Webinar Q&A Report:

Utilizing Nerve Cuff Electrodes for in vivo Peripheral Nerve Research

1. Is the de-sheathing of the nerve necessary for electrostimulation?

   T. Tsaava: The removal of the layer of epineurium of peripheral nerves (i.e. desheathing) significantly improves the signal-to-noise ratio of electrically recorded compound action potentials. During the electrical stimulation of peripheral nerves, desheathing may affect stimulation thresholds, where thresholds can be lower in a deasheathed nerve compared to non-desheathed one. In our practice we have not used desheathing method for stimulation experiments.

   C. Langdale: No, I do not feel it is not necessary. Cleaning connective tissue around the nerve will help decrease impedance and potentially decrease current needed for stimulation. If it is not clean (i.e. connective tissue etc...), you may need to use higher amplitudes.

2. One graph showed that VN electrostimulation per se increases cytokine production? Is it always the case?

   T. Tsaava: Vagus nerve stimulation decreases serum TNFα levels in a murine endotoxemia model. The graph showed the effects of the VNS using specific pulse parameters in naïve mice. We observed increase in cytokine levels in naïve mice at specific amplitudes and frequencies of the applied electrical pulse.

3. Do you spray/squirt KCl on top of the vagus nerve and/or electrode cuff?

   T. Tsaava: KCL is applied to the nerve directly without flooding the electrode to avoid shunting.

4. Once the vagus nerve is separated from the carotid, how was the nerve treated? Did you say that it was de-sheathed? How extensively does the nerve to be cleared of connective tissue?

   T. Tsaava: Once the vagus nerve is separated from the carotid, a thin layer of connective tissue is removed. Desheathing should be performed carefully using fine forceps so that the fibers of the nerve are not damaged.

5. For the cuff on the vagus nerve, what lead orientation do you use--parallel or perpendicular to the long axis of the cuff?

   T. Tsaava: For the electrical stimulation and recording experiments we use AirRay Miro Cuff Sling electrode with the cable entry on the top side. The lead orientation is perpendicular to the nerve.

6. Do you use commercial silicone-coated forceps, or do you coat your own? How thick is the silicone coating?

   C. Langdale: No, I do not use silicone treated forceps. I use inox 5/45 and number 5 superfine when handling. It is sharp, so great care must be taken when handling cuffs. I have not found suitable silicone forceps that are small enough to use with the 300 uM and smaller diameter cuffs.

7. Do you use computational modelling to optimize/design electrodes? Like Electromagnetic-Coupled simulations?

   M. Schüttler: We have done this internally in the past and plan to offer this as a service to customers in the future.

8. How can be the time-dependent changes in the tissue impedance be modelled?

   M. Schüttler: We recommend expecting an increase of the tissue impedance by a factor of up to 3 within the first ~ 10 days after implantation, followed by a slow drop, perhaps stabilizing at twice the initial value. This should be considered e.g. for the specifications of stimulator electronics that need to be able to generate higher voltages than initially calculated to drive a desired stimulation current.

9. How can you predict the effects of inter-subject variability of tissue parameters and nerve structures on stimulation parameters?

   M. Schüttler: Expect a subject-individual variability of diameters of +/- 50%. The thicker the nerve is, the higher stimulation impulse it requires. Make sure that your stimulator system has enough power to adapt to varying stimulation parameters.

10. what is your control group when using microelectrode stimulation e.g. your TNF levels w/o stimulation how they were achieved? Just opening and not manipulating vagus?

    T. Tsaava: In stimulation experiments the control group undergoes the same surgical procedures, except the vagus nerve manipulation. TNF levels in unmanipulated animals in normal state are negligible.

11. Was testing of the nerve cuff done in rats?

    C. Langdale: Yes, I tested my cuffs in rats.

12. If 3 electrodes are placed along the nerve, wouldn't they all record the same data, but with a slight delay from e1 to e2 to e3?

    M. Schüttler: That's correct for the case that all contacts are amplified against the same reference electrode. The 'trick' of using 3 equally spaced electrode contacts within a cuff is based on double-differential amplification of the three contacts which helps rejecting artifacts originating from outside the cuff (e.g. electrical muscle activity). So-called true-tripolar amplification is done by amplification of the voltage across contact e1 and e2 (calling the result: d1) and amplification at same gain of the voltage across contact e2 and e3 (calling the result: d2).

    Amplifying the voltage across d1 and d2 results in a single signal with dramatically reduced artifact content.

    Similar artifact reduction can be achieved with a quasi-tripolar amplification, which only requires one differential amplifier: The two outer contacts e1 and 3 of the cuff are electrically shorted and amplified against e2.
    Critical for the success of tripolar amplification is a cuff electrode with a closure mechanism that provides an excellent electrical seal against the environment along the length of the cuff.

13. We have observed some mechanical effect after vague nerve manipulation in mice, such as loss of size. How to avoid the mechanical effects of electrode implantation?

    T. Tsaava: Our acute electrical stimulation or recording experiments are less than one hour long. During the experiment the exposed nerve area can be covered with a small piece of parafilm to avoid drying and subsequent shrinkage of the nerve.

14. Are these single cuff electrodes of a single use?

    T. Tsaava: AirRay Miro Cuff Sling Electrodes if properly maintained can be reusable.

    M. Schüttler: Cuffs used in acute experiments can be re-used after careful washing and disinfection. We do not recommend using re-used cuffs in chronic experiments.

15. I've been told that the nerve should not be touched with a metal tool, is this true? What kind of tools do you use to isolate the nerve and place the cuff?

    T. Tsaava: In our practice, metal tools are used to manipulate nerves. When isolating peripheral nerves in vivo the speed and precision of the surgery is important.

16. What is the highest electrode cuff that CorTec currently manufacture and is there a capability to make cuffs to order with different electrode patterns or spacings?

    M. Schüttler: So far, we have built cuffs that fit nerves in the diameter range of 50 micrometers to 9 millimeters, carrying between two and 16 contacts. We are more than happy to extend this range according to customer requirements!

17. What is a good alternative to 0.9% saline?

T. Tsaava: 1X PBS

M. Schüttler: 0.9% saline is very often used for measurement of impedance etc. in-vitro at room temperature. However, this concentration of saline provides a slightly to high conductivity compared to the situation in-vivo. We found a better match for peripheral nerve cuffs using 0.36% saline when measured at 21°C, corresponding to 0.26% saline when used at 37°C.

18. What is everyone's experience on recording or stimulation shunting due to liquid build up at the implantation site? How can we limit it? What are some strategies for acute/chronic preparation to avoid shunting?

T. Tsaava: In acute experiments the liquid build-up at the electrode site is a common occurrence. To avoid shunting the excess liquid is periodically drained using delicate blotting wipes (e.g. Kimwipe), without touching the nerve.

19. What is a good cuff electrode length?

T. Tsaava: In mouse acute recording and stimulation experiments we use AirRay Micro Cuff Sling electrode of 2mm length.

M. Schüttler: Typically, the length of the cuff is typically restricted by the available space at the implantation site.

For bipolar recording, two contacts inside the cuff should ideally be spaced matching the spatial distribution of a propagating action potential. The wavelength of an action potential travelling along a 10 micrometers fiber is about 20-30 millimeters and increases/decreases approximately linear with the fiber diameter. Typically, there is not enough space for cuffs this length and one must use shorter contact distances that will result in reduced signal amplitude.

For bipolar stimulation, the ideal contact distance, and hence: cuff length, depends on many factors such as cuff diameter, nerve fiber diameter, etc. As a guideline, 2-3 mm contact distance will give lowest stim threshold, beyond, threshold is not reduced. Very large diameter cuffs might require larger contact distances.

20. What is a good cuff contact size?

T. Tsaava: In mouse acute recording and stimulation experiments we use AiRay Micro Cuff Sling electrode with 100um and 200um inner diameter.

M. Schüttler: For recording: The larger the contact, the lower the noise originating from the electrode. For stimulation: The larger the contact, the higher is the electrical charge (current amplitude x pulse width) that can safely be injected through the electrode into the tissue.

21. How do I know whether or not I have damaged a nerve during implantation?

    M. Schüttler: A changed electrophysiology can indicate that you have damaged the nerve

22. With all the different choices to customize cuffs, how do you choose which one?

    C. Langdale: It is, unfortunately, trial and error. I first perform dissections of the nerve of interest and determine what the diameter of the nerve is. I then determine how much nerve I can realistically isolate, which will help determine the width of my cuff. For example, if I estimate that my nerve is 275 uM in diameter and I can expose 3 mm of the nerve, then I will most likely focus on 300 uM cuff designs that are 2 mm in width. The lead exit from the cuff will be determined by the orientation. I am sure CorTec representatives can help narrow down a few choices for you once you have some basic knowledge of the nerve of interest.

23. How does the impedance change after use the same cuff several times?

    T. Tsaava: With proper maintenance of the electrode (i.e. rinsing in normal saline after each experiment, dipping in 3% hydrogen peroxide, checking for mechanical damage, etc.) the impedance should not change too much over a long period of time.

24. What is the signal-to-noise ratio if you don't de-sheath the nerve?

    T. Tsaava: As mentioned above, desheathing significantly improves the signal-to-noise ratio of electrically recorded compound action potentials. In mouse acute electrical recording experiments with un-desheathed nerve we have observed significant reduction in the compound action potential counts.

25. Do the platinum-iridium leads get oxidized?

    M. Schüttler: During production, CorTec does not intentionally oxidize electrode contacts, however, when using platinum-iridium as recording electrode material, the surface is expected to be oxidized by exposure to oxygen during storage and implantation.

    When using platinum-iridium as stimulation electrode material, the predominant mechanism of electrical charge transfer is oxidizing and reducing the surface of the contacts.

If you have additional questions for CorTec regarding content from their webinar or wish to receive additional information about their products and laboratory services, please contact them by email or phone.

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