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Cellular mechanisms of developmental and sex differences in the rapid hormonal modulation of a social communication signal

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ABSTRACT

Some gymnotiform electric fish species rapidly modify their electric signal waveforms by altering the action potential (AP) waveforms of their electrocytes, the excitable cells that produce the electric organ discharge (EOD). The fish *Brachyhypopomus gauderio* alters EOD amplitude and pulse duration as a social signal in accordance with the prevailing social conditions, under the dual regulation of melanocortins and androgens. We show here that *B. gauderio* uses two distinct cellular mechanisms to change signal amplitude, and its use of these two mechanisms varies with age and sex of the signaler. EOD amplitude and waveform are regulated by the coordinated timing and shaping of two APs generated from two opposing excitable membranes in each electrocyte. The two membranes fire in sequence within 100 μs of each other with the second AP being broader than the first. We have shown previously that mature males increase EOD amplitude and duration when melanocortin peptide hormones act directly on electrocytes to selectively broaden the second AP and increase the delay between the two APs by approximately 25 μs. Here we show that females selectively broaden only the second AP as males do, but increase amplitude of both APs with no change in delay between them, a previously unreported second mechanism of EOD amplitude change in *B. gauderio*. Juvenile fish broaden both APs and increase the delay between the APs. Cellular mechanisms of EOD plasticity are therefore shaped during development, presumably by sex steroids, becoming sexually dimorphic at maturity.

Introduction

Many species of gymnotiform electric fish display sexual dimorphism in their electric communication signals. These sex differences in the electric organ discharge (EOD) waveform are shaped by steroid hormones that determine sex differences in the action potential (AP) waveforms of electrocytes, the excitable cells of the electric organ that produce the EOD (Bass and Hopkins, 1983; Hagedorn and Carr, 1985). Some of these species also modulate the EOD waveform on a circadian rhythm (Franchina and Stoddard, 1998; Hagedorn, 1995) and within minutes of changes in the social environment (Franchina et al., 2001; Gavassa et al., 2012; Hagedorn and Zelick, 1989). These short-term modulations are regulated by circulating melanocortin peptides that modulate electrocyte AP characteristics within minutes to reshape the EOD waveform (Markham and Stoddard, 2005; Markham et al., 2009b). Perhaps more interesting is the interaction of these steroid and peptide hormone effects: beyond determining developmental changes and sex differences in tonic signal waveform, steroid hormones also influence the particular pattern of rapid waveform plasticity induced by peptide hormones (Allee et al., 2009; Goldina et al., 2011).

The EOD waveform of the gymnotiform *Brachyhypopomus gauderio* is particularly interesting in this regard because EODs in this species are sexually dimorphic and show pronounced short-term plasticity in response to environmental events. The EOD in *B. gauderio* is a biphasic pulse (Fig. 1) with an initial head-positive phase (P1) followed by a head-negative second phase (P2). This EOD waveform is sexually dimorphic at maturity: in females P1 and P2 are approximately equal in duration, while males show a pronounced extension of P2 (typically measured as its time constant of relaxation, τ_{P2}). Superimposed on these sex differences in the EOD waveform are rapid EOD modulations induced by circadian and social stimuli. Both males and females can increase their EOD amplitude and τ_{P2} rapidly, but the magnitude of these changes is larger in males. As we show here, sexually immature juveniles can also quickly augment their EOD waveforms.

The biphasic EOD is produced by the simultaneous discharges of electric organ cells (electrocytes) with dual excitable membranes that generate two opposing action potentials in close succession (Bennett, 1961) (Fig. 1). The summed action potentials from each cell produce a biphasic extracellular potential (the μEOD) and the simultaneous μEODs of electrocytes within the electric organ produce the biphasic EOD, with the EOD waveform determined primarily by the waveform and timing of the electrocytes' two APs (Bennett, 1961). We have shown in males that rapid increases in amplitude and τ_{P2} are initiated by circulating melanocortin peptides through two primary cellular mechanisms at

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Table 1
Restricted Maximum Likelihood Analysis (RMLA) results for the effect of Group on body morphology, electrocyte morphology, and electrocyte electrophysiology.

			Pairwise comparisons					
	Omnibus RMLA		Juvenile *vs.* female		Juvenile *vs.* male		Female *vs.* male	
Variable	Test statistic	*p*	d.f.	*p*	d.f.	*p*	d.f.	*p*
Weight[a]	F (2, 18) = 75.46	0.000	18	0.000	18	0.000	18	0.14
Total length[a]	F (2, 18) = 56.78	0.000	18	0.001	18	0.000	18	0.000
Filament length[a]	F (2, 18) = 44.66	0.000	18	0.01	18	0.000	18	0.000
Filament: length ratio[a]	F (2, 18) = 13.13	0.000	18	0.83	18	0.000	18	0.000
Electrocyte width	F (2, 17.14) = 36.04	0.000	17.92	0.81	16.45	0.000	16.87	0.000
Electrocyte diameter	F (2, 16.27) = 12.43	0.001	16.81	0.002	15.78	0.001	16.07	0.15
Electrocyte diameter: width ratio	F (2, 16.88) = 34.65	0.000	18.31	0.000	15.70	0.003	16.43	0.000
AP1 amplitude	F (2, 16.35) = 4.16	0.039	17.22	0.03	15.46	0.13	16.25	0.67
AP2 amplitude	F (2, 13.46) = 2.02	0.17	14.33	0.07	12.62	0.30	13.35	0.56
AP1 width	F (2, 16.86) = 1.49	0.25	17.88	0.10	15.85	0.44	16.71	0.52
AP2 width	F (2, 16.79) = 0.67	0.53	17.28	0.38	16.24	0.32	16.67	0.80
AP1 rise slope	F (2, 17.70) = 4.83	0.02	18.54	0.05	16.82	0.01	17.62	0.21
AP2 rise slope	F (2, 16.91) = 9.91	0.001	17.98	0.01	15.87	0.001	16.74	0.18
V_{rest}	F (2, 17.34) = 0.18	0.84	18.20	0.88	16.44	0.64	17.24	0.56
AP1–AP2 delay	F (2, 14.63) = 8.85	0.003	15.13	0.02	14.08	0.001	14.60	0.04
Membrane resistance	F (2, 15.91) = 29.13	0.000	16.58	0.000	15.29	0.000	15.77	0.26
Time constant	F (2, 15.89) = 0.16	0.86	16.18	0.73	15.57	0.60	15.85	0.83
μEOD amplitude	F (2, 14.55) = 9.16	0.003	15.12	0.02	13.95	0.001	14.47	0.04
P1 amplitude	F (2, 15.73) = 7.72	0.01	16.07	0.22	15.33	0.001	15.71	0.01
P2 amplitude	F (2, 14.87) = 2.14	0.15	15.20	0.14	14.47	0.80	14.85	0.57
P1 width	F (2, 17.34) = 8.24	0.003	18.02	0.16	16.61	0.001	17.29	0.01
P2 width	F (2, 16.00) = 0.35	0.71	17.25	0.85	16.69	0.43	16.98	0.52
τ_{P2}	F (2, 16.90) = .036	0.71	17.22	0.45	16.52	0.547	16.88	0.98

[a] Only one measurement per individual, so degrees of freedom are equivalent to ANOVA.

the electrocyte. Amplitude enhancements occur through an increased delay between the electrocytes' dual APs, a change that reduces the overlap of the opposing APs thereby increasing the contribution of AP1 to μEOD P1. Increases in the μEOD τ_{P2} are a function of the selective broadening of the second AP (Markham and Stoddard, 2005). Both effects are mediated by cAMP/PKA activation under the control of a G-protein coupled melanocortin receptor. No studies to date have examined the cellular mechanisms of EOD waveform in females and juveniles.

In addition to shaping sex differences in the tonic EOD waveform, steroid hormones also regulate the degree of melanocortin-induced plasticity in at least two EOD waveform parameters, amplitude and P2 duration. Administration of the non-aromatizable androgen DHT in females enhances τ_{P2} responsiveness to melanocortin injection *in vivo*, but only extremely high DHT doses enhance amplitude responsiveness (Allee et al., 2009). In mature males, implants that produce supraphysiological levels of T and 11-KT increase responsiveness of τ_{P2} to melanocortin injections, but only 11-KT increases response of EOD amplitude to α-MSH (Goldina et al., 2011). Oxygenated androgens thus regulate the degree and nature of rapid EOD plasticity, and electrocytes appear to be the convergence point where long-term effects of steroid hormones regulate the short-term effects of peptide hormones.

If rapid EOD plasticity is regulated by androgens and the locus of this regulation is the electrocyte, then developmental and sex differences in electrocyte discharge plasticity presumably reflect the historical and current effects of steroid hormones that regulate maturation and sexual differentiation. We therefore predicted that differences in the degree and plasticity of electrocyte discharge waveform should be evident if responsiveness is compared across developmental stage (reproductively immature *vs.* mature fish), and between reproductively mature female and male fish. We examined this possibility in the present experiments by comparing circadian and melanocortin induced changes in EOD waveform in juvenile, male, and female fish *in vivo*. We then compared electrocyte μEOD and AP waveform characteristics as well as melanocortin-induced changes in these parameters. We found that males, females, and juveniles show similar patterns of EOD waveform plasticity *in vivo*, but different cellular mechanisms of μEOD modulation *in vitro*, arising from different underlying changes in AP waveform and timing modulations. Thus whole animal signal modulation patterns appear to be produced by different cellular mechanisms of action potential regulation that vary with sex and sexual maturity.

Materials and methods

Animals

We studied captive-bred *B. gauderio* from colonies maintained at Florida International University (Miami, FL) and The University of Oklahoma. Experiments were approved in advance by the Institutional Animal Care and Use Committee of Florida International University and the Institutional Animal Care and Use Committee of the University of Oklahoma.

Table 2
ANOVA results for *in vivo* effects of Treatment (ACTH, saline, circadian swing) and Group (juvenile, female, male) on EOD waveform parameters.

	Group		Treatment		Group × Treatment	
	d.f. = (2, 59)		d.f. = (2, 59)		d.f. = (4, 59)	
Variable	F	p	F	p	F	p
Amplitude	3.81	0.03	60.24	.000	1.77	0.15
P1 amplitude	1.26	0.29	47.66	.000	2.88	0.03
P1 dur 50	7.39	0.001	41.92	.000	1.36	0.26
P2 amplitude	1.69	0.19	71.66	.000	3.90	0.007
P2 dur 50	2.67	0.08	81.67	.000	8.99	0.000
τ_{P2}	1.17	0.32	45.62	.000	0.88	0.48