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Electroshock Induced Seizures in Adult *C. elegans*

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[Abstract] The nematode *Caenorhabditis elegans* is a useful model organism for dissecting molecular mechanisms of neurological diseases. While hermaphrodite *C. elegans* contains only 302 neurons, the conserved homologous neurotransmitters, simpler neuronal circuitry, and fully mapped connectome make it an appealing model system for neurological research. Here we developed an assay to induce an electroconvulsive seizure in *C. elegans* which can be used as a behavioral method of analyzing potential anti-epileptic therapeutics and novel genes involved in seizure susceptibility. In this assay, worms are suspended in an aqueous solution as current is passed through the liquid. At the onset of the shock, worms will briefly paralyze and twitch, and shortly after regain normal sinusoidal locomotion. The time to locomotor recovery is used as a metric of recovery from a seizure which can be reduced or extended by incorporating drugs that alter neuronal and muscular excitability.
Keywords: Epilepsy, Seizure, *C. elegans*, Electroshock, Electroconvulsion, Antiepileptic drugs, AEDs

[Background] We were interested in using the powerful genetic model, *Caenorhabditis elegans*, to develop an electroconvulsive seizure assay that can be easily manipulated by pharmacology. Invertebrate models have been used in seizures research for decades (Lee and Wu, 2002) however there were no protocols specifically investigating electroconvulsive seizures in *C. elegans.* In the past, multiple groups have developed methods of analyzing paralysis in response to chemical proconvulsants such as the $GABA_A$ receptor blockers, pentylentetrazol (PTZ) and picrotoxin (PTX), as well as an acetylcholinesterase inhibitor, aldicarb (Williams *et al.*, 2004; Vashlishan *et al.*, 2008). While these methods typically analyze the time to paralysis, our method quantifies the time it takes to recover from an electric shock-induced seizure (Risley *et al.*, 2016).

Materials and Reagents

1. 60 x 15 mm Petri dishes (Excel Scientific, catalog number: D-901)
2. Pipette tips (Thermo Fisher Scientific, Thermo Scientific™, catalog number: 0094300120)
3. Vacuum filter (Corning, catalog number: 430320)
4. 2, 20 gauge insulated copper wire, cut to 8 cm segments (Del City, catalog number: 1120101)
5. Plastic tubing, cut into 9 mm segments (Emurdock, catalog number: AAQ04127)
   Note: This product has been discontinued.
6. 2 x Alligator clip wires (United Scientific Supplies, catalog number: WAG024-PK/6)

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7. *C. elegans* wild type strain N2 (obtained from *Caenorhabditis* Genetics Center)
8. LB broth powder (Fisher Scientific, catalog number: BP1426-500)
9. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S3014)
10. Agar (Sigma-Aldrich, catalog number: A7002)
11. Peptone (Fisher Scientific, catalog number: BP1420)
12. Calcium chloride solution ($CaCl_2$) (Sigma-Aldrich, catalog number: 21115)
13. Magnesium sulfate solution ($MgSO_4$) (Sigma-Aldrich, catalog number: M3409)
14. Cholesterol (Sigma-Aldrich, catalog number: C8667)
15. Ethanol (Fisher Scientific, catalog number: 04-355-226)
16. Potassium phosphate dibasic (K_2HPO_4) (Sigma-Aldrich, catalog number: P3786)
17. Potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: P5655)
18. Sodium phosphate dibasic (Na_2HPO_4) (Sigma-Aldrich, catalog number: 795410)
19. LB broth (see Recipes)
20. Nematode growth medium (NGM) agar plates (see Recipes)
21. KP buffer (see Recipes)
22. *E. coli* strain OP50 (see Recipes)
23. M9 solution (see Recipes)

Equipment

1. 2 L flask
2. Pipettes
3. Stir bar
4. Dissection stereo microscope (Tritech Research, model: SMT1)
5. Dissecting stereoscope (AmScope, model: SM-1TSX)
6. Timer output stimulator (Grass Instruments, model: S44)
7. Stimulator (Grass Instruments, model: SD9)
8. CCD color microscope camera (Hitachi, model: KP-D20BU)
9. Television monitor (RCA, model: 19LA30RQ)
10. HDD and DVD recorder (Magnavox, catalog number: MDR535H/F7)
11. DVD-Recordable discs (Verbatim, catalog number: 95032)
12. Digital oscilloscope (OWON Technology, catalog number: PDS5022T)
13. Ethanol lamp (Carolina, catalog number: 706604)
14. Water bath (50 °C) (Corning, model: Corning® LSE™ Digital Water Bath, catalog number:6783)
15. Incubators (37 °C) (Thelco, model: 4)
16. Incubator (20 °C) (Cuisinart, catalog number: CWC 1200DZ)
17. Timer (VWR, catalog number: 62344-641)
18. A metric ruler (Fisher Scientific, catalog number: 09-016)
19. Infrared Temperature Gun (J-1 Trading Wholesale, Nubee, catalog number: NUB8380)

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Software

1. Open source image processing program, we use VLC media player (version 2.2.4)
2. SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA)

Procedure

1. Set up the microscope camera to the TV and digital video recorder as per the camera instructions (Figures 1 and 2A).

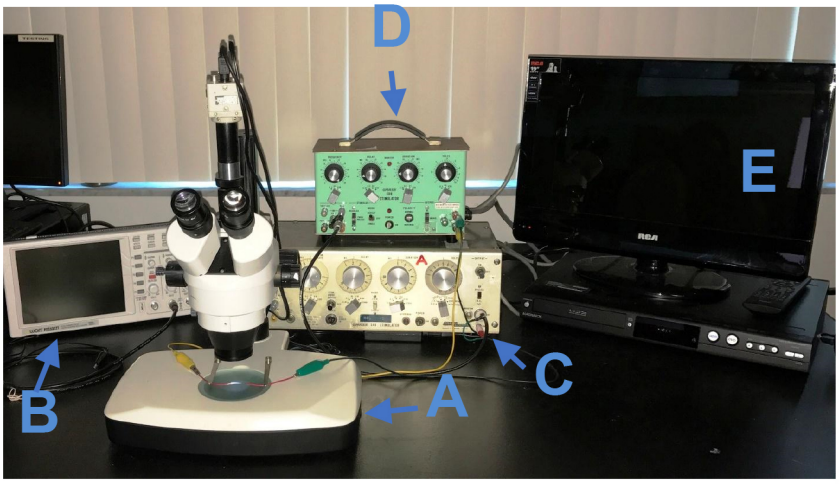

Figure 1. The experimental setup. The experimental setup includes a stereoscope (A), oscilloscope (B), timer output stimulator (S44) (C), stimulator (SD9) (D), and the recording equipment (E).

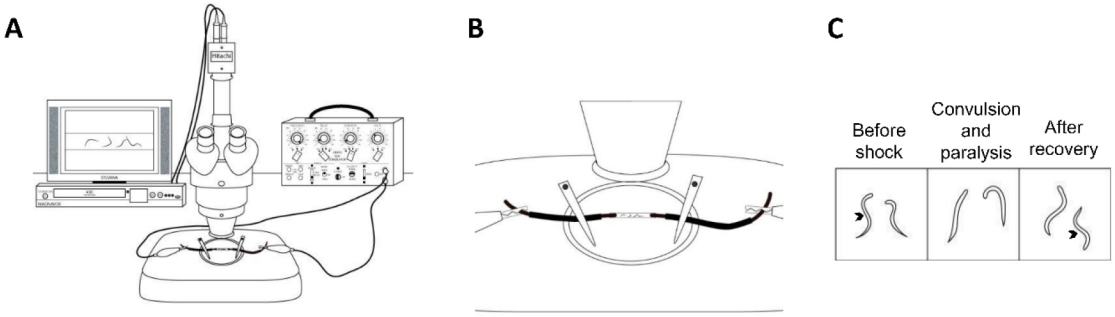

Figure 2. A simplified schematic of the experimental setup and analysis. A. The stereoscope (center), the recording equipment (left), and a stimulator (right) represent a simplified schematic of a basic experimental setup. To control duration, our setup includes an additional stimulator (S44); however, timing can be controlled by many different methods. B. A close up schematic of the experimental tube plugged on either end with copper wires; C. This schematic represents the normal, sinusoidal body position (arrow) of a worm before the shock.

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During and after the shock, the worms generally exhibit convulsions and paralysis, and then typically resume normal sinusoidal locomotion. This figure was adapted from Risley *et al.*, 2016.

2. Connect the output on the S44 stimulator via a cable with a banana plug splitter and connect it to the ground and mod banana plug inputs on the SD9 stimulator. The goal here is to connect the S44 to the SD9 stimulator so that the S44 can be used to modulate duration.
   Note: Be sure the polarity of the banana plugs is consistent.
3. Set the S44 to at least 5 V and desired stimulus duration; we use 3 sec.
4. Clip one of the alligator clips onto the positive (+) output on the SD9 and the other onto the negative (-) output. These alligator clip wires will connect the SD9 stimulator to the experimental tube (Figures 3 and 2B).

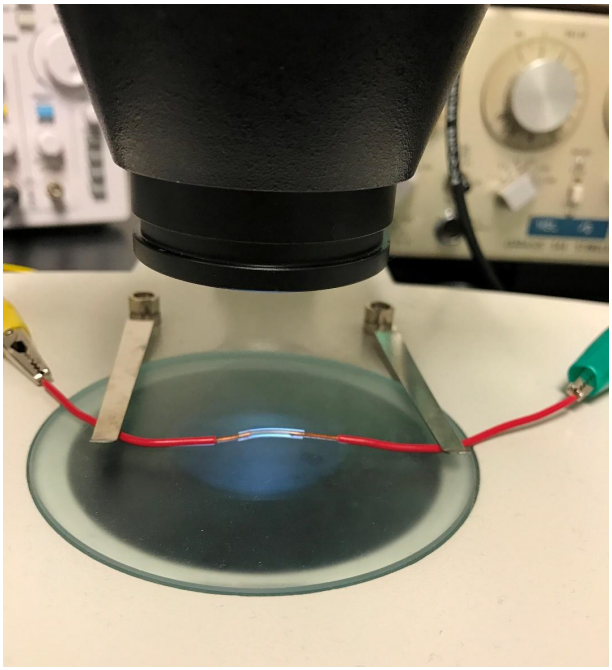

Figure 3. Close-up image of the experimental tube. The tube is plugged on either end with the insulated copper wires that are attached to the stimulator. The distance between the copper wire should be exactly 10 mm.

5. Next clip each of the copper wires to the open end of each alligator clip. The open end of each copper wire will later be plugged directly into experimental tube when the experiment is ready to begin. Set up the microscope camera to the TV as per the camera instructions (Figures 1 and 2A).
   Note: It is very important to set up the experimental tubes consistently to obtain a consistent.
6. 1-day old adult *Caenorhabditis elegans* are used for the experiments. 24-h before experimentation, approximately 30 L4 stage worms per genotype are transferred to fresh NGM plates seeded with OP50 *E. coli*. The worms are placed in a 20 °C incubator overnight; and will

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mature into 1-day old adults in approximately 18 h. During experimentation the following day, the worm plates are left on the lab bench at room temperature (23 °C).

7. Typically, the drug solutions are prepared on the morning of experimentation. The drugs are dissolved directly into a vial of M9 solution with a total volume of 3-5 ml, depending on the number of experimental trials. Sham controls are also prepared at this time. The prepared reagents can be stored, at 4 °C or based on the specifications of the drug, for experiments performed on consecutive days.

   Note: Water-soluble drugs were dissolved directly into M9 (Risley et al., 2016); however, a DMSO concentration curve was conducted in Risley et al. (2016) and it was reported that DMSO concentrations up to 0.5% had no significant effect on wild-type locomotor recovery after electric shock.

8. Next, load a blank DVD+R into the HDD/DVD recorder and turn on the TV screen. Also, verify that the settings are correct on the timer output stimulator and stimulator using the oscilloscope. We typically set the electroshock parameters to 47 V, 3 sec duration. This voltage was selected based off a voltage-response curve. The maximum voltage in which wild-type worms recovered was about 60 V and they recovered, on average, in 85 sec. 47 V was selected as worms recover in approximately one half the time of maximum limit for recovery (Figure 4).

   Note: The timer output stimulator (S44) is used only as a trigger for the SD9 stimulator, which sends current to the worms. The duration, typically 3 sec, is set on the S44 and used as a trigger to regulate the duration. There are many other ways to control for the duration including using a digital trigger via a computer or an Arduino.

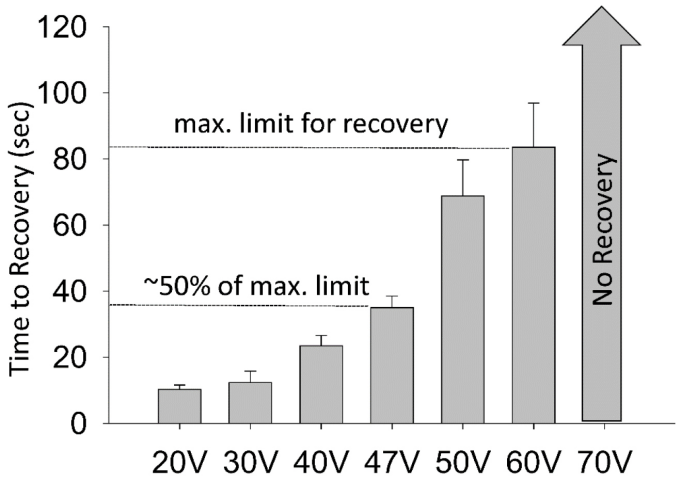

Figure 4. A voltage response curve demonstrates locomotor recovery times in wild-type worms. Wild-type worms did not recover at voltages ≥ 70 V. The maximum tested limit for recovery was 60 V where worms recovered in approximately 80 seconds. 47 V was the voltage in which recovery time was 50% of the maximum limit. This figure was adapted from Risley *et al.*, 2016.

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9. Load approximately 20 µl of control solution or drug solution. Immediately following, load 6-8 worms into the tube using a platinum pick and set a timer for 30 min. 30 sec before the timer expires, gently place the tube under the microscope/camera setup with the light base turned on, and plug each copper wire into the two ends of the tube; the wires should be 10 mm apart (Figures 3 and 2B). Verify the distance between the copper wires is precise (a ruler will suffice) and initiate the electroshock. Allow the experiment to record for 10 min. After the 10 min recording, the tube and its contents are discarded.
   Note: Generally, a tube is loaded every 10 min. Each tube is shocked and recorded for 10 min before it is discarded and another tube is immediately prepared. Also, OP50 transferred from the platinum pick to the tube should be minimized as it could impede the view of the worms.

Data analysis

1. The analysis is manually scored using a video player. Each worm is scored individually in each tube. An example screenshot of a raw video file can be seen in Figure 5 and a schematic representation in Figure 2C. The time of the shock onset (depicted by the appearance of bubbles) is recorded in a notebook and subsequent recovery times of each worm are recorded.

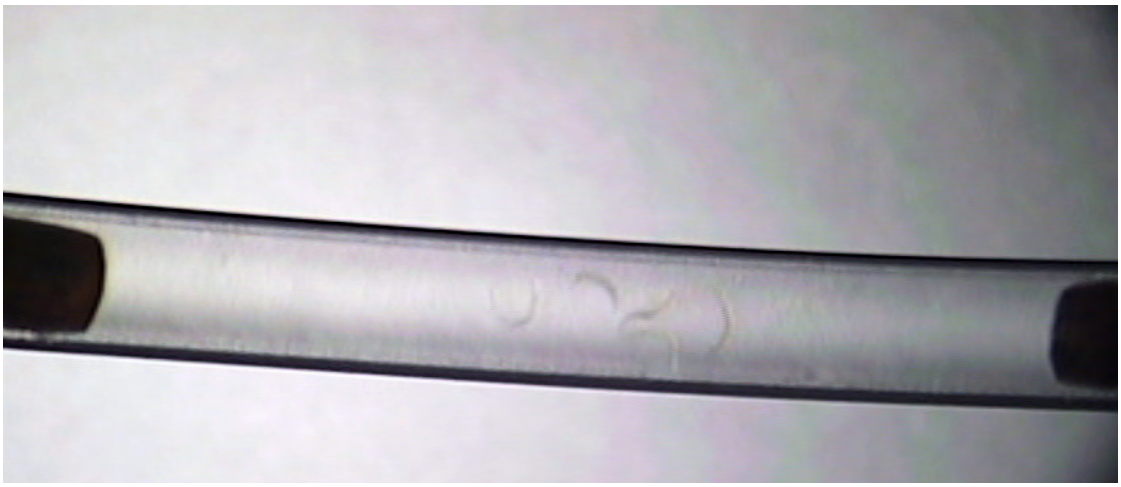

Figure 5. Example screenshot of recorded raw data. The image is a screenshot from a raw data video file of an experiment. There are five worms centered inside the middle of the tube. The tube is plugged by the copper wire on either end. This is an example of the video file that is analyzed by the researcher.

2. Recovery is defined as a worm regaining its ability to move in a sinusoidal movement, similar to the locomotion it exhibited prior to the shock. Partial recovery of only head or tail is not considered recovery. (Video 1)
3. Recovery time of each worm was considered n = 1 and the total number of experimental tubes was N = 1. For all experiments, a full data set is considered n ≥ 10 and N ≥ 6.

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4. Data is analyzed in SigmaPlot using One-Way ANOVA followed by a post-hoc Multiple Comparison's test or a Student's *t*-test; however, appropriate statistical analysis should be determined by the researcher. Data was typically represented by a bar graph ± SEM. An example of the data bar graphs can be seen in the voltage-response curve in Figure 4.

Video 1. Example video of the experimental setup and analysis. This video shows another visual of the experimental setup, followed by a raw data video file. The video is annotated with notes describing when each worm is considered recovered. This figure was adapted from Risley *et al.*, 2016.

Notes

1. Due to electrolysis during the stimulus, bubbles will form on the edge of the tube. Worms that are covered by the bubbles are excluded from analysis. We have used an infrared temperature gun to record temperature fluctuations over 3 sec. The temperature fluctuations are acute and minimal, 1 ± 0.5 °C, however temperature should be recorded to be sure increased temperature will not compound the effect of the electroshock. If the shock is administered for more than 3 sec, care should be taken that the temperature does not increase substantially as this could confound the results.
2. The speed of the sinusoidal locomotion was not taken into consideration during analysis.
3. Worms that did not recover were excluded from analysis.

Recipes

1. LB broth
   Add 25 g LB broth powder and 1 L dH_2O to a 2 L flask
   Autoclave for 30 min

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2. Nematode growth medium (NGM) agar plates
   a. Add 3 g NaCl, 17 g agar, 2.5 g peptone, and 975 ml dH_2O to a 2 L flask
   b. Autoclave for 30 min
   c. Let cool to 50 °C in the water bath
   d. Add 1 ml 1 M $CaCl_2$, 1 ml 1 M $MgSO_4$, 5 mg cholesterol dissolved in 1 ml 95% ethanol, 25 ml KP buffer to the agar solution, mixing well after each addition
   e. Pipette 10 ml into each 60 x 15 mm Petri dish
   f. Cover and store at 4 °C
   g. When needed, seed plates with 50 µl of OP50 *E. coli* and incubate overnight at 37 °C
3. KP buffer
   Add 2.46 g KH_2PO_4 and 1.205 g K_2HPO_4, to 25 ml diH_2O with a stir bar
   Adjust pH to 6.0 and vacuum filter
4. OP50 *E. coli*
   Suspend 1 colony of OP50 *E.coli* from a stock plate in LB broth
   Incubate overnight at 37 °C with the lid loosely on
   Store at 4 °C
5. M9 solution
   Add 3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$ to a 2 L flask and fill with diH_2O to 1 L
   Autoclave for 15 min and store at room temperature

Acknowledgments

We have published this protocol in (Risley *et al.,* 2016; Opperman *et al.*, 2017). Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

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