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Classic Clinical Technique Adapted to Demonstrate Autonomic Nervous System Physiology in an Undergraduate Laboratory Course

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The sympathetic skin response can be measured across the hands and feet with a simple bio amplifier. Controlled by the sympathetic nervous system, this response results from the activation of the eccrine sweat glands by many types of stimuli. This classical clinical test is used to evaluate peripheral neuropathies caused by a wide range of diseases, and can easily be adapted to teach a range of physiology applicable to neuroscience curricula.

Key words: sympathetic skin response, autonomic nervous system, neuropathy, non-myelinated fibers, habituation, physiology.

The autonomic nervous system

Made up of the sympathetic and parasympathetic nervous system, the autonomic nervous system's (ANS) primary function is to maintain homeostasis. A variety of organs have dual parasympathetic and sympathetic supply. In such situations, the two systems often have opposite effects. For example, in the heart, parasympathetic stimulation causes slowing of the heart, whereas sympathetic stimulation increases heart rate. In the intestine, parasympathetic stimulation increases smooth muscle contractility, whereas sympathetic stimulation decreases it. However, the description of the sympathetic division as orchestrating the 'fight or flight' responses is an over-simplification. Generally, both divisions function together to regulate the daily activities of the body (Silverthorn, 2013).

Some tissues are only supplied by one division of the ANS. For example, sweat glands and certain blood vessels have only a sympathetic supply, and the ciliary muscle in the eye has only parasympathetic innervation.

Physiology of the sympathetic skin response

The sympathetic skin response (SSR) is a change in potential recorded from the surface of the skin, and is seen when the eccrine sweat glands are activated by sympathetic nerve activity. Many different types of stimuli can activare the SSR.

This response is a result of polysynaptic reflex arc activation. The efferent part of the SSR reflex arc consists of myelinated sympathetic fibers of neurons. Postganglionic fibers are non-myelinated (type C) and innervate the eccrine sweat glands. Many different terms for the SSR have been used, such as electrodermal response, psychogalvanic reflex, galvanic skin response. An extensive review of the phenomena and its clinical implications can be found in Vetrugno et al (2003) and Kucera et al. (2004).

Technique can be added to an existing laboratory

Computer based data acquisition systems have become quite common in undergraduate physiology laboratories. These powerful systems can be used to record and measure any number of physiological parameters of interest to neurobiology students. Biological signals such as electrocardiography, electromyography (EMG), electroencephalography, and visual evoked potential are commonly measured with a differential amplifier. These procedures are easy for students to record and analyze during a single laboratory period. We have adapted a traditional clinical technique to extend the capability of this equipment that allows for assessment of autonomic nervous system function; specifically skin potentials generated during stressful stimuli, the SSR.

For this exercise we use an AC coupled human-safe bio amplifier. This measures the transitory potential generated as the eccrine sweat glands are activated. The positive electrodes are attached to the active measurement sites (palm and sole). The negative electrodes are positioned at the negative active position (top of foot and back of hand). A 5th 'body' electrode must be placed at a convenient point on the body. This is an isolated ground connection and not a reference. This allows for the measurement of the potential between the positive and negative electrodes and not an artificial potential between the subject and the amplifier.

Traditional polygraph-style recordings measure the SSR with a device that monitors skin conductance, often referred to as galvanic skin response amplifier. These devices apply a low constant-voltage alternating current across the leads. This allows for continuous measurement in skin moisture caused by the eccrine sweat glands. We can however, record the the transient potential caused by the applied stimuli with a general purpose bio amplifier and computer-based recording system without purchasing additional equipment.

MATERIALS AND METHODS

This procedure is derived from clinical tests used to assess peripheral and autonomic nerve functions. Students will document a function of the ANS by stimulating sweating using stressful stimuli.

A dual channel bio amplifier capable of recording signals in the 20 mV range and a computer-based data recording system can be used to record the SSR. Suggested recording parameters are: Sampling rate of 1000 samples/second, low pass filter (hardware or digital)

at 2Hz. A high pass filter at 0.5 Hz will remove DC artifacts from the signal. Appropriate cables and disposable Ag/AgCl electrodes to connect to the amplifier will also be necessary. Electrode paste, abrasive gel and alcohol swabs may be useful. For the electrical stimulation exercises, an isolated, constant current stimulator rated and approved for use with human subjects under IEC 60601-1 2000 is required. A tendon hammer with a mechanism to interface with the recording is ideal but not strictly necessary.

The students work in groups of three, a volunteer, one to run the software and one to apply the stimuli.

Attachment to the subject:

Skin on the palms and back of the hands, and the sole and top of the foot should be cleaned with alcohol wipes and lightly abraded if necessary. The differential leads from the bio amplifier should be placed on either side of the hand and foot as shown in Figure 1.

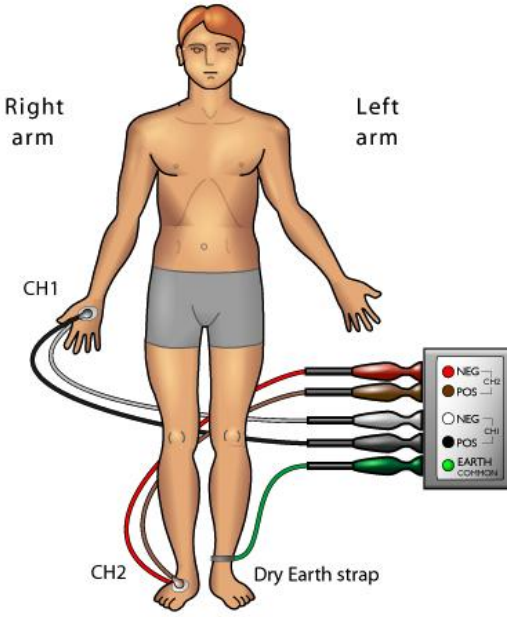

Figure 1. Proper electrode placement to record skin potentials.

Activation of the ANS with a stressful stimulus

The volunteer should relax and especially keep the arms and legs still throughout the exercise. Postural changes will cause noisy data.

Start the recording software and record baseline skin potential for 30 seconds.

Begin with stimulator amplitude set to 4 mA. Apply stimulation to the median nerve at the wrist on the arm **opposite** of the hand and foot attached to the bio amplifier (Fig. 2).

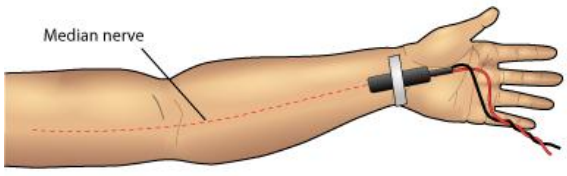

Figure 2. Location of the Median Nerve for stimulation.

Be sure to annotate the record to indicate exactly when stimulation occurs.

If the response does not resemble the one shown in Figure 3, increase the stimulator amplitude by 0.5 mA and repeat the stimulation at random intervals about 60 seconds apart. Continue to do this until a satisfactory response is obtained.

Once you have a reliable response from both hand and foot (Fig. 3), record three in a row, approximately 1 minute apart.

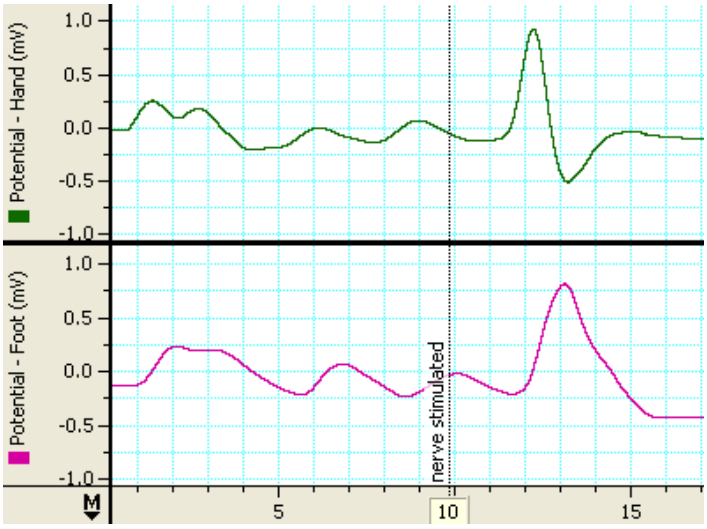

Figure 3. Increases in hand (green) and foot (pink) skin potentials after peripheral nerve stimulation (indicated by the Comment) recorded with PowerLab® data acquisition system and LabTutor® software.

Other stimuli for students to test

Make sure each recording is annotated as each different stimulus is applied. Allow a recovery time of at least a minute between each stimulus.

1) Have a member of the group unexpectedly make a loud sound by hitting something with the tendon hammer to startle the volunteer.

2) Instruct the volunteer to take a deep, gasping breath..

3) After notifying the volunteer that a physical stimulus is about to be applied, have the third member of the group apply a firm thump the sternum with the tendon hammer when the volunteer does not expect this.

Repeat these procedures with another volunteer.

Analysis – Nerve Stimulation

Students quantify the three responses to nerve stimulation by measuring the latency and amplitude of the skin potentials and calculating the mean response.(Fig. 4).

Analysis – Other Stimuli

Students measure the response to the startle, gasp and sternal thump in the same manner as the nerve stimulation recording. They can then compare the latencies and amplitudes of the response to each type of stimuli.

DISCUSSION

Biological variables can differ greatly between individuals.

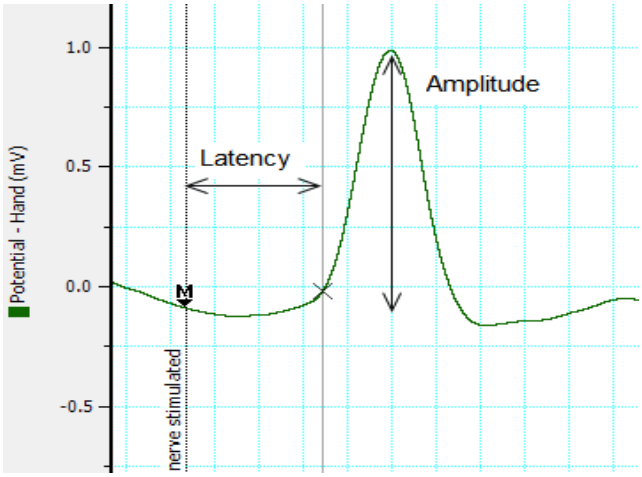

Figure 4. Procedure for measuring the latency and amplitude of each recorded skin potential response.

In healthy subjects the latency from the hands is significantly shorter than from the feet, and the amplitude is significantly higher from the hands compared to the feet. Many different studies have looked at the variation in amplitude and latency. Age, height, body temperature, as well as gender have been studied. The Kucera et al. (2004) paper provides a thorough review of the literature on the subject.

Student-generated data can be compared to patient case-study data. Then students can compare their responses to those of patients with documented neuropathies. The LabTutor® teaching suite has a comprehensive exercise including patient case studies available for students to analyze along side their own data. Student-designed and inquiry-based exercises are possible, as any number of different stimuli can be tested by the students. The phenomena of habituation can be studied using this procedure. The SSR will diminish rapidly with repeated stimuli. Different individuals will adapt to different stimulation types and stimulation frequencies. This can be a usefull demonstration of human physiological variability.

Combining the SSR with other measurements of autonomic nervous system functions such as heart rate variability, the dive response, and adding the Valsalva maneuver can further demonstrate the functions of the autonomic nervous system.

Troubleshooting

A possible problem that could arise in this experiment is noise in the signal. Noisy skin potential signals are usually due to poor connection of the recording electrodes. Make sure the skin has been abraded with an abrasive pad/gel. You may also use a small amount of electrode cream on the recording electrodes.

Suggestions for integration with existing curricula

If you use the human-safe isolated stimulator portions of the exercise, these are easily combined with an evoked EMG exercise. Stimulation is applied to the median nerve and the resulting evoked EMG is recorded from the abductor pollicis brevis. Comparisons can then be made between conduction velocities of motor neurons, and the unmylinated C-fibers that control the eccrine sweat glands.

CONCLUSION

If you are already recording biopotentials from human subjects, this clinical technique can easily be added to your laboratory with no additional equipment. This can be combined with other exercises to enhance the student experience in your course.

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