

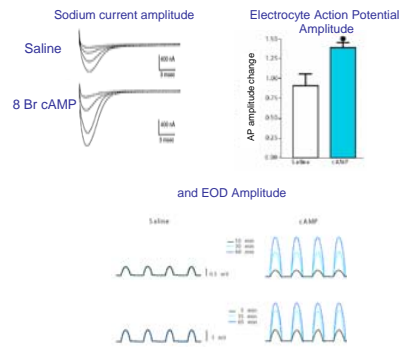
## Introduction

The electric organ discharge (EOD) of weakly electric fish functions (in addition to electrolocation) as an intraspecific communication signal, and conveys information about species and gender identity as well as reproductive readiness or dominance status. The basic component of this unique communication signal is the electrocyte action potential (AP). Synchronous firing of the electrocytes results in a compound AP which equates to one pulse of the EOD. Variable features of the EOD are frequency, determined by the brainstem pacemaker, and EOD pulse duration and amplitude, controlled by the electrocyte. Any of these features can be modulated in response to change in the physical or social environment. For example, in *Sternopygus macrurus* EOD frequency and pulse duration exhibit sexual dimorphism which becomes more pronounced during breeding season. Recent work has documented diurnal variation in the EOD amplitude of *Brachyhyppopomus pinnicaudatus*.

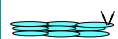
Activation of PKA with 8 Br cAMP increases the sodium current (INa) amplitude in *Sternopygus macrurus*, and injections of 8Br cAMP evoke rapid increases in the EOD amplitude while in *Brachyhyppopomus* injection of ACTH increases EOD amplitude.

We propose that diurnal variation in EOD amplitude is a general phenomenon in weakly electric fish and that the effect of ACTH on amplitude occurs via activation of PKA which in turn phosphorylates ion channels. We examined whether EOD amplitude of *Sternopygus* varies diurnally and looked at the effect of ACTH on the EOD and sodium current in this species.

### Activation of PKA in *Sternopygus* increases ...



## Methods



**Voltage Clamp** Recordings were made in an *in vitro* electric organ preparation. We used the Axon Laboratory 2B amplifier, Digidata 1320A data acquisition system and Pclamp 8 software in the two electrode voltage clamp configuration to isolate the sodium currents. ACTH was bath applied at 100 nM and 60 mM TEA was used to block the outward delayed rectifying potassium current.

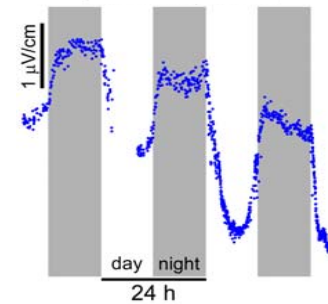


**EOD Modulation** The "fish in a tube" configuration previously developed by Stoddard and colleagues for *Brachyhyppopomus* was modified for the larger *Sternopygus*. EOD amplitude was measured peak-to-peak. RMS and other related measures of waveform power correlated with peak-to-peak amplitude. EOD frequency remained stable throughout the experiments.

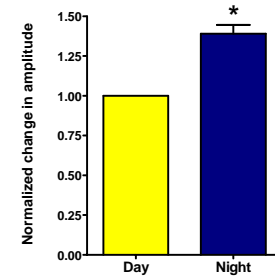
**Diurnal Variation in EOD.** Individual fish were placed into the recording tank and allowed to equilibrate for one day under a 12:12 light:dark cycle. Recordings were made over three days beginning on Day 2.

**Effect of ACTH on EOD.** The fish were placed into the recording tank and baseline measures taken for at least three days. Intramuscular injections of either saline or ACTH (25 nM/g) were made at midday. The following day the fish was injected with the alternate solution and order of treatment was counterbalanced across fish. Order of treatment had no effect.

## 1 Diurnal Variation in *Sternopygus* EOD Amplitude

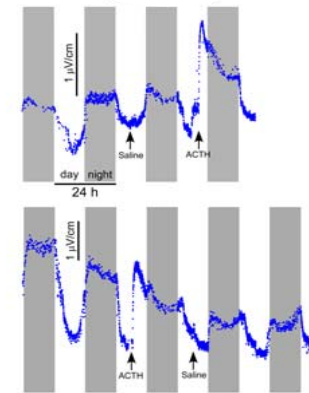


EOD amplitude reaches a maximum during the night, begins to drop with the onset of the light cycle and declines throughout the day until a minimum is reached at around mid-day (approximately 1:00 pm). Note that the EOD amplitude begins to increase in advance of the dark cycle, suggesting a true circadian rhythm.



Increase in EOD amplitude during the night compared to day time minimum amplitude. Mean peak night time EOD amplitude was 39 ( $\pm 5.5$ )% greater than mean minimum daytime amplitude, a difference that is significant at  $p = 0.0203$  (paired t test,  $n = 8$ ).

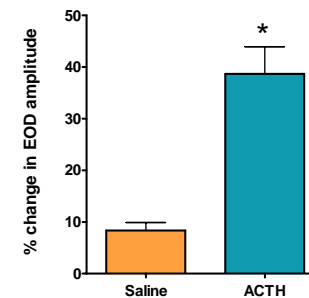
## 2 ACTH Injections Increase EOD Amplitude



Top panel: The fish was injected first with saline about halfway through the light cycle and then about 24 hours later with ACTH.

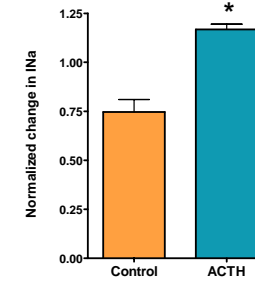
Bottom panel: A second fish in which the order of injections was reversed.

EOD amplitude increased rapidly following injection; half maximal amplitude was achieved within 8.4 min ( $\pm 1.362$  min) and peak amplitude by 59.08 min ( $\pm 4.428$  min) Order of injection did not affect the response.



ACTH injections evoked a robust increase in EOD amplitude. The mean amplitude increase produced by ACTH was 38.66% ( $\pm 5.288$ ),  $p < 0.0001$  (t test of saline v ACTH, two-tailed, unpaired,  $n = 8$  in each group).

## 3 ACTH Increases Sodium Current Amplitude



Application of ACTH to electrocytes *in vitro* increases sodium current amplitude (mean increase in INa compared to control = 42% ( $\pm 0.0278$ )  $N=5$ , t test of control v ACTH  $p = 0.0003$ , two-tailed, unpaired.)

## Conclusions

- Sternopygus* shows a clear diurnal variation in EOD amplitude. The increase in night time amplitude ( $39 \pm 5.5\%$ ) is similar in scale to that seen in male *Brachyhyppopomus* ( $24 \pm 9\%$ ). The EOD amplitude increase anticipates the onset of the dark cycle, supporting the idea that *Sternopygus* EOD amplitude variation is a true circadian rhythm as is the case for *Brachyhyppopomus*. It is reasonable to propose that diurnal amplitude modulation may be a general phenomenon in the weakly electric fish.
- ACTH acts on melanocortin receptors and melanocortin receptors act via PKA. The results presented here in conjunction with earlier studies suggest that ACTH increases *Sternopygus* EOD amplitude either by directly phosphorylating sodium channels or indirectly by phosphorylating an intermediary that acts on the channels. Work is underway to determine whether ACTH also affects the electrocyte ion currents in *Brachyhyppopomus*.
- Amplitude modulation has many possible advantages for the fish. Increased amplitude during the night when the fish are active likely expands broadcast and detection range. Conversely the ability to turn down amplitude during the day may serve a vital function making the animals less visible to electroreceptive predators. In addition decreased daytime amplitude probably allows for significant energy savings.

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