

Webinar Q&A Report:

Microneurography: Recording Nerve Traffic Via Intraneural Microelectrodes in Awake Human Subjects

Q: Is it possible to record from a population of afferent fibres simultaneously?

Yes, one can record from a population of, say, muscle spindle afferents at the same time. You will know which muscle fascicle you are in by the twitch responses to intraneural electrical stimulation and/or the afferent responses to muscle stretch. The same is true for a cutaneous fascicle, but one usually wants to record from a single, type-identified, afferent. A multi-unit recording is easier than a single-unit recording, of course, which is why most researchers studying sympathetic nerve activity record multi-unit activity.

Q: When quantifying MSNA as the number of spikes/cardiac cycles, if the microelectrode moves in such a manner that the baseline of the integrated signal changes, are the number of spikes/cardiac cycle still a comparable measure before and after the movement of the microelectrode?

This depends on what is causing the baseline shift. If the electrode tip has moved then, no, you cannot compare recordings before and after the baseline shift. However, if the baseline shift is temporary, due to infiltration of EMG or afferent activity, then one can compare signals before and after (and during, if one uses spike extraction to discriminate the spikes of MSNA).

Q: Should we quantify and report MSNA site quality (i.e. signal to noise ratio)? Can this influence MSNA burst frequency change within an individual between visits?

Generally, one doesn't report signal-noise ratio, but pragmatically it makes sense to have an optimal recording. If you imagine a burst of MSNA as being like an iceberg floating in the sea, the more that is exposed the easier to quantify amplitude. Counting bursts is facilitated by converting the nerve signal to a mean-voltage neurogram (integrated or root-mean-square processed). You cannot compare burst amplitudes in the same subject between recording sessions, because the amplitude depends on the proximity of the microelectrode tip to the active sympathetic axons. This is why most investigators report the burst incidence (bursts per 100 heart beats) and burst frequency (bursts per minute), which is very stable over time for a given individual.

Q: Sometimes MSNA sites fade/amplitude changes over time without obvious sounds of a site change. Is there a way to take this into consideration when analysing the neurogram?

Yes, an abrupt site change is usually indicated by an artifact and can be readily detected, but some site changes are slower and more sinister. If you performed a standard manoeuvre (e.g. a maximal inspiratory breath-hold or end-expiratory breath-hold) at the beginning and end of the recording you would know, and could correct for a slight site change by dividing the burst amplitude by the amplitude of the largest burst recorded in each manoeuvre.

Q: Do we need to relocate the coupled areas for different subjects?

If you mean ensuring that the same fascicle (e.g. that supplying tibialis anterior or extensor digitorum longus) is recorded from across subjects then the answer is no: MSNA is the same regardless of the fascicle you record from, and similar across nerves on either side of the body.

Q: How does the NeuroAmpEX compare to concentric or needle EMGs? Are the differences mainly in SNR?

The NeuroAmp EX is designed for use with monopolar metal microelectrodes, which are insulated down to the tip; the reference electrode is a separate monopolar electrode inserted into inert tissue (i.e. the skin) nearby. A concentric needle, by definition, comprises a central active electrode separated from the surrounding sheath by insulation; the outer electrode serves as the reference. Some intramuscular needle electrodes use two (or more) central electrodes. The primary difference lies in the electrode material. Microneurography needles are made of tungsten, rather than stainless steel, because it is tougher, allowing it to penetrate the tough nerve sheath. As for signal-to-noise ratio, the two types of recordings (monopolar or bipolar) can be similar.

Q: Is the software bundled with NeuroAmp EX? What is that software called?

The Neuro Amp EX connects to a PC or Mac via an ADInstruments [PowerLab](#) Data Acquisition System. All PowerLabs ship with [LabChart](#) software. Purchasing the 'Pro' version of LabChart unlocks the specialised [Spike sorting](#) and Peak Analysis components of the software.

Q: What are the benefits of using microelectrodes while simultaneously recording fMRI?

This is a niche development that allows one to use the recorded bursts of sympathetic nerve activity to identify areas in the brain responsible for their generation.

Q: Can we use surface electrodes?

ADInstruments offers a range of [Bio-Amplifiers](#) that can be used to take surface bio-potential measurements of, for example, EMG or skin potential. However, these signals are much larger than those generated by nerve fibres (which are also deeper), which is why microneurography was developed: to directly tap into the nerve signals through intraneural metal microelectrodes. And, because the nerve signals are so small they need to be amplified close to the recording site, via the headstage (pre-amplifier). The NeuroAmp EX headstage pre-amplifies the nerve signal 100 x, which is then further amplified in the NeuroAmp EX amplifier.

Q: I use an A-M 1700 amplifier to record from dorsal roots in rats. I don't use a headstage presently but am not getting the best signals. SNR is typically around 1.5 or so. The A-M amplifier has a driven ground option. Have you used this in your recordings or is your reference the same as earth ground?

The reference electrode used in microneurography is passive, not driven, and the amplifier has differential inputs. The reference electrode is inserted a few mm into the skin, and is basically connected to the subject ground (surface electrode on the subject's skin), not to any equipment (or chassis) ground. By having the reference and active electrodes as close to each other as possible (~1 cm) means that the common-mode rejection will be higher, thereby limiting interference from signals common to both electrodes (e.g. EMG); we also use the Spike Histogram software in LabChart to extract nerve spikes from the nerve recording and thereby isolate the neural signal from any EMG interference. The A-M amplifier does not have a headstage so your noise will be higher: when you need a high signal-to-noise ratio it is important that the recording leads are as short as possible. This is why a headstage (preamplifier) is used. The NeuroAmp EX headstage has a gain of 100, so the signal is already amplified before it travels through the cables to the amplifier. If you want to get better recordings you should use a headstage. For example, you could connect hook wires directly to the NeuroAmpEX headstage and get great recordings, because you will be pre amplifying the signal.

Q: Where is your reference electrode going in the 3rd or 4th slide where you show the tungsten needles penetrating the subject's leg?

The reference electrode, which has a larger exposed recording surface (and hence a lower impedance) is inserted into inert tissue as close as possible to the active electrode; in practice, it is inserted a few mm into the skin about 1 cm away. A surface Ag/AgCl electrode, connected to the green lead, was placed on the subject's knee and serves as the ground electrode.

Q: What is the fuzz of positive spikes that permeate the recording in the GVS slide?

Ah, that was a concurrently recorded muscle spindle afferent, firing spontaneously. Note that myelinated axons generate positive-going spikes, while unmyelinated axons generate negative-going spikes.

Q: Approximately what is the area of your exposed electrode tips?

Microelectrode needles are electrolytically sharpened, with the highest impedance electrodes (~10 MOhm) typically having an exposed surface of a few microns. Further details can be obtained from a company that makes these electrodes, such as [FHC](#).

Q: What impedance are you using for the needle?

This depends on whether it is a multi-unit or a single-unit recording. For multi-unit recordings of sympathetic nerve activity, the impedance typically ranges from 50-150 KOhm. For single unit recordings from myelinated axons, the impedance is typically >300 KOhm, with impedances of 2-5 MOhms being typically used for single-unit recordings from C-fibres.

Q: Is this a differential amplifier and what amplifier settings are you using (filter settings and gain)?

Yes, the Neuro Amp EX is a differential amplifier. It offers total gains as high as 500,000X. There is 100x Gain in the headstage with the rest taking place in the amplifier and PowerLab. It also has hardware low and high pass filters built in which can be controlled in the software. 1, 2 and 5 kHz low-pass filters are

available as are 100, 300 and 500 Hz high pass filters. The LabChart software also provides software filters that can be set to any frequency post-recording. Most recordings in this webinar were recorded using the 50uV range (gain 20,000 X) with a 5 kHz low-pass filter and a 300 Hz high-pass filter.

Q: What is the bin size (msec) of the cross-correlation histogram?

Bin size for all the different types of histograms are fully configurable in the LabChart Software. Mostly I use 50 ms bins, occasionally 20 ms.

Q: Is spike software available for LabChart Mac?

Yes, the Spike Histogram module is only available on LabChart 7 for Mac, but has not yet been implemented in LabChart 8. The version of Spike Histogram on LabChart 7 for Mac is also missing a number of features when compared to the Windows version of the software. Using LabChart 8 on Windows gives the best experience, as it has template matching.

Q: How would one connect an internal stimulation to the micro setup?

The outputs from an isolated stimulator (such as the FE180 Stimulus Isolator, which is certified for human connection) can be connected directly to the leads going to the input terminal pin of the recording electrodes, via a mini-alligator clip attached directly to the input pin. Note, this should be the black (cathodal) lead. The anode can simply be attached to a surface Ag/AgCl electrode on the skin. The headstage of the NeuroAmp EX was designed with input protection circuitry, meaning it will not be damaged by currents up to 1 mA (0.2 ms pulses).

Q: Can you record and stimulate one nerve simultaneously? Does the software provide any signal processing option to separate stimulus signal from recording signal?

No, because the preamplifier would require a relay to disconnect the stimulating leads from the preamplifier during stimulation. Leaving the stimulating leads connected means they act as long antennae, bringing in too much noise.

Q: How would you recommend training on determining path of nerves and placement of micro-electrodes?

Microneurography is a highly skilled and invasive technique that requires 1-2 months of training in a laboratory that uses it routinely. Institutional Review Boards (Human Research Ethics Committees) will require evidence of proficiency before granting approval.

Q: Is it possible for micro-stimulation to evoke sensory or physiological responses?

Yes, indeed. Microstimulation can be used to selectively stimulate one sensory axon (which may or may not generate a percept, depending on the type of afferent) or motor axon (which will generate a uniform EMG and twitch response). Microstimulation requires careful titration of the current, and fine manipulation of the microelectrode, to produce a uniform response at the lowest current.

Q: What has been your experience on recording proprioceptive signals? In patients with an amputation?

It is straightforward to record from muscle spindle endings, but I have not attempted to do this from amputees. While it would be possible to record from the nerve proximal to the amputation, it would be very difficult to identify an afferent, given the lack of parent tissue which the axon supplies. Nevertheless, it would be possible to microstimulate within a muscle or cutaneous fascicle to study somatosensory reorganization.

Q: My team is working on nerve signal acquisition at the surface of the skin ultimately to create a device that will be able to detect and create a 3D model of muscle activity of the entire hand. We were thinking of using the microneurography technique to help us get a better sense of the range of nerve signals we might get at the surface of the skin. Can you help us get a better sense of the metrics we should be testing for using microneurography given our goal? For example, should we focus on single or multi-unit recordings, how does MSNA relate, and should which metrics are most relevant in a histogram (GVS, RMS EMG, EMG, ECG, etc.)?

This will be very difficult using surface electrodes, because the nerve signals are so small, but if you are interested in motor activity then intramuscular wire electrodes would be ideal for this purpose, as you can compare the activity from small areas within specific muscles with the surface-recorded EMG.

Q: How many re-insertions during microneurography are acceptable?

The more you reinsert a microelectrode through the skin the more insulation you lose from the recording tip and hence the lower the impedance the electrode becomes, reducing its selectivity. We try not to remove the microelectrode from the skin, but will often explore several fascicles in a given experiment. Naturally, this too may reduce the impedance, but usually not as much as inserting through the tough skin. It has also been shown that the number of post-experiment signs (related to slight swelling within the nerve) is lower when one limits the amount of searching to one hour.

Q: How we can set the threshold (noise floor value) in spike histogram software?

The settings dialogue found under the 'Spike Histogram' menu in LabChart allows you to set a noise threshold. Typically a user will have a brief scroll through their data, read off an appropriate value from the Chart view. This is typically set at, say, 8-10 μV – just above the noise level. This can then be adjusted using the Discriminator settings.

Q: Is it possible to do frequency analysis in multiunit recording?

It is possible to use the Spike Histogram software to differentiate between multiple units in the discriminator and then analyse each unit individually. All the analysis features shown work similarly for both single and multi-unit recordings. In addition, spectral analysis can be performed on a multi-unit nerve signal, but – depending on the type of recording – it would usually be better to analyse the RMS-processed nerve signal.

If you have additional questions for [ADInstruments](#) or Professor Macefield regarding content from their webinar or wish to receive additional information about solutions for microneurography research, please contact them by email:

Technical Support – ADInstruments

support@adinstruments.com

Prof. Vaughan Macefield – Professor, Physiology

Mohammed Bin Rashid University of Medicine and Health Sciences

vaughan.macefield@mbru.ac.ae



ADInstruments – North America

2205 Executive Circle
Colorado Springs,
Colorado, 80906
USA

Tel: +1 719 576 3970

ADInstruments – Europe

Unit B, Bishops Mews,
Transport Way
Oxford, OX4 6HD
UNITED KINGDOM

Tel: +44 1865 332 050

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