Social dominance regulates androgen and estrogen receptor gene expression

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Abstract

In Astatotilapia burtoni, dominant males have higher levels of sex steroid hormones than subordinate males. Because of the complex regulatory interactions between steroid hormones and receptors, we asked whether dominance is also associated with variation in sex steroid receptor gene expression. Using quantitative PCR, we compared the expression of specific subtypes of androgen (AR) and estrogen (ER) receptor genes between dominant and subordinated males in 3 divisions of the brain, the pituitary, and the testes. We measured mRNA levels of AR-α, AR-β, ER-α, ER-βα, and ER-ββ, gonadotropin-releasing hormone 1 (GnRH1), and GnRH receptor 1 (GnRH-R1) relative to 18S rRNA. In the anterior part of the brain, we found that dominant males had higher mRNA expression of AR-α, AR-β, ER-βα, and ER-ββ, but not ER-α, compared to subordinate males. This effect of dominance was reflected in a positive correlation between testes size and AR-α, AR-β, ER-βα, and ER-ββ in the anterior brain. In addition, mRNA levels of all ARs and ERs in the anterior brain were positively correlated with mRNA level of GnRH1. In the middle and posterior portions of the brain, as well as the testes, steroid receptor mRNA levels were similar among dominants and subordinates. In the pituitary, ER-α mRNA level was positively correlated with testes size and AR-α mRNA was positively correlated with GnRH-R1 mRNA level. These data suggest that dominant male brains could be more sensitive to sex steroids, which may contribute to the increased complexity of the behavioral repertoires of dominant males.

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In the cichlid fish Astatotilapia (Haplochromis) burtoni, social status regulates male fertility by controlling the brain–pituitary–gonad (BPG) axis. Consequently, dominant males have 40% larger testes relative to body size compared with subordinate males. Ultimately, dominance affects testes size through regulation of gonadotropin-releasing hormone 1 (GnRH1) production by neurons in the anterior parvocellular preoptic nucleus (aPPn) of the hypothalamus (Davis and Fernald, 1990; Francis et al., 1993; White et al., 2002). The enlarged testes of dominant males contain a higher proportion of mature sperm (Fraley and Fernald, 1982), and produce higher levels of testicular hormones, including 11-ketotestosterone and testosterone (Parikh et al., 2006). The higher levels of circulating androgens in dominant males have behavioral (Francis et al., 1992) and physiological (Soma et al., 1996) consequences. To more fully understand the potential influence of differences in sex steroid hormone levels, it is important to understand whether and how sex steroid receptors might also be regulated by social status. For example, if the higher androgen levels of dominant males are accompanied by lower expression of steroid receptors in the brain due to negative feedback regulation, higher androgens might not have behavioral consequences for the dominant males. If, on the other hand, increased levels of androgens are accompanied by similar or higher levels of steroid receptor expression, the tissue might be more sensitive to steroid levels. Because effects of androgens are sometimes mediated by estrogen receptors following aromatization, we were interested in both androgen and estrogen receptor expression. Thus, we asked whether dominant and subordinate males differ in the expression of androgen (AR) or estrogen (ER) receptor genes at multiple points of the BPG axis.

Among teleosts, two androgen receptors (AR-α, AR-β) and three estrogen receptors (ER-α, ER-βα, ER-ββ) of the regulatory transcription factor type have been identified. This elaboration of the number of steroid receptor gene paralogs, like in other gene families, was the result of an early genome duplication in
the lineage leading to teleosts (Hoegg et al., 2004). The three ERs have distinct pharmacological characteristics (Hawkins and Thomas, 2004), and they have different tissue distributions (Choi and Habibi, 2003; Halm et al., 2004; Pinto et al., 2006). All ERs are expressed in the brain, pituitary, and gonad (Choi and Habibi, 2003) and in the female brain all three ERs are expressed in the hypothalamus, in overlapping but distinct patterns (Hawkins et al., 2000; Menuet et al., 2002), suggesting that each receptor type has a role in some aspects of neuroendocrine function. Direct comparison of neural expression levels of the three ERs outside of the hypothalamus has not been reported. Less is known about the functional attributes or tissue distribution of the two androgen receptors of teleosts. Like the ERs, the two ARs show distinct binding profiles (Sperry and Thomas, 2000) and their expression varies with seasonal changes in gonadal growth (Sperry and Thomas, 1999). Less is known about the tissue distribution of the AR subtypes. More generally, little is known about how these receptors are regulated by reproductive capacity in males.

To address this question, we compared the relative levels of gene expression for these receptor subtypes in the brain, pituitary, and testes of dominant and subordinate males using quantitative polymerase chain reaction (PCR). In addition, we determined whether variation in steroid receptor gene expression corresponded to different measures of reproductive capacity in the brain (GnRH1 expression), the pituitary (GnRH receptor 1 expression), and the testes (testes size relative to body size). Although three forms of GnRH are expressed in A. burtoni, we did not investigate GnRH2 or GnRH3 because their expression does not vary with reproductive capacity in A. burtoni (White et al., 2006). Likewise, although there are two receptors for GnRH, we focused on GnRH-R1 because it is expressed in gonadotropes (Chen and Fernald, 2006), and its expression is regulated by reproductive capacity (Au et al., 2006).

Methods

All work was performed in accord with accepted standards of humane animal care and was approved by the Stanford University Administrative Panel on Laboratory Animal Care committee.

Identification of ER-αa and ER-βb cDNA sequence

Although A. burtoni-specific sequences for AR-α, AR-β, and ER-α cDNA were available through GenBank (see Table 1), at the start of the study sequence for A. burtoni ER-αa and ER-βb were unknown. To identify the cDNA sequence for these transcripts, we used a combination of PCR and library screening as follows. To identify ER-βa cDNA sequence, we used PCR followed by a phage library screen. To prepare the cDNA for PCR, we extracted total RNA from estriol-primed female liver using Trizol (Invitrogen, Carlsbad CA) and synthesized cDNA using an anchored poly-T primer and Superscript II reverse transcriptase (Invitrogen). To amplify ER-βa cDNA, we designed the following primers based on the sequence of Nile tilapia ER Type II: forward 5′-GCC CAT CTG TAT CCG CTC AC-3′ and reverse 5′-ATC ATG ACG CCT TCT GTC GTC-3′. We amplified an 800 base pair fragment on a thermal cycler (PCR Express, Thermo Electron, Waltham, MA) using the following program: 1 min at 94°C followed by 5 cycles of 30 s at 94°C, 30 s at 60°C, 90 s at 72°C, followed by 10 cycles of 30 s at 94°C, 30 s at 60–51°C (decreasing 1 degree per cycle), 90 s at 72°C, followed by 20 cycles of 30 s at 94°C, 30 s at 50°C, 90 s at 72°C, and a final step of 5 min at 72°C. We gel extracted the amplicon and reamplified it before cloning and sequencing. We identified the fragment as ER-βa based on sequence similarity to known ER-βa sequences using BLAST.

To obtain a full-length ER-βa clone, we screened an A. burtoni brain cDNA phage library (Stratagene, La Jolla CA) using a 174-bp probe corresponding to nucleotides 906–1079 of the full length clone. Duplicate membranes were incubated with the 32P-deoxy CTP-labeled probe produced by random priming (DECAprime II, Ambion, Austin, TX) overnight at 65°C in hybridization buffer (30% formamide, 0.6 M NaCl, 0.04 M sodium phosphate, 2.5 mM EDTA, 1% SDS). For the primary screen, we washed the membranes twice at room temperature and twice at 55°C with 2× SSC containing 0.1% SDS and exposed the membranes to film to identify potential clones. These potential clones were excised and screened a second time as above, yielding three positive full-length cDNA clones (GenBank accession no. DQ862128).

To isolate a subclone of ER-βb, we designed the following degenerate primers to regions that differentiate ER-βa and ER-βb in other teleosts: forward 5′-GGG GAT GCA GAC GGT C-3′, reverse 5′-GAC CTG AAG AAT CCN TA-3′. RNA extraction, cDNA synthesis, and PCR reaction assembly were carried out as described above for the cloning of ER-βa. To amplify the 1410-bp fragment, we used the following PCR cycling program: 1 min at 94°C, followed by 5 cycles of 30 s at 94°C, 30 s at 60°C, 90 s at 72°C, followed by 10 cycles of 30 s at 94°C, 90 s at 60–50°C (decreasing 1 degree per cycle), 90 s at 72°C, followed by 20 cycles of 30 s at 94°C, 30 s at 50°C, 90 s at 72°C, and final elongation for 5 min at 72°C. The PCR product was sequenced and determined to have high similarity to ER-βb sequences. We then used rapid amplification of cDNA ends (SmartRACE, Clontech, Palo Alto, CA) with the A burtoni gene-specific primer 5′-GTA AGG GTT GGA CTG TCT CGG TG-3′ to isolate the 3′ end of the cDNA (GenBank accession no. DQ862129).

Animals and tissue collection

Adult male and female A. burtoni were housed together in aquaria maintained under conditions mimicking those of their natural habitat (12:12 L: D, full spectrum lights, pH 8.0, 26°C; Fernald and Hira, 1977). We used focal observations to determine the dominance status of individual males over a greater than 3-week period as follows. We observed each male in a tank every 1–3 days for a 3-min period to determine whether a male was dominant or subordinate. We categorized a male as dominant if he defended a territory and

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank accession no.</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Amplicon length</th>
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<tr>
<td>AR-αa</td>
<td>AF121257</td>
<td>CGC TGT ATC TGG TAC GGT AG</td>
<td>TGA GGA ATC GCA CTT GG</td>
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<td>AR-β</td>
<td>AY082342</td>
<td>TTC GGC GAC AAG TAC TC</td>
<td>ACT GTT CAC GGC GCA TTA</td>
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<td>ER-αa</td>
<td>AY422089</td>
<td>CGG TTT CCC AGA GAC GAC CAG</td>
<td>CTC GCC CAA GCC GTA T</td>
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<td>ACA AGA AGG TTT GCC GTGTC C</td>
<td>GCC GTG CTC CTA GTA TTC A</td>
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<tr>
<td>ER-βb</td>
<td>DQ862129</td>
<td>AAT CTT AAG GAG CGG AAG G</td>
<td>CTA GCG CAG ATG ACG AT</td>
<td>51</td>
</tr>
<tr>
<td>GnRH1</td>
<td>U31865</td>
<td>CAG ACA CAC TGG GCA ATA TG</td>
<td>GCC CAC ACT CGG AAG A</td>
<td>128</td>
</tr>
<tr>
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<td>TCA GTA CAG CGG CGAAAG</td>
<td>GCA TCT AGG GGC ATG AGT</td>
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<tr>
<td>18S</td>
<td>U67333</td>
<td>CCC TTC AAA CCC TCT TAC CC</td>
<td>CCA CGG CTA AGA GTC GTA TT</td>
<td>82</td>
</tr>
</tbody>
</table>
displayed dominant coloration (eyebear and body coloration). We categorized a male as subordinate if he did not defend a territory and did not display dominant coloration. Only males whose status was consistent and unambiguous for at least 3 weeks were included in the study. This period of time is sufficient to identify males whose status-related physiological characteristics (e.g., GnRH1 neuron size) are stable (White et al., 2002). Using the above criteria, we chose three subordinate and three dominant males from three different community tanks (total N=18), and we accounted for potential variation due to community tank in our statistical analysis (see Statistics). We weighed subjects, killed them by rapid decapitation, and then collected their brains, pituitaries, and testes. Testis weight was used to determine gonadosomatic index (GSI; ratio of testes mass to body mass multiplied by 100). Using a scalpel, we divided the brain into anterior, middle, and posterior parts by transversely sectioning the tissue at the anterior commissure and between the optic tectum and cerebellum. As a result, the anterior portion contained the entire telencephalon and a portion of the aPpN. The middle portion contained the diencephalon, including the remainder of the aPpN, the pretectum, and the midbrain; the posterior portion contained the hindbrain. Post facto we determined that the anterior portion of the brain included part, but not all, of the aPpN because both the anterior and middle portions show GnRH expression (see Results) and GnRH1-expressing neurons are located in the most anterior part of the aPpN (Davis and Fernald, 1990; Fernald and Shelton, 1985).

Following dissection, tissue was immersed in Trizol, frozen in an ethanol–dry ice bath, and stored at −80°C until RNA extraction. To extract RNA, we homogenized the tissue and followed the Trizol protocol. Because of the large number of samples (total=90), we extracted RNA in multiple groups organized by tissue and tank (e.g., we extracted RNA from all the testes of males from tank 1 simultaneously). We accounted for possible variation introduced by RNA extraction group in our statistical analysis (see Statistics). RNA integrity and concentration was estimated from spectrophotometric absorbance at 260 nm and 280 nm. We treated the RNA with DNase (Turbo DNA-free, Ambion, Austin TX) to remove contaminating genomic DNA before synthesizing cDNA using random hexamer primers and Transcriptor reverse transcription (Roche Applied Science, Indianapolis IN).

**Quantitative PCR**

We used quantitative PCR to measure mRNA expression of two ARs, three ERs, and the reference transcript 18S in all 5 tissues. In addition, we measured mRNA expression of GnRH1 in the anterior and middle portions of the brain as well as GnRH-R1 mRNA from the pituitary. We designed primers (Table 1) using a strategy described in Greenwood et al. (2003) that maximizes reaction efficiency. For the real-time PCR reaction, we used iQ Sybr Green Supermix (Bio-Rad Laboratories, Hercules CA) and our primers at 0.5 μM concentration. The concentration of cDNA included in the reaction varied, and this variation was accounted for by our measurement of 18S for each sample (see below). The PCR parameters were 3 min at 95°C followed by 40 cycles of 95°C, 60°C, and 72°C for 30 s each. We used PCR Miner (Zhao and Fernald, 2005) to calculate the reaction efficiencies and cycle thresholds from the fluorescence readings of individual wells during the reaction. The reaction efficiencies were 1.9–2.1. For each sample, we calculated a mean cycle threshold of three PCR reactions. We calculated the expression of each target gene of interest relative to 18S using the equation: relative target gene expression = 100 × (Etarget ^ CTtarget) / (E18S ^ CT18S), where E was reaction efficiency and CT was cycle threshold (Pfafl, 2001). Thus, expression of the target genes are expressed as a percent of 18S rRNA expression.

**Statistics**

In our comparison of dominant to subordinate males, the factor of interest in the experiment was social status. In addition to this factor, we accounted for error attributable to the confounded variables of community tank and RNA extraction group by including the factor “tank/group” in the analyses. To test for effects of status on mRNA expression of ARs, ERs, GnRH1, GnRH-R1, and on GSI, we used multiple two-way analysis of variance (ANOVA) tests with status and tank/group as between subject factors although we did not include the interaction between status and tank/group in the model. We do not report or discuss effects of tank/group, and in all ANOVA models we used Type III sums of squares. When comparing dominants to subordinates in our graphical representations, we used the estimated marginal means and standard errors, which represent the effect tested by the ANOVA model (i.e., status with effect of group/tank removed); we present graphs of data for which p<0.1.

In addition to the effects of social status, we were interested in whether the ARs and ERs covaried with our different measures of reproductive capacity, namely, GnRH1 expression level in the brain, GnRH-R1 expression level in the pituitary, and GSI. Specifically, we examined the following relationships: within the anterior and middle portions of the brain, covariation of each SR mRNA expression with GSI; within the anterior and middle portions of the brain, covariation of each SR mRNA expression with GnRH1 expression; within the pituitary, covariation of each SR mRNA expression with GnRH-R1 expression; and within the testes, covariation of each SR mRNA expression with GSI. For our analysis of covariation with GnRH1, we used the sum of the expression level in the anterior and middle brain parts to represent GnRH1 expression by the entire population of GnRH1 neurons. Because we wanted to examine all of these relationships independently of the influence of tank/group, we used multiple regression analysis with tank/group coded by dummy variables. Specifically, the model included three independent variables (two dummy variables representing tank/group and either GSI, GnRH1, or GnRH-R1) and one dependent variable (the AR or ER). Using this model, we ignored the overall explanatory power of the combination of independent variables and focused on the independent contribution of the variable representing the reproductive axis (GSI, GnRH1, or GnRH-R1) on AR or ER expression. We used the significance test of the beta weights (also called the standardized coefficients) to evaluate whether the reproductive axis variable influenced a specific receptor’s expression, and the partial correlation to assess the direction of the relationship. Finally, to represent the relationships, we graphed the partial plots, which were the unstandardized residuals of each variable after the effect of tank/group is removed; we present graphs of data for which p<0.1.

Sample sizes were 18 for each tissue and gene except for the testes. During the PCR reaction for the testes, air bubbles were inadvertently introduced into a small number of wells which resulted in abnormal fluorescence curves and necessitated the exclusion of these samples from the analyses; the resulting sample sizes were 16 (ER-α), 16 (ER-βa), 15 (ER-βb), 15 (AR-α), and 14 (AR-α).

**Results**

Replicating previous results (e.g., White et al., 2002), the dominant males in our study had larger testes than subordinate males, as measured by GSI (F1,14=5.5, p=0.034), and higher expression levels of GnRH1 mRNA in the brain (F1,14=7.3, p=0.017). However, unlike a previous study which found higher GnRH-R1 expression in dominant males (Au et al., 2006), we found similar GnRH-R1 in the pituitaries of dominants and subordinates (estimated marginal means±SE: dominant males, 23.5±7.0; subordinate males, 21.2±7.0; F1,14=0.6, p=0.82). Further examination suggested that data from one subordinate male were an outlier with respect to GnRH-R1 expression; this male had GnRH-R1 expression levels that were 95% of 18S compared to a range from 3.0% to 29.0% for all other males in the study. This male did not appear to be an outlier on any other measure, including GSI and GnRH1 expression. When this subject was removed from the analysis of pituitary GnRH-R1 mRNA levels, dominant males had slightly higher mean levels than subordinate males, although the difference was not statistically significant (estimated marginal means±SE: dominant males, 21.2±2.6; subordinate males, 14.4±2.8; F1,13=3.1, p=0.1). All subsequent analyses that involved GnRH-R1 expression exclude this subordinate male.

Effects of social status on expression of AR and ER mRNA levels are reported in Table 2. In the anterior portion of the
bran, dominant males had higher expression of AR-α, AR-β, ER-βα, and ER-ββ (Figs. 1A–D), whereas ER-α was similar in dominants and subordinates. In the middle portion of the brain, AR-α was higher in dominants, but this difference was not statistically significant (Fig. 1E); mRNA expression of the other SRs was similar in dominants and subordinates. In the posterior part of the brain, dominant and subordinate males had similar levels of receptor expression (all \( p > 0.1 \)). In the pituitary, dominant males had higher ER-α expression than subordinates, but this difference was not statistically significant (Fig. 1F); all other SRs had similar expression levels. Status had no detectable effect on any steroid receptor mRNA expression in the testes (all \( p > 0.2 \)).

Given that dominance is associated with enhanced activity of the reproductive axis, we asked whether variation in receptor expression level was correlated with variation in GSI, GnRH1 expression in the brain, or GnRH-R1 expression in the pituitary. For GSI, the pattern of correlations with receptor expression level generally matched the pattern for the effect of status (Fig. 2). Specifically, we found that, in the anterior part of the brain, GSI was positively correlated with AR-α (Fig. 2A; \( r = 0.73, p = 0.001 \)), AR-β (Fig. 2B; \( r = 0.57, p = 0.02 \)), ER-βα (Fig. 2C; \( r = 0.66, p = 0.005 \)), and ER-ββ (Fig. 2D; \( r = 0.55, p = 0.028 \)). In the middle part of the brain, GSI was weakly positively associated with AR-α (Fig. 2E; \( r = 0.44, p = 0.085 \)). In the pituitary, we found a strong positive correlation between GSI and AR-α (Fig. 2F; \( r = 0.74, p = 0.001 \)). In the testes, we did not detect any relationship between any steroid receptor mRNA expression and GSI (all \( p > 0.2 \)). We also examined the correlation between GnRH1 mRNA and SR mRNA expression levels. We found significant positive correlations for all SRs in the anterior portion of the brain with GnRH1 (Fig. 3; AR-α: \( r = 0.75, p = 0.001 \); AR-β: \( r = 0.74, p = 0.001 \); ER-α: \( r = 0.71, p = 0.002 \); ER-βα: \( r = 0.72, p = 0.002 \); ER-ββ: \( r = 0.71, p = 0.002 \)), including ER-α, which did not show an effect of status or GSI. In the middle portion of the brain, we did not detect any significant correlations, including for AR-α, for which we detected weak relationships with status (Fig. 1E) and GSI (Fig. 2E).

Since regulation of GnRH receptors in the pituitary may be an important part of social regulation of the BPG axis and SRs may contribute to mediating this relationship, we examined their correlation within the pituitary. We detected a strong relationship between GnRH-R1 and AR-α (\( r = 0.73, p = 0.002 \)) and a weaker relationship with AR-β (\( r = 0.48, p = 0.07 \)) that appears to be weakened statistically primarily by a single outlying point (Fig. 4). In addition, ER-α was correlated with GnRH-R1 (Fig. 4; \( r = 0.54, p = 0.038 \)) although the data are quite scattered, thus reducing our confidence in this conclusion.

### Discussion

In *A. burtoni*, social dominance determines reproductive capacity of males through regulation of the brain–pituitary–gonad (BPG) axis. The increased activity of the BPG axis results

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**Table 2**

Effects of social status on mRNA expression level of androgen and estrogen receptor subtypes

<table>
<thead>
<tr>
<th></th>
<th>AR-α F</th>
<th>p</th>
<th>AR-β F</th>
<th>p</th>
<th>ER-α F</th>
<th>p</th>
<th>ER-βα F</th>
<th>p</th>
<th>ER-ββ F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior brain</td>
<td>17.53</td>
<td>0.001</td>
<td>8.19</td>
<td>0.013</td>
<td>2.43</td>
<td>0.142</td>
<td>9.67</td>
<td>0.008</td>
<td>6.39</td>
<td>0.024</td>
</tr>
<tr>
<td>Middle brain</td>
<td>4.34</td>
<td>0.056</td>
<td>2.38</td>
<td>0.145</td>
<td>2.63</td>
<td>0.127</td>
<td>0.66</td>
<td>0.430</td>
<td>1.02</td>
<td>0.331</td>
</tr>
<tr>
<td>Posterior brain</td>
<td>0.08</td>
<td>0.787</td>
<td>0.10</td>
<td>0.755</td>
<td>2.02</td>
<td>0.177</td>
<td>0.01</td>
<td>0.934</td>
<td>0.63</td>
<td>0.441</td>
</tr>
<tr>
<td>Pituitary</td>
<td>0.35</td>
<td>0.565</td>
<td>0.19</td>
<td>0.670</td>
<td>3.96</td>
<td>0.066</td>
<td>0.01</td>
<td>0.934</td>
<td>0.20</td>
<td>0.663</td>
</tr>
<tr>
<td>Testes</td>
<td>&lt;0.01</td>
<td>0.976</td>
<td>1.62</td>
<td>0.231</td>
<td>0.02</td>
<td>0.882</td>
<td>0.62</td>
<td>0.446</td>
<td>1.02</td>
<td>0.331</td>
</tr>
</tbody>
</table>

Degrees of freedom were 1,14 for all \( F \) statistics with the exception of the testes, which were 1,12 (ER-α, ER-βα), 1,11 (ER-ββ, AR-α), and 1,10 (AR-β).

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![Fig. 1. Effects of reproductive status on expression of steroid receptor genes in subordinate (sub) and dominant (dom) males. Asterisks indicate \( p < 0.05 \). (A–D) Estimated marginal means (±SE) of AR and ER gene expression in subordinate and dominant males in the anterior portion of the brain. (E) Estimated marginal means (±SE) of AR-α gene expression in subordinate and dominant males in the middle portion of the brain. (F) Estimated marginal means (±SE) of ER-α gene expression in subordinate and dominant males in the pituitary.](image-url)
in increased production of sex steroid hormones (Parikh et al., 2006) which in males are primarily androgens. Using quantitative PCR, we found that dominance and reproductive capacity were associated with increased mRNA expression of two androgen receptors and two estrogen receptors in the brain. In the pituitary, only ER-α was regulated by reproductive capacity, although expression of pituitary AR-α was correlated with GnRH-R1. We did not find a difference in GnRH-R1 mRNA expression in the pituitary between dominant and subordinate males unlike in a previous study (Au et al., 2006). The reasons for the discrepancy between the studies is not clear, although it suggests that GnRH-R1 mRNA expression is highly labile and may be regulated by factors in addition to social status. In the testes, we found no evidence that reproductive capacity was
associated with changes in expression of ARs or ERs, in spite of the fact that enlarged testes are associated with increased production of mature sperm (Fraley and Fernald, 1982) and increased androgen production (Parikh et al., 2006).

One of the striking aspects of our results was the fact that, in the anterior brain, expression of all ARs and ERs were regulated by reproductive capacity: all but ER-α were expressed at higher levels in dominant males and were positively correlated with GSI, and expression of all ARs and ERs were positively correlated with GnRH1 expression. ER-α was the only receptor, among those measured, that did not show a robust relationship between reproductive capacity and expression levels, suggesting that ER-α may have a different role in the brain than the other ERs or ARs. In addition, this increase in expression of the SRs, if reflected in increased protein levels (You and Yin, 2000), implies that the anterior part of the brain could have increased sensitivity to steroid hormones. Such increased sensitivity may enhance the well described negative feedback responses of GnRH1 neurons to circulating steroid levels (e.g., Soma et al., 1996), or increase steroid sensitivity of brain regions involved in behavior. Given that dominant males have higher, not lower, androgen levels, it seems unlikely that GnRH1 neurons, or the brain regions regulating them, have enhanced negative feedback, a conclusion supported by castration studies (Soma et al., 1996). Recent data describing the neuroanatomical distribution of AR-α and AR-β in dominant males shows that AR-β has a wider distribution than AR-α, with AR-β expressed in the pallium and subpallium in addition to hypothalamic regions (L.K. Harbott, S.S. Burmeister, R. White, M. Vagell, and R.D. Fernald, unpublished). This distribution pattern suggest that, at least for AR-β, increased expression in dominant males has the potential to influence brain regions involved in sensory processing, behavioral motivation, as well as regulation of the BPG axis. Information about receptor distribution (particularly the ERs) and the relative binding capacity of the receptors will further inform the implications of the current study. Without additional studies that incorporate tissue localization, our conclusions must remain cautious given our relatively imprecise anatomical resolution.

We were surprised to find robust effects of differences in reproductive capacity on AR and ER mRNA expression in the anterior, but not middle, portions of the brain since all but part of one hypothalamic nucleus (the aPPn) were included in the middle portion whereas the anterior portion consisted primarily of the telencephalon. The hypothalamus is generally known to express high levels of steroid hormone receptors and is likely to be a central player in generating behavioral and physiological responses to changes in steroid hormone levels. Studies from A. burtoni, as well as other teleost, suggest that all AR and ER subtypes are expressed in regions of the preoptic area and hypothalamus (Harbott et al., unpublished; Hawkins et al., 2000; Menuet et al., 2002) and distinct steroid binding sensitivities (Hawkins and Thomas, 2004; Olsson et al., 2006) of the SR subtypes suggest they serve different functions. Thus, our data contribute to the growing evidence that the steroid receptor paralogs have diverged to serve related functions. Although the distinction between the evolution of novel functions versus subfunctionalization cannot be determined without additional evidence from a representative of the common ancestor of teleosts, the patterns, on the whole, suggest subfunctionalization. Such an elaboration in function is consistent with recent theories on the important role of steroid hormone receptors in the evolution of vertebrates (Baker, 2003).

In conclusion, we have found that in the anterior part of the brain, the two ARs and two ER-βs are regulated by social status and reproductive capacity. Although it has long been established that teleosts have elaborated the number of AR and ER genes through genome duplication, the functional role of these steroid hormones in reproductive behavior is unclear. Here we show that the expression levels of the genes for these receptors are regulated by dominance and reproductive capacity in males. Although much more work remains before we fully understand the role of these receptors in reproductive behavior, these data provide the first direct evidence that these steroid receptors are regulated by social context in males.
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References


