Chronic recruitment of primary afferent neurons by microstimulation in the feline dorsal root ganglia

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Abstract

Objective. This study describes results of primary afferent neural microstimulation experiments using microelectrode arrays implanted chronically in the lumbar dorsal root ganglia (DRG) of four cats. The goal was to test the stability and selectivity of these microelectrode arrays as a potential interface for restoration of somatosensory feedback after damage to the nervous system such as amputation. Approach. A five-contact nerve-cuff electrode implanted on the sciatic nerve was used to record the antidromic compound action potential response to DRG microstimulation (2–15 μA biphasic pulses, 200 μs cathodal pulse width), and the threshold for eliciting a response was tracked over time. Recorded responses were segregated based on conduction velocity to determine thresholds for recruiting Group I and Group II/Aβ primary afferent fibers. Main results. Thresholds were initially low (5.1 ± 2.3 μA for Group I and 6.3 ± 2.0 μA for Group II/Aβ) and increased over time. Additionally the number of electrodes with thresholds less than or equal to 15 μA decreased over time. Approximately 12% of tested electrodes continued to elicit responses at 15 μA up to 26 weeks after implantation. Higher stimulation intensities (up to 30 μA) were tested in one cat at 23 weeks post-implantation yielding responses on over 20 additional electrodes. Within the first six weeks after implantation, approximately equal numbers of electrodes elicited only Group I or Group II/Aβ responses at threshold, but the relative proportion of Group II/Aβ responses decreased over time. Significance. These results suggest that it is possible to activate Group I or Group II/Aβ primary afferent fibers in isolation with penetrating microelectrode arrays implanted in the DRG, and that those responses can be elicited up to 26 weeks after implantation, although it may be difficult to achieve a consistent response day-to-day with currently available electrode technology. The DRG are compelling targets for sensory neuroprostheses with potential to achieve recruitment of a range of sensory fiber types over multiple months after implantation.

Keywords: sensory stimulation, neuroprostheses, chronic implantation, dorsal root ganglia

1. Introduction

There has recently been a great deal of progress in the mechanical design of prosthetic limbs for amputees, with
development of high degree-of-freedom devices, such as the Modular Prosthetic Limb (Johns Hopkins University, Applied Physics Laboratory, Baltimore, MD, USA) and the DEKA arm (DEKA Research and Development Corp, Manchester, NH, USA) [1, 2]. These devices have the potential to reproduce much of the functionality of the intact limb and may also be instrumented with sensors that monitor joint position, torque, and fingertip pressure to allow restoration of sensory feedback. Sensory feedback is crucial for motor control, and one of the major impediments to adoption of advanced prosthetic technologies is this lack of sensation [3, 4]. Remarkably, simple body-powered prostheses are often preferred over the more advanced devices, in large part because of the sensory feedback provided by the harness and control cable [5]. Without cutaneous and proprioceptive feedback from the limb, users of these advanced devices must rely mainly on visual feedback for information about limb orientation and grip force [5].

There is a growing body of evidence that suggests it may be possible to restore sensory function by electrically stimulating the peripheral nerves that remain intact in the limb above the level of amputation. Multiple studies have demonstrated that stimulation of peripheral nerves results in activation of neurons in primary somatosensory cortex (S1). In one study, Utah slant electrode arrays were implanted in the median, ulnar, and radial nerves of a monkey, and somatosensory evoked potentials were recorded in S1 via an electrode grid on the surface of the brain [6]. While this method did not provide information on the modality or perceived location of the stimulus, it did demonstrate that short latency (less than 20 ms) cortical responses occurred after stimulation in the peripheral nerves. In a recent study from our lab, Utah electrode arrays were implanted into S1 and the lumbar dorsal root ganglia (DRG) of a cat, and stimulus pulses were patterned to replicate the firing of action potentials recorded in the DRG during leg movements [7]. In some cases, multi-unit cortical responses to patterned electrical stimulation were similar to the cortical responses recorded during passive leg movement.

Additional studies in humans have demonstrated that peripheral nerve microstimulation evokes sensory percepts that are localized to specific regions of the hand and forearm [8–10]. In one study, longitudinal infraspinacular electrodes (LIFEs) were implanted in the median nerve stumps of three amputees. In all three individuals, electrodes were identified that could selectively elicit distally referred sensations of either thumb pressure or elbow flexion/extension [8]. Further, the magnitude of those sensations was related directly to the frequency of stimulation. This study suggested that there is great potential for restoring sensory function to amputees via electrical stimulation. However, long-term studies have not been performed to test the chronic stability of LIFE or other peripheral nerve interfaces for sensory restoration.

This paper describes the testing of microelectrode arrays implanted chronically in the DRG to stimulate primary afferent neurons to restore sensory function. The DRG are attractive targets for achieving a stable peripheral neural interface for a number of reasons. The spinal roots provide the potential for a mechanically stable anatomical location for the implantation of penetrating electrodes because they may be less prone to the stretching and movement that occurs in the more distal regions of peripheral nerves [11, 12]. Further, they can be accessed by minimally invasive surgical techniques that are used commonly during procedures such as neuroma removal or spinal root decompression [13, 14]. Additionally, because the dorsal and ventral spinal roots offer complete segregation of sensory and motor functions, respectively, electrodes placed in the DRG will activate sensory neurons. Anatomical selectivity can be achieved through the use of multiple microelectrodes, implanted in the spinal roots at different vertebral levels [15]. Through the use of multi-electrode arrays and varied stimulation parameters, it may also be possible to activate proprioceptive or cutaneous fibers in isolation to elicit distinct sensations [16]. Previous work from our lab has demonstrated that it is possible, in an acute preparation, to selectively activate either Group I, Group II, or Aβ primary afferents with penetrating microelectrode arrays in the L6 and L7 DRG, and to produce cortical responses that resemble the natural response to changes in limb position [7, 17].

The goal of this study was to examine the chronic stability and selectivity of the response to microstimulation via penetrating microelectrodes in the DRG. Floating microelectrode arrays (Microprobes for Life Science, Gaithersburg, MD, USA) were implanted chronically in the L6 and L7 DRG of four cats and the stability and selectivity of the response to stimulation was monitored for up to six months after implantation. Initially, many electrodes elicited both Group I and Group II/Aβ responses with low thresholds. Over time, thresholds increased, the number of electrodes that elicited a response decreased, and there was fluctuation in the conduction velocity and shape of the responses elicited by individual channels. There were, however, still nine implanted electrodes across three arrays that continued to evoke a response at 24 weeks after implantation, suggesting that it is possible to achieve a long-term chronic interface with the DRG as part of a sensory neuroprosthesis.

2. Methods

The ability to activate sensory neurons in the DRG was assessed by measuring the electroneurogram (ENG) in the sciatic nerve in response to microstimulation of the L6 and L7 DRG using floating microelectrode arrays (FMAs). All procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

2.1. Implanted microelectrodes and nerve-cuff

Four adult male cats (4.3–5.2 kg) were included in this study. Prior to implantation of stimulating and recording electrodes, anesthesia was induced with ketamine, followed by intubation and administration of isoflurane (1–2%) for the duration of the procedure. Blood pressure, oxygen saturation, expired CO₂, and body temperature were monitored throughout the procedure and kept within normal physiological ranges.

Figure 1 shows a schematic of the implanted electrodes and hardware used during this study. In each cat, a five-contact spiral nerve-cuff electrode (Ardiem Medical, Indiana,
had slightly longer electrode shanks (2.4, 2.6, 2.8 and 3.0 mm) than all other arrays, although care was taken to ensure that the electrodes were implanted appropriately into the DRG. During implantation of all arrays, a custom vacuum holder attached to a micromanipulator was used to position the array over the DRG during visualization with a surgical microscope. The array was positioned so that its long axis was aligned with the proximal/distal axis of the spinal root. A pneumatic inserter with 1.5 mm of travel (Blackrock Microsystems, Salt Lake City, UT) was used to rapidly insert the array through the epineurium into the DRG. Following initial insertion, electrophysiological recordings were performed to verify sensory responses evoked by manipulation of the left hindlimb. Single-unit action potential recordings were acquired from the implanted FMA, sampled at 25 kHz, and digitized via a neurophysiology recording system (RZ2, Tucker-Davis Technologies, Alachua, FL). Based on the quality of these recordings and visualization of the array under a surgical microscope, repeated impacts were applied with a pneumatic inserter that had 1 mm of travel until the array was sufficiently inserted into the DRG. This procedure was necessary because many of the electrodes were longer than the available travel of the inserter, and therefore required multiple impacts to achieve full insertion into the DRG. Rapid insertion was required as the DRG is covered in a tough perineurium that is difficult to penetrate using slow insertion methods. Lead wires were tacked to the spinal dura with 8–0 silk sutures and routed to a percutaneous port between the iailiac crests. A stainless steel ground wire (AS636, Cooner Wire Company, Chatsworth, CA), which acted as the stimulation return, was fixed to the iliac crest with a bone screw. In some animals, a layer of silicone rubber was poured over the arrays to minimize adherence of connective tissue (Kwik-Cast, WPI, Sarasota, FL). A baseplate was attached to the dorsal fascia and iliac crests and all lead wires and external connectors were passed into a protective plastic backpack mounted to the baseplate via percutaneous posts.

2.2. Stimulation and data acquisition

At regular intervals after implantation, the stability and selectivity of the response to stimulation was measured during anesthetized recording sessions. During these sessions, anesthesia was induced and maintained by intramuscular injection of dexmedetomidine (0.04 mg kg$^{-1}$). Stimulus pulses were applied at 33 pulses per second through randomly selected individual electrodes within each FMA while antidromic propagation of compound action potentials (CAPs) was recorded via the nerve-cuff. All stimuli were 200 $\mu$s cathodic-leading, charge-balanced biphasic pulses, with current amplitudes of 2, 5, 7, 10, or 15 $\mu$A. The anodic phase of each stimulus pulse was 400 $\mu$s and half the amplitude of the cathodic phase. Current amplitudes were chosen based on results of our previous study of the acute response to DRG microstimulation, as well as to maintain stimulation safety, to avoid activating spinal reflexes that could cause muscle contraction and movement artifact, and to constrain the duration of experiments and minimize the time under anesthesia.

Figure 1. (a) Rendering of the 32-channel FMAs implanted in the L6 and L7 DRG of 4 cats. Electrode shank lengths spanned the depth of the DRG and exposed tip lengths varied from 30 to 120 $\mu$m. Each array also included two ground electrodes and two reference electrodes at the corners (3.0 mm length). (b) Diagram of the experimental setup. Microstimulation in the DRG generated compound action potentials which propagated antidromically along the sciatic nerve and were recorded differentially via a nerve-cuff electrode.

PA, USA) was implanted around the left sciatic nerve to record the antidromic ENG response to microstimulation in the DRG. Contacts were 1 mm wide platinum bands spaced 4 mm apart along the length of the nerve. The proximal, distal, and central contacts were tied together to act as a reference, and ENG signals were recorded from the second and fourth contacts differentially with respect to the reference. Two sutures were tied loosely around the cuff to ensure that it remained in place for the duration of chronic experiments and the lead wires were tunneled under the skin to a percutaneous port centered on the back between the iliac crests.

A dorsal laminectomy was performed to expose the left L6 and L7 DRG, and 32-channel FMAs was implanted in each one. The platinum-iridium electrodes of each FMA (figure 1(a)) had a variety of lengths designed to span the depth of the DRG (1.2, 1.4, 1.6, and 2.0 mm) and exposed tip sizes of 30, 60, 90, and 120 $\mu$m (mean ± std pre-implant impedance for each site size: 144 ± 128 k$\Omega$, 62 ± 57 k$\Omega$, 31 ± 28 k$\Omega$, 14 ± 13 k$\Omega$), allowing us to examine the effect of tip size and impedance on the selectivity and stability of microstimulation. The array implanted in the L6 DRG of Cat C...
Figure 2. The procedure used to analyze ENG data and identify the presence of CAPs. (a) Stimulus-triggered averaging and subsampling were performed to reduce the noise in the ENG signal and quantify the variability in the CAPs. Based on the differences in conduction velocity of CV_{fast} and CV_{slow} fiber types, as well as the propagation delay between the second and fourth contacts within the nerve-cuff electrode, time windows (gray boxes) were created to differentiate CV_{fast} and CV_{slow} fiber types. (b) A sliding 0.5 ms window of signal from the fourth contact within the cuff was cross-correlated with signal from the second contact. If the local cross-correlation surpassed the mean plus three standard deviations of the cross-correlation of the noise, a CAP was identified. (c) For signals that included a CAP, the RMS of the signal was calculated at each current amplitude. RMS signals above the mean plus 1.5 standard deviations of the noise signal were identified as supra-threshold. Linear interpolation was used to determine the threshold current amplitude.

anesthesia [17]. ENG signals were amplified (gain = 10 000) and band-pass filtered (300–10 000 Hz) with a differential amplifier (Model 1800, A-M Systems, Sequim, WA, USA), digitized and sampled at 300 kHz with a data acquisition board (USB-6259, National Instruments, Austin, TX, USA).

At the end of most experimental sessions, electrode impedances were recorded at 1 kHz using one of two multi-channel potentiostat systems (niPOD, NeuroNexus Technologies, Ann Arbor, MI, USA or CompactStat, Ivium Technologies, Eindhoven, The Netherlands). Similarity of the impedance results for these two systems was confirmed via unpublished in vitro testing of all electrodes in an FMA placed in a saline bath. Electrode impedances were used to track changes in the electrode tissue interface. We expected that changes to the electrode surface as a result of electrochemical reactions during stimulation (e.g. pitting) would result in a higher electrode surface area and a corresponding decrease in impedance. Alternatively, increases in electrode impedance might correspond to encapsulation of the electrode tip.

2.3. ENG analysis

This study relied on the antidromic propagation of CAPs along the sciatic nerve as a measure of recruitment in the DRG and a surrogate for the orthodromic action potentials traveling into the central nervous system. Stimulus-triggered averaging (figure 2) was performed on the filtered ENG signals to isolate evoked responses. A set of automated techniques was used to determine the presence or absence of CAPs within the signal. The conduction velocity for each CAP was measured from its propagation delay between the stimulating and recording electrodes. Based on these conduction velocities, the fiber type of the recorded axons was inferred, and selectivity was determined based on the ability to activate only Group I or Group II and A\textsubscript{\beta} fibers.

2.3.1. Stimulus-triggered averaging. ENG signals typically have low signal-to-noise ratio with a great deal of contamination from both neural and non-neural bioelectric sources. To reduce the amplitude of this noise and reveal the underlying CAPs evoked by stimulation, stimulus-triggered averaging (figure 2(a)) was performed on all ENG recordings. For every electrode within the FMA, 600 repetitions of each stimulus amplitude were applied. A subsampling procedure was performed to generate a distribution of average responses to these stimuli by repeatedly averaging (100 times) random selections of 500 of the 600 responses to stimulation. These distributions were used to perform statistical tests and
determine differences in the magnitude of the ENG response under different stimulus conditions. All of the methods described below rely on these subsampled stimulus-triggered averages, rather than the raw ENG signal.

2.3.2. Measurement of conduction velocity and fiber type. For a neural interface to successfully restore sensory function, it should selectively activate multiple sensory modalities, especially proprioceptive and cutaneous sensations, in a controlled manner. Primary afferents can be loosely segregated into separate populations based on their axonal diameters and corresponding conduction velocities, though those populations have some overlap [18–20]. In the cat, Group I proprioceptive afferents, which are sensitive to muscle length, stretch velocity, and force, typically have conduction velocities between 75 and 120 m s⁻¹, while Group II proprioceptive afferents, which are sensitive primarily to muscle length, typically have conduction velocities ranging from 33 to 60 m s⁻¹ [18, 19]. For Aβ cutaneous afferents, conduction velocities typically range between 45 and 80 m s⁻¹ [20]. For this study, primary afferents were segregated into two groups based on these conduction velocities and corresponding sensory modalities: fibers with fast conduction velocities between 75 and 120 m s⁻¹ (CVfast), representing mainly Group I proprioceptive afferents, and fibers with slower conduction velocities between 33 and 75 m s⁻¹ (CVslow), representing a mix of Group II proprioceptive and Aβ cutaneous afferents. Other primary afferents, such as Group III, Aδ and C fibers have smaller diameter axons and produce lower amplitude ENG signals that are difficult to discriminate, and were therefore not considered in this study [17]. By measuring the conduction velocity of CAPs traveling through the nerve-cuff electrode, it is possible to infer the most likely sensory modalities of the activated neurons. To measure conduction velocity, the distance between the stimulating and recording electrodes was estimated using a supra-threshold stimulus pulse (15 μA) in the DRG that elicited an obvious ENG response. By measuring the time delay between the peaks in the recorded ENG signals for the second and fourth contacts of the nerve-cuff electrode (which were separated by 8 mm), it was possible to calculate the conduction velocity of the stimulated fibers. Further, by measuring the time delay between the stimulus onset and the peak of the CAP, it was possible to estimate the distance from the stimulating electrode to the nerve-cuff electrode. Based on the conduction velocities for CVfast and CVslow fibers, this distance was used to estimate time windows (gray boxes in figures 2(a), 3, 6) in which those fibers would conduct CAPs through the nerve-cuff electrode. For all subsequent recordings, ENG responses were classified as either CVfast or CVslow based on the time window in which they occurred.

2.3.3. Estimation of stimulus threshold. Throughout this study, the threshold current required to elicit a response to stimulation was used as a means of quantifying selectivity and stability. To quantify threshold, it was necessary to detect the presence or absence of CAPs in the noisy ENG signal. All steps in the detection process were performed on the subsampled, stimulus-triggered averaged data. First (figure 2(b)), the local cross-correlation (LCC) was calculated between the signals recorded from the second and fourth contacts of the nerve-cuff electrode. The process for calculating LCC is described in detail elsewhere [17], but briefly, the cross-correlation was calculated between the signal recorded from the second contact and a 0.5 ms window of the signal recorded from the fourth contact. The 0.5 ms window for the fourth contact was moved through either the CVfast or CVslow time window in 50 μs steps, and the peak of the cross-correlation was calculated. This peak was compared to the cross-correlation of a 0.5 ms window of noise with another window of noise that was the size of the CVfast or CVslow time window. If the peak of the LCC exceeded three standard deviations above the cross-correlation of the noise, the trial was identified as containing a CAP. Only electrodes that demonstrated CAPs in response to 15 μA stimulation were flagged as responding to stimulation. For those electrodes that elicited CAPs in response to 15 μA stimulation, the average signal power (RMS) was calculated (figure 2(c)) for the time windows corresponding to CVfast or CVslow fibers at all tested current amplitudes. A one-tailed Student’s t-test was used to determine whether the RMS of the signal within the CVfast or CVslow time window was above threshold. Threshold was defined as 1.5 standard deviations above the RMS of a 1 ms window of unstimulated noise. The accuracy of the method was determined by calculating the false positive and false negative rates when comparing the results with those of an expert human observer for a randomly selected subset of 200 responses. The threshold value was chosen because it produced low rates of both false positives (2%; mainly from CAPs that straddled the CVfast and CVslow time windows) and false negatives (6%; mainly from very low amplitude CAPs). Because only a limited number of stimulus current amplitudes were tested during this study, we estimated the threshold value by linearly interpolating (figure 2(c)) between the highest subthreshold current amplitude and the lowest supra-threshold amplitude.

2.3.4. Selectivity and stability of recruitment. Chronic stability and selectivity of the response to stimulation are especially important for a sensory stimulation neuroprosthesis. Tuning of stimulus parameters to achieve meaningful sensory feedback is likely to be a time-intensive process involving psychophysical metrics. As such, an ideal sensory interface should activate a distinct sensory modality, localized to the same peripherally referred location, with the same current amplitude from day to day. While it is impossible to infer what perceptions might have been evoked by a given stimulus in this study, the threshold response to stimulation and the type of fiber activated by that stimulation provide useful metrics for assessing efficacy. Selectivity for a given electrode was quantified as the difference in stimulation thresholds for activation of CVfast and CVslow fibers. Counts were made of the number of electrodes that selectively activated either CVfast or CVslow fibers and statistical analyses were performed to determine the effects of factors such as implant location (L6 versus L7 DRG) and electrode size on the stability of recruitment.
To quantify the stability of the response to stimulation, the threshold for activation of CV\textsubscript{fast} and CV\textsubscript{slow} afferents was monitored over time. However, it was difficult to make a statistical comparison of threshold values across factors that may affect the stability of stimulation (i.e. time, cat, implant site, electrode size, fiber type, electrode impedance) because of a ceiling effect caused by limiting stimulation to 15 \( \mu \)A. In the case when an electrode did not generate a response at or below 15 \( \mu \)A, it was treated as a missing observation. Because of their dependence on the factors of interest, missing observations were not distributed randomly throughout the data set. The probability of observing a missing value depended clearly on time post-implantation, making simple statistical techniques like analysis-of-variance unreliable. To circumvent this problem, a related quantity was analyzed: the probability (\( P_{15} \)) of electrodes generating CAPs when stimulated at or below 15 \( \mu \)A. This model assumes that, on the logit scale, the probability of a CAP is linear in time, with different intercepts, \( \alpha_{s,g} \), and different slopes, \( \beta_{s,g} \), for each of the 4 \( \times \) 2 combinations of tip size and fiber type. Likelihood ratio (LR) tests revealed that the slopes were not significantly different from one another, suggesting the relative effect of time was the same for all electrodes and fiber types. Furthermore, the intercepts could be decomposed into separate additive effects of fiber type and tip size. Hence, the probability of a CAP at \( t \) time points between 64 and 134 days after implantation, there is also a response in the CV\textsubscript{fast} time window.

2.3.5. Logistic regression model of \( P_{15} \). To study the change in \( P_{15} \) of each implanted electrode array with respect to various factors of interest such as time, tip size, and fiber type, a logistic regression was performed on the data set, using the model:

\[
\text{logit}(P_{15}(s, g, t)) = \log \left( \frac{P_{15}(s, g, t)}{1 - P_{15}(s, g, t)} \right) = \alpha_{s,g} + \beta_{s,g} \times t;
\]

where \( P_{15}(s, g, t) \) is the probability that an electrode with tip size \( s \) elicits a CAP for fibers of type \( g \), when stimulated with a current less than or equal to 15 \( \mu \)A. This model assumes that, on the logit scale, the probability of a CAP is linear in time, with different intercepts, \( \alpha_{s,g} \), and different slopes, \( \beta_{s,g} \), for each of the 4 \( \times \) 2 combinations of tip size and fiber type. Likelihood ratio (LR) tests revealed that the slopes were not significantly different from one another, suggesting the relative effect of time was the same for all electrodes and fiber types. However, the intercepts were significantly different (\( p < 0.05 \)), suggesting that different tip sizes and fiber types had different effects on the probability of producing a CAP at the time of implantation. Furthermore, the intercepts could be decomposed into separate additive effects of fiber type and tip size.
size, i.e. there was no interaction effect between these factors. Therefore, the final model for $P_{15}(s, g, t)$ was

$$\text{logit}(P_{15}(s, g, t)) = \alpha_g + (\gamma_0 + \gamma_1 s) + \beta t,$$

(2)

where $\alpha_1 = 0$ so that $\alpha_2$ measured the differential effect between $CV_{\text{fast}}$ and $CV_{\text{slow}}$ fibers, $\gamma_1 s$ was the linear effect of tip size $s$, and $\beta t$ was the linear effect of time $t$, which was the same for all electrodes and all fiber types.

Impedance values were also considered as a factor that may affect $P_{15}$, however we found no relationship between changes in impedance and changes in threshold. As such, that factor was not included in the final formulation of the response rate model.

3. Results

The goal of this study was to examine the chronic stability and selectivity of microstimulation in the lumbar DRG of anesthetized cats using penetrating microelectrode arrays. FMAs were implanted in the left L6 and L7 DRG of four cats, and the threshold response to stimulation and number of responding electrodes were tracked over time.

3.1. Typical responses to stimulation

Figure 3 shows the responses to stimulation through two electrodes from one L6 DRG array. Figure 3(a) shows the response to stimulation at different current amplitudes through a single electrode on a single day. In this case, a $CV_{\text{fast}}$ response (in the light gray box) was first observed at a stimulation amplitude of 10 $\mu$A, followed by a more complex combination of $CV_{\text{fast}}$ and $CV_{\text{slow}}$ responses (in both light and dark gray boxes) at 15 $\mu$A. Figure 3(b) shows the response evoked by a different electrode at 15 $\mu$A at times ranging from 9 to 134 days after implantation. On many days, a $CV_{\text{fast}}$ response was visible, while on some later days (e.g. days 51, 64, and 119), there was an additional slower response that straddled the $CV_{\text{fast}}$ and $CV_{\text{slow}}$ time windows. These types of responses were typical, with recruitment of one fiber type and, for some electrodes, additional recruitment of a second fiber type at higher amplitudes. In many cases, as in figure 3(b), a response was visible at early time points, but that response changed or disappeared and returned again at later time points. Figure 4 shows plots of the threshold response to stimulation within the first four weeks after implantation for each electrode in the L6 arrays of cats B, C, and D. These plots were typical of the response for both L6 and L7 arrays, and show a high degree of variability in thresholds as well as a significant reduction in the number of electrodes that responded to stimulation at amplitudes up to 15 $\mu$A over the first month after implantation.

3.2. Threshold stability over time

Figure 5 shows, for each cat and each implant site, the expected probabilities that electrodes would generate CAPs when stimulated at or below 15 $\mu$A, as functions of the number of weeks post-implantation, electrode tip size, and fiber type. The shaded areas are 95% pointwise confidence bands for the expected $P_{15}$. For clarity, only the smallest and largest tip sizes are shown. Table 1 shows the time windows over which responses were tracked in each of the four cats. Note that not all electrode arrays were tested at each time point after implantation, and that testing with Cat A did not begin until four weeks after implantation. Typically, longitudinal tracking ended either as a result of gross array failure caused by damage to the leads near the connector or because of a lack of response to stimulation (limited to 15 $\mu$A in most cats). Electrode impedances provided a useful tool for diagnosing lead damage and gross electrode failure, as impedances for all electrodes in an FMA would suddenly exceed 2 M$\Omega$ after leads...
Figure 5. $P_{15}$ for the largest and smallest electrodes in each implanted array across the time post-implantation. The implant periods ranged from 4 weeks (Cat D) to 26 weeks (Cat A). Shaded bands are 95% pointwise confidence intervals. The $P_{15}$ value for all arrays except the L7 DRG array for Cat A decreased over time, suggesting that the threshold for stimulation was increasing. For all arrays, larger electrodes had a higher $P_{15}$ than smaller electrodes. Also, CV$_{fast}$ fibers had a higher $P_{15}$ than CV$_{slow}$ fibers, suggesting CV$_{fast}$ fibers were activated more often.

Table 1. Summary of data collection intervals for all cats and reasons for terminating experiments.

| Array location | First time point (weeks after implantation) | Last time point (weeks after implantation) | No. of sessions | Reason for termination |
|----------------|---------------------------------------------|---------------------------------------------|-----------------|------------------------|
| Cat A L6       | 4                                           | 26                                          | 13              | No response to stimulation |
| Cat A L7       | 4                                           | 11                                          | 10              | Lead broke             |
| Cat B L6       | 1                                           | 6                                           | 9               | Lead broke             |
| Cat B L7       | 1                                           | 6                                           | 9               | Lead broke             |
| Cat C L6       | 1                                           | 24                                          | 12              | End of study           |
| Cat C L7       | 1                                           | 24                                          | 12              | End of study           |
| Cat D L6       | 1                                           | 4                                           | 5               | Infection              |
| Cat D L7       | 1                                           | 4                                           | 3               | Infection              |

In all cats, there were multiple electrodes on each array that evoked a CAP in the nerve-cuff electrode. In the first week post-implantation, there was typically a high number of electrodes that evoked responses (3 cats; CV$_{fast}$: 105 out of 192 electrodes; CV$_{slow}$: 83 out of 192 electrodes) with average thresholds of $5.1 \pm 2.3 \mu A$ for CV$_{fast}$ and $6.3 \pm 2.0 \mu A$ for CV$_{slow}$ fibers. Four weeks after implantation, the number of electrodes that elicited a response in both the CV$_{fast}$ and CV$_{slow}$ time windows decreased (4 cats; CV$_{fast}$: 64 out of 256 electrodes; CV$_{slow}$: 30 out of 256 electrodes) and the mean threshold current amplitude for responsive electrodes increased to $9.2 \pm 3.6 \mu A$ for CV$_{fast}$ fibers and $11.2 \pm 2.6 \mu A$ for CV$_{slow}$ fibers. Cumulatively across Cats A and C, there were 9 electrodes that continued to evoke a response to stimulation at $15 \mu A$ up to 24 weeks after implantation.

Overall, $P_{15}$ decreased with time for all cats, both CV$_{fast}$ and CV$_{slow}$ fiber types, and all electrode tip sizes, except for the L7 DRG in Cat A (figure 5(b)), where the $P_{15}$ remained constant. The rates of decrease in $P_{15}$ were remarkably different for different cats, as were the initial $P_{15}$ values after implant. However, there were systematic patterns in the change in $P_{15}$. First, larger electrodes had higher $P_{15}$ values than smaller electrodes, as illustrated by the dotted and solid curves in each panel of figure 5, for all 16 combinations of cat, fiber group, and implant site. This suggests that larger electrodes achieved activation of both CV$_{fast}$ and CV$_{slow}$ fibers more often than smaller electrodes. Although this result was statistically significant only for the L6 and L7 arrays for Cat A (two-sided test p-values: $p < 0.001$), the L7 array for Cat B ($p = 0.014$), and borderline significant for the L7 array for Cat C ($p = 0.073$), the probability that this systematic difference happened by chance is very low. Comparing the curves in each pair of adjacent panels, the $P_{15}$ of the electrodes for Cats A broke. In Cat D, the experiment was terminated at four weeks post-implantation because of an infection at the percutaneous connector site.
Figure 6. (a) The ENG signal recorded in response to stimulus pulses with current amplitudes of 2, 5, 7, 10, and 15 μA, applied through a single FMA electrode at time \( t = 0 \), 4 weeks after implantation. There is a large CV\(_{\text{fast}}\) CAP in response to stimuli at 10 and 15 μA. (b) At 23 weeks, the same electrode did not elicit a response at 15 μA but a CV\(_{\text{fast}}\) response was evoked at 25 μA.

Figure 7. Summary of selectivity data for all electrodes implanted in all cats. (a), (b) The percentage of responding electrodes and the average thresholds for electrodes that only elicited either a CV\(_{\text{fast}}\) or CV\(_{\text{slow}}\) CAP. (c), (d) The percentage of responding electrodes that elicited both CV\(_{\text{fast}}\) and CV\(_{\text{slow}}\) CAPs, as well as the difference in threshold for their responses. (e) The total percentage of responding electrodes that selectively elicited either a CV\(_{\text{fast}}\) or a CV\(_{\text{slow}}\) response.

and C were significantly higher for CV\(_{\text{fast}}\) than CV\(_{\text{slow}}\) fibers (two-sided test: \( p < 0.001 \) for L6 and L7 arrays for Cat A, \( p < 0.001 \) and \( p = 0.006 \) for L6 and L7 arrays, respectively, for Cat C). The estimated effects for Cats B and D were not statistically significant. These results suggest that more electrodes recruited CV\(_{\text{fast}}\) than CV\(_{\text{slow}}\) fibers. Finally, for all four cats, the P\(_{15}\) of electrodes implanted in L7 decreased less sharply than the P\(_{15}\) of electrodes implanted in L6. Notably, there was no significant decrease for the L7 implant in Cat A. This result was statistically significant for Cats A and B (one-sided \( p < 0.001 \)), and borderline significant for Cats C and D (\( p = 0.053 \) and \( p = 0.067 \), respectively). Impedances remained constant over the course of the study, and there was no relationship between decreased P\(_{15}\) values and changes in impedance.

In Cat C, further testing at higher current amplitudes was performed at 23 weeks after implantation to determine if the reduced response rate was a result of increased thresholds or some other problem such as lead breakage. At that time point, only two electrodes were still responding to stimulation at 15 μA. However, when the current amplitude was increased to 30 μA, an additional 23 electrodes elicited responses to stimulation. As can be seen in figure 6, many of those responses at higher amplitudes appeared similar in shape to the responses elicited by lower amplitude stimulation at earlier time points. Because of the time constraints of the experiment, this testing procedure at higher amplitudes was only performed once in Cat C.

3.3. Selectivity of stimulation

To assess the selectivity of stimulation of CV\(_{\text{fast}}\) and CV\(_{\text{slow}}\) fibers, the threshold current amplitudes for eliciting those responses were compared for each electrode. Figures 7(a) and (b) show the percentage of electrodes that elicited a response and selectively activated only CV\(_{\text{fast}}\) or CV\(_{\text{slow}}\) fibers and the threshold for activation. Figures 7(c) and (d) show the percentage of electrodes that elicited a response and activated both CV\(_{\text{fast}}\) and CV\(_{\text{slow}}\) fibers, but activated one fiber type at a lower current amplitude than the other. Also shown is the difference in threshold for activation of CV\(_{\text{fast}}\) or CV\(_{\text{slow}}\) fibers for those electrodes. These plots demonstrate the dynamic range for selectively stimulating one fiber type when the threshold for the other
were selective for either CV_fast or CV_slow fibers. These results demonstrate that it was more common for electrodes to selectively activate only CV_fast fibers than to activate only CV_slow fibers. For those channels that activated both fiber types, CV_slow fibers were more commonly activated at lower current amplitudes than CV_fast fibers. The mean difference in estimated stimulation threshold (i.e. dynamic range) between CV_fast and CV_slow fibers was 2.6 ± 2.3 μA for electrodes that activated CV_fast fibers first, and 3.2 ± 2.8 μA for electrodes that activated CV_slow fibers first. Overall, there was a high degree of variability in the number of CV_fast and CV_slow fibers activated selectively, with approximately the same proportion of each fiber type activated selectively at early time points, but with more CV_fast than CV_slow fibers activated at time points after 6 weeks.

4. Discussion

The goal of this study was to assess the chronic stability and selectivity of primary afferent recruitment using penetrating microelectrode arrays in the DRG. Based on the results of this study, it is clear that it is possible to achieve selective activation of both CV_fast and CV_slow fiber types with chronically implanted electrodes. However, the likelihood of recruiting only CV_slow fibers declined over time. We also found that, within the first week after implantation, thresholds for activating both CV_fast and CV_slow fibers were very low, averaging between 5.0 and 6.5 μA. These thresholds are higher than those from our previous work in acute experiments, where activation of DRG neurons occurred at an average of 2.7 ± 1.3 μA [17]. This difference in thresholds can likely be attributed to differences in stimulating electrode technology, variations in data analysis methodology, and the assumed tissue response that likely occurred during the one week delay before the first set of data was collected in the current study. These thresholds are generally similar to those reported for activating DRG neurons in acute experiments with LIFEs or microneurography needle electrodes [21, 22].

During the first month after implantation, mean thresholds increased and the number of electrodes that elicited a response to stimulus pulses up to 15 μA decreased. Further testing in one animal at higher amplitudes (up to 30 μA) demonstrated continued responses to stimulation on many electrodes (23 out of 64) at 23 weeks after implantation. The decreased P15 and the restored response at higher current amplitudes suggest that a ceiling effect was occurring as a result of the limit that was chosen for the maximum stimulus amplitude to test in this study. We chose to limit the maximum amplitude to 15 μA for several reasons. First, we wanted to reduce the risk of tissue damage associated with high amplitude stimulation. While safety limits have not been determined for DRG microstimulation, studies of intracortical microstimulation in cat brain suggest that intensities as low as 20 μA (4 nC/phase) may cause neuronal damage [23]. Second, we wanted to avoid recruitment of spinal reflexes during testing. Lastly, we needed to impose a limit on the number of stimulus amplitudes tested on each of the 64 electrodes to limit the total time each animal spent under anesthesia. In future testing, it may be beneficial to use closed-loop algorithms such as binary search to intelligently choose stimulation parameters and avoid the ceiling effects of the current experimental protocol. Such an intelligent algorithm would need to identify the presence or absence of CAPs in real-time, and could potentially be designed to perform only enough stimulus repetitions to confidently identify a CAP from the noisy ENG signal. Alternatively, it may be appropriate to rely on a behavioral response from awake animals as a means to detect stimulation thresholds [24].

A variety of factors may have contributed to the decrease in P15 over time, such as migration of cells away from the electrodes, mechanical failure of electrode leads, accumulation of a tissue encapsulation layer around the electrodes, or damage to the electrodes as a result of electrochemical reactions during stimulation. Additionally, the variability observed between the P15 values in L6 and L7 DRG may be attributed to differences in anatomical structure, surgical access, and implant technique. The lack of a relationship between impedances and P15 values suggests that there were not significant changes in the electrode-tissue interface over time, and the observed changes in threshold may be a function of changes in the location or density of neurons near the electrode tips. Future studies should include histological analysis of neural tissue near the electrodes to explore the consistency of the placement and orientation of the electrodes with respect to the DRG as well as the biological response to the electrodes. As with microelectrode implants at other locations in the nervous system (e.g. periphery, cortex, etc.), the longevity and stability of neural interfaces with the DRG may be improved by further development of technologies such as flexible electrodes or chemical agents to suppress immune response and promote neural growth [25].

From the ENG data it is not possible to identify whether the same set of neurons were activated by each electrode from day to day. When the response to stimulation disappeared and reappeared over time, the activated neuron pool certainly changed, but in cases such as in figure 3(b), where a CV_fast response occurred at each time point from 64 to 134 days after implantation, it is unclear whether those responses were always from the same set of neurons. However, for the CV_fast response in figure 3(b), as well as many other examples across all animals, the size, shape, and time delay of the CAP appears to be consistent over time. As figure 4 makes clear, though, there is a great deal of fluctuation in the response to stimulation within the first month after implantation. With respect to the implementation of a sensory neuropaethesis, it is unclear how these fluctuations would translate to perceived sensation. It seems likely that, over the first month after implantation, the rapid changes in response would lead to variability in sensation, but, as in the case of figure 3, the perceived sensation may stabilize over time. Future work will require significant effort to characterize the perceived sensation as a result of stimulation over time.

Throughout this study, activation of CV_fast or CV_slow fibers was used as a representation of the ability to selectively recruit
different sensory modalities. Based on the data in figures 5 and 7, it seems that, initially, the thresholds for CV_fast and CV_slow activation were similar and that approximately equal numbers of electrodes selectively activated CV_fast and CV_slow fibers. At later time points, however, more electrodes elicited CV_fast than CV_slow responses. Further, those electrodes that elicited a CV_slow response were more likely to elicit a CV_fast response at slightly higher amplitudes, suggesting the dynamic range for selectively activating CV_slow fibers may be limited. Models of the response to stimulation in the DRG predict that medium diameter (i.e., CV_slow) fibers will be recruited in a slightly higher proportion than large diameter (i.e., CV_fast) fibers [26]. Additionally, our previous work with acute implantation of penetrating electrodes in the DRG demonstrated that approximately equal proportions of CV_fast and CV_slow fibers were activated [17]. Those results largely agree with the initial phase of this study, although further work should be devoted to studying the difference in CV_fast and CV_slow responses at later time points after implantation.

Overall, this study represents the first example of a chronic microstimulation interface with the DRG and suggests that the DRG is an appropriate target for interfacing with the peripheral nervous system to restore sensory function after injury, though it will likely be necessary to develop electrode technologies that can provide a more stable interface with the nerve. There are, however, some important limitations that should be noted with respect to this study. First, the study relies on antidromic propagation of CAPs to evaluate the effectiveness of sensory stimulation, and classifies sensory modality based on conduction velocity. While this approach provides a good first approximation for the efficacy of a sensory neuroprosthesis, further testing in awake animals is needed to determine if the animals can detect stimulation at the threshold amplitudes found in this study. It will be impossible to evaluate with certainty the qualia of sensory percepts experienced by the animals, but ultimately we would like to assess sensations evoked by stimulation of proprioceptive and cutaneous afferents. Human studies will be needed to definitively answer these and other questions about the percepts evoked by DRG microstimulation.

A second limitation of the study is the relatively small sample size. Out of eight FMAs implanted in the DRG of four cats, only three lasted to the end of the study without suffering gross array failure such as lead breakage or infection. This certainly limits the predictive power of the study and does not allow for generalization of the ability to evoke responses in other animals. It should be noted, however, that in all of the arrays that did not suffer gross mechanical failure (Cat A: L6; Cat C: L6 and L7), there remained multiple electrodes that could elicit CAPs at least 24 weeks after implantation. These results demonstrate that it is possible to evoke responses via microstimulation of the DRG with chronically implanted penetrating electrode arrays for an extended period of time after implantation.

An additional limitation of this study results from the intrinsic characteristics of the ENG signal. The CAPs recorded from the ENG signal often contain multiple overlapping responses, which can sum both constructively and destructively. This summation may result in the appearance that the response to stimulation is actually either larger or smaller than the individual superimposed responses would be. Because the procedure for estimating threshold relied on the RMS of the ENG signal, this may have resulted in slight under- or overestimation of the stimulation threshold. Since this effect would only affect the linear interpolation procedure used to estimate threshold, but likely would not affect the LCC procedure for detecting CAPs, it is unlikely to have a significant effect on the results presented here.

A final limitation occurs because of the anatomical structure and innervation of the hindlimb. In this study, stimulating electrodes were implanted in the L6 and L7 DRG, and ENG recordings from the sciatic nerve were used to detect the response to stimulation. While many neurons whose cell bodies are in the L6 and L7 DRG have axons that project through the sciatic nerve, some may have projections through the femoral nerve or may exit the sciatic nerve above the level of the cuff, making them undetectable with this method. This may have contributed to the observed differences in P15 for electrodes implanted in the L6 and L7 DRG, or may have resulted in a slight underestimation in the overall number of electrodes that elicited a response to stimulation. Despite these limitations, this study presents the first example of a chronic neural interface with the DRG for sensory stimulation. These results demonstrate that it is possible to activate multiple populations of primary afferents independently with penetrating arrays implanted in the DRG, and that responses can be elicited by microstimulation up to 26 weeks after implantation. These findings suggest that the DRG are potentially feasible sites for sensory neural interfaces. Future work will include detailed histological analysis of the tissue near the tips of the implanted electrodes in order to gain additional insight into the failure modes and to develop improvements to the electrode design that may extend the usable life and functionality of these devices. Future future work should focus on behavioral testing of the response to stimulation, as well as testing of alternative stimulus waveforms, pulse widths, and paradigms that might further improve selectivity and allow for tuning of the type of fibers activated by individual stimulating electrodes [27].

5. Conclusions

This study presents results from the first chronic implantation of stimulating electrodes in the DRG as part of a neuroprosthesis for sensory stimulation. Over 26 weeks after implantation, a subset of electrodes elicited CAP responses in the sciatic nerve at stimulation amplitudes of 15 μA or below, though the number of electrodes that responded to stimulation decreased and threshold for eliciting a response increased over time. In one cat, only two electrodes still elicited a response at 23 weeks after implantation at 15 μA, but when the stimulation amplitude was increased to 30 μA, over twenty additional electrodes elicited a response. Based on CAP conduction velocity, it was possible to selectively activate both CV_fast and CV_slow primary afferents via many of the implanted electrodes,
though the percentage of electrodes that selectively activated CV\textsubscript{slow} fibers decreased over time. These results suggest that the DRG is a viable anatomical location for chronic stimulation as part of a sensory neuroprosthesis, and that the approach merits further study.

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Appendix. Analysis of P\textsubscript{15}

Below is a detailed description of the statistical analysis performed on P\textsubscript{15} data to determine effects of factors such as time since implant, animal, DRG (L6 versus L7), electrode tip size, and stimulated fiber type (CV\textsubscript{fast} versus CV\textsubscript{slow}) on threshold. We found that electrode impedances remained constant over the course of the study, and as such could not account for changes in P\textsubscript{15}. Therefore, we did not include them in the following models.

\textbf{A.1. Logistic regression models of P\textsubscript{15}}

Let \(P_{15}(s, g, t)\) be the probability at time \(t\) that electrodes with tip size \(s\) for fibers of type \(g\) generate a CAP when stimulated at or below 15 \(\mu\text{A}\), for an implanted array in a particular DRG in a particular cat. We consider \(P_{15}(s, g, t)\) separately for each implant because their estimates are visibly different across cats (A, B, C, D) and DRG (L6, L7); see figure 5. We estimate \(P_{15}(s, g, t)\) using a logistic regression fitted to the observed proportions of electrodes with tip size \(s\) that activate fibers of type \(g\) at time \(t\). We start by modeling the logit of \(P_{15}(s, g, t)\) according to

\[
\logit(P_{15}(s, g, t)) = \log \left( \frac{P_{15}(s, g, t)}{1 - P_{15}(s, g, t)} \right) = \alpha_{s,g} + \beta_{s,g} \times t; \quad \text{(Model 1)}
\]

i.e. we assume that, on the logit scale, the probability of a CAP is linear in time, with different intercepts and different slopes for each of the \(4 \times 2\) combinations of tip size and fiber type. This is (Model 1); it only assumes that given a particular cat, implant, electrode tip size, and fiber type, the time course of \(P_{15}(s, g, t)\) is a smooth function of time that is linear on the logit scale. (Model 2) is the same as (Model 1), but with equal slopes, \(\beta_{s,g} = \beta\), i.e.

\[
\logit(P_{15}(s, g, t)) = \log \left( \frac{P_{15}(s, g, t)}{1 - P_{15}(s, g, t)} \right) = \alpha_{s,g} + \beta \times t; \quad \text{(Model 2)}
\]

and implies that the effect of time is the same for all electrodes on an array. Sequential analysis of variance (ANOVA) tests performed at the 5\% significance level revealed that (Model 2) provides a better fit than (Model 1), from which we conclude that there are no significant interactions between time since implant, and tip size or fiber type.

Next, we investigate if tip size and fiber type have baseline effects on \(P_{15}\) values, and if those factors interact. We consider (Model 3):

\[
\logit(P_{15}(s, g, t)) = (\alpha_{g} + \gamma_{s}) + \beta \times t, \quad \text{(Model 3)}
\]

an extension of (Model 2) that assumes no interactions between tip size and fiber type; i.e. the intercepts \(\alpha_{s,g}\) in (Model 2) can be decomposed into additive effects of electrode size, \(\gamma_{s}\), and fiber type, \(\alpha_{g}\). We set \(\alpha_{1} = 0\) so that parameters are uniquely defined. Sequential ANOVA tests suggest that (Model 3) fits the data on all implants better than (Model 2).

Finally, (Model 4) specifies that the effect of tip size on \(P_{15}\) is proportional to tip size, i.e.

\[
\logit(P_{15}(s, g, t)) = \alpha_{g} + (\gamma_{1} + \gamma_{ arg \times s} + \beta \times t, \quad \text{(Model 4)}
\]

where \(\alpha_{1} = 0\), \(\alpha_{arg} \) measures the differential effect between CV\textsubscript{fast} and CV\textsubscript{slow} fibers, \(\gamma_{\arg} \times s + 25\) is the linear effect of tip size, and \(\beta \times t\) is the linear effect of time. We rescaled the tip size by 25, so that the magnitude of the corresponding coefficient \(\gamma_{s}\) is comparable to the other regression coefficients. (Model 3) and (Model 4) are not embedded so we compare them using Akaike Information Criterion (AIC) scores rather than sequential ANOVA tests. (Model 4) provides better fits to the data of all implants, except for the L7 DRG for Cat A, for which (Model 3) has a slightly better AIC score. This difference is because the tip size effect is not linear for that implant. However, we verified that the effect was ordered, i.e. the larger the tip size is, the higher the \(P_{15}\) of the electrode.

\textbf{A.2. Results}

Table A.1 reports the estimated coefficients of (Model 4) for all implants. The \(P_{15}\) values predicted by that model are plotted in figure 5 in the main text. (Model 4) is not the best model for the L7 DRG implant for Cat A, but \(\gamma_{1}\) nevertheless captures qualitatively the \(P_{15}\) increase associated with the increase in the tip size, which facilitates comparisons between cats and implant locations.

We conclude from Table A.1 that:

1. With the exception of the time coefficient \(\beta\) for the L7 DRG for Cat A, which is not significantly different from 0 at the 5\% significance level, all \(\beta\)'s are negative and significant: \(P_{15}\) for all electrodes decayed with time. Using two-samples \(t\)-tests, we determined that \(P_{15}\) decayed significantly faster for the two implants of Cat B, and that Cats A and C had the same rates of decay at L6, and also at L7.

2. For all cats, the estimate of \(\beta\) at L7 is significantly larger than the estimate of \(\beta\) at L6: \(P_{15}\) decayed with time more slowly for the electrodes implanted at L7.

3. We detect a fiber type effect in Cats A and C: the estimate of \(\alpha_{arg}\) is negative, which means that \(P_{15}\) for all electrodes on these implants are lower for CV\textsubscript{slow} fibers. We detect no fiber type effects for Cats B and D, possibly because the samples are too small.
There is a clear effect of electrode tip size for the implants of Cat A, and a smaller effect for the implants of Cat C, and the L7 DRG of Cat B: the estimate of $\gamma_1$ is positive, i.e. the larger the tip size is, the higher the P15 is.

We fitted a model with common slope and common intercept to the data of Cats B and D. We found that model provided a better fit than (Model 4); these data have no significant effects of tips size and fiber type.

### A.3. Model diagnostics and outliers

On particular days, the electrodes on specific implants all differed from 0; p-values less than 10^-3 are coded as **. Parameters: $\alpha_2$ measures the mean differential electrode P15 between CV_{fast} and CV_{slow} fibers; a negative value means that P15 of all electrodes on an implant is lower for CV_{slow} fibers. $\gamma_1$ measures the effect of electrode tip size; a positive value means that electrodes’ P15 values increase with their tip sizes, linearly on the logit scale. $\beta$ measures the effect of time; a negative value means that P15 decreases over time, linearly on the logit scale.

| Array Location | $\alpha_2$ (SD) | $\gamma_1$(SD) | $\beta$(SD) |
|----------------|----------------|----------------|------------|
| Cat A L6       | -1.95 (0.19) **| 0.40 (0.07) **| -0.10 (0.01) ** |
| L7             | -1.76 (0.28) **| 0.52 (0.10) **| 0.06 (0.05) ** |
| Cat B L6       | -0.05          | 0.09           | -1.03 (0.11) ** |
| L7             | 0.29           | 0.19 (0.08)    | 0.01 (0.45) ** |
| Cat C L6       | -2.59 (0.41) **| 0.10 (0.06)    | -0.14 (0.02) ** |
| L7             | -1.00 (0.37) 0.006 | 0.24 (0.13)    | 0.07 (0.08) (0.03) 0.003 |
| Cat D L6       | 0.20           | 0.13           | -1.06 (0.13) ** |
| L7             | 0.26           | 0.17           | -0.79 (0.12) ** |

(4) There is a clear effect of electrode tip size for the implants of Cat A, and a smaller effect for the implants of Cat C, and the L7 DRG of Cat B: the estimate of $\gamma_1$ is positive, i.e. the larger the tip size is, the higher the P15 is.

(5) We fitted a model with common slope and common intercept to the data of Cats B and D. We found that model provided a better fit than (Model 4); these data have no significant effects of tips size and fiber type.

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