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Chronic Vagus-Nerve Activity with Carbon Nanotube Sensors in Freely Moving Rodents

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We use this protocol and it's working

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Abstract

This study presents the first chronic recording and decoding of activity in the vagus nerve of freely moving animals. The majority of vagal afferent fibers come from the gut and abnormal vagal activity has been linked to eating and metabolic disorders. The study uses carbon nanotube yarn electrodes, which have small size, high flexibility, and low impedance, to provide a stable, high-signal-to-noise ratio interface for chronic recording in small autonomic nerves in rats, with high-quality signals continuing up to four months after implantation. The technology was successfully applied to make the first direct measurements of vagal tone in freely moving animals, and the neural-recording data was synchronized with continuous video recording of the subjects. The results show several spike clusters that show tuning to animal eating, and the firing dynamics of multiple decoded spike clusters can be used to classify eating compared to drinking, grooming, and resting behaviors.

Materials

CNT yarns as manufactured in the following:

Protocol

NAME

Carbon nanotube (CNT) yarn fabrication for chronic interfacing with the autonomic nervous system

CREATED BY

Aaron Rodrigues

Preview

35NLT®-DFT® wire (Fort Wayne Metals, Fort Wayne, IN, USA)

Silver conductive epoxy (H20E, EPO-TEK)

Silicone elastomer (MED-4211/MED-4011, NuSil Silicone Technology, Carpinteria, CA, USA)

11-0 nylon suture (S&T 5V33)

pyralene-C (5 µm thick vapor deposition coating, SMART Microsystems, Elyria, OH, USA)

Laser spot welder (KelanC Laser, set to 1A current, 0.3 ms pulse width, and 300 µm diameter)

Male Sprague Dawley rats (RRID:RGD_70508) between 7–12 weeks of age

fibrin glue (Tisseel, Baxter International Inc., Deerfield, IL, USA)

5-pin Omnectics connector (Omnetics Connector Corporation MCP-5-SS)

Troubleshooting

Protocol for CNTY Electrode Manufacture:

- 1 Carbon nanotube yarns (CNTYs) were manufactured at Case Western Reserve University using a previously described method (<https://doi.org/10.1038/s41598-017-10639-w>).
- 2 CNTYs were connected to 35NLT®-DFT® wire using silver conductive epoxy, creating a CNTYDFT® junction.
- 3 Dacron mesh and silicone elastomer were added to seal the junction, and the impedance of the junction was measured at 1kHz in a saline bath to confirm the seal.
- 4 The free end of the CNTY was tied to the end of an 11-0 nylon suture using a fisherman's knot, as shown in **Figure 1A**.

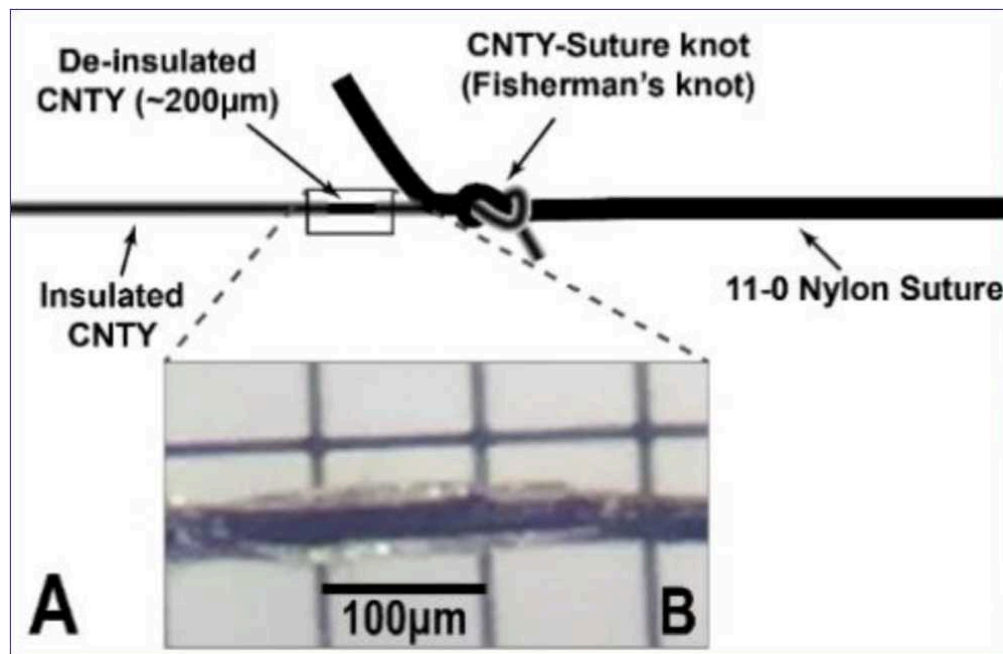


Figure 1. Electrode implantation **(A)** Diagram of CNTY electrode mated with an 11-0 nylon suture with a fisherman's knot. **(B)** Section of CNTY electrode deinsulated by laser.

- 5 The entire CNTY was coated with parylene-C (5 µm thick vapor deposition coating) on a custom rack.
- 6 A small section (~200 µm long) of parylene-C was removed approximately 500 µm behind the CNTY-suture knot using a laser spot welder, as shown in **Figure 1B**.

- 7 Electrode viability was confirmed by measuring the impedance of the recording site before and after using the laser.

Surgical procedure for implanting the electrodes in rats:

- 8 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.
- 9 Electrodes were implanted in male Sprague Dawley rats between 7–12 weeks of age
- 10 A midline incision was made along the neck to expose the left cervical vagus nerve.
- 11 The muscles and salivary glands were separated and held in place, revealing the carotid sheath which contains the carotid artery and vagus nerve.
- 12 The vagus nerve was carefully separated from the carotid artery using blunt dissection and held in slight tension using a glass hook.
- 13 CNTY electrodes were implanted by sewing the suture through the nerve for ~2 mm.
- 14 After the implantation, the signals travel from the implants to the headcap connector mounted on the animal's skull, where they are digitized and amplified by the custom amplifier board. These signals are then routed through a commutator, which allows the animal to move freely without twisting or pulling on the cable.
- 15 The signals are sent to an Intan USB interface board, which sends the signals to a computer, where they are saved and can be viewed in real time.