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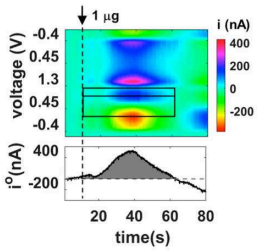
Cyclic Voltammetry in the Spleen for In Vivo Recording of Stimulus-Evoked Norepinephrine



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We use this protocol and it's working

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Disclaimer

Abstract

This protocol describes methodology for in vivo recording of norepinephrine concentration in the spleen of mice during a stimulus (i.e electrical nerve stimulation, drug injection, etc.). Part 1 describes the preparation of surgical and recording equipment. Part 2 describes the surgery and recording.

The typical voltammetric signal for norepinephrine in the spleen is characterized in Figure 1 of the referenced manuscript. Stimulation of the splenic, splanchnic, or vagus nerves with a 10-second electrical train should illicit a rising signal that plateus in ~1min and comes back to baseline in ~1-2 minutes.

Materials

For Preparation (Part 1)

Fast-Scan Cyclic Voltammetry Recording Package by Pinnacle
- Voltage Driver
- Headboard
- Pinnacle Reference Electrode
- Pinnacle Carbon Fiber Micro-Electrode (Pinnacle 7001-CFE)
- Pinnacle FSCV Recording Software

Silver Wire
4% Bleach Solution
ParaFilm
Wooden Popsicle Sticks or Cotton Tip Applicators
Surgical Tape
Soldering Iron
Solder
Scalpel
Surgical Stereotax

For Surgery (Part 2)

Isoflurane
Oxygen
Heating pad
Surgical Microscope
Surgical Gauze
Surgical Tape
Cotton tips
Saline

Vagus, Splanchnic or Splenic Nerve Stimulation

Stimulating Electrode (Cortec AirRay Micro Sling Cuff 0.1mm - 2 contact Pt/Ir)
Kwik-Sil Elastomer (World Precision Instruments)
2X 7S Dumont Surgical Forceps (Roboz RS-4935)
Dumont Microhook (Roboz RS-6142)

Splanchnic Nerve Stimulation

ChaT-TdTomato Mice (See note in splanchnic nerve electrode placement, step 9)
Fluorescence Dissecting Microscope with Green Laser and Red Filter for visualization of TdTomato

Vein Catheterization

6-0 silk suture
1French polyurethane catheter (Instech C10PU-MFV1301)
30 gauge needle, bent

Norepinephrine bitartrate
Injectable saline

Fast-Scan Cyclic Voltammetry Setup
MicroPoint CottonTips (Digi-Key CCT2425)
Surgical Tape

Troubleshooting

Safety warnings

Carbon Fiber Electrode is highly fragile, especially to sheer forces. Always face electrode tips away from movement as loose clothing brushing against it can destroy it.

FSCV recordings are sensitive to movement. Breathing motions can create artifacts, spleen must be stabilized as much as possible before inserting electrodes.

Once punctured, FSCV electrodes cannot be removed from the spleen without major bleeding, so remove electrodes only once animal can be sacrificed.

Ethics statement

This protocol involves procedures done on live animals and therefore requires IACUC approval from the user's institution. All work done in this protocol was approved by the Feinstein Institutes for Medical Research IACUC.

Before start

For Part 1:
Purchase Pinnacle fast-scan cyclic voltammetry system and consult set-up instructions. Purchase disposable Pinnacle carbon fiber micro-electrodes, silver wire, and bleach solution. Gather wooden cotton-tip rods or popsicle sticks, ParaFilm, and adhesive.

For Part 2:
Check isoflurane and oxygen tank levels in anesthesia vaporizer, decontaminate surgical field and instruments, allow heating pad to come to temperature.

Setting Up Voltammetry Recording Equipment

1 Cut off wire lead from any used or broken Pinnacle electrode or reference to obtain headboard pin
connector. Cut 10cm of silver wire and solder it to exposed wire from headboard pin connector to obtain custom Pinnacle reference electrode. Sharpen silver wire tip using a scalpel, than submerge in fresh bleach solution for 1 hour to obtain custom Ag/Cl reference. (Figure 1 – Component A)

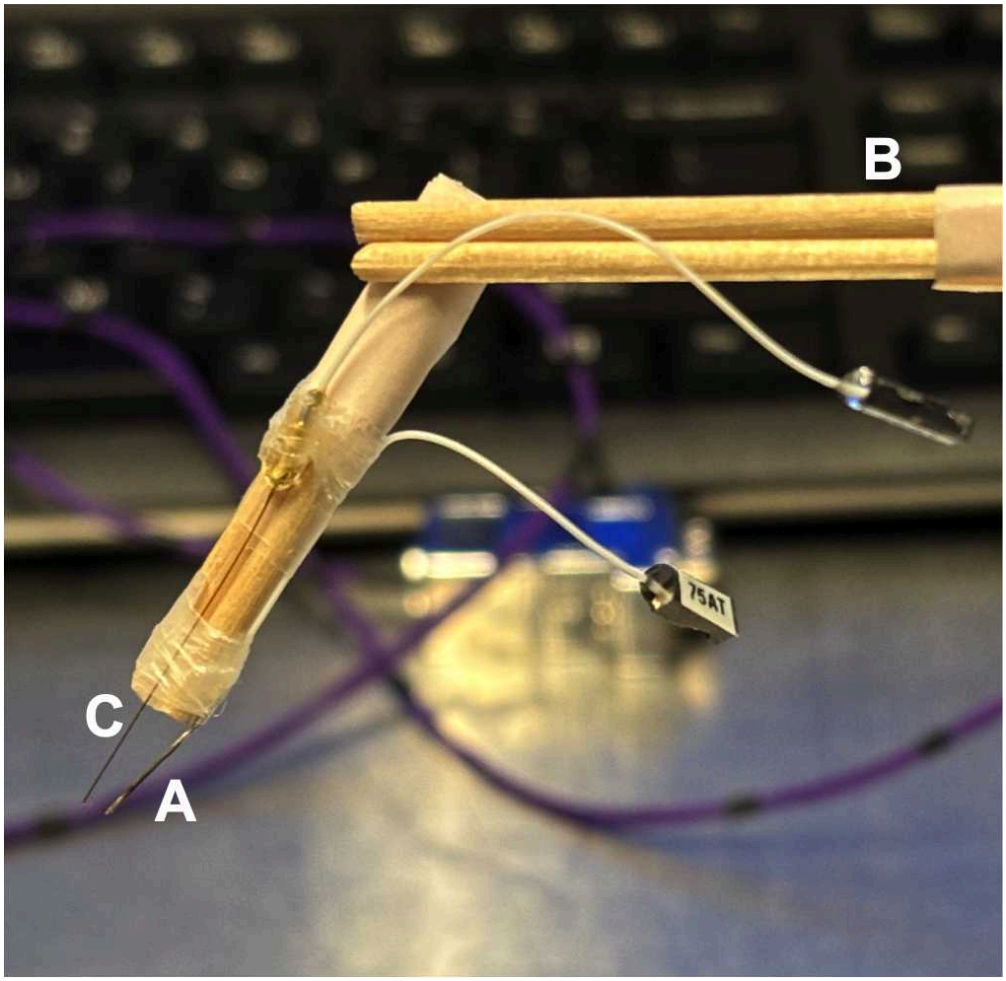

Figure 1: Fast-scan cyclic voltammetry implant components. **Component A**: Ag/AgCl electrode, sharpened to pierce splenic capsule. **Component B**: Wooden rig to stabilize electrodes. **Component C**: Carbon-fiber microelectrode from Pinnacle.

2 Assemble electrode support rig using any suitable materials and adhesives, such as wooden applicators sticks and tape (Figure 1 – Component B)

3 Attach custom sharpened Ag/Cl reference electrode to tip of support rig using ParaFilm, ensure at least 5cm of clearance between tip of support rig and sharpened tip of reference electrode. Wrap ParaFilm at two attachment points, one near the tip of the support rig, such that reference electrode is rigidly bound to the support rig.

4 Gently remove Pinnacle carbon-fiber electrode from packaging and attach to support rig using ParaFilm, same as reference electrode. (Figure 1 – Component C) Tip of sharp Ag reference electrode should be protruding just ahead of the tip of carbon fiber and 1 cm apart from each other. Both electrodes should be rigidly attached to support rig. (Figure 1)

5 Mount Pinnacle headboard to stereotax so that it can be moved precisely during implantation procedure. (Figure 2A). Then attach electrode support rig to sterotax and connect reference and carbon fiber electrodes to headboard (Figure 2B)

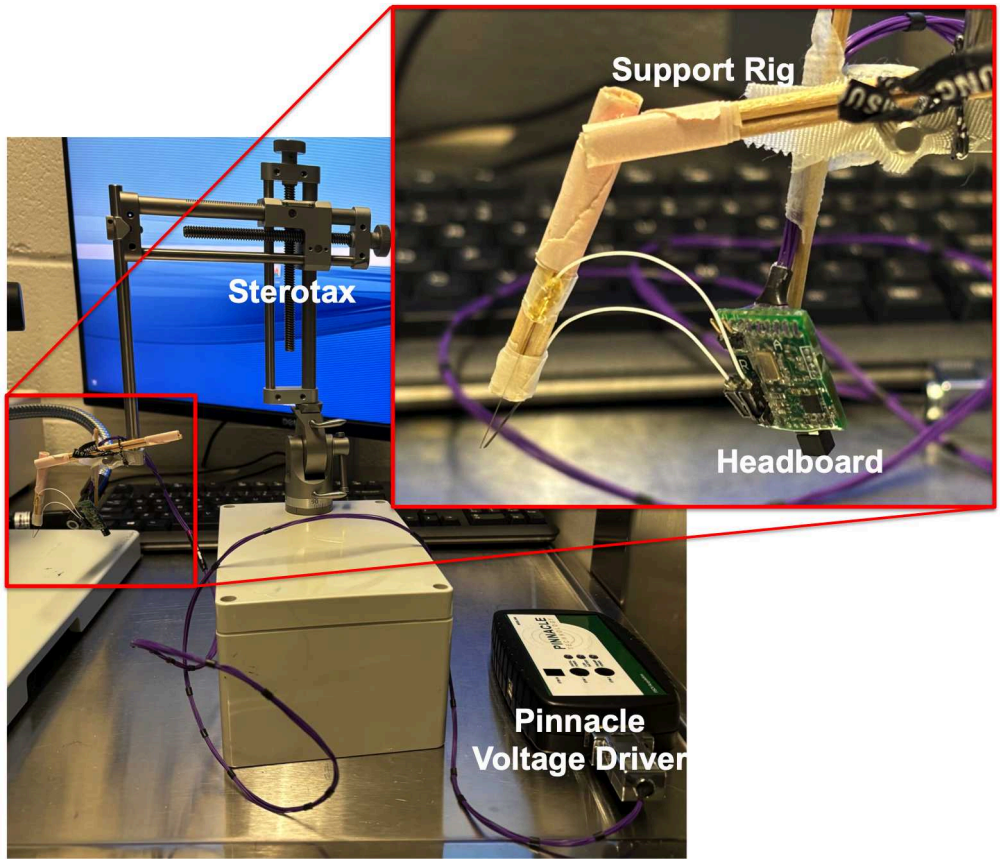

Figure 2: Fast-scan cyclic voltammetry rig for recording and implantation. Stereotax for implant manipulation. Pinnacle voltage driver and headboard for recording. Support rig to stabilize electrodes during implantation.

6 Connect headboard to Pinnacle voltage driver device (see Pinnacle instructions for set up).

Surgical Preparation

7 Induce anesthesia with 4% isoflurane. Lay animal dorsum on heating pad and maintain anesthesia at 1.5%.

8 The following instruction split depending on the type of stimulation that will be used to evoke NE release in the spleen.

STEP CASE

Vagus Nerve Stimulation 9 steps

Implantation of vagus nerve stimulation cuff

Vagus Nerve Electrode Placement

9 Shave cervical region and make midline incision.

10 Lift left parotid gland to expose left anterior triangle of the neck. Left sternocleidomastoid and esophagus should be visible.

11 Retract sternocleidomastoid to expose the carotid sheath. Gently blunt dissect the carotid sheath between internal carotid and left vagus nerve to separate left vagus nerve.

12 Use microhook to lift vagus nerve up and over a Cortec stimulating electrode cuff. Seal vagus nerve on cuff with Quik-Sil elastomer to prevent movement or current leakage.

13 Make incision under left costal margin through skin and external and internal oblique muscles to reach peritoneal cavity. (shown in Figure 3A) Gently massage spleen out of the peritoneal cavity using wet cotton tips.

Fast-Scan Cyclic Voltammetry Set Up

14 Place three cotton tips under the spleen, taped down to the heating pad as shown in Figure 3A. Elevate the middle cottontip by inserting a wooden stick between the cottontips until the splenic neurovascular bundle is taught. This rig should dampen the movement of the spleen with the animal's breathing and hold it rigidly in place. Ensure that spleen surface is dry.

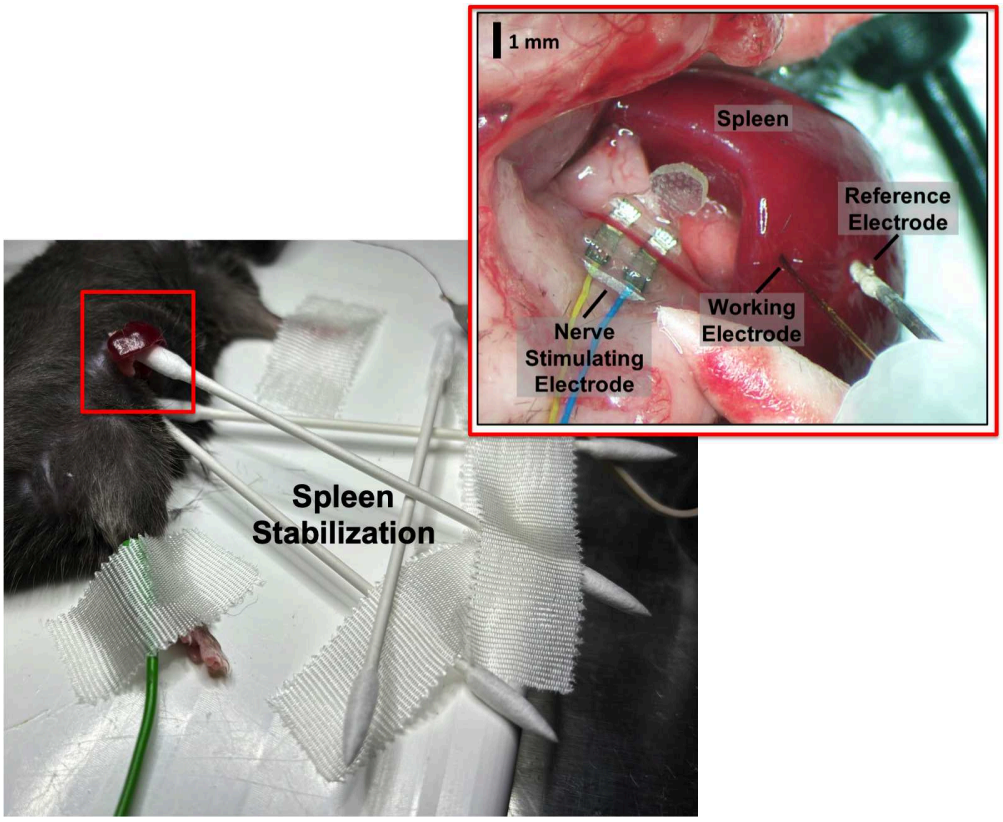

Figure 3: A) Stabilization of spleen using micropoint cottontips. B) Exposed spleen with FSCV electrodes inserted and Cortec stimulation electrode on splenic nerve.

15 Position the electrode tips above the spleen. Use stereotax to slowly descend electrodes until they touch the splenic capsule. Then alternate moving electrodes downward and midline until the reference and carbon fiber pierce the splenic capsule (Figure 3B). Once pierced, depress electrodes slightly more and do not move any further. If pierced spleen begins bleeding uncontrollably, procedure will have to be restarted. Otherwise, cover spleen and exposed abdominal organs in damp KimWipe, carefully avoiding any motion of submerged electrodes.

16 Open Pinnacle FSCV application and turn on continuous cycling. Allow electrodes to record for 10 minutes, undisturbed, for conditioning.

17 Record voltammetry signal continuously while delivering electrical stimulation trains to electrode leads (30Hz, bipolar, 10-200µA, 5-100 seconds). Allow voltammetry signal to return to baseline before delivering next stimulus.