ice, and stored at −80 C.

Hormone assays

Free testosterone, 17β-estradiol, and progesterone were measured for each individual using ELISA (Enzo Life Sciences, Farmingdale, NY); Intraassay variation was 9.43, 2.67, and 4.06%,

respectively; interassay variation was 4.96, 3.38, and 10.5%, respectively. Plasma samples were diluted 1:30 and processed as previously described (27).

Laser microdissection and RNA isolation

Brains were sectioned at 18 μm, thaw mounted onto membrane-covered slides (P.A.L.M. Microlaser Technologies AG, Bernried, Germany), and hydrated for 1 min in cold 95, 70, and 50% ethanol, stained with toluidine blue (0.5% toluidine blue, 1% phenol, 20% ethanol in water) for 2 min, rapidly dehydrated in an ascending ethanol series, incubated in xylene for 5 min, and air dried for 2 min. Sections were visualized on a laser-microdissection microscope (P.A.L.M. Microlaser Technologies), and an area corresponding to the POA (including parvocellular, magnocellular, and some gigantocellular cells) (28) was excised and captured with the laser (Supplemental Fig. 3). Some technical variation likely resulted from the capture of the gigantocellular POA due to the sporadic distribution of these neurons. Thirty microliters of Trizol (Invitrogen, Carlsbad, CA) was added to the sample and stored at −80 C until further processing. Total RNA was isolated with Trizol according to the manufacturer's instructions.

RNA amplification, reverse transcription, and quantitative PCR (qPCR)

Isolated RNA was subjected to one round of amplification using the MessageAmp II system (Ambion, Austin, TX) according to manufacturer's instructions. Additionally, samples from animals that received either vehicle only or ER antagonist treatment were divided after the first round of amplification, with one half being immediately reverse transcribed and the other half processed for another round of RNA amplification to be used for microarray analysis. Each RNA sample was treated with deoxyribonuclease I (Ambion) according to the manufacturer's instructions. The RNA was reverse transcribed using Superscript III reverse transcriptase (Invitrogen) and gene-specific primers (see Supplemental Table 1). Positive controls used 1 ng of whole-brain RNA in place of RNA derived from laser-microdissected samples and in negative controls the reverse transcriptase was omitted. Excess primers and salts from the transcription reaction were removed in Microcon YM30 columns (Millipore, Bedford, MA). An aliquot of the sample was used for Ribogreen (Invitrogen) analysis to determine total cDNA concentration of each sample. qPCR primers (Supplemental Table 1) were designed to flank exon boundaries using the zebrafish genome as a reference. For each sample, target gene abundance was measured in triplicate in an ABI PRISM 7900HT real-time PCR cycler (ABI SDS version 2.2.1 software) using SYBR Green (Invitrogen). Standard curves were constructed using known dilutions of cDNA, and amplification efficiency was calculated. For each individual, median values from the reference and target gene triplicates were used to calculate the relative transcript abundance of the target gene using the mean normalized expression formula of Simon (29). Each sample was normalized to total cDNA as measured by Ribogreen.

Transcriptome profiling

Microarray samples were prepared as previously described (18) and hybridized in a loop design (Supplemental Fig. 4) to a 19K *A. burtoni* microarray (GEO platform GPL6416) constructed from brain-specific and mixed tissue libraries representing a total of 17,712 cichlid-specific features (30, 69). DOM and SUB males were examined in separate loops, and each array was competitively hybridized with a vehicle- or ER antagonist-treated sample. Two technical replicates were performed, where each sample was labeled with both Cy3 and Cy5 to control for dye bias. The slides were scanned on an Axon 4000B array scanner using GenePix version 6.0 software (Molecular Devices, Sunnyvale, CA) and filtered for microarray printing errors, hybridization artifacts, and signals with low intensity. Intensities were lowess-normalized within arrays in R/Bioconductor; only features with average intensities 2 SD or more above average background were considered for further analysis (18). All raw and processed data are available at the GEO database (accession number GSE28508).

Data analysis

All analyses, with the exception of the microarray data, were conducted in PASW (IBM, Somers, NY). To determine differences between DOM and SUB, *t* tests were used with vehicle-treated animals. In all other cases, statistics for DOM and SUB were run separately. For behavioral data, a generalized estimating equations (GEE) (31) model was used with the behavioral measure as the dependent variable, and baseline behavior (d 1 and 2) was compared with drug treatment (d 4–6) as a within-subject variable; the model was run separately for each drug treatment. Hormone measurements and qPCR data were not normally distributed and were log-transformed, resulting in normally distributed residuals. Hormone data were analyzed with ANOVA using hormone as the dependent variable and drug as the independent variable followed by a Bonferroni *post hoc* test. qPCR gene expression data from the experimental groups were compared with the control gene expression levels using ANOVA followed by Dunnett's *t* test, which corrects for multiple testing. Significance was considered as $P < 0.05$. Because most of our data were nonparametric, we calculated correlations using Spearman rank correlation coefficients to explore covariation patterns. The microarray data were analyzed in R/Bioconductor. Between-group analyses were conducted using the LIMMA package (18, 32).

Results

Distinct roles of sex steroids in a social hierarchy

We used a within-subject design by treating DOM and SUB with specific sex steroid receptor agonists or antagonists (Supplemental Fig. 1) and found that sex steroid receptors differentially modulate aggression and courtship in the two phenotypes (Fig. 1). Although teleost fish have a single ERα and PR, there are two isoforms of AR (ARα and ARβ) and ERβ (ERβa and ERβb) due to a genome duplication event in this lineage (33). We selected drugs that targeted all three ER isoforms or both AR isoforms.

Manipulation of ER changed aggressive behavior, but not courtship behavior, independent of social phenotype. DOM males display two kinds of aggression: chases are

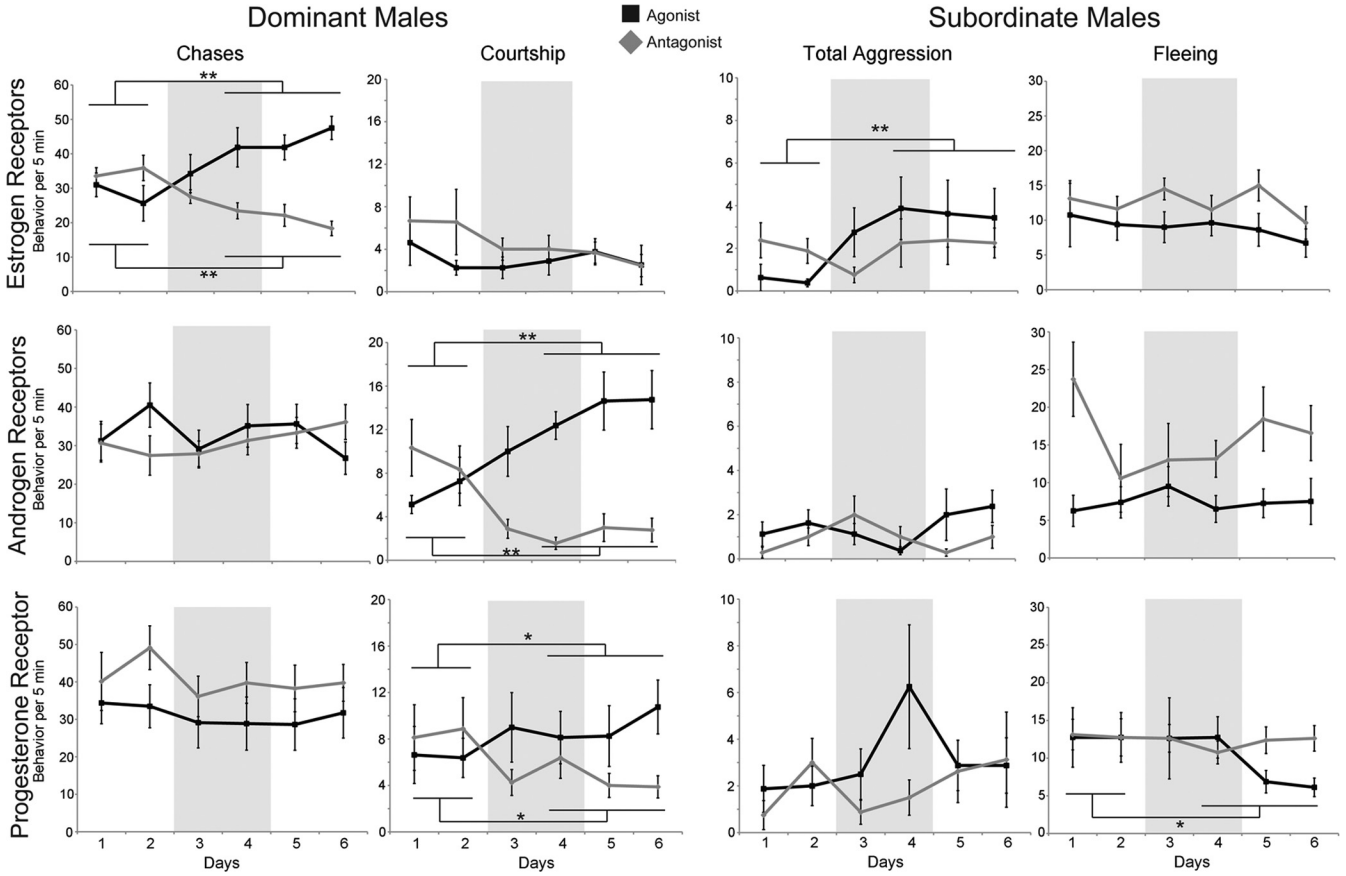

FIG. 1. Effects of sex steroid receptor manipulation vary by social status. The mean behavioral observations of animals given ER (*top row*), AR (*middle row*), or PR (*bottom row*) manipulations are shown with agonist (*black lines*) and antagonist (*gray lines*) treatments for DOM and SUB males (n = 8 per status per treatment). Behavior per 5 min is represented on the *vertical axis*, and day of treatment is on the *horizontal axis*. *Gray background shading* indicates days of drug administration. Data are represented as the mean ± SEM; GEE: **, $P < 0.0001$; *, $P < 0.05$.

typically directed toward SUB and females in the school, whereas territorial defense behavior is typically directed toward other DOM in the community. Estradiol treatment increased chases in DOM (GEE, $P = 9.3 \times 10^{-9}$; Wald $\chi^2 = 32.984$), whereas treatment with the ER-specific antagonist ICI182780 decreased chases (GEE, $P = 1.4 \times 10^{-6}$; Wald $\chi^2 = 23.284$). Estradiol treatment also decreased territorial defense behavior (GEE, $P = 0.045$; Wald $\chi^2 = 4.036$; data not shown), although ICI182780 treatment had no effect (GEE, $P = 0.283$; Wald $\chi^2 = 1.151$; data not shown). ER manipulations in DOM did not affect courtship displays (GEE: estradiol, $P = 0.864$; Wald $\chi^2 = 0.03$; ICI182780, $P = 0.06$; Wald $\chi^2 = 3.539$), although there is a nonsignificant trend for the antagonist to decrease courtship displays. Aggressive displays (a sum of chases and bites) were also increased in SUB treated with estradiol (GEE, $P = 1.8 \times 10^{-4}$; Wald $\chi^2 = 13.993$), which was unexpected given the severe social suppression SUB are subjected to by DOM. Not surprisingly, treatment of SUB with an ER antagonist did not result in a behavioral change (GEE, $P = 0.8$; Wald $\chi^2 = 0.064$), possibly due to a floor effect, because SUB rarely display aggressive behavior even under control conditions. ER manipulation did not affect fleeing behavior in SUB (GEE: estradiol, $P = 0.24$; Wald $\chi^2 = 1.382$; ICI182780, $P = 0.86$; Wald $\chi^2 = 0.033$). Courtship and territorial defense behavior are almost never observed in SUB and thus are not reported.

AR manipulations exclusively affected courtship behavior and had no effect on aggressive behavior in either social phenotype. Instead of the teleost-specific androgen 11KT, we used the nonaromatizable androgen DHT for pharmacological manipulation of AR, because both 11KT and DHT produce similar effects in other teleosts (34, 35), yet DHT has a higher binding affinity to both ARα and ARβ (23, 24). DOM treated with DHT increased courtship displays (GEE, $P < 0.0002$; Wald $\chi^2 = 13.794$), whereas treatment with an AR antagonist, cyproterone acetate (CA), decreased courtship behavior (GEE, $P = 8.5 \times 10^{-6}$; Wald $\chi^2 = 19.823$). Manipulation of AR did not alter chases (GEE: DHT, $P = 0.22$; Wald $\chi^2 = 1.502$; CA, $P = 0.20$; Wald $\chi^2 = 1.624$) or territorial defense behavior (GEE: DHT, $P = 0.20$; Wald $\chi^2 = 1.653$; CA, $P = 0.09$; Wald $\chi^2 = 2.890$; data not shown) in DOM. Similarly,

no behavioral change in total aggression (GEE: DHT, $P = 0.55$; Wald $\chi^2 = 0.360$; CA, $P = 0.55$; Wald $\chi^2 = 0.355$) or fleeing (GEE: DHT, $P = 0.81$; Wald $\chi^2 = 0.058$; CA, $P = 0.75$; Wald $\chi^2 = 0.100$) was observed in SUB.

PR manipulations showed effects similar to those of AR, as we observed changes in courtship behavior displayed by DOM. We used 17α-20β-P as a PR agonist because it cannot be converted readily into testosterone and has higher binding affinity to the teleost PR than progesterone (24). DOM treated with 17α-20β-P increased courtship displays (GEE, $P = 0.040$; Wald $\chi^2 = 4.233$), whereas the PR antagonist ZK112993 decreased courtship displays (GEE, $P = 0.041$; Wald $\chi^2 = 4.168$). PR manipulation in DOM did not affect chasing (GEE: 17α-20β-P, $P = 0.148$; Wald $\chi^2 = 2.091$; ZK112993, $P = 0.053$; Wald $\chi^2 = 3.751$) or territorial defense behavior (GEE: 17α-20β-P, $P = 0.08$; Wald $\chi^2 = 3.100$; ZK112993, $P = 0.752$; Wald $\chi^2 = 0.100$; data not shown). Similar to DOM, total aggression in SUB did not change with PR manipulation (GEE: 17α-20β-P, $P = 0.14$; Wald $\chi^2 = 2.173$; ZK112993, $P = 0.68$; Wald $\chi^2 = 0.172$). Interestingly, SUB treated with 17α-20β-P displayed less fleeing behavior (GEE, $P = 0.001$; Wald $\chi^2 = 11.195$), but their behavior did not change after treatment with the PR antagonist (GEE, $P = 0.52$; Wald $\chi^2 = 0.409$).

Dissociation of behavior from hormones and physiology

To investigate whether our manipulations of sex steroid receptor signaling affected circulating levels of sex steroid hormones in relation to social status, we measured free circulating 17β-estradiol, testosterone, and progesterone in the plasma of animals that received a receptor antagonist or vehicle only (Fig. 2); individuals that received an agonist were not used for hormone measurements or gene expression studies. As expected, control DOM had higher circulating testosterone ($t_{11} = 3.7$; $P = 0.005$) and 17β-estradiol ($t_{12} = 3.8$; $P = 0.003$) levels than control SUB as previously reported (36). Progesterone levels did not differ between social states, although there was a nonsignificant trend to higher levels in DOM ($t_{11} = 2.053$; $P = 0.065$). Surprisingly, we found that in DOM treated with a receptor antagonist, circulating levels did not change for any of the sex steroids compared with controls [ANOVA: 17β-estradiol, $F_{(3,34)} = 1.796$, $P = 0.169$; testosterone, $F_{(3,32)} = 0.289$, $P = 0.83$; progesterone, $F_{(3,33)} = 0.289$, $P = 0.436$], despite the clear behavioral responses we observed to receptor antagonist manipulation. Even more striking, SUB displayed distinct changes in circulating hormone levels with treatment of receptor antagonists [ANOVA: 17β-estradiol, $F_{(3,30)} = 7.106$, $P = 4.23 \times 10^{-4}$; testosterone, $F_{(3,30)} = 7.106$, $P = 0.001$; progesterone, $F_{(3,30)} = 3.471$, $P = 0.03$]. Specifically, SUB treated with ER antagonists had lower levels of 17β-estradiol (Dunnett's t test $P = 0.017$) and testosterone (Dunnett's t test $P = 0.032$) compared with controls. Thus, on a physiological level, SUB responded even though they showed little behavioral response to steroid receptor manipulations within a social community.

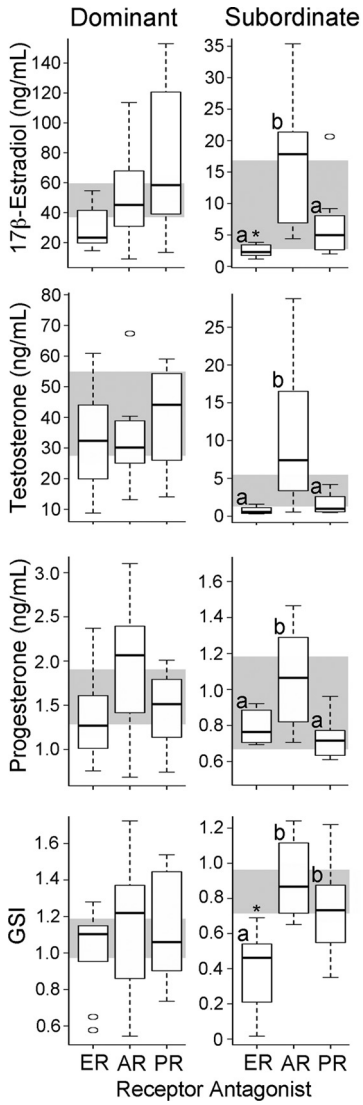

FIG. 2. Physiology and circulating hormone levels change in SUB but not DOM males after steroid receptor manipulation. Free 17β-estradiol, testosterone, and progesterone levels, as well as GSI, are depicted by *box and whisker plots* for DOM (*left panels*) and SUB (*right panels*) males (n = 8 per status per treatment). Each *box* represents groups receiving a sex steroid receptor antagonist (x-axis), and the *gray background bar* depicts the first and third quartiles for control animals. *Letters* indicate significance between groups; *, significant difference from control with Bonferroni *post hoc* test.

The GSI, a measure of relative testes mass, was higher in control DOM compared with control SUB ($t_{14} = 2.3$; $P = 0.039$). Steroid receptor antagonists did not affect the GSI of DOM [$F_{(3,34)} = 0.231$; $P = 0.853$], yet in SUB, ER antagonist treatment resulted in a significant reduction in GSI [$F_{(3,32)} =$

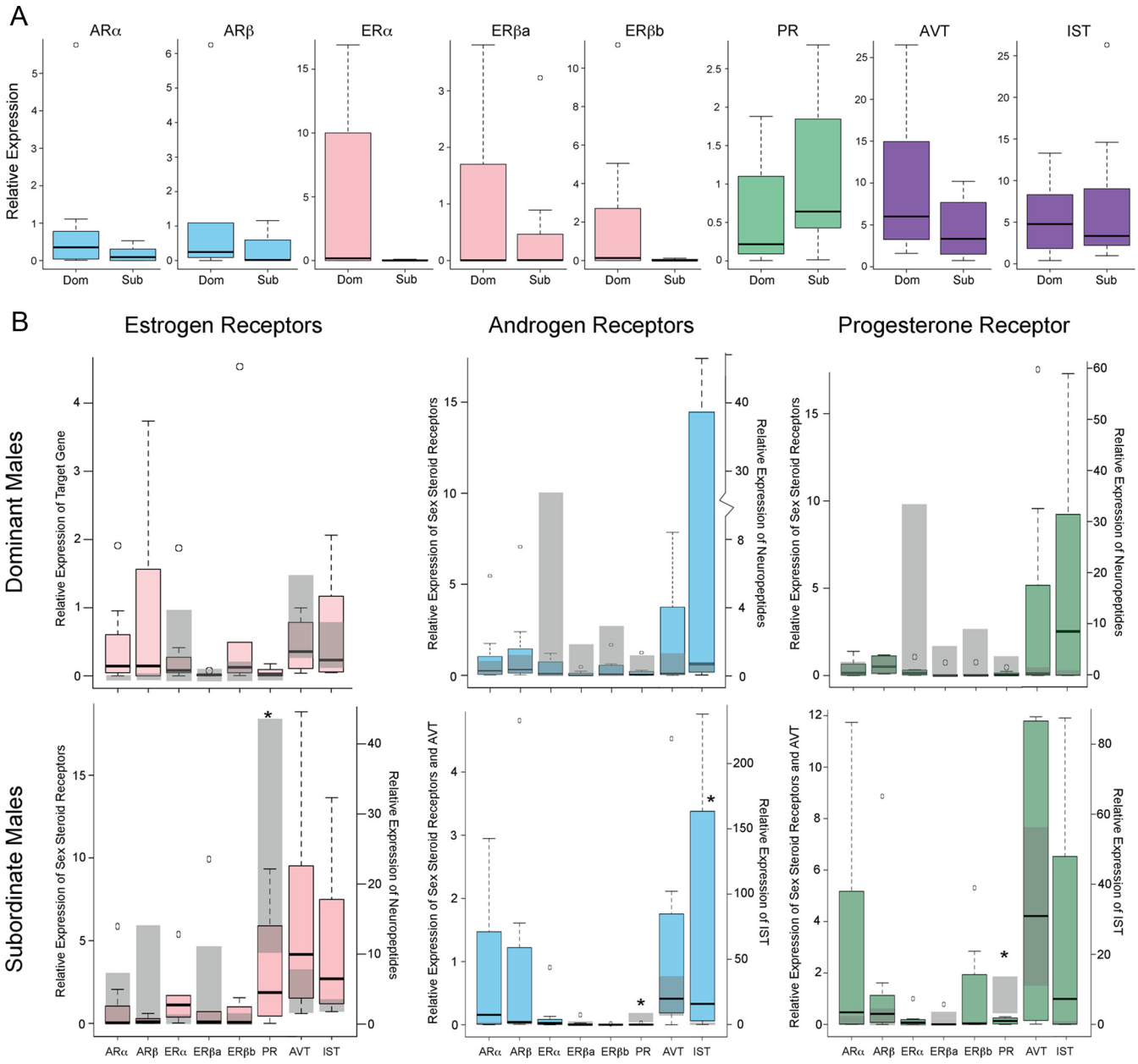

FIG. 3. Expression of candidate genes in the POA. A, Expression levels of steroid hormone receptors and neuropeptides in control treated DOM and SUB males are shown as *box and whisker plots*. B, Changes in gene expression after treatment of an ER (*left panel*), AR (*middle panel*), or PR (*right panel*) antagonist in DOM (*top row*) and SUB (*bottom row*) males is shown with *colored box and whisker plots*. *Gray background bars* depict the first and third quartiles for control animals. *, $P < 0.05$, Dunnett's *t* test.

8.521; $P = 0.0004$; Bonferroni *post hoc* test: $P < 0.033$; mean ± SEM: ICI182780 0.391 ± 0.080; control 0.872 ± 0.066], a remarkable physiological change in such a short time.

Sex steroid receptor-mediated gene expression impinges on social status

To better understand how sex steroid receptors regulate social behavior in a hierarchy, we used qPCR to analyze the expression of these receptors and neuropeptides in the POA of males that received either a sex steroid receptor antagonist or vehicle (Fig. 3). We measured mRNA levels of all sex steroid receptors as well as the nonapeptides arginine vasotocin (AVT) and isotocin (IST), because all these neuroendocrine pathways play important roles in modulating social behavior across vertebrates (reviewed in Ref. 37).

To our surprise, control and experimental groups of DOM did not differ in the expression of any of these candidate genes despite the robust changes in social behavior we had observed, whereas in SUB, mRNA levels of several candidate genes differed significantly between control and experimental groups. These results

are consistent with our findings described above of an apparent dissociation, at least in DOM, of the behavioral from the physiological responses to sex steroid antagonist treatment. However, it should be noted that microdissection of the POA did not include all gigantocellular neurons and thus may have masked potential differences (see also Ref. 34). In SUB, PR and IST levels were sensitive to antagonist treatment [PR: ANOVA, $F_{(3,28)} = 5.321$ $P = 0.006$; IST: ANOVA: $F_{(3,28)} = 3.820$, $P = 0.022$]. Specifically, SUB given an ER, AR, or PR antagonist decreased expression of PR (Dunnett's *t* test: ER antagonist $P = 0.038$; AR antagonist $P = 0.005$; PR antagonist $P = 0.006$), whereas expression of IST increased after exposure to the AR antagonist (Dunnett's *t* test $P = 0.02$).

ER regulation of the social transcriptome

Because perturbation of ER exhibited consistent and significant effects on aggression in both DOM and SUB, we then asked to which extent the POA gene network regulated by ER differed between social phenotypes. To investigate the molecular consequences of ER perturbation on a genomic scale, we compared the POA transcriptomes of the individuals from the behavioral trials above after administration of either ER antagonist or vehicle. In DOM, 12,676 (71.6%) of 17,712 array features with cichlid sequences yielded above background intensities, whereas in SUB, there were only 8,132 (45.9%) such features, indicating that the POA expresses about 56% more genes in DOM compared with SUB ($\chi^2 = 2405.002$; $P < 0.001$). This difference is not a technical artifact, because normalized intensity distributions (as a measure of dynamic range in gene expression) did not differ substantially between the DOM and SUB experiments (Supplemental Fig. 5A). We then compared (separately for DOM and SUB) the POA transcription profiles of ER antagonist-treated animals with those of vehicle controls, although none of the differences survived false-discovery correction for $P < 0.05$ in either social phenotype, which is not surprising given such a subtle perturbation. However, when we examined the *P* value distributions for DOM and SUB, we discovered that small *P* values were considerably overrepresented in the DOM dataset (Supplemental Fig. 5B), indicating widespread gene regulation. We chose $P < 0.05$ as an acceptable significance threshold, because our aim was to compare broad-scale patterns of gene regulation across phenotypes. Unexpectedly, despite the almost complete absence of physiological or candidate gene expression responses to ER antagonist treatment in DOM males, 1,047 (8.25%) of the 12,676 array features that provided signal above background were differentially regulated between control *vs.* ER antagonist (LIMMA, $P < 0.05$). An even more striking picture emerged in SUB, where the *P* value distribution was surprisingly devoid of small values (Supplemental Fig. 5B), suggesting a genome-wide suppression of expression variation in this phenotype. At a $P < 0.05$ threshold, only 48 (0.59%) of the features were differentially regulated between the control and ER antagonist groups (LIMMA, $P < 0.05$), even though SUB demonstrated significant changes in physiological and candidate gene expression measures, as described above. Finally, the POA transcriptome responses to ER perturbation showed very little overlap between DOM and SUB, because only four genes were regulated at $P < 0.05$ by ER in both datasets (Supplemental Table 2). These sequences represent novel genes for which no annotations could be found in the databases.

Integration of genes, physiology, and behavior

To examine the relationship between behavior, hormone levels, gonadal state, and gene expression in DOM and SUB, we used the network analysis platform Cytoscape (38) to create association networks based on Spearman correlation coefficients (Supplemental Tables 3 and 4) between measures of behavior, physiology, hormones, and POA candidate gene expression of neuroendocrine genes determined by qPCR in DOM (n = 32) and SUB (n = 32). As can be seen in Fig. 4, only four significant correlations are shared between DOM and SUB males: ERα and ERβa are highly correlated, as are AVT and IST, 17β-estradiol levels and GSI, and 17β-estradiol and progesterone levels.

This analysis enabled us to propose network modules that may contribute to specific aspects of *A. burtoni* sociality, based on what is known about behavior, physiology, and life history of this species. In DOM, border disputes and threat displays are directed only toward other DOM (39). Our results suggest that these behavior patterns are tightly regulated by ERβa and ERβb signaling and together form a territory defense module. The most striking differences between DOM and SUB were the interactions of hormones and physiology with behavior and expression of sex steroid receptors in the POA. In DOM, testosterone, in association with chasing and courtship, represents a community interaction module, because these behavior patterns are directed exclusively at the community of females and SUB. However, in SUB, testosterone is associated with the other steroid hormones and GSI as well as AR subtype expression, suggesting that circulating steroid hormones are reflective of gonadal state. Finally, we found strong

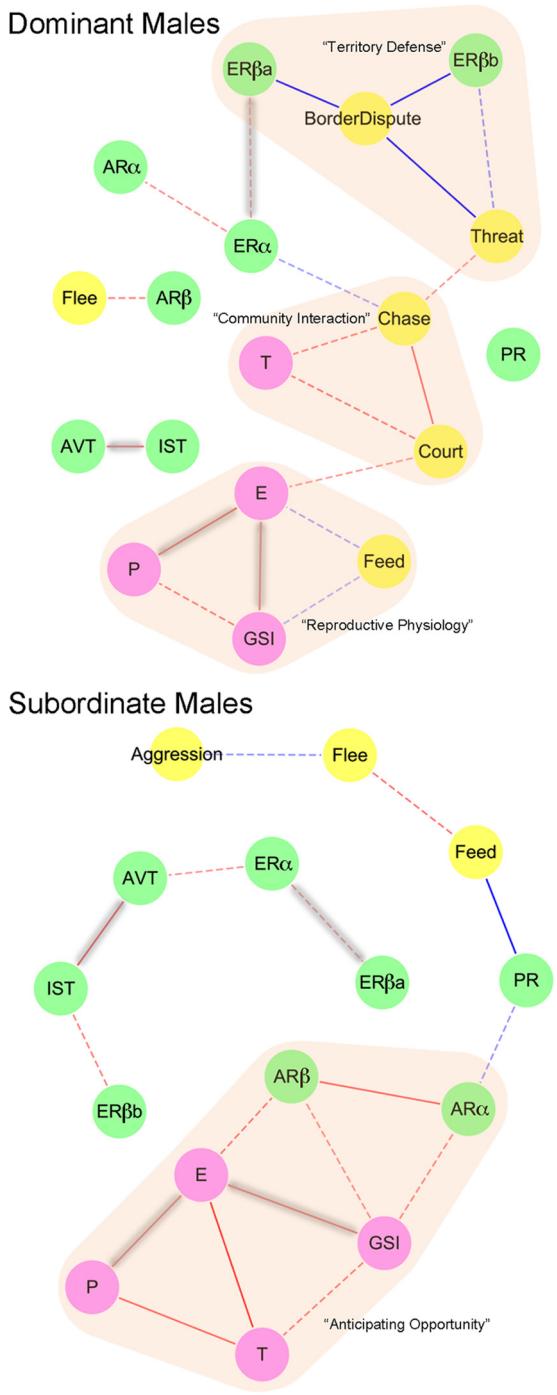

FIG. 4. Integrating across levels of biological organization: behavior, physiology, hormones, and gene expression. A covariance network is represented in DOM (*top*) and SUB (*bottom*) males. Edges represent significant positive (*red*) or negative (*blue*) correlations between behavior (*yellow*), POA gene expression (*green*), and physiology (*purple*). Edges *highlighted in gray* are common across social states, and *dashed lines* did not survive false discovery rate correction. Functional modules are *highlighted in orange*. T, Testosterone; E, estradiol; P, progesterone.

correlations between 17β-estradiol, progesterone, GSI, and feeding in DOM, which could be indicative of a module governing reproductive physiology.

Discussion

Distinct roles for steroid hormone pathways in social behavior

Androgens and estrogens have been intensively studied for decades in the context of sexual behavior and aggression because they dynamically influence behavior (40–42). Within teleosts, the role of androgens in regulating sexual behavior appears to be consistent across species (43, 44). Of particular relevance to our study is recent work in mammals by Juntti *et al.* (45), who showed that mice with a conditional neuronal AR knockout displayed deficits in sexual behavior, yet the number of aggressive displays did not differ compared with wild-type mice. Research in other nonmammalian tetrapods also suggests that AR is important in male-typical sexual behavior, including amphibians (46), birds (47), and reptiles (48). The conversion of testosterone to estrogen by aromatase is necessary for male aggression in mammals and birds (49, 50) as well as for reproductive behavior in some species (51). Male ERα knockout mice also display deficits in sexual behavior (52), although this may be due to organizational effects of estrogen in the developing male brain (53).

Estrogenic and androgenic modulation of behavior varies based on environmental cues (9, 54). However, in *A. burtoni* males, which breed year-round, variation in AR and ER modulation of behavior likely arises from social rather than seasonal cues. We have shown here that testosterone modulates sexual behavior in DOM and that estrogens regulate aggression independent of social status. Although we cannot rule out the conversion of DHT to 3β-diol (55), we are confident that the androgenic regulation of sexual behavior is AR dependent, because the effects of the AR antagonist were opposite to those of DHT. We obtained these results using males that were well established in their respective social states and by treating them with an agonist or antagonist for several days. However, when SUB are provided with an opportunity to ascend in social status, aggressive behavior and androgens rise within 30 min of transition, although estrogens do not increase for several days (7). Androgens may play a short-term role in modulating aggressive behavior during social instability (see also Refs. 8 and 11), whereas estrogens may maintain aggressive motivation over the long term if and when the social environment has stabilized. These different temporal roles of androgens and estrogens may also be a consequence of the relatively slow up-regulation of brain aromatase by AR (56). Interestingly, we found that treatment of SUB with DHT did not change aggressive behavior, although different results may have been obtained if SUB were treated with 11KT rather than DHT. Furthermore, the dose used to treat SUB was determined based on

a dose-response curve conducted in DOM, and therefore an alternate dose may also have produced a different behavioral response. Nonetheless, our results suggest that to ascend in social status, SUB need both an androgen surge as well as a social opportunity, such as a vacant territory and/or the removal of suppression by DOM males.

The role of progesterone in the adult male brain is less understood than the actions of androgens and estrogens, although several studies indicate that PR is important in male-typical sexual behavior in mammals (57, 58) and reptiles (59). Our results also show that PR facilitates courtship behavior in *A. burtoni* DOM. In SUB, however, treatment with a PR antagonist unexpectedly decreased fleeing behavior, which might suggest that the perception or evaluation of social (*i.e.* threatening) cues is modulated by PR. Work in humans has shown that progesterone, along with cortisol, increases in avoidance situations that signify fear of rejection (60). Furthermore, treatment with 17α-20β-P has antianxiety effects in rodents (61). It is thus possible that PR regulates social cognition in a manner that is conserved across vertebrates, although more studies across a wide range of taxa will be needed to test this hypothesis. The work in humans also points to the importance of the stress axis in social status. Although we did not measure cortisol levels or glucocorticoid receptor expression, this pathway does indeed play a pivotal role in *A. burtoni* behavior (62).

Integrating brain gene expression with behavior and physiology

We have used a covariation network analysis to organize data across levels of biological organization and propose functional modules that allow us to generate new hypotheses about the neuroendocrine and molecular basis of social behavior. Here we discuss correlations between behavior, physiology, and gene expression in the POA, a brain region highly conserved in function across vertebrates (20), although these patterns may well differ when other brain regions are analyzed. Our results suggest distinct modules that may govern different aspects of behavior within a social group. DOM display behavior patterns toward other DOM that are closely associated with expression of ER, which is supported by our pharmacological results where ER manipulations altered these territory defense behaviors. Interactions with other community members, including females and SUB, appear to involve a different module that is dominated by androgens. We hypothesize that this is due to the dynamic response of androgens to social cues (6, 11, 12) that may then modulate courtship or chasing behavior. SUB are quite different, in that their hormones are tightly linked to gonadal state and AR expression, which may enable them to quickly seize social opportunities to ascend to DOM status, as previous work has shown a rapid increase in circulating androgens in response to an opportunity to become territorial (6). Additionally, several interactions are shared by DOM and SUB (*e.g.* AVT and IST or ERα and ERβa), suggesting that some genes are coregulated in a similar manner independent of social status. Finally, although this kind of network analysis is clearly useful for integrating data across levels of biological organization to visualize large-scale biological patterns, it is important to emphasize that correlation of nodes does not necessarily imply causation and that it will be necessary to experimentally manipulate the proposed modules to fully characterize their function in regulating behavior in a complex social environment.

We found several interesting patterns with steroid receptor antagonist treatment and downstream gene expression in the POA, particularly the suppression of IST by AR in SUB. Neuropeptides of the oxytocin family have drawn a lot of attention in the mammalian literature by regulating trust (63) and affiliative behavior (64). In *A. burtoni*, SUB school (*i.e.* affiliate) with other SUB and females, yet when given the opportunity to ascend to dominance (a relatively solitary station), schooling behavior is suppressed, possibly via down-regulation of the IST pathway. This idea is consistent with a study in goldfish that demonstrated a role for IST in regulating social approach behavior (65).

Another interesting relationship is that of androgens, estrogens, and expression of their receptors in the POA, which seem to play very different roles depending on an individual's position in the social hierarchy. SUB have lower expression of ER compared with DOM, and the lack of a transcriptome response in SUB compared with DOM follows this pattern. Inhibition of ER mechanisms in SUB after administration of an ER antagonist, in combination with low circulating testosterone levels, and the resulting absence of brain AR activation, appears to lead to a remarkable genome-wide suppression of both transcriptional activity and variation in the POA.

Searching for patterns across levels of biological organization

In an era when molecular tools have become available for nontraditional model systems to explore the underpinnings of complex behavioral phenotypes, few researchers have analyzed a particular phenotype across many levels of biological organization. Our results show for the first time how in individuals of the same species, biological responses to a perturbation can differ across levels of biological organization as well as between phenotypes. We are not aware of any other study to date that has integrated behavior, hormones, physiology, candidate gene expression, and transcriptome profiling within the same individ-

uals, yet a complete understanding of the mechanisms underlying behavioral variation across individuals might require the kind of integration across multiple levels of biological organization we have attempted here. Furthermore, given that mRNA and protein need not always be concordant (66), it will be important to include analyses of the proteome and small RNAs in future studies as well.

Our results show that phenotypic changes at one level of biological organization do not predict changes of comparable magnitude at another level, although it is important to note that gene expression may be more reflective of behavior than circulating hormone levels. Our expectation at the beginning of this study was that a behavioral response to sex steroid receptor manipulations would be accompanied by corresponding changes in circulating hormone levels, candidate gene expression, and transcriptome activity, regardless of social status. However, we found marked differences in DOM and SUB responses at various levels. These discrepancies could, in part, be explained by differences in gene expression of sex steroid receptors in some brain regions (67) or in the testes (68), by effects of the community structure on the behavioral expressions of DOM and SUB males, or most likely a combination of all these factors.

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References

1. **Newman ML, Sellers JG, Josephs RA** 2005 Testosterone, cognition, and social status. Horm Behav 47:205–211
2. **Parikh VN, Clement TS, Fernald RD** 2006 Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*. Behav Brain Res 166:291–295
3. **Burmeister SS, Jarvis ED, Fernald RD** 2005 Rapid behavioral and genomic responses to social opportunity. PLoS Biol 3:e363
4. **Greenwood AK, Wark AR, Fernald RD, Hofmann HA** 2008 Expression of arginine vasotocin in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. Proc Biol Sci 275:2393–2402
5. **Gesquiere LR, Learn NH, Simao MC, Onyango PO, Alberts SC, Altmann J** 2011 Life at the top: rank and stress in wild male baboons. Science 333:357–360
6. **Maruska KP, Fernald RD** 2010 Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. Horm Behav 58:230–240
7. **Oliveira RF, Hirschenhauser K, Carneiro LA, Canario AV** 2002 Social modulation of androgen levels in male teleost fish. Comp Biochem Physiol B Biochem Mol Biol 132:203–215
8. **Trainor BC, Rowland MR, Nelson RJ** 2007 Photoperiod affects estrogen receptor α, estrogen receptor β and aggressive behavior. Eur J Neurosci 26:207–218
9. **Goymann W** 2009 Social modulation of androgens in male birds. Gen Comp Endocrinol 163:149–157
10. **Wingfield JC, Hegner RE, Dufty AM, Jr., Ball GF** 1990 The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 136: 829–846
11. **Hirschenhauser K, Oliveira RF** 2006 Social modulation of androgens in male vertebrates: meta-analyses of the "challenge hypothesis." Anim Behav 71:265–277
12. **Gleason ED, Fuxjager MJ, Oyegbile TO, Marler CA** 2009 Testosterone release and social context: when it occurs and why. Front Neuroendocrinol 30:460–469
13. **Wahli W, Martinez E** 1991 Superfamily of steroid nuclear receptors: positive and negative regulators of gene expression. FASEB J 5:2243–2249
14. **Remage-Healey L, Bass AH** 2006 A rapid neuromodulatory role for steroid hormones in the control of reproductive behavior. Brain Res 1126:27–35
15. **Mani SK, Portillo W, Reyna A** 2009 Steroid hormone action in the brain: cross-talk between signalling pathways. J Neuroendocrinol 21:243–247
16. **Hofmann HA** 2003 Functional genomics of neural and behavioral plasticity. J Neurobiol 54:272–282
17. **Robinson GE, Fernald RD, Clayton DF** 2008 Genes and social behavior. Science 322:896–900
18. **Renn SC, Aubin-Horth N, Hofmann HA** 2008 Fish and chips: functional genomics of social plasticity in an African cichlid fish. J Exp Biol 211:3041–3056
19. **Newman SW** 1999 The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. Ann NY Acad Sci 877:242–257
20. **O'Connell LA, Hofmann HA** 2011 The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. J Comp Neurol 519:3599–3639
21. **Munchrath LA, Hofmann HA** 2010 Distribution of sex steroid hormone receptors in the brain of an African cichlid fish, *Astatotilapia burtoni*. J Comp Neurol 518:3302–3326
22. **Fernald, RD** 1977 Quantitative behavioural observations of Haplochromis burtoni under semi-natural conditions. Anim Behav 25: 643–653
23. **Wells K, and Van Der Kraak G** 2000 Differential binding of endogenous steroids and chemicals to androgen receptors in rainbow trout and goldfish. Environ Toxicol Chem 19:2059–2065
24. **Sperry TS, Thomas P** 1999 Characterization of two nuclear androgen receptors in Atlantic croaker: comparison of their biochemical properties and binding specificities. Endocrinology 140:1602–1611
25. **Hawkins MB, Thomas P** 2004 The unusual binding properties of the

third distinct teleost estrogen receptor subtype ERβa are accompanied by highly conserved amino acid changes in the ligand binding domain. Endocrinology 145:2968–2977
26. **Pinter J, Thomas P** 1997 The ovarian progestogen receptor in the spotted seatrout, *Cynoscion nebulosus*, demonstrates steroid specificity different from progesterone receptors in other vertebrates. J Steroid Biochem 60:113–119
27. **Kidd CE, Kidd MR, Hofmann HA** 2010 Measuring multiple hormones from a single water sample using enzyme immunoassays. Gen Comp Endocrinol 165:277–285
28. **Braford Jr MR, Northcutt RG** 1983 Organization of the diencephalon and pretectum of ray-finned fishes. In: David RG, Northcutt RG, eds. Fish neurobiology. Vol 2. Ann Arbor, MI: University of Michigan Press; 117–163
29. **Simon P** 2003 Q-Gene: processing quantitative real-time RT-PCR data. Bioinformatics 19:1439–1440
30. **Salzburger W, Renn SC, Steinke D, Braasch I, Hofmann HA, Meyer A** 2008 Annotation of expressed sequence tags for the East African cichlid fish *Astatotilapia burtoni* and evolutionary analyses of cichlid ORFs. BMC Genomics 9:96
31. **Liang K, Zeger SL** 1986 Longitudinal data analysis using generalized linear models. Biometrika 73:13–22
32. **Smyth GK** 2005 Limma: Linear models for microarray data. In: Gentleman R, Carey V, Dudoit S, Irizarry R, Huber W, eds. Bioinformatics and computational biology solutions using R and Bioconductor. New York, NY: Springe; 397–420
33. **Hoegg S, Brinkmann H, Taylor JS, Meyer A** 2004 Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. J Mol Evol 59:190–203
34. **Zakon HH, Dunlap KD** 1999 Sex steroids and communication signals in electric fish: a tale of two species. Brain Behav Evol 54:61–69
35. **Margiotta-Casaluci L, Sumpter JP** 2011 5α-Dihydrotestosterone is a potent androgen in the fathead minnow (*Pimephales promelas*). Gen Comp Endocrinol 171:309–318
36. **Maruska KP, Fernald RD** 2010 Steroid receptor expression in the fish inner ear varies with sex, social status, and reproductive state. BMC Neurosci 11:58
37. **Goodson JL, Thompson RR** 2010 Nonapeptide mechanisms of social cognition, behavior and species-specific social systems. Curr Opin Neurobiol 20:784–794
38. **Kohl M, Wiese S, Warscheid B** 2011 Cytoscape: software for visualization and analysis of biological networks. Methods Mol Biol 696:291–303
39. **Baerends GP, Baerends-van Roon JM** 1950 An introduction to the study of the ethology of cichlid fishes. Behavior Suppl 1:1–242
40. **Beach FA** 1948 Hormones and behavior. New York: Paul B. Hoeber
41. **Lehrman DS** 1965 Interaction between internal and external environments in the regulation of the reproductive cycle of the ring dove. Sex and Behavior. Beach FA, ed. New York: Wiley; 355–380
42. **Ball GF, Balthazart J** 2004 Hormonal regulation of brain circuits mediating male sexual behavior in birds. Physiol Behav 83:329–346
43. **Salek SJ, Sullivan CV, Godwin J** 2001 Courtship behavior of male white perch, *Morone americana*: evidence for control by androgens. Comp Biochem Physiol A Mol Integr Physiol 130:731–740
44. **Sebire M, Allen Y, Bersuder P, Katsiadaki I** 2008 The model antiandrogen flutamide suppresses the expression of typical male stickleback reproductive behaviour. Aquat Toxicol 90:37–47
45. **Juntti SA, Tollkuhn J, Wu MV, Fraser EJ, Soderborg T, Tan S, Honda S, Harada N, Shah NM** 2010 The androgen receptor governs the execution, but not programming, of male sexual and territorial behaviors. Neuron 66:260–272
46. **Behrends T, Urbatzka R, Krackow S, Elepfandt A, Kloas W** 2010 Mate calling behavior of male South African clawed frogs (*Xenopus laevis*) is suppressed by the antiandrogenic endocrine disrupting compound flutamide. Gen Comp Endocrinol 168:269–274
47. **Fusani L, Day LB, Canoine V, Reinemann D, Hernandez E, Schlinger BA** 2007 Androgen and the elaborate courtship behavior of a tropical lekking bird. Horm Behav 51:62–68
48. **Tokarz RR** 1986 Hormonal regulation of male reproductive behavior in the lizard *Anolis sagrei*: a test of the aromatization hypothesis. Horm Behav 20:364–377
49. **Schlinger BA, Callard GV** 1990 Aromatization mediates aggressive behavior in quail. Gen Comp Endocrinol 79:39–53
50. **Trainor BC, Kyomen HH, Marler CA** 2006 Estrogenic encounters: how interactions between aromatase and the environment modulate aggression. Front Neuroendocrinol 27:170–179
51. **Adkins-Regan E, Garcia M** 1986 Effect of flutamide (an antiandrogen) and diethylstilbestrol on the reproductive behavior of Japanese quail. Physiol Behav 36:419–425
52. **Wersinger SR, Sannen K, Villalba C, Lubahn DB, Rissman EF, De Vries GJ** 1997 Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor alpha gene. Horm Behav 32:176–183
53. **Kudwa AE, Michopoulos V, Gatewood JD, Rissman EF** 2006 Roles of estrogen receptors alpha and beta in differentiation of mouse sexual behavior. Neuroscience 138:921–928
54. **Wingfield JC, Lynn S, Soma KK** 2001 Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. Brain Behav Evol 57:239–251
55. **Mouriec K, Gueguen MM, Manuel C, Percevault F, Thieulant ML, Pakdel F, Kah O** 2009 Androgens upregulate cyp19a1b (aromatase B) gene expression in the brain of zebrafish (*Danio rerio*) through estrogen receptors. Biol Reprod 80:889–896
56. **Abdelgadir SE, Resko JA, Ojeda SR, Lephart ED, McPhaul MJ, Roselli CE** 1994 Androgens regulate aromatase cytochrome P450 messenger ribonucleic acid in rat brain. Endocrinology 135:395–401
57. **Witt DM, Young LJ, Crews D** 1994 Progesterone and sexual behavior in males. Psychoneuroendocrinology 19:553–562
58. **Phelps SM, Lydon JP, O'malley BW, Crews D** 1998 Regulation of male sexual behavior by progesterone receptor, sexual experience, and androgen. Horm Behav 34:294–302
59. **Lindzey J, Crews D** 1988 Effects of progestins on sexual behaviour in castrated lizards (*Cnemidophorus inornatus*). J Endocrinol 119:265–273
60. **Wirth MM, Schultheiss OC** 2006 Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol. Horm Behav 50:786–795
61. **Picazo O, Fernández-Guasti A** 1995 Anti-anxiety effects of progesterone and some of its reduced metabolites: an evaluation using the burying behavior test. Brain Res 680:135–141
62. **Fox HE, White SA, Kao MH, Fernald RD** 1997 Stress and dominance in a social fish. J Neurosci 17:6463–6469
63. **Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E** 2005 Oxytocin increases trust in humans. Nature 435:673–676
64. **Insel TR, Shapiro LE** 1992 Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci USA 89:5981–5985
65. **Thompson RR, Walton JC** 2004 Peptide effects on social behavior: effects of vasotocin and isotocin on social approach behavior in male goldfish (*Carassius auratus*). Behav Neurosci 118:620–626
66. **Vogel C, Abreu Rde S, Ko D, Le SY, Shapiro BA, Burns SC, Sandhu D, Boutz DR, Marcotte EM, Penalva LO** 2010 Sequence signatures and mRNA concentration can explain two-thirds of protein abundance variation in a human cell line. Mol Syst Biol 6:400
67. **Burmeister SS, Kailasanath V, Fernald RD** 2007 Social dominance regulates androgen and estrogen receptor gene expression. Horm Behav 51:164–170
68. **Maruska KP, Fernald RD** 2011 Plasticity of the reproductive axis caused by social status change in an african cichlid fish. II. Testicular gene expression and spermatogenesis. Endocrinology 152:291–302
69. **Renn SCP, Aubin-Horth N, Hofmann HA** 2004 Biologically meaningful expression profiling across species using heterologous hybridization to a cDNA microarray. BMC Genomics 5, 42