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Sex steroid hormones modulate responses to social challenge and opportunity in males of the monogamous convict cichlid, *Amatitliana nigrofasciata*

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ABSTRACT

Steroid hormones play an important role in modulating behavioral responses to various social stimuli. It has been suggested that variation in the hormonal regulation of behavior across species is associated with social organization and/or mating system. In order to further elucidate the interplay of hormones and behavior in social situations, we exposed males of the monogamous convict cichlid *Amatitliana nigrofasciata* to three social stimuli: gravid female, intruder male, and a nonsocial stimulus. We used a repeated measure design to create behavioral profiles and explore how sex steroid hormones respond to and regulate social behavior. Results show distinct behavioral responses to different social situations, with circulating 11-ketotestosterone increasing in response to social stimuli. Pharmacological manipulations using specific androgen and estrogen receptor agonists and antagonists exposed complex control over digging behavior in the social opportunity context. In the social challenge context, aggressive behaviors decreased in response to blocking the androgen receptor pathway. Our results extend our understanding of sex steroid regulation of behavioral responses to social stimulation.

1. Introduction

The endocrine mechanisms that underlie behavioral responses to social stimulation have been studied in much detail over the last three decades (Goncalves and Oliveira, 2011; Munakata and Kobayashi, 2010). Many such studies have focused on steroid hormone responses in a single social context, such as male–male competitive interactions (Oliveira et al., 2002) but see (Amstislavskaya and Popova, 2004; O'Connell et al., in press) for androgen responses in males exposed to reproductive females. Thus, the physiological and behavioral responses to specific social contexts are well known, yet few studies have manipulated sex steroid hormone pathways and examined responses to different social contexts in the same individuals.

The role of sex steroid hormones in regulating social behavior has been well studied (Goncalves and Oliveira, 2011; Munakata and Kobayashi, 2010). Circulating androgen levels in particular are well known to be involved in male social behavior and vary with season (Wingfield, 1990), conspecific behavior (Oliveira et al., 2002; Villars, 1983), and mating system (Oliveira et al., 2001). Their involvement in affiliative and sexual behavior has been shown in mammals (Pasch et al., 2010), birds (Wingfield et al., 2001), and fish (Desjardins et al., 2008; O'Connell and Hofmann, 2012) and androgen levels can be affected either by aggressive conspecifics (Villars, 1983) or reproductive opportunities such as gravid or receptive females (Amstislavskaya and Popova, 2004; Dulka et al., 1987; O'Connell et al., in press). Additionally, reproductive behaviors such as courtship signals in lizards (Cooper et al., 1987), mounting in house mice (James and Nyby, 2002), and nesting in fish (Pall et al., 2002) can be elicited by the administration of androgens either directly or induced via female pheromones. While the classic association between androgens and increased aggression has been confirmed in many species (Nelson and Trainor, 2007), including cichlid fish (Fernald, 1976; Munro and Pitcher, 1985; Ros et al., 2003), there is increasing evidence that estrogen can also elicit aggressive behavior (mice: Ogawa et al., 1997; song sparrows: (Soma et al., 2000); cichlid fish: (O'Connell and Hofmann, 2012), likely via the aromatization of testosterone to estradiol (Trainor et al., 2006).

Pharmacological manipulations of sex steroid hormone receptors allow us to clarify whether androgens are influencing behavior directly via the androgen receptor or indirectly via the estrogen receptor. For example, the administration of the androgen receptor (AR) antagonist flutamide is sufficient to block reproductive behaviors in stickleback fish (Sebire et al., 2008). In the model cichlid fish

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Astatotilapia burtoni, ARs modulate courtship behavior, but estrogen receptor (ER) pathways modulate aggression (O'Connell and Hofmann, 2012). Importantly, the dynamics of pathway manipulations can be complex, involving factors such as social status and mating system (Hegner and Wingfield, 1987; O'Connell and Hofmann, 2012), and the resulting behavioral changes are typically examined in different animals and at the level of particular behavioral displays rather than entire suites of behavior (Vagell and McGinnis, 1998). However, how steroid hormone pathways regulate behavior across different social contexts in the same individuals has rarely been studied.

Here, we use the convict cichlid, *Amatitlania nigrofasciata* in order to better understand the interplay between sex steroid hormones and behavioral response across different social contexts. This teleost fish is native to lakes and streams from Guatemala to Panama (Mackereth and Keenleyside, 1993) and has become a useful model system in behavioral ecology (Kortmulder, 1998; Munro and Pitcher, 1985). Males and females form monogamous pairbonds and defend a nesting territory where the gravid female will attach her eggs to the substrate to be fertilized by the male. Both males and females participate in parental care which can last up to six weeks (Mackereth and Keenleyside, 1993; Noakes, 1991; O'Connell et al., 2012). The well-described behavior patterns of the convict cichlid together with recent insights into the endocrine regulation of its social behavior (Brown et al., 2003; O'Connell et al., 2012; Oldfield and Hofmann, 2011) make it an ideal model for studying the complex interplay of hormones and behavior in a monogamous species.

In the present study, we examined the role of androgens and estrogen in regulating the behavior of male convict cichlids in two social contexts as well as a nonsocial situation. We predicted that these hormones would differ between contexts and the resulting behavioral responses would be context-appropriate, such as aggression when exposed to a male intruder or courtship when exposed to a gravid female. We first examined behavior in untreated males across contexts to create behavior profiles (suites of behavior that represent how an animal responds to particular social contexts). Additionally, we measured circulating levels of testosterone (T), 17β-estradiol (E2) and 11-ketotestosterone (11-KT), an oxidized form of T that has been shown to act as the active androgen in this species (O'Connell et al., 2012) and hypothesized that hormone responses vary with social context. Finally, we carried out pharmacological manipulations of AR and ER signaling separately to test the hypothesis that these pathways directly regulate aggressive, sexual, and maybe even certain non-social behavior patterns.

2. Methods

2.1. Animals

Animals were kept on a 12:12 light:dark cycle, fed once a day with Omega One African cichlid flakes (Arcata Pet, Product No. 11154) and kept in sex-specific group tanks until the start of the experiment. On day zero, at approximately 15:00 h, focal animals were measured for standard length (SL) and body mass and then tagged in the dorsal muscle using colored beads for individual identification. To ensure consistent treatment of all experimental animals, each fish was then placed into one of four compartments in a 114 L home tank for the duration of the experiment (except for the time spent in the test tank). To avoid any behavioral abnormalities due to social isolation we allowed each test subject to interact with another experimental male across a transparent and perforated divider. All experiments were carried out with the approval of the Institutional Animal Care and Use Committee at UT Austin.

2.2. Experiment 1: hormone measures and behavior

On day four, at 15:00 h, animals ($n = 8$) were placed into a 38 L (liter) compartment of a 227 L test tank overnight (approximately 21 h). At 11:30 h, one of three stimuli was introduced: 15 cm section of PVC pipe (nonsocial), a gravid female (reproductive opportunity), or a conspecific male (challenge). Female gravidity was based on a rotund belly and has been shown to affect male mate choice in convict cichlids (Nuttall and Keenleyside, 1993). In order to avoid immediate subordination when exposed to a relatively larger individual in a novel environment, we made sure that in every case the focal animal was larger (by 20 ± 0.8% on average) than the stimulus animal. The focal animal was exposed to the stimulus for 60 min and recorded using a multi-channel video surveillance system (Altec, Austin, TX) with VideoInsight software (Enterprise IP). We then harvested approximately 50 μL of blood from the dorsal aorta of each focal male using a heparinized butterfly infusion set with a 26 gauge needle (Becton Dickinson) before returning it to its home tank. We also removed the stimulus animal from the experimental tank and measured its standard length. Blood was centrifuged at 4000 rpm for 10 min and plasma was isolated and stored at −80 °C. Circulating hormone levels were measured according to (Kidd et al., 2010) using commercial ELISA systems to measure free 11-KT (Cayman Chemical, Product No. 582751), T (Assay Designs, Product No. 900-065) and E2 (Assay Designs, Product No. 900–008) using a dilution of 1:100, 1:30 and 1:30, respectively. Due to the small size of the animals the blood collection procedure did not always yield sufficient plasma for all three assays.

Focal animals were maintained in their home tank for eight days prior to the subsequent stimulus treatment, so that each animal was exposed to all three stimuli in a randomized order, with blood drawn after each exposure. Focal animals were tested in each context and subsequently returned to the community tanks at the end of the experiment (Fig. 1A).

Video recordings were scored by an observer blinded to the treatment. We chose the 30–40 min time window of the 60 min stimulus exposure for scoring and analysis, as preliminary observations had shown that animals reliably engaged in a behavioral response within approximately 20–50 min of stimulus onset. Based on previously described ethograms (Baerends and Baerends-Van Roon, 1950; Oldfield and Hofmann, 2011), behavior patterns were categorized into the indices of aggressive, affiliative and neutral to allow a more general analysis of suites of behavior. Aggressive behavior included bite, charge and chase while affiliative and neutral behavior consisted of only kiss and foraging respectively. Behaviors such as tailbeat (when an individual rapidly waves its tail to a conspecific's face or side), frontal and lateral displays are context dependent (Baerends and Baerends-Van Roon, 1950; Oldfield and Hofmann, 2011) and thus counts for each were added into the aggressive index when expressed in a challenge situation and into the affiliative index when expressed in the opportunity situation. Approach was considered affiliative in the social contexts and neutral in the nonsocial context. Due to their ambiguous meaning, digging and vertical display (an individual puts itself into a vertical position with its rostral end up and caudal end down) behavior were analyzed separately (Baerends and Baerends-Van Roon, 1950; Oldfield and Hofmann, 2011).

2.3. Experiment 2: hormone manipulation

Forty new males were taken from the sex-specific group tanks, weighed, measured and tagged on day 0 as described above. On days 4 and 5, fish received an intraperitoneal injection of 10 μL per gram body weight (gbw) with one of five treatments: dihydrotestosterone (DHT) (Sigma), 17β-estradiol (Steraloids), ER

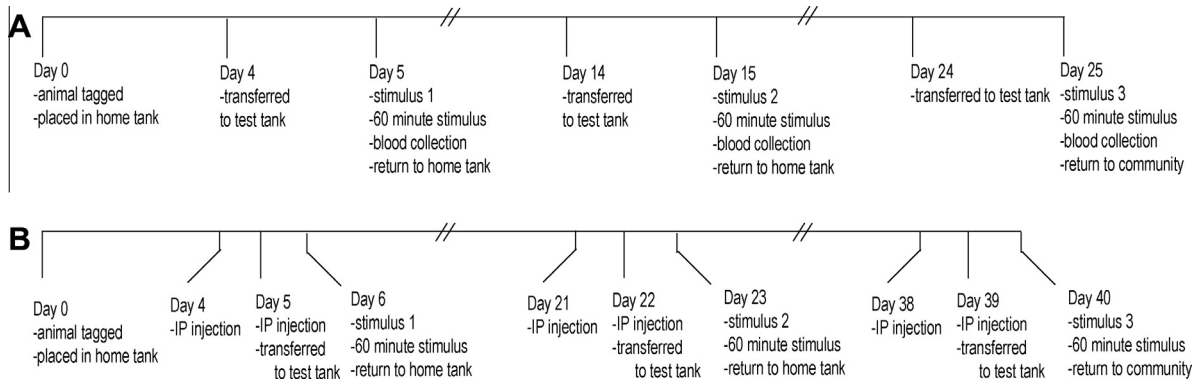

Fig. 1. Timelines for behavioral testing and pharmacological manipulation. (A) Timeline indicates order of transfers between tanks, observation periods, and blood collections for (A) Experiment 1 and (B) Experiment 2, respectively.

antagonist ICI182780 (Sigma), AR antagonist cyproterone acetate (Sigma) or a vehicle (mineral oil) as a control. The multiple-day regimen avoided measuring non-genomic responses of the steroids (Remage-Healey and Bass, 2006). DHT is not aromatizable and binds AR with even higher affinity than 11-KT (Sperry and Thomas, 1999; Wells and van der Kraak, 2000). Antagonists used have been shown to bind the appropriate teleost sex steroid receptors (ICI182780: Hawkins and Thomas, 2004; cyproterone acetate: Wells and van der Kraak, 2000). The antiestrogen binds both ERα and ERβ receptor subtypes (Howell et al., 2000). Doses and the repeated-injection scheduled were based on previous studies in a related cichlid (O'Connell and Hofmann, 2012): 0.13 μg/gbw DHT, 0.4 μg/gbw E2, 1.6 μg/gbw ICI182780, and 0.83 μg/gbw cyproterone acetate (1 mg/gbw: Kramer et al., 1969; Oliveira et al., 2009). Each fish received the same drug treatment throughout the experiment and the researcher was blinded to the treatment.

After the first injection, animals were returned to their home tank. After the second injection, animals were put into the test tank overnight with stimuli presented the next day (as described in Section 2.2) and recorded for 60 min. After this time, the stimulus was removed and measured while the focal animal was returned to its home tank. After a recovery period of two weeks, the process was repeated on days 21–23 and again on days 38–40 with the remaining stimuli (Fig. 1B). Video recordings were scored by an observer blinded to the treatment for behavior patterns that occurred during the time window of 30 and 40 min after stimulus onset, as described above.

2.4. Statistical analysis

All statistical analyses were conducted using Predictive Analytics SoftWare (PASW) Statistics 18.

2.4.1. Behavioral response to stimulus

A repeated measure ANOVA was run to determine significant differences in behavior between social contexts. When sphericity was not assumed for the dependent variable, Greenhouse–Gaussian was used. A univariate ANOVA with Tukey post hoc test was run to explore the effects of each context on behavior and behavior index. Because the frequency distributions of aggressive behavior across all contexts were not normal, we employed non-parametric statistics using the Related-Samples Friedman's Two-Way Analysis of Variance by Ranks and then further investigated between each pair of contexts using Related-Samples Wilcoxon Signed Ranks Test.

2.4.2. Hormonal response to stimulus

E2 (Shapiro–Wilk normality test; $n = 23$; $W_{before} = 0.8952$, $p = 0.0202$; $W_{after} = 0.9467$, $p = 0.2496$) and 11-KT ($n = 22$; $W_{before} = 0.7933$, $p = 0.0004$, $n = 22$; $W_{after} = 0.9915$, $p = 0.9991$) levels were not normally distributed and were therefore log transformed. For differences between contexts and hormone measures, a repeated measures ANOVA was run with week order (order in which stimulus were presented) as a covariate. T levels showed a bimodal distribution and were analyzed across all three contexts using a Related-Samples Friedman's Analysis of Variance by Ranks. In addition to exploring each social context individually, analysis was carried out on nonsocial category (PVC alone) versus social category (challenge and opportunity combined) using the student's *t*-test.

2.4.3. Hormone–behavior correlations

Pearson correlations were calculated between E2, 11-KT, individual behavior, affiliative and neutral behavior indices and size measures. Correlations between aggressive behavior, T and aforementioned variables were carried out using Kendall's tau due to non-normally distributed data. Additional analyses were explored on indices of social versus nonsocial context with the appropriate correlation algorithm. After correcting for false discovery rate (Benjamini and Hochberg, 1995), correlations reported had significance of $p < 0.05$.

2.4.4. Hormone receptor manipulations

A general linear model was designed for each drug–behavior combination using a stepwise selection procedure of elimination. Covariates included the week order and the size asymmetry between focal and stimulus animal with all two-way interaction effects included. The models were run between all drugs and control (mineral oil), between each agonist–antagonist pair for behavioral indices and also each individual behavior. A Mann–Whitney *U* test for independent samples was used for inter-drug analysis of aggressive behavior due to a bimodal distribution of aggressive behavior.

In order to analyze the effect of drug injection versus no drug/untreated animals with regards to vertical display behavior, counts from mineral oil-treated animals in Experiment 2 (control animals) and untreated (i.e., all) animals from Experiment 1 were collapsed together into a non-drug treated group. A univariate ANOVA was used to detect significant differences in the number of vertical behavioral patterns expressed between non-drug treated and drug treated animals.

Table 1
ANOVA statistics for behavior counts and hormone levels across all social and non-social contexts.

Response variable	*n*	df	Statistic	*p*
Aggressive behavior[a]	8	2	12.08	2.00E-03
Affiliatiative behavior[b]	8	1	14.874	0.006
Neutral behavior[b]	8	1.122	14.88	0.004
Total behaviors[b]	8	2	4.827	2.90E-02
Testosterone[a]	8	2	0.75	0.687
Estradiol[b]	7	1.053	1.864	0.229
11-Ketotestosterone[b]	6	2	2.174	0.176

[a] Related-Samples Freidman's Two-Way ANOVA by Ranks.
[b] Repeated measures ANOVA.

3. Results

3.1. Experiment 1: male behavioral and hormonal responses to different stimuli

Neutral behavior was different between contexts (ANOVA $F_{1.12,8} = 14.880$, $p = 0.004$); Table 1) with more neutral behavior in the nonsocial context than in the two social contexts (Tukey HSD, $p = 0.0009$; Fig. 2A). Across-context effects were also seen in affiliative behavior (ANOVA, $F_{1,8} = 14.874$, $p = 0.006$; Table 1) with more affiliative behavior when exposed to a gravid female than to a male intruder or nonsocial stimulus ($p = 0.0004$; Fig. 2B). Also, aggressive behavior varied significantly across contexts (Friedman's $T(2) = 12.08$, $p = 0.002$; Table 1) with significantly more aggressive behavior when exposed to an intruder than in either the nonsocial context (Wilcoxon signed rank test: $T_8 = 36.0$, $p = 0.012$) or when exposed to a gravid female ($T_8 = 35.0$, $p = 0.017$; Fig. 2C). Digging behavior varied across contexts ($F_{2,8} = 4.849$, $p = 0.025$) with a gravid female eliciting more digs than nonsocial exposure ($p < 0.038$), and a trend compared to male intruder ($p < 0.052$). Vertical display behavior also was affected by stimulus ($F_{2,8} = 5.830$, $p = 0.014$) with significantly more displays in the reproductive context than in the intruder and nonsocial stimuli ($p < 0.02$).

There were no significant effects of stimulus on circulating levels of *T* [Friedman's $T_{2,8} = 0.750$, $p = 0.687$], E2 [$F_{1.053,8} = 1.864$, $p = 0.229$], or 11-KT levels [$F_{2,8} = 2.174$, $p = 0.176$] (Fig. 3A–C; Table 1), although 11-KT levels appeared to be higher in social contexts (Fig. 3C). Indeed, when we combined the data from the two social contexts, 11-KT was significantly higher in a social versus a nonsocial situation ($t_{21} = 5.463$, $p = 2.03 * 10^{-5}$; Fig. 3D).

We found that across all three stimulus contexts there was a significant correlation between 11KT levels and digging behavior (Pearson's $r = 0.462$, $p = 0.030$, $n = 22$) and between *T* and E2 (Kendall's tau-b = 0.494, $p < 0.001$, $n = 23$). No stimulus-specific correlations remained significant after adjustment for multiple hypothesis testing.

3.2. Effects of pharmacological manipulation on behavior

There were no significant differences in behavioral indices or in individual behaviors when comparing drug-treated to control animals. However, in the non-social context, approach behavior occurred less frequently in DHT-treated animals compared to animals treated with an AR antagonist ($F_{1,8} = 16.34$, $p = 0.001$; Fig. 4A).

There were no significant differences in behavioral indices or individual behaviors when comparing either agonist to its antagonist. However, we found that in the social opportunity context digging behavior increased with DHT ($F_{1,8} = 5.03$, $p = 0.042$), E2 ($F_{1,8} = 10.41$, $p = 0.009$), and ER antagonist ($F_{1,8} = 10.646$, $p = 0.007$) treatment (Fig. 4B). Interestingly, the week order also significantly affected digging behavior of ER antagonist-treated animals ($F_{1,8} = 10.239$, $p = 0.008$) when compared to control, and there was a significant interaction effect between drug treatment and week order ($F_{1,8} = 9.90$, $p = 0.008$).

When comparing an agonist and its antagonist, there were no significant differences in any behavioral index or individual behavior. However, in the social challenge context, the number of aggressive displays was significantly lower in the AR antagonist treatment compared to the controls (Mann–Whitney $U = 52.0$, $n_1 = n_2 = 8$, $p = 0.036$; Fig. 4C1). The number of vertical displays showed a non-significant increase in all drug-treated groups compared to vehicle-treated control (Fig. 4C2). This difference was significant when all drug treatments (D) were combined and compared to non-drug (ND) treated animals (ANOVA, $F_1 = 6.55$, $n_D = 32$, $n_{ND} = 16$, $p = 0.014$).

4. Discussion

In the present study, we exposed convict cichlid males to both reproductive and intruder contexts in addition to a nonsocial treatment. Our experimental paradigm included two of the most important social contexts that all animals face: challenges, such as competition for resources, and opportunities, such as reproduction. We observed distinct behavioral and hormonal responses to these

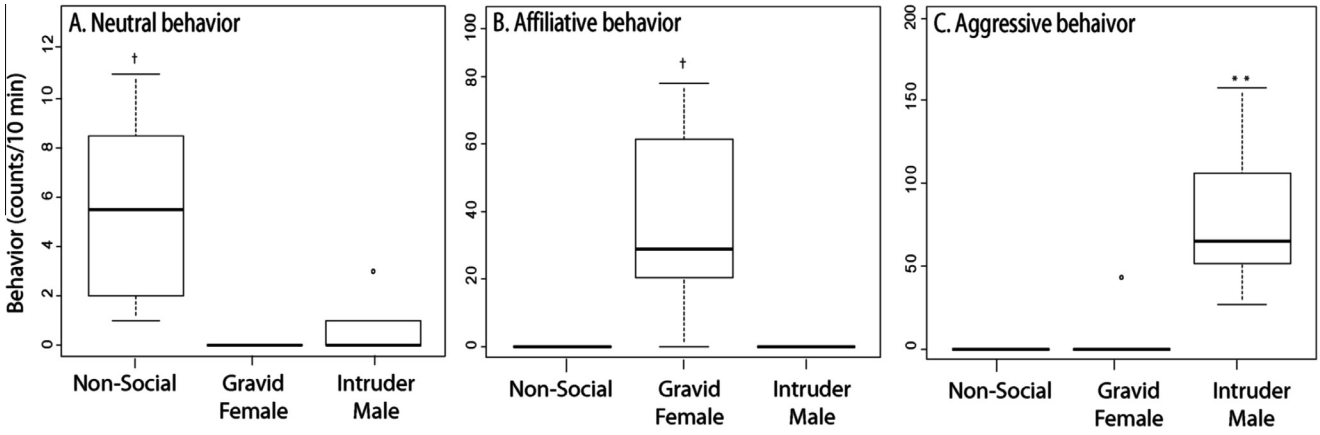

Fig. 2. Convict cichlid males exhibit context-specific behavioral profiles. Box-and-whisker plots show that (A) neural behavior counts are highest in a non-social context, (B) affiliative behaviors counts are highest when exposed to a gravid female, and (C) aggressive behavior counts are highest when exposed to a male intruder. $n = 8$ for all groups. †: $p < 0.001$, **: $p < 0.01$.

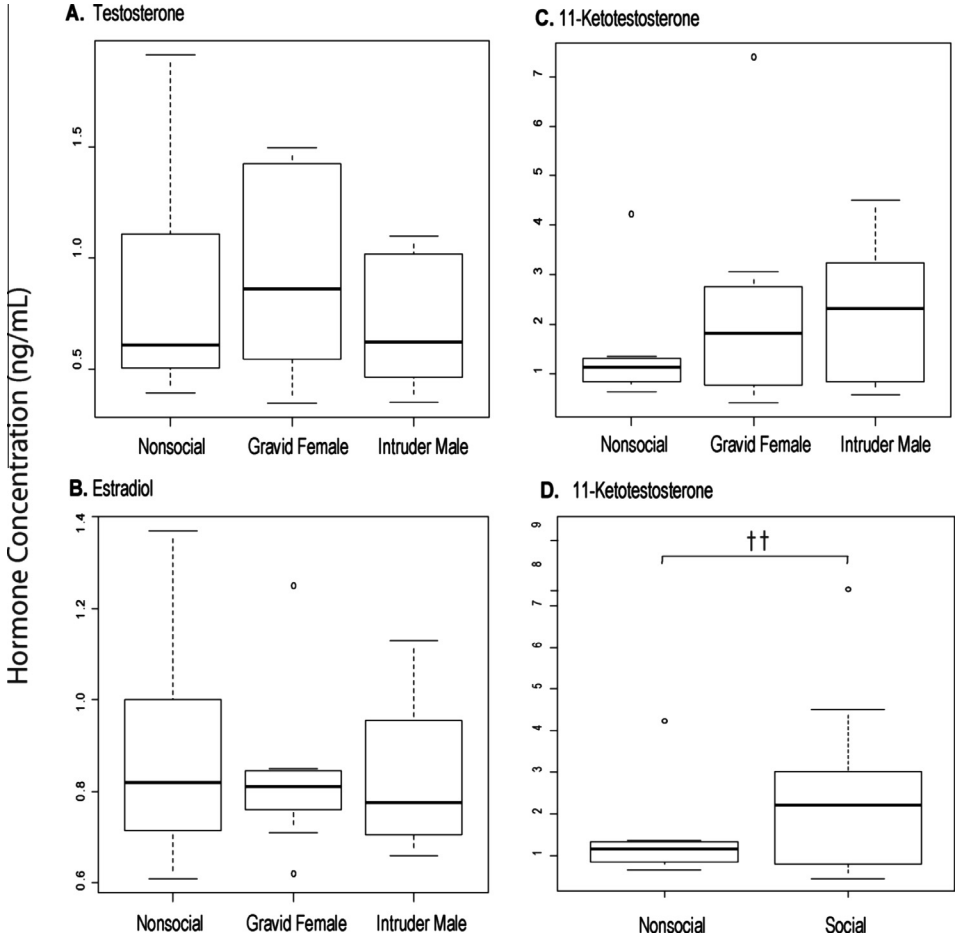

Fig. 3. Circulating hormone levels vary between non-social and social contexts. Box-and-whisker plots show free (A) testosterone, (B) estradiol, and (C) 11-ketotestosterone levels across three social contexts and (D) 11-ketotestosterone levels between non-social and social situations. ††: $p < 1 * 10^{-4}$.

stimuli. Furthermore, we determined the role of estrogen and androgen receptor pathways in regulating these responses.

When exposed to a social or non-social stimulus, males exhibited clear context-appropriate responses. Not surprisingly, social situations induced an 11-KT response, confirming previous studies showing that 11-KT acts as an active androgen in convict cichlid males (O'Connell et al., 2012; Wong, 2008). Androgen responses to social situations have been measured in the circulation as soon as 30 min after stimulus onset (Maruska and Fernald, 2010) and in urine and fish-holding water as long as four hours later (Hirschenhauser et al., 2004). We do not know when the hormone response reaches its maximum in our study species or whether the 60 min time point used in our experiment is close to it. Additionally, variation in the behavior of the stimulus animals (i.e., the intensity of the challenge) could have influenced the focal males' androgen responses as well (Villars, 1983). Our finding that presenting a male with a gravid female causes an 11-KT response is consistent with similar findings in other species (Amstislavskaya and Popova, 2004; Nomura et al., 2002) and provides further evidence that androgens regulate male sexual behavior.

In the presence of a female, digging likely presents a reproductive behavior in terms of preparing a breeding site (Baerends and Baerends-Van Roon, 1950). We can only speculate about the function of digging behavior in the nonsocial situation. While it could be interpreted as displacement activity in response to a stressor (Kortmulder, 1998), it might also serve the preparation of a nest site in case a gravid female becomes available, or it could serve to provide shelter considering that much time was spent in these dugouts (data not shown).

The association between estrogen and social behavior is quite complex, involving factors such as different ER sub-types, social experience, genotype and age (Nomura et al., 2002; Ogawa et al., 1998a, 1998b, 1999). We have shown here that in the convict cichlid circulating levels of E2 and *T* were correlated, as has previously been observed in other species (Kidd et al., 2010; O'Connell and Hofmann, 2012; Phillips et al., 1996; Renn et al., 2012). Estrogen administration affected digging behavior specifically, but not other aspects of reproductive behavior. This finding is similar to the situation in mice, where particular behaviors but not entire suites of behavior were abolished with the knockout of the ERα gene (Ogawa et al., 2000).

Exogenous androgens can increase aggressive behavior (Fernald, 1976; Munro and Pitcher, 1985; Nelson and Chiavegatto, 2001), either directly or via aromatization to estrogen (Trainor et al., 2006; Wu et al., 2009). Our results show that activating the AR pathway did not result in any significant behavioral changes in the social contexts, while blocking the pathway decreased aggression, suggesting that the AR pathway may mediate aggression in some fashion. Surprisingly, androgen and estrogen manipulation did not affect courtship or aggressive behavior, respectively as is seen in other species (Hull et al., 2002; Nelson and Trainor, 2007). These interactions clearly require additional experimentation, such as altering drug doses or duration of drug administration.

In the nonsocial context, AR pathway activation resulted in a decrease of approach behavior, which can be considered a measure of overall locomotor activity. This would, however, be contrary to previous studies, which showed that androgen pathway activation

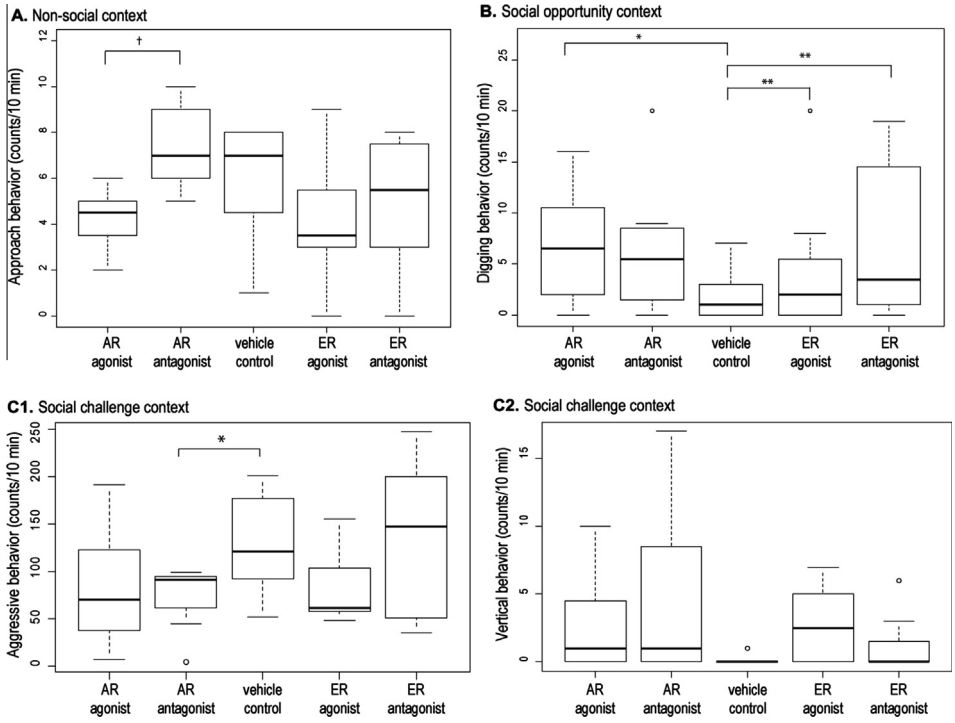

Fig. 4. Effects of steroid hormone manipulations on behavior vary depending on experimental context. Androgen receptor (AR) and estrogen receptor (ER) manipulations alter the frequency of (A) approach behavior in the non-social context and (B) digging behavior in the social opportunity context (C1) aggressive behavior and (C2) vertical displays in the social challenge context, as depicted by box-and-whisker plots. AR agonist: dihydrotestosterone; AR antagonist: cyproterone acetate; Vehicle control: mineral oil; ER agonist: 17β-estradiol; ER antagonist: ICI82780, ∗: $p < 0.05$, ∗∗: $p < 0.01$, †: $p < 0.001$.

increases locomotor activity (Ellis and Turek, 1983; Wada, 1982). Alternatively, this behavioral change could also indicate anxiety-like processes as approach behavior was typically preceded and followed by hiding behind the PVC pipe. Although it is currently speculative, results in rats also show an increase in anxiety-like behavior in response to an AR antagonist (Edinger and Frye, 2006) and a decrease in response to androgen administration (Osborne et al., 2009).

Interestingly, the AR agonist, ER agonist, and ER antagonist elicited an increase in digging behavior in the reproductive opportunity context but had no influence on this behavior in the other contexts. Moreover, the effect of ER antagonist depended on the temporal order of drug administration. These results suggest that the function and the hormonal control of this behavior are both context-dependent. Further investigation is needed to explain the increase in digging behavior with both ER agonist and antagonist, which could be partially due to the involvement of different ER subtypes, as seen in mice (Ogawa et al., 2000), or altered ratios between androgens and estrogens in the system as a consequence of drug treatment.

The pharmacological effects on vertical display behavior were maybe most striking. This behavior has classically been interpreted as appeasement behavior (Baerends and Baerends-Van Roon, 1950), especially in reproductive interactions between males and females. Consistent with this interpretation, we observed this behavior pattern in non-treated animals when exposed to a gravid female but never in the male intruder context. Drug treatment did not affect this behavior in the gravid female context but, to our great surprise, significantly increased it in the intruder context. Importantly, drug-induced vertical displays in the intruder context were immediately preceded and followed by very aggressive chasing and biting, something that was almost never observed in conjunction with this behavior in animals exposed to a gravid female (personal observation). Future work will need to examine this association more closely in order to determine whether this alternation between putative appeasement and aggressive behaviors might reflect a motivational conflict in males whose sex steroid receptor pathways have been perturbed.

5. Conclusion

The experiments presented here disentangle the context-specific relationships between sex steroid hormones and certain social behaviors in male convict cichlids. We report novel effects of these pathways on social and, potentially, anxiety-like behavior. Males displayed context-specific behaviors along with increased circulating 11-KT in response to social stimuli. Also, approach, aggressive, and reproductive behaviors were regulated by androgen and estrogen receptor pathways in a context-dependent manner. Taken together, our work provides insights into steroid regulation of behavior in response to reproductive opportunity and intruder challenge in a natural model system and suggests numerous avenues for future research.

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References

Amstislavskaya, T.G., Popova, N.K., 2004. Female-induced sexual arousal in male mice and rats: behavioral and testosterone response. Horm. Behav. 46, 544–550.

Baerends, G.P., Baerends-Van Roon, J.M., 1950. An introduction to the study of the ethology of cichlid fishes. Behav. Suppl. 1, 1–243.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57, 289–300.

Brown, G.E., Adrian Jr., J.C., Naderi, N.T., Harvey, M.C., Kelly, J.M., 2003. Nitrogen oxides elicit antipredator responses in juvenile channel catfish, but not in convict cichlids or rainbow trout: conservation of the ostariophysan alarm pheromone. J. Chem. Ecol. 29, 1781–1796.

Cooper, W.E., Mendonca, M.T., Vitt, L.J., 1987. Induction of orange head coloration and activation of courtship and aggression by testosterone in the male broad-headed skink (*Eumeces laticeps*). J. Herpetol. 21, 96–101.

Desjardins, J.K., Stiver, K.A., Fitzpatrick, J.L., Millican, N., Kraak, G.J.V.D., Balshine, S., 2008. Sex and status in a cooperative breeding fish: behavior and androgens. Behav. Ecol. Sociobiol. 62, 785–794.

Dulka, J.G., Stacey, N.E., Sorenson, P.W., Kraak, G.J.V.D., 1987. A steroid sex pheromone synchronizes male–female spawning readiness in goldfish. Nature 325, 251–253.

Edinger, K.L., Frye, C.A., 2006. Intrahippocampal administration of an androgen receptor antagonist, flutamide, can increase anxiety-like behavior in intact and DHT-replaced male rats. Horm. Behav. 50, 216–222.

Ellis, G.B., Turek, F.W., 1983. Testosterone and photoperiod interact to regulate locomotor activity in male hamsters. Horm. Behav. 17, 66–75.

Fernald, R.D., 1976. The effect of testosterone on the behavior and coloration of adult male cichlid fish (*Haplochromis burtoni*, Gunther). Horm. Res. 7, 172–178.

Goncalves, D.M., Oliveira, R.F., 2011. Hormones and sexual behavior of teleost fishes. In: Norris, D.O., Lopez, K.H. (Eds.), Hormones and Reproduction of Vertebrates. Academic Press, New York, pp. 119–147.

Hawkins, M.B., Thomas, P., 2004. The unusual binding properties of the third distinct teleost estrogen receptor subtype ERbetaa are accompanied by highly conserved amino acid changes in the ligand binding domain. Endocrinology 145, 2968–2977.

Hegner, R.E., Wingfield, J.C., 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. The Auk 104, 462–469.

Hirschenhauser, K., Taborsky, M., Oliveira, T., Canario, A.V.M., Oliveira, R.F., 2004. A test of the 'challenge hypothesis' in cichlid fish: simulated partner and territory intruder experiments. Anim. Behav. 68, 741–750.

Howell, A., Osborne, C.K., Morris, C., Wakeling, A.E., 2000. ICI 182,780 (Faslodex): development of a novel, "pure" antiestrogen. Cancer 89, 817–825.

Hull, E.M., Meisel, R.L., Sachs, B.D., 2002. Male sexual behavior. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), Hormones, Brain and Behavior. Academic Press, New York, pp. 3–137.

James, P.J., Nyby, J.G., 2002. Testosterone rapidly affects the expression of copulatory behavior in house mice (Mus musculus). Physiol. Behav. 75, 287–294.

Kidd, C.E., Kidd, M.R., Hofmann, H.A., 2010. Measuring multiple hormones from a single water sample using enzyme immunoassays. Gen. Comp. Endocrinol. 165, 277–285.

Kortmulder, K., 1998. Displacement behaviour: solving a silent contradiction. Acta Biotheor. 46, 53–63.

Kramer, B., Molenda, W., Fiedler, K., 1969. Behavioural effect of the antiandrogen cyproterone acetate (Schering) in Tilapia mossambica and Lepomis gibbosus. Gen. Comp. Endocrinol. 13, 515.

Mackereth, R.W., Keenleyside, M.H.A., 1993. Breeding, territoriality, and pair formation in the convict cichlid (Cichlasoma nigrofasciatum; Pisces, Cichlidae). Can. J. Zool. 71, 960–967.

Maruska, K.P., Fernald, R.D., 2010. Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. Horm. Behav. 58, 230–240.

Munakata, A., Kobayashi, M., 2010. Endocrine control of sexual behavior in teleost fish. Gen. Comp. Endocrinol. 165, 456–468.

Munro, A.D., Pitcher, T.J., 1985. Steroid hormones and agonistic behavior in a cichlid teleost, Aequidens pulcher. Horm. Behav. 19, 353–371.

Nelson, R.J., Chiavegatto, S., 2001. Molecular basis of aggression. Trends Neurosci. 24, 713–719.

Nelson, R.J., Trainor, B.C., 2007. Neural mechanisms of aggression. Nat. Rev. Neurosci. 8, 536–546.

Noakes, D.L.G., 1991. Ontogeny of behaviour in cichlids. In: Keenleyside, M. (Ed.), Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman and Hall, London.

Nomura, M., Durbak, L., Chan, J., Smithies, O., Gustafsson, J.A., Korach, K.S., Pfaff, D.W., Ogawa, S., 2002. Genotype/age interactions on aggressive behavior in gonadally intact estrogen receptor beta knockout (betaERKO) male mice. Horm. Behav. 41, 288–296.

Nuttall, D.B., Keenleyside, M.H.A., 1993. Mate choice by the male convict cichlid (Cichlasoma nigrofasciatum; Pisces, Cichlidae). Ethology 95, 247–256.

O'Connell L.A., Rigney M.M., Dykstra D.W., Hofmann H.A., 2013. Neuroendocrine mechanisms underlying sensory integration of social signals. J Neuroendocrinol, in press. http://dx.doi.org/10.1111/jne.12045. [Epub ahead of print].

O'Connell, L.A., Hofmann, H.A., 2012. Social status predicts how sex steroid receptors regulate complex behavior across levels of biological organization. Endocrinology 153, 1341–1351.

O'Connell, L.A., Matthews, B.J., Hofmann, H.A., 2012. Isotocin regulates paternal care in a monogamous cichlid fish. Horm. Behav. 61, 725–733.

Ogawa, S., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1997. Behavioral effects of estrogen receptor gene disruption in male mice. Proc. Natl. Acad. Sci. USA 94, 1476–1481.

Ogawa, S., Eng, V., Taylor, J., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1998a. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. Endocrinology 139, 5070–5081.

Ogawa, S., Washburn, T.F., Taylor, J., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1998b. Modifications of testosterone-dependent behaviors by estrogen receptor-alpha gene disruption in male mice. Endocrinology 139, 5058–5069.

Ogawa, S., Chan, J., Chester, A.E., Gustafsson, J.A., Korach, K.S., Pfaff, D.W., 1999. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. Proc. Natl. Acad. Sci. USA 96, 12887–12892.

Ogawa, S., Chester, A.E., Hewitt, S.C., Walker, V.R., Gustafsson, J.A., Smithies, O., Korach, K.S., Pfaff, D.W., 2000. Abolition of male sexual behaviors in mice lacking estrogen receptors alpha and beta (alpha beta ERKO). Proc. Natl. Acad. Sci. USA 97, 14737–14741.

Oldfield, R.G., Hofmann, H.A., 2011. Neuropeptide regulation of social behavior in a monogamous cichlid fish. Phys. Behav. 102, 296–303.

Oliveira, R.F., Ros, A.F.H., Hirschenhauser, K., Canario, A.V.M., 2001. Androgens and mating systems in fish: intra- and interspecific analyses. In: Goos, H.J.T., Rastogi, R.K., Vaudry, H., Pierantoni, R. (Eds.), Perspectives in Comparative Endocrinology: Unity and Diversity. Monduzzi Editore, Sorrento, pp. 985–993.

Oliveira, R.F., Hirschenhauser, K., Carneiro, L.A., Canario, A.V., 2002. Social modulation of androgen levels in male teleost fish. Comp. Biochem. Physiol. 132, 203–215.

Oliveira, R.F., Silva, A., Canario, A.V., 2009. Why do winners keep winning? Androgen mediation of winner but not loser effects in cichlid fish. Proc. Biol. Sci. 276, 2249–2256.

Osborne, D.M., Edinger, K., Frye, C.A., 2009. Chronic administration of androgens with actions at estrogen receptor beta have anti-anxiety and cognitive-enhancing effects in male rats. Age (Dordrecht, Netherlands) 31, 191–198.

Pall, M.K., Mayer, I., Borg, B., 2002. Androgen and behavior in the male three-spined stickleback, Gasterosteus aculeatus. II. Castration and 11-ketoandrostenedione effects on courtship and parental care during the nesting cycle. Horm. Behav. 42, 337–344.

Pasch, B., George, A.S., Hamlin, H.J., Guillette Jr., L.J., Phelps, S.M., 2010. Androgens modulate song effort and aggression in Neotropical singing mice. Horm. Behav. 59, 90–97.

Phillips, G.B., Pinkernell, B.H., Jing, T.Y., 1996. The association of hyperestrogenemia with coronary thrombosis in men. Arterioscler. Thromb. Vasc. Biol. 16, 1383–1387.

Remage-Healey, L., Bass, A.H., 2006. A rapid neuromodulatory role for steroid hormones in the control of reproductive behavior. Brain Res. 1126, 27–35.

Renn, S.C., Fraser, E.J., Aubin-Horth, N., Trainor, B.C., Hofmann, H.A., 2012. Females of an African cichlid fish display male-typical social dominance behavior and elevated androgens in the absence of males. Horm. Behav. 61, 496–503.

Ros, A.F., Canario, A.V., Couto, E., Zeilstra, I., Oliveira, R.F., 2003. Endocrine correlates of intra-specific variation in the mating system of the St. Peter's fish (*Sarotherodon galilaeus*). Horm. Behav. 44, 365–373.

Sebire, M., Allen, Y., Bersuder, P., Katsiadaki, I., 2008. The model anti-androgen flutamide suppresses the expression of typical male stickleback reproductive behaviour. Aquat. Toxicol. (Amsterdam, Netherlands) 90, 37–47.

Soma, K.K., Tramontin, A.D., Wingfield, J.C., 2000. Oestrogen regulates male aggression in the non-breeding season. Proc. Biol. Sci. 267, 1089–1096.

Sperry, T.S., Thomas, P., 1999. Characterization of two nuclear androgen receptors in Atlantic croaker: comparison of their biochemical properties and binding specificities. Endocrinology 140, 1602–1611.

Trainor, B.C., Kyomen, H.H., Marler, C.A., 2006. Estrogenic encounters: how interactions between aromatase and the environment modulate aggression. Front. Neurondocrinol. 27, 170–179.

Vagell, M.E., McGinnis, M.Y., 1998. The role of gonadal steroid receptor activation in the restoration of sociosexual behavior in adult male rats. Horm. Behav. 33, 163–179.

Villars, T.A., 1983. Hormones and aggressive behavior in teleost fishes. In: Svare, B.B. (Ed.), Hormones and Aggressive Behavior. Plenum Press, New York, pp. 407–433.

Wada, M., 1982. Effects of sex steroids on calling, locomotor activity, and sexual behavior in castrated male Japanese quail. Horm. Behav. 16, 147–157.

Wells, K., van der Kraak, G.J., 2000. Differential binding of endogenous steroids and chemicals to androgen receptors in rainbow trout and goldfish. Environ. Toxicol. Chem. 19, 2059–2065.

Wingfield, J.G., 1990. The challenge hypothesis: theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. Am. Nat. 136, 829–846.

Wingfield, J.C., Lynn, S., Soma, K.K., 2001. Avoiding the 'costs' of testosterone: ecological bases of hormone–behavior interactions. Brain Behav. Evol. 57, 239–251.

Wong, S., 2008. The Bidirectional Effects of Hormones on Aggressive Behavior in the Convict Cichlid Fish (*Amatitliana nigrofasciata*). California State University.

Wu, M.V., Manoli, D.S., Fraser, E.J., Coats, J.K., Tollkuhn, J., Honda, S., Harada, N., Shah, N.M., 2009. Estrogen masculinizes neural pathways and sex-specific behaviors. Cell 139, 61–72.