Behavioural Brain Research 237 (2013) 318–324

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Social status, breeding state, and GnRH soma size in convict cichlids (*Cryptoheros nigrofasciatus*)

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HIGHLIGHTS

- We examine social influences on gonadotropin-releasing hormone in hypothalamus.
- Male social status affects GnRH transcribing neuron soma size in the preoptic area.
- Female breeding state affects GnRH transcribing neuron soma size.
- Territorial males and spawning females had larger GnRH neuron soma.
- We find no effects on number of GnRH transcribing neurons.

ARTICLE INFO

Article history:
Received 5 July 2012
Received in revised form 8 September 2012
Accepted 14 September 2012
Available online xxx

Keywords:
Convict cichlid
Cryptoheros nigrofasciatus
Gonadotropinreleasing hormone
Territoriality
Social status
Breeding state

ABSTRACT

Gonadotropin-releasing hormone (GnRH) expressing neurons in the preoptic area (POA) of the hypothalamus plays a key role in regulating reproductive function through the control of gonadotropin release. Several studies have illustrated the importance of the social environment in modulating the size of GnRH expressing neurons. In the African cichlid fish *Astatotilapia burtoni*, the size of the soma of GnRH expressing neurons in the POA varies with social status in males, and with breeding state in females. Territorial males have larger GnRH+ cells than non-territorial males, while brooder females have smaller GnRH+ cells than control females. The lek-like breeding system of *A. burtoni* is, however, only one type of social system within the diverse assemblage of cichlids. To gain a better understanding of GnRH neuronal plasticity in response to the changes in the social environment, we tested whether similar effects occur in the monogamous New World cichlid, the convict cichlid (*Cryptoheros nigrofasciatus*), a model species for the study of social behaviour. Our results indicate that, indeed GnRH expressing neuron soma size, and not cell number, varies with both male territorial status, and manipulations of female breeding state in this monogamous, biparental, New World cichlid.

1. Introduction

Reproduction is arguably the most important phase of any organism's life [1]. In vertebrates, gonadotropin-releasing hormone (GnRH) plays a critical role in regulating reproduction and sexual maturation via the hypothalamic-pituitary-gonadal (HPG) axis [2,3]. Although over 20 different forms of GnRH have been described [4], the primary function of GnRH is highly conserved across species. In teleost fishes, GnRH released from cells in the pre-optic area of the hypothalamus (POA) controls reproductive physiology and behaviour by stimulating the production and secretion of gonadotropins. These gonadotropins increase gametogenesis and sex steroid synthesis [3,5] as well as the expression of sexual behaviour. In most vertebrates, GnRH1 is delivered to the anterior lobe of the pituitary through the hypophyseal portal system, however, in fish, GnRH-secreting neurons directly innervate the anterior lobe of the pituitary [2,3].

In many fish, sexual maturation and reproduction are influenced by social factors, which ultimately exert their effects on GnRH secreting neurons of the POA [6]. Different forms of GnRH can have varying distributions and functions [5], but in many teleosts GnRH1 expressing cells (hereafter simply referred to as GnRH+ cells) show

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http://dx.doi.org/10.1016/j.bbr.2012.09.023

a remarkable plasticity with respect to the social environment [7–9]. This phenomenon has been particularly well studied in the African cichlid *Astatotilapia burtoni*. *A. burtoni* breed on contiguous territories in a lek-like system [10,11]. Males exhibit two distinctive phenotypes based on social status [12]. Territorial (T) males are brightly coloured, highly aggressive, and reproductively active; non-territorial males (NT) are, like females, cryptically coloured, are less aggressive and do not reproduce until a breeding site becomes available, at which point they transform into the T phenotype [12,13]. These T and NT social states correspond with the soma size of GnRH+ neurons in the POA. T males have significantly larger GnRH+ cells than NT males and this reflects their reproduction condition; T males have well-developed testes that readily release milt while NT males are hypogonadal [14]. Mature *A. burtoni* males can change between T and NT states depending on the social environment [13,15–17]. These changes from T to NT are similarly reflected in GnRH+ soma size and reproductive state. Thus, GnRH+ soma size and GSI decrease when T males become NT, and GnRH+ soma size and GSI increase when NT males become T [16]. Females, in contrast, exhibit differences in GnRH+ soma size with both age and reproductive state. First and experienced brooders have smaller GnRH+ neurons than virgins, experienced spawners or postreproductive females, but this difference is partially confounded by size. Older females are larger and have larger GnRH+ soma sizes and when body size is controlled for, the differences among reproductive states are no longer significant [18]. Whether manipulations in social status affect GnRH+ neuromorphology in females is unknown because female *A. burtoni* do not show the same levels of aggression and territoriality as males.

Although *A. burtoni* has proven to be a model system for understanding the importance of social environment in modulating GnRH+ neurons and the HPG axis [12], cichlids display a wide range of mating and parental care systems, of which the haremic *A. burtoni* system is only one [19]. The convict cichlid (*Cryptoheros nigrofasciatus)*, a teleost from Central America, is highly aggressive and sequentially monogamous, and unlike *A. burtoni*, both sexes engage in similar levels of territorial defense [20]. This makes convict cichlids an ideal comparison with *A. burtoni* for investigating the effects of social status manipulations on intra- and intersexual GnRH+ neuromorphology. Here, we test, (1) whether GnRH+ soma size is regulated by reproductive state in females convict cichlids, and (2) whether GnRH+ cells show the same plasticity with respect to social status in male convicts as in *A. butoni* males.

2. Materials and methods

2.1. Subjects

Sexually mature convict cichlids were purchased from a local pet supplier and housed in a stock communal aquarium (196 cm × 30 cm × 29 cm; $L \times W \times H$) for at least 10 months (Experiment 1) or at least three weeks (Experiment 2) before experimental manipulations (see below). The stock aquarium contained a 2 cm layer of sand and multiple terracotta flowerpots scattered throughout the bottom to serve as refuge sites. Water temperature was maintained at 26 ± 2 °C and lighting conditions were set to a 12:12 light:dark cycle. Fish were fed Tetramin fish flakes daily. Animal housing and experimental procedures were conducted according to the guidelines and principles of the Canadian Council on Animal Care. The University of Alberta Biological Sciences Animal Policy and Welfare Committee approved all protocols (BSAPWC protocol #392601).

2.2. Standard length, somatic and gonadal mass

Fish were euthanized in a solution containing 1 mL of 2-phenoxy-ethanol diluted in 500 mL of water and subsequently decapitated. Their standard lengths (to the nearest 0.1 mm), somatic and gonadal weights (both to the nearest 0.0001 g) were measured. Gonadosomatic indices were calculated as follows:

$$\text{GSI} = \frac{\text{gonad weight (g)}}{\text{body weight (g)}} \times 100$$

Testes and ovaries were also removed and briefly examined.

2.3. Histology

Immediately after decapitation, the heads were submerged in 4% paraformaldehyde (pH 7.4) and stored at 4 °C for at least 48 h. Following extraction, the brains were post-fixed in 4% paraformaldehyde for 24 h before cryoprotection in 30% sucrose for 30 h. Brains were frozen at −21 °C and sliced into 40 μm serial sagittal sections using a cryostat (Leica CM 1900 UV). Sections were mounted onto electrostatic slides (Fisherbrand Superfrost/Plus) and left to dry at room temperature for 24 h. The mounted sections were then rinsed three times with 0.1 M phosphate buffered saline (PBS, pH 7.4) and treated with 10% goat blocking serum and 0.4% Triton-X in 0.1 M PBS for 1 h. Next, following Bushnik and Fernald [2], we used a polyclonal rabbit anti-GnRH primary antibody raised against human luteinizing hormone releasing hormone (1:1625 anti-LHRH; AB1567, Chemicon, Temecula, CA), which is the alternate name for GnRH1 in mammals. The sections were incubated with the primary antibody and 0.4% Triton-X in 0.1 M PBS for 48 h. After three rinses with 0.1 M PBS, the sections were incubated with a fluorescent secondary antibody (1:100 Cy3 goat anti-rabbit, Jackson ImmunoResearch, West Grove, PA, USA) in 0.1 M PBS for 2 h. The sections were then rinsed with 0.1 M PBS for at least 5 min. In addition, we processed four female *A. burtoni* brains (kindly donated by N.E. Stacy) in parallel with the convict cichlid brains. Although not shown, the distribution of GnRH+ cells in the *A. burtoni* brains was identical to that of previous reports [18]. Thus, the antibody we used was labelling the same populations of GnRH+ cells. We also ran controls, which consisted of the omission of the primary or secondary antibody and, in all cases, no GnRH+ cells were observed.

2.4. Quantification of GnRH soma size

Previous research indicates that the mean soma size of GnRH-ir neurons outside the POA (i.e., in the terminal nerve and mesencephalon) do not vary as a function of social status [14]. Therefore, all GnRH+ neurons measured in the present study were limited to those located within the POA. Cross sectional areas of all GnRH+ cells were viewed with a compound light microscope (Leica DMRE) equipped with fluorescence filters (rhodamine and FITC). Images were captured with a Retiga EXi *FAST* Cooled mono 12-bit camera (Qimaging, Burnaby, BC, Canada) and analyzed with OPEN-LAB (Improvision, Lexington, MA). All measurements in an experiment were made by a single observer blind to the sex and status of the individual fish. Total soma area was measured in ImageJ [21] by tracing around the entire labelled cell body in the same manner as previous studies in *A. burtoni* [18]. All labelled cells that had a clearly demarcated perimeter and a visible nucleus in the plane of section were measured (see Fig. 1). At least 22 cells were measured per fish to calculate the mean GnRH+ soma size for that particular fish. On average, this amounted to 48.1 cells in males (range = 22–99 cells) and 63.9 cells in females (range = 22–142 cells), from an average of 3.7 (±1.6, S.D., range = 1–7) sections per fish.

2.5. Statistics

Shapiro–Wilk tests for normality indicated that mean soma size, GSI, body mass and standard length were normally distributed within both sexes (p's > 0.05), with the exception of GSI in females ($W = 0.8897$, $p = 0.002$). The GSI residuals from treatment groups (see below) were, however, normally distributed ($W = 0.9731$, $p = 0.534$). We therefore performed parametric tests on all of our data. Correlation coefficients were obtained using the Pearson product-moment correlation test. ANCOVA and t-tests were used to detect differences between treatment groups. All statistics were computed using R.

3. Experiment 1: Reproductive state manipulations in convict cichlid females

3.1. Reproductive state manipulations

To induce breeding, two males and one female were moved from the communal stock aquarium into a 114 L tank (91 × 31 × 35 cm; $L \times W \times H$); one male (the breeder) was larger than the female while the other male was smaller. (The addition of a smaller male improved the chances of a successful mating, as he seemed to attract the initial aggression the larger male would have otherwise directed at the female novel tank-mate). All aquaria contained a 2 cm layer of sand and a terracotta flowerpot to serve as a substrate for egg-laying. The smaller male was removed if it was in poor condition (i.e., it had shredded fins, light coloured vertical bars, and/or was hovering near the surface), or if the breeding pair had bonded. If the breeding male exhibited aggression towards the female after the smaller male was removed, both were returned to the stock tank and another trio of fish selected. Males were left with the females

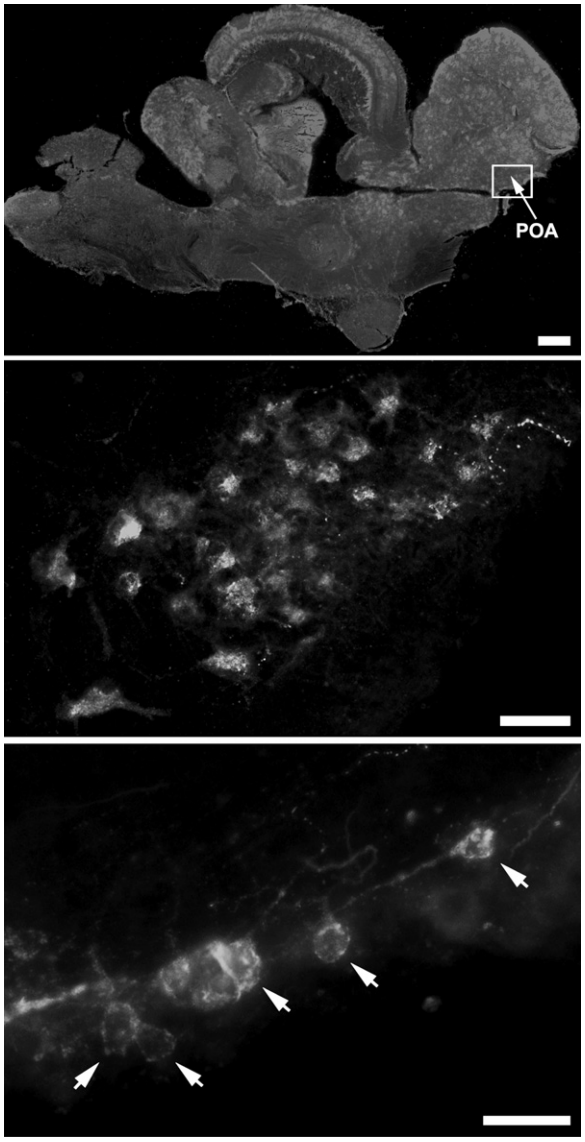

Fig. 1. Gonadotropin-releasing hormone immunoreactive (GnRH+) cells in the preoptic area (POA) of the Convict cichlid (*Amatitlania nigrofasciata*). (A) shows a mid-sagittal section through the brain indicating the POA. (B) shows a cluster of GnRH-ir cells in the POA. (C) shows a higher magnification photomicrograph with the white arrows indicating some of the labelled cell bodies. Scale bars: 300 μm in (A); 35 μm in (B); 25 μm in (C).

until her sacrifice, (the eventual mean length of cohabitation for females with the male they paired with was 19.7 days (S.D. 6.6) for brooders and 9.6 days (S.D. 5.3) for spawners).

3.2. Reproductive state classification

Fish were observed twice daily (5 min each) to determine their reproductive status. Spawners ($n = 12$) were females that had laid eggs within a 24 h period. Brooders ($n = 12$) were those whose fry have reached the free-swimming stage, about a week after spawning. Note that this is in contrast to the two-week brooding period in *A. burtoni* females because convict cichlids are substrate spawners and do not carry eggs or fry in their mouths [22]. Controls ($n = 12$) were females isolated from males and cohoused in a separate aquarium (114 L) for 10 days.

4. Experiment 2: Social status manipulations in male convict cichlids

4.1. Social status manipulations

To determine the effects of social status on GnRH soma size in males, we manipulated the social environment of 11 experimental individuals ($n = 6$ Territorial, 5 Non-territorial). Each fish was tagged at the base of the dorsal fin and caudal peduncle with injectable elastomer dye (Northwest Marine Technologies). Their standard lengths (to the nearest 0.1 mm) and weights (to the nearest 0.01 g) were measured. Fish were subsequently size matched according to weight. Territorial status was achieved by housing the experimental fish with smaller conspecifics (at least 15% weight difference), while non-territorial status was obtained by housing the experimental animal with larger conspecifics (again, a minimum of no less than 15% weight asymmetry). On average, the larger fish were 2.70 times the size of the smaller fish on average, with asymmetries ranging from 1.156:1 to 4.22:1). Subjects were housed in 114L aquaria (91 cm × 31 cm × 35 cm; $L \times W \times H$) containing a 2 cm layer of sand and a terracotta flowerpot. In total, seven fish were housed in each aquarium (three males, three females, and the experimental animal). They remained in their altered social environment for 14 days, as previous research has shown that this amount of time was sufficient in producing detectable changes in GnRH soma size in *A. burtoni* [5].

4.2. Social status classification

At the end of the 14 days, subjects were categorized as territorial or non-territorial based on a set of behavioural and morphological characteristics. Each experimental fish was observed for 7 min. Fish were considered territorial if they met the following criteria: (1) they resided in and defended the terracotta flowerpot placed in the aquarium; (2) they chased all conspecifics and never backed down from a challenge; and (3) they exhibited dark vertical bars. In contrast, fish were deemed non-territorial if they met at least two of the following criteria: (1) constant fleeing from larger conspecifics; (2) exhibition of light coloured vertical bars; or (3) hovering near the surface of the aquarium.

5. Results

5.1. Experiment 1: Reproductive state manipulations in convict cichlid females

Female body mass was strongly correlated with body length ($r(34) = 0.92$, $p < 0.00001$, Fig. 2A). The residual from this least squares linear regression was used as a measure of condition [23,24]. Condition showed a non-significant trend towards an effect of treatment group ($F(2,33) = 2.76$, $p = 0.08$) and tended to be higher among fish in the control group, although post hoc tests were non-significant (Fig. 2B). Neither body mass ($F(2,33) = 1.016$, $p = 0.373$) nor standard length ($F(2,33) = 0.778$, $p = 0.468$) differed significantly among the three groups.

5.2. Gonadal state

All ovarian eggs were round and orange and spawners had larger eggs than brooders. GSI and condition were not, however, significantly correlated with one another ($r(33) = 0.27$, $p = 0.12$). GSI varied significantly among the treatment groups ($F(2,32) = 4.8$, $p = 0.015$, Fig. 3A); when condition was included as a co-variate, the treatment group effect remained significant, ($F(2,31) = 3.4$, $p = 0.046$, Fig. 3B). Females in the control group had the highest GSI, which was significantly higher than brooders ($t(12.3) = 2.69$, $p = 0.019$,

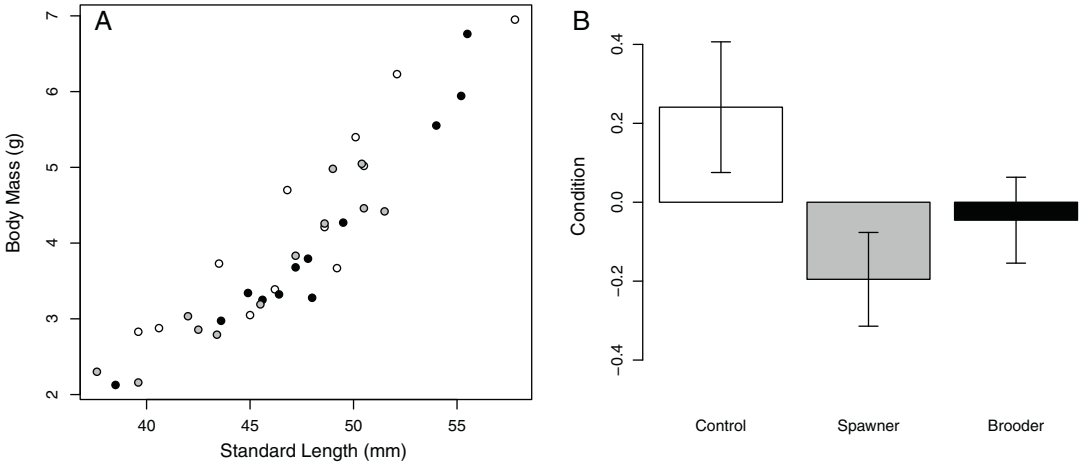

Fig. 2. Relationship between mass and length of females as a function of treatment group. Panel A shows Control group with white dots, Spawners with grey dots and Brooders with black dots. Panel B shows the non-significant ($p = 0.08$) trend towards differences in condition (the residuals from the mass–length regression line) between treatment groups.

and marginally higher than spawners ($t(15.5) = 1.87$, $p = 0.081$). Brooders and spawners did not have significantly different GSIs ($t(18.467) = 1.12$, $p = 0.28$).

5.3. POA GnRH-ir number

GnRH+ cell bodies and fibres were detected in the pre-optic area of the hypothalamus (Fig. 1). These fibres appeared to project directly to the anterior lobe of the pituitary. The number of GnRH+ cells in the POA was independent of body size ($r(34) = -0.042$, $p = 0.81$), even after including treatment group as a co-factor ($F(1,30) = 0.043$, $p = 0.89$). Similarly, condition had no effect on the total number of GnRH+ cells across all fish ($r(34) = 0.07$, $p = 0.68$), nor when treatment group was included as a co-factor ($F(1,30) = 1.25$, $p = 0.27$).

5.4. POA GnRH-ir soma size

Mean GnRH+ soma size was significantly correlated with standard length ($r = 0.55$, $n = 34$, $p < 0.0005$) and body mass ($r = 0.63$, $n = 34$, $p < 0.0001$), but not with condition ($r = 0.32$, $n = 34$, $p = 0.056$). An ANCOVA on mean GnRH+ soma size as a function of treatment group, body length and condition showed significant effects of body length ($F(1,31) = 18.0$, $p < 0.0005$), condition ($F(1,31) = 14.17$, $p < 0.001$) and treatment group ($F(2,31) = 10.6$, $p < 0.005$, condition and treatment effects plotted in Fig. 4). The significant treatment effects on mean GnRH+ somas size, after correcting for body length and condition effects, are shown in Fig. 4B; spawners had significantly larger GnRH+ soma sizes than the controls or brooders. An analysis of covariance found no correlation between the condition-corrected mean GnRH+ soma size in the POA and GSI when treatment group included as a co-factor ($F(1,31) = 0.03$, $p = 0.87$), or between condition-corrected mean GnRH+ soma size and GSI in the sample as a whole ($r(33) = -0.01$, $p = 0.97$).

5.5. Experiment 2: Social status manipulations in convict cichlid males

Male body mass was highly correlated with body length ($r(9) = 0.89$, $p < 0.0005$). The residual from this linear regression was used as a measure of condition. T and NT males did not differ from one another in body length ($t(6.57) = 0.5854$, $p = 0.5778$), body mass ($t(5.228) = 0.5665$, $p = 0.5945$) or condition ($t(8.971) = 0.0759$, $p = 0.94$), Fig. 5A).

Territorial males had significantly larger GSI than non-territorial males (T: 0.76 ± 0.11; NT: 0.36 ± 0.13; $t(8.5) = 2.3$, $p < 0.05$, Fig. 5B).

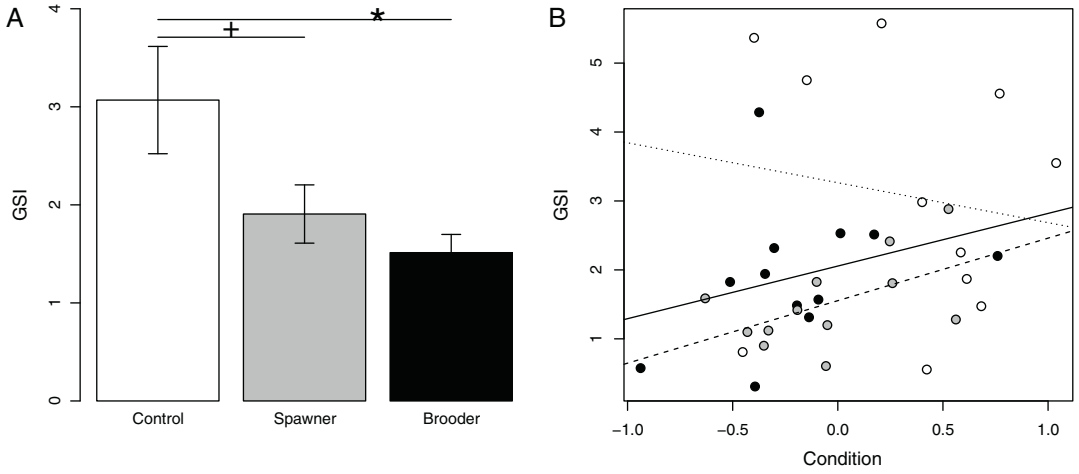

Fig. 3. Gonadosomatic index in females as a function of (A) by treatment group, and (B) by treatment after with condition included as a covariate (Control group, white dots and dotted line; Spawners, grey dots and dashed line; Brooders, black dots and solid line) as a covariate. There was no significant GSI vs. treatment correlation, but there was a significant effect of treatment group on GSI in both analyses (see text for statistical details).

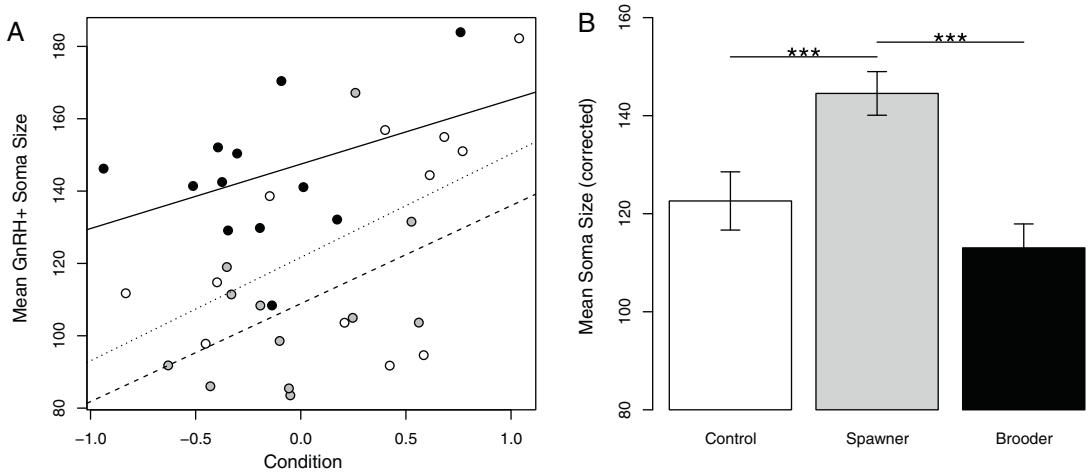

Fig. 4. Mean GnRH+ cell soma size in females as a function of (A) condition with treatment included as a covariate (Control group, white dots and dotted line; Spawners, grey dots and dashed line; Brooders, black dots and solid line), and (B) by treatment after removing the effects of body condition and size. Females in the Spawner treatment had significantly larger GnRH+ soma sizes than those in the other two groups (see text for statistical details).

There was no significant relationship between GSI and condition ($r(9) = -0.11$, $p = 0.73$) in males.

5.6. POA GnRH+ cell number

Neither body size nor condition had an effect on the number of GnRH+ cells in the male POA (standard length: $r(8) = -0.30$, $p = 0.36$; condition: $r(8) = -0.33$, $p = 0.31$), even when territorial status was included as a covariate (standard length: $F(1,8) = 0.61$, $p = 0.45$; condition: $F(1,8) = 1.07$, $p = 0.33$). The number of POA GnRH+ cells also did not differ between T and NT males (T: 41.5 (S.E. 8.1), NT: 56 (S.E. 13.4), $t(6.727) = 0.92$, $p = 0.38$, Fig. 5C).

5.7. POA GnRH+ soma size

Mean GnRH+ soma size in males was not significantly correlated with body mass ($r(9) = 0.08$, $p = 0.83$), body size ($r(9) = 0.03$, $p = 0.93$), or condition ($r(9) = 0.11$, $p = 0.75$). Territorial males had larger mean

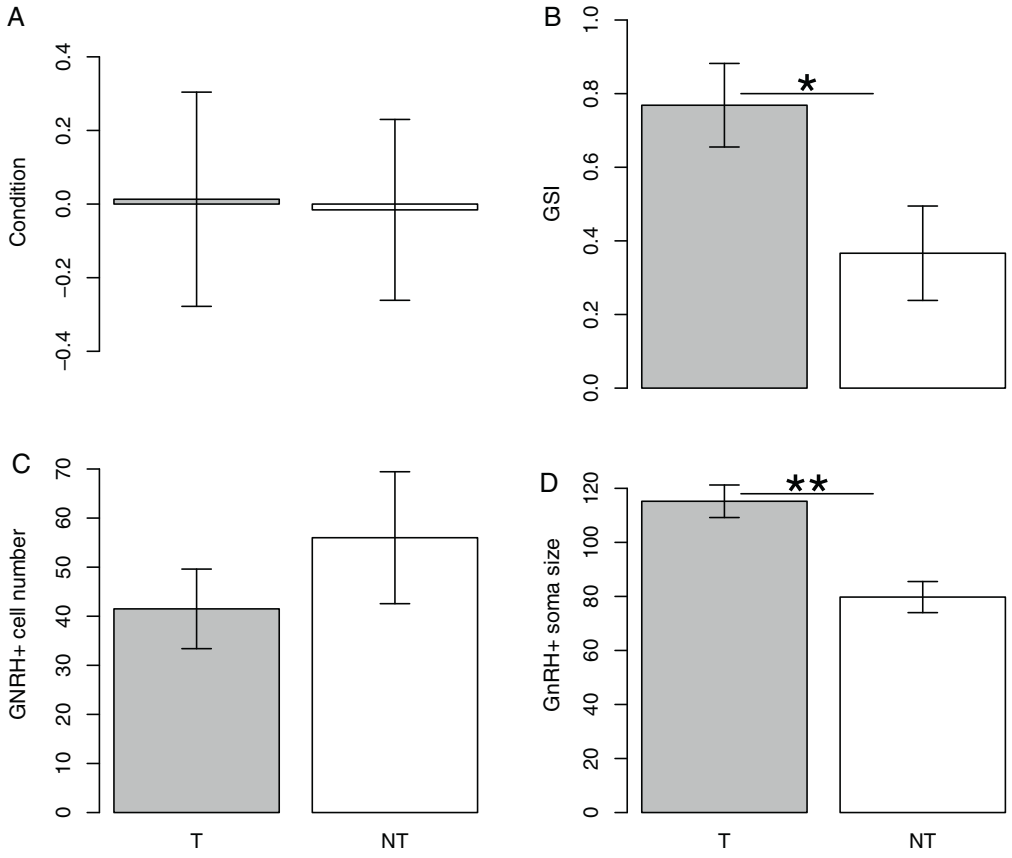

Fig. 5. Body size, Gonadosomatic index and GnRH+ cell soma size in males. All bars are means ± SEM, territorial (T) males shown with grey bars on the left, and non-territorial (NT) males are open bars on the right. Panel A shows no evidence of any difference in condition (mass-length residuals) between NT and T males. Panel B shows the higher gonadosomatic index (GSI) in T males. Panel C shows no evidence of a difference in the number of GnRH+ cells in the POA between NT and T males. Panel D) shows the greater GnRH+ soma size in T males (see text for statistical details).

GnRH+ soma sizes than NT males ($t(8.96) = 4.26$, $p < 0.005$, Fig. 5D). This effect remains significant in an ANCOVA including body length and condition ($F(1,7) = 14.8.$, $p < 0.01$) while the effects of body length ($F(1,7) = 0.31$, $p = 0.60$), and condition ($F(1,7) = 0.17$, $p = 0.70$) remained non-significant. Mean GnRH+ cell soma size was correlated positively with GSI (all males, $r(9) = 0.81$, $p < 0.005$), even after including treatment as a cofactor ($F(1,8) = 6.7$, $p < 0.05$).

Mean soma size was smaller in the males than in the females from Experiment 1, $t(20.43) = 3.3$, $p = 0.003$).

6. Discussion

Here, we show that the soma size of GnRH+ cells in the convict cichlid POA is plastic with respect to social status in males, and varied between reproductive state treatment groups in females. More specifically, territorial (T) males have significantly larger GnRH+ cells than non-territorial (NT) males. We also found that GSI is linked to social status; T males have significantly larger GSIs than NT males, and control females had larger GSI than brooders.

These size differences in GnRH+ cells are not due to mass, body length, or condition effects, although there was a trend towards an effect of condition on GnRH+ cell size in females. That smaller GnRH+ cells were found in brooder females could have reflected changes in condition rather than reproductive state itself as females eat less during the brooding period [25]. However, when body length and condition were included as covariates in an ANCOVA, T males still had substantially larger GnRH+ cells than NT males, and breeding state still influenced GnRH+ soma size in females.

In *A. burtoni*, as females get larger, GnRH+ soma size increases and this is overlaid with changes in GnRH+ soma size that vary with reproductive state [18]. In other words, body size and reproductive state interact to affect GnRH+ soma size. Convict cichlids, in contrast, appear to have a more robust difference in GnRH+ soma size among reproductive states and this difference is independent of body condition or size. Despite the significant difference in GnRH+ soma size between spawners and controls or brooders (Fig. 4), we found no correlation between GnRH+ cell soma size and GSI in females. There are a few possible explanations for the lack of a significant relationship between GnRH+ soma size and GSI in females. First, the measurement of GSI in spawners varied greatly because we did not cull all spawning females at the moment they initiated spawning (we classified "spawners" as females that had laid eggs within 24 h). Ovaries from females that had already laid all their eggs within that time period would have no eggs remaining, while ovaries from those that had just initiated spawning would still retain most of the eggs. Thus, spawners that had completed spawning before culling would have had very low GSIs while GSIs from those that had just begun spawning would have been high. This should have resulted in greater variability in GSI and/or soma size among spawners, but this is not what we observed in our data (Figs. 3 and 4). A second possibility to explain the relationship between GnRH+ soma size and GSI is that soma size more closely reflects oestrogen levels than it does GSI. Oestrogen levels vary with reproductive state in female fish (see references in [18]) and oestrogen exerts effects on GnRH soma size [26,27]. We did not measure steroid hormone levels in fish from these experiments, but this could explain why we failed to detect a relationship between GnRH+ soma size and GSI and why our control females were more variable than the brooders and spawners in both soma size and GSI. Without following the specific reproductive cycle of our control fish, we likely included females that were in varying stages of reproductive onset such that both GSI and GnRH soma size were more variable. Future studies should specifically track and measure steroid hormone levels of fish in order to control for this effect on GnRH+ soma size. If we had euthanized spawners the moment they began laying eggs, then GSI data could have correspond with GnRH+ soma size across the groups; that is, spawners would have the largest GSIs, brooders would have the smallest, and controls would be somewhere in between.

It may be that rather than plasticity in soma size, our results reflect different subsets of neurons in the POA, some with smaller somas and others with larger somas, that express GnRH under different social settings or reproductive stages. To address this issue, we measured the total number of POA GnRH-ir cells across experimental conditions. A disparity in GnRH+ numbers would indicate that the differences in GnRH+ soma size could, in part, reflect different cell populations expressing GnRH under different conditions [17]. We did not find any differences in GnRH+ numbers between treatment groups, body length, or condition in either experiment. While we cannot directly rule out that differently sized GnRH+ neurons express GnRH at different times, the general consensus is that these cells are plastic with respect to soma size in *A. burtoni* [6,12,18].

GnRH ultimately induces gametogenesis and sex steroid synthesis [3,5]. Therefore, the enlargement of these cells in reproductively active animals (i.e., T males and spawning females) likely reflects the increased production and release of GnRH. Indeed, mRNA levels for GnRH1 are significantly higher in territorial *A. burtoni* males than in NT males [6]. Similarly, GnRH1 expression is substantially higher in spawning *A. burtoni* females compared to brooding females [6]. Whether the same pattern exists in convict cichlid males/females is currently unknown. In terms of GnRH release, social status influences the basic electrical properties of GnRH+ cells [28]. For example, GnRH-secreting neurons in NT males have significantly longer action potential durations and show delayed after-hyperpolarization compared to the same neurons in T males [28]. These properties could limit the cells' maximum firing rate; in a variety of neuroendocrine cells, secretion is accomplished via action potential bursts, which synergistically enhance the efficacy of release [28–30]. Therefore, a capped maximum firing rate in neurons from NT males could decrease the amount of GnRH released per burst and reduce the overall efficiency of GnRH release [28]. Accordingly, neurons from NT males tend to fire less rapidly in response to an injected current [28]. Additional work is needed to determine whether the electrical properties of GnRH-ir neurons differ between spawning and brooding females, and whether the electrophysiological properties of GnRH-ir cells in convict cichlid males also differ depending on social status.

Despite the different reproductive strategies between convict cichlids and *A. burtoni*, the results of our study mostly mirror those from past *A. burtoni* studies. In both species, T males have larger GnRH+ cells and GSIs than NT males [14,16]. Given that the most aggressive/dominant convict males (and females) are usually the first to pair-bond and spawn [31], it is unsurprising that territorial convict males have the largest GnRH+ cells and GSIs. Whether social status affects GnRH+ soma size in female convicts is currently unknown. We found that spawning convicts, like their *A. burtoni* counterparts, have larger GnRH+ cells than brooders [18]. Notably, while *A. burtoni* spawners had higher GSIs than brooders [18], our data on convict females did not match this pattern, for reasons already detailed above. Because the most aggressive convict females are normally the first to pair-bond [31], aggression clearly plays a role in female convict reproduction. One possibility is, like males, social status regulates GnRH+ soma size in females. That is, after a female gains social dominance, the GnRH-secreting cells in her POA enlarge, and her eggs begin to develop. Another possibility is that GnRH+ soma size does not depend on social state. Instead, either other exogenous factors (e.g. food availability, water, presence of males) prime the enlargement of GnRH-secreting cells, or these cells change size according to some internal cycle and are not affected by exogenous signals. In any case, only when the female

eggs' are fully (or close to) developed and she is ready to spawn does her aggression and territoriality increase. Evidence from laboratory studies seems to support the latter possibility. Several days before a female is ready to spawn, she follows the male, exchanges courtship displays with him, and aggressively chases away any females that approach him [31]. After a pair bond is formed, the female and male together look for spawning sites and on the day of spawning, they aggressively defend their chosen site [31]. As egg production requires more time and is more energetically expensive than sperm [32], it may be more advantageous for the female to focus her energy on egg reproduction rather than on agonistic interactions, and find male only when she is ready to spawn. Regardless, additional experiments are needed to determine whether social status manipulations will change GnRH+ soma size in convict females.

Our results indicate that, indeed GnRH+ neuron soma size, and not cell number, varies with both male territorial status, and female breeding state, in this monogamous, biparental, New World cichlid. Further work, on this and other species with varying mating strategies, will be required to fully elucidate the relationship between social status, sex and neuronal plasticity of GnRH expressing POA cells.

Acknowledgements

ANI and JMPP supported by NSERC and Alberta Ingenuity Fund, PLH and DRWW supported by NSERC Discovery Grants. The authors than Cristian Gutiérrez-Ibáñez for his help with the preparation of the manuscript.

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