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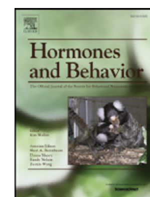

Food deprivation reduces and leptin increases the amplitude of an active sensory and communication signal in a weakly electric fish

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ABSTRACT

Energetic demands of social communication signals can constrain signal duration, repetition, and magnitude. The metabolic costs of communication signals are further magnified when they are coupled to active sensory systems that require constant signal generation. Under such circumstances, metabolic stress incurs additional risk because energy shortfalls could degrade sensory system performance as well as the social functions of the communication signal. The weakly electric fish *Eigenmannia virescens* generates electric organ discharges (EODs) that serve as both active sensory and communication signals. These EODs are maintained at steady frequencies of 200–600 Hz throughout the lifespan, and thus represent a substantial metabolic investment. We investigated the effects of metabolic stress (food deprivation) on EOD amplitude (EODa) and EOD frequency (EODf) in *E. virescens* and found that only EODa decreases during food deprivation and recovers after restoration of feeding. Cortisol did not alter EODa under any conditions, and plasma cortisol levels were not changed by food deprivation. Both melanocortin hormones and social challenges caused transient EODa increases in both food-deprived and well-fed fish. Intramuscular injections of leptin increased EODa in food-deprived fish but not well-fed fish, identifying leptin as a novel regulator of EODa and suggesting that leptin mediates EODa responses to metabolic stress. The sensitivity of EODa to dietary energy availability likely arises because of the extreme energetic costs of EOD production in *E. virescens* and also could reflect reproductive strategies of iteroparous species that reduce social signaling and reproduction during periods of stress to later resume reproductive efforts when conditions improve.

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Introduction

The moment-to-moment metabolic costs of animal communication signals range from insignificant to staggering (reviewed by Stoddard and Salazar, 2011). Communication signals with the highest instantaneous costs, such as the vocal calls of Carolina wrens and the mechanical trilling of orthopteran katydids are typically intermittent in nature and can be stopped entirely to reduce their ongoing metabolic demands. During metabolic stress, reducing or ceasing signaling in these cases means losing only the benefits associated with signaling (e.g., territorial defense, mate attraction) while allowing a shift to other beneficial activities such as foraging. When metabolically expensive communication signals are coupled to active sensory systems, however, reducing or ceasing signaling effort in response to metabolic stress would incur the additional cost of degrading or eliminating sensory performance, potentially interfering with navigation and foraging for food or prey items.

This is the case for weakly electric fish that both sense and communicate with brief electric organ discharges (EODs). The EOD is produced by summation of synchronized action potentials from ~1000 electric organ cells (electrocytes) within the electric organ. Electric fish analyze distortions of the resulting electric fields caused by nearby objects to image their surroundings (Caputi and Budelli, 2006; Caputi et al., 1998; Marsat et al., 2012; von der Emde, 1999, 2006). These fish also communicate with transient changes in EODf (Hagedorn and Heiligenberg, 1985; Hopkins, 1974; Hupé and Lewis, 2008). EOD waveform can encode information such as species (Hopkins and Bass, 1981), sex (Hopkins, 1972), and even individual identity (McGregor and Westby, 1992). Additionally, in some species EOD waveform and rate indicate social status, reproductive status, and body condition (Gavassa et al., 2011, 2013).

The metabolic cost of each EOD is driven primarily by the costs associated with producing the electrocyte action potentials, specifically the energy required by the Na^+/K^+ ATPases to restore the necessary ionic gradients after each action potential (Lewis et al., 2014). The ongoing cost of EOD production is then a function of the cost per discharge and the discharge frequency. Pulse-type electric fish generate EODs at relatively low frequencies (1–100 Hz) with long irregular intervals between each EOD, while wave-type fish produce higher

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frequency EODs at regular uniform intervals (~100–2000 Hz) (Crampton and Albert, 2006). Under normal conditions, both pulse fish and wave fish control the metabolic demands of EOD production by circadian reductions in EODa (Markham et al., 2009b; Salazar and Stoddard, 2008). Pulse fish achieve additional energetic savings by large circadian reductions in EODf (Silva et al., 2007; Stoddard et al., 2007).

The high and steady discharge frequencies of wave fish create significant energetic demands (Lewis et al., 2014), likely making the EODs of these fish sensitive to metabolic stress. Consistent with this hypothesis, wave fish tend to occupy high-oxygen habitats whereas pulse fish generally are more tolerant of low-oxygen environments (Crampton, 1998). Reducing either EODa or EODf could reduce metabolic demand during periods of metabolic stress. Under acute metabolic stress (hypoxia) the wave species *Apteronotus leptorhynchus* and *Eigenmannia virescens* diminish EODa, but not EODf, to reduce the energetic costs of EOD production (Reardon et al., 2011), suggesting that, during short-term metabolic stress, wave fish may be unable to rapidly reduce EODf or that reductions in EODa are more effective in reducing EOD costs. It is not known if wave fish would respond similarly during longer periods of metabolic stress such as that would occur during food deprivation over several days.

In the pulse gymnotiform *Brachyhypopomus gauderio*, metabolic stress associated with food restriction does not cause measurable changes in EODa, and fish subjected to food restriction actually show larger socially-induced increases in EODa (Gavassa and Stoddard, 2012). These authors further found that exogenous cortisol reduces EODa, though food restriction does not alter levels of circulating endogenous cortisol. Here we report direct experimental tests investigating the effects of food deprivation on EODa and EODf in the high-frequency wave-type fish *E. virescens* (~250–600 Hz). We also investigated the endocrine mechanisms coupling food deprivation to changes in the EOD. Comparing *E. virescens* to *B. gauderio* in this regard is important both because of their different EOD rates and patterns, but also because of their different reproductive strategies. *B. gauderio* are semelparous single-season breeders (Silva et al., 2003), while *E. virescens* are longer-lived iteroparous breeders (Hagedorn and Heiligenberg, 1985; Kramer, 1987) that continue to reproduce for at least three years in laboratory conditions (Kirschbaum, 1979). Semelparous species typically continue reproductive behaviors (including costly advertisement signals) during periods of stress, while iteroparous species typically reduce reproductive efforts during stressful periods to survive then resume reproduction when environmental conditions are again favorable (Wingfield and Sapolsky, 2003). Given both the higher energetic demand of EOD production in *E. virescens* and their iteroparous life history, we predicted that *E. virescens* would reduce signaling effort during food restriction and restore signal strength when dietary energy sources are again available.

Materials and methods

Animals

Fish were wild-caught male and female *E. virescens* (Glass knife fish) from tropical South America (Fig. 1), obtained through tropical fish importers. In some experiments *E. virescens* were exposed to male and female *B. gauderio* taken from breeding colonies maintained at The University of Oklahoma. Because animals were not in reproductive condition we could not determine sex non-lethally. Fish were housed in groups of 4–10 in 40-liter or 10-liter tanks and fed live oligochaete blackworms *ad libitum* in a recirculating aquarium system at 28 ± 1 °C with water conductivity of 200–400 μS/cm. In *E. virescens*, each EOD is a positive-going pulse approximately 1–2 ms in duration, and these EODs occur at regular intervals under the control of a medullary pacemaker nucleus, producing a 200–600 Hz sinusoidal wave (Hopkins, 1974) (Figs. 1B–D). All experimental methods were approved by the Institutional Animal Care and Use Committee of The University of Oklahoma, and have complied with the guidelines given in the Public Health Service policy on humane care and use of laboratory animals (National Institutes of Health (U.S.) et al., 2002)

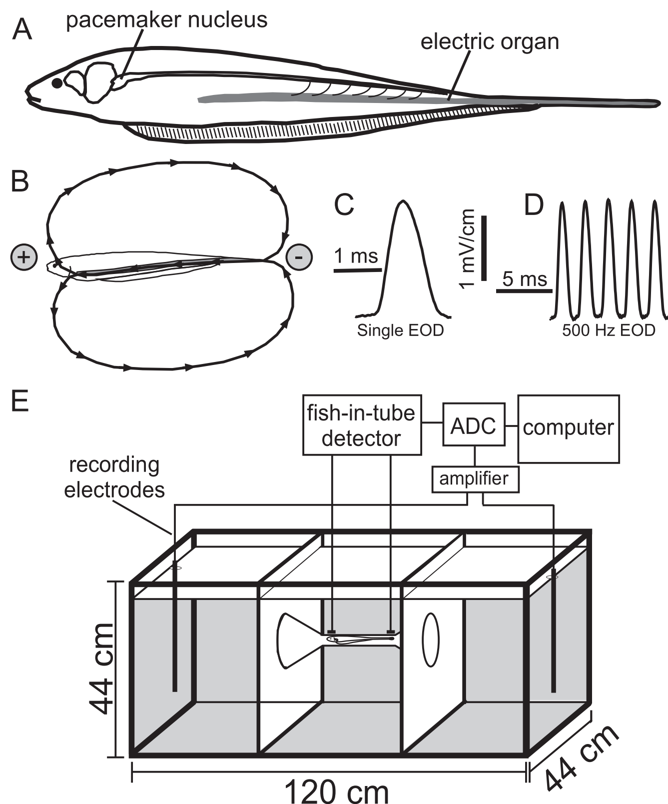

Fig. 1. The electric organ discharge in *Eigenmannia virescens* and experimental tank configuration. A) The electric organ discharge (EOD) is produced by the coordinated action potentials of the electrocytes in the electric organ. A medullary pacemaker nucleus synchronizes electrocyte action potentials via spinal electromotor neurons that innervate each electrocyte. B) The simultaneous action potentials of all electrocytes sum to produce current that moves forward toward the head, following a return path through the water to the tail. By convention, current toward the head measured as positive (upward). C) A single EOD is a positive-going voltage pulse approximately 1 ms in duration. D) The EOD waveform is a sinusoidal wave with frequency set by the pacemaker nucleus. E) Experimental preparation for recording calibrated EODs of free-swimming fish. EODs were digitized from nichrome recording electrodes at the ends of the tank only when custom circuitry detected that the fish was centered within an electrically transparent mesh tube and therefore centered between the recording electrodes at the ends of the tank. ADC: analog-to-digital-converter.

Solutions and reagents

The physiological saline for injections contained (in mM): 114 NaCl, 2 KCl, 4 $CaCl_2 \cdot 2H_20$, 2 $MgCl_2 \cdot 6H_20$, 5 HEPES and 6 glucose; pH to 7.2 with NaOH. We purchased adrenocorticotropic hormone (ACTH 1–39 from porcine pituitary) and hydrocortisone (98% HPLC grade) from Sigma-Aldrich (St. Louis, MO), and purchased leptin (human recombinant) from Enzo Life Sciences (Farmingdale, NY). We dissolved ACTH in water at a stock concentration of 1 M, and then stored this solution in single-use aliquots at − 20 °C. Stock solutions were thawed and diluted in saline to working concentrations immediately before use. Hydrocortisone was dissolved in 90% ethanol to a concentration of 15 mg ml^{-1} at the time of use. We dissolved leptin in saline at the working concentration of 1 mg/ml then stored it at −20 °C in single-use aliquots that were thawed to room temperature just before use.

EOD recordings

Our automated system for recording calibrated EODs from freely swimming fish and procedures for injecting fish are described in detail

elsewhere (Stoddard et al., 2003). Fish were placed in an automated measurement tank, 120 × 44 × 44 cm, located in a light- and temperature-controlled room on a 12L:12D light cycle (Fig. 1E). The tanks are divided by a screen mesh into three compartments, and an electrically-transparent mesh tube connects the two outer compartments. The fish can move between the two outer compartments via the tube but cannot access the central compartment. A 50-ms sample of the EOD waveform was amplified and digitized from nichrome wire electrodes at opposite ends of the tank only when the fish passed through or was resting in the mesh tube centered between the recording electrodes. EODa was measured peak-to peak and EODf was computed from waveform zero crossings during each 50-ms sample. EODs were recorded throughout each experiment at intervals of approximately 1 min. Fish were held in the recording tanks for at least 3 days with *ad libitum* food before beginning experiments and we recorded baseline EODs for at least 24 h before beginning any experimental manipulations.

Food and environmental manipulations

We tested the effects of food availability by providing blackworms *ad libitum* (Ad-lib Food condition) in the recording tank or completely removing all worms from the recording tank (Food Deprivation condition) for a maximum of seven days. Worms were placed in a 10-cm diameter PVC cup (3 cm deep) to prevent worms from moving within the tank. In some experiments, we controlled the amount of food delivered to food-deprived fish by weighing and delivering 10 g of worms. As a control for non-food changes in environmental complexity, we arranged three 56-cm lengths of PVC pipe diagonally across each of the recording tank's outer chambers and left these obstacles in place for 24 h (Obstacles condition). As further control for the effects of sensory cues associated with food apart from the fish's consumption of the food, we placed 20 g of worms in a nylon mesh bag from which the worms could not escape and placed these bagged worms in the feeding cup (Inaccessible Food condition).

Injections and social challenges

We prepared injection solutions to produce the desired dose when injected intramuscularly at 1 μL g^{-1} body weight. Saline injections (μL g^{-1}) served as a control condition for handling and injection effects. All ACTH injections (25 nM g^{-1} body weight) were administered mid-day (12:00–15:00) at or near the circadian minimum of EODa. We have shown previously that EOD response to ACTH saturates at this dose (Markham and Stoddard, 2005; Markham et al., 2009a). Leptin injections (1 μg g^{-1} body weight) were administered in late afternoon to early evening (16:00–18:00) when fish sometimes begin to leave their daytime refugia to begin searching for food within the tank. To perform the injections, fish were quickly netted from the recording tank, injected in the hypaxial muscle, and then returned to the recording tank where EOD recordings continued at ~1 min intervals. Handling time from capture to replacement in the tank was usually less than 2 min.

To expose fish to social challenges, we added a second fish of comparable size to the center compartment of the recording tank in late afternoon (16:00–18:00). This allowed the two fish to interact electrically and chemically but not physically. During conspecific social challenges the intruder fish was an *E. virescens*. During heterospecific challenges the intruder fish was *B. gauderio*, a distantly-related gymnotiform pulse electric fish. The heterospecific challenge condition was included here to control for any effects due only to the presence of a second fish in the tank, independent of interspecific social interactions. We removed the intruder fish 24 h later.

Plasma collection and cortisol assays

We assayed plasma cortisol from both food-deprived fish (7 days deprived) and fish fed *ad libitum*. Plasma samples were collected by caudal severance of fish anesthetized by immersion for 1 min in an overdose of fast-acting eugenol anesthesia (1.5 mg L^{-1}). Following severance of the caudal filament with a sterile scalpel, blood was collected from the sub-vertebral sinus using heparinized glass capillary tubes. The blood was expelled from the capillaries into 0.5 ml microcentrifuge tubes chilled on ice and centrifuged for 10 min at 13,000 RPM in a refrigerated centrifuge. We then separated the plasma and stored it at −80 °C until all the samples were sent as a group to the Biomarkers Core Laboratory at the Yerkes National Primate Research Center for assays of cortisol concentrations by high performance liquid chromatography (HPLC) followed by mass spectroscopy (MS). Briefly, 50 μL of homogenized sample was diluted with 100 μL of HPLC grade methanol and 50 μL of d4-cortisol. When plasma samples were less than 50 μL, 25 μL of plasma was diluted with 25 μL of HPLC grade methanol to obtain the required 50 μL sample volume. The isotopically diluted sample was vortexed for 30 s and then centrifuged at 13,200 RPM for 7 min. The supernatant was diluted 80:20 with HPLC grade H_2O for HPLC–MS/MS analysis. Calibrators, a matrix blank, and 4 levels of quality control plasma were prepared similarly, according to the Yerkes National Primate Center Biomarkers Core Lab protocol, "Cortisol Analysis using HPLC–MS/MS." Plasma calibrators were prepared in a commercially available mouse serum; water calibrators were prepared in Type II H_2O. Samples were analyzed using a Phenomenex Kinetex C18 column in a Shimadzu NexeraX2 30 series HPLC system in tandem with an AB Sciex Triple Quad 6500 Mass Spectrometer, according to the protocol "Cortisol Analysis using HPLC–MS/MS."

Cortisol administration

To evaluate the effects of cortisol on EODa, we used established methods for noninvasive manipulation of plasma cortisol in gymnotiform fish (Gavassa and Stoddard, 2012). Briefly, we added hydrocortisone (15 mg ml^{-1}) in 90% ethanol to the fish's recording tank at a volume sufficient to achieve a water concentration of 150 ng ml^{-1} cortisol, and producing a final ethanol concentration of 10 μL L^{-1}. This cortisol concentration is the upper limit of circulating cortisol in the related *B. gauderio* (Salazar and Stoddard, 2009). As a control condition we added 90% ethanol to the tank water to achieve the same ethanol concentration as for the cortisol-treated tanks. We recorded EODs while allowing 24 h for cortisol levels to equilibrate before any further experimental manipulations and continued EOD recordings for at least 48 h after any experimental manipulation. To confirm that water cortisol levels remained at or near their target concentrations during the experiments, we sampled tank water at the end of each experiment and assayed water cortisol concentrations in the same manner as that for plasma samples.

Data treatment and analysis

The calibrated EOD recorded in vivo is a quasi-sinusoidal wave of constant frequency that varies in amplitude which we measured peak-to-peak. Rapid and transient changes in the EODa resulting from in vivo injections of ACTH and from social challenges in other gymnotiforms are superimposed upon ongoing circadian cycles in EODa (Allee et al., 2008; Markham et al., 2009b). We therefore mathematically isolated ACTH-induced changes in EODa from the underlying circadian changes as described previously (Markham and Stoddard, 2013; Stoddard et al., 2003). We quantified the ACTH-induced EODa changes as the peak increase in signal amplitude within 4 h following injection (Stoddard et al., 2003). During social challenges, EODa measurements were unreliable because of interference between the EODs of the two fish in each tank. We therefore

controlled for underlying circadian EOD changes by comparing the focal fish's EODa just prior to the social challenge to its EODa immediately after the removal of the second fish 24 h later.

To assess the effects of manipulations with effects lasting over many days, we first transformed the EODa and EODf data with irregular intervals to a regular time series as described previously (Stoddard et al., 2007). Briefly, we fit a smoothing cubic spline to the data (MATLAB R2010b Curve Fitting Toolbox v3.0, Mathworks, Natick, MA) and resampled on this cubic spline fit at 1 min intervals. To allow comparisons across fish with different baseline amplitudes and frequencies, we then normalized EODa and EODf in each time series to the values at the time of treatment.

Statistical analyses and data plotting were performed with MATLAB, Prism (Graphpad, La Jolla, CA), and SPSS 19 (IBM, Armonk, New York). Averaged data are reported as mean ± SEM. All statistical analyses were compared to a significance level set at $p < 0.05$. Experiments with only two treatment conditions were analyzed with Student's t-tests. Our experiments examining changes in EODa and EODf were pretest–posttest designs comparing changes from baseline for both measures. To control for differences in baseline values across individuals, data from these experiments were analyzed with ANCOVAs testing differences in post-treatment measures after baseline measures were entered as the covariate (Bonate, 2000; Vickers, 2001). Significant omnibus ANCOVAs were further analyzed with post-hoc comparisons using Tukey's HSD to maintain experiment-wise alpha at 0.05. Effect sizes were computed as Cohen's d for t-test analyses and η^2 for all ANCOVAs.

Results

EODa but not EODf decreases during food deprivation and recovers after feeding

EODa decreased over seven days of food deprivation. Restoring feeding by delivering 10 g of blackworms (which the fish consumed within 24 h) produced a slow and sustained increase in EODa that lasted for 3 days before EODa again declined due to food deprivation (Figs. 2A–C). Following feeding, EODa increased by 38.7 ± 12.8% at 24 h post-feeding and increased further by 57.8 ± 17.6% at 48 h post-feeding (Figs. 2A–C). To ensure that post-feeding amplitude changes were due to consumption of the food and not simply foraging or the presence of new objects in the tank, we also presented inaccessible food and novel obstacles (PVC pipe) to food deprived fish. EODa in these conditions continued a slow decline similar to EODa declines in fish that continued under food deprivation with no treatment at all (Figs. 2A–C) Only feeding led to an increase in EODa and a significant interaction of time and treatment was due to the increase in EODa from 24 h to 48 h only in the feeding condition (ANCOVA time effect [24 h vs 48 h]: $F_{1,19} = 0.835$, $p = 0.372$, $\eta^2 = 0.042$, treatment effect: $F_{1,19} = 3.682$, $p = 0.03$, $\eta^2 = 0.389$, interaction: $F_{3,19} = 4.037$, $p = 0.022$, $\eta^2 = 0.389$).

EODf did not change in response to feeding or any of the other experimental conditions (Fig. 2D). The data suggest a slight decline in EODf in all groups but these changes were not statistically significant (ANCOVA time effect [24 h vs 48 h]: $F_{1,19} = 0.004$, $p = 0.947$, $\eta^2 = 0.0$, treatment effect: $F_{1,19} = 0.298$, $p = 0.826$, $\eta^2 = 0.045$, interaction: $F_{3,19} = 1.791$, $p = 0.183$, $\eta^2 = 0.220$).

ACTH increases EODa and decreases EODf in food-deprived and well-fed fish

The decline in EODa observed in food-deprived fish could result from a peripheral shortage of energy in the electric organ or instead from a centrally-mediated endocrine mechanism for proactively reducing EODa during metabolic energy shortages. We tested whether decreased EODa in food deprived fish resulted from limitations at the electric organ by administering exogenous ACTH, a hormone known to act directly on electrocytes to increase EODa in related gymnotiforms (Markham and Stoddard, 2005; Markham et al., 2009b). Injections of ACTH produced comparable rapid EODa increases of 64.8 ± 21.9% and 43.0 ± 11.5% in food-deprived and well-fed fish, respectively (Figs. 3A, B) (ANCOVA Feeding State effect [food-deprived vs. ad-lib]: $F_{1,20} = 0.004$, $p = 0.952$, $\eta^2 = 0.0$, hormone effect: $F_{1,20} = 6.085$, $p = 0.023$, $\eta^2 = 0.233$, interaction: $F_{1,20} = 1.943$, $p = 0.089$, $\eta^2 = 0.089$). In contrast, ACTH injections decreased EODf by 1.17 ± 0.13% and 1.57 ± 0.39% in food-deprived and well-fed fish, respectively (Fig. 3C). These changes were small but statistically significant (ANCOVA Feeding State effect [food-deprived vs. ad-lib]: $F_{1,19} = 3.088$, $p = 0.095$, $\eta^2 = 0.140$, hormone effect: $F_{1,19} = 27.020$, $p < 0.001$, $\eta^2 = 0.587$, interaction: $F_{1,19} = 0.595$, $p = 0.450$, $\eta^2 = 0.030$).

Effects of social challenges on EODa and EODf

Conspecific social challenges caused EODa to increase by 46.9 ± 16.0% in food-deprived fish and increase by 44.0 ± 12.4% in well-fed fish (Figs. 3D, E). These changes in EODa were not different for food-deprived versus well-fed fish, and conspecific social challenges produced much larger increases in EODa than heterospecific challenges (Fig. 3E) (ANCOVA Feeding State effect [food-deprived vs. ad-lib]: $F_{1,17} = 1.574$, $p = 0.227$, $\eta^2 = 0.085$, treatment effect [conspecific vs heterospecific]: $F_{1,17} = 7.824$, $p = 0.012$, $\eta^2 = 0.315$, interaction: $F_{1,17} = 1.273$, $p = 0.275$ $\eta^2 = 0.070$). Surprisingly, we found that EODf changed only in response to heterospecific challenge in food deprived fish, increasing by 4.63 ± 2.48%, while EODf did not change in any other experimental condition (Fig. 3F) (ANCOVA Feeding State effect [food-deprived vs. ad-lib]: $F_{1,17} = 2.643$, $p = 0.122$, $\eta^2 = 0.135$, treatment effect [conspecific vs. heterospecific]: $F_{1,17} = 2.505$, $p = 0.132$, $\eta^2 = 0.128$, interaction: $F_{1,17} = 4.689$, $p = 0.045$ $\eta^2 = 0.216$).

Exogenous cortisol does not influence EODa nor EODf and feeding state does not change plasma cortisol levels

Cortisol levels increase in many taxa during stressful conditions including metabolic stress and elevated cortisol decreases activity and social signaling effort in many animals (reviewed by McEwen and Wingfield, 2003). Additionally, cortisol treatment decreases EODa in the pulse-type gymnotiform *B. gauderio* (Gavassa and Stoddard, 2012). We therefore investigated whether cortisol treatment would prevent EODa increases associated with feeding or further suppress EODa in food-deprived fish. After treating food-deprived fish with exogenous cortisol or EtOH as a carrier control for 24 h, we either delivered 10 g of food or left fish unfed for an additional two days. After delivery of 10 g of food, EODa showed nearly identical increases over 48 h in both cortisol-treated and carrier-control fish (Figs. 4A, C) while EODa did not change in fish that remained unfed (Fig. 4C) (ANCOVA timepoint effect [24 h vs 48 h]: $F_{1,16} = 0.254$, $p = 0.621$, $\eta^2 = 0.016$, hormone effect [cortisol vs. carrier]: $F_{1,16} = 0.221$, $p = 0.645$, $\eta^2 = 0.014$, treatment effect [food vs no-food]: $F_{1,16} = 11.319$, $p = 0.004$, $\eta^2 = 0.414$, interaction [timepoint × cortisol], $F_{1,16} = 0.157$, $p = 0.697$, $\eta^2 = 0.010$, interaction [timepoint × treatment], $F_{1,16} = 12.339$, $p = 0.003$, $\eta^2 = 0.435$, interaction [cortisol × treatment]: $F_{1,16} = 1.087$, $p = 0.313$, $\eta^2 = 0.064$, interaction [time × cortisol × treatment], $F_{1,16} = 1.734$, $p = 0.206$, $\eta^2 = 0.098$). We also found that 48 h of cortisol treatment did not change EODa in well-fed fish compared to carrier-control fish (Fig. 4B) (timepoint effect [24 h vs 48 h]: $F_{1,6} = 3.889$, $p = 0.106$, $\eta^2 = 0.437$, condition effect [cort vs. carrier]: $F_{1,6} = 0.012$, $p = 0.917$, $\eta^2 = 0.002$, interaction [timepoint × condition], $F_{1,6} = 4.256$, $p = 0.094$, $\eta^2 = 0.460$).

EODf also did not change in response to cortisol or feeding in food deprived fish (Fig. 4D) (timepoint effect [24 h vs 48 h]: $F_{1,16} = 2.330$, $p = 0.146$, $\eta^2 = 0.127$, cortisol effect [cort vs. carrier]: $F_{1,16} = 1.295$, $p = 0.272$, $\eta^2 = 0.075$, treatment effect [food vs no-food]:

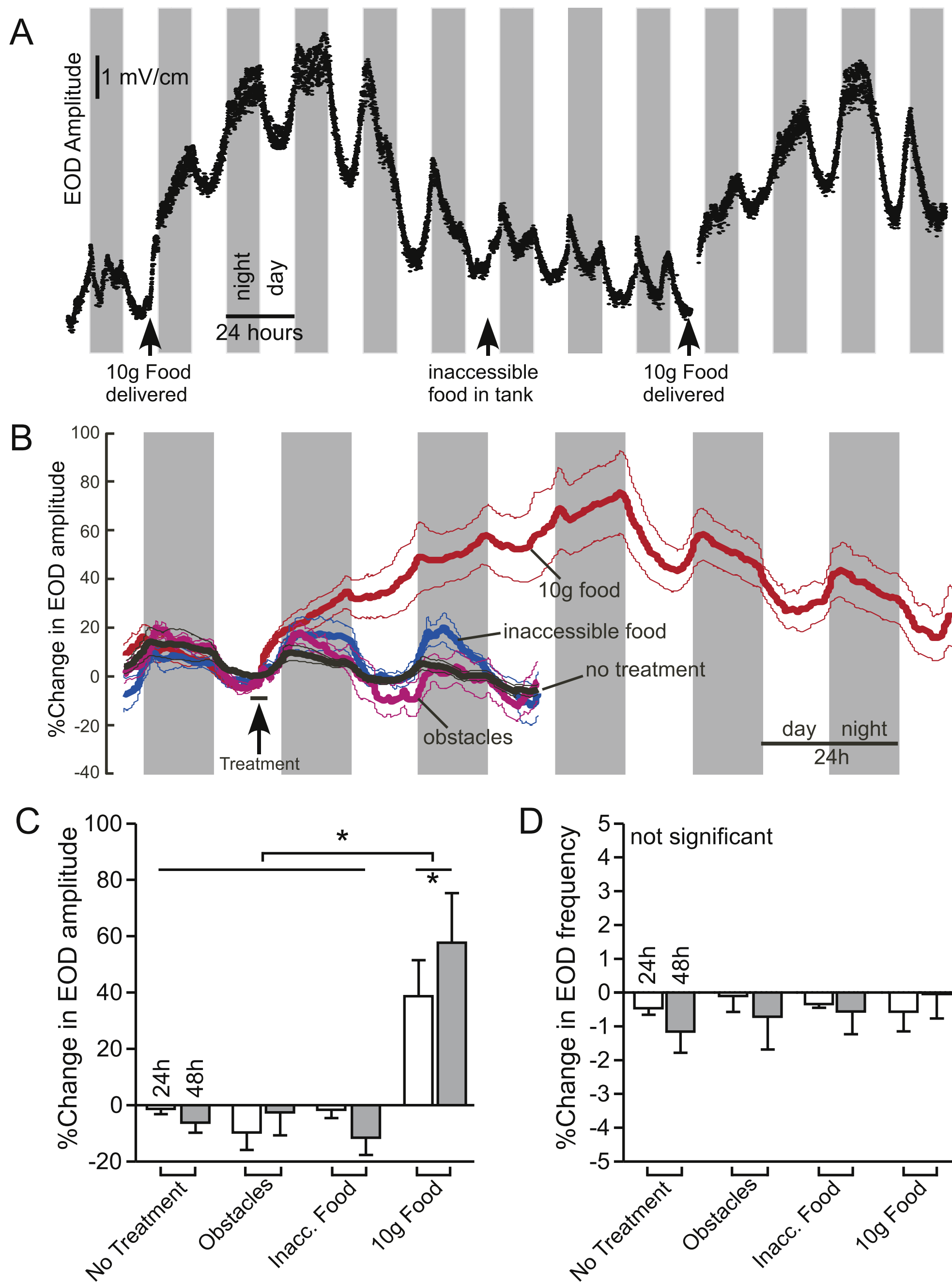

Fig. 2. EOD amplitude decreases during food deprivation and recovers after feeding. A) Representative data from a single fish recorded over 13 days. Each data point represents one EOD recorded at ~1 min intervals and gray bars indicate periods of diurnal darkness. Initial condition is 5 day food deprivation. Following delivery of food, EODa increases over 3 days then decreases sharply over the subsequent 3 days. Introducing inaccessible food to the tank does not change EODa. B) Changes in EODa following feeding ($n = 6$; red lines), introduction of novel obstacles in the tank ($n = 6$; magenta lines), introduction of inaccessible food ($n = 4$; blue lines), and no-treatment controls (continued food deprivation, $n = 14$; black lines). Baseline condition is 5 days food deprivation for all groups. Values are normalized to the time of treatment, thick lines indicate means and thin lines indicate ±SEM. C) Percent change in EODa at 24 h and 48 h post-treatment. Bars indicate means and error bars are SEM. White and gray bars represent change at 24 h and 48 h, respectively. Only delivery of food increased EODa ($p = 0.030$), and within this condition, EODa increased from 24 h to 48 h post-treatment ($p = 0.016$). D) Percent change in EODf at 24 h and 48 h post-treatment. Bars indicate means and error bars are SEM. White and gray bars represent change at 24 h and 48 h, respectively. No statistically significant differences in EODf were found in any of the conditions ($p > 0.183$).

$F_{1,16} = 0.199$, $p = 0.661$, $\eta^2 = 0.012$, interaction [timepoint × cortisol], $F_{1,16} = 0.017$, $p = 0.897$, $\eta^2 = 0.001$, interaction (timepoint × treatment), $F_{1,16} = 2.521$, $p = 0.132$, $\eta^2 = 0.136$, interaction [cortisol × treatment]: $F_{1,16} = 0.322$, $p = 0.578$, $\eta^2 = 0.020$, interaction [time × cortisol × treatment], $F_{1,16} = 4.219$, $p = 0.057$, $\eta^2 = 0.209$).

Water samples taken from the cortisol and control tanks at the end of each experiment confirmed that cortisol levels remained at 143 ± 15.8 ng/ml in the cortisol-treated tanks and 1.12 ± 0.04 in the control tanks (Fig. 5) ($t = 13.29$, $p < 0.0001$, $df = 39$; Cohen's $d = 5.67$). As a final test for any role of cortisol in mediating diet-related changes in EODa we first fed fish *ad libitum* for seven days then either removed all food or continued *ad libitum* food for an additional seven days before collecting plasma samples for cortisol assays. Plasma cortisol levels in food deprived fish (45.3 ± 23.2 ng/ml) were not different from cortisol levels in well-fed fish (43.83 ± 11.1 ng/ml) (Fig. 5; $t = 0.06$, $p = 0.96$, $df = 14$; Cohen's $d = 0.03$).

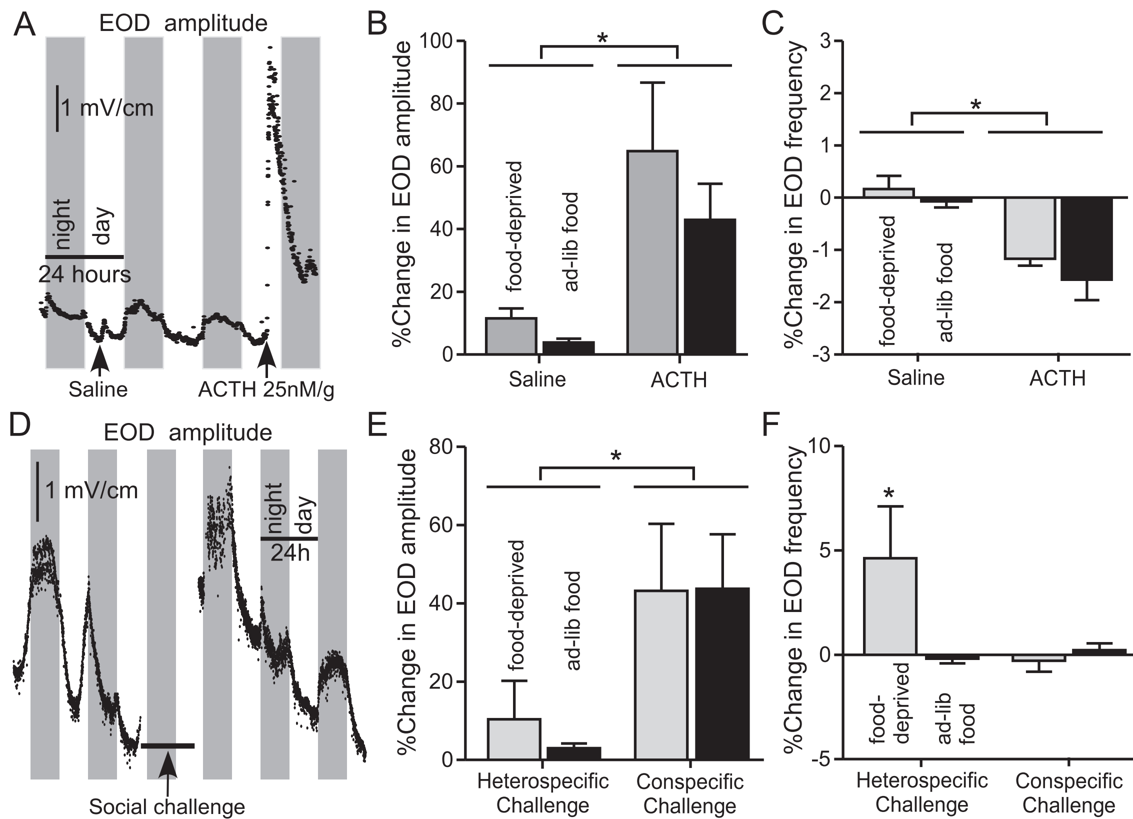

Fig. 3. ACTH injections and social challenges cause transient increases in EOD amplitude in both food-deprived and well-fed fish. A) Representative EODa data for one food-deprived fish showing the effects of saline and ACTH injections. Each data point represents one EOD and gray bars indicate periods of diurnal darkness. Saline injection caused only a small transient change in EODa while ACTH injection (25 nM g^{-1}) caused a rapid and dramatic enhancement of EODa. B) Percent change in EODa following saline or ACTH injections. Bars indicate means and error bars are SEM. Gray bars indicate food-deprived fish and black bars represent fish fed *ad libitum*. ACTH injections increased EODa regardless of feeding state ($p = 0.023$). C) Percent change in EODf following saline or ACTH injections. Bars indicate means and error bars are SEM. Gray bars indicate food-deprived fish and black bars represent fish fed *ad libitum*. ACTH injections caused a small but reliable decrease in EODf regardless of feeding state ($p < 0.001$). D) Representative data for one food-deprived fish showing the effects of the 24 h social challenge with a conspecific fish. Social challenge caused a pronounced increase in EODa that decayed over ~2 days after removal of the social stimulus fish. E) Percent change in EODa following 24 h exposure to a heterospecific (electric fish of different species) or conspecific challenger (same species). Bars indicate means and error bars are SEM. Gray bars indicate food-deprived fish and black bars represent fish fed *ad libitum*. EODa increased in response to a conspecific challenger regardless of feeding state but did not change in response to a heterospecific challenger ($p = 0.012$). F) Percent change in EODf following 24 h exposure to a heterospecific or conspecific challenger. Bars indicate means and error bars are SEM. Gray bars indicate food-deprived fish and black bars represent fish fed *ad libitum*. EODf showed a moderate increase only in food-deprived fish exposed to a heterospecific challenger ($p = 0.045$).

Leptin causes sustained increases in EODa only in food-deprived fish and decreases EODf regardless of feeding state

Given this evidence that cortisol is not involved in mediating feeding-related changes in EODa, we screened in pilot experiments a number of endocrine factors involved in energy homeostasis for possible effects on EODa (cholecystokinin, polypeptide y, ghrelin, leptin, and neuropeptide Y). Of these, only leptin appeared to exert any influence on EODa or EODf. We therefore carried out a full experimental examination of leptin's effects on EODa and EODf. Midday injections of leptin (1 μg g^{-1}) in food-deprived fish caused slow and sustained increases in EODa over at least 48 h compared to saline-injected controls (we resumed feeding 48 h post-injection). EODa increased by 25.5 ± 7.9% after 24 h and by 29.8 ± 5.3% after 48 h. In contrast, when leptin was injected in well-fed fish, EODa showed little to no change, decreasing only slightly by 3.3 ± 2.1% and 3.9 ± 2.3% at 24 h and 48 h, respectively (Figs. 6A, C) (timepoint effect [24 h vs 48 h]: $F_{1,17} = 2.270$, $p = 0.150$, $\eta^2 = 0.118$, condition effect [leptin_{min} vs. leptin_{max} vs saline]: $F_{2,17} = 21.255$, $p < 0.001$, $\eta^2 = 0.714$, interaction [timepoint × condition], $F_{2,17} = 5.709$, $p = 0.013$, $\eta^2 = 0.402$). The timecourse of EODa changes was much slower following leptin injections than that after ACTH injections (Fig. 6B); EODa increased within minutes of ACTH injections and peaked within 2 h, while changes in amplitude associated with leptin injection developed over hours and continued for days.

Regardless of feeding state, leptin injections caused a decrease in EODf compared to saline-injection controls (Fig. 6D) (timepoint effect [24 h vs 48 h]: $F_{1,17} = 0.043$, $p = 0.839$, $\eta^2 = 0.002$, condition effect [leptin_{min} vs. leptin_{max} vs saline]: $F_{2,17} = 6.691$, $p = 0.007$, $\eta^2 = 0.714$, interaction [timepoint × condition], $F_{2,17} = 0.713$, $p = 0.504$, $\eta^2 = 0.440$). Leptin injections in food-deprived fish decreased EODf by 1.37 ± 0.47% at 24 h and decreased EODf by 2.57 ± 0.82% at 48 h. In fish fed *ad -libitum*, leptin injections decreased EODf by 1.55 ± 0.30 and 1.90 ± 0.30 at 24 h and 48 h, respectively.

Discussion

The core finding of the present study is that the amplitude of electric sensory and communication signals in *E. virescens* is reduced under food deprivation and recovers with feeding. This response to mild but sustained metabolic stress is most likely a centrally-mediated endocrine response to declining energy reserves that proactively reduces EODa to conserve energy. The present results suggest that reduced EODa during food deprivation is not a result of ATP depletion in the electric organ, as EODa increases within minutes of treatment with melanocortin peptides in both food-deprived and well-fed fish. Social interactions also increase EODa in food-deprived fish, further evidence that the electric organ retains the capacity for high-amplitude signaling.

Somewhat surprisingly, cortisol does not appear to play any role in the response of EODa to food deprivation. Instead, we found that leptin

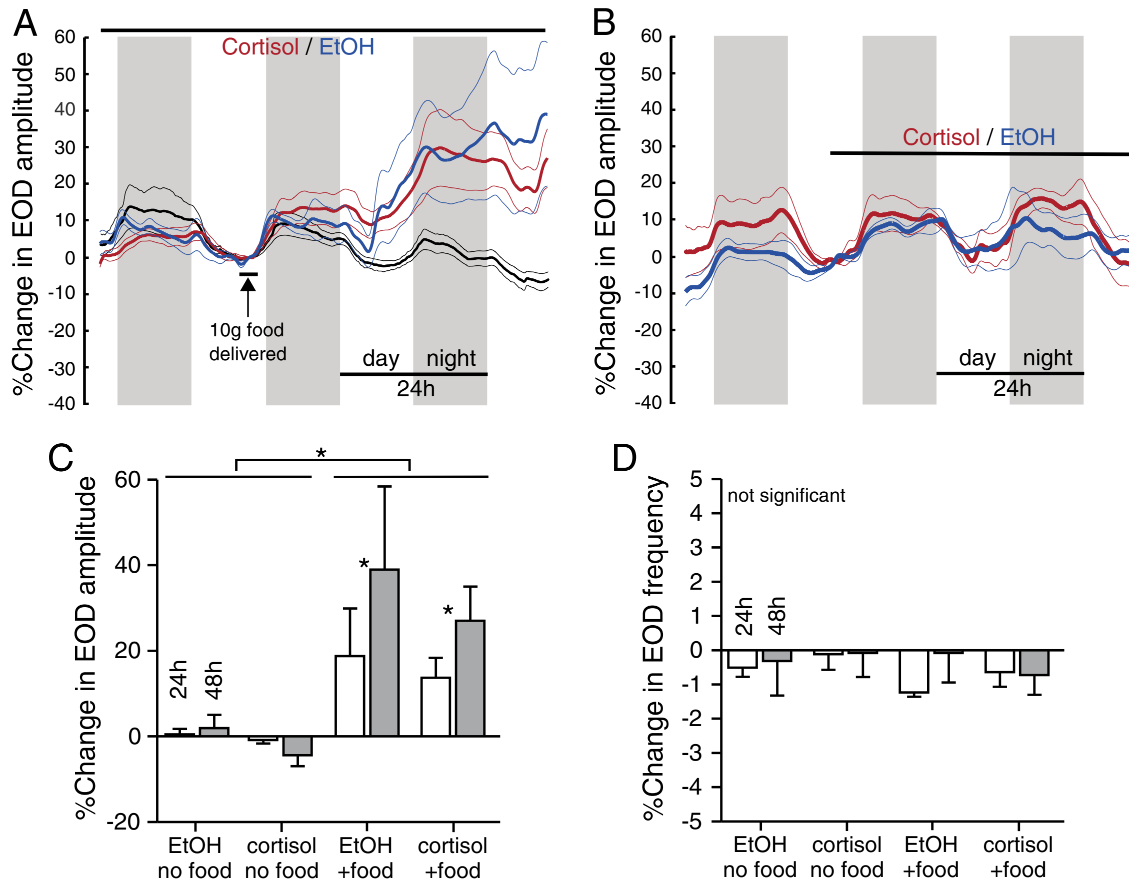

Fig. 4. Cortisol does not change EOD amplitude. A) Treatment with cortisol (red) or EtOH carrier control (blue) did not prevent increases in EODa following feeding in food-deprived fish (compare to effects of no treatment, black lines). No-treatment data are the same as those shown in Fig. 2B. Thick lines indicate means and thin lines are ± SEM. Vertical gray bars indicate periods of diurnal darkness. Baseline condition is 5 days of food deprivation. Values are normalized to zero at the time of food delivery. B) Treatment with cortisol (red) or EtOH carrier control (blue) did not change EODa in fish fed *ad libitum*. Thick lines indicate means and thin lines are ± SEM. Vertical gray bars indicate periods of diurnal darkness. Values are normalized to zero at the time of cortisol or EtOH treatment. C) Percent change in EODa at 24 h and 48 h for food-deprived fish pretreated with cortisol or EtOH carrier only, then given 10 g food or no food. Bars indicate means and error bars are SEM. White and gray bars represent change at 24 h and 48 h, respectively. Food delivery increased EODa for cortisol and EtOH treated fish, while EODa was unchanged in fish that did not receive food ($p = 0.004$). Within fish that received food, EODa increased from 24 h to 48 h post-treatment ($p = 0.003$). D) Percent change in EODf at 24 h and 48 h for food-deprived fish pretreated with cortisol or EtOH carrier only, then given 10 g food or no food. Bars indicate means and error bars are SEM. White and gray bars represent change at 24 h and 48 h, respectively. No statistically significant differences in EODf were found in any of the conditions ($p > 0.146$).

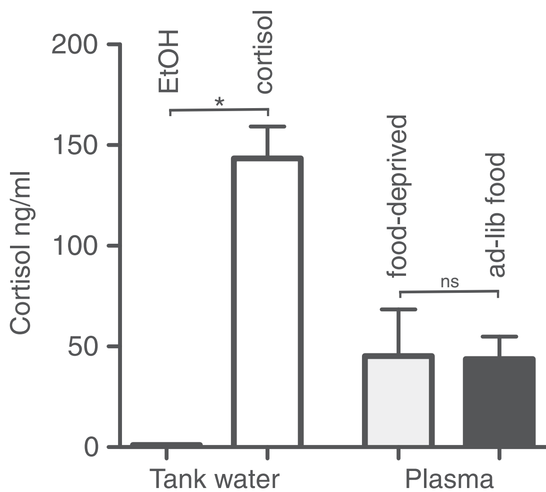

Fig. 5. Left: Addition of cortisol to tank water produced target concentrations that persisted through the end of each experiment. Water samples taken from tanks after the experiments in Fig. 4 show that cortisol levels remained elevated in cortisol-treated tanks (white bar) and at or near zero in EtOH control tanks (black bar) ($p < 0.001$). Right: Plasma cortisol levels in food deprived fish (gray bar) and well-fed fish (black bar) were not different ($p = 0.96$).

is potentially the endocrine factor regulating EODa in response to food availability, while acknowledging that additional hormones could be involved. We did not directly assay circulating leptin levels because antibody-based detection methods did not effectively quantify leptin in *E. virescens*. This outcome is understandable as leptin exhibits low amino acid sequence identities across different teleost species (Ronnestad et al., 2010). This, however, raises the question of how recombinant human leptin could have such pronounced effects in the present experiments. Although the primary structure of leptin is highly variable across taxa, its predicted tertiary structure and functionality are broadly conserved (Ronnestad et al., 2010) even to such an extent that amphibian leptin effectively activates human leptin receptors and vice-versa (Crespi and Denver, 2006).

In the absence of direct assays of plasma leptin in this study, several features of the present results nonetheless support a direct role for leptin in EODa regulation. First, leptin injections had no effect in well-fed fish, presumably because circulating leptin levels in these fish were already at saturating levels. This lack of leptin effects in well-fed fish was not because of an absolute limit on EODa because ACTH injections and social challenges in well-fed fish both increased EODa by more than 40%. Second, the timecourse of EODa changes following leptin injections was similar to the timecourse of increasing EODa following feeding. Finally, leptin's well-established role in increasing energy

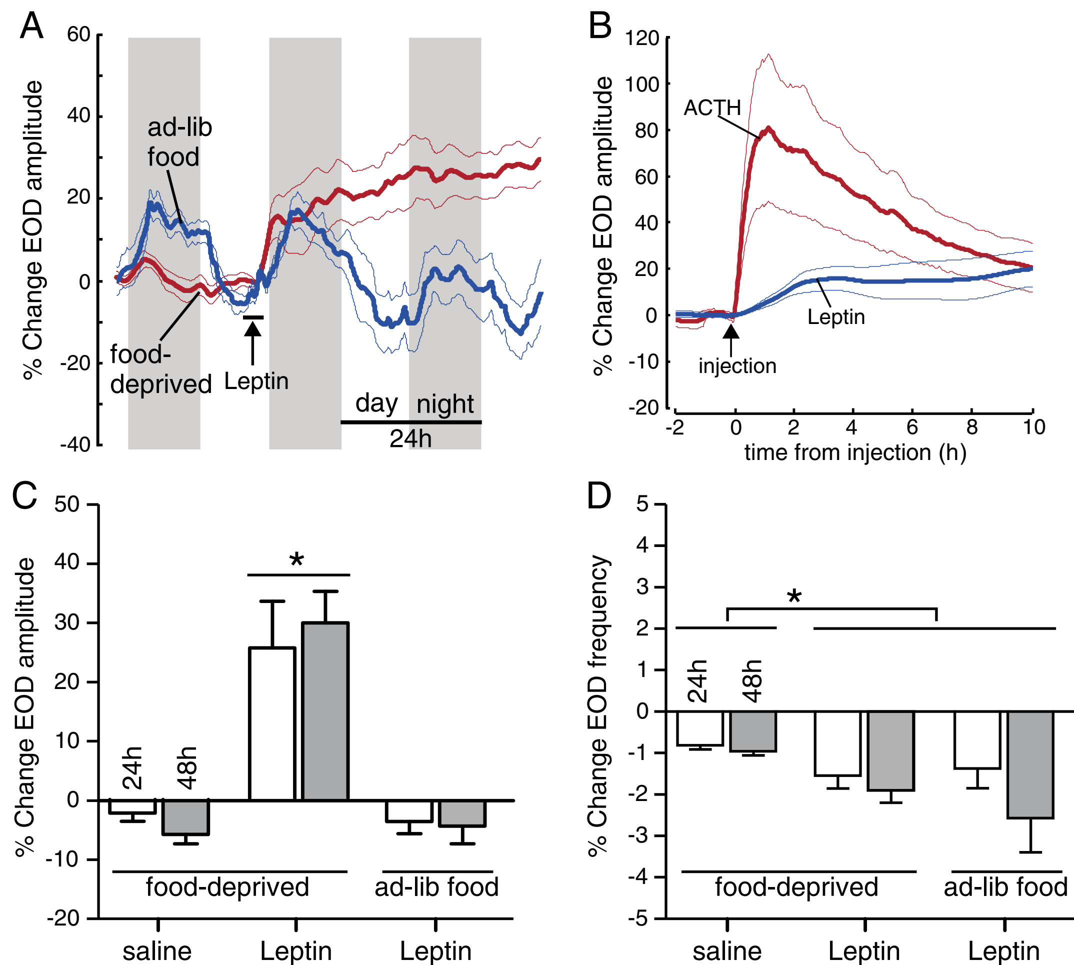

Fig. 6. Leptin injections cause sustained increases in EOD amplitude only in food-deprived fish. A) Changes in EODa following injection of leptin (1 μg g^{-1}) in food-deprived fish (n = 7; red lines) or well-fed fish (n = 8, blue lines) show that leptin increased EODa only in food-deprived fish. Thick lines indicate means and thin lines are ± SEM. Vertical gray bars indicate periods of diurnal darkness, and values are normalized to zero at the time of leptin injection. B) EODa increased more slowly following leptin injections (blue) than following ACTH injections (red). Thick lines indicate means and thin lines are ± SEM. Values are normalized to zero at the time of leptin or ACTH injection. EODa increased within minutes of ACTH injection while the effects of leptin injection were only apparent after ~1 h. C) Percent change in EODa at 24 h and 48 h in response to injection of saline in food-deprived fish, injection of leptin in food-deprived fish, or injection of leptin in fish fed *ad libitum*. Bars indicate means and error bars are SEM. White and gray bars represent change at 24 h and 48 h, respectively. EODa increased only in food-deprived fish that received leptin injections ($p < 0.001$). D) Percent change in EODf at 24 h and 48 h post-treatment. Bars indicate means and error bars are SEM. White and gray bars represent change at 24 h and 48 h, respectively. Leptin injections caused a small decrease in EODf compared to saline injections, regardless of feeding state ($p = 0.007$).

expenditure in other systems (Ahima and Osei, 2004) is consistent with its effects on EODa in the present experiments.

Consistent with earlier findings (Reardon et al., 2011) fish did not reduce EODf in response to food deprivation or increase EODf after restoration of feeding. Interestingly, we did observe small but reliable reductions in EODf in response to hormone treatments with EODf sometimes decreasing as EODa increased (ACTH injections), but in other cases EODf decreased whether EODa increased or remained unchanged (leptin injections). The only other condition in which EODf changed was a moderate increase in EODf observed when food-deprived fish were exposed to a heterospecific electric fish, a condition where no increase in EODa was observed. The only clear conclusion from these outcomes is that EODa and EODf are, at to least some degree, independently regulated. Additional experimental work is clearly needed to determine the causes and significance of these EODf changes. The increased EODf in food-deprived fish exposed to a heterospecific challenger seems counterproductive as this would amplify the EOD metabolic cost. Again, only additional investigation will clarify the causes and consequences of enhanced EODf in food-deprived fish in these conditions.

A central question to be addressed in future work is how, exactly, leptin is regulating EODa. Leptin is a peptide hormone secreted primarily from adipose tissue in mammals to produce circulating plasma levels proportional to body fat mass (Maffei et al., 1995). High leptin levels reduce feeding and increase energy mobilization and expenditure, while low leptin levels cause the opposite (reviewed by Schwartz et al., 2000). In ancestral vertebrates such as amphibians and teleost fish, leptin is secreted by a much wider range of tissues (Ronnestad et al., 2010) but its physiological effects are still centered on regulation of energy balance.

In the course of regulating EODa, leptin could be acting directly on electroctyes to increase action potential amplitude when energy stores are high and conversely allowing action potential amplitude to decrease as circulating levels of leptin decline with depletion of energy stores. Leptin does regulate neuronal excitability in other systems via membrane-bound leptin receptors (LepRs) that target ATP-sensitive K^{+} channels (K_{ATP}) (Spanswick et al., 1997), Ca^{2+}-activated K^{+} channels (Shanley et al., 2002), or voltage-gated K^{+} channels (Baver et al., 2014) to alter resting membrane potential and firing rate. We do not yet know if *E. virescens* electrocytes express LepRs, but this is a distinct possibility as electrocytes are developmentally derived from skeletal myocytes (Kirschbaum and Schwassmann, 2008) which express LepRs in other teleosts (Ronnestad et al., 2010). A second possibility is that leptin is exerting

central effects to regulate circulating levels of one or more secondary hormone(s) that then target electrocytes to control EODa. In other taxa, leptin acts in the hypothalamus to upregulate circulating levels of melanocortin peptides including ACTH and alpha-melanocyte stimulating hormone (reviewed by Shimizu et al., 2007; Williams et al., 2011), both of which increase EODa in pulse and wave fish (Markham et al., 2009a, 2009b). These potential central and peripheral effects are not mutually exclusive so one or both of these mechanisms could be at work in *E. virescens*. Direct experimental tests of these hypotheses will be the focus of future work.

E. virescens also reduces EODa but not EODf under metabolic stress caused by hypoxia, changes that occur on timescales spanning tens of minutes (Reardon et al., 2011). In the present experiments, EODa declined over the course of days during food-deprivation, while the timecourse for recovery of EODa following feeding spanned tens of minutes to hours. Directly comparing the timecourse of EODa changes caused by hypoxia and food deprivation/feeding is difficult as we could control the time of food delivery but not the timing of food consumption in the present experiments. Nonetheless, hypoxia and food-deprivation effects on EODa both follow timecourses roughly consistent with the rate at which leptin treatment increased EODa, suggesting the possibility that leptin mediates EODa responses to metabolic state in both cases. Indeed, leptin mediates hypoxia responses in several species of fish (Bernier et al., 2012; Chu et al., 2010; MacDonald et al., 2014), but does so by increasing leptin expression which in turn inhibits feeding. Additional research to determine the endocrine cascades that reduce EODa in hypoxic conditions and during food deprivation will provide a more complete picture of the neuroendocrine mechanisms for managing the energetic costs of EOD production during metabolic stress in *E. virescens*.

The effects of food deprivation on EODa in *E. virescens* contrast with those observed in the pulse fish *B. gauderio*, where food restriction had no effect on baseline EODa and actually magnified the transient increases in EODa caused by social challenges (Gavassa and Stoddard, 2012). Additionally, cortisol suppresses EODa in *B. gauderio* whereas it has no effect on EODa in *E. virescens*. The reasons for such different responses to food-deprivation could stem from differences between *E. virescens* and *B. gauderio* in the energetic demands of EOD production. Respirometry studies for both species have directly measured oxygen consumption attributable to EOD production (Lewis et al., 2014; Salazar and Stoddard, 2008). Standardizing to the number of ATP molecules required to support EOD generation, male *B. gauderio* expend 4.1×10^{20} ATP d^{-1} g^{-1} on EOD production while *E. virescens* expends approximately two orders of magnitude more energy on EOD generation (1.08×10^{22} ATP d^{-1} g^{-1}) primarily due to the higher EOD frequencies in *E. virescens*. This difference in EOD energy demand alone could account for the divergent physiological and behavioral responses to dietary energy shortfalls between these species.

It is also possible that differences in life history and reproductive strategy could be responsible for the different EODa responses to metabolic stress in *E. virescens* and *B. gauderio*. *B. gauderio* are semelparous single-season breeders (Silva et al., 2003), while *E. virescens* are longer-lived iteroparous breeders (Hagedorn and Heiligenberg, 1985; Kramer, 1987) that continue to reproduce for at least three years in laboratory conditions (Kirschbaum, 1979). Semelparous species typically continue reproductive behaviors (including costly advertisement signals) during periods of stress, while iteroparous species typically reduce reproductive efforts during stressful periods to survive then resume reproduction when environmental conditions are again favorable (Wingfield and Sapolsky, 2003). As suggested by Gavassa and Stoddard (2012), *B. gauderio* may be responding to a dietary energy shortfall with a "go for broke" strategy of maintaining EODa and even increasing it during social encounters as a terminal investment in reproduction. In contrast, during periods of food scarcity an iteroparous breeder such as *E. virescens* would be expected to reduce energy expenditure (as we found here) to await better food availability.

What are the sensory and social consequences of reducing EODa in response to metabolic stress? The ~40% reduction in EODa that occurs during food deprivation could impair sensory performance as the active space of the EOD would be reduced accordingly. Very little evidence is currently available on the effects of changing EODa on electrosensory performance (c.f., Stoddard et al., 2006). In the social context, diminished EODa during food deprivation suggests that the EOD is an honest indicator of body condition. However, our social challenge experiments demonstrate that food-deprived *E. virescens* can, at least temporarily, reduce signal honesty by increasing EODa as happens also in *B. gauderio* (Gavassa et al., 2012). We do not yet know, however, how long this strategy can be maintained.

It is well documented that EODa and waveform are regulated by melanocortin peptides over minutes to hours (Markham et al., 2009a, 2009b) and regulated by steroid hormones over days to weeks (Allee et al., 2009; Dunlap and Zakon, 1998; Few and Zakon, 2001), largely in response to prevailing social conditions (Gavassa et al., 2013; Salazar and Stoddard, 2009). The present findings have identified a novel neuroendocrine pathway that regulates EODa in accordance with dietary energy availability and metabolic stress during periods of energy shortfall, further highlighting the central importance of energetic constraints in shaping the communication and sensory signals of weakly electric fish (Salazar et al., 2013). Further research directed toward a full account of the behavioral consequences of EODa changes as well as the physiological pathways and mechanisms regulating EODa will provide an integrated understanding of how evolutionary forces have shaped energetically costly communication signals in electric fish.

Beyond the context of communication in electric fish, the present results might have more general implications for understanding the cost–benefit tradeoffs inherent in animal communication systems and how these tradeoffs might alter signaling behavior in response to metabolic stress. The cost–benefit structure of signaling is potentially different for animals that rely only on passive sensory systems than it is for animals where signaling is coupled to active sensory systems. Specifically, assuming signals of equal metabolic cost, the overall costs of signaling would be higher for passive sensing animals, while the costs of reducing or stopping signaling are higher for active-sensing animals. During signaling, passive-sensing animals incur metabolic, risk, and foraging opportunity costs, while active-sensing animals incur only metabolic and risk costs. In contrast, reducing or ceasing signaling restores the opportunity to forage for prey or food items in passive-sensing animals, but could have the opposite effect of impairing or eliminating the ability to locate food items in active-sensing animals.

From this perspective it seems reasonable to predict different responses to metabolic stress in passive- versus active-sensing animals. Passive-sensing animals should more readily reduce signaling effort during periods of food restriction or deprivation in order to reduce metabolic costs while regaining foraging opportunity. Active-sensing animals, in contrast, would need to strike a balance between reducing metabolic costs while retaining sufficient sensory performance to support foraging. The present results and those of Gavassa et al. (2012) suggest that, for active-sensing electric fish, that balance depends in part on reproductive strategy. Direct experimental comparisons between passive- and active-sensing animals face the major methodological challenges of normalizing signaling effort, opportunity costs, and metabolic stress. Nonetheless, such experiments could provide important advances in understanding the potentially different optimization strategies through which animal communication signals respond to food shortages and metabolic stress.

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References

Ahima, R.S., Osei, S.Y., 2004. Leptin signaling. Physiol. Behav. 81, 223–241.

Allee, S.J., Markham, M.R., Salazar, V.L., Stoddard, P.K., 2008. Opposing actions of 5HT(1A) and 5HT(2)-like serotonin receptors on modulations of the electric signal waveform in the electric fish *Brachyhypopomus pinnicaudatus*. Horm. Behav. 53, 481–488.

Allee, S.J., Markham, M.R., Stoddard, P.K., 2009. Androgens enhance plasticity of an electric communication signal in female knifefish, *Brachyhypopomus pinnicaudatus*. Horm. Behav. 56, 264–273.

Baver, S.B., Hope, K., Guyot, S., Bjørbaek, C., Kaczorowski, C., O'Connell, K.M.S., 2014. Leptin modulates the intrinsic excitability of AgRP/NPY neurons in the arcuate nucleus of the hypothalamus. J. Neurosci. 34, 5486–5496.

Bernier, N.J., Gorissen, M., Flik, G., 2012. Differential effects of chronic hypoxia and feed restriction on the expression of leptin and its receptor, food intake regulation and the endocrine stress response in common carp. J. Exp. Biol. 215, 2273–2282.

Bonate, P.L., 2000. Analysis of Pretest–Posttest Designs. Chapman & Hall/CRC, Boca Raton.

Caputi, A., Budelli, R., 2006. Peripheral electrosensory imaging by weakly electric fish. J. Comp. Physiol. A. 192, 587.

Caputi, A.A., Budelli, R., Grant, K., Bell, C.C., 1998. The electric image in weakly electric fish: physical images of resistive objects in *Gnathonemus petersii*. J. Exp. Biol. 201, 2115–2128.

Chu, D.L., Li, V.W., Yu, R.M., 2010. Leptin: clue to poor appetite in oxygen-starved fish. Mol. Cell. Endocrinol. 319, 143–146.

Crampton, W.G.R., 1998. Effects of anoxia on the distribution, respiratory strategies and electric signal diversity of gymnotiform fishes. J. Fish Biol. 53, 307–330.

Crampton, W.G., Albert, J.S., 2006. Evolution of electric signal diversity in gymnotiform fishes. In: Ladich, F., Collin, S.P., Moller, P., Kapoor, B.G. (Eds.), Communication in Fishes. Science Publishers, Enfield, NH, pp. 647–731.

Crespi, E.J., Denver, R.J., 2006. Leptin (ob gene) of the South African clawed frog *Xenopus laevis*. Proc. Natl. Acad. Sci. U. S. A. 103, 10092–10097.

Dunlap, K.D., Zakon, H.H., 1998. Behavioral actions of androgens and androgen receptor expression in the electrocommunication system of an electric fish, *Eigenmannia virescens*. Horm. Behav. 34, 30–38.

Few, W.P., Zakon, H.H., 2001. Androgens alter electric organ discharge pulse duration despite stability in electric organ discharge frequency. Horm. Behav. 40, 434–442.

Gavassa, S., Stoddard, P.K., 2012. Food restriction promotes signaling effort in response to social challenge in a short-lived electric fish. Horm. Behav. 62, 381–388.

Gavassa, S., Silva, A.C., Stoddard, P.K., 2011. Tight hormonal phenotypic integration ensures honesty of the electric signal of male and female *Brachyhypopomus gauderio*. Horm. Behav. 60, 420–426.

Gavassa, S., Silva, A.C., Gonzalez, E., Stoddard, P.K., 2012. Signal modulation as a mechanism for handicap disposal. Anim. Behav. 83, 935–944.

Gavassa, S., Goldina, A., Silva, A.C., Stoddard, P.K., 2013. Behavioral ecology, endocrinology and signal reliability of electric communication. J. Exp. Biol. 216, 2403–2411.

Hagedorn, M., Heiligenberg, W., 1985. Court and spark: electric signals in the courtship and mating of gymnotoid fish. Anim. Behav. 33, 254–265.

Hopkins, C.D., 1972. Sex differences in electric signaling in an electric fish. Science 176, 1035–1037.

Hopkins, C.D., 1974. Electric communication: functions in the social behavior of *Eigenmannia virescens*. Behaviour 50, 270–305.

Hopkins, C.D., Bass, A.H., 1981. Temporal coding of species recognition signals in an electric fish. Science 212, 85–87.

Hupé, G.J., Lewis, J.E., 2008. Electrocommunication signals in free swimming brown ghost knifefish, *Apteronotus leptorhynchus*. J. Exp. Biol. 211, 1657–1667.

Kirschbaum, F., 1979. Reproduction of the weakly electric fish *Eigenmannia virescens* (Rhamphichtyidae, Teleostei) in captivity. 1. Control of gonadal recrudescence and regression by environmental-factors. Behav. Ecol. Sociobiol. 4, 331–355.

Kirschbaum, F., Schwassmann, H.O., 2008. Ontogeny and evolution of electric organs in gymnotiform fish. J. Physiol. Paris 102, 347–356.

Kramer, B., 1987. The sexually dimorphic jamming avoidance-response in the electric fish *Eigenmannia* (Teleostei, Gymnotiformes). J. Exp. Biol. 130, 39–62.

Lewis, J.E., Gilmour, K.M., Moorhead, M.J., Perry, S.F., Markham, M.R., 2014. Action potential energetics at the organismal level reveal a trade-off in efficiency at high firing rates. J. Neurosci. 34, 197–201.

MacDonald, L.E., Alderman, S.L., Kramer, S., Woo, P.T., Bernier, N.J., 2014. Hypoxemia-induced leptin secretion: a mechanism for the control of food intake in diseased fish. J. Endocrinol. 221, 441–455.

Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., et al., 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1, 1155–1161.

Markham, M.R., Stoddard, P.K., 2005. Adrenocorticotropic hormone enhances the masculinity of an electric communication signal by modulating the waveform and timing of action potentials within individual cells. J. Neurosci. 25, 8746–8754.

Markham, M.R., Stoddard, P.K., 2013. Cellular mechanisms of developmental and sex differences in the rapid hormonal modulation of a social communication signal. Horm. Behav. 63, 586–597.

Markham, M.R., Allee, S.J., Goldina, A., Stoddard, P.K., 2009a. Melanocortins regulate the electric waveforms of gymnotiform electric fish. Horm. Behav. 55, 306–313.

Markham, M.R., McAnelly, M.L., Stoddard, P.K., Zakon, H.H., 2009b. Circadian and social cues regulate ion channel trafficking. PLoS Biol. 7, e1000203.

Marsat, G., Longtin, A., Maler, L., 2012. Cellular and circuit properties supporting different sensory coding strategies in electric fish and other systems. Curr. Opin. Neurobiol. 22, 686–692.

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15.

McGregor, P.K., Westby, G.W.M., 1992. Discrimination of individually characteristic electric organ discharges by a weakly electric fish. Anim. Behav. 43, 977–986.

National Institutes of Health (U.S.), Office of Laboratory Animal Welfare, United States. Public Health Service, 2002. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Office of Laboratory Animal Welfare, National Institutes of Health, Dept. of Health and Human Services, Bethesda, MD (6705 Rockledge Dr., Bethesda 20892-7982).

Reardon, E.E., Parisi, A., Krahe, R., Chapman, L.J., 2011. Energetic constraints on electric signalling in wave-type weakly electric fishes. J. Exp. Biol. 214, 4141–4150.

Ronnestad, I., Nilsen, T.O., Murashita, K., Angotzi, A.R., Gamst Moen, A.G., Stefansson, S.O., Kling, P., Thrandur Bjornsson, B., Kurokawa, T., 2010. Leptin and leptin receptor genes in Atlantic salmon: cloning, phylogeny, tissue distribution and expression correlated to long-term feeding status. Gen. Comp. Endocrinol. 168, 55–70.

Salazar, V.L., Stoddard, P.K., 2008. Sex differences in energetic costs explain sexual dimorphism in the circadian rhythm modulation of the electrocommunication signal of the gymnotiform fish *Brachyhypopomus pinnicaudatus*. J. Exp. Biol. 211, 1012–1020.

Salazar, V.L., Stoddard, P.K., 2009. Social competition affects electric signal plasticity and steroid levels in the gymnotiform fish *Brachyhypopomus gauderio*. Horm. Behav. 56, 399–409.

Salazar, V.L., Krahe, R., Lewis, J.E., 2013. The energetics of electric organ discharge generation in gymnotiform weakly electric fish. J. Exp. Biol. 216, 2459–2468.

Schwartz, M.W., Woods, S.C., Porte Jr., D., Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. Nature 404, 661–671.

Shanley, L.J., Irving, A.J., Rae, M.G., Ashford, M.L.J., Harvey, J., 2002. Leptin inhibits rat hippocampal neurons via activation of large conductance calcium-activated K+ channels. Nat. Neurosci. 5, 299–300.

Shimizu, H., Inoue, K., Mori, M., 2007. The leptin-dependent and -independent melanocortin signaling system: regulation of feeding and energy expenditure. J. Endocrinol. 193, 1–9.

Silva, A., Quintana, L., Galeano, M., Errandonea, P., 2003. Biogeography and breeding in Gymnotiformes from Uruguay. Environ. Biol. Fish 66, 329–338.

Silva, A., Perrone, R., Macadar, O., 2007. Environmental, seasonal, and social modulations of basal activity in a weakly electric fish. Physiol. Behav. 90, 525–536.

Spanswick, D., Smith, M.A., Groppi, V.E., Logan, S.D., Ashford, M.L., 1997. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature 390, 521–525.

Stoddard, P.K., Salazar, V.L., 2011. Energetic cost of communication. J. Exp. Biol. 214, 200–205.

Stoddard, P.K., Markham, M.R., Salazar, V.L., 2003. Serotonin modulates the electric waveform of the gymnotiform electric fish *Brachyhypopomus pinnicaudatus*. J. Exp. Biol. 206, 1353–1362.

Stoddard, P.K., Zakon, H.H., Markham, M.R., McAnelly, L., 2006. Regulation and modulation of electric waveforms in gymnotiform electric fish. J. Comp. Physiol. A. 192, 613.

Stoddard, P.K., Markham, M.R., Salazar, V.L., Allee, S., 2007. Circadian rhythms in electric waveform structure and rate in the electric fish *Brachyhypopomus pinnicaudatus*. Physiol. Behav. 90, 11–20.

Vickers, A.J., 2001. The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. BMC Med. Res. Methodol. 1, 6.

von der Emde, G., 1999. Active electrolocation of objects in weakly electric fish. J. Exp. Biol. 202, 1205–1215.

von der Emde, G., 2006. Non-visual environmental imaging and object detection through active electrolocation in weakly electric fish. J. Comp. Physiol. A. 192, 601.

Williams, K.W., Scott, M.M., Elmquist, J.K., 2011. Modulation of the central melanocortin system by leptin, insulin, and serotonin: co-ordinated actions in a dispersed neuronal network. Eur. J. Pharmacol. 660, 2–12.

Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. 15, 711–724.