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Social instability promotes hormone–behavior associated patterns in a cichlid fish

Olinda Almeida [a,1], Eliane Gonçalves-de-Freitas [b,1], João S. Lopes [a,c], Rui F. Oliveira [a,c,*]

[a] Unidade de Investigação em Eco-Etologia, ISPA - Instituto Universitário, Rua Jardim do Tabaco, 34, 1149-041 Lisboa, Portugal
[b] Instituto de Biociências, Letras e Ciências Exatas, UNESP, and Aquaculture Center of UNESP (CAUNESP)., Rua Cristóvão Colombo, 2265, 15054-000 São José do Rio Preto, SP, Brazil
[c] Champalimaud Neuroscience Programme, Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras, Portugal

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ABSTRACT

Androgens are known to respond to social challenges and to control the expression of social behavior and reproductive traits, such as gonadal maturation and sperm production, expression of secondary sex characters and reproductive behaviors. According to the challenge hypothesis variation in androgen levels above a breeding baseline should be explained by the regime of social challenges faced by the individual considering the trade-offs of androgens with other traits (e.g. parental care). One prediction that can be derived from the challenge hypothesis is that androgen levels should increase in response to social instability. Moreover, considering that a tighter association of relevant traits is expected in periods of environmental instability, we also predict that in unstable environments the degree of correlations among different behaviors should increase and hormones and behavior should be associated. These predictions were tested in a polygamous cichlid fish (Mozambique tilapia, *Oreochromis mossambicus*) with exclusive maternal care. Social instability was produced by swapping dominant males among groups. Stable treatment consisted in removing and placing back dominant males in the same group, in order to control for handling stress. Cortisol levels were also measured to monitor stress levels involved in the procedure and their relation to the androgen patterns and behavior. As predicted androgen levels increased in males in response to the establishment of a social hierarchy and presence of receptive females. However, there were no further differential increases in androgen levels over the social manipulation phase between social stable and social unstable groups. As predicted behaviors were significantly more correlated among themselves in the unstable than in the stable treatment and an associated hormone–behavior pattern was only observed in the unstable treatment.

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Introduction

Hormones play a key role in the physiological adaptation of organisms to their environments. On one hand hormones are physiological integrators that interact with multiple systems and with each other, therefore controlling suites of morphological and behavioral traits. On the other hand, they respond to different environmental cues, hence adjusting phenotypic form and function to predictable (e.g. seasonal) and unpredictable (e.g. stressor) environmental changes (Cohen et al., 2012). Androgens are known to regulate spermatogenesis, the expression of secondary sex characters and reproductive and territorial behavior on one hand, and to respond to predictable changes that signal the breeding season in temperate species, therefore linking the expression of reproductive traits to the appropriate environmental context (Adkins-Regan, 2005). Additionally, androgens also respond to short-term changes in the social environment (e.g. mating opportunity, territorial intrusion; Wingfield et al., 1990; Hirschenhauser and Oliveira, 2006; Goymann, 2009) and these have been interpreted as a mechanism to fine-tune androgen-dependent traits to acute and transient changes in the environment (Oliveira, 2009; Oyegbile and Marler, 2005). The challenge hypothesis (Wingfield et al., 1990) has been proposed as an explanatory framework for the observed variation in androgen responsiveness to the environment. According to this hypothesis androgen levels rise above a constitutive baseline during the breeding season triggered by environmental cues (e.g. photoperiod), and this breeding baseline is both necessary and sufficient for successful breeding (i.e. spermatogenesis, expression of secondary sex characters and reproductive behavior). Further increases in androgen levels above this breeding baseline would be explained by the degree of social stimulation that males are exposed to, and would not be required for breeding. Given the detrimental effects of androgens on male parental care behavior, this androgen responsiveness to social stimulation would also have a trade-off with paternal care (Wingfield et al., 1990, 2001). Therefore, the challenge hypothesis has predicted that androgen responsiveness to social challenges would vary with mating systems and

* Corresponding author at: Unidade de Investigação em Eco-Etologia, ISPA - Instituto Universitário, Rua Jardim do Tabaco, 34, 1149-041 Lisboa, Portugal.
E-mail address: ruiol@ispa.pt (R.F. Oliveira).
[1] These authors contributed equally to this work.

parental type. Androgen responsiveness should be higher in monoga-mous (that are expected to face less social challenges) than in polyga-mous species, and in species with biparental or paternal care than in species with maternal care only (Wingfield et al., 1990). These predic-tions have been supported by comparative data in different vertebrate taxa (fish: Oliveira et al., 2002; Hirschenhauser et al., 2004; birds: Hirschenhauser et al., 2003; Goymann, 2009). Further hypotheses have been subsequently proposed in an attempt to explain notable cases of lack of androgen modulation by the social environment. For example, the lack of androgen responsiveness in males with short-breeding seasons or single brooded has been interpreted as an adapta-tion to facilitate a rapid transition from the mating to the parental care phase (Goymann, 2009; Landys et al., 2007; Wingfield and Hunt, 2002). Similarly, the exceptions to the predictions derived from the costs of androgens on parental care have been tentatively explained by discriminating species in which male parental care is critical for off-spring survival (i.e. essential paternal care hypothesis, Lynn et al., 2002).

Despite the adaptive hypotheses described above for patterns of hormone–behavior association there are many cases in which hor-mones become dissociated from putatively hormone-dependent traits. For example, aggressiveness and androgen levels may become season-ally dissociated in species that express aggressive behavior outside the breeding season when androgen levels are low (e.g. Apfelbeck et al., 2013; Canoine and Gwinner, 2002; Dittami and Reyer, 1984; Landys et al., 2010; Logan and Wingfield, 1990; Wingfield, 1994). It has also been noted that the strength of the correlations between androgens and behavior seems to be higher at periods of social instability, such as during the establishment of territories or dominance hierarchies, the response to territorial intrusions or the competition with other males for access to mating opportunities, than during periods of social stability when behavioral output drops to baseline levels (Oliveira et al., 2002 and references therein). This socially-driven temporal varia-tion in the association between hormones and behavior within the same species may reflect the dual role of hormones on phenotypic inte-gration (i.e. when multiple functionally-related traits are correlated with each other) and independence (i.e. when multiple functionally-related traits are independent of each other; McGlothlin and Ketterson, 2008; Ketterson et al., 2009). As described above for andro-gens, the co-regulation of different traits by the same hormone gives rise to correlations among traits, which become linked in their response to the environment hence promoting phenotypic integration. However, in certain conditions the independent response of some of the correlat-ed traits could be advantageous (e.g. need to express androgen-dependent aggression outside the breeding season). Therefore, it would be adaptive if the pleiotropic effects of hormones could vary be-tween different life-history stages, or even within the same life-history stage depending on the regime of environmental challenge, in order to offer the best compromise given the environmental demands faced by the organism. Indeed, heterogeneous environments have been shown to favor plasticity in the correlation structures among phenotypic traits, and patterns of integration may vary across environments (Earley et al., 2012; Schlichting, 1989). Therefore, we predict that plasticity in hormone-driven phenotypic integration should vary with social stabili-ty, such that unstable social environments characterized by a high rate of social challenges that require a tighter phenotypic integration would promote associated hormone–behavior patterns, whereas in sta-ble social environments (with low rates of social challenges) where phenotypic integration can be relaxed hormone–behavior patterns can become dissociated.

Here we tested this hypothesis in a lek-breeding African cichlid fish (Mozambique tilapia, Oreochromis mossambicus). In this species males establish territories in breeding aggregations to which they attract fe-males to spawn with, and parental care is exclusively provided by the females (Baerends and Baerends-van Roon, 1950). Territorial males adopt a dark nuptial coloration which may vary in intensity and that can be turned on and off within minutes. Androgen responsiveness to

social challenges has been previously reported in this species (Oliveira et al., 1996) and its magnitude in comparison to that observed in other cichlid species with different mating systems and parental care types matched the predictions of the challenge hypothesis (Hirschenhauser et al., 2004). After the establishment of mixed-sex breeding groups social manipulations created either unstable or stable social environments. For social instability dominant males were swapped among groups. For social stability the dominant male of each group was removed and placed back in its own group, in order to con-trol for handling stress. It was predicted that group formation would trigger an androgen response and that hormone and behavior would become correlated. After the social manipulations it was predicted that the unstable treatment would promote hormone–behavior associ-ated patterns, whereas the stable treatment would promote a dissociat-ed hormone–behavior pattern. Apart from the males that were part of the social groups, bystander males that were visually exposed to the so-cial groups but prevented from engaging in social interactions were also tested, in order to assess if the mere perception of social context is enough to promote the predicted responses.

Methods

Animals and housing

Adult Mozambique tilapia (O. mossambicus) from a stock held at ISPA - Instituto Universitário (Lisbon, Portugal) was used. Stock fish were kept in mixed-sex groups (3–4 males to 4–5 females per tank) in glass tanks ($120 \times 40 \times 50$ cm), with fine gravel substrate, a double filtering system (both sand and external biofilter, Eheim) and constant aeration. Water quality was monitored once a week for nitrites (0.2–0.5 ppm), ammonia (<0.5 ppm) (Palintest kit®) and pH (6.0–6.2). Water temperature was kept at 26 ± 2 °C and photoperiod was 12L:12D. Fish were fed ad libitum daily with commercial cichlid floating and sinking sticks.

Experimental procedures

Mixed-sex groups (2 males + 2 females) and 1 bystander male were tested in social stable or unstable social environments. Fish were observed in aquaria with two compartments, a larger one (40 cm $\times 75$ cm $\times 50$ cm) to receive males and females, and a smaller one (40 cm $\times 25$ cm $\times 50$ cm) to house the bystander male (Fig. 1A). The two compartments were divided by a one-way mirror, which allowed the bystander to observe the social group without being seen by them, and the water to circulate between the two compartments. To improve the unidirectional properties of the one-way mirror a light bulb was placed on top of the group compartment, and direct illumina-tion of the bystander's compartment was prevented by using a black opaque plastic around it (except in the frontal wall of the tank to allow observing the fish), so that the bystander's compartment was illu-minated through the unidirectional glass. The one-way mirror formed a mirror image for fish in the group compartment with which they could interact. In fact, in the first day dominant males interacted aggressively with their mirror image in the one-way-mirror. However, they subse-quently habituated to the mirror and such interactions were greatly re-duced in the following days, when males occasionally approached the one-way mirror without displaying any aggressive behavior.

The experimental procedure consisted of the following 4 phases (Fig. 1B):

Phase 1   Social isolation — forty five males were isolated in 40 l aquaria for 7 days to dilute previous social experience.

Phase 2   Group formation — afterwards, two size-matched males [mean $\pm$ standard deviation (SD) of standard length (SL); difference between dominant and subordinate = 0.38 + 0.26 cm; stable group: SL = 12.88 $\pm$ 2.44 cm, coefficient of variation (CV) =