TECHNICAL PAPER
An Electrophysiological Investigation of Power-Amplification in the Ballistic Mantis Shrimp Punch

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Mantis shrimp are aggressive, burrowing crustaceans that hunt using one the fastest movements in the natural world. These stomatopods can crack the calcified shells of prey or spear down unsuspecting fish with lighting speed. Their strike makes use of power-amplification mechanisms to move their limbs much faster than is possible by muscles alone. Other arthropods such as crickets and grasshoppers also use power-amplified kicks that allow these animals to rapidly jump away from predator threats. Here we present a template laboratory exercise for studying the electrophysiology of power-amplified limb movement in arthropods, with a specific focus on mantis shrimp strikes. The exercise is designed in such a way that it can be applied to other species that perform power-amplified limb movements (e.g., house crickets, *Acheta domesticus*) and species that do not (e.g., cockroaches, *Blaberus discoidalis*). Students learn to handle the animals, make and implant electromyogram (EMG) probes, and finally perform experiments. This integrative approach introduces the concept of power-amplified neuromuscular control; allows students to develop scientific methods, and conveys high-level insights into behavior, and convergent evolution, the process by which different species evolve similar traits.

Our power-amplification laboratory exercise involves a non-terminal preparation which allows electrophysiological recordings across multiple days from arthropods using a low-cost EMG amplifier. Students learn the principles of electrophysiology by fabricating their own electrode system and performing implant surgeries. Students then present behaviorally-relevant stimuli that generate attack strikes in the animals during the electrophysiology experiments to get insight into the underlying mechanisms of power amplification. Analyses of the EMG data (spike train burst duration, firing rate, and spike amplitude) allow students to compare mantis shrimp with other power-amplifying species, as well as a non-power-amplifying one. The major learning goal of this exercise is to empower students by providing an experience to develop their own setup to examine a complex biological principle. By contrasting power-amplifiers with non-power-amplifiers, these analyses highlight the peculiarity of power amplification at multiple levels of analysis, from behavior to physiology. Our comparative design requires students to consider the behavioral function of the movement in different species alongside the neuromuscular underpinnings of each movement. This laboratory exercise allows students to develop methodology, problem-solving and inquisitive skills crucial for pursuing science.

Key words: electrophysiology, behavior, convergent evolution, power amplification, motor control, muscle activity

The opportunity to discover insights into the natural world through behavior can inspire undergraduates to select a neuroscience laboratory course or choose neuroscience as a major. Laboratory exercises that investigate the extraordinary behaviors of animals can capture the imagination of students and promote a deeper engagement with the field (Pokala and Glater, 2018). For decades, the amazing hunting abilities of mantis shrimp (stomatopods) have attracted the attention of the public (e.g., British Broadcasting Corporation Television, 1985). While measuring only a few inches long, mantis shrimp can throw the fastest punch of any animal with a peak impact force equivalent to a tiger's bite (Patek et al., 2004; Kagaya and Patek, 2016; Crane et al., 2018). Smaller mantis shrimp are popular with aquarium hobbyists and can be easily obtained for relatively low cost (~$30-50). Teachers can harness the novelty of these animals to motivate students to learn concepts in behavioral neuroscience and electrophysiology.

Undergraduate and high school labs must often make use of available organisms and low-cost resources to bring ideas from neurobiology into the classroom (Gage, 2019). Invertebrates are an asset to the biology/neuroscience teaching arsenal, as they are widely available, affordable, and offer insights into how neural activity encodes information that would otherwise be too invasive in human or other vertebrate systems (Linder and Deyrup-Olsen, 1991). Cockroaches and crickets have an established history as tractable and exemplary systems of invertebrate nervous systems for classroom laboratory experiments (Ramos et al., 2007; Dagda et al., 2013; Nguyen et al., 2017). Unlike labs done with humans, these systems are not limited to non-invasive investigations. One such lab uses grasshoppers to demonstrate the role of the descending contralateral movement detector circuit in eliciting muscular responses to looming visual cues (Nguyen et al., 2017). As more sophisticated techniques in teaching laboratories gain momentum, it has become increasingly important to make neurobiology as inexpensive and accessible as possible to minimize educational inequities. An excellent overview of cost-saving suggestions is

provided in Wyttenbach et al. (2018).

A range of arthropods, including crickets, mantis shrimp, trap-jaw ants, and other jumping insects use a latch and spring mechanical process to create power-amplification, a phenomenon that allows some arthropods to perform tasks with strength disproportionate to their size. These feats include the trap jaw ant's mandible strike, the locust's jump, and the mantis shrimp's appendage strike (Patek et al., 2004; Sutton and Burrows, 2011; Kayaga and Patek, 2016; Burrows, 2016; Ilton et al., 2018). Though the underlying mechanisms of power amplification across all species are multifarious and not fully characterized, they all generally use the same resilient properties of chitin (exoskeleton) as a spring to load potential energy via muscle contraction. This potential energy is translated into kinetic energy and fast appendage motion with the removal of a latch that holds the spring in place. Though the muscular and electrophysiological control of power-amplified movements are well characterized within certain species, such as locusts (Burrows, 2016; Bayley et al., 2012), much remains to be understood about the electrophysiology of these movements across different taxa. Of particular interest is the question of whether the convergent evolution of muscular power-amplification yields similar or different mechanisms of energy buildup and release. A carefully designed laboratory exercise for bringing high school or undergraduate students in contact with these questions could place them on the scientific frontier alongside professional scientists, sparking enthusiasm and curiosity for the scientific process.

Here we present a method for recording EMGs in arthropods with and without power-amplification. While the focus of the surgical protocol described is specific to mantis shrimp, our design provides a low-cost way to teach muscle electrophysiology in other common organisms such as cockroaches and crickets. Using common materials, we made a chronic, modular implant, or "backpack", capable of recording EMG activity reflecting the buildup of energy preceding cricket leg movement or the famed mantis shrimp strike. It is simple enough to be fabricated by students during the laboratory exercise and readily modified for new uses. We provide students an opportunity to quantify muscle bursts in power-amplified behaviors that can be used to compare muscle activity across different types of limb locomotion. We provide a sample analysis investigating the muscle-driven movements of the cockroach, and power-amplified kicks and strikes.

Hands-on fabrication of lab equipment has been shown to improve students' engagement with the laboratory exercise (Crisp et al., 2016). Making the equipment allows for an intuitive understanding of how the electronics and physiology interact, and builds a foundation of skills for investigating other questions about physiology and behavior in the future. This laboratory exercise emphasizes student participation, in particular animal handling, making and implanting EMG probes, and hands-on behavioral experimentation.

Arthropod electrophysiological investigations are usually terminal (Kayaga and Patek, 2016), which can serve as an obstacle to bring this technique into the classroom. This device is chronically implanted, which means the recording subject can return to its tank at the end of the exercise and be recorded again after days or weeks.

Analyses used in this paper are available as Python scripts online, and described in the Mantis Shrimp Punch Instructor's Guide, available in the supplemental materials. Results we report here are intended to serve as a scaffold for student- and instructor-driven exploration of EMG and audio data.

MATERIALS AND METHODS

The following methods are reported as an easy protocol to guide students when performing this lab. The entire lab protocol has three distinct sections: fabrication, surgery, and experimentation. These sections can be completed in a single 3-4 hour session; however, if time is limited, each section can be separated across consecutive labs. Due to the cost and time devoted to managing each station, we recommend creating groups of 2-4 students each to reduce these factors. Each section of the materials and methods can lead the instructor through the laboratory exercise, from fabrication of each part, to surgery, to experimentation. Instructors should have all groups complete each part of the fabrication before moving on so students who finish first can help groups that may be struggling; facilitating cooperativity between groups simulates collaboration between labs for troubleshooting methods. The Mantis Shrimp Punch Instructor's Guide explains the procedure more narratively and includes a table with all the materials necessary for the exercise. We have also provided a video of the experimentation process is available at https://youtu.be/NKdECK_3sJQ.

While we used a Muscle SpikerBox Pro (Backyard Brains, Ann Arbor, MI) for these experiments, we note that any EMG amplifier will be able to transduce mantis shrimp muscle activity. Also, we use silver wire throughout this procedure, but platinum, iridium, or steel wire would be acceptable substitutions.

Fabrication

Previous labs have used hands-on fabrication steps as a pedagogical device for facilitating a deeper understanding of electrophysiology rigs for EMG (Crisp et al., 2016). In this lab, students develop a plug-and-play recording system with electrodes that stay positioned in muscles throughout the probe's lifetime. The fabrication process takes 30-60 minutes and allows the students to experience the development of EMG methods by creating the probe from scratch (including the backpack itself and the backpack plug, which connects the backpack to the SpikerBox). These electrodes could be made in advance and re-used in later courses. The electrode system consists of an electrode backpack which will remain on the animal, and a connection adapter that will connect the recording system to the backpack before each experiment.

Electrode Backpack

To make the chronic implant (the backpack), cut three ~1.5" lengths of insulated silver wire. Deinsulate a few millimeters of both ends of the silver wire by holding the end above a

flame, causing the insulation to retract from the end. Cut three pins from a line of dip sockets and solder each female lead to one end of each length of stripped silver wire. Using liquid electrical tape, completely insulate the soldered leads, and allow 10 minutes to dry. Make sure the entire insulated length is covered. If the metal inside of the dip socket pins are exposed on the side when cut from the line, they must be covered in liquid electrical tape as well. Finally, the flame deinsulation process can leave little balls, or prills, of silver at the tips of the wires, which come together as a result of the liquified metal's surface tension. Cut off the prills, leaving deinsulated silver wire.

Backpack Plug
The backpack plug and hydrophone will be connected to the recording system through a Muscle SpikerBox electrode cable. This electrode cable is a 1m cord that has a 3.5mm stereo audio connector on one end which terminates to three cable wires with alligator clips: two red (Left and Right audio) and one black (ground). The backpack plug is made of two parts: a three-pin row of dip sockets, and a muscle electric cable composed of three separate cable wires. Each pin and cable wire is connected by a deinsulated silver wire. For a visual, see the Mantis Shrimp Punch Instructor's Guide.

Cut three pins from a line of dip sockets and sand the female side with coarse sandpaper until metal is exposed on each socket. Then, cut three more ~8" lengths of insulated silver wires, deinsulating both ends of each silver wire with flame as described above. Solder the metal part of each dip socket to a deinsulated silver wire end using flux paste.

On the electrode cable, cut off the alligator clips and strip off a few millimeters of insulation on each cable wire. Solder the remaining ends of the deinsulated silver to each cable wire, and ensure the black wire is not soldered to the middle socket. Insulate all exposed and uninsulated metal with liquid electrical tape.

Hydrophone
To capture precise timestamps of the strike, EMG events are cross-indexed with corresponding audio 'pop' waveforms, recorded with a custom hydrophone (Figure 1). Another Muscle SpikerBox electrode cable is used to make the hydrophone, alligator clips removed and wires stripped. The microphone is a passive ~4W speaker as might be found in a toy speaker.

To prepare the speaker, solder one lead of the 4W microphone to one of the cable's red wires. Then, solder the other speaker lead to both the black and the remaining red cable wire. Insulate all metal and soldered parts with liquid electrical tape. Finally, place the microphone in a plastic glove.

Stereotax
For the surgery, the animal is restrained on a stereotax (Figure 2a) with at least one degree of freedom, allowing the slab to be tipped downward into a shallow pool of water.

Make a slab measuring approximately 0.1" x 1.5" x 7" out of a strong material like acrylic. Affix a 7" x 1.25" x 0.75" piece of Styrofoam to the bottom of the slab with superglue
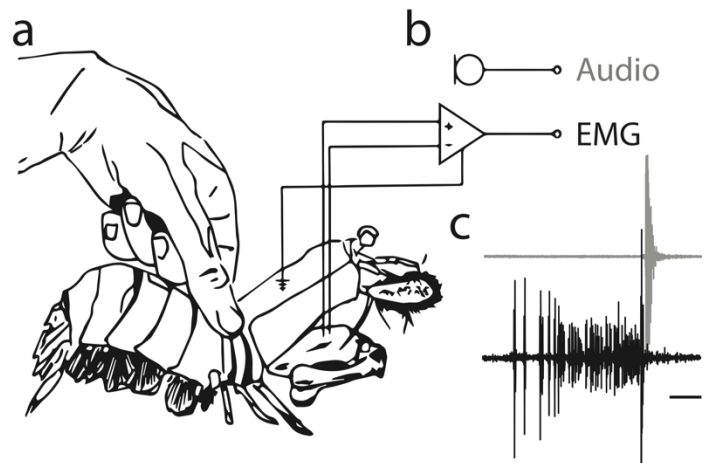

Figure 1. Experimental setup. *a)* demonstration of the proper grasping technique to minimize injury from animal strike. The backpack is placed on the carapace. The EMG wires are implanted in the merus, targeting the extensor muscle. The antennal scale is the feather-like appendage extending away from the carapace. *b)* hydrophone and amplifier recording locations. *c)* example traces of audio (gray) and EMG (Black). Large punch artifact in EMG (large spike end of trace) was clipped for visibility. Scale bar = 250ms.

or marine epoxy. The Styrofoam acts as a pliant "corkboard" material into which pipe cleaner or jumper wire restraints can be securely plugged.

The body of the stereotax must support the weight of the slab and be able to rotate. 3D printed parts for the stereotax as well as CAD and 3D print files for printing (https://backyardbrains.com/products/micromanipulator) and instructions for building your own (https://backyardbrains.com/products/files/SearcherBuildingInstructions.pdf). Alternatively, a magnetic gimballed stand such as the Nootle Magnetic foot mini ball head camera stand sold by Grifiti (http://www.grifiti.com/nootle/grifiti-nootle-magnetic -mini-ball-head-camera-stand.html) can be securely mounted to a slab. Using a magnetic stereotaxic mount also requires a thin slab of iron or steel to function as a wide and heavy base.

Surgery
Mantis shrimp are available for purchase from most aquarium supply stores and should be maintained in a cycled saltwater tank with aeration, filtration, and feeding every other day with frozen and/or live bait (e.g., snails, small crabs). We used *Odontodactylus scyllarus*, *Gonodactylus smithii*, and *Squilla empusa* ranging in size from 3" to 8" long. A 6" length of 2" diameter PVC piping should be included in the tank for the mantis shrimp to reside in.

Anesthesia and Restraint
The size of the mantis shrimp dictates the method of restraint. Large animals are defined as being greater than 5" long, whereas small animals are defined as less than 5" in length. To anesthetize a small animal, place it in a cup containing ice; for large animals, use a bucket containing a

layer of ice at the bottom. Allow the animal to cool for approximately two minutes, or until the animal stops moving. Ice should be kept nearby throughout the surgery to chill the animal if it becomes too active.

For large mantis shrimp, at least three points along the body need to be restrained: below the carapace, in the middle of the abdominal region, and at the bottom of the abdominal segment, above the uropods. These points of restraint can be held with either pipe cleaners or jumper wires, inserted in the Styrofoam glued to the bottom of the slab (Figure 2a).

For small mantis shrimp, assemble two pieces of silly putty on both sides of the slab's bottom. Place the shrimp at approximately 45° to the slab on its rostrocaudal axis. Gently pinch the silly putty against its sides. The silly putty will hold the animal in this angled position. Again, at least three points along the body are restrained with pipe cleaners/jumper wires inserted in Styrofoam (Figure 2a). Once restrained, dip the slab into a shallow pool of water so that the mantis shrimp's pleopods are mostly immersed in aerated water.

Adhering the Backpack
Lightly score the carapace on the side of the raptorial appendage to be implanted with coarse sandpaper. Apply superglue on top of the scored carapace using a needle. Place the backpack on the superglue, the female face of the dip socket toward the anterior. Use either dental cement or marine epoxy to further adhere the backpack to the carapace. The sides, top, and back of the backpack should be covered by adhesive, but the holes in front must not be obstructed.

Dental cement is mixed in a glass or silicone container, cleaned after each mixing with ethanol. Dental cement fully hardens after five to ten minutes. If dental cement is unavailable, marine epoxy (Loctite; Düsseldorf, Germany) can be substituted. Marine epoxy begins to set after ten minutes and dries over the course of a few hours, reaching maximum hardness after 24 hours.

Implanting Wires
To implant the ground, place the higher gauge (smaller tip) needle against the carapace, caudal to the backpack. To open the cuticle, roll the needle between thumb and forefinger, gently keeping it against that same spot on the cuticle. Fingers will periodically travel down the syringe due to the pressure exerted. To fix this, hold the syringe in place with the non-dominant hand (i.e., touching the top) while the rolling hand is brought back toward the top, and again grasped between thumb and forefinger. If the needle cannot pierce the carapace, the lower gauge (larger tip) needle may work better. The higher gauge needle might poke through into the flesh, in which case it may be gently removed. The lower gauge needle will make a hole from which the animal will bleed slightly.

Using two pairs of forceps in each hand, make a millimeter-long bend "anchor" in the silver wire ground, which should be the most medial lead (Figure 2b). This anchor ensures the ground will stay in the tissue. Angle the tip of the bent anchor into the hole in the cuticle, making sure

the entire length of bare silver is below the surface. Apply superglue at the hole, followed by a thin layer of dental cement or marine epoxy. Adhesive must not be allowed to harden and should be quickly wiped away from the animal's joints.

The same technique for implanting the ground is applied to implanting the two wires of the EMG electrode leads. Score the merus with sandpaper, and open a single hole in the cuticle above the extensor muscle using the technique described above. To locate the extensor and carapace, see the placement of the wires in Figure s 1a, 2b, and 2c. For the remaining silver wires, make an anchor and angle the wire into the hole, making sure to insert them in different directions to avoid shorting. Apply adhesive to the hole using the technique described above. The wires must not be covered with dental cement or marine epoxy except at the base, as they may become brittle. The antennal scale (Figure 1) is wont to fold back against the merus. Thus, it may be held out of the way with a needle skewering into the silly putty or with a pipe cleaner/ jumper wire.

At the end of the surgery, return the animal to its tank.

Data Acquisition
Connect the backpack plug to channel 1 on the Muscle SpikerBox Pro and the hydrophone to channel 2. EMG and audio data are acquired with SpikeRecorder, an open-source desktop app for Windows, MacOS, and Linux (Backyard Brains, Ann Arbor, MI). To make the mantis shrimp's restraint, cut a strip of fabric, 2" long, 0.5" wide, with a notch in the middle, and wrap it around the mantis shrimp (Figure 2c,d). For a large animal, the restraint should be bigger: 3" long, 1" wide. Position the hydrophone half-in, half-out of the water.

The animal's tail should have enough space to maneuver and stretch. The anesthetized animal's anterior abdomen should be wrapped such that the legs are pointing away from the merus and clamped using a helping hands tool. Lower the restrained animal into an aerated salt water bath with its pleopods in the water and the carapace out of the water (Figure 2c,d).

Stimulus Delivery
The stimulus can be a pen, pencil, cotton swab, frozen or live bait, or a rolled-up piece of paper towel. The stimulus can be presented in a variety of ways, the simplest of which is to place it in front of the mantis shrimp, close enough for it to strike. This is particularly suitable for soft stimuli such as frozen bait or a rolled up paper towel. Rigid stimuli such as a pencil can be placed in front of the animal, or run rapidly back and forth against the pleopods three or four times to elicit a strike. This second method will induce stress, so employ it as a last resort. During stimulus presentation, the experimenter's hand should not touch the salt water. Additionally, the experimenter should use electrically inert objects as stimuli. We found a three-inch long length of rolled up paper towel to be the best stimulus for eliciting a strike for *Gonodactylus smithii*. Space stimulus presentations out by 5-10m, and make a single recording for each stimulus presentation.

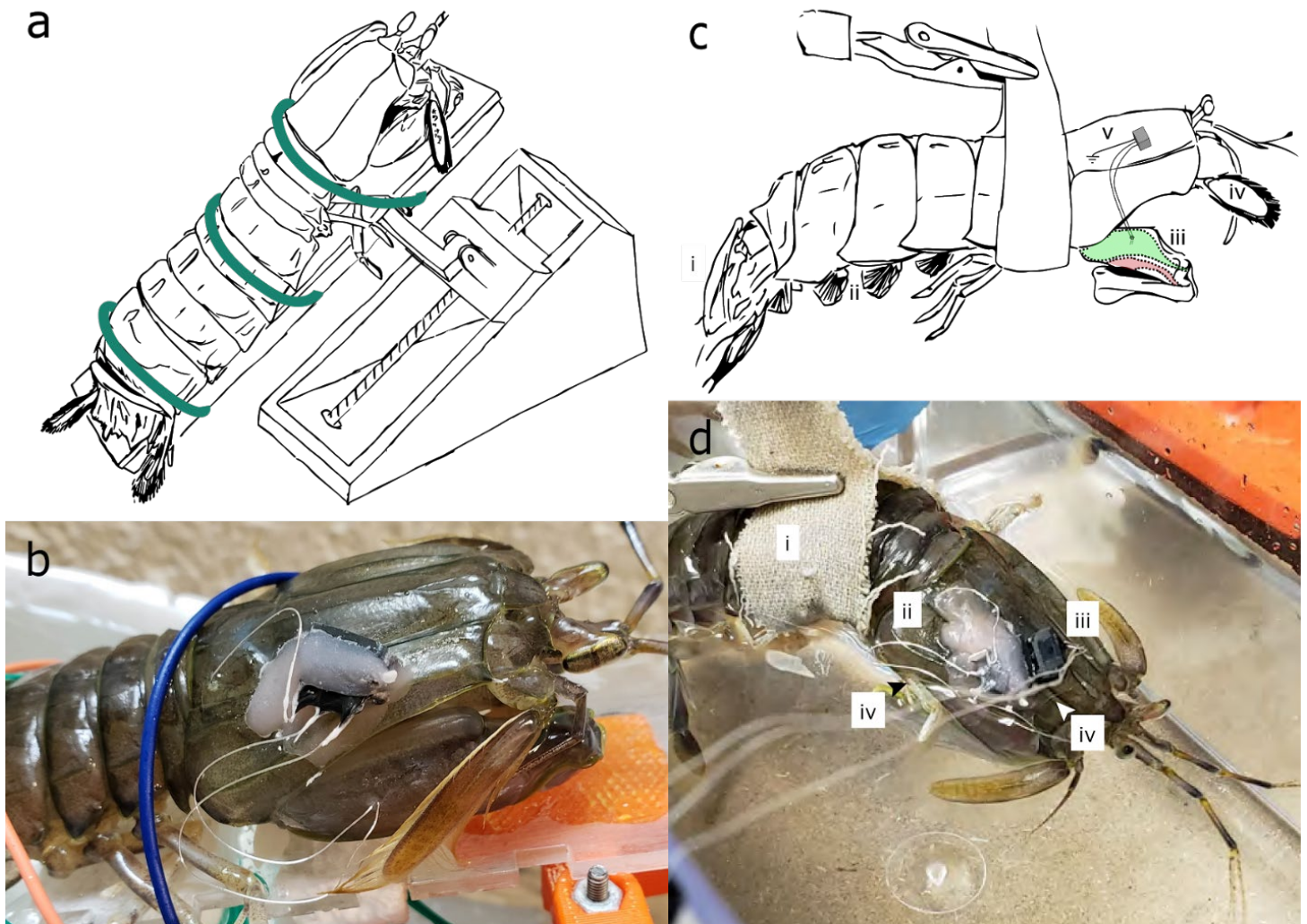The trace of a backpack with a short will have a large

Figure 2: Mantis shrimp surgical and recording restraints. *a)* a large mantis shrimp (*Squilla empusa*) in restraint during surgery. The stereotaxic apparatus tilts the animal into a pool of water, allowing it to breath, while minimizing movement. *b)* Close-up of placement of the backpack with dental cement and placement of wires in merus. *c)* Small mantis shrimp (*Gonodactylus smithii*) in restraint during experimentation with callouts highlighting anatomical features: *i* Telson. *ii* Pleopods. *iii* Merus. The extensor muscle is shown in below the cuticle in green and the flexor muscle is shown in red. *iv* Antennal scale. *v* Carapace with backpack and ground. *d)* Photo of experimental setup with callouts highlighting individual parts: *i* Cloth restraint. *ii* Dental cement encasing the backpack with wires going below the water into the merus. *iii* The plug connected to the backpack with wires. *iv* Marked with black and white arrowheads, these callouts demonstrate that the water covers most of the dorsal surface of the animal, leaving only the backpack dry. Note that only the pleopods need to be submerged.

noise band, obscuring the true EMG signal. When the backpack recording is shorted by water, dry it with paper towels.

Data Analysis

Analysis was done in Python, using some of the analyses from Kayaga and Patek (2016) for mantis shrimp data. For all organisms, we examined spike shapes in addition to spike times, as well as the number of spikes in the first and second halves of each burst.

RESULTS

We present analyses of EMG data from one individual of each species. These analyses should serve as a template for more rigorous analyses, as opposed to true novel findings. Code for running these analyses is available at https://github.com/backyardbrains/MantisShrimpEMG. We hope that students and instructors alike will adopt and expand on the ideas and techniques shown here.

EMGs were acquired from two species with power-amplified movements: the mantis shrimp (*Gonodactylus smithii*, N=1, n=21 and the cricket *Acheta domesticus*, N=1, n=10) and in one non-power-amplifying organism (the cockroach *Blaberus discoidalis*, N=1, n=16). Figure 3 shows example EMG traces from each species. Implants were successful for other mantis shrimp species (*S. empusa*

Mantis Shrimp

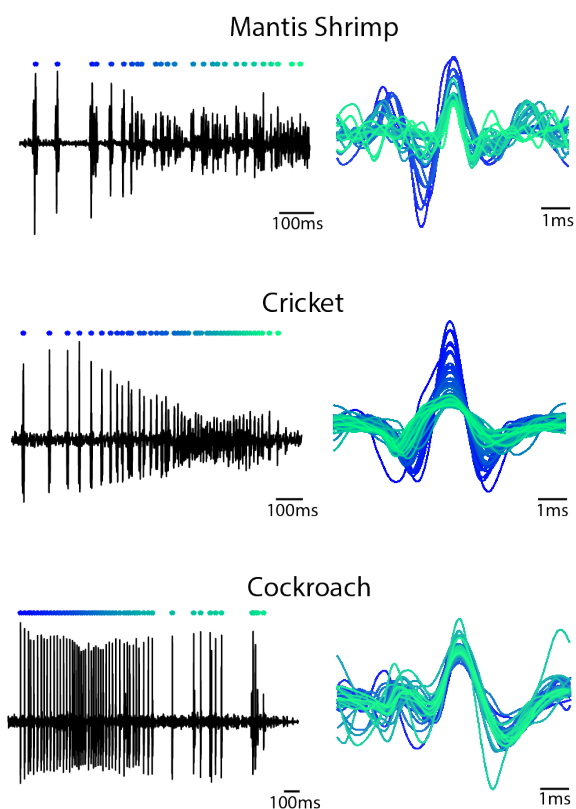

Cricket

Cockroach

Figure 3. Examples of EMG bursts across three species. Burst events in mantis shrimp were distinguished by the presence of a sufficient audio signal at the end of the burst epoch (Figure 1c), in addition to unpublished video evidence and notetaking. Bursts in crickets and cockroaches did not correspond to behavioral criteria, instead they were selected based on the presence of bursting. On the left, example EMG trace recordings with spike times are indicated by colored stars above each trace. The color changes from blue to teal linearly across time, and this chronological color gradient is reflected in the peak-aligned spike waveforms on the right, with earlier spikes dark blue and later spikes teal.

and *O. scyllarus*); however, we generated insufficient data for statistical analyses. Therefore, behavior and associated muscle twitches were characterized in the *G. smithii* mantis shrimp during the ballistic strike; however, behavior was not quantified from the cricket and cockroach. Instead, we included EMG activity from the hindleg extensor muscles from crickets and cockroaches that took the form of short bursts (<3.5s) for our analysis (Figure 3), as bursting is indicative of power-amplified movement in crickets (Burrows and Morris, 2003). Power-amplified EMG bursts from the extensor muscle from the second raptorial appendage of *G. smithii* were included based on their resemblance to "canonical" power-amplified bursts in mantis shrimp (Kayaga and Patek, 2016).

We found anecdotal distinctions between power-amplifying and non-power-amplifying animals. First, EMG spike shape changed within bursts (Figure 3). Early spikes tended to have larger waveforms which diminished in size over the course of the burst in power-amplifying subjects (Figure 3a,b), but tended to stay the same size in non-power-amplifying animals (Figure 3c).

The firing rate of spikes within the EMG traces changed

over the course of each EMG burst. Figure 4 compares burst lengths (time between first spike and last spike) and spike counts within the first and last half of the burst across 3 different animals (Figure 4a). Power amplifying animals such as mantis shrimp and crickets had shorter average burst durations than cockroaches. Tukey's post-hoc test showed that though there was a significant difference between mantis shrimp and cockroaches, there was no significant difference between cockroaches and crickets, and there was a significant difference between mantis shrimp and crickets (Kruskal-Wallis test: $\chi2(2)=16.85$, p=0.0002, (Figure 4b). The variance in data between mantis shrimp and cockroaches differed across organisms as well (Levene's test for equal variance: F(2,43)=9.72, p=0.00033).

One salient feature of power-amplifying EMG bursts in our data is the tendency to increase in firing rate from the beginning to the end of the recording (Figure 4c). This increasing trend held across 87% of all recordings of power-amplifying animals, and exhibits a stark difference from cockroaches, in which only 25% of the recordings have a net increase in spikes between the beginning and end of the burst. Individual changes in spiking rate between the first and second half of a burst have not been examined in the literature.

DISCUSSION

Here we have outlined a multi-phase teaching laboratory that introduces several concepts in behavioral neuroscience. The mantis shrimp is only one of many power-amplification examples found in nature. Students should be encouraged to consider similar mechanisms of energy build up and release with exoskeletons (trap jaw ants, grasshoppers, froghoppers, crickets) or without (Achilles tendons, frog legs, salamander tongues; see: Roberts and Azizi, 2010), and how this capability relates to behavior.

This lab requires students to first make and then use the equipment necessary for discovering these principles. Fabricating probes enables the class to delve into the electrical properties of muscle movement by implementing the devices that measure them. Students learn practical skills for animal surgery, from anesthetizing and restraining the animals to actual operations. In the final phase of the lab, students integrate insights from the preceding steps to acquire recordings from muscles that drive an innate behavior.

Since the laboratory exercise is broken into three parts, there are several natural break points for instructors. These could be used as stopping points for shorter labs (<1hr), to ensure that longer lab sessions are on schedule, or to deepen engagement with the material through discussion. The first break point occurs after probe fabrication. After developing a functional EMG electrode, there could be a discussion on how basic electronics concepts (e.g., operational amplifiers) apply to biological processes. Animal surgery represents the second breakpoint. If an animal breaks its restraints, it can be easily re-anesthetized and placed back on the stereotaxic apparatus, and the surgery can continue as normal. The surgery phase affords students the opportunity to make fixable mistakes, a rare

accommodation for surgeries. It also incorporates tactile learning of mantis shrimp anatomy (i.e., gills, eyes, antennae, reproductive organs). The experimentation and analysis phase is the third break point. This phase explicitly leaves room for students to troubleshoot and innovate, two crucial skills in STEM. Because students design their own stimuli for eliciting a strike, they can reason in real time about why certain features of a stimulus are more salient to the animal than others. Given the sophisticated visual system of some mantis shrimp, behavioral contexts for striking, and individual differences, students are faced with a highly tractable puzzle in behavioral neuroscience. Therefore, groups will have to collaborate, sharing methods and ideas to successfully evoke striking behavior and collect data.

We present this EMG setup as a low-cost alternative to expensive electrophysiology suites; however, a significant limitation to our design is that it is not waterproof, which we believe makes it an ideal candidate for innovation. We found it more than feasible to collect data while monitoring for shorts, but a waterproof design would allow for experiments that take place underwater, perhaps even in burrows. Gruhn and Rathmayer (2002) implemented a chronic and modular crustacean electrophysiology system for crayfish and used a waterproof socket. We hope researchers and students will build upon our and Gruhn and Rathmayer's designs to develop a DIY technique that is waterproof and inexpensive.

In addition to a teaching laboratory, we present a novel paradigm for doing electrophysiology in mantis shrimp and other arthropods. Non-terminal chronic implants allow for greater return on investment per individual and for more robust statistical analysis of data, namely within-subject experimental designs.

Finally, we present preliminary analyses of inter-species comparisons of power-amplification. Low subject counts render our findings anecdotal, but we found evidence of commonalities in EMG traces between mantis shrimp and crickets. In particular, the variances of the mantis shrimp and cricket burst durations were closer to each other than to that of the cockroach. The mantis shrimp and cricket tended to increase firing rates from the beginning to the end of the burst or co-contraction, while the cockroach showed no pattern of firing rate modulation across the recording. The difference between the mantis shrimp and the cricket burst durations were, however, statistically significant, while the burst durations of the cockroach were not significantly different from those of either power-amplifying species.

Power amplification is usually investigated in terms of the interplay between a set of extensor and flexor muscles in a particular organism. Mantis shrimp have two extensors and two flexors; we only recorded from the largest, the lateral extensor. Our design could be easily expanded to record from both the lateral extensor and the lateral flexor, providing a near complete view of the power-amplification system, though analysis of extensor co-contractions between power-amplifying and non-power-amplifying species suggests that examination of extensor spikes may be sufficient to differentiate between these categories. These similarities between the mantis shrimp and the cricket suggest a possible commonality in muscular mechanisms for power-amplification.
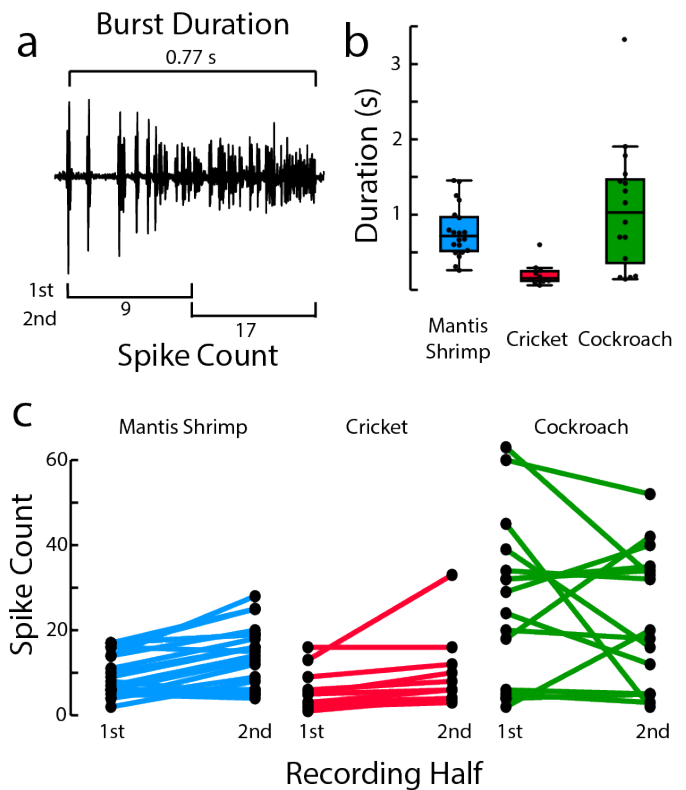

Figure 4. Burst analysis of recording sessions across three species. *a)* Burst of extensor spikes in the Mantis Shrimp. Burst duration is time difference between first and last spikes. To show temporal change in spiking, we calculate the number of spikes in the first vs second half of each burst, in this example 9 and 17 respectively. *b)* Characteristic EMG bursts from each organism. Individual spike times are highlighted by stars. *c)* Waveform summary of spikes for each organism.

The mantis shrimp species used in this study, *Gonodactylus smithii*, is not necessarily representative of all mantis shrimp species. To test for variation among smashing type mantis shrimp species, we performed a meta-analysis of EMG data reported from a second species, *N. bredini* (Kayaga and Patek, 2016) against our data from *G. smithii*. We found differences in muscular activity during the strikes of these two species. Though the number of extensor spikes in *G. smithii's* burst (mean= 23.9 spikes) falls within the range of means of its counterpart (mean range: 13.4 – 68.8 spikes), its average extensor co-contraction duration (mean=722 ms) was far above the mean range (mean: 243-383 ms) of values in the literature. The EMG spike analysis performed on *N. bredini* measured the number of spikes in the first and last 100 ms of the strike (Kagaya and Patek, 2016). For our study, we used an alternative measure that counted the number of spikes in the first and second halves of the strike. This method was developed because we found that it better predicted differences between power-amplifying and non-power-amplifying species. This distinction between power-amplifiers and non-power-amplifiers lies at the center of this laboratory exercise and the data analysis approach was tailored to highlight this distinction. Burst spike count and burst duration provide a sense of the power these species can muster to store energy for the spike. Assuming

approximately equal energy, a longer duration and an equal number of spikes would indicate a slower rate of energy storage (lower power) in the meral saddle. However, to measure power, strike force must be measured as well (Patek et al., 2004).

Variations in ecological and local environmental factors often give rise to morphological and physiological phenotypes among closely related species. The stomatopod strike system and its variability among species specialized for feeding on different categories of prey provides an excellent system in which to study this biological principle (DeVries et al 2016). Given the simplified and inexpensive methods laid out here, we provide a framework in which the muscular physiology of stomatopods with different feeding morphologies and ecological requirements can be evaluated. Our study is in no way an exhaustive comparison between the strikes of different mantis shrimp species, though we did observe evidence for a significant difference in strike parameters between two mantis shrimp species. Different strike parameters among species will likely reflect specializations particular to each species' ecology, representing an unexplored scientific milieu in comparative ecology that is now highly accessible in the classroom. These analyses are easy to perform at the undergraduate level, requiring a low level of programming skills. Additionally, code for performing these analyses is available online.

Again, the analyses presented here are meant to be examples for the kinds of insights students can make from this preparation. They may also produce hypotheses students can confirm or reject for themselves. Groups in the classroom can pool their data and yield a high N. Indeed, data from multiple years of teaching different class cohorts can be saved and further compiled to produce a robust and publishable data set.

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