Simulation Software

Experimental Physiology Manual

for Computer Based Amphibian Experiments

K Sri Nageswari
Rajeev Sharma
M Syamala Devi

2nd Edition

JAYPEE

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Amphibian Nerve-muscle Experiments

INTRODUCTION

Gastrocnemius muscle-sciatic nerve preparation of frog is used for demonstration of properties of skeletal muscle contraction and nerve function. Living tissue of frog is preferred because:

- Frog is a cold-blooded animal and so no temperature adjustments are needed.
- Living tissue of frog gets oxygen from atmospheric air and hence no oxygenation required.
- Isolated nerve muscle preparation of frog can be used for long duration if handled with care and kept suitably moist.

LEARNING OBJECTIVES

- To identify and describe the use of each instrument used for nerve muscle experiments.
- To describe the composition of Ringer's solution and function of each component.
- To describe the steps of dissection of gastrocnemius muscle-sciatic nerve preparation.

Steps to Follow for Carrying Out Any Nerve-muscle Experiment

Step 1

Move the cursor over to the Nerve-muscle icon.

Step 2

A show box appears that contains four submodules:

1. Apparatus
2. Reagents
3. Dissection
4. Contents
    - Select any Amphibian Experiment

The first three submodules display the details of how the experiments are carried out in laboratories with the set up of the apparatus and dissection procedures that are common to nerve-muscle experiments. The "Contents" module opens another screen, which lists all the nerve-muscle experiments simulated.

Step 3

The users need to click "Apparatus", "Reagents" and then "Dissection" to know the details of each before proceeding onto contents, which includes details of each experiment.

When you click on "Apparatus," following screen appears **(Fig. 3)**.

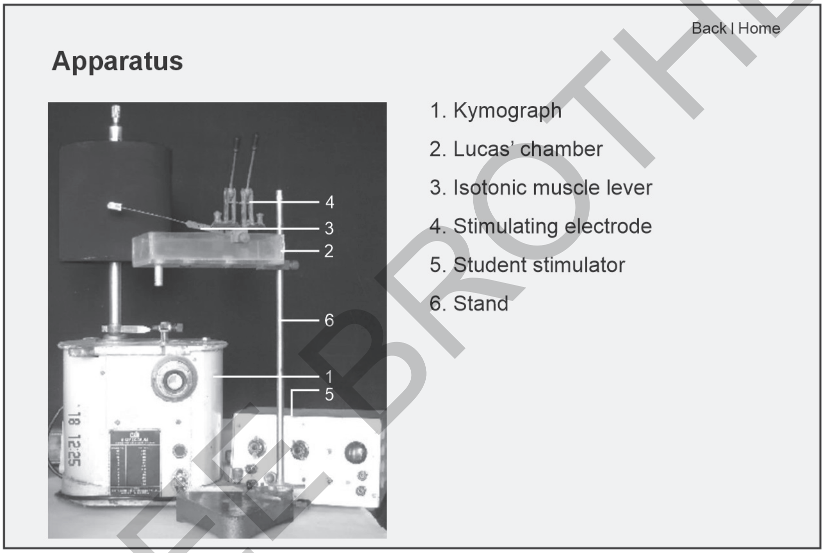

FIG. 3: Apparatus for carrying out amphibian nerve-muscle experiments.

On clicking the serial number against each apparatus, the details about the same appear:

1. **Kymograph:**
   - A recording instrument which records tissue movements on a moving surface
   - Runs with an inbuilt electric motor at 220 V, AC
   - Has a metal gear box to which a rotating shaft is connected
   - Speed of the shaft is controlled by gears in gear box
   - Range of speed is 2.3 mm/sec to 560 mm/sec
   - Clutch lever is present on the body, used to change the gears
   - Contact block, bearing contact knob, is present on the body of the kymograph
   - Projecting strikers are present at the base of the rotating shaft
   - Drum used for obtaining the graph is mounted on the shaft and fixed with a drum grip lever.
2. **Lucas chamber/Muscle trough:**
   - Rectangular perspex/plastic chamber used to put the isolated nerve muscle preparation
   - Filled with Ringer's solution to keep the preparation viable for longer period.
3. **Isotonic muscle lever/Writing lever:**
   - Attached to Lucas chamber and used for recording muscle contraction
   - Has a vertical arm and horizontal arm
   - Muscle tendon is attached to vertical arm with a thread
   - Horizontal arm has holes to hang weights and a writing point, to which a triangular peace of photographic film or an ink writing pen is attached to obtain the tracing.
4. **Stimulating electrodes:**
   - Used for electrical stimulation of tissues
   - One type (a) is a pair of ball and socket type silver electrodes fitted on the muscle trunk
   - Another type (b) is a bipolar electrode, having a long insulated handle with uninsulated prongs at one end to deliver the stimulus.
5. **Student stimulator:**
   - Electronic stimulator with a DC output of 0–25 V
   - All the stimulus parameters like strength (V), frequency (pulses), various modes of stimulation like single, multiple or external are controlled on the same instrument.
6. **Myograph stand:**
   - Vertical rod with a heavy triangular base
   - Used to fix Lucas chamber/myograph board.

When you click on reagents, following screen appears **(Fig. 4)**.

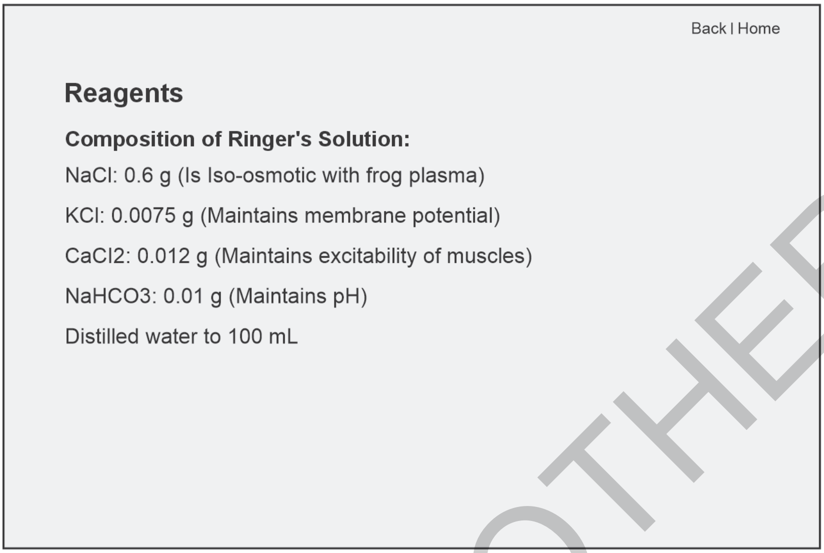

FIG. 4: List of reagents.

When you click on dissection, following procedure is displayed **(Fig. 5)**.

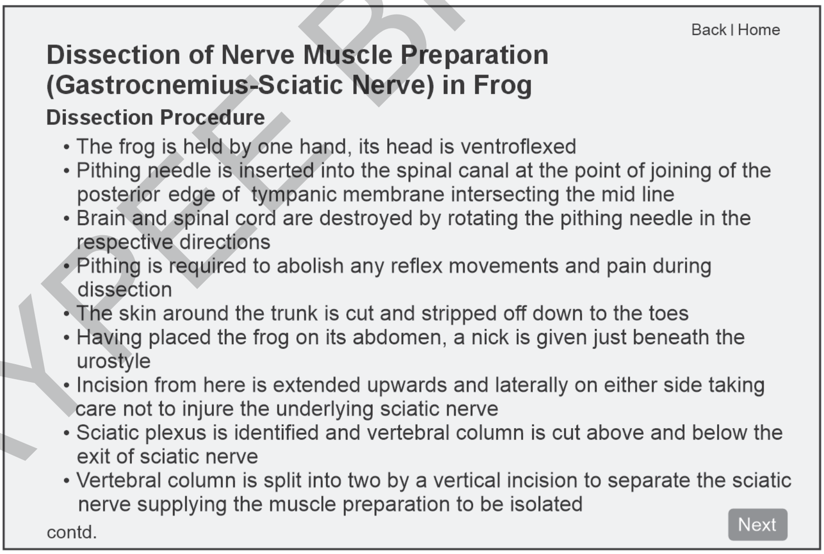

FIG. 5: Dissection procedure for nerve-muscle preparation (gastrocnemius-sciatic nerve) in frog.

On clicking the 'Contd' icon, rest of the procedure is displayed on next screen **(Fig. 5)**.

Step 4

Once you are through with them, the “Contents” icon is clicked and the content screen displaying the list of all the nerve-muscle experiments appears **(Fig. 6)**.

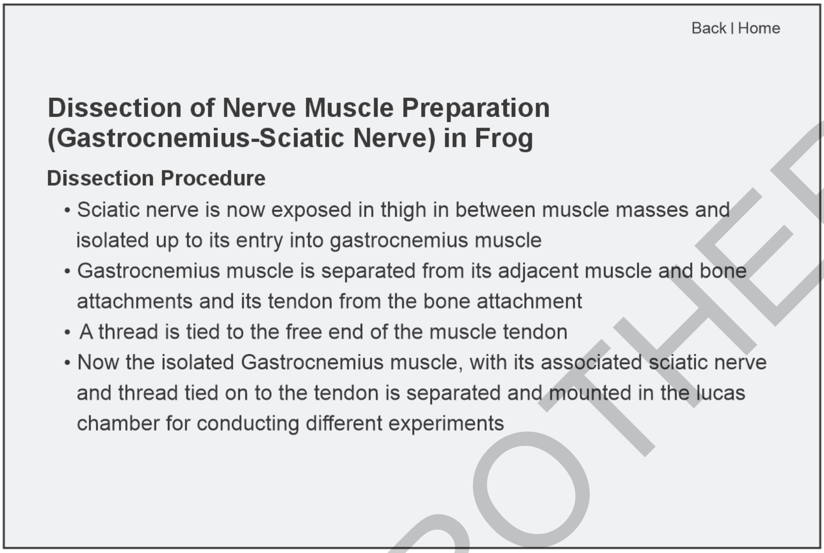

FIG. 6: Dissection procedure (continued).

Step 5

User can select any experiment from the given list. Once the selection is made, the user is provided with the details of the experiment in the form of Procedure, Show Graph, Observations and Inference icons highlighted sequentially on subsequent screener. Clicking the Next icon closes the Experiment and takes back to List of Experiments.

Step 6

Now click on the experiment you want to carry, and then click on the procedure icon to study its theoretical part **(Fig. 7)**.

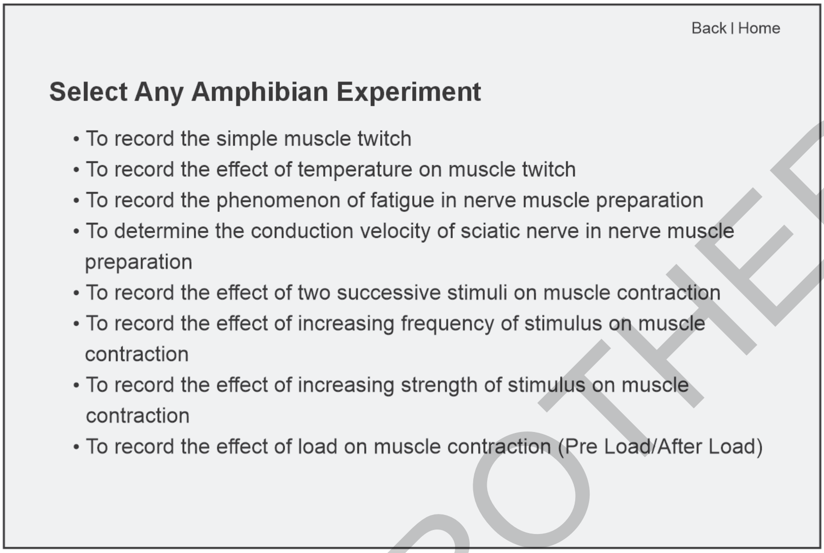

FIG. 7: Selection of a particular experiment.

Step 7

Once you are through with the procedure, click the "Draw graph" icon. An "Experiment screen" will open as per the experiment selected in "Contents screen".

Step 8

Click on the Draw Curve icon. A curve is drawn. Once the curve is drawn, click on the "Observations icon" to read the derived calculations of the drawn curve.

2.1 To record a simple muscle twitch

INTRODUCTION

When the nerve in a muscle nerve preparation is stimulated with a single induction shock, it results in a single twitch like contraction followed by relaxation of the muscle. This recorded event is called a simple muscle twitch.

LEARNING OBJECTIVES

- To identify and comment upon the graph of simple muscle twitch.
- To calculate Latent period, Contraction period and Relaxation period in the given graph.

Procedure

- After mounting the nerve-muscle preparation in the Lucas chamber filled with Ringer solution (28°C) the muscle tendon is tied to the hook of vertical arm of writing lever
- Horizontal arm of writing lever is supported with a weight and vertical arm is supported with after-load screw
- "External" terminals of student stimulator are connected with contact buttons of kymograph to include it in the circuit
- "Stim out" terminals of student stimulator are connected to stimulating electrodes
- Nerve is placed on to the stimulating electrodes
- Point of stimulus is marked manually on the recording drum at a point where projecting strikers touch the contact button
- With clutch "On", drum speed of 560 mm/sec, a single stimulus is applied to the nerve while the "Standby" knob of stimulator is kept at "External"
- A single muscle twitch is obtained when the projecting strikers touch the contact buttons and the circuit is completed **(Fig. 8)**
- A tuning fork of 100 Hz is made to vibrate after striking it on the dorsum of hand and is gently touched to the moving drum below the obtained tracing
- The duration of muscle twitch and the duration of its various components are calculated.

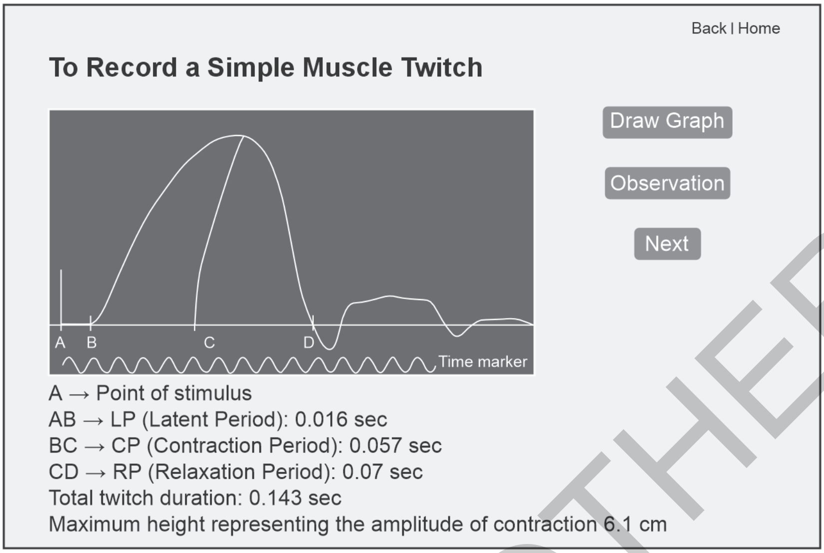

FIG. 8: Simple muscle twitch screen with observations.

Discussion

Normal twitch duration is around 0.1 seconds that includes latent period, contraction period and relaxation period:

- Latent period normally is of 0.01 seconds that represents the time taken for the stimulus to travel from the point of stimulation to recording electrode. This includes:
  - Time taken to travel along the nerve → neuromuscular junction → motor end plate → excitation contraction coupling → muscle contraction
  - Time taken to overcome inertia of lever
  - Time taken to overcome viscous resistance of muscle
- The duration of contraction period is 0.04 seconds that represents period from start of contraction to peak of contraction
- Relaxation period represents time taken by muscle to relax and come back to resting state. Its duration is around 0.05 seconds.

Duration of different components of simple muscle twitch can either be obtained by making a tuning fork of 100 Hz vibrate and record its vibrations in the form of tracings on the kymograph below the simple muscle curve or it can be deduced from speed of the moving drum which is normally kept at 560 m/sec.

Experimental Physiology Manual
for Computer Based Amphibian Experiments

Experimental Physiology Manual for Computer Based Amphibian Experiments has been prepared for phase 1 MBBS students across the country. The idea behind designing these computer-based experiments was to enable the students to get a feel of doing the amphibian experiments on their own as these experiments are no longer done by students using live frogs. After thorough understanding of input parameters/values, output parameters, graphical forms of output, and static or dynamic type of recording conditions, we converted the procedures followed in live experiments to computer algorithms and designed the simulation software. The students can carry this manual to the experimental laboratory and also write down the proceedings of the laboratory in the blank page provided on the left side of each experiment. As each experimental detail has been given, this will save the time consumed in writing down the experimental details on their own.

K Sri Nageswari retired as Professor and Head in the Department of Physiology at Government Medical College, Chandigarh, Punjab, India (1998–2006). After superannuation, she served as Professor and Head of Physiology and as advisor academics, research, and administration till 2020 at various private medical institutes. She has around 38 years of teaching and research experience, 68 publications including abstracts, 5 technical reports, 2 chapters in books, and 3 books in physiology. She served as reviewer and examiner for various MD and PhD theses and prestigious journals and projects. She is an International Fellow in Medical Education (ECFMG, now known as FAIMER, USA) and worked at Department of Physiology and Biophysics at University of Illinois Chicago (UIC), Chicago, USA, in 2001. She had been a regular member of the American Physiological Society since 2001, Emeritus Member since 2014, and member of Physiological Society, UK, since 2016. She is recognized as living history in physiology, and her life history is posted in the "Living History of Physiology series" in the American Physiological Society's website https://www.the-aps.org in 2018. She is Fellow of Indian Association of Biomedical Scientists since 2003. She conducted IUPS workshop for medical teachers at College of Medicine, Arabian Gulf University, Kingdom of Bahrain on Medical Education, in 2012. Her research interests are Biological and Immunological Effects of Electromagnetic Radiation, Medical Education and Obesity with many research projects in this area. She had introduced a number of innovative teaching–learning strategies into the course curriculum in Physiology for medical students. She is a recipient of Professor Puthuraya Award for Best Teacher in Physiology, Dev Raj Bajaj Research Award for invention of new research technique/ instrumentation in the field of Physiology, and ML Gupta Award for significant contribution in the field of Medical Education by Association of Physiologists and Pharmacologists of India in the years 2009, 2006, and 2005, respectively.

Rajeev Sharma is working as Professor and Head in the Department of Physiology at Guru Gobind Singh Medical College, Faridkot in Punjab, India. He has also served the institute in the capacity of Principal (2020–2023), Professor In-Charge, Academics and Research (2014–2017) and Joint Controller of Examinations in Baba Farid University of Health Sciences, Faridkot. Previously, he served in Government Medical College, Chandigarh from 2002 to 2010. He has more than 24 years of experience of teaching Physiology to various undergraduate and postgraduate students. He has many research papers published in National and International Journals and has been an invited speaker in various conferences. He has also completed the Advance Course in Medical Education (ACME). He along with Dr K Sri Nageswari has written another book "Practical Workbook of Human Physiology" for undergraduate Medical students which is running its second edition. He is the Life Member of Association of Physiologists and Pharmacologists of India (APPI) and member of Executive body of Association of Physiologists of India (ASSOPI). His key areas of interest are Medical Education and Neurophysiology.

M Syamala Devi is an Ex-professor in the Department of Computer Science and Applications at Panjab University, Chandigarh, Punjab, India. Before joining Panjab University, she served Indian Space Research Organization, Sriharikota, and National Institute of Technical Teachers' Training & Research, Chandigarh. Her areas of expertise include algorithms, image processing, distributed artificial intelligence, and educational computing. Nine students were awarded PhD under her supervision. She has published about 60 papers in national and international research journals, proceedings of various national and international seminars, and conferences. She has adjudicated a number of PhD theses and reviewed research papers.

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