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HASKELL LABORATORY
FOR
TOXICOLOGY AND INDUSTRIAL MEDICINE

Haskell Laboratory
Newark, Delaware 19711
June 8, 1965

Dr. Sven G. Eliasson
Department of Neurology
Washington University School of Medicine
St. Louis, Missouri

Dear Dr. Eliasson:

We certainly appreciate your interest in the problems which we are encountering in our nerve conduction experiments. If these can be solved we hope to apply your method to nerves, obtained at autopsy, from rats used for toxicological tests. Since our telephone conversation last week, we have tried nerves obtained from untreated rats which were stunned then given a pneumothorax. To our surprise these nerves behaved much as those obtained from the chloroformed animals; this suggests that our techniques, as well as the chloroform, are causing trouble. As a result, I am taking advantage of your kindness and describing, as briefly as possible, our techniques and equipment.

Recording Equipment - Tektronix

Type 561 A oscilloscope, single beam, with Type 2A63 differential amplifier and Type 2B67 time base. Voltage sensitivity: 1 mV to 20 V per division (1 cm) oscilloscope screen; sweep speed: 1 μSec. to 5 seconds per division oscilloscope screen. As a rule we operate at 2-5 mV and 0.2 mSec. per division. Sweep triggered by stimulus.

Stimulator -

Grass SD 5 square wave stimulator with built in isolation unit. Fresh nerve: threshold stimulus 0.2 to 0.4 V when stimulus duration is 0.06 mSec. Maximal stimulus: about 2-4 V but is frequently difficult to determine when response is superimposed on shock artefact.

Conduction Chamber -

Harvard Apparatus Company, with platinum electrodes rearranged so as to be 5 mm apart.

All of the equipment, with the exception of the tank containing 95 per cent oxygen and 5 per cent carbon dioxide, is in a constant temperature room maintained at 98.5 ± 0.5°F. The humidity, however, is low. The Ringer-Locke used to moisten the nerve during dissection,

or in which nerves are "stored", is oxygenated with the 95 per cent oxygen and 5 per cent carbon dioxide mixture for 30 minutes or longer prior to use. Incidently, the sodium chloride in the Ringer-Locke is Merck preparation for biological work, and glass-distilled water was used. Some of the Ringer-Locke is placed in the conduction chamber almost to the height of the electrodes, and the oxygen-carbon dioxide mixture passed through it to provide a moist atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide for the nerve. The chamber is kept covered. As an alternative to the Ringer-Locke in the chamber we have tried mineral oil through which the oxygen-carbon dioxide mixture is bubbled; enough oil to cover the nerve is used.

Nerve Dissection -

To avoid jerking and stretching, I do most of the dissection with a Bard-Parker scapel, use fine forceps to pass ligatures (black mercerized cotton) under the nerve at either end, and scissors to free the main trunk from branches and underlying tissue. To avoid slipping, I tie the ligatures rather tight. My fastest time, from removing rat from chloroform or stunning it, to mounting nerve in chamber, is 4 to 5 minutes. I can try to shorten this time. Even those nerves taken from stunned rats and mounted immediately in the chamber have failed to show any spontaneous activity.

The following questions trouble us.

1. Why do apparently "healthy" nerves show marked decreases in spike height and conduction velocity 10 to 15 minutes after mounting? This deterioration may be accompanied by progressively increasing influence of shock artefact as though the artefact were prolonged on the nerve response. Can drying of nerve cause this?

2. Would monophasic recording be better than diphasic? I have found it difficult to crush an area without stretching the nerve as the tissue sticks to the hemostats or to limit a burn to an adequately localized area, especially when recording electrodes are close together. Is there a more reliable method of killing the area under the distal electrode?

3. Is there any way to mark or identify on the nerve tissue the locations of the stimulus cathode and the proximal recording electrode so that conduction distance can be measured accurately?

4. Is it correct to measure conduction time from the start of the shock artefact (we realize that this includes excitation time)? The conduction velocities which we are recording, nerves just mounted:

	Inflection Velocity M/sec	Peak Velocity M/sec
Stunned Rats	50-60	30-35
Chloroformed Rats	55+	33-34

Our "peak" velocity measurements are more consistent than our inflection velocity measurements.

5. Is it possible to reduce the duration of the shock artefact so that measurements can be made on conduction distances shorter than 1 centimeter? At times the artefact is so prolonged as to interfere with the response when the conduction distance is 1 centimeter; this may be associated with a deteriorating nerve.

6. Is epoxy cement, well set, neurotoxic? With the idea that we could save time and nerve damage by leaving the nerve in position, we made a platinum electrode set, including both stimulating and recording electrodes, which could be slipped under the nerve. The wires were held in position and insulated by epoxy cement. They have been disappointing but need further checks.

I am enclosing copies of a few of our photographs of nerve responses in case they may provide information. I apologize for the length of this letter but I have been unable to reduce it and still describe our methods.

Sincerely yours,

Mary E. Maxfield, Ph.D.
Physiologist

MEM/dyb
Enclosures