🌐 Glossopharyngeal Nerve Chronic Recording In Anesthetized Rat

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| 1 | Works for me | dx.doi.org/10.17504/protocols.io.wssfeee |

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ABSTRACT

We propose to develop a neural interface based on carbon nanotube (CNT) yarns with a size similar to large axons (10 μm) that has a mechanical and molecular compatibility with the nerve. The mechanical properties of the interface will be optimized by matching flexural rigidity of the implant to that of the glossopharyngeal nerve (GLN). Since the wire is very flexible, a custom-inserting technique has been developed for this purpose and tested in rats. Following insertion, the signal-to-noise ratio and impedance of the wires will be measured for 3 months. The biocompatibility of the implant will be assessed using histological techniques.

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MATERIALS TEXT

MATERIALS

22 gauge needle **Sigma Aldrich Catalog #Z192473**

25 mg [Des-Pro2]-Bradykinin **biorbyt Catalog #orb321454**

10 g 4-Dimethylaminopyridine **biorbyt Catalog #orb321037**

100 mg Atipamezole **biorbyt Catalog #orb341888**

5 g Carprofen **biorbyt Catalog #orb321211**

1 g Isoflurane **biorbyt Catalog #orb322211**

1 mg Ketamine (BSA) **biorbyt Catalog #orb61131**

1 g Lidocaine HCl **biorbyt Catalog #orb341990**

1 g L-NAME HCl **biorbyt Catalog #orb134513**

1 g Phenylephrine hydrochloride **biorbyt Catalog #orb341924**

1 g Xylazine hydrochloride **biorbyt Catalog #orb341967**

1 ml syringe **Contributed by users Catalog #9161406V**

Histamine **Bio Basic Inc. Catalog #HB0500.SIZE.5g**

Sodium nitroprusside dihydrate **Bio Basic Inc. Catalog #SB0867.SIZE.50g**

N-Acetyl-5-bromo-3-indoxyl-2,3,4-tri-O-acetyl-beta-D-glucuronic acid methyl ester **Gold Biotechnology Catalog #A-790**

Collagenase Type II 100 mg **Stemcell Technologies Catalog #7418**

Lipopolysaccharide (1 microgram/ml in LPS stored -20decC) **Sigma Aldrich Catalog #L4391**

Bayer Contour Blood Glucose Monitoring system **Amazon**

Humulin 70/30 insulin **Contributed by users Catalog #3 0002-8715-01 7**

Hypoxia Challenge- Rats will be lightly sedated with either Isoflurane or Ketamine/Xylazine. Impedance will be

1   taken via the port. Neural recordings will be taken next. We will expose the rat to 10% O, 90%N (1L/min flow rate) until the oxygen saturation rate is reduced to 70%. This takes about 30 seconds. We allow 5 minutes between the three hypoxia challenges. We will perform the hypoxia challenges once a week for 6 weeks.

2   **Chronic In vivo Implation procedure**.
     All surgical and experimental procedures were done with the approval and oversight of the Case Western Reserve University, Institutional Animal Care and Use Committee to ensure compliance with all federal, state and local animal welfare laws and regulations.

3
     Adult male Sprague Dawley rats (350–450 grams, Charles River Laboratories) were used for all peripheral nerve implants discussed. Prior to surgery, all surgical instruments and implant assemblies were autoclave sterilized at 250 °C for 1 hour. During surgery, the animals were gas anesthetized under 2% isoflurane in 1 L/min oxygen. For each of the nerves, a section approximately 3–4 mm was completely separated from and devoid of all connective tissue. A custom made hook was attached to a three-axis micromanipulator and positioned to provide a small amount of tension and suspend the nerve in the air. The slip knot was then removed, thereby releasing the silicone tube junction from the microneedle. The CNT yarn electrode assembly was then advanced into the nerve until all of the uninsulated CNT yarn segment was within the perineurium. Micro tweezers were then used to apply a small amount of pressure at the implant site while the Tungsten mirconeedle was carefully removed leaving the CNT yarn inside the nerve. The hook was then advanced along the nerve to the next implant site, approximately 2 mm away, and the implant process was repeated. The CNT yarns and the silicone tube junctions were then carefully positioned within the incision site and completely encapsulated with 1 mL of fibrin glue (Tisseel, Baxter International Inc.) to secure the implant in place while the normal physiological process of collagen encapsulation took place while the fibrin glue was absorbed by the body.

4   The free DFT® wire ends are then tunneled and externalized at a location on the back between the shoulder blades. The free DFT® wire ends and the ground wire are then passed through a custom made, percutaneous silicone plug and soldered to an electrical connector (MCP-05-SS, Omnetics Connector Corporation). The connector was then fitted inside the silicone tube of the percutaneous plug and the tube was filled with UV curable, medical grade epoxy (OG603, Epoxy Technology Inc.).

5   Blunt dissection is then performed to create a pocket in the back between the skin and muscle. The implanted wires and ground wire are placed within this pocket. The percutaneous plug's mesh base was then sutured to the underlying muscle fascia using biodegradable 3-0 sutures (Vicryl Plus, Ethicon). Finally, all skin incision sites were closed using 4-0 sutures (Webpro™, Patterson Veterinary).

6   Carprofen (5mg/kg) SQ at surgery than again once a day for 4 days. hour post-op

7   Lidocaine (4mg/kg) + Bupivacaine (2mg/kg) SQ at Surgery (at least 30 sec) prior to incision

8   **Chronic autonomic nerve recordings**
     The recording system was designed to accommodate two, differential recording channels. Each recording channel has eight amplifier hardware averaging for increased signal-to-noise recording results. This was achieved by creating, two custom, printed circuit boards (PCB). The first PCB contains the mating connector (MCS-05-DD, Omnetics Connector Corporation) to the connector which resides in the implanted percutaneous plug. DFT® wires are soldered to make electrical connections between the two PCB boards for all the CNT yarn electrode implants (up to four total) and the implanted ground wire.

9   The second PCB routes each differential electrode pair to an electrical connector (NPD-36-AA-GS, Omnetics Connector Corporation) that connects directly to a commercially available 16-amplifier, neural instrumentation amplifier board (C3313/RHD2216, Intan Technologies LLC). The electrode connections are routed such that one differential channel is simultaneously recorded with amplifiers 0–7 and the other is captured with amplifiers 8–15. The RHD2216 performs the signal filtering, analog signal multiplexing, and analog to digital conversion and is controlled via a digital SPI

connection to a commercially available acquisition system (C3100/RHD20000 Evaluation System, Intan Technologies, LLC) e . Neural signal acquisition was performed and stored to a laptop computer using the following system configurations: 20 k samples/sec/amplifier, DSP offset removal was enabled with the high-pass filter cutoff frequency set at 100 Hz, the amplifier bandpass filters were configured to be between 100 Hz and 9 kHz.

10 A manual trigger switch signal is recorded via a separate analog-to-digital input line on the RHD2000 evaluation system. This trigger signal is synchronized with the recorded neural amplifier data and is also sampled at a rate of 20 k samples/sec. Holding down a simple push button switch, produces a high logic-level trigger signal which is used to mark different events during the various experiments. Releasing the push button returns the trigger signal to a low logic-level).