



Supplementary Material for
The shocking predatory strike of the electric eel

Kenneth Catania*

*Corresponding author. E-mail: ken.catania@vanderbilt.edu

Published 5 December 2014, *Science* **346**, 1231 (2014)
DOI: 10.1126/science.1260807

This PDF file includes:

Materials and Methods

Supplementary Text

Figs. S1 to S4

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencemag.org/content/346/6214/1231/suppl/DC1)

Movies S1 to S7

Supplementary Materials:

Materials and Methods

Figures S1-S4

Movies S1-S7

Supplementary Text

Materials and Methods

Animals

All procedures were approved by the Vanderbilt Institutional Animal Care and Use Committee. Four eels (*Electrophorus electricus*) were purchased from a commercial fish supplier and housed individually in custom-made Plexiglas aquariums ranging in size from 80 to 120 gallons (300-480 liters) with aerated water, gravel bottom, rocks, plastic imitation branches, and plastic plants with water temperature maintained between 24 and 28 °C with thermostatically controlled aquarium heaters and pH between 6.5 and 7.5. Lighting was on a 12/12 light-dark cycle and eels were fed earthworms, fish, and crayfish. Eels ranged in size from 50 cm (2 specimens) to 70-80 cm (1 specimen), and 115 cm (1 specimen).

Recordings of Eel Predatory Behavior

For recordings of predatory behavior and the effects of the electric organ discharge on prey animals were transferred to either a 75 cm x 30 cm x 12 cm or 150 cm x 56 cm x 25 cm (LxWxH) custom made Plexiglas aquarium. Water temperature was 25-26 °C, pH. 7.1-7.4. The electric organ discharges were recorded using carbon electrodes (1.4 cm x 30 cm) in the water connected on their exposed tips to wire leads from a split BNC cable that connected directly to 1 channel of either a PowerLab 8/35 or PowerLab 4/30 data acquisition unit (ADInstruments) sampling at a minimum of 100k per second and in turn connected to a MacPro laptop running LabChart 7 software (ADInstruments). Video was collected with a MotionPro HS-3 camera (Redlake) at 1000 frames per second with 2 RPS Studio CoolLED 100 RS-5610 for lighting at 704 microsecond shutter speed using the circular recording mode for capturing events. The camera's synchronization output was recorded on a separate PowerLab channel allowing precise coordination of each frame with other recorded events (e.g. the EOD). Video was transferred to a MacPro laptop using MotionProX software (Integrated Tools Design). To illustrate the relationship of each high-voltage EOD to behavior in the supplementary movies, each frame during which an EOD occurred (at 1000 fps each EOD peak corresponded to a unique frame) was colorized in Photoshop CS 6 (Adobe Systems Incorporated). The tiff format image files were then opened in sequence in QuickTime Player 7 Pro (Apple Inc) and the sequence was exported as a QuickTime movie. To illustrate the effectiveness of the EOD in figure 1c, an arbitrarily recorded c-start was retrospectively identified in a different, but size-matched fish, and the positions of their bodies and timing of movements were matched at the 40 ms plate in figure 1B, and then subsequently illustrated 160 ms later in 'C'.

Prey Fish Muscle Tension Experiments

To investigate the effect of the eel's EOD on muscle tension, prey fish were anesthetized with 2% buffered MS222 and pithed to destroy the brain. The pithing hole was sealed with cyanoacrylate (Vetbond, 3M) and the fish was clamped at the tail with a plastic clamp and attached rostrally to a MLTF500/ST force transducer (ADInstruments) using nylon thread sutured through the mandible and premaxillary. The pithed-fish was separated from the eel by a 1% agar barrier that was suspended in a rectangular Plexiglas frame with a thin nylon screen in the center for added support and to ensure the eel did not break through the barrier. The output of the force transducer was connected to a channel of the PowerLab to allow comparison of

tension to EOD timing. 20 cases of tension onset latency from the PowerLab traces were compared to 20 trials of first twitch movement from high speed video as described in the text. The latter measures were obtained by magnifying the slow-motion playback of eel attacks on fish and marking the first frame during which the characteristic, visible muscle contractions associated with the EOD occurred. These initial, subtle movements could be confidently attributed to the EOD by backtracking the video from the more overt movements that followed. For both the tension onset and video-twitch measures, latency to effect was measured from the peak of the first EOD in the volley. Comparison of the mean latency for tension onset (n=20, mean 3.35 ms, Std 0.42 ms) was significantly longer than video derived twitch onset (n=20, mean 2.92 ms, Std 0.85 ms) as indicated by t-test (t ratio 2.016, p=0.027) but not discussed given that tension development for the force transducer trials, through nylon fibers, could be expected to lag first visible muscle response.

Pithing that included spinal cord (double-pithing) was accomplished using the stainless steel plunger of a Hamilton syringe that was bent to match the curvature of the fish spinal column. The procedure was perfected using dead fish and success confirmed through dissection with the rod in place. Successful use in brain-pithed fish with viable muscles was further perfected prior to experiment and a correlation between successive segmental twitching and proper pithing signaled the rod traversed the spinal cord. After experiment this was confirmed on gross microscopic inspection of the hemisected fish.

To provide a rough comparison of eel-generated tension to maximal pithed-fish tension, a subset of pithed fish were directly stimulated by connecting the leads of an SD9 grass stimulator to the head and tail with alligator clips with settings as described and illustrated in figure S1. To investigate the effect of curare on muscle tension, two force transducers were used for two pithed fish preparations and 20 microliters of 1% curare was injected at a shallow angle IP in one fish, whereas 20 microliters of saline was injected into the other. Un-normalized tension responses were collected (and illustrated) over time as the eel periodically shocked and consumed earthworms behind the agar barrier at a distance of roughly 5 to 15 centimeters depending on eel movements.

Analysis of Interpulse Intervals During Electric Organ Discharge

To examine interpulse intervals during eel attack volleys, high-voltage EOD's were recorded from each eel as it attacked prey or moving objects. Each volley in the analysis was separated by at least one minute from any other high voltage EOD. Secondary volleys (Fig. S4B) were those that occurred with at least a 50 ms gap between it and the preceding volley (but no more than 1000ms - such volleys often occurred when eels were manipulating captured prey). Interpulse intervals were measured by first using the "Find Local Maximum" (or minimum, depending on eel orientation relative to electrodes) command in LabChart, then visually confirming that the program had identified a high-voltage EOD peak, and then using the Datapad function to collect and export the times to Microsoft Excel. IPI's were then calculated in Excel, and imported into the JMP statistical program (SAS), and the intervals were assessed with an ANOVA followed by Tukey's HSD.

Experiments Simulating Hidden Prey

To investigate the use of doublets during hunting, prey were placed below a 1% agar barrier in the larger Plexiglas aquarium previously described and filmed from below with camera settings as described above. In some cases, the agar barrier was perforated to facilitate the transfer of mechanosensory cues (e.g. Fig. 3G). To simulate the effect of the eel's doublet (fish twitch) in the experimental preparation illustrated in Figure 4, the "Fast Response Output" feature of LabChart was used, triggering a brief square wave when the eel's EOD crossed threshold typical for the eel's high-voltage output. The output of the PowerLab was in turn connected to the triggering input of an SD9 Grass stimulator set for output of 5 volts, 1 ms, and no delay. Recordings through a monitoring channel on the PowerLab confirmed that this output was essentially instantaneous once triggered. The leads of the SD9 stimulator were connected through the fish pithing hole and the fish rectum. The fish was then placed into a small Ziploc bag that was sealed around the electrodes.

As evident on the supplementary video, after a slight physiological delay, fish twitch was generated in this paradigm by the SD9 output. Because it was not always obvious when and where an exploring eel would emit a doublet, it was often most expedient in early trials to trigger the SD9 for every high-voltage EOD. For example setting the LabChart to trigger the SD9 only once would require resetting the software if the eel emitted a doublet on the far side of the tank. Thus in some supplementary videos the pithed fish responds to both the doublet and the subsequent volley as a result of SD9 stimulation. However pithed fish inside the plastic bag never independently responded to the eel's EOD in the absence of SD9 stimulation (see movie S6, clip 6).

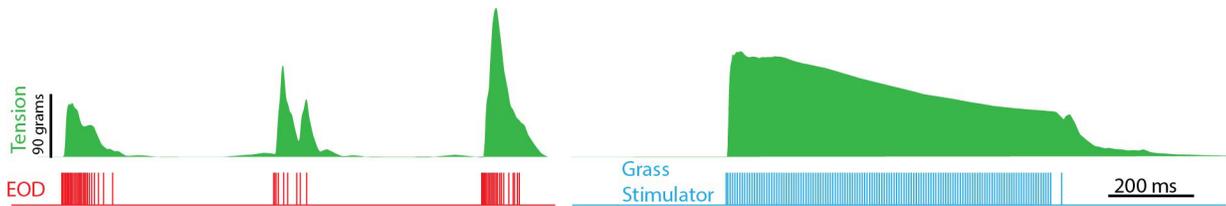


Figure S1 Tension in a pithed fish (24.8 grams) induced by eel EOD compared to tension produced by direct stimulation. Left shows the tension (green) developed in relationship to the eel EOD (red). The same fish preparation (2 minutes later), in the same configuration (Fig. 2a) was then stimulated with leads connected directly to the tail and head with alligator clamps from a Grass SD9 stimulator set at 50 volts, 200 Hz, with 1ms square waves (blue). Similar results were obtained in other examples indicating that eels likely induce a substantial proportion of maximal whole body fish tension with their EOD. The amount of tension developed under this condition supports the conclusion that eels induce sufficient whole-body tension to prevent voluntary movement.

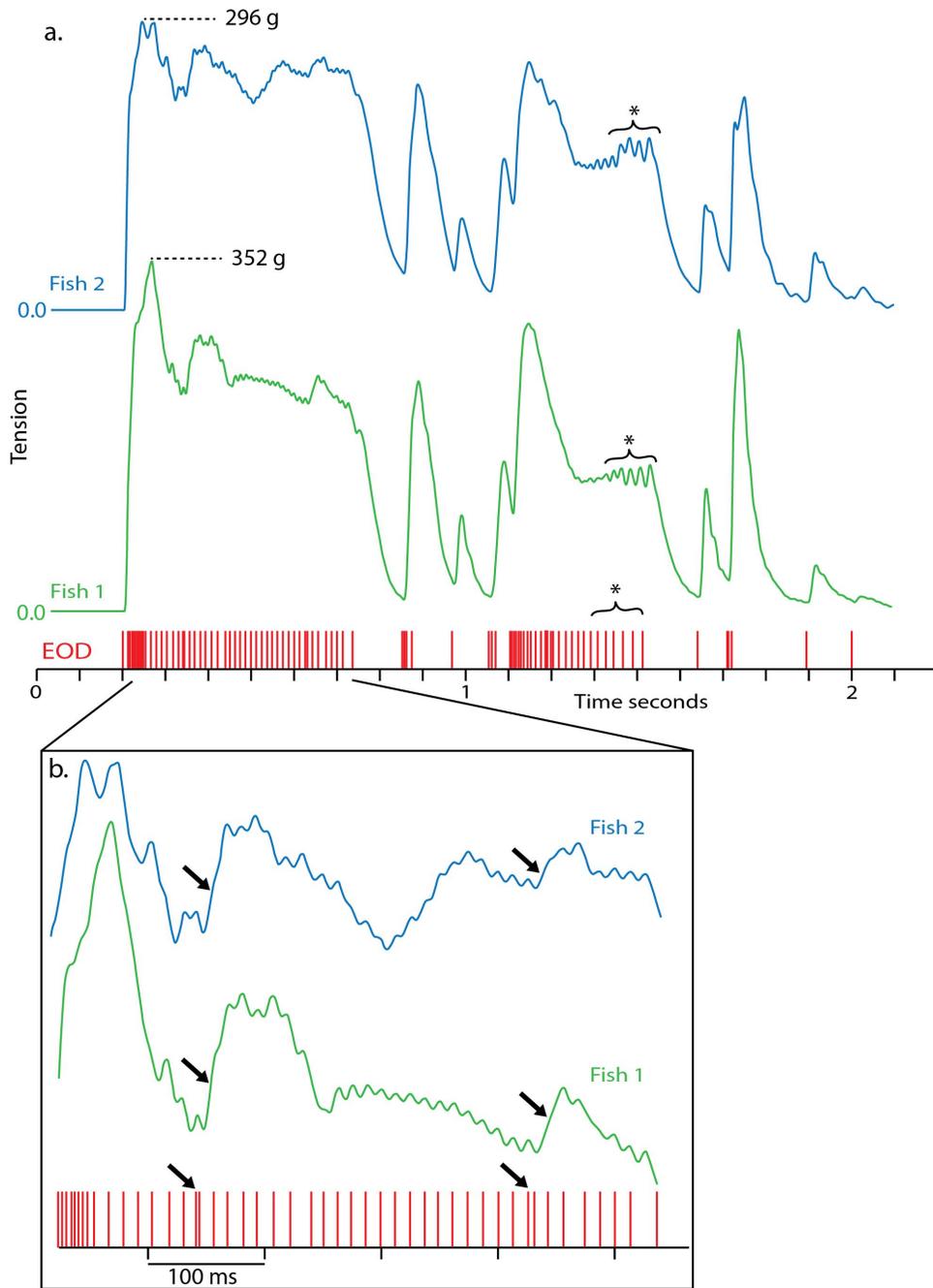


Figure S2 Coactivation of muscles in 2 adjacent fish during an eel's strong EOD. **A.** Green and blue traces show tension in a pithed (Fish 2, 23.8 g koi) and double-pithed (Fish 1, 23.6 gram koi) respectively. Red tick-marks below mark the time of emission for each strong EOD pulse from a 50 cm eel as it was fed night crawlers while separated from the fish preparations by an agar barrier. A similar pattern of overall tension was induced in each fish during each volley, and individual fish twitches were evoked at longer interpulse intervals. This is evident as subtle peaks during the first volley, and as larger peaks during the longer intervals in the second volley (asterisk). **B.** Expanded time frame for a section of the initial volley reveals more details. Note in particular the effect of closely spaced EOD's that occur in the midst of an otherwise decreasing rate (arrows). These cause a marked increase in tension for both fish preparations.

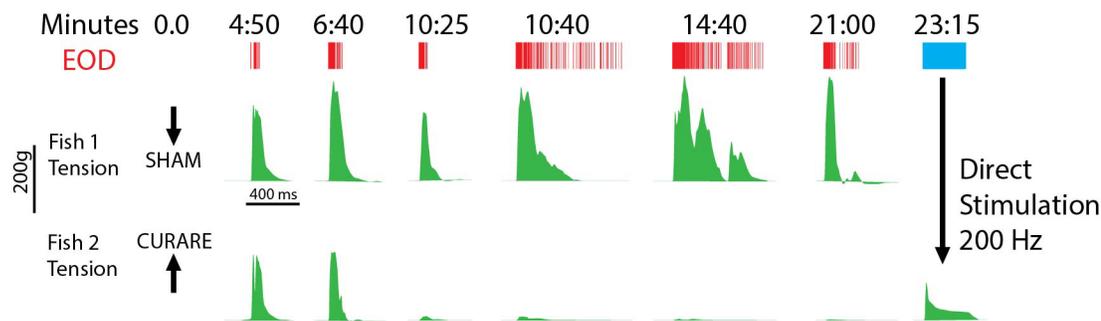


Figure S3 Effect of curare. Red trace indicates the strong EOD matched in time to un-normalized tension in two pithed fish preparations (green). Fish 1 = 37.7 g, fish 2 = 37.2 g. Arrows indicate the time of sham (fish 1, 20 microliters of saline ip) or curare (fish 2, 20 microliters of 1% curare ip) injection. The initial, unnormalized tension responses were similar in time-course and magnitude, but the curare injected fish-tension responses dropped to near zero over a short time. The far right shows the tension response to direct stimulation of the curarized preparation with the SD9 Grass stimulator with leads connected directly to the head and tail of the fish, at 100 volts, 200 Hz, 1ms square wave (blue at 23:15 time).

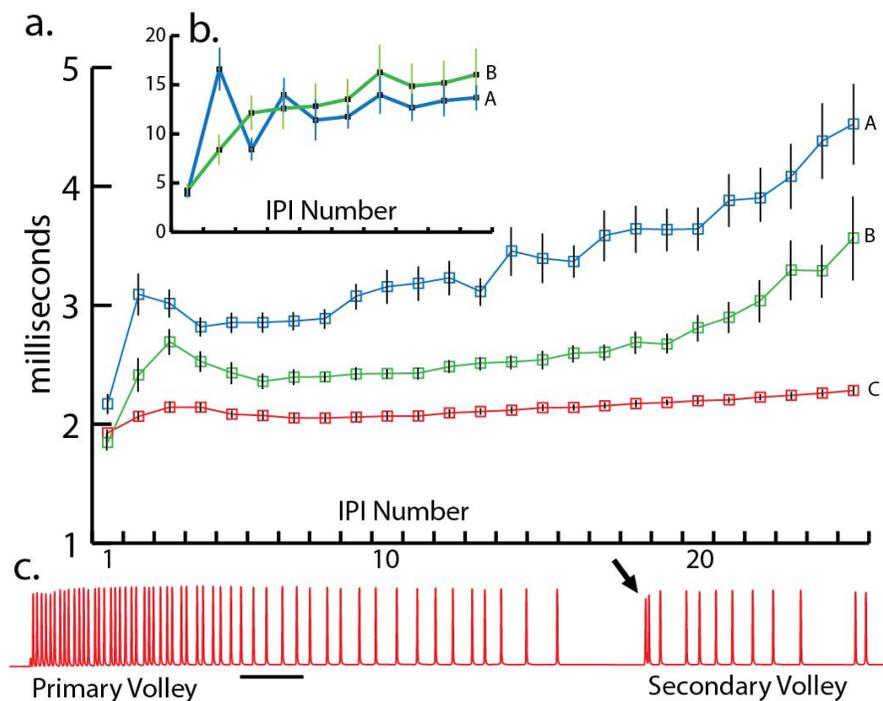


Figure S4 Interpulse intervals for eel strong EOD volleys. **A.** Interpulse interval (IPI) number and length illustrated for 3 of 4 eels investigated in this study (4th omitted for clarity). Blue, Green and Red range in order from smallest eel (50 cm) to largest eel (115 cm) showing a trend toward shorter IPI's for larger eels. The first IPI was significantly shorter than the next 9 (ANOVA followed by Tukey-Kramer HSD) with $F=6.795$, $P<0.0001$ for eel A ($n=40$ volleys), $F=5.677$, $P<0.0001$ for eel B ($n=20$ volleys), and $F=4.882$, $P<0.0001$ for eel C ($n=75$ volleys). Although the distinction between the first IPI and later intervals was subtle for the first volleys of an attack, the short, first IPI (doublet) was more obvious in secondary and later volleys as shown in "c" (arrow). **B.** IPI number and length for volleys that occurred later in an attack (shown for 2 eels for clarity). **C.** Example of a primary volley at attack onset, followed shortly by a secondary volley. Secondary volleys were common when prey were captured and being manipulated by the eel.

Movie Captions

Movie S1. Eels emit two different magnitudes of electric organ discharge. The low amplitude discharge is used for exploring their environment and does not function in offense or defense. This video shows an eel emitting its low voltage electric organ discharge followed by an example of a high-voltage volley during prey capture.

Movie S2 This movie shows examples of an eel capturing fish in slow motion. Red frames have been colorized to indicate each time a high-voltage pulse was emitted. In some cases, when the eel discharge frequency slows, fish regain the ability to move and escape.

Movie S3 This movie shows eels emitting “doublets” or pairs of high-voltage pulses while investigating their surroundings and various objects. In particular, eels often explore conductors with doublets. These sound like short chirps on the audio.

Movie S4 This movie shows a pithed fish (under perforated, thick agar) being investigated by an eel using doublets. Note the effect of the doublets on the pithed-fish muscles and the subsequent transition of the eel from exploratory to attack behavior.

Movie S5 This movie shows examples of eels hunting prey (below agar) using an initial doublet that evokes prey movement.

Movie S6 This movie shows examples of experiments and controls that use stimulation of a pithed fish preparation to mimic the effect of an eel doublet on prey. See Figure 4 for more details on each variation.

Movie S7 This movie shows an eel, filmed from the side, as it uses doublets while hunting a tadpole below agar. As the tadpole moves in response to each doublet the eel followed and eventually attacks following a doublet (see trace of EOD at end of movie).

SI Text

For the experiments illustrated in Figure 4C, the latency from fish twitch to attack was 27.6 ms (Std 6.3) for eel 1 and 24.8 ms (Std 4.6) for eel 2. These values were not significantly different from latencies from doublet to attack volley for actual prey for each eel (mean 26.6 ms, Std 5.2 eel 1; mean 25.1ms, Std 4.1 eel 2), nor were they significantly different from responses to twitch generated in the absence of doublets (Figure 4D) with latencies of 35.2 ms, Std 5.1 (eel 1) and 25.5 ms Std 5.6 (eel 2). Significance assessed with ANOVA; F Ratio 3.2 ($p=0.056$) for eel 1 and F Ratio 0.053 ($p=0.95$) eel 2. Exceptions to the twitch-triggered attack behavior were trials during which doublets triggered twitch when the eel was distant from the preparation.