



Sex differences and similarities in the neuroendocrine regulation of social behavior in an African cichlid fish ☆,☆☆



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ABSTRACT

An individual's position in a social hierarchy profoundly affects behavior and physiology through interactions with community members, yet little is known about how the brain contributes to status differences between and within the social states or sexes. We aimed to determine sex-specific attributes of social status by comparing circulating sex steroid hormones and neural gene expression of sex steroid receptors in dominant and subordinate male and female *Astatotilapia burtoni*, a highly social African cichlid fish. We found that testosterone and 17 β -estradiol levels are higher in males regardless of status and dominant individuals regardless of sex. Progesterone was found to be higher in dominant individuals regardless of sex. Based on pharmacological manipulations in males and females, progesterone appears to be a common mechanism for promoting courtship in dominant individuals. We also examined expression of androgen receptors, estrogen receptor α , and the progesterone receptor in five brain regions that are important for social behavior. Most of the differences in brain sex steroid receptor expression were due to sex rather than status. Our results suggest that the parvocellular preoptic area is a core region for mediating sex differences through androgen and estrogen receptor expression, whereas the progesterone receptor may mediate sex and status behaviors in the putative homologs of the nucleus accumbens and ventromedial hypothalamus. Overall our results suggest sex differences and similarities in the regulation of social dominance by gonadal hormones and their receptors in the brain.

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Introduction

In many social species, members of a community form dominance hierarchies where social status profoundly affects an individual's behavior and physiology through interactions with community members (Sapolsky, 2005). The physiological basis of social dominance is often associated with differences in reproductive status and sex steroid hormone levels (Wingfield et al., 1991), which make studying the molecular determinants of social dominance difficult to dissect from reproductive physiology. Moreover, little is known about how these physiological and behavioral differences are integrated within the brain.

In order to disentangle sex-specific physiology from the neuroendocrine mechanisms of social dominance, we utilized a highly social fish that has plastic behavioral phenotypes and readily forms dominance hierarchies within community tanks. Males of the cichlid fish *Astatotilapia burtoni* display phenotypic plasticity in social status, alternating between dominant (DOM) and subordinate (SUB) phenotypes depending on the social environment. DOM males are conspicuously colored, reproductively active, and aggressively defend territories where they court and spawn with females. SUB males are dull in coloration, school with females, and are reproductively inactive. Although neuroendocrine differences between DOM and SUB males have been described in various contexts (Maruska and Fernald, 2010a; O'Connell and Hofmann, 2012), disentangling the neural basis of social dominance from differences in reproductive status is difficult. Female *A. burtoni* provide an excellent opportunity to dissect the mechanisms of social status from reproductive state, as females will also form dominance hierarchies in the absence of males, but both DOM and SUB females are reproductively active (Renn et al., 2012). By comparing DOM and SUB males and females, we have a unique opportunity to determine to which extent the neuroendocrine underpinnings of behavior within a social hierarchy are due to either reproductive state or social status.

In order to function in a social community that is based on a dominance hierarchy, individuals must integrate external social information

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with internal physiology into an appropriate behavioral response to conspecific cues. Steroid hormones and their receptors are crucial in this process, as steroid hormones relay acute social information as well as reproductive status. The influence of social status on steroid hormone levels has been extensively studied in *A. burtoni*, as DOM males have higher levels of androgens and 17 β -estradiol compared to SUB males (O'Connell and Hofmann, 2011, 2012, O'Connell and Hofmann, 2012). Furthermore, gonadal hormones seem to mediate distinct components of male social behavior, as manipulation of the estrogen receptors (ERs) alters aggression in both DOM and SUB males, whereas manipulation of the androgen and progesterin receptors (AR and PR, respectively) alters courtship behavior in DOM males only (O'Connell and Hofmann, 2012). In SUB males, PR seems to mediate social cognition, as PR antagonists decrease anxiety-related behavior in response to threatening DOM males (O'Connell and Hofmann, 2012). Importantly, hormones can play very dynamic roles, as androgens and estrogens rise in SUBs within 30 min of being given the opportunity to transition to DOM status (Maruska and Fernald, 2010a, 2010b; Huffman et al., 2012; Maruska et al., 2013). Even more striking is that brain gene expression of steroid hormone receptors can change within 30 min of providing *A. burtoni* males with an opportunity to ascend in social status (Maruska and Fernald, 2010b). DOM *A. burtoni* females also have higher androgen levels compared to SUB females (Renn et al., 2012), although this difference is not as pronounced as in males. Despite a great deal of work on hormonal differences between male phenotypes, little is known about where and how sex steroid receptors act to mediate social dominance behavior in the brain. An understanding of how sex steroids mediate social dominance behavior in female *A. burtoni* is also lacking.

Our goal in the design of the present study was to test the hypothesis that sex steroid hormones and their receptors regulate some aspects of social dominance behavior independent of reproductive state and/or sex. Specifically, we hypothesized based on results from our previously published studies described above that androgens would influence social dominance behavior in *A. burtoni* individuals regardless of sex, whereas estrogens and progestins would influence sex-specific behaviors. We quantified mRNA abundance of the androgen receptors (AR α and AR β), estrogen receptor α (ER α), and the progesterone receptor (PR) using quantitative radioactive *in situ* hybridization. Our quantification efforts focused on five core brain regions involved in social behavior (O'Connell and Hofmann, 2011; putative mammalian homologs are in parentheses, although note that some of these homologs are tentative and not necessarily one-to-one): the ventral part of the ventral telencephalon (Vv, lateral septum-like), the dorsal part of the ventral telencephalon (Vd, putative nucleus accumbens); POA, the anterior tuberal nucleus (aTn, putative ventromedial hypothalamus), and the periventricular part of the posterior tuberculum (TPp, putative ventral tegmental area/substantia nigra). These regions are important in evaluating the salience of an external stimulus as well as regulating sexual behavior and aggression across vertebrates (Goodson, 2005; O'Connell and Hofmann, 2011).

Methods

Study organism and behavior

A. burtoni males and females descended from a wild-caught stock population were kept in aquaria as described previously (Munchrath and Hofmann, 2010). DOM and SUB males were randomly selected for observation within mixed sex communities containing 8 males and 8 females in sixteen 110 liter tanks. All focal males had been in their respective social states for at least two weeks prior to observation. Sixteen additional 110 liter tanks were populated with 12 females per tank with no males present to stimulate the formation of female dominance hierarchies as previously described (Renn et al., 2012). All focal females were stable in their social status for at least one week prior to observation. Each male in the mixed sex tanks and all females in the

female-only tanks were marked with a colored bead attached to a plastic tag inserted just below the dorsal fin, allowing identification of individual animals. Gravel substrate and five terracotta shelters were also placed in each tank to provide the substrate that facilitates the establishment and maintenance of territories. In both mixed sex and female-only communities there were usually two to four DOM individuals, while the remaining animals were of SUB status. DOMs were identified as aggressively defending a territory within the tank and presence of a dark lachrymal stripe (eye bar) across the head, which is characteristic of territory holders. SUBs were identified by absence of a territory, schooling with females in the tank, fleeing from DOMs, dull coloration and lack of an eye bar. There were no observable differences in status within SUB individuals (high-ranking SUBs versus low ranking SUBs), as SUBs rarely display aggressive behavior. Every effort was made to minimize pain or discomfort of the animals and all work was carried out in compliance with the Institutional Animal Care and Use Committee at The University of Texas at Austin.

One DOM (males: $n = 24$; body mass (BM) = 6.45 ± 0.92 g, standard length (SL) = 5.98 ± 0.31 cm; females: $n = 16$; BM: 3.20 ± 0.18 g; SL: 4.91 ± 0.10 cm) and one SUB (males: $n = 24$; BM = 5.44 ± 1.01 g, SL = 5.7 ± 0.37 cm; females: $n = 16$; BM: 2.30 ± 0.10 g; SL: 4.51 ± 0.07 cm) individual from the same community were observed between 09:00 and 11:00 h for 5 min each on three days for one week. As expected, DOM males were larger both in length ($t_{46} = 3.201$, $p = 0.002$) and mass ($t_{46} = 3.637$, $p = 0.003$) compared to SUB males. Similarly, DOM females were larger than SUB females (standard length: $t_{30} = 3.259$, $p = 0.003$; mass: $t_{30} = 4.264$, $p = 1.84 \times 10^{-4}$). A single observer quantified the behavior of DOM and SUB dyads within each community tank. Aggressive (chases, bites, threats, border disputes), sexual (quivers, leads), and fleeing behavior patterns were observed as described in Fernald (1976). On the last day of behavioral observations, weight and length of each focal individual were recorded and blood was drawn from the dorsal aorta for hormone assays. The gonadosomatic index (GSI) was calculated as the ratio of gonad weight to body weight multiplied by 100. Ovaries were fixed in Bouin's solution for ovarian histology (see supplementary methods). Brains were rapidly dissected and fresh frozen for *in situ* hybridization ($n = 16$ male dyads; $n = 8$ female dyads). To avoid the potentially confounding effects of ovarian stage, brain analyses were carried out on a subset of females ($n = 8$ per social status) where the DOM and SUB dyads had similar GSI.

Hormone assays

Free (bioavailable rather than total) circulating testosterone, 17 β -estradiol, and progesterone were measured for most individuals using ELISA (Enzo Life Sciences, NY, USA) where inter and intra-assay variations were 5.19% and 3.14%, 3.18% and 4.25%, and 2.94% and 4.41%, respectively. Plasma samples were diluted 1:30 and processed as in Kidd et al. (2010) and according to manufacturer's instructions. As *A. burtoni* are small cichlids, the amount of blood plasma isolated from each individual was sufficient to only measure three hormones in most individuals. We chose to measure testosterone rather than 11-ketotestosterone, as testosterone levels are consistently a magnitude higher than 11-ketotestosterone levels in this species (Trainor and Hofmann, 2006; Maruska et al., 2013; O'Connell et al., 2013). More generally, our recent comparative analyses suggest that testosterone is the active androgen in haplochromine cichlids (Dijkstra et al., 2012).

Radioactive *in situ* hybridization (ISH)

Brains ($n = 16$ males per social status and $n = 8$ females per social status) were fresh frozen in O.C.T. (Tissue-Tek) and stored at -80°C until sectioning at $20\ \mu\text{m}$ into four series. For male dyads, one set of brains ($n = 8$ per social status) was used to quantify expression of

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