Hormones and Behavior 58 (2010) 230–240

Contents lists available at ScienceDirect

Hormones and Behavior

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

Behavioral and physiological plasticity: Rapid changes during social ascent in an African cichlid fish

Karen P. Maruska *, Russell D. Fernald

Biology Department, Stanford University, 371 Serra Mall, Stanford, CA 94305, USA

ARTICLE INFO

Article history:
Received 13 January 2010
Revised 10 March 2010
Accepted 10 March 2010
Available online 18 March 2010

Keywords:
11-ketotestosterone
Androgen
Astatotilapia burtoni
Challenge hypothesis
Dominance
Reproductive behavior
Social ascension
Territorial behavior

ABSTRACT

In many vertebrates, reproduction is regulated by social interactions in which dominant males control access to females and food. Subordinate males that displace dominant individuals must rapidly adopt behavioral and physiological traits of the higher rank to gain reproductive success. To understand the process of phenotypic plasticity during social ascent, we analyzed the temporal expression pattern of dominance behaviors and circulating androgen levels when socially-suppressed males of an African cichlid fish *Astatotilapia burtoni* ascended in status. These experiments tested a prediction of the 'challenge hypothesis' that, during periods of social instability, male androgen levels are higher than during socially stable times. We found that socially and reproductively suppressed males perform territorial and reproductive behaviors within minutes of an opportunity to ascend in status, and that animals switch from initial expression of territorial behaviors to more reproductive behaviors during territory establishment. Following this rapid response, social stability may be achieved within 1–3 days of social ascent. Consistent with predictions of the 'challenge hypothesis', circulating 11-ketotestosterone (11-KT) levels were elevated within 30 min following social opportunity, coincident with increased aggressive behavior. However, territorial behaviors and serum 11-KT levels were then dissociated by 72 h after social ascent, suggesting either rapid social stability and/or increased physiological potential for androgen production. This behavioral and physiological plasticity in male *A. burtoni* suggests that perception of social opportunity triggers a suite of quick changes to facilitate rapid transition towards reproductive success, and reveals important features of social ascent not previously recognized.

Introduction

Social interactions can have profound effects on the behavior, physiology, and reproductive capacities of animals, especially in species where a dominance hierarchy is socially regulated. Typically in vertebrates, low social rank is associated with submissive behaviors and reduced reproductive opportunities, while high-ranking individuals show dominant behaviors and have high reproductive success (Creel et al., 2002; Ellis, 1995; Fernald, 2009; Ryder et al., 2009; Sapolsky, 2005). However, dynamic social interactions and changing physical habitats due to disturbance or variable environmental conditions offer subordinate individuals opportunities to displace higher-ranking dominant ones. Such shifts in social status can be accompanied by dramatic changes in the brain that may occur more slowly than changes in behavior and physiology (Burmeister et al., 2005; White et al., 2002). What is the precise timing of the changes in these phenotypic traits? Here we asked how quickly behavioral and physiological changes occur during social transitions. We exploited a particularly useful model system, the highly social African cichlid fish, *Astatotilapia burtoni*, to test how perception of social opportunity influences changes in behavior and circulating steroid levels.

A. burtoni is endemic to Lake Tanganyika, Africa where it lives in shallow shore pools in a lek-like social system. Males exist in one of two phenotypes: 1) dominant territorial males (~10–30% of population) are brightly colored, aggressively defend a spawning territory, and actively court and spawn with females; and 2) subordinate non-territorial males resemble and school with females, express submissive behaviors, and do not court females (Fernald and Hirata, 1977). Males can rapidly and reversibly switch between dominant and subordinate states depending on the composition of the social environment, and such transformations produce a suite of behavioral and physiological changes (Fernald, 2009). When subordinate males ascend in social status, they intensify their body coloration and increase dominance behaviors within minutes (Burmeister et al., 2005). Further, within 20 min, the immediate early gene *egr*-1 is up-regulated in the gonadotropin-releasing hormone neurons (GnRH1) located in the preoptic area of the brain (Burmeister et al., 2005). These neurons are the neural gateway to the entire reproductive axis in all vertebrates. In contrast to this rapid behavioral and brain genomic response, other physiological changes such as increases in

* Corresponding author. Fax: +1 650 724 4980.
E-mail address: maruska@stanford.edu (K.P. Maruska).

0018-506X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2010.03.011

GnRH1 neuron size, GnRH1 synthesis, and testis growth can take up to a week or more to achieve dominant male levels (White et al., 2002). It may be advantageous for subordinate males to quickly display dominance coloration and behaviors to secure a territory in a dynamic social environment even though their reproductive axis may be up-regulated more slowly.

What role do androgens play in this status transformation? Androgens are known as critical regulators of male reproduction in all vertebrates, typically increasing concomitantly with reproductive-related behavior and physiology. However, circulating androgen levels are also influenced by social interactions with conspecifics, or by watching the interactions of others (Cardwell and Liley, 1991; Dzieweczynski et al., 2006; Hirschenhauser and Oliveira, 2006; Hirschenhauser et al., 2004; Oliveira et al., 2002; Oliveira et al., 2001). Generally, androgen levels are higher in dominant compared to subordinate individuals within a population (Oliveira, 2009; Oliveira et al., 2002; Parikh et al., 2006), but the relationship between androgen levels and social status also depends on other factors. For example, Wingfield et al. (1990) proposed the 'challenge hypothesis' which predicts that androgen levels also depend on aggressive interactions over status rank and the stability of the social environment. In *A. burtoni*, dominant males have higher circulating levels of both testosterone (T) and the important fish androgen 11-ketotestosterone (11-KT) compared to subordinates (Parikh et al., 2006). Moreover, intramuscular injections of T can increase aggressive behaviors among males (Fernald, 1976). These data are consistent with the prediction that stable dominant males involved in more aggressive interactions defending their territory will have higher circulating androgens that can also directly influence behavior. However, previous studies measured androgen levels only in stable male phenotypes and little is known about how dominance behaviors and androgen levels change during times of social transition and instability.

Another prediction of the 'challenge hypothesis' is that male androgen levels should be higher during periods of social instability (i.e., during territory establishment and status changes within a dominance hierarchy) when compared to more stable conditions. We therefore predicted that aggression levels would be correlated with circulating androgen levels when fish recognize an opportunity to ascend in social rank, but that aggressive behaviors and androgens will be dissociated as the social environment temporally stabilizes. In *A. burtoni*, we can control precisely social stability and the timing of social transitions, making it an ideal model to test predictions of the 'challenge hypothesis'. However, *A. burtoni* also differs from many previous models used to examine the 'challenge hypothesis' because 1) it is not a seasonal breeder, but rather, reproductive fitness is dictated by social status, and the ability to reproduce depends on both male–male territorial and male–female sexual interactions; and 2) subordinate males have small testes in relation to their body size, which poses a potential physiological constraint to androgen production. These variables represent two of the recently proposed factors to include in future tests of the 'challenge hypothesis', namely, the androgen response to male–female interactions, and the physiological potential to produce and secrete androgens (Goymann et al., 2007).

Although Burmeister et al. (2005) showed previously that ascending subordinate *A. burtoni* males display dominance behaviors within minutes, these data were sampled only during the 20 min following the first dominance behaviors because the newly ascended males were then sacrificed to measure immediate early gene mRNA levels. Thus, the expression of territorial and reproductive behaviors over subsequent days as the ascended males' status stabilized was unknown. Burmeister et al. (2005) also described for the first time that even dominant males apparently began their typical aggressive behaviors anew each day. Therefore, the goal of this study was to examine in detail the temporal expression pattern of dominance behaviors and circulating androgen levels when socially-suppressed *A. burtoni* males ascended in status. This allowed us to test a prediction of the 'challenge hypothesis' that during periods of social instability and territory establishment, male androgen levels are higher than during times of social stability.

Methods

Animals

Laboratory-bred male cichlid fish *A. burtoni*, derived from wild-caught stock collected in Lake Tanganyika, Africa, in the 1970's were maintained in aquaria under environmental conditions that mimic their natural equatorial habitat (28 °C; pH 8.0; 12 h light:12 h dark with full spectrum illumination; constant aeration), and fed cichlid pellets and flakes (AquaDine, Healdsburg, CA, USA) each morning. Aquaria contained gravel-covered bottoms with half terra cotta pots that served as spawning territories. All experimental procedures were approved by the Stanford Administrative Panel for Laboratory Animal Care.

Social manipulation

We used a paradigm similar to that described by Burmeister et al. (2005) to create an opportunity for social ascent by subordinate males, except that our subjects were previously suppressed for 4–5 weeks rather than 2 weeks (Fig. 1). The 4–5 week period was chosen to ensure full suppression of the entire reproductive axis, and mimics social situations in the wild and the laboratory (Fernald and Hirata, 1977; Hofmann et al., 1999). All subject males used in this study were dominant for at least 3 weeks prior to use in experiments, but other details on past breeding history was unknown. To create socially and reproductively suppressed males for the ascension paradigm, larger dominant males were used to suppress smaller subject males. Specifically, dominant subject males (standard length: 6.23 ± 0.52 cm; body mass: 7.52 ± 0.21 g) from community tanks were placed into aquaria for 4–5 weeks with several larger dominant suppressor males (2–4; SL ~ 7.5–9.0 cm), females (6–10; SL ~ 4.5–6.5 cm), and subordinate males (3–4; SL ~ 5.0–6.5 cm). Suppression of subject fish was verified by focal observations to confirm subordinate behaviors (e.g., fleeing) and submissive coloration as previously described (Burmeister et al., 2005). At the end of the suppression period, subjects were moved into the central compartment of an experimental tank that contained one larger resident dominant male (~7.5–9.0 cm) and 4 females (~5.0–6.5 cm). This central compartment was isolated from community tanks on either side that contained multiple dominant males, subordinate males, and females, with transparent acrylic barriers so that fish could interact visually but not physically. All dominant males in adjacent community tanks were smaller in size than the suppressed subject male to ensure his ascension upon presentation of social opportunity.

Subject males remained in the experimental tank for 2 days during which time we confirmed with behavioral observations that they remained suppressed by the larger resident male (e.g., performed no territorial or reproductive behaviors, and fled from suppressor male). On the day of ascension, the resident suppressor male was removed with a net in the dark 1 h prior to light onset using infrared night vision goggles. This protocol minimized disturbance in the tank and meant that visual absence of the suppressor occurred consistently only at light onset for all tested individuals.

Stable dominant and stable subordinate males were also used as control comparisons to males ascending in social status. Stable subordinate males were suppressed in community tanks for 4–5 weeks and transferred to the experimental tank as described above. On the day of ascension, the net was dipped into the tank prior to light onset to replicate any disturbance caused by catching the resident male. The dominant resident was not removed however, which kept the subject male in subordinate status. Stable dominants