RECORDING FROM EFFERENT SYMPATHETIC FIBRES IN HUMANS USING MICRONEUROGRAPHY

ETHICS-SOP\_METHODS\_Microneurography-024

# Document Sign-offs

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In draft

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# Glossary of Terms

Microneurography - a neurophysiological method employed to visualize and record the normal traffic of nerve impulses that are conducted in peripheral nerves of waking human subjects.

# Purpose

Microneurography is used to record biological signals from peripheral nerves in awake, human subjects

# Scope

For use by REB members, the Ethics Office, and members of P. Millar’s laboratory.

# Responsibility

The PI holds an MSc and PhD in Cardiovascular Physiology and Postdoctoral training in Clinical Cardiovascular Physiology and Microneurography. It is anticipated that graduate students will be trained to perform microneurography following 1) reading the most recent SOP and scientific literature on the technique; 2) 1-2 months of observing the PI perform microneurography; 3) 1-2 months of practice externally locating the nerve using the stimulator pen; and 4) successful demonstration of appropriate and safe techniques during a trial run. At the onset, graduate students will be allotted 15-30 minutes to locate the nerve after which the PI will take over a ensure nerve site is located and the participant can continue with the study.

# Distribution of Copies

Ethics Website

# Procedure

1. Subjects will remain in an adjustable treatment chair or bed for the entirety of the microneurography procedure. They will be sitting upright or positioned in the supine posture depending on the study protocol.The nerve recorded from is the common peroneal (fibular) nerve (located at the side of the knee.
2. Subjects will be required to remove their shoes and socks and wear shorts or loose pants that will allow access to the leg until just above the knee.
3. The microneurography procedure begins with the use of surface stimulation.
	1. A stimulator "probe" with a tip the size of a pencil eraser will be used to stimulate the nerve through the skin (using electrode gel to reduce the impedance and make the search procedure more comfortable for the subject). The stimulus is generated by Grass Instruments S48 Square Pulse Simulator. An isolation unit (SIU5, Grass Instruments) is used to isolate the signal from ground and deliver a constant current stimulus to the subject.
	2. The stimulus will begin very low (30 V) and will be increased until the subject reports tingling from the skin or the experimenter begins to see muscle twitches. This stimulation is not uncomfortable and will feel like tingling or twitching in the leg of the subject.
	3. Once the investigator is certain of the location at which the biggest twitch is evoked with the smallest amount of current, the leg is marked with a washable pen to indicate the location at which the electrode will be inserted.
	4. The location of insertion is then thoroughly swabbed with alcohol for a few seconds and then allowed to air dry.
4. The next steps involve the insertion of the tungsten microelectrodes (FHC, USA). There will be two electrodes inserted, a reference and a recording electrode. Before being shipped, electrodes are sterilized using a STERRAD® Sterilization Systems, 100s procedure using hydrogen peroxide gas plasma. Prior to use with human participants, electrodes are individually packed in reference-active electrode pairs and sterilized a second time. The second round of sterilization occurs at the Toronto General Hospital, using a steam sterilization procedure following provincially developed standards and guidelines. Confirmation letter is provided at the end of this document.
	1. After swabbing the location of insertion with alcohol, a small sterilized electrode (low impedance-200 microns diameter, 20-30mm length) will be inserted just under the skin and then advanced inward 2-3mm. This electrode serves as the reference for the following stimulation and recording procedures. The insertion of the electrode may be accompanied with a sharp pin prick sensation. This is brief and dissipates after a second or two (as with a temporary pin prick).
	2. A second electrode which is also sterilized and is of higher electrical impedance is then inserted at the location indicated with the marker.
5. The experimenter will begin to **search for the nerve** by moving the electrode towards the nerve while relying on audio feedback of the neural signal as well as feedback from the subject. Depending on the depth of the nerve, the electrode could be advanced from <1 cm to 2.5 cm. Subjects may perceive mildly ‘sharp’ sensations as the electrode is advanced and withdrawn. These sensations are generally associated with tenting and dimpling of the skin around the insertion site. These sensations are mildly uncomfortable and only last for 1-2 seconds.
	1. When the electrode is near the nerve the subject will again feel muscle twitches or tingling (pins and needles). To ensure the correct nerve has been located, information from the subject on the location of tingling is used. The advancement of the electrode into the nerve may cause a spray, or sensation such as tingling.
	2. Once the investigator enters the nerve only very small manipulations (<1-5 mm) are made to isolatesympathetic efferent fibres directed towards skeletal muscle. The search for muscle sympathetic nerve is aided by an auditory signal of the neural activity, and/or by performing 3 common tests: mechanical flexion of the toes to induce afferent feedback; light stroking or touching of the skin to stimulate sympathetic skin fibres; and completion of a breath-hold known to increase muscle sympathetic activity.
6. In rare cases, the removal of the microelectrode is accompanied by a tiny drop of blood on the surface of the leg. In this case
	1. The insertion area is re-sterilized with alcohol
	2. If the experiment continues, the electrode is re-sterilized with alcohol and inserted in a slightly different location to avoid the capillary and to minimize subject discomfort.
7. Based on standard practices to reduce the risk of participant discomfort, a 60 minute time limit is imposed on internal searching for the nerve site. In addition, the same nerve will only be studied once per month (every 4 weeks). A subject/nerve will be used a maximum of 12 times in a year. There is currently no limitation on life-time participation.

# Wording for Consent Forms



Figure 1. The fibular (peroneal) nerve on the outside part of the knee.

To locate the fibular nerve, we will palpate the surface of the outside part of the knee. We will next touch a small stimulating pen to your leg and look for a muscle twitch. This will be a strange feeling as your leg will twitch on its own. We will mark with a pen the position of the nerve on your leg. Once the nerve is located, we will then proceed to place the microelectrode into the nerve. Proper placement in the nerve will be determined by listening for specific sounds while we move the microelectrode. Placement may take up to 60 minutes. After this is completed, a 10 minute rest period will be completed (with the upper arm blood pressure cuff).

Risk: *Microneurography*: The microneurography technique is used in research to directly measure nerve activity. The sterilized microelectrode will be inserted into one your fibular (peroneal) nerve on the outside part of your knee (see Figure 1). The microelectrode is very tiny in diameter and will very rarely draw blood upon insertion. During the placement of the electrode, there may be tingling sensations in parts of the leg. This is normal and will subside once we stop moving the electrode. In order to find the best recording site, we will move the electrode to different sites in the nerve. This movement may cause mechanical microdamage to the nerve and surrounding tissue. The damage is minor and nerves in the periphery are able to repair themselves. There are no reported cases of permanent nerve injury as a result of microneurography. In addition, the principle investigator, Dr. Philip Millar, has 4 years’ experience (in over 200 people) with this procedure with no complications. Dr. Philip Millar will be extensively training and supervising student investigator, Anthony Incognito, in this procedure to ensure that it is conducted in a safe and effective manner. To minimize risk, we will limit the time of search for the nerve fibre (electrode manipulation) to 1 hour. If the nerve is not found by this time then the experiment will be end and we will invite you back to complete the experiment at a later date. If the nerve is found within an hour then the remainder of the visit will continue, taking approximately 2 additional hours. To further reduce risk, we do not record from the same nerve more than once a month.

# Wording for Website

n/a

# Documentation/Record Keeping

Click on here to describe documentation used in this procedure, or record keeping requirements

# External Regulatory Requirements

Click on here and describe any regulatory requirements applicable to SOP

# Internal Related, or Referenced Policies, Procedures

Click on here to list in-house procedures, other SOP's, manuals referenced in document; provide locations of materials

# References

Eckberg DL et al. *Acta Physiol Scand.* 1989; 137: 567-569.

# Revision History

| Revision # | Reviewer | Reason | Date Last Reviewed | Next Review Date |
| --- | --- | --- | --- | --- |
| 1.0 |  |  |  |  |

# Review Cycle

Annual

# Appendix

Click on here to enter sources of documents used to write the procedure