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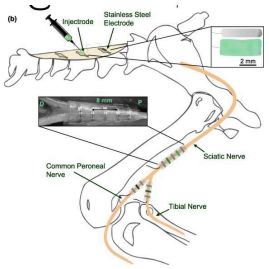
The Injectrode - A Truly Injectable Electrode for Dorsal Root Ganglion Stimulation to Treat Pain Protocol



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We use this protocol and it's working

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Disclaimer

Abstract

This is a protocol for an experiment to compare afferent fiber recruitment by dorsal root ganglion (DRG) stimulation using an injectable polymer electrode (Injectrode®) and a more traditional cylindrical stainless steel electrode. This protocol will result in data describing the recruitment rate and evoked compound action potentials (ECAP) of Aα, Aβ, and Aδ fibers due to stimulation at different pulse widths by the stainless steel electrode versus the Injectrode. This protocol will also result in data on the impedance values of both electrodes.

Image Attribution

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Materials

	A	B	C	D	E	F	G	H
	Electronic Equipment	Vital Signs Monitoring	Pre-surgery Tools	Surgical Tools	Surgical Accessories	Implant Tools	Drugs	Misc. Hardware/ Docs
	Ripple Stim headstage	Monitor	Heating bag	Disposable scalpel	Marker	Surgical microscope	Ketamine or dexdomitor	Electrical tape
	Ripple Grapevine	Pulse oximeter	Surgical mats on table	Scissors - small pointy	Beakers (saline, water)	Microrulers	Isoflurane	Scissors
	Ripple recording headstage	Temperature probe	O2 tank full, hooked up	Scissors - small round tip	Stainless steel bowl (trash)		Saline	Drug Dosage sheet
	Ripple cables	Respiration monitor	Lube	Scissors - large round tip	Cotton balls		Euthasol	Notes doc
	Magnetic holders for headstages	Extra heart rate montior	Gloves	Forceps - small straight (x2)	Gauze, large			Camera
	Nerve cuffs	Extra respiration monitor	Turnikey	Forceps - small curved (x2)	Gauze, small			Streaming setup
	Injectrode material	Stethoscope	IV catheters	Forceps - normal teeth	Syringes (for irrigation)			Calipers
	Injectrode injector	Emergency vent bag	PRN adapters	Forceps - normal no teeth	Suction beaker (enzyme)			
	DRG electrodes		Surgical tape	Needle driver	Suction tube with beaker plug			
	EMG electrodes		Line for IV bag	Periosteal elevator	Suction large tube to vacuum			
	Cooner wire		Clippers	Rongeurs (S, L)	Suction tip			
	Electrode for stim testing		Vacuum	Hooks	Suction stylet			

	A	B	C	D	E	F	G	H
			Surgical light	Michel clips	Saugers			
			Towels and saline in incubator	Michel clip tool	Bone wax			
			Magnetic board on table	Cauterizer	Cotton buds			
			Ties for legs	Cautery return electrode	Sutures 3-0 (X4)			
				Cautery gel (signa gel)				
				Cautery BP tip				
				Piezo drill				
				Drill bit				
				Towel clips				
				Microscissors				
				Microspatula				
				Wooden handled bone scraper				
				Blunt pokers				

Experiment Model (Animal Anesthetization)

1 Anesthetized adult male cats with ketamine (intramuscular, 10 mg kg^{-1}) and acepromazine (intramuscular, 0.1 mg kg^{-1}) or dexdomitor (intramuscular, 0.04 mg kg^{-1}).

2 Intubated cat and administered isoflurane (inhalation, 2%-2.5%) for the duration of the experiment.

3 Used antisedan (intramuscular, 1:1 dexdomitor dose) to reverse the effects of dexdomitor following the
administration of isoflurane.

4 Administered atropine (intramuscular, 0.05 mg kg^{-1}) to reduce saliva output.

5 Shaved the back, limbs, and ears of the cat.

6 Continuously monitored vital signs including heart rate, SpO_2, core temperature, respiratory rate, and $ETCO_2$ throughout the experiment.

7 Continually administered IV fluids (saline, 0.9% sodium chloride) throughout the experiment.

NOTE: Ventilator Settings Used:

Flow	2 L/min
Respiration Rate	8 breaths/min
PIP	7
Assist	-0.3

Implant Nerve Cuffs

8 NOTE: Implanted the nerve cuff electrodes in the hind limb ipsilateral to the stimulated dorsal root ganglion (DRG) around the sciatic, common peroneal, and tibial nerves (Figures 1 and 2).

Placed the cat on the table in supine position.

9 Lifted and tied the hind limb ipsilateral to the stimulated DRG to the bar of a stereotaxic frame set up on the table.

10 Made an incision behind the knee.

11 Dissected tissue bluntly to locate sciatic, common peroneal, and tibial nerves.

12 Placed one spiral nerve cuff electrode with five contacts (3 mm diameter, contacts 4 mm apart, center-to- center; Ardiem Medical, Indiana, PA, USA) made of silicone with platinum contacts on the sciatic nerve. (Figure 2)

13 Shorted the proximal, center, and distal contacts together to use as a reference electrode. The second and fourth contact were used for recording. (created two tripolar configurations)

14 Placed one spiral nerve cuff electrode with three contacts (2 mm diameter, contacts 4 mm apart, center-to-center) around both the tibial nerve and the common peroneal nerve (Figure 2). Shorted the proximal and distal contacts together to create a tripolar configuration with the center electrode used for recording.

15 Took photos of cuff placement.

16 Tested stimulation through each electrode on each cuff to verify function. Determined the motor threshold
for each electrode contact.

NOTE: Motor threshold is determined by observing twitches in the hindlimb.

17 Sutured incision closed.

NOTE: Can staple or suture the incision closed.

18

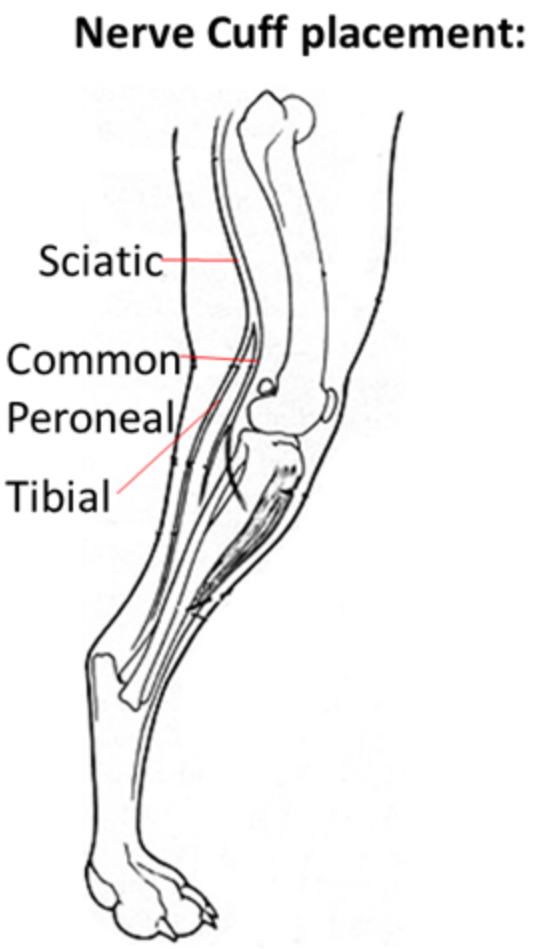

Figure 1: Diagram of cat hind limb with sciatic, common peroneal, and tibial nerve locations denoted for nerve cuff placement.

Implant Electrodes

19 Injectrode Information:

Injectrodes were manufactured by Neuronoff, Inc. (Cleveland, OH, USA) using a similar polymer-conductor variant of the Injectrode as described previously by Trevathan *et al* (**2019**). Briefly, we mixed two parts of Pt-curing silicone elastomers (World Precision Instruments, FL, USA) with metallic silver particles (Sigma-Aldrich, MO, USA) and loaded the mixture into a syringe that was calibrated to hold 10 μL of Injectrode material. An insulated silver wire (AS-766-36; Cooner Wire Company, Chatsworth, CA, USA) with de-insulated ends was placed inside the syringe such that it became embedded in the Injectrode material upon curing on one end, while the other end was connected to the stimulator for DRG stimulation (Figure 2). Based on measurements taken from explanted Injectrodes, the lengths and diameters of the Injectrodes ranged from 4.0–6.0 (4.4 ± 0.7)

mm and 1.3–3.1 (1.8 ± 0.6) mm, respectively. These parameters correspond to an average surface area of 30.8 ± 12.5 mm^2.

20 Stainless Steel Electrode Information:
We manufactured stainless steel DRG electrodes in-house (University of Pittsburgh) using 1 mm diameter hypodermic tubing (McMaster-Carr, Elmhurst, IL, USA) cut to a length of 4.5 mm and crimped to a Teflon-insulated lead wire (AS632; Cooner Wire Company, Chatsworth, CA, USA). The surface area of the stainless steel electrode was 15.7 mm^2, which is 1.96 times smaller than the average surface area of the Injectrode.

21 After we implanted the nerve cuffs, we repositioned the cat to prone.

22 Used cautery to create an incision along spinal processes L6-L7.

23 Implanted electrode through either a partial laminectomy or a burr hole.

24 Partial Laminectomy

24.1 Perform a partial laminectomy of L6 and L7 to expose the DRG.

24.2 Place the electrodes on the dorsal surface of the crown of the DRG for stimulation.

25 Burr Hole

25.1 Expose the contralateral side of the DRG through a partial laminectomy as noted above.

25.2 Guide the burr hole by matching the locations of the DRG on the exposed contralateral side.

25.3 Drill a burr hole through the laminae overlying the DRG (Figure 2).

25.4 For Stainless Steel Electrode:
Place vertically in the hole.

For Injectrode:
Inject into the hole by placing the tip of the blunt needle into the hole (implantation video available on the corresponding SPARC dataset: DOI: 10.26275/qjai-jsin)

26 Filled the open cavity with 5 mL of saline to keep the tissue from drying.

27 Placed a stimulation ground electrode (AS636, Cooner Wire Company, Chatsworth, CA, USA) with ~5 cm exposure in between the skin and lumbodorsal fascia ipsilateral to the DRG electrodes for monopolar
stimulation.

28 Took photos of spinal cord, DRG, and electrode placement with transparent ruler and reference in place.

29 Measured Injectrode with calipers.

30

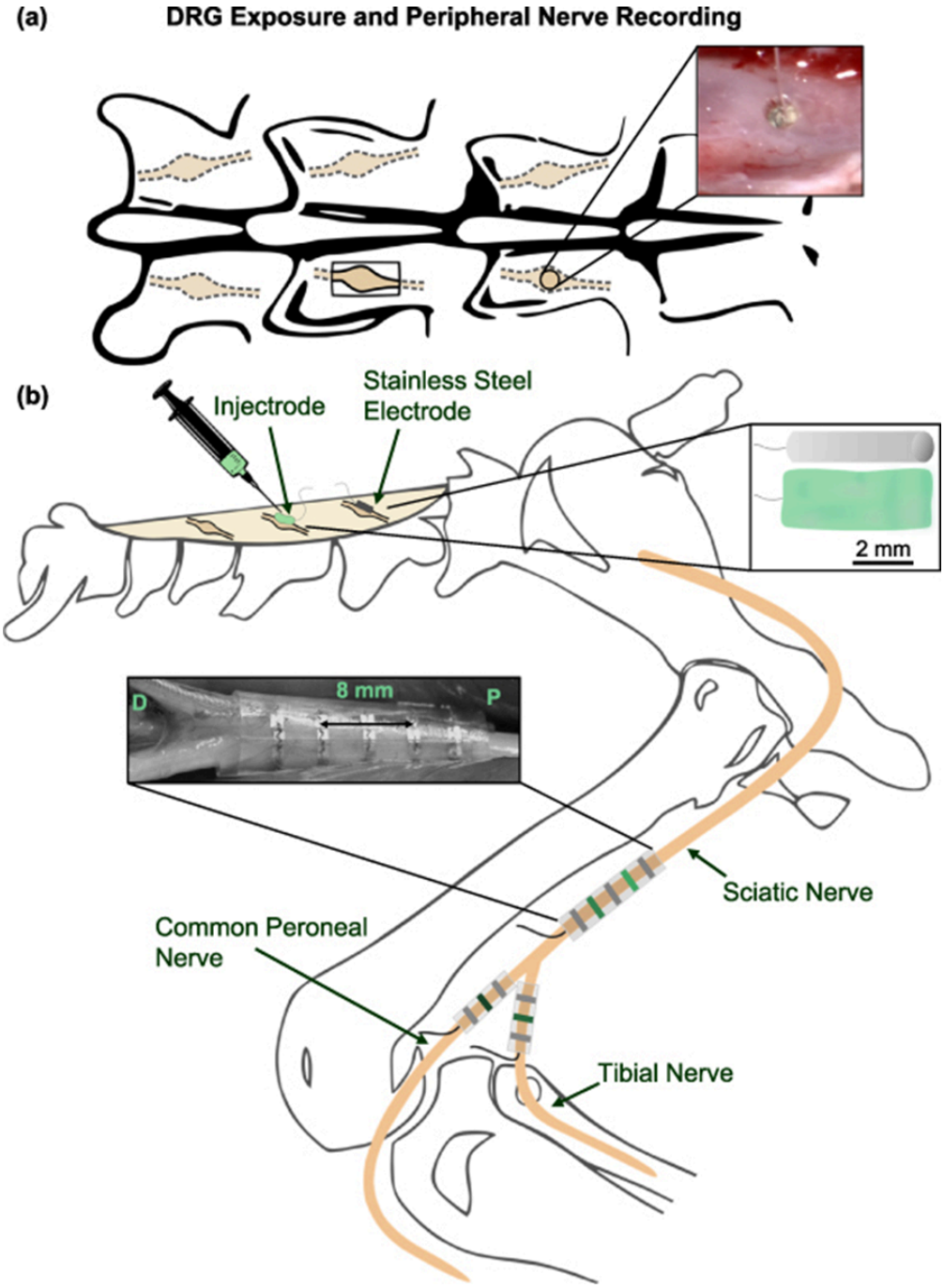

Figure 2: Experimental setup. (a) Exposure of dorsal root ganglion (DRG) by either a partial laminectomy or a burr hole. Inset: Injectrode delivered through a burr hole over the DRG. (b) An Injectrode or a stainless steel electrode were placed on top of a DRG for delivering stimulation. Antidromic evoked compound action potentials were recorded using spiral nerve cuffs placed
on the sciatic, tibial, and common peroneal nerves. Inset: five-contact nerve cuff placed on sciatic nerve. Cuff contacts were 4 mm apart. Gray nerve cuff contacts (1, 3, 5 for sciatic or 1, 3 for tibial and common peroneal) were shorted together and used as a reference. Green nerve cuff contacts (2, 4 for sciatic or 2 for tibial and common peroneal) were used for recording. P = proximal; D = distal. Inset: Scaled illustration of the stainless steel electrode and Injectrode.

Data Acquisition and Stimulation

31 Determined the motor threshold by delivering a train of five stimulation pulses to the electrode in the DRG.

32 Recorded electroneurogram (ENG) signals using Grapevine Neural Interface Processor (Ripple, Salt Lake City, UT, USA) and a single-ended headstage (Surf S2).

33 Filtered the ENG using a 0.1 Hz high pass filter and a 7.5 kHz low pass filter, followed by digitization at 30 kS s^{-1}.

34 Delivered monopolar stimulation using a high current ECOG + Stim front end (Ripple, Salt Lake City, UT, USA) or an A-M Systems 4100 Stimulator (A-M Systems, Sequim, WA, USA) at 58 Hz using biphasic, symmetric, cathode-leading pulses.

35 Stimulation pulse widths were either 80, 150, or 300 μs per phase depending on desired stimulation tests.

36 Constructed recruitment curves for all cats, electrodes, and DRG locations by varying the stimulation amplitude from below sensory threshold up to motor threshold in steps of 20–250 μA (for coarse and fine
steps).

37 Visualized responses to select the stimulation amplitude range for the recruitment curves.

37.1 Interpolated over a 1 ms window to blank stimulation artifacts.

37.2 Filtered the ENG using a second-order high-pass filter with a cutoff frequency of 300 Hz, and stimulation-triggered average the responses to the 600 stimulus pulses.

38 Randomized the order of stimulation amplitudes in the recruitment curve. Repeated 600 pulses at each amplitude with 5–10 s pauses between changes in amplitude.

39 Repeated steps 19 and 20 for each pulse width.

Electrochemical Impedance Study (EIS)

40 Conduct after stimulation trials have been run.

41 Used a three-electrode setup:

41.1 Working Electrode: The DRG stimulation electrode (either stainless steel or Injectrode)

41.2 Counter Electrode: A platinum wire (CHI115, CH Instruments, Inc., Austin, TX, USA; 32 mm long, 0.5 mm
diameter) placed distally in between the skin and muscle of the leg ipsilateral to the active (working) electrode.

41.3 Reference Electrode: An Ag|AgCl wire (CHI111, CH Instruments, Inc., Austin, TX, USA; 40 mm long, 0.5 mm
diameter) placed in between the skin and muscle of the back near the active electrode.

42 Used a potentiostat (CompactStat, Ivium Technologies, Eindhoven, The Netherlands) to perform EIS measurements.

43 Tested EIS frequency values ranging from 1 to 100 000 Hz at eight points per decade and a peak-to-peak voltage of 25 mV.

After Data Collection

44 Following testing with the electrodes delivered through the burr hole, removed the lamina to confirm the location of the Injectrode.

45 Injected 86 mg kg^{-1} euthasol intravenously (IV) to euthanize animal.

NOTE: Euthosol is made of 390 mg ml^{-1} pentobarb and 50 mg ml^{-1} phenytoin (anti-convulsant).

46 Confirmed that animal is deceased.

ENG Analysis

47 Used MATLAB code to analyze data (can find code on corresponding SPARC data repository: (insert link to repository)).

48 Determined the presence of an evoked compound action potential (ECAP) at a particular stimulation amplitude by comparing the root-mean-square (RMS) amplitude of the ENG to a threshold value (shown in
Ayers *et al* **2016**, Nanivadekar *et al* **2019**).

49 Used a bootstrapping method to verify consistency of the evoked responses:
For each stimulation amplitude, we generated a stimulus-triggered average of the ENG on each nerve cuff electrode using a random sample of 80% of the 600 total stimulus repetitions. We repeated this step 200 times.

50 Rectified and smoothed the average waveforms by calculating the RMS of each averaged ENG signal using a 100 μs sliding window with a 33 μs overlap with the previous window.

51 From each averaged RMS ENG signal, we calculated the ECAP threshold. This is defined as one standard deviation above the upper limit of the 99% confidence interval of the baseline RMS amplitude during a 1 ms period before each stimulation pulse. The threshold is the lowest stimulation amplitude that evoked a response in any of the four nerve cuff electrodes.

52 Determined if an ECAP is detected. An ECAP is considered detected if 95% of the subsets of random samples were supra-threshold during the same 100 μs window of the RMS ENG signal.

53 Confirmed the accuracy of the automated approach by visually identifying stimulation thresholds.

54 Calculated the conduction velocity of the ECAPs using the ENG from the sciatic nerve cuffs by following a procedure described in Fisher *et al* **2014**:

54.1 Measure the time difference between the peaks of the first, short-latency ECAP recorded from the proximal and distal tripoles in the five-contact cuff (contacts 2 and 4).

54.2 Contacts 2 and 4 are 8 mm apart, center-to-center. Divide that distance by the time difference (dt) between peaks of the ECAPs to obtain the conduction velocity for the lowest latency ECAP.

54.3 If multiple ECAPs are present, calculate the conduction velocity of the longer latency ECAP(s):

54.4 Determine the distance between the DRG electrode and the proximal tripole using the conduction velocity of the first ECAP and the latency between the onset of the stimulus

and the peak of that ECAP.

54.5 Divide the distance between the stimulating and recording electrodes by the latency of the later ECAP(s) to obtain their conduction velocity.

55 Used the calculated distance between the DRG electrode and cuff electrode, and the known conduction velocity ranges for primary afferent axons, Aα (80–120 m s^{-1}), Aβ (35–80 m s^{-1}), or Aδ fibers (5–35 m s^{-1}) to define latency windows from the onset of stimulation for each fiber type.

56 Used these latency windows to find the ECAP threshold for the different fiber types (Aα, Aβ, and Aδ) and construct recruitment curves for the different fiber type thresholds and each pulse width.

57 Depicted the peak-to-peak amplitude of the ECAP as a function of stimulation charge (current amplitude × pulse width).

58 Determined the recruitment rate for the different fiber types by fitting a line to the linear region of the recruitment curves using least squares regression (MATLAB's polyfit function, first order). The recruitment rate is the slope of the best-fit line.

NOTE: ECAPs from Aδ fibers were only detected when stimulation amplitude was very high (close to motor
threshold) in the corresponding paper (Dalrymple *et al* **2021**). This may lead to not collecting enough data points to create recruitment curves for Aδ fibers.

59 Compared the recruitment rates across all trials by normalizing all slope values to the slope of the recruitment curve obtained for the stainless steel electrode and a pulse width of 300 μs.

NOTE: 300 μs is the pulse width typically used in clinical applications of DRG stimulation (Liem *et al* **2013**, Deer *et al* **2017**, Kent *et al* **2018**, Graham *et al* **2019**).

Statistical Methods

60 Used the Shapiro–Wilk test to test for normality and Levene's test to assess the homogeneity of variance.

61 Used a student's *t*-test to compare threshold and recruitment rate means between the L6 and L7 DRG.

62 Performed a two-way analysis of variance (ANOVA) to test for effects of material (Injectrode or stainless steel) and fiber type (Aα, Aβ, or Aδ) on ECAP thresholds.

63 Performed two-way ANOVAs to test for significant effects of material and pulse width (80, 150, or 300 μs) on ECAP thresholds and recruitment rate.

64 Used the Bonferroni correction for multiple comparisons to adjust the resulting *p*-value.

NOTE: ANOVA and the post-hoc tests were performed using SPSS Statistics (version 26; IBM, Armonk, NY, USA).

65 Used a paired *t*-test to compare ECAP thresholds over time using Excel (version 2108; Microsoft Corporation, Redmond, WA, USA).

66 Performed equivalence testing using the two one-sided *t*-test (TOST) method (Lakens **2017**) in MATLAB using the TOST function (Rastogi **2017**) to compare the thresholds of the Injectrode versus the stainless steel electrodes across fiber types and over time.

NOTE: A p-value $\leqslant$0.05 indicated significance in this analysis.

Protocol references

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