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Androgens enhance plasticity of an electric communication signal in female knifefish, *Brachyhypopomus pinnicaudatus*

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ABSTRACT

Sex steroids were initially defined by their actions shaping sexually dimorphic behavioral patterns. More recently scientists have begun exploring the role of steroids in determining sex differences in behavioral plasticity. We investigated the role of androgens in potentiating circadian, pharmacological, and socially-induced plasticity in the amplitude and duration of electric organ discharges (EODs) of female gymnotiform fish. We first challenged female fish with injections of serotonin (5-HT) and adrenocorticotropic hormone (ACTH), and with social encounters with female and male conspecifics to characterize females' pre-implant responses to each treatment. Each individual was then implanted with a pellet containing dihydrotestosterone (DHT) concentrations of 0.0, 0.03, 0.1, 0.3, or 1.0 mg 10 g^{-1} body weight. We then repeated all challenges and compared each female's pre- and post-implant responses. The highest implant dose enhanced EOD duration modulations in response to all challenge types, responses to male challenge were also greater at the second highest dose, and responses to ACTH challenge were enhanced in females receiving all but the smallest dose (and blank) implants. Alternatively, amplitude modulations were enhanced only during female challenges and only when females received the highest DHT dose. Our results highlight the differential regulation of EOD duration and amplitude, and suggest that DHT enhanced the intrinsic plasticity of the electrogenic cells that produce the EOD rather than modifying behavioral phenotypes. The relative failure of DHT to enhance EOD amplitude plasticity also implies that factors other than androgens are involved in regulating/promoting male-typical EOD circadian rhythms and waveform modulations displayed in social contexts.

Females and males frequently differ in their responses to inter- and intrasexual social encounters with conspecifics and in their sensitivities to hormones that regulate social behaviors. These sexual dimorphisms are often the behavioral accompaniment to a species' reproductive system (Andersson, 1994; Kelley, 1988) where they augment sex-typical behavioral responses by exaggerating physiological or morphological trait differences or by imposing limits on such trait expression in one sex or the other. Gonadal steroid hormones play a major role in shaping these behavioral patterns both during development and at sexual maturity (Adkins-Regan, 1981, 1998; Arnold and Breedlove, 1985; Balthazart et al., 1996; Crews and Silver, 1985; Goncalves et al., 2008; Goy and McEwen, 1980; Kelley, 1988; Kendrick and Schlinger, 1996; Kime, 1993; Rhen and Crews, 2000). Artificially altered levels of sex steroids can masculinize or feminize physiology and morphology depending on which hormones are introduced, the sex and developmental stage of the individuals exposed, and sensitivity of particular tissues to the hormones (Brenowitz and Lent, 2002; Cooke et al., 1998; Herfeld and Moller, 1998; Lund et al., 2006; Staub and Beer, 1997; Wilson and Davies, 2007). These changes in structure are often accompanied by changes in behavior. These roles of androgens in sexual differentiation are well documented, but their function in potentiating behavioral plasticity, or the range of responsiveness to various stimuli, is an active area of investigation. Recent studies on teleost fishes, however, suggest that androgens may be more efficient at masculinizing morphologies and physical traits or a fraction of male typical behaviors than they are at activating the totality of male behavioral repertoires (Lee and Bass, 2005; Oliveira et al., 2001, 2005).

Among teleosts, the electric communication systems of electric fishes are excellent models for investigating the roles of androgens in potentiating sexually dimorphic behavioral plasticity versus organizing morphology. Gymnotiform and mormyrid fishes produce weak electric organ discharges (EODs) for electrolocation and electrocommunication (Bennett, 1961; Hopkins, 1983; Lissman, 1958) and the ontogenetic development of sexually dimorphic EODs is controlled by sex steroids (Zakon, 2000). Extended durations in male EOD waveforms compared to female EODs is a commonly recurring sexual dimorphism in pulse-type fish (Bass and Hopkins, 1983, 1985; Franchina, 1997; Hagedorn and Carr, 1985; Mills and Zakon, 1991; Stoddard et al., 2006). When sexual dimorphisms in EOD waveform exist, extended durations of male

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EODs have been linked to high social status, higher levels of circulating androgens, and increased conspicuousness to predators (Carlson et al., 2000; Franchina et al., 2001; Hagedorn and Zelick, 1989; Hanika and Kramer, 1999, 2000; Kramer, 1997). Additionally, in species with sexually dimorphic EODs, plasma levels of testosterone and 11-ketotestosterone are often correlated with masculine waveforms, estradiol profiles are correlated with female and juvenile waveforms, and sexually mature female EODs can be 'masculinized' by administering exogenous androgens (Bass and Hopkins, 1983, 1985; Dulka and Maler, 1994; Dulka et al., 1995; Dunlap et al., 1997; Dunlap and Zakon, 1998; Hagedorn and Carr, 1985; Hagedorn and Heiligenberg, 1985; Meyer, 1983; Silva et al., 1999; Zakon et al., 1991). The composite literature makes it clear that androgens are correlated with production of male-like EOD waveforms, yet our insight into how higher androgen levels interact with various physiological and social environments is limited.

The weakly electric gymnotiform fish, *Brachyhypopomus pinnicaudatus* (Hopkins, 1991) produces sinusoidal biphasic EODs with prominent sexual dimorphism in the duration of the second phase (P2) (Hagedorn, 1988; Hopkins et al., 1990; Westby, 1988). Waveform amplitude and duration of P2 oscillate with circadian rhythms that free-run under constant light or constant dark and both EOD parameters increase over minutes in response to social interactions (Franchina et al., 2001; Franchina and Stoddard, 1998; Hagedorn, 1995; Silva et al., 1999; Stoddard et al., 2003; Stoddard et al., 2007). Previous studies of short-term waveform flexibility and circadian oscillations have focused on male waveforms and their responses to social and pharmacological challenges (Allee et al., 2008; Hagedorn and Zelick, 1989; Markham and Stoddard, 2005; Stoddard et al., 2003); however both sexes modulate their EODs in response to stressors and social stimuli (Franchina et al., 2001; Markham and Stoddard, 2005; Salazar and Stoddard, 2007; Silva et al., 1999; Stoddard et al., 2003) and express circadian rhythms in EOD waveform structure (Franchina and Stoddard, 1998; Hagedorn, 1995; Silva et al., 2007; Stoddard et al., 2007).

In every context studied to date, females' EOD waveform modulations are smaller and less consistent than those of males, suggesting androgens could be responsible for the sexually dimorphic EOD flexibility expressed in this fish. Social isolation attenuates EOD amplitude and P2 duration and social stimulation by the addition of a conspecific stimulus fish can restore waveform characters to pre-isolation levels (Franchina et al., 2001). Male tankmates are more potent than females in restoring the magnitudes of circadian oscillations of previously isolated males. These findings, combined with literature showing males of many taxa respond to same-sex social challenges by increasing circulating androgens (Oliveira et al., 2002; Wingfield et al., 1990), suggest that androgens might regulate differences in behavioral responses to social interactions as well as the magnitude of circadian oscillation in EOD waveform (Stoddard et al., 2003).

However, sex steroids are not the sole humoral regulators of the EOD waveform, which suggests plasticity in behavioral displays may be controlled entirely by other factors or by interactions between androgens and these factors. Melanocortin peptides act directly on electrocytes to increase amplitude and P2 duration of the EOD waveform over a time scale of minutes (Markham et al., 2009; Markham and Stoddard, 2005). Melanocortin injections produce waveform changes identical in shape and time course to modulations initiated by social interactions (Franchina et al., 2001; Hagedorn, 1995). Injections of three upstream regulators of circulating melanocortin levels, serotonin, CRF, and TRH, also elicit these changes in EOD waveform (Markham et al., 2009). Our goals in this study were: (1) to determine whether androgens enhance circadian rhythmicity in female EOD waveform amplitude and/or duration and (2) to determine the role of androgens in regulating behavioral plasticity in the EOD waveform in response to social challenges and to pharmacological challenges known to elicit responses mimicking normal social responses.

Methods

Animals and measurement system

Animals were adult female *B. pinnicaudatus* maintained in mixed-sex groups in 450-liter outdoor stock pools (dimensions: $185\times95\times26$ cm) on Florida International University grounds in Miami, Florida. We randomly selected mature fish from the outdoor pools, brought them indoors, and held females individually in 284 l automated EOD measurement tanks ($120\times44\times44$ cm) throughout each experiment. Test fish were fed oligochaete blackworms *ad libitum* under constant photoperiod (12L: 12D) and temperature ($28\ ^\circ C\pm1\ ^\circ C$). Experiments were approved in advance by the FIU IACUC and complied with the "Principles of Animal Care" publication No. 86-23, revised 1985, of the National Institutes of Health.

We recorded calibrated EODs from the freely swimming females at intervals of ~1 min around the clock for the *entirety* of each trial using an automated system described in detail elsewhere (Stoddard et al., 2003). Briefly, the system amplifies and digitizes EOD waveforms only when the fish swims through an unglazed ceramic tube centered in the tank between two recording electrodes. The EOD waveform of *B. pinnicaudatus* is a sinusoidal wave that varies in its amplitude and in the duration of the second phase (P2; Fig 1A); waveform features that reach their maxima during the nighttime hours. We measured the amplitude of the EOD waveform peak-to-peak and P2 duration as τ_{P2}, the time constant of an inverse exponential function fit to the decay segment of the 2nd phase of the EOD waveform (Fig. 1A).

Design overview

Experimental data were collected during January 2003–December 2008 in twelve separate trials ($n=3$–8 females per trial; median trial duration = 28 days; mean trial duration = 28.6 days). A total of 58 female subjects (not including female social challengers) were used; however data for all female-challenge combinations are not necessarily included in statistical analyses. Complications with individual fish (e.g. illness) as well as technical problems (e.g. power outages, electrode failure, etc.) sometimes resulted in missing data for particular females and/or female-challenge combinations. We therefore continued running new trials with new fish until we collected data from six females for each DHT dose and challenge type.

For each trial, we acclimated females to the testing tanks for a minimum of 24 h then ran a series of pharmacological and social challenges known to elicit EOD modulations. Each challenge was presented on a different day and provided the individual's pre-DHT implant responses to each challenge. After all pre-implant challenges had been administered; we implanted females with silicone pellets containing the non-aromatizable androgen 5α-dihydrotestosterone (DHT) and resumed continuous EOD recordings while leaving the subjects undisturbed. We re-challenged the females with the same series of challenges as before, starting on the day after the nightly EOD τ_{P2} had crested (3–7 days post-implant depending on DHT dose administered). Implant doses of 0.0, 0.03 mg, 0.1 mg, 0.3 mg, or 1.0 mg 10 g^{-1} body weight were pseudo-randomly assigned to individuals until we had minimum sample sizes of six females per DHT dose with complete before- and after-implant challenge data.

The order of challenge presentation was randomly assigned for both pre-implant and post-implant portions of the trial series prior to the start of each trial. We shuffled challenge orders when necessary to prevent unnecessary interruptions to the time-sensitive trial. Florida is the lightning capital of the United States and if a social challenge was scheduled for a day when it was unsafe to sample challengers from the pools located on the roof, we substituted a pharmacological