**Grasshopper DCMD: an undergraduate electrophysiology lab for investigating single-unit responses to behaviourally-relevant stimuli**

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**Abstract**

Observing neural responses to visual stimuli can provide compelling neuroscience laboratories for undergraduates. However, experimental setup can be highly complex and expensive. In this paper, we describe three related hands-on experiments using the grasshopper and affordable technology to bring introductory concepts of neurophysiology to life and enhance student understanding and interest. We simplified a vision-related procedure using open-source and low-cost electrophysiology equipment, the Backyard Brains Spikerbox bioamplifier and an iPad oscilloscope app, to extracellularly record and analyze the descending contralateral movement detector (DCMD) neuron that underlies the grasshopper's motor sensitivity to looming monocular visual cues. In the natural environment, this visual and motor response serves as an escape mechanism for the grasshopper, a prey for a variety of animals including birds and larger insects. With our protocol, students can record and visualize DCMD firing frequencies whilst grasshoppers are exposed to a range of simulated objects on direct collision courses, and understand the neuroanatomy of a single neuron and its related neural units that contribute to the escape behavior in grasshoppers. In addition to the single-unit electrophysiology experiment, we provide two follow-up experiments to encourage further scientific inquiry: finding an ‘ideal’ intertrial interval to avoid habituation of the DCMD response, and investigating whether visibility of the stimulus affects DCMD activity. We also discuss the enthusiastic and constructive feedback received from undergraduates who performed the experiments in a classroom laboratory.



**Introduction**

Undergraduate neuroscience programs often face the challenge of incorporating meaningful and engaging hands-on learning experiences to their curricula on limited budgets and resources. Aiming to build enthusiasm for the principles of neurophysiology in the undergraduate classroom, many have proposed teaching via wet labs at the undergraduate level, such as observation of *Xenopus* tadpole swimming to learn about vertebrate sensory and motor systems, and neurophysiology using cricket or cockroach legs to demonstrate electrical signaling within an animal’s nervous system (Li et al. 2014, Dagda et al. 2013, Marzullo & Gage 2012, Land et al. 2001).

In this paper, we present a tractable electrophysiology procedure using a low-cost, open-source electrophysiology kit called the SpikerBox (Marzullo & Gage 2012) from Backyard Brains (BYB, backyardbrains.com) that extracellularly records neurons to analyze the firing rate of the grasshopper descending contralateral movement detector (DCMD) neuron. The DCMD underlies the locust’s motor responses to looming monocular visual cues perceived by their eyes, and provides an excellent systems-level view of a decoding problem being computed by the grasshopper brain in an escape response. By studying the DCMD system, students also have a chance to learn the role of natural selection on the evolution of the nervous system.

The lab activities presented here are designed to guide students from an observational question like “why are grasshoppers hard to catch?” to quantifiable questions regarding the visual detection of dangerous objects using electrophysiology. Students can investigate the electrical transmission of highly processed visual information for this life-saving “see-and-jump” escape mechanism.

We choose our local grasshoppers for ease of obtaining and handling, but many species of locust can be used **(List of Species and References needed, Ask Rob Olberg).** In some locations outside the USA, grasshoppers are available for purchase as feeder insects and are inexpensive. Grasshoppers can be found and caught in nearby grass fields by the students, which will also provides context for the experiment as the activity allows for direct observation of the insects’ escape mechanism.

The ability to avoid collision with surrounding objects or escape a predator is crucial to the survival of an animal and typically requires the visual detection of approaching stimuli and a motor reaction (Pearson & O’Shea 1984). In the grasshopper visual system (Fig. 1), two pairs of well-studied monocular motion-detecting neurons, the lobula giant movement detectors (LGMDs) and the descending contralateral movement detectors (DCMDs), underlie the animal’s ability to jump away to escape a potential predator or avoid collision with a looming object. The LGMDs reside in the optic lobe and are excited by approaching visual stimuli. In turn, they send the neural signals to their postsynaptic targets, the DCMDs, which respond to object movements detected by the contralateral eye and activate motoneurons in the thoracic ganglia appropriate to produce the jumping reaction. The LGMDs and DCMDs together make up an early warning system to prepare the escape behavior in the face of possible collision with approaching objects (Rind & Simmons 1992, Hatsopoulos et al. 1995, Gabbiani et al. 1999). **[Greg Stopped here]**

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| **Fig 1. Neuroanatomy of the grasshopper’s motion detector neurons.** The grasshopper optic lobes (lamina, medulla and lobula) lie in the central brain beneath each of the compound eyes and processes visual information. The lobula giant motion detectors (LGMDs) receive visual signals from the lobula and pass these inputs to the descending contralateral motion detectors (DCMDs). The LGMDs respond to motion of an object seen moving across the insect eye as well as to the looming effect, where an object increases in size as it approaches the eye. The DCMD activates motor neurons in the thoracic ganglia. These primary motion detector neurons underpin the animal’s detector and motor response to a looming object, forming the see-and-jump escape mechanism. |

We performed extracellular recordings of the DCMD response to approaching stimuli in grasshoppers using the SpikerBox (Marzullo & Gage 2012) and an Apple iPad. The SpikerBox provides connections to electrodes for recording electrophysiological responses from a target animal, and a bioamplifier that connects to a smartphone, tablet, or computer. We also developed the included software package, the SpikeRecorder, which is currently available for iOS systems and provides an application to generate and display the visual stimuli, as well as an oscilloscope application to record, visualize and analyze neuronal activity. This software, along with open source information on the SpikerBox, is available on the Backyard Brains website.

Here, we present three consecutive experiments for undergraduate labs: 1) Recording of DCMD signals when the grasshopper is exposed to approaching stimuli, 2) Finding the ideal inter-trial interval, the duration between stimulus presentations to prevent habituation and lack of DCMD responses, and 3) Testing the effect of contrast of the stimulus on DCMD response by adjusting screen brightness.

With this simple and affordable apparatus, students can observe neural responses to visual stimuli in an invertebrate model, and educators can, in addition to traditional lectures, enhance undergraduates’ understanding of the fundamental concepts of neuroscience.

**Materials & Methods**

*Animals & Electrophysiology Preparation*

Depending on location in the world, grasshoppers are available and inexpensive to purchase, or easily found in grass fields in summer. Adult grasshoppers are best, for their relative large size and are therefore easier to handle. If possible, the animals should be refrigerated (suggested temperature: 3ºC) overnight until the experiments.

To prepare for extracellular recording of the DCMD neuron, further anesthetize the grasshopper in ice for 15 min or until the insect is inactive after storage in the fridge. Then, use masking tape to tape the animal ventral (belly) side up onto the corkboard on the SpikerBox apparatus (see Fig. 3), with its head and part of the thorax exposed. Pull back its head using standard sewing thread and tape the thread to the SpikerBox to expose the neck connectives (Fig. 2). If the thread pulling back the grasshopper’s head does not stay in place, heat up a small mixture of beeswax and rosin (a teaspoon total) on a glass petri dish on a magnetic stirrer and use a sewing needle or similar to place a dab of the warm liquid on the thread, gluing it to the grasshopper’s neck. This mixture will dry and secure the thread restraint, and is quick and easy to remove from the animal after the experiment.

The neck connectives, containing the contralateral nerve cord and DCMD axons, can be seen with the naked eye, but a binocular microscope (suggested magnification: 20X) should be used for more precise visualization and electrode placement. Using a 0.3mm insect pin, make a small incision in the middle of the neck. Modify the straight silver wire recording electrode (0.127mm) into a hook by using tweezers and bending the tip. Then, guide the recording hook electrode with a micromanipulator (see the 3D printed micromanipulator Backyard Brains, Ann Arbor, MI)) into the incision and around the neck connective contralateral to the eye that will be exposed to the visual stimuli (Fig. 3). When the electrode is in place, put a small dab of Vaseline on the incision to keep it from drying out. Ground the reference electrode (sewing needle, 0.6mm diameter) in the grasshopper’s thorax. To check for proper electrode position, record DCMD responses (listen for popcorn-like pops on the speaker connected to the Spikerbox) when the grasshopper is exposed to an approaching plastic stick.

Finally, for the experiment to begin, the longitudinal axis of the grasshopper’s body should be oriented parallel to an Apple iPad 10cm away such that the angle between the eye and the center of the screen is as minimal as possible. A spirit level can be used to easily minimize the angle by lining the level edges to the center of the iPad and the grasshopper’s eye and adjusting until the bubble in the horizontal tube is centered. To achieve the upright position of the iPad, a possible method is to simply tape it against a wall. If possible, recordings should be done in the dark for maximal surrounding darkness, and therefore better contrast intensity of the experimental stimuli. Placing the apparatus inside a cardboard box can easily achieve this.

This setup (Fig. 3) is the initial step for all three experiments described below.

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| **Fig 2. Ventral view of the grasshopper neck showing the neck connectives from the head to the thoracic ganglia. (Left)** Neck connectives (pointed to by arrows) are visible as white stripes under the neck skin. Sewing thread is used to hold the grasshopper’s head back and in place during the experiments. **(Right)** A simple neck cut exposes the connectives, two translucent tubes, where the part of the ventral nerve cord can be found. Placing a hook electrode around one connective and exposing the contralateral eye to looming visual stimuli allows DCMD activity to be recorded. |

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| **Table 1. Materials** | |
| **Animal Preparation** | Ice  Microscope (20X or higher)  Vaseline  Sewing thread (or similar)  Masking tape (or other types)  Insect pin (0.3mm)  BYB 3D printed micromanipulator  Spirit level with horizontal tube  Standard ruler  **Included with BYB SpikerBox:**  Recording electrode (silver wire, 0.127mm, modify into hook with tweezers)  Reference electrode (sewing needle, 0.6mm diameter)  **Optional:**  Beeswax  Rosin  Magnetic stirrer |
| **Spike Recording & Analysis** | Apple iPad  BYB SpikerBox  Green cable (Computer/tablet/phone to SpikerBox)  Blue cable (Speaker to SpikerBox)  Speaker (Suggested: RadioShack mini audio amplifier)  SpikeRecorder application (currently available for iOS) |

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| **Fig 3. Experimental setup to measure descending contralateral movement detector (DCMD) activity during simulated stimulus presentation.** **(Top)** Schematic representation of the experimental setup, grasshopper is taped down onto the corkboard on top of the SpikerBox. The reference electrode is grounded at the thorax and the recording hook electrode, guided by the 3D printed micromanipulator for precision, is placed around the neck connective where the ventral nerve cord and DCMD neuron’s axon is found. The iPad presenting the visual stimuli is placed 10cm from the grasshopper’s eye and the angle between the grasshopper’s eye and the center of the iPad is minimized. **(Bottom left)** Photograph of the apparatus. **(Bottom right)** Visual stimuli are displayed on the iPad to simulate the looming effect of an object approaching the grasshopper. The radius S’, subtending angle θ, of a filled black dot can be manipulated to simulate a fixed sized object (S) approaching at a particular constant velocity (v) on the retina of the insect. DCMD recordings can be made during visual simulations of different sized objects moving at different velocities. |

*Visual Stimuli & Spike Amplification, Recording, and Sorting*

Electrophysiological measurements are carried out using the low-cost and open-source bioamplifier, the Spikerbox v.1.3c, developed by BYB. The SpikerBox has a 4x gain instrumentation amplifier, 220x gain amplifier bandpass-filtered 340-1300Hz, and a 20x gain audio amplifier (Marzullo & Gage 2012). It also contains an output port for a Radioshack mini speaker (blue port and cable) and an output port for laptops, tablets, and smartphones (green port and cable) to make neural activity audible, visual, and recordable. During all experiments, the blue and green cables from the SpikerBox should be connected to the speaker and iPad respectively.

The BYB SpikeRecorder iPad application provides both the monocular visual stimuli and recording of DCMD activity. Visual stimuli consist of expanding dark circles (of various radii, S, and velocities, v) on a white background to simulate an approaching and colliding object on a direct collision course. Adjustable parameters in the application include: number of trials, distance between subject and stimulus/screen (cm), object size (cm) and velocity (m/s), inter-trial interval or ITI (s). The ITI is the amount of time between the end of the previous visual display and the commencement of the next looming trial. The iPad displays a blank white screen during this interval. Stimuli of particular parameters S and v are pseudo-randomly presented by the application such that each parameter is repeated an equal amount of times during a single experimental session.

Spikes can be sorted from noise after recording in the iPad application (Fig. 4). Noise is identified as false spikes with constant and consistent amplitudes (mV) over time. These spikes have noticeably lower amplitude than the real DCMD spikes. The app allows the student to set minimum and maximum threshold values (mV), and spikes within the range are counted as spikes for further analysis. After sorting the spikes from one recording for one trial (one S, one v), the app automatically performs global sorting for the rest of the trials conducted during one experimental session using these threshold parameters.

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| **Fig 4. Spike sorting in the SpikeRecorder iPad app.** SpikeRecorder provides both the looming visual stimuli and recording and analysis of DCMD activity. DCMD spikes can be sorted from noise after recording by providing minimum and maximum spike threshold values. These values can be applied to all recordings made within a single grasshopper’s experimental session. The image above shows the app’s output of the DCMD recordings during a looming experiment (object size S = 6cm, velocity v = -2m/s) with a minimum spike amplitude of approximately 0.30mV, maximum of 0.65mV. Red dots are spikes recorded within the thresholds. |

*Experiment 1: Looming Stimuli DCMD Experiments*

**Objective:** This experiment, coupled with instructors’ lectures and introduction to neuroanatomy of the grasshopper visual-motor system, demonstrate to students the basic electrical properties of the brain and how sensory input stimulates a particular neuronal system to drive a certain animal behavior, using a simplified version of electrophysiology and model invertebrate. Students can follow the procedure below, with modifications appropriate for a classroom lab or other learning settings, after developing their own hypotheses, such as when the DCMD neuron would peak in firing frequency when the object approaches the grasshopper’s eye, or how different object sizes and velocities may affect DCMD response.

**Procedure:** In the SpikeRecorder application, keep the following parameters constant: 10cm distance between the grasshopper’s eye and the iPad and intertrial intervals, ITI, of 45s to reduce habituation of the DCMD response to visual stimuli. Enter required values of choice for S (object radius) and v (object approaching velocity), to begin, we suggest choosing S of 6cm and a range of v values (-2, -4, -6, -8m/s). Each combination of S and v should be repeated for 3 simulation trials. Such parameters will create an experiment, approximately 10 minutes in duration, that allows students to observe the differences in DCMD response to objects of the same size but approaching at different velocities. Once the application is set up, check again for the correct iPad positioning and connected cables as described above and press ‘Start’ in the iPad app to begin. Observe the approach of the black dotsand listen for DCMD spikes in form of popcorn popping sounds through the speakers.

*Experiment 2: Intertrial Interval Experiments*

**Objective:** In this experiment, students will be exposed to the concept of intertrial intervals (ITIs), often used in behavior studies, such as the literature DCMD experiments (Rind & Simmons, 1992). ITIs, the time between trials, ensure that each trial’s stimulus is distinct and thus the animal would not develop habituation to the presented stimuli, preventing accurate DCMD responses. Instructors can discuss the use of ITIs of at least 40s long used in literature papers (e.g. Rind & Simmons 1992). Students can hypothesize the effect of different ITIs on DCMD activity and subsequently implement this experiment to investigate their predictions, and determine a sufficiently long interval to avoid habituation to the stimuli.

**Procedure:** With the same setup as Experiment 1, enter parameters to be kept constant in the application: 10cm distance between eye and iPad, chosen number of trials per ITI, and one pair of S and v values. We recommend using three ITIs to produce distinctly different DCMD responses: 45s, 22.5s, and 1s.

*Experiment 3: Screen Brightness Experiments*

**Objective:** This experiment aims to engage students in further experimental inquiry after they have performed the previous two activities. Here, they can hypothesize and test the effect of screen brightness, which affects the contrast intensity and thus visibility of the black stimuli against the white background, on DCMD response.

**Procedure:** Adjust brightness of the screen in the Settings of the iPad, whose brightness is approximately 425 cd/m2 at maximum and 5 cd/m2 at minimum. Constant parameters include 10cm distance between eye and iPad, 45s ITI, number of trials per brightness level, and one pair of S and v values. Students may choose different brightness levels, each is a separate experiment. We recommend initially observing the effects of the maximum and minimum screen brightnesses (the iPad screen will not be completely black at the minimum setting, but very dim).

*Data Analysis*

The SpikeRecorder app includes data analysis and visualization functionality. DCMD activity in conjunction with change in angular size of the visual stimulus over time can be visualized in perievent histograms and raster plots, with time of collision defined as the 0 second point. Students may save the data graphs from the app and engage with the biostatistics as a post-lab assignment.

For more in-depth analysis, data, recorded and contained within the SpikeRecorder application can be exported to JSON files, which can then be imported into MatLab (MathWorks, Inc.) using the open-source JSONlab toolbox. The MatLab code files are available for download from the Backyard Brains website. A database of the experimental data (recording and spike timestamps, stimulus angles, time of collision) will be created by the code. Perievent time histograms and raster plots can then be plotted to visualize firing rate and timing of spikes in relation to stimulus.

*Student Laboratory & Evaluation*

During the Fall 2016 semester, students in the Molecular and Cellular Biology 81 course taught by David Cox at Harvard University participated in a classroom laboratory performing the first experiment, as described above. Students were sent an online survey a week after the lab to rate the effectiveness of the various aspects of the experiment, such as the ease of the grasshopper preparation and whether the lab section improved the students’ understanding of the use of electrophysiology to study brains and behavior. The answers were on the scale of ‘1=Strongly agree’ to ‘5=Strongly disagree.’ The overall rating at the end of the survey asked students to rate the whole lab as ‘1=Terrible’ to ‘10=Excellent.’ The last question asked students for ideas for improvements.

**Results**

*Technical considerations*

To confirm that the SpikeRecorder accurately records the simulated time of collision, we performed tests to check for the synchronization between the recorded and simulated time of collision (0s on the perievent and raster plots, Fig. 5).

First, the refresh rate of the iPad screen was measured. A 5mm phototransistor (EverLight, PT334-6C model) was placed 10cm from the screen, to ensure that it would get light from the whole screen, and a single trial of the approaching stimulus (S: 6cm, v: -2m/s, at maximum screen brightness) was run. The recording output of the phototransistor was observed on an oscilloscope (Tektronix, TDS2000 model). The duration of one frame was 16.8ms, corresponding to 60Hz refresh rate (60 frames per sec) of the iPad screen. Trials with stimuli of various speeds were completed. We then calculated the delay between the recorded time of collision on the software and the actual time when the stimulus filled up the whole iPad screen for each S and v parameter (see above). A delay of up to 60ms for the fastest stimuli (e.g. velocities of -6 and -8m/s) was recorded. This result does not have a significant effect on DCMD response recordings, as data visualized in Fig. 5 to 7 show results in accordance with literature findings. But this delay issue is still taken into account when determining the accuracy of future experimental results and for future improvements to the app.

*Animals*

We performed the three experiments above-described to verify the efficacy of our equipment and to refine the protocol, as well as provide results as points of reference. In our experiments, due to dependence on local availability and catching abilities, both adult and nymph, male and female, grasshoppers (*Melanoplus femurrubrum* and *Melanoplus differentialis*) were caught in Nichols Arboretum, MI, USA, in June and July 2016. The animals were refrigerated (average temperature 3ºC) until the experiments (average 4 days).

*Experiment 1: DCMD response to simulated approaching stimuli*

In this replication of literature DCMD studies by Rind & Simmons (1992) and Hatsopoulos et al. (1995), 19 grasshoppers were tested and 11, whose recordings were complete and not plagued by disruptions such as excessive noise and death of the insects during the experiments, were selected for analysis. Each grasshopper was individually exposed to visual stimuli on the iPad while the SpikeRecorder application recorded DCMD activity amplified by the SpikerBox. Stimuli approach the grasshopper’s eye on a direct collision course. Parameters kept constant across all DCMD experiments were: 10cm distance between the grasshopper’s eye and the iPad; 16 trials per pair of S and v; and intertrial intervals, ITI, of 45s to reduce habituation of the DCMD response to visual stimuli. Each stimulus is a pair of S (6 and 8cm) and v (-2, -4, -6, -8m/s). Initially, two separate experiments of stimuli with constant S and varying v were performed on the same grasshopper. However, to prevent the effect of habituation of the DCMD to the stimuli presented later in a second experiment, the SpikeRecorder application was modified to pseudo-randomly present stimuli of varying S and v throughout a continuous experiment of approximately 2 hours, 128 trials. Because we were not limited to a classroom timescale, we allowed the experiments to run longer than the amount of trials suggested in Methods to obtain more data for analysis. Students may set up a similar, longer experimental session if desired.

Here, we show results of data analysis in both the SpikeRecorder app and MatLab (Fig. 5 and Fig. 6). Example extracellular recordings of DCMD response for circles 6cm and 8cm in radius approaching at four velocities (-2, -4, -6, -8m/s) are shown in the graphs generated by the SpikeRecorder app in Fig. 5.

Close to Hatsopoulos et al.’s 1995 and Rind and Simmons’ 1992 results, which show silence in DCMD activity during the first 2s of a 2.75s approach, our results find that DCMD firing frequency increased approximately 1.8s after the onset of the stimulus (S: 6cm, v: -2m/s) and peaked around the time of collision (0s) between the object and the eye, when the object's image on the grasshopper's retina reached a certain angular size (80º) (Fig. 5A). The faster the velocity of the stimulus, the later the DCMD peak firing occurred, and as Rind and Simmons found in 1992, the lower the firing frequency, as shown in Fig. 6 comparing stimuli approaching at -2, -4, -6m/s. As shown in Fig. 5B, with the circles at the slowest velocity (-2m/s), peak firing occurred approximately before the time to collision between the eye and the stimuli. With increasingly faster velocities (-4, -6, -8m/s), DCMD firing peaked up to approximately 0.05s after collision, with possible technical delay above-discussed. The MatLab code we developed allows data analyses similar to the SpikeRecorder app, with a wider range of tools. As shown in Fig. 6, DCMD activity patterns of a circle of the same size approaching at various velocities can be better visualized, compared to the overall graph from the app (Fig. 5B). Students may simply use the data analyses function of the app, or use the MatLab code to gain experience in data analysis and visualization.

From a neuroethological perspective, instructors can discuss these results as explaining how the form of the DCMD neuron contributes to its function in the grasshopper’s escape behavior. The more rapid the approaching object (seen in our stimuli at v of -6 and -8m/s), the slower and less the DCMD fires, does this imply a lower chance of successful avoidance of collision, or does a higher firing rate of slower objects inform the grasshopper it has more time to make a final decision?

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| **Fig 5. Analysis of DCMD response to approaching objects in SpikeRecorder app, with additional annotations.** Constants include: d: 10cm; S: 6, 8 cm; v: -2, -4, -6, -8 m/s; trials S and v: 30; ITI: 45s. Collision at 0s. **(A)** Graph showing DCMD activity over time and change in angular size of stimulus for a single trial (S: 6cm, v: -2m/s). DCMD firing frequency increased approximately 1.8s after the onset of the stimulus (-2s, not shown) and peaked (106Hz) around the time of collision (0s, red dash line) between the object and the eye, when the object's image on the grasshopper's retina reaches a maximum angular size (80º). **(B)** After spike sorting for a particular experiment, SpikeRecorder also produces a perievent histogram (PETH) and raster plot in one graph to show DCMD firing activity over the course of the collision (-2s to 0s) to 2s after, with the peak in firing at 92Hz around collision. Raster plot shows combinations of S and v ordered top to bottom by large to small S, then slow to fast v. Similar patterns of DCMD activity are seen for circles 6cm and 8cm in size, with peak firing frequency before collision when the circles are approaching at slower velocities (-2 and -4m/s) compared to those at faster velocities (-6 and -8m/s). |

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| **Fig 6. Analysis of DCMD response to approaching objects in MatLab.** Constants include:d: 10cm; S: 6cm; v: -2, -4, -6m/s; trials per pair of S and v: 30; ITI: 45s. Collision at 0s. **(Top)** Importing data from the SpikeRecorder app into MatLab allows additional data visualizations, such as color-coding and overlaying the DCMD activity patterns of various S and v combinations to compare differences in firing frequency over the collision course. **(Bottom)** Color-coded raster plot of DCMD spiking pattern across each pair of S and v over time. DCMD firing peaks around collision for objects approaching at -2m/s, and after collision for objects approaching faster. |

*Experiment 2: DCMD activity during various intertrial trials*

ITI experiments were carried out with 2 grasshoppers. The setup was the same as Experiment 1. Constant parameters included 10cm distance between eye and iPad, 30 trials per ITI, and one pair of S (6cm) and v (-2m/s). Three ITIs were chosen: 45s, 22.5s, and 1s and the total experiment consisted of 90 trials.

In line with previous studies (refs), an ITI of 1s yields low and late firing frequency (approximately 20Hz), compared to the frequencies with ITIs of 22.5s (45Hz) and 45s (75Hz), shown in Fig. 7. The 45s ITI showed the most consistent and frequent firing, and is the suggested minimum for the other experiments.

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| **Fig 7. Varying the inter-trial-interval reveals DCMD response needs time to recover.** Peak firing frequency is around 20, 45, and 75Hz for 1, 22.5, 45s ITI, respectively. The 45s ITI shows the most consistent and frequent firing of the DCMD, and is the ITI of choice for other experiments. |

*Experiment 3: Effect of screen brightness on DCMD response to approaching stimuli*

Maximum and minimum brightness levels were set for two separate experiments. Constant parameters included 10cm distance between eye and iPad, 45s ITI, 20 trials per brightness level for 40 trials total, and one pair of S (6cm) and v (-2m/s). As shown in Fig. 8, the DCMD activity profiles at maximum and minimum iPad screen brightness are not substantially different, with 90Hz and 95Hz peak frequency, respectively. This result suggests that light level does not affect how visual stimuli evoke DCMD responses. In dim background lighting, the grasshopper’s eyes can still detect the edges of the expanding circles and react to avoid the looming collision - an advantageous escape behavior in natural settings.

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| **Fig 8. DCMD shows little difference in response to varying iPad screen brightness.** **(Top)** At maximum brightness, DCMD firing rate is consistent and peaks at 95Hz. **(Bottom)** At minimum brightness, firing rate peaks at 90Hz and is not significantly different from activity pattern at full screen brightness. |

*Student Evaluation*

Students generally enjoyed the Looming Stimuli DCMD experiment and responded positively. Post-lab survey data were collected from 18 undergraduates. For the technical aspects of the experiment, 72% ‘strongly agreed’ and ‘agreed’ that the grasshopper preparation mounting using tape and thread was easy to learn and user friendly (Fig. 9). 50% or more students ‘strongly agreed’ and ‘agreed’ that the visual stimulus worked reliably, that they were able to clearly hear a neural response to the looming stimuli displayed on the SpikerBox, and that they were able to sort spikes from the recordings and display graphs on a mobile device to show relative timing of spikes to the virtual moment of collision. The most challenging part of the protocol might be electrode placement, which 44% of the students thought was easy.

As for the evaluation of the lab’s effect on student understanding and interest in neurophysiology, at least 60% of the students ‘strongly agreed’ and ‘agreed’ that the lab increased their interest in studying the neural basis of animal behavior, that they are able to understand how electrophysiology can be used to study brains and behavior, that they understood how the DCMD works as an escape response, that the lab encouraged them to generate and test their own hypotheses and increased their interest in becoming a neuroscientist.

Overall, 16 out of the 18 students rated the lab 7 or higher out of 10, with 10 being ‘Excellent.’ Ideas for improvements include providing a clearer diagram for electrode placement in the grasshopper’s neck and improving the user interface of the SpikeRecorder app by framing the data analysis steps as a sequence (e.g. spike sorting before graph viewing).

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| **Fig 9. Student evaluation of the Looming Stimuli DCMD Experiment.** Survey data from 18 undergraduates in the Molecular & Cellular Biology 81 course taught by David Cox at Harvard University. Most students were able to perform the protocol, including grasshopper preparation and data analysis on a mobile device, with electrode placement possibly being the most difficult step. The lab enhanced student understanding and interest in electrophysiology as a tool and in neuroscience overall. Answers with 0% responses are not shown on the bars. |

**Discussion**

The three experiments presented in this paper aim to provide classroom laboratories to introduce undergraduates to the concepts of neurophysiology using a manageable grasshopper model and a simplified essential tool of neuroscience: electrophysiology. Our results demonstrate the efficacy of the tools, as the SpikerBox and SpikeRecorder app successfully reproduced grasshopper DCMD responses seen in the current literature (refs). Thus, the affordable and simple experiments discussed in this paper could be implemented in undergraduate laboratories to provide students with hands-on activities to better understand the neurophysiology concepts of neuroanatomy, sensory responses, and the electrical properties of the brain.

**Effectiveness of the experiments**

Overall, the students who performed the Looming Stimuli DCMD experiment at Harvard University during the Fall of 2016 provided enthusiastic feedback and evaluated the hands-on activity as contributing to their understanding of electrophysiology as a tool to investigate the neural basis of a particular animal behavior, as well as to their general interest in neuroscience as a potential academic pursuit. The students also made valuable suggestions for improvements, such as using distant relatives of the grasshoppers for more variations, and providing clearer protocol to reduce the difficulty of placing the recording electrode into the grasshopper’s neck, which we have addressed in this publication and are discussed below.

**Procedure challenges**

Our results serve as points of reference for students to compare their results against, though variations are expected. Possible procedural challenges may arise, especially given the limited time in a classroom lab, and here we discuss several potential problems and solutions. First, noise signals are likely to be present during neural recordings and may obscure the desired signals. Since a noise-free environment is not realistic in a classroom, simple Faraday cages may be used. This provides the instructor with an opportunity to briefly explain how a closed conductive container, like a wire mesh box, can shield the materials inside from outside electromagnetic interference. If desired, this can also be an additional experiment, in which students can observe the differences in noise and neural signals during various levels of interference. Another challenge students may encounter is the animal preparation. If the grasshopper wakes up early from the anesthesia and becomes active during the surgery, it should be replaced into ice until it is inactive once more. Electrode placement, especially the hook recording electrode around the neck connective, is relatively simple but does demand using a microscope and micromanipulator for precision. Adult grasshoppers should be used if possible, as their neck connectives are larger and more visible. If the electrode placement around a neck connective proves too difficult, students may modify the hook electrode into a simple straight wire and place it between the two neck connectives. This is too imprecise for full DCMD analysis, but the amplification would be sufficient for DCMD spikes to be visualized and recorded.

**Further experiments**

We encourage modifications and experiments that may arise from our procedures. Example research ideas using the SpikerBox amplifier and grasshoppers or similar invertebrates include: somatotopy with grasshopper legs, the effect of temperature on neuronal responses or spikes, and the effect of drugs (e.g. nicotine) on the nervous system. Detailed experiments can be found on the Backyard Brains website.

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Dear Editor:

I am writing to submit our attached manuscript entitled: "Grasshopper DCMD: an undergraduate electrophysiology lab for investigating single-unit responses to behaviourally-relevant stimuli," for consideration for publication as an article in Journal of Undergraduate Neuroscience Education.

Relevant to the scope and objective of your journal, our paper describes a series of experiments that instructors of undergraduate neuroscience/physiology courses can implement as in-class laboratories to introduce basic concepts of neurophysiology and enhance student understanding. We provide a detailed protocol for tractable recording of the descending contralateral movement detector (DCMD) neuron that underlies the grasshopper's motor sensitivity to looming visual cues. In addition to using a manageable grasshopper model, to solve the problem of high cost and complexity of electrophysiology equipment, we describe the use of an affordable and open source electrophysiology kit called the SpikerBox from Backyard Brains. With this bioamplifier coupled with an oscilloscope app we have developed for Apple iPad tablets, students can investigate the neural basis of the escape behavior grasshoppers exhibit in nature. In the manuscript, we also discuss the feedback received from undergraduates who have performed our experiments as a classroom lab session.

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