Demonstrating the Stimulus Strength-Duration Relationship Using the Cockroach Leg

California Lutheran UNIVERSITY

Kenneth Long, Ph.D
Department of Biology

Abstract

The stimulus strength-duration (S-D) relationship is typically demonstrated in physiology labs using the frog sciatic nerve preparation. An invertebrate model can also be used to demonstrate the S-D relationship. The built-in 10V stimulator of the Biopac MP36 and needle electrodes are used to stimulate movement of the cockroach tibia and tarsus. Students determine the necessary voltages that produce threshold responses for a range of stimulus durations (0.1 to 1 msec). Classic hyperbolic S-D curves are generated and rheobase and chronaxie are determined. This experimental setup can also be used to demonstrate summation and tetanus.

Theory

The strength of a stimulus and its duration determine the threshold of excitable membranes. As stimulus strength increases, the time required to excite the membrane decreases, and vice versa. Because of membrane capacitance, the relationship between stimulus strength and duration is not a simple product of the two, but rather shows a hyperbolic relationship – below a certain stimulus strength (the "rheobase," there is no stimulation duration long enough to bring a membrane to threshold. Chronaxie, the duration of a threshold stimulus at twice the rheobase, can be used as a measure of membrane excitability – the lower the chronaxie, the greater the membrane excitability.

Animals

American cockroaches, *Periplaneta americana*, were purchased from Carolina Biological and maintained in plastic terrariums; food (dog and cat kibble along with miscellaneous fruit and vegetable pieces) and water were supplied *ad libitum.* Roaches were immobilized by cooling in a refrigerator (3-6 °C) for 20-30 minutes prior to the removal of their legs.

Materials

Computer with BSL 4.1 Software
Biopac MP36 Data Acquisition Unit
OUT3: Low Voltage Stimulator Adaptor
ELSTM2 Needle Electrodes
Plastic 100 mm x 15 mm Petri Dishes
Modeling Clay
Small Surgical Scissors
Fine Forceps (e.g., Dumont #5)
Refrigerator

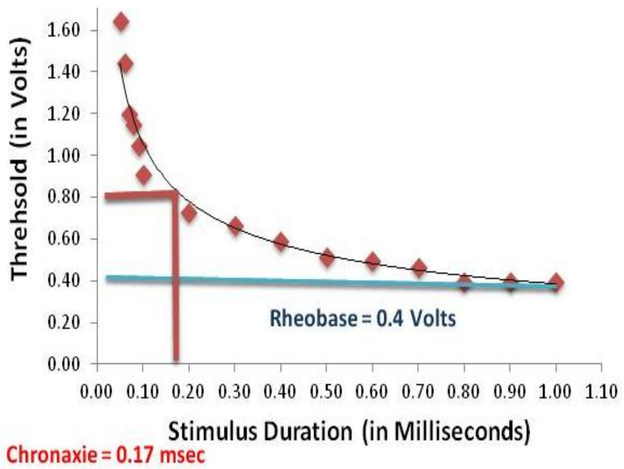
Introduction

The stimulus strength-duration (S-D) relationship and the concepts of rheobase and chronaxie are over 100 years old[3,4]. While employed far less frequently today compared with 20-40 years ago, the concepts are still used in neuromuscular research, and in studies of cardiac pacemakers[2] and deep brain stimulation[6]. For example, knowing chronaxie values for neural tissue can help prevent damage from excessive stimulation of implanted electrodes.

While the S-D relationship is rarely covered in introductory physiology and neuroscience texts, it is often covered in upper division neurophysiology labs, but not typically in lower division A & P labs. Hands-on neurophysiology exercises that are simple (not technically demanding), reliable, and relatively inexpensive should be valuable additions to both lower and upper division physiology lab curricula.

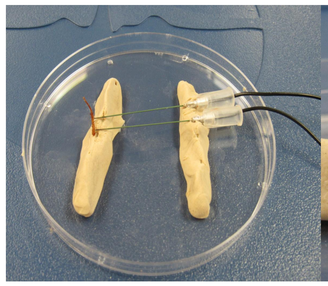
Invertebrates such as earthworms[1] and cockroaches[5] are useful model organisms and can replace vertebrates in some exercises. The S-D relationship is typically tested using the frog sciatic nerve/compound action potential preparation. I developed this cockroach-based lab exercise a few years ago to demonstrate the S-D relationship to physiology students. I used Biopac equipment, but it can also be preformed using other data acquisition systems (AD Instruments and iWorx) that have built-in or separate stimulators.

Example Student Data



Procedure

Metathoracic and mesothoracic legs are removed using small surgical scissors. Prothoracic legs are usually too small to use in the experiment. Legs are positioned and stabilized using forceps and modeling clay in the top of a plastic Petri dish. The tips of needle electrodes (ELSTM2) are placed in the femur and the electrodes are stabilized using modeling clay. The ELSTM2 is connected to an OUT3 (an adaptor for the built-in low voltage stimulator in the Biopac MP36). The stimulator settings can be set during the experiment or graph template (*.gtl*) files that have the settings preconfigured can be used.



Cockroach leg and electrodes are stabilized with modeling clay in top of Petri dish

Cockroach leg with two stimulating electrodes placed in the femur

Stimulus Strength vs. Stimulus Duration

The stimulus S-D relationship is examined by determining the threshold voltage for stimuli of 0.1 to 1.0 msec durations. Threshold is defined as the first noticeable twitch of the tarsus. Rheobase and chronaxie are determined from the data. If chronaxie is less than 0.1 msec, threshold voltage should be determined for durations between 0.05 and 0.1 msec. Example student results are shown on the left.

Twitch Summation

Using the 2X rheobase voltage and the chronaxie determined in the strength-duration experiment, students next test the summation of muscle twitches. Beginning with a 200 msec between pulses, two distinct twitches should be observed. Students then reduce the delay in increments of 5 msec until only one visible twitch is seen – the largest delay between two pulses that results in one twitch is considered complete summation – typically 60-80 msec.

Demonstrating Tetanus

With a stimulator set on continuous pulses, the frequency of stimulation is increased in 1 Hz steps from 1 Hz up to 20 Hz until tetanus is observed (typically 12-15 Hz).

Advantages

Simple, low cost preparation that demonstrates a basic physiological concept.
Reliable results: Most students generate usable data in a short amount of time.
Allows simple mathematical modeling along with the generation and interpretation of graphs.
Uses an invertebrate instead of a vertebrate animal.

Disadvantages

Not amenable to experimental variables, e.g., effect of drugs.
No record of responses other than visual observations.

HAPS Learning Outcomes Addressed

#7.Demonstrate laboratory procedures used to examine anatomical structures and evaluate physiological functions of each organ system.
#8: Interpret graphs of anatomical and physiological data.

References

1) Bähring R. and Bauer C.K. (2014) Easy method to examine single nerve fiber excitability and conduction parameters using intact nonanesthetized earthworms. *Adv Physiol Educ.* 38(3):253-64. doi: 10.1152/advan.00137.2013
2) Coates, S and Thwaites, B. (2000) The strength-duration curve and its importance in pacing efficiency: a study of 325 pacing leads in 229 patients. *Pacing Clin Electrophysiol.* 23(8):1273-7.
3) Irnich, W. (2010) The terms "chronaxie" and "rheobase" are 100 years old. *Pacing Clin Electrophysiol.* 33(4):491-6. doi: 10.1111/j.1540-8159.2009.02666.x.
4) Lapique L. (1909) Definition experimentalie de l'excitablilité. *Proc Soc: Biol* **77**:280–285.
5) Ramos, R.L. Moiseff, A. and Brumberg, J.C. (2007) Utility and versatility of extracellular recordings from the cockroach for neurophysiological instruction and demonstration. *J Undergrad Neurosci Educ.* 5(2): A28–A34.
6) Rodríguez-Fernández ÁL, Rebollo-Roldán J, Jiménez-Rejano JJ, Güeita-Rodríguez J (2016) Strength-Duration curves of the common fibular nerve show hypoexcitability in people with functional ankle instability. *PM & R.* 8(6):536-44. doi: 10.1016/j.pmrj.2015.09.009.

Online Resource

The McGill Physiology Virtual Lab has an excellent overview of the strength-duration relationship in the context of the compound action potential.
https://www.medicine.mcgill.ca/physio/vlab/CAP/S-D.htm

Acknowledgements

Thanks to the students in my Human Physiology, Vertebrate Physiology, and Introduction to Neuroscience courses and their cockroach subjects. Thanks to the Office of the Provost for travel funding (I hope), CLU's Biology Department, and Joe Crump whose undergraduate research project laid the groundwork for this exercise.

Cockroach Image top of poster: http://pestcontrolcanada.com/cockroaches/