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Social competition affects electric signal plasticity and steroid levels in the gymnotiform fish *Brachyhypopomus gauderio*

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ABSTRACT

Sexually-selected communication signals can be used by competing males to settle contests without incurring the costs of fighting. Steroid regulation of these signals can render them as reliable indicators of a male's physiological state. We investigated how plasticity in electrocommunication signals is driven by social competition for mates, mediated by steroid hormones, and subject to the effects of past social experience. We measured the electric waveform's amplitude and duration and steroid hormone levels of male gymnotiform electric fish (*Brachyhypopomus gauderio*) following week-long periods of social isolation, and low or high social competition. To quantify the effect of social history on the modulation of the electric signal, six groups of six males experienced all three social conditions but in different order. We found that males differentially modulate their electric signals depending on the order they experienced these conditions. Thus, past social interactions affect both present and future social electric signals. Cortisol levels and the amplitude of the electric signal appeared to track the intensity of competition, while androgen levels and the duration of the electric signal only responded to the presence (low and high competition) or absence (isolation) of a social environment (low and high androgens respectively). In addition, cortisol levels were related to the body size of the males at high social competition. Taken together, these findings suggest that the capacity of males to modulate their signals in response to social competition is regulated by steroids.

Social experiences influence an animal's motivational state during present and future social interactions. Males compete fiercely or adopt alternative mating strategies when fewer mates are available, resources are limiting, and the reproductive period is short (Andersson, 1994). But contests between males consume time and energy in the best case, or can lead to injury in the worst case (Neat et al., 1998). Ritualized behaviors and reliable signals facilitate the resolution of contests while minimizing their costs (Grafen, 1990; Parker, 1974; Smith, 1973; Zahavi, 1975). Furthermore, male aggressive interactions regulate and are regulated by steroid hormones such as glucocorticosteroids and androgens (Abbott et al., 2003; Elofsson et al., 2000; Goymann and Wingfield, 2004; Oliveira et al., 2002; Overli et al., 1999; Summers and Winberg, 2006; Wingfield et al., 1987, 1990). Yet, this bidirectional relationship can result in collateral costs such as suppression of immune function, depletion of energy stores, and reduced parental care (Romero, 2004; Sapolsky et al., 2000; Wingfield et al., 1990).

Some organisms have evolved innovative adaptations to balance the benefits and costs of energetically-demanding signals and displays. Such is the case of the gymnotiform fish *Brachyhypopomus gauderio*[1] (Giora and Malabarba, 2009), which, by coupling its electric signal waveform to endocrine systems with circadian, seasonal, and behavioral drivers, can direct its expensive signal displays to the times when it might derive the greatest benefit (Salazar and Stoddard, 2008). Four features of the life history of the nocturnal gymnotiform fish *B. gauderio* make it an excellent candidate to understand the adaptive role of the circadian regulation of communication signals. First, these fish generate an electric organ discharge (EOD) to navigate, locate prey, and communicate with conspecifics in the dark. During the breeding season, not only do males have larger amplitude and longer duration EODs, but they further enhance their EODs by increasing these sex differences at night while decreasing them during the day (Franchina and Stoddard, 1998; Stoddard et al., 2007b). Second, these sexually dimorphic characters are associated with reproductive success. Gravid female *B. gauderio* preferentially associate with bigger males with larger amplitude and longer duration EODs (Curtis and Stoddard, 2003). Third, EOD circadian rhythm plasticity is sensitive to changes in the social environment.

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[1] Recently, *B. pinnicaudatus* was divided into two species. Specimens found in the northern range of its distribution remain as *B. pinnicaudatus*, while those found in the southern range are now classified as *B. gauderio*. Drs. William Crampton and David de Santana have confirmed that our laboratory colony originated from the southern species *B. gauderio*.

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Social isolation decreases the EOD circadian rhythm, and addition of a social companion to the tank of an isolated male restores the reduced EOD circadian rhythm of the isolated male to levels observed in males sampled from social groups (Franchina et al., 2001). Nevertheless, this effect is sex-specific: a male social companion induces a bigger and faster effect than a female social companion (Franchina et al., 2001). Fourth, the EOD is modulated by melanocortins (Markham et al., 2009; Markham and Stoddard, 2005) and by steroid hormones (Mills and Zakon, 1991; Stoddard et al., 2006).

The EOD operates in the same modality as the nervous system's action potentials, making it a tractable system to investigate the role of hormones in the regulation of signal production mechanisms in the context of sexually-selected communication. The EOD can be deconstructed into its waveform amplitude (voltage in mV, Fig. 1A), its waveform duration (time in ms, Fig. 1A), and its repetition rate or frequency (EODs per second). The contributions of the central and the peripheral components of the electrocommunication neural network can be dissected out (Zakon, 1998): the EOD repetition rate is controlled by the medullary pacemaker nucleus (Dye and Meyer, 1986; Kawasaki and Heiligenberg, 1989, 1990; Keller et al., 1991), while the EOD waveform's amplitude and duration are determined by the intrinsic properties of the peripheral electric organ (EO)'s electrocytes (Bennett, 1970; Bennett et al., 1967). Androgens can alter the EOD centrally (EOD repetition rate) and peripherally (EOD waveform) (Bass and Volman, 1987; Mills and Zakon, 1991; Mills et al., 1992; Stoddard et al., 2006; Zakon et al., 1991). The effects at either level are independent from each other (Few and Zakon, 2001). Furthermore, 11-ketotestosterone (11-KT) and cortisol are positively related to modulations of the EOD repetition rate in taxa with sex differences in this parameter (Dunlap, 2002; Dunlap et al., 2002).

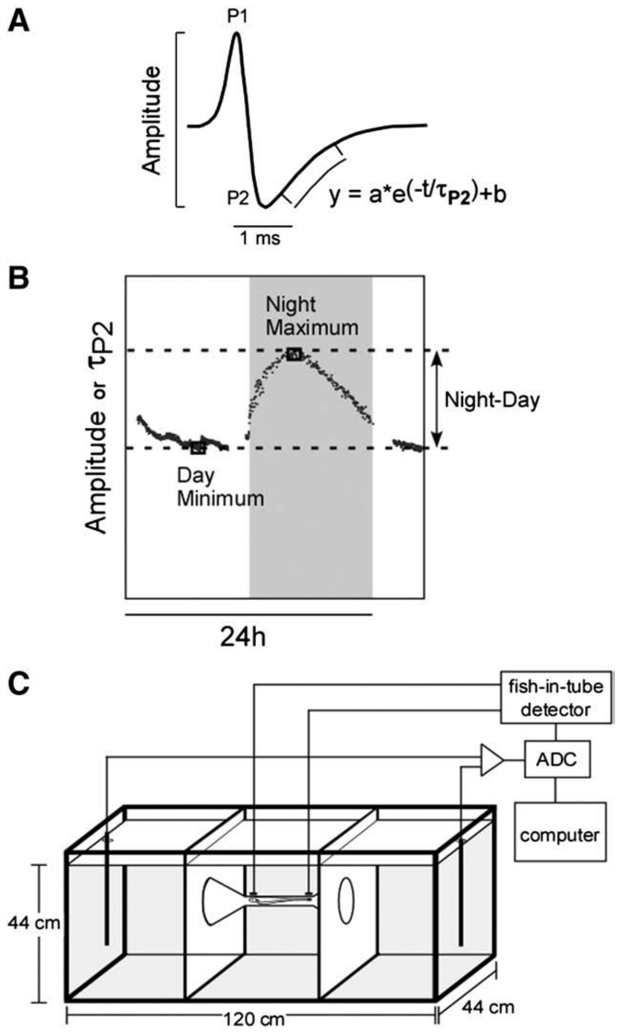

Fig. 1. EOD data acquisition. (A) The EOD of *B. gauderio* is a biphasic waveform composed of a positive phase (P1) and a negative phase (P2). We measured the peak-to-peak amplitude and the time constant of P2 repolarization (τ_{P2}). (B) To determine changes in the EOD circadian rhythm, we measured daytime low, nighttime high, and the night-to-day difference for the amplitude and τ_{P2}. (C) The tank set-up in the EOD machine automated system (Stoddard et al., 2003) allowed us to record the EOD of free-swimming fish continuously and accurately.

Increasing the density of males in a population has been shown to increase the incidence of aggressive encounters and the levels of androgens in teleost fish (Oliveira et al., 2002; Pankhurst and Barnett, 1993). Nevertheless, the effect of changing social group dynamics on steroid-regulated, condition-dependent communication signals is not well understood. In this study, we measured the EOD and the steroid hormone levels of isolated males, and males at low and high competition. Males experienced all three conditions but in different order which allowed us to quantify the effect of social history on the modulation of the EOD.

Methods

Animals

Our subjects were male *B. gauderio*, a gymnotiform pulse-type weakly electric fish native to marshes and slow waters of South America. Fish were selected randomly from a captive-reared, 11th generation breeding colony located at Florida International University, Miami, Florida. Males' body length ranged from 13.0 to 24.6 cm and females' body length ranged from 14.1 to 17.9 cm. We categorized juveniles by length (7 cm or smaller) and by the absence of sexually-mature characters (e.g., long and thick tails indicative of breeding males or swollen abdomens indicative of gravid females). Fish were reared and housed in 450-liter (185 × 95 × 26 cm) outdoor pools with water conductivity at 90 ± 10 $\mu S\ cm^{-1}$ and mean ambient temperature at 27 ± 2 °C. The water surface of each pool was covered 80–100% with water hyacinths (*Eichhornia crassipes*). Each breeding pool contained 10–20 fish. All fish were fed live oligochaete blackworms (Gulfstream Tropical Aquarium, Dania, Florida) three times per week. Experiments took place during the reproductive months, typically from May to September.

Before the beginning of the experiment, we tagged male subjects with fluorescent visible implant elastomer (VIE, Northwest Marine Technology, Inc.) for individual identification. For individual tagging, we anesthetized each fish using 0.075% 2-phenoxyethanol for 2–3 min and injected the elastomer tags on the same side of each fish caudal to the pectoral fin. The elastomer tags were injected subcutaneously following a numerical code consisting of a combination of orange, yellow and green vertical and horizontal lines approx. 2–3 mm in length (Supplementary Materials). Experiments complied with NIH ‘Principles of Animal Care’ publication no. 86-23, rev. 1985, and were approved by the FIU IACUC (protocol approval no. 07-004).

The EOD machine

This method has been described in detail by Stoddard et al. (2003). In brief, this automated system allowed us to record and perform online analysis of calibrated EODs in free-swimming fish. We placed male fish into one of the outer compartments of the recording tank (Fig. 1C) and recorded the fish's EOD only when it was positioned in the center of the tank. Every 60s, the peak-to-peak amplitude and τ_{P2} (time constant of repolarization of the 2nd phase, a measure of EOD duration) of nine consecutive EODs were recorded provided that the fish was in the center of the tank (Stoddard et al., 2003) (Fig. 1A). During the night, the EOD was sampled at irregular intervals since the fish were more active and did not necessarily swim through the center