Preparation of Rat Sciatic Nerve for *Ex Vivo* Neurophysiology

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Abstract

Ex vivo preparations enable the study of many neurophysiological processes in isolation from the rest of the body while preserving local tissue structure. This work describes the preparation of rat sciatic nerves for *ex vivo* neurophysiology, including buffer preparation, animal procedures, equipment setup and neurophysiological recording. This work provides an overview of the different types of experiments possible with this method. The outlined method aims to provide 6 h of stimulation and recording on extracted peripheral nerve tissue in tightly controlled conditions for optimal consistency in results. Results obtained using this method are A-fibre compound action potentials (CAP) with peak-to-peak amplitudes in the millivolt range over the entire duration of the experiment. CAP amplitudes and shapes are consistent and reliable, making them useful to test and compare new electrodes to existing models, or the effects of interventions on the tissue, such as the use of chemicals, surgical alterations, or neuromodulatory stimulation techniques. Both conventional commercially available cuff electrodes with platinum-iridium contacts and custom-made conductive elastomer electrodes were tested and gave similar results in terms of nerve stimulus strength-duration response.

Introduction

The current understanding of fundamental nerve function as modeled *in silico* is lacking in several aspects, notably with respect to the effects of nerve tissue compartmentalization outside of the soma, axon, and dendrites. Axon-myelin interactions are still poorly

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A complete version of this article that includes the video component is available at http://dx.doi.org/10.3791/63838.

Disclosures
The authors have no conflicts of interest.

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understood as evidenced by the fact that even detailed computational nerve models such as MRG[1] (for mammalian nerves) that adequately capture conventional electrical stimulation response, do not capture other experimentally observed behaviors such as high-frequency block carryover[2] or secondary onset response[3].

This protocol provides a method to efficiently investigate neurophysiological processes at the nerve level in an acute small laboratory animal model, using a standardized preparation protocol to isolate the nerve, control its environment, and remove it from an *in vivo* context to an *ex vivo* context. This will prevent other body processes or anesthetics used by *in vivo* nerve stimulation protocols to alter nerve behavior and confound measured results or their interpretation[4, 5]. This enables the development of more realistic models focusing solely on effects specific to nerve tissues that are poorly understood. This protocol is also useful as a testbed for new nerve stimulation and recording electrode materials and geometries, as well as new stimulation paradigms such as high-frequency block[2, 3]. Variations of this technique have been used previously to study nerve physiology in tightly controlled conditions[6], for example, to measure ion channel dynamics and properties or the effects of local anesthetics[7].

This technique provides several advantages compared to alternatives such as acute *in vivo* small animal experimentation[8]. The technique obviates the need to maintain anesthesia depth as the tissue has been extracted from the body, reducing the amount of required equipment such as an anesthetic diffuser, oxygen concentrator, and heating pad. This simplifies the experimental protocol, reducing the risk of mistakes. As anesthetics can potentially alter nerve function[4], this technique ensures that measures will not be confounded by side effects from these anesthetic compounds. Finally, this technique is more appropriate than acute *in vivo* experiments when studying the effects of neurotoxic compounds such as tetrodotoxin, which would kill an anesthetized animal by paralysis.

Peripheral nerve sections are a unique *ex vivo* system since there is a high chance that the fibers responsible for recorded neural signals do not contain any soma. As these would normally be located, for motor neurons, in the spine, and for sensory neurons in the dorsal root ganglia next to the spine, the preparation of a section of the mammalian nerve can be roughly modeled as a collection of tubular membranes with ion channels, open at both ends[9]. Metabolism is maintained by the mitochondria located in the axon at the time of tissue dissection[10]. Suturing of the open ends of the axolemma is encouraged after extraction to close them and thereby help maintain existing ionic gradients across the membrane, which are essential for normal nerve function.

To maintain tissue homeostasis outside the body, several environmental variables must be tightly controlled. These are temperature[11], oxygenation[12], osmolarity, pH[13, 14], and access to glucose to maintain metabolism. For this protocol, the approach is to use a modified Krebs-Henseleit buffer[15, 16] (mKHB) continuously aerated with a mixture of oxygen and carbon dioxide. The mKHB is in the family of cardioplegic buffers[6, 17] used to preserve dissected tissues outside of the body, for example, in *ex vivo* experiments. These buffers do not contain any hemoglobin, antibiotics, or antifungals and are, therefore, only suitable for preparations involving small amounts of tissue for a limited time. pH control was achieved with the carbonate and carbon dioxide redox pair, requiring constant aeration of the buffer

with carbon dioxide to maintain pH equilibrium. This is to avoid using other common buffering agents such as HEPES, which can modify nerve cell function[18]. To oxygenate the buffer and provide pH control, a mixture of 5% carbon dioxide in oxygen called carbogen (95% O_2, 5% CO_2) was used. A heating stirrer was used for temperature control of a buffer container, and the buffer was perfused through a nerve bath, and then recirculated to the starting container. A typical experiment would last 6-8 h before the nerve loses its viability and no longer responds sufficiently to stimulation for measures to be representative of healthy tissue.

To optimize the signal-to-noise ratio, silver-chloride electrodes were used for recording, which were prepared according to previously described methods[19]. For stimulation, a combination of commercial off-the-shelf platinum cuff electrodes and custom-made conductive polymer cuff electrodes can be used. Conductive polymer cuff electrodes have notably higher charge capacities, which are useful when stimulating the nerve using high amplitude waveforms[20].

The stimulator used in this protocol has been previously described[20]. Documentation, design files, and software scripts to use it are publicly available[21]. Other stimulators can be used to execute this protocol; however, the custom stimulator is also capable of high-frequency alternative current (HFAC) block[2, 20], which enables a wider range of neurophysiology experiments. To use HFAC block, conductive elastomer cuffs are recommended to avoid damage to the nerve. Conductive elastomer nerve cuffs are soft and fully polymeric electrode arrays produced from conductive elastomers as the conductive component and polydimethylsiloxane as the insulation[22]. Devices were manufactured in a bipolar configuration using conventional laser microfabrication techniques.

Protocol

All animal care and procedures were performed under appropriate licenses issued by the UK Home office under the Animals (Scientific Procedures) Act (1986) and were approved by the Animal Welfare and Ethical Review Board of Imperial College London.

1. Preparation of buffers

NOTE: This part of the protocol can be carried out well in advance of the rest of the protocol, except for the final steps involving the preparation of modified Krebs-Henseleit Buffer (mKHB) at 1x concentration.

1. Prepare 1 M $CaCl^2$ stock solution
    1. Add 14.701 g of $CaCl^2$ dihydrate to a clean 100 mL beaker. Add ~75 mL of deionized water and stir until complete dissolution of the salt.
    2. Transfer the solution to a 100 mL graduated flask and add deionized water until 100 mL volume has been reached. Transfer the solution to a bottle and store it in a refrigerator at 4 °C.
2. Prepare 10x concentrated mKHB stock

1. Add 66.03 g of sodium chloride (NaCl), 3.57 g of potassium chloride (KCl), 1.63 g of potassium dihydrogen phosphate (KH_2PO_4), and 1.44 g of magnesium sulfate to a 2 L beaker.
2. Add ~750 mL of deionized water to the beaker and stir until the salts have dissolved (there may be small salt crystals left at the bottom). Add 25 mL of 1 M $CaCl^2$ stock solution (step 1.1) and stir; ensure this is the last salt added.
3. Transfer the solution to a 1 L graduated flask and add deionized water to reach a total volume of 1 L. Transfer the solution to a 1 L bottle. Store concentrated 10x mKHB at 4 °C in a refrigerator. NOTE: Concentrated mKHB stock can be stored for approximately 1 month before replacement.

3. Prepare dissection Petri dish (coating)
    1. Prepare a clean glass Petri dish (120 mm diameter) for coating by washing and drying the dish carefully.
    2. Following the manufacturer's instructions for usage and curing of the conformal alkoxy coating, coat the bottom of the Petri Dish with ~3-5 mm of coating by carefully pouring the conformal coating mixture into the dish until the desired thickness has been reached.
    3. Cure the coating in an oven at 60 °C until it is firm to the touch. The dissection Petri dish is now ready. NOTE: Gentle cleaning of the dish after each use will ensure that the coating lasts for years before replacement is needed. When replacing the coating, ensure to remove all the coating material before applying a new coating. Note that the conformal alkoxy coating (Table of Materials) used in this protocol has a shelf life shorter than a year.

2. Pre-dissection preparations

NOTE: This step starts the experiment. The below steps must be carried out on the same day, in this order.

1. Prepare 1x mKHB
    1. Prepare a clean 2 L beaker for the buffer. Transfer 200 mL of 10x mKHB stock to the 2 L beaker. Add 2.1 g of sodium carbonate ($NaHCO_3$) and 0.99 g of anhydrous Dextrose (D-glucose) to the 2 L beaker.
    2. Add approximately 1 L of deionized water to the beaker. Stir until salts have been completely dissolved. Transfer the solution to a 2 L graduated flask and add deionized water to reach a total volume of 2 L.
    3. Transfer the solution to a 2 L glass bottle placed on a heating stirrer with the temperature set to 37 °C. NOTE: Smaller heating stirrer will often be unable to reach the target 37 °C temperature due to the thermal

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inertia of large containers full of water. Adjust the temperature upward so the contents of the bottle reach 37 °C with stirring. Monitor the temperature during the experiment and lower the set temperature in the event of overshoot.

4. Place a thermometer in the 2 L bottle to monitor the temperature, using grippers to prevent the thermometer from being impacted by the stirring flea. Aerate the buffer with carbogen for a minimum of 30 min to oxygenate the solution. This should set the pH to 7.4. Measure the pH with a pH meter to ensure it is within 0.1 pH units of 7.4 (at 37 °C). NOTE: To adjust the pH to 7.4, use hydrochloric acid or sodium hydroxide when needed.

5. Fill two 15 mL centrifuge tubes and one 100 mL bottle with mKHB and place them on ice to cool. NOTE: The centrifuge tubes and the bottle can be cleaned and reused after each experiment without autoclaving.

6. For later parts of the experiment, continue to aerate the buffer in the 2 L bottle with carbogen at 37 °C with continuous stirring.

   NOTE: Unsupervised use of carbogen may be dangerous if no automatic systems are in place to shut off gas flow in the event of a leak. An automated sensor and electronic shutoff valve can mitigate this; otherwise, a person must be left to supervise the equipment if dissection takes place in a different room. In all cases, an oxygen sensor should be used near the setup to warn operators when ambient oxygen concentration rises above 25%. Use a room with active ventilation if possible.

2. Turn on the signal acquisition device, low-noise preamplifier, line noise filter, and oscilloscope for recording and stimulation, to allow enough time for temperature stabilization.

3. Ensure all electrical recording equipment is correctly configured

   1. Set the low-noise preamplifier to AC-coupled input with the input band-pass filter set to 6 dB roll-off per decade, and cutoff frequencies set to 30 Hz and 3 kHz for the high-pass and low-pass filters, respectively.

   2. Set the gain of the low-noise preamplifier to 100.

3. Animal anesthesia and euthanization

NOTE: Female rats between 250 and 330 g (**Table of Materials**) were used for the studies.

1. Prepare surgical tools and consumables: 12 cm straight scissors (blunt); 2 mm cutting edge angled spring scissors; 4 cm fine scissors, sharp or semi-sharp; #7 Dumont forceps; 45° angled fine forceps and 6-0 suturing silk or thread.

2. Place the rat in an anaesthetization container or chamber. Connect oxygen and the anesthetic diffuser to the container. Set anesthetic (isoflurane) concentration

to 3.5% and wait approximately 10 min or until the animal shows signs of anesthesia such as loss of righting reflex.

3. After the rat shows signs of anesthesia, confirm the loss of consciousness with a toe pinch withdrawal reflex test. Proceed only when there is no toe withdrawal; otherwise, check all the connections and anesthetic levels in the diffuser and repeat step 3.2.

4. Take the animal out of the container and proceed with cervical dislocation, followed by incision of a femoral artery for confirmation of death.

4. Dissection protocol

NOTE: Place the animal with its belly down on the dissection table. Repeat the following steps for both legs. Typically, the right leg is dissected first.

1. Holding the ankle firmly between the thumb, index finger, and middle finger, sever the calcaneal tendon using 12 cm straight blunt scissors.

2. With fine sharp scissors, make a skin incision from the calcaneal tendon along the back of the leg all the way up to the base of the spine, taking care not to dissect the muscle tissue below.

3. Using fine forceps and fine scissors, make careful incisions through the muscle layers near the middle of the back of the leg until the sciatic nerve is exposed. As soon as the sciatic nerve is visible, moisturize the cavity using ice-cold mKHB to prevent the nerve from drying out.

4. Using hemostats, pull the flaps of skin on each side apart, and maintain the incision open for finer dissection work. Starting from the location of the calcaneal tendon incision, with fine scissors, interrupt the muscle on the medial side of the leg to free the nerve. Continue to maintain moisture levels in the area with ice-cold mKHB.

5. As the nerve is exposed while moving up the leg, dissect the overlying muscle tissue. Free the nerve nearer to the spine from connective tissues until reaching the spinal cleft at which point there is a kink in the nerve. Do not attempt to clean the nerve yet as speed is essential at this stage.

6. Sever the nerve as close to the spine as possible with fine scissors. To make the dissection easier, very gently pull the end of the nerve near the ankle using forceps. Never pinch the nerve in the middle, but only the ends. Never pull a nerve taut.

   OPTIONAL: At this point, if time allows, suturing of both ends of the nerve can be carried out to help maintain viability. Keep handling to a minimum to prevent damage. If there is insufficient time (see step 4.5), skip this step and follow step 5.2 later on.

7. Place the dissected nerve in the 15 mL centrifuge tube filled with mKHB (step 2.1.5), close the tube, and place the tube back on ice until the start of the cleaning procedure.

8. Repeat steps 4.1-4.7 for the other leg. Ideally, each nerve should take 5-10 min to extract, in order to maximize tissue viability.

5. Nerve cleaning procedure

1. Fill the coated Petri dish approximately halfway with chilled oxygenated mKHB. Place one of the dissected sciatic nerves in the dish and pin both ends of the nerve to the dish such that the nerve is straight without kinks, torsion, or twists. Pin the nerve as close to the ends as possible.
2. Using 6-0 silk sutures or fine thread, tie a double knot around each end of the nerve to prevent cytosol leakage into the buffer. Place the knots just next to the insect pins on the side closer to the center of the nerve, as this will prevent leakage from nerve tissue into the buffer. Do not carry out this step if the nerve has been previously ligated.
3. Using the microscope for precision and the 2 mm angled spring scissors, remove fat, blood vessels, and muscle tissue from the nerve. Prune any nerve branches that will not be used in the stimulation and recording protocol.
    1. Every 5 min of cleaning, replace the buffer with fresh chilled oxygenated mKHB. Use fine forceps to pull on connective tissues, fat, and blood vessels to ease dissection.
4. Place the nerves back in the transport tubes filled with fresh oxygenated chilled mKHB and place the tubes on ice.

6. Equipment setup

NOTE: The equipment setup used to carry out experiments is illustrated in Figure 1. Briefly, it consists of a dualcompartment nerve bath, a 2 L bottle placed on a heating stirrer, a source of carbogen for buffer aeration, and tubing to allow the buffer to flow from the bottle to the bath, and back to the bottle using a peristaltic pump. The bath can be machined out of plexiglass or 3D-printed from watertight materials. It has a depth of approximately 2 cm, and the partition separating the two chambers of the bath features a 1.5 mm diameter hole to allow the threading of a peripheral nerve across both chambers. One chamber is large, must be at least 4 or 5 cm long, and will be filled with buffer. The other chamber should be at least 3 cm long and will be filled with silicone or mineral oil. The bath must not be made too large as this will degrade control of perfusion, temperature, and pH. Different bath sizes may be required depending on the size of the nerve tissue being studied.

1. Prepare a clean dual-chamber nerve bath (see Supplementary File for design specifications). Place the nerve bath below the level of the 2 L bottle placed on the heating stirrer, using standard laboratory boss heads and grippers. Connect the drain of the bath to the peristaltic pump inlet.
2. Connect the outlet of the peristaltic pump to a tube leading back to the 2 L buffer bottle. Connect the bath inlet to a tube with an adjustable flow valve and put the tube inside the 2 L bottle. Use a three-way valve with a syringe connected to the

middle outlet to help with priming the tube as a siphon for gravity-assisted buffer inflow.

3. Prime the siphon by drawing the syringe until buffer flows into it. Configure the valve such that the flow rate of buffer into the bath is ~5-6 mL·min^{-1}. The flow can be increased initially to fill the bath. Once the bath buffer level has reached the drain, place the nerves in the bath.

4. Using an insect pin, secure the end of the nerve at the corner of the buffer-filled bath chamber. Using 45° angled fine forceps and pinching the nerve only at the ends, carefully thread the nerve to be stimulated through the hole in the partition between the two bath chambers.

5. Secure the other end of the nerve in the oil chamber of the bath with an insect pin, ensuring that the nerve is straight without being stretched and is free of kinks and twists. Using silicone grease, make a seal to prevent buffer leakage from the buffer chamber into the oil chamber. Fill the oil chamber with silicone or mineral oil.

6. Place the Ag/AgCl recording electrode hooks in the oil bath chamber and secure them using boss heads and grippers. Drape the portion of the nerve in the oil bath over the hooks without pulling the nerve taut. Do not pinch the nerve; use angled forceps to lift the nerve without pinching.

7. Adjust or repair the silicone grease seal if any leakage is observed after moving the nerve.

8. Connect the reference Ag/AgCl electrode to the amplifier ground and place the electrode in the buffer-filled bath chamber by securing it using a laboratory gripper.

7. Electrode implantation on the nerve in the bath

1. Prepare a clean nerve cuff electrode for stimulation according to reference[21]. Place the electrode in the buffer-filled bath chamber. Using forceps or fine tweezers with blunt or angled tips, open the electrode in the bath to wet the inside of the cuff.

2. If bubbles remain, use a fine syringe to draw buffer from the bath and force the bubbles out of the cuff. With a tweezer under the nerve, gently open the cuff and slide it under the nerve. Close the cuff around the nerve, taking care to avoid any kinking or twisting of the nerve.

3. Connect the stimulation electrode to the stimulator and secure the stimulation electrode lead with tape. Connect the current return electrode to the stimulator and secure the lead with tape. If using a square platinum sheet as the current return electrode, position the sheet away from the nerve in the bath.

8. Stimulation and recording

1. Connect the stimulator TTL signal output to channel 4 of the oscilloscope, which will be used to trigger the oscilloscope. On the oscilloscope screen, press the **Trigger** channel tab, and specify Channel 4 as the triggering channel. Set the trigger level to 1 V using the **Level** knob.

2. On the oscilloscope, set time resolution to 1 ms/division and voltage resolution to 10 mV/division. Center the trigger reference in time and set the trigger level to 1 V. NOTE: Follow steps 8.3 to 8.6 if using the custom stimulator (see **Table of Materials**). It is assumed that the MATLAB software and device drivers have been installed on the laboratory computer using instructions provided freely online for the custom neural stimulator[21]. Otherwise, follow the manufacturer's instructions for the use of a commercially available stimulator as an alternative.

3. Connect the stimulator to the laboratory computer. Turn on the stimulator by connecting the battery power supply to the power input. Start the MATLAB software on the laboratory computer.

4. Execute the custom MATLAB script: **HFAC_4ch_Stimulator_Initialization.m**(**Table of Materials**).

   NOTE: The USB communication cable between the computer and stimulator should flash green. If it does not, then there is a configuration error, and the power supply and connections must be verified.

5. Open the MATLAB script: **HFAC_4ch_Monophasic_Stimulation.m** (**Table of Materials**). By directly editing the MATLAB script, set the parameters as follows: stimulator pulse amplitude = -300 μA, stimulator pulse width = 300 μs, stimulator number of pulses = 10 and stimulator time between pulses = 1 s.

6. Start the stimulation protocol by clicking on **Run** in the MATLAB software.

Representative Results

Representative results that can be obtained with this protocol are the consistent compound action potentials from A-type nerve fibers within the sciatic nerve. These action potentials typically have a peak-to-peak amplitude of approximately 1 mV at the electrode and therefore 100 mV once amplified (Figure 2). Similar stimulation amplitudes and pulse widths should yield similar CAP amplitudes. Conductive elastomer cuff electrodes will generally require slightly higher stimulation amplitudes in order to obtain the same CAP amplitude compared to commercially available platinum cuff electrodes. This difference is generally small compared to the variation in stimulation amplitude required to stimulate nerves coming from different animals. This is because small differences in nerve size and cuff fit have a large effect on the required stimulation amplitude to obtain a specific CAP amplitude, regardless of the cuff material. This can be used to test the effects of different buffer compositions, such as different ion concentrations or the addition of nerve excitatory or inhibitory substances such as tetrodotoxin. If the buffer waste pipe is routed to an extra

container, the addition of nerve excitability-altering substances can be made temporary for the experiment, with wash-out rates dependent on the rate of buffer inflow.

The minimum current density, calculated as the stimulation amplitude divided by the surface area of the stimulation electrodes, required to activate the A-type fibers, and obtain an observable compound action potential of the oscilloscope was plotted versus pulse width in Figure 3. The results shown in Figure 3 represent typical nerve excitability for both commercially available standard platinum nerve cuffs and custom-made conductive elastomer nerve cuffs.

The extracted nerves should remain viable for approximately 6 h after extraction and, therefore, experiments must fit within this time window. Loss of nerve viability leads to a progressive decline in CAP amplitude and conduction speed. After action potential amplitude declines below 50% of initial amplitude (at the start of recording), the nerve should be considered no longer viable as results will be significantly skewed. Representative results with respect to nerve longevity are shown in Figure 4. The right and left sciatic nerves were extracted from one animal between 10:00 AM and 11:00 AM on a given day. Initial CAPs were obtained from the right sciatic nerve during initial tests before experiments, and standard CAPs were obtained from both left and right sciatic nerves, which had been kept alive using this protocol, at the end of the experiments. Minimal CAP amplitude reduction was observed with the right sciatic nerve, while the left sciatic nerve CAP amplitude at approximately 3 mV was similar to that of the right sciatic nerve at the start of the experiment more than 6 h after nerve extraction.

Discussion

In this work, we described a protocol to prepare rat sciatic nerves for *ex vivo* neurophysiology. Tissue extraction takes approximately 30 min, including animal handling, anesthesia, culling, and dissection, while nerve cleaning, placement in the bath, and electrode implantation should require an additional 30 min before recording can be started. Buffer preparation can be carried out in 30 min, though this can be done ahead of the rest of the experiment. This type of preparation and experiment has been used and described in the past[7, 12], using similar buffers and for the same tissue type. To the authors' knowledge, however, this is the first time a description of the buffer preparation, dissection, equipment set up, and subsequent recording has been given in the same document.

This protocol can enable a wide variety of experiments in neurophysiology that would not be possible in either *in vitro* or *in vivo* contexts. For example, an advantage of *ex vivo* preparations is that they preserve the macro and microstructure of the extracted tissue while isolating this tissue from the rest of the body. This results in a simpler setup as anesthesia does not have to be maintained, which is otherwise a requirement in *in vivo* experiments. In terms of enabling experiments, *ex vivo* preparations allow the use of substances such as tetrodotoxin, which are difficult to justify in an *in vivo* context[23] as they carry a high risk for the animal. When the use of such substances benefit investigation, they are easier to use in *ex vivo* preparations. The use of the custom stimulator[20] enables experiments using HFAC block for neuromodulation using this experimental setup.

The most critical step in the protocol is the dissection step, because even a small mistake using the dissection scissors can damage the nerve if enough care is not taken. The speed at this stage is also essential as the tissue must be rapidly extracted from the body and placed in a chilled buffer to maximize viability at the start of the recording. After tissue extraction, while care should be taken when cleaning the nerve and implanting any electrodes, the protocol is more flexible with respect to time, and the risk of operator error is, therefore, lower. As nerve diameters and placement of fascicles within the nerves will vary from animal to animal, some variability in results should be expected even if using the same electrodes and stimulation protocol. The effect of this variability can be seen in the error bars for stimulation thresholds in Figure 3. It is important not to pinch the nerve at any stage of the preparation as this can cause irreversible damage to the tissue. Handle the nerve only by its ends using forceps and with great care not to pull the nerve taut.

Several aspects of the protocol in its current form can be improved by increasing the amount of equipment and setup time. To help with the diagnosis of potential issues with this experimental setup, automated measurements of pH and dissolved oxygen in the bath could be useful but have not been implemented here. Both measurements can be achieved using amperometric or potentiometric methods[12, 19]. Equipment that will require regular maintenance is the tubing and glassware, which accumulates salt deposits over time. The AgCl recording hooks will also require regular re-coating or replacement, along with the AgCl reference. Stimulation electrodes should be cleaned after each use but will generally not require replacement for many experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge Dr. Gerald Hunsberger of GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA, and Galvani Bioelectronics (Stevenage, UK) for sharing their original nerve preparation technique with us. The authors acknowledge Robert Toth for the Dual-Chamber nerve bath design. The authors acknowledge funding from the Healthcare Technologies Challenge Awards (HTCA) grant of the Engineering and Physical Sciences Research Council (EPSRC). The authors acknowledge the High Performance Embedded and Distributed Systems Centre for Doctoral Training (HiPEDS CDT) of Imperial College London for funding Adrien Rapeaux (EP/L016796/1). Adrien Rapeaux is currently funded by the UK Dementia Research Institute, Care Research and Technology Centre. The authors gratefully acknowledge Zack Bailey of Imperial College, in the Department of Bioengineering, for help with experiments and access to animal tissues during the production of the JoVE video article.

Data Sharing

The raw data used in the figures of this article will be made available by the authors, without undue reservation.

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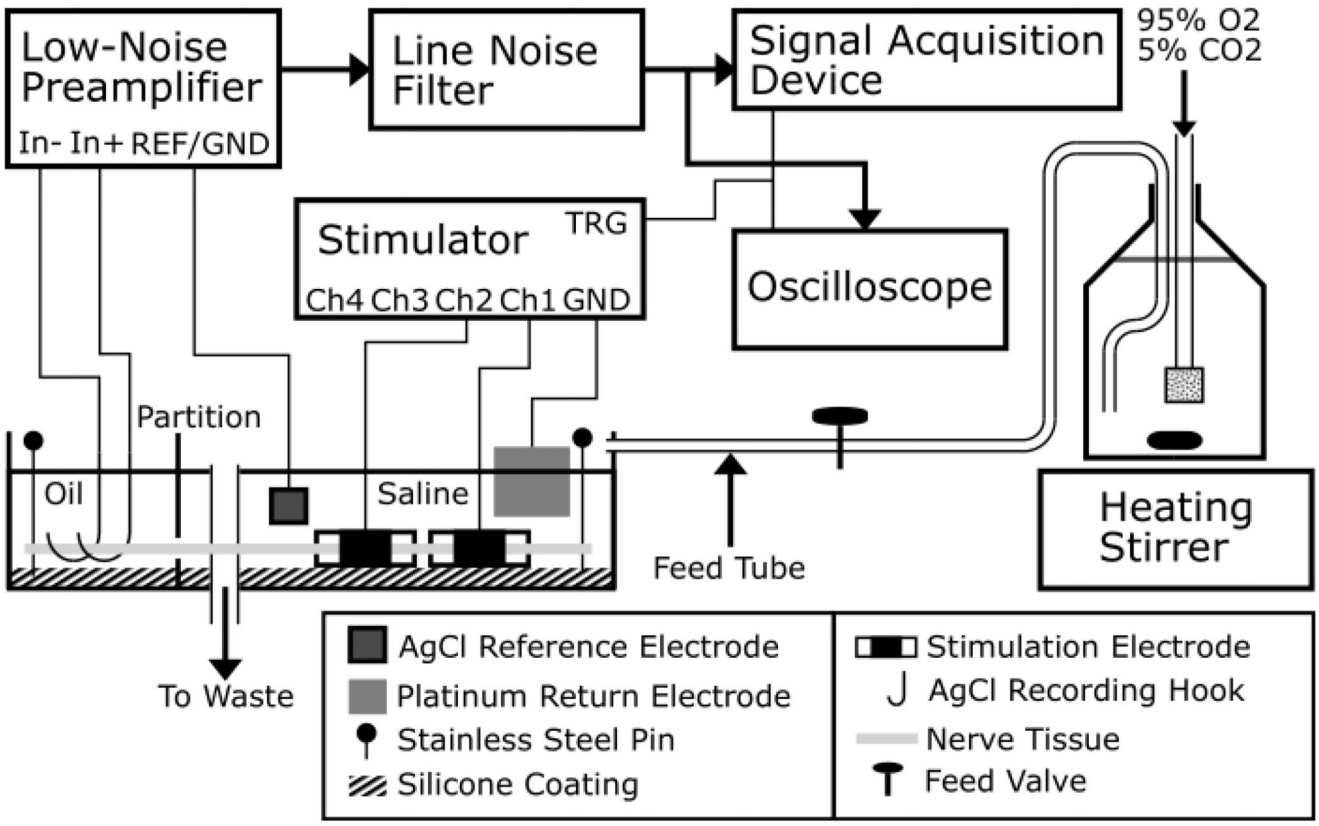

Figure 1. Schematic representation of the experimental setup used in the protocol.
This figure has been modified from Rapeaux, A. et al. (2020)[20]. Please click here to view a larger version of this figure.

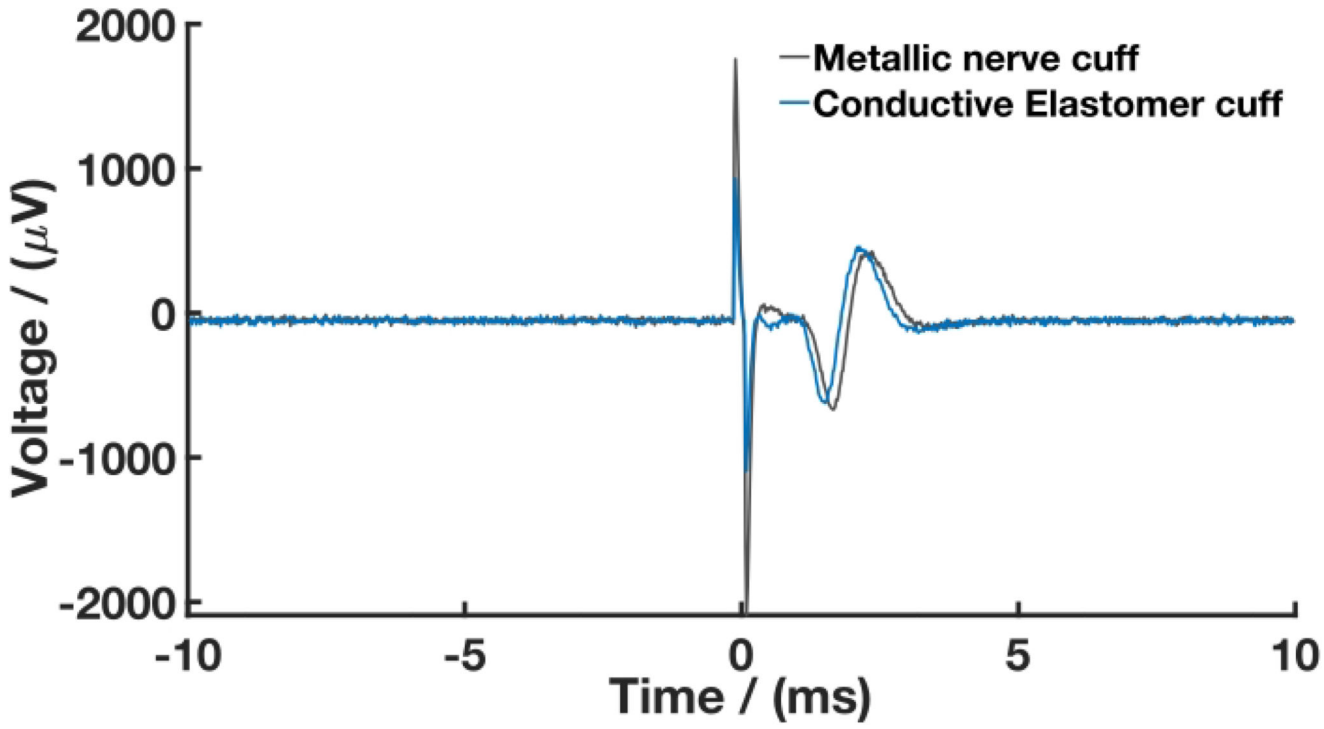

Figure 2. Representative CAPs obtained *ex vivo* following stimulation by metallic and conductive elastomer nerve cuff arrays.

Reproduced with modifications from Cuttaz, E. A. et al. (2021)[22]. Please click here to view a larger version of this figure.

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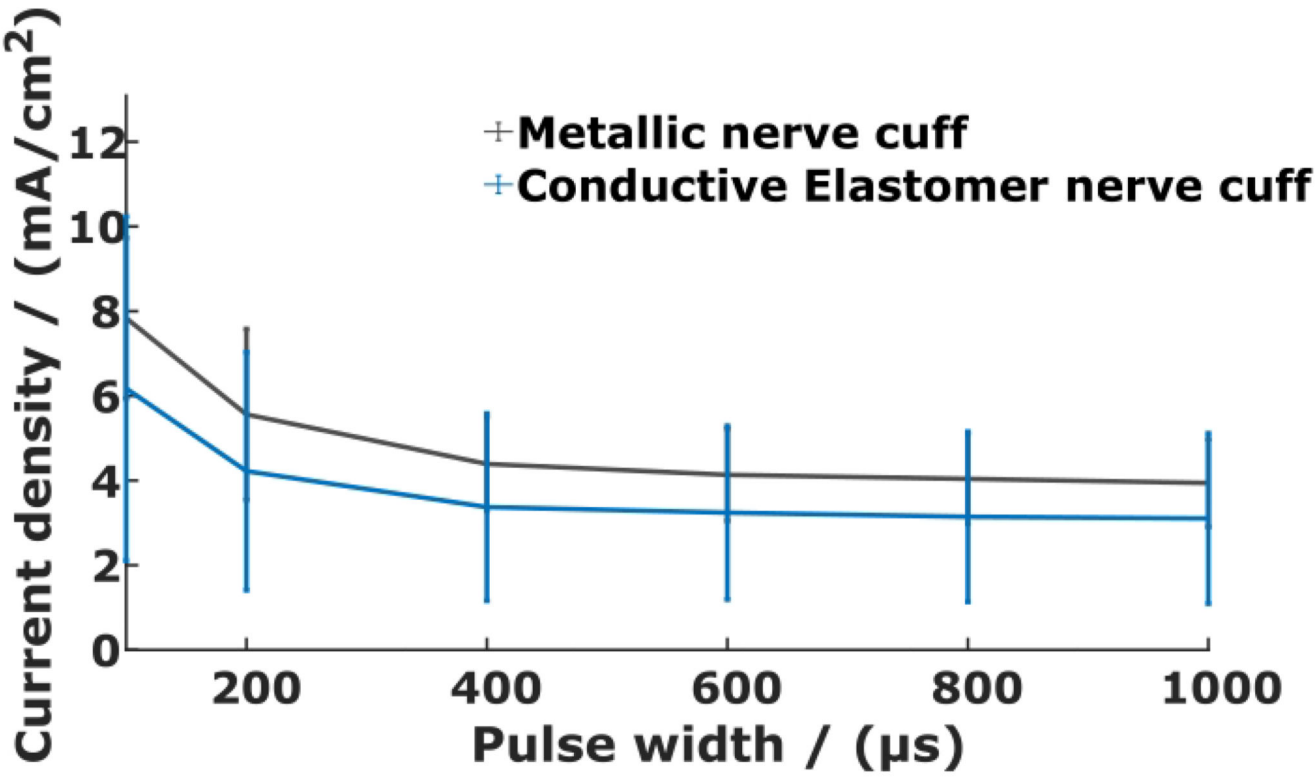

Figure 3. A-fibre activation threshold of metallic and conductive elastomer cuff arrays.
Error bars represent ±1 standard deviation from the mean. Reproduced with modifications from Cuttaz, E. A. et al. (2021)[22]. Please click here to view a larger version of this figure.

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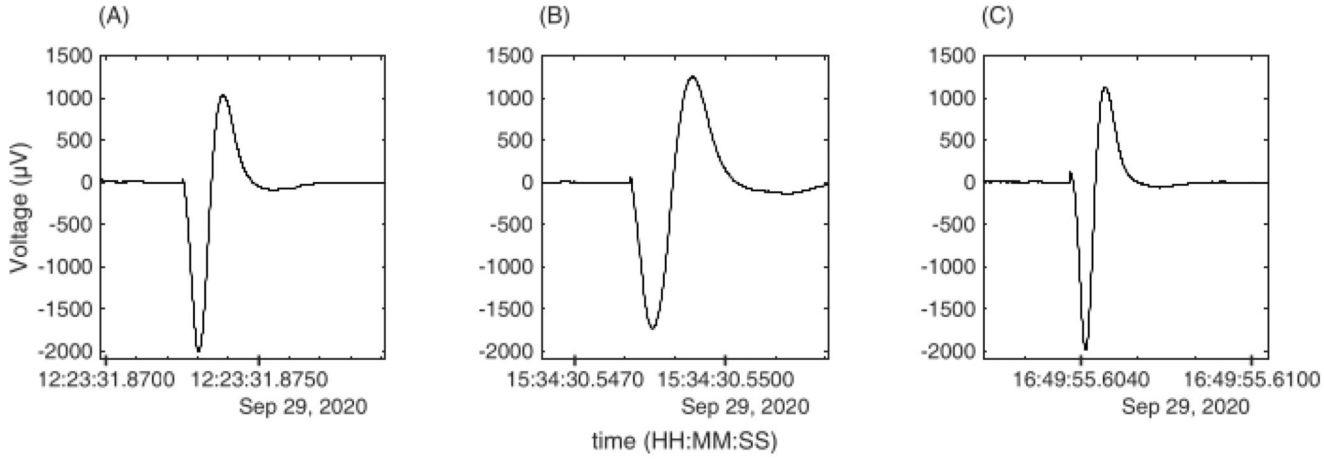

Figure 4. Representative CAPs obtained *ex vivo* over a day of experiments and using both sciatic nerves from one animal.

(**A**) A-type fiber CAP obtained near midday from the right sciatic nerve. (**B**) A-type fiber CAP obtained mid-afternoon from the left sciatic nerve. (**C**) A-type fiber CAP obtained at the end of experiments with the same left sciatic nerve in (**B**). The x-axis corresponds to the time of day at which the recordings were taken. Please click here to view a larger version of this figure.

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