Published in final edited form as:

J Neural Eng. 2017 February ; 14(1): 016008. doi:10.1088/1741-2552/14/1/016008.

RODENT MODEL FOR ASSESSING THE LONG TERM SAFETY AND PERFORMANCE OF PERIPHERAL NERVE RECORDING ELECTRODES

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Abstract

Objective—In the U.S. alone, there are approximately 185,000 cases of limb amputation annually, which can reduce the quality of life for those individuals. Current prosthesis technology could be improved by access to signals from the nervous system for intuitive prosthesis control. After amputation, residual peripheral nerves continue to convey motor signals and electrical stimulation of these nerves can elicit sensory percepts. However, current technology for extracting information directly from peripheral nerves has limited chronic reliability, and novel approaches must be vetted to ensure safe long-term use. The present study aims to optimize methods to establish a test platform using rodent model to assess the long term safety and performance of electrode interfaces implanted in the peripheral nerves.

Approach—Floating Microelectrode Arrays (FMA, Microprobes for Life Sciences) were implanted into the rodent sciatic nerve. Weekly in vivo recordings and impedance measurements were performed in animals to assess performance and physical integrity of electrodes. Motor (walking track analysis) and sensory (Von Frey) function tests were used to assess change in nerve function due to the implant. Following the terminal recording session, the nerve was explanted and the health of axons, myelin and surrounding tissues were assessed using immunohistochemistry (IHC). The explanted electrodes were visualized under high magnification using scanning electron microscopy (SEM) to observe any physical damage.

Main Results—Recordings of axonal action potentials demonstrated notable session-to-session variability. Impedance of the electrodes increased upon implantation and displayed relative stability until electrode failure. Initial deficits in motor function recovered by 2 weeks, while

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sensory deficits persisted through 6 weeks of assessment. The primary cause of failure was identified as lead wire breakage in all of animals. IHC indicated myelinated and unmyelinated axons near the implanted electrode shanks, along with dense cellular accumulations near the implant site. Scanning electron microscopy (SEM) showed alterations of the electrode insulation and deformation of electrode shanks.

Significance—We describe a comprehensive testing platform with applicability to electrodes that record from the peripheral nerves. This study assesses the long term safety and performance of electrodes in the peripheral nerves using a rodent model. Under this animal test platform, FMA electrodes record single unit action potentials but have limited chronic reliability due to structural weaknesses. Future work will apply these methods to other commercially-available and novel peripheral electrode technologies.

Introduction

In the U.S.A. alone, there are approximately 185,000 cases of limb amputation each year (Nghiem et al., 2015). Studies of this patient population have demonstrated that residual peripheral nerves in chronic amputees remain viable long after amputation, and motor axons produce detectable action potentials (Davis et al., 2016, Dhillon et al., 2004, Rossini et al., 2010). Similarly, electrical stimulation of the residual nerves can elicit realistic sensory percepts (Davis et al., 2016, Raspopovic et al., 2014, Tan et al., 2014). For these patients, neuroprosthetic devices that interface with the peripheral nervous system (PNS) can provide new movement capabilities with intuitive control strategies. In comparison with central nervous system interfaces, PNS neuroprosthetics offer several advantages, including ease of surgical access, the ability of PNS neurons to regenerate following surgical manipulation (Faroni et al., 2015, Vasudevan et al., 2014, Vasudevan et al., 2013), presence of both afferent and efferent axons in close proximity (Saal and Bensmaia, 2015, Schmalbruch, 1986) and high specificity for interfacing with individual axons (Navarro et al., 2005). Taken together, this evidence suggests that the PNS holds promise for further investigation as the preferred site for closed-loop neuroprosthetic devices to restore movement capability to amputees.

Interfacing with the peripheral nerves can be performed either by placing the electrodes around the nerve trunk (extraneural electrodes) or inserting electrodes into the nerves (intraneural electrodes) (Branner and Normann, 2000). Extraneural electrodes, such as the spiral cuff electrodes, allow the epineurium to remain intact, and have a long history of use (Fisher et al., 2009, Naples et al., 1988, Polasek et al., 2007). However, the electrode contacts are separated from the nerve fascicles by the epineurium and perineurium, which reduces spatial selectivity for both recording and stimulation. Extraneural flat interface nerve electrodes (FINE) address this issue by using gentle mechanical force to flatten the nerve, in order to improve specificity through better access to individual fascicles (Durand et al., 2014, Leventhal et al., 2006, Tyler and Durand, 2002, Tyler and Durand, 2003), but these are currently used primarily for electrical stimulation, rather than recording applications (Tan et al., 2015, Tan et al., 2014). Alternative approaches use intraneural electrodes, such as microelectrode arrays and thin films electrodes, to penetrate the nerve and access individual axons, allowing for higher selectivity (Badia et al., 2011, Branner and Normann, 2000).

Examples include longitudinal intrafascicular electrodes (LIFE) (Lawrence et al., 2004, Rossini et al., 2010), transverse intrafascicular multichannel electrode (TIME) (Boretius et al., 2010), Utah arrays (Branner and Normann, 2000, Clark et al., 2011), and floating microelectrode arrays (FMA) (Debnath et al., 2014). A major challenge with intraneural electrodes has been poor long-term stability due to multiple factors, including device reliability and nerve fiber damage due to electrode motion inside the nerves (Grill et al., 2009, Saal and Bensmaia, 2015). Yet, the potential for restoring lost function using PNS interfaces encourages development efforts to increase long-term reliability and performance of these devices (Durand et al., 2014).

The present study aims to establish a test platform using a rodent model for assessing the safety and performance of peripheral nerve interfaces. Such a platform would allow for standardized assessment strategies of current and next generation peripheral nerve interfaces. We report a set of protocols designed to assess the recording capabilities of novel peripheral interface devices over chronic timescales, and then we applied these to the evaluation of an existing PNS interface used for scientific research.

To assess the longitudinal performance of an intraneural electrode, we implanted Floating Microelectrode Arrays (FMA, Microprobes for Life Sciences) into the rodent sciatic nerve. *In vivo* electrophysiological measurements were performed weekly in awake, behaving animals, and *in vivo* electrical impedance measurement was conducted weekly under anesthesia to determine the physical integrity of the implanted electrodes. The effects of FMA implantation on motor and sensory function was assessed using walking track analysis and Von Frey tests respectively. Following the terminal recording experiment, local neuroinflammation, axonal and myelin integrity were assessed using immunohistochemistry (IHC) and the explanted electrodes were visualized under high magnification using scanning electrode microscopy (SEM) to observe any physical damage. Taken together, this set of analyses revealed minor tissue damage and functional impairment over the duration of implantation, but with large session-to-session variability in recording quality and ultimate device failure due to a mechanical mismatch between the electrode lead wire and the forces exerted on the electrode *in vivo*. These analyses present a summary of the materials, electrophysiological, behavioral and biological performance of an intraneural electrode for interfacing with the PNS.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the Food and Drug Administration, White Oak campus. Experiments were performed on female Lewis rats, purchased from Charles River Laboratories International Inc. All animals were individually housed in plastic cages with 12-hour light and dark cycle before and after experiments. Animals were randomly assigned to one of three groups: (1) Control, n=6, (2) Sham, n=3 and (3) FMA implants, n=7. The control group animals were not subjected to any surgical procedures.

Implant Design

Eighteen channel (16 recording, 1 ground, and 1 reference) high density FMA electrodes with Parylene-C insulated shanks were purchased with custom specifications from Microprobes for Life Science, U.S.A. The channels were arranged as shown in figure 1C, with rows 1 and 4 at 1 mm shank length, and rows 2 and 3 with 0.8 mm shank length. The unimplanted array used figure 6 was designed with slightly different shank lengths, row 1 1mm, row 2 0.8 mm, row 3 1.3 mm and row 4 1.1 mm, but is otherwise identical to the implanted arrays. The electrode and the headstage connector (A73098-001, Omnetics Connector Corporation, U.S.A.) were linked via a 5.5 cm long gold lead wire bundle encased in a 2.5 cm long silicone tube starting at the connector level.

To record electromyography (EMG) signals from gastrocnemius and tibialis anterior muscles during gait, EMG implants (electrodes) were custom manufactured by Microprobes for Life Science, using 6 insulated wires (AS 632, Cooner Wire Company, U.S.A.) connected to a second headstage connector (A73098-001, Omnetics Connector Corporation, U.S.A.). The two headstage connectors were housed in a 3D printed connector mount designed using SolidWorks (Dassault Systèmes SolidWorks Corp., France) made with 420 Stainless Steel infused with bronze (Shapeways.com) as shown in figure 1B and 1D. A 2' x 2' section of Mersilene mesh (Ethicon Inc., U.S.A.) was attached to the bottom of the connector mount using acrylonitrile butadiene styrene dissolved in acetone. The mesh was attached to promote tissue ingrowth following implantation, increasing chronic stability of the connector mount attachment to the skin. A 5.5 cm ground wire (AS 631, Cooner Wire Company, U.S.A.) with 1.3 cm distal de-insulation was attached to the connector mount, to be implanted in the muscle near FMA implantation site, as shown in figure 1A.

Surgical Procedure

All surgical procedures were performed under aseptic conditions. Adult rats (200 – 280 g) were deeply anesthetized with a ketamine (80 mg/kg) and Dexmedetomidine (0.5 mg/kg) cocktail, administered through intraperitoneal injection. After confirming the loss of toe pinch reflex, the surgical site was shaved and sterilized with iodine and alcohol. The skin over the lower thoracic/upper lumbar area was incised to expose the underlying fascia, which served as a region to secure the connector mount. The right sciatic nerve was exposed using blunt dissection methods, followed by exposure of gastrocnemius and tibialis anterior muscles through skin incision between the muscles. The suture tabs of the connector mount were sutured to the lumbar fascia using 4-0 Polypropylene sutures (Oasis, U.S.A.) and the electrodes were carefully tunneled under the skin to the implant site as show in figure 1A. For FMA implantation, a 2 mm long incision of the epineurium was made 5 mm proximal to the sciatic trifurcation. The base of the FMA electrode was carefully handled using fine forceps and the shanks were manually inserted through the epineurium incision, until the base was flush with the nerve (Fig 1A). To secure the FMA, fibrin sealant (Tisseel, Baxter, U.S.A.) was applied around the implant site, and then further secured by 4 mm long, 3.2 mm (ID) silicone tubing placed around the implant/fibrin glue construct and filled with additional fibrin sealant. The ground wire was inserted into the muscles next the FMA electrode implant (Fig 1A). Two EMG wires per muscle (placed 2 mm apart) were implanted in the gastrocnemius and tibialis anterior muscles using an 18 G needle, followed

by removal of ~1 mm Teflon insulation using a scalpel to create a recording site, as described elsewhere (Courtine et al., 2009). The wires were bundled at the proximal EMG wire entry point and distal end (EMG wire exit) and secured by suture. Wounds were closed in layers and at the end of surgery, animals were administered Atipamezole (0.5 mg/kg) for reversal, Gentamycin (8 mg/kg) for antibiotics and Meloxicam (2 mg/kg) for analgesia. Animals were further administered Meloxicam (1 mg/kg) for the two days post-surgery. For sham procedures, a connector mount was implanted as following identical procedures, the sciatic nerve and muscles were exposed, and then the wounds were closed in layers, followed by post-surgery drug administration as mentioned for the FMA implanted animals.

Electrical Characteristics

Impedance of the individual channels pre- and post- implantation, until electrode failure was recorded (1 Hz-1 MHz, Gamry Instruments Inc., U.S.A.). Pre-implant measurements were obtained by immersing the electrodes in phosphate buffered solution (PBS) and measuring impedance between the ground wire and the channel of interest (Fig 2A, labeled “PRE”). Measurements were obtained immediately after implantation (Fig 2A, labeled “POST”), again at 2 weeks following implantation, and weekly thereafter until device failure. A device is considered to have failed when all the channels have impedance of greater than 2.0 MΩ. This threshold was selected post-hoc, based on experimental results demonstrating that no channels with impedance >1.6MΩ recorded any neural activity. In addition, arrays with all channels >2 MΩ were observed to have visible signs of failure, such as cable breakage.

Electrophysiological Assessment

Recording of spontaneous neural activity was performed weekly, starting at two weeks following implantation, and maintained until device failure, defined as an increase in impedance of all the electrode channels above 2 MΩ. Animals were connected to the recording system (Neuralynx, U.S.A.) under anesthesia (3% isoflurane for induction, 1.5% maintenance). Recordings were obtained for a 15 minute session, with the first 3 minutes under anesthesia, followed by ~3 minutes as the animal recovered from anesthesia and 9 minutes as the animal walked freely inside the home cage. Events from channels (>2 standard deviations) were extracted using Igor Pro (Wavemetrics, Inc., USA) and the electrophysiological data was analyzed using Neuraview and SpikeSort 3D (Neuralynx, U.S.A.) to confirm the presence of neural activity in individual channels. Channels with neural activity were segmented into anesthetized state (first 3 mins of recording) and awake state (last 3 minutes of recording) for comparison. Action potentials were identified with automatic spike sorting in SpikeSort 3D using KlustaKwik algorithms, followed by manual refinement of individual clusters. On a given channel, the baseline noise clusters were grouped and typically a single neural waveform cluster was identified. However, in case when two neural waveform clusters were identified, the cluster with higher peak-to-peak voltage was selected for further analysis. A sample baseline noise cluster (red) and neural signal cluster (green) is shown in figure 3B. Signal-to-noise ratio (SNR) was calculated as the peak-to-peak voltage of the average neural waveform divided by the peak-to-peak voltage of baseline noise elements from that same channel as shown in figure 3A. Recording yield of individual electrodes over time was calculated for both anesthetized state and awake

state as the number of channels with neural activity to the number of functional channels (between 10 k Ω - 2 M Ω , Fig 3C).

Implant Response on Function

To assess the effects of array implantation on impairment and recovery of motor function, we performed walking track analysis. Walking track videos were recorded at post-implantation weeks 1, 2, 4 and 6 or until device failure. Animals were placed on a plexiglass platform (8 x 40 cm path) and allowed to walk freely, while the footprints were imaged with a high-resolution video camera (720p) placed underneath the platform (FreeWalk, Cleversys Inc., U.S.A.). Images of consecutive footsteps were extracted and measurements of toe spread, intermediary toe spread and print length using ImageJ were used to calculate the sciatic function index (SFI) (Bain et al., 1989). SFI for 5 consecutive data sets were calculated and the average from each session was used for data analysis. ANOVA followed by Dunnett's test was used for comparing Sham and FMA implant groups to Control (GraphPad Prism, U.S.A.).

To assess the effects of electrode implantation on the impairment and recovery of sensory function, we tested mechanical allodynia measured by hind paw withdrawal thresholds using the Von Frey test (Pitcher et al., 1999). Von Frey test was performed at post-implantation weeks 1, 2, 4 and 6, or until device failure. Animals were placed in a custom 10' x 10' cage with perforated floor and given 5 minutes before commencing the measurements. While the animals were stationary on all four paws, the rigid tips of the electronic Von Frey anesthesiometer (IITC Life Science, U.S.A) were introduced to the central region of the plantar section of the foot, carefully avoiding the foot pads on the experimental leg. Withdrawal response, in grams, was recorded only when the hind paw was completely retracted by the animal, and averaged across 5 trials. Sham and FMA implant groups were compared to Control at a given time point using ANOVA followed by Dunnett's test (GraphPad Prism, U.S.A.).

Tissue Histology

After electrode failure was identified using impedance measurements, animals were anesthetized and the implants were surgically exposed. Any mechanical dislocation or migration of the electrode was noted, and the array and cable were assessed for breakage. Electrodes were carefully removed from the nerve, and the nerve segment from ~2 mm proximal to 2mm distal to the FMA implant location was dissected and immersion fixed in 4% paraformaldehyde at 4 °C. Following fixation, samples were washed in phosphate-buffered saline (PBS) and embedded in paraffin (ASP300 S, Leica Biosystems, Germany). Thin sections (~15 μ m) were cut and deparaffinized by incubating the slides at 65 °C for 30 min, followed by rehydration with xylene and graded alcohol and heat-induced epitome retrieval using citric acid buffer. Tissues were blocked with 4% goat serum (Thermo Fisher Scientific, U.S.A) and 0.5% Triton X-100 (Sigma Aldrich, U.S.A.) in PBS for 1 h at room temperature and stained for primary antibodies against Anti- β -Tubulin (Sigma Aldrich, U.S.A.) and Anti-P0 (EMD Millipore, U.S.A.) overnight at 4 °C. Secondary antibodies against Anti- β -Tubulin and Anti-P0 (Jackson Immuno Research, U.S.A.) were used at room temperature for 1 h, followed by nuclear staining using DAPI (Life Technologies, U.S.A.).

Uninjured nerves were harvested for comparison and processed similarly. Images of the nerves were acquired with 10x lens under an Olympus confocal microscope.

Electrode Imaging

Electrodes were immersed in a solution containing 1% Tergazyme (Alconox, U.S.A.), 0.5% Triton X-100 in deionized (DI) water for 3 days post-harvest, to remove cellular debris. Following decellularization, electrodes were washed in DI water and dried in a vacuum desiccator for scanning electron microscopy (SEM) imaging (fig 6). High magnification SEM images were captured under low vacuum mode, with 15 kV of electron acceleration potential at 30 Pa pressure, to analyze the overall integrity of the shanks and the insulating material (Jeol 6390LV, U.S.A.). Unimplanted electrodes (with different shank length, but otherwise identical physical parameters) used to demonstrate intact shanks and insulation before implantation.

Results

These results demonstrate a method for chronic assessment of the performance and safety of a penetrating electrode array implanted in the PNS, and profiles the performance of a single electrode type. Long-term implantation of invasive recording electrode array, the FMA, produced nerve injury and short-term functional deficits, while allowing single neuron action potentials recordings with variability across sessions, and ultimately was subject to mechanical failure of the lead wire.

Implant Design and Surgical Procedure

One key element for performing chronic electrophysiological recordings in the peripheral nerve is the stability of the connector mount secured to the lumbar fascia. In these experiments, the Mersilene mesh attached to the bottom side of the connector mount supported tissue ingrowth, which in turn reinforced its stability over time (Fig 1D). Both FMA and EMG electrodes were successfully tunneled under the skin to their respective implant locations, without causing mechanical damage to the fragile components. The FMA was inserted into the sciatic nerve (Fig 1A) and secured in place using fibrin sealant, followed by silicone cuff filled with fibrin sealant. This method ensured stability at the time of implant and through the experimental period, which was confirmed by the presence of fibrotic encapsulation anchoring the electrode to the nerve at the time of harvest. The EMG implantation procedure was found to be effective in mobilizing the recording sites inside the target muscles over chronic time periods, as observed at the time of harvest.

Electrical Characteristics of implanted FMAs

All impedance measurements used for data analysis were extracted at 1 kHz, from broadband measurements (Fig 2A). Electrode channels were considered to be functional only if their impedance ranged between 10 k Ω and 2 M Ω , as anything below 10 k Ω could result from insulation failure at the electrode shanks, lead wire or connector, and anything above 2 M Ω is likely to be an open circuit. The pre-implant impedance ranged between 123 k Ω - 1.10 M Ω , while the post-implant (~1 h) impedance of functional channels ranged between 60 k Ω - 1.26 M Ω . The average post-implant to pre-implant impedance of functional

channels increased by $153 \pm 50\%$ (pre-implantation impedance = $0.40 \pm 0.16 \text{ M}\Omega$; post-implantation impedance = $0.58 \pm 0.22 \text{ M}\Omega$, $n=7$). *In vivo* impedance results from each animal are represented in a heat map in Figure 2A, where the color of each block represents impedance value of a channel during a given measurement session, and white represents an open circuit ($> 2 \text{ M}\Omega$). Some arrays showed slow decline in the number of functional channels (i.e. FMA-2), while other displayed longer periods of stability (i.e. FMA-3). Abrupt reduction in impedance followed by recovery was also observed in electrodes (see FMA-3 at 5 weeks and FMA-4 at 4 weeks) and it is not clear if these changes were due to biological or abiotic variability, or measurement error. For all arrays, at least some channels remained functional for 6 weeks post implantation. When all electrodes reached above $2 \text{ M}\Omega$, visual inspection of the array found that the lead wire was damaged either near the connector mount or the muscle exit over the sciatic nerve, indicated by blue arrows in figure 1D. Of 7 implanted animals, FMA wire lead breakage occurred at the base of the connector mount ($n=3$ animals), at the point proximal to where the wire exited the muscle ($n=3$), between the connector and muscle exit ($n=1$), and due to damage inflicted by the animal ($n=1$). EMG wires broke at the base of the connector mount ($n=7$). Damage to the lead wire at the connector mount interface can be attributed to high stresses at this region. Two additional animals (data not shown, arrays FMA-6 and FMA-7) experienced lead wire breakage earlier than 2 weeks post implantation. FMA-6 lead wire breakage can be attributed to mechanical forces, while FMA-7 lead wire breakage resulted from damage caused by the animal.

Chronic electrophysiology from awake, freely-moving animals

Propagating action potentials were recorded during 15 minute weekly sessions, during which the animal was anesthetized for the first three minutes, and then allowed to recover and was fully awake by minute six. Only recordings from the fully anesthetized (first 3 minutes of the session) and fully awake (last 3 minutes of the session) animal were further analyzed for single units. Single unit spikes were identified through offline cluster-cutting analysis using SpikeSort 3D (Neuralynx Inc) (Fig 3B). Channels with at least one identifiable neural spike waveform were classified as having neural activity and the SNR on a given recording session was calculated as the peak-to-peak voltage of the average neural waveform divided by the peak-to-peak voltage of baseline noise elements from that same channel (Fig 3A). The average SNR for all electrodes in the awake state was 2.15 ± 0.60 , with a range between 1.41 and 3.96, while in the anesthetized state the average SNR was 2.17 ± 0.98 , with a range between 1.45 and 5.61. Recording channels showed variability in both SNR and neural activity across sessions. The yield of each array was calculated as the ratio of number of channels with neural activity over the number of functional channels at a given time point. The array yield fluctuated over time, with an average yield of 9% (awake) and 6% (anesthetized) over all implanted arrays prior to device failure. The impedance of channels with neural activity (anesthetized and awake state combined) was $675 \pm 354 \text{ k}\Omega$ (range $24 \text{ k}\Omega$ - $1.5 \text{ M}\Omega$), roughly $75 \text{ k}\Omega$ lower than the impedance of all functional channels between 2 weeks and device failure ($744 \pm 462 \text{ k}\Omega$, range $20.7 \text{ k}\Omega$ - $1.99 \text{ M}\Omega$), suggesting lower impedance may confer benefits for detections of neural signals using FMA electrodes. EMG implants failed within the first two weeks, prior to EMG recording. Failure was attributed to observed wire breakage at the exit of the connector interface, which can be due

to high mechanical stress in that region, and possible remedies include the use of more durable wire.

Behavioral assessment of the effect of electrode array on motor and sensory function

To assess functional impairment following electrode implantation, we performed locomotor and sensory behavioral assessments until device failure. Walking track analysis used to determine motor function deficits, as indicated by changes in the sciatic function index (SFI). SFI was calculated through measurements in toe spread length, intermediary toe spread and print length imaged through a plexiglass floor as the animal traversed a track. The FMA implanted cohort (n=5) displayed an acute reduction in SFI compared to the Control cohort (n=6) at 1 week, with complete recovery by week 4 post-implantation (Fig 4A). No changes in motor function were observed in those animals exposed only to surgical procedures (Sham, n=3). These results suggest that early, transient motor deficits are related to electrode array implantation.

Alterations to sensory function were determined by measuring the withdrawal reflex using Von Frey test in both the FMA implant and sham surgery cohorts compared to the naïve control (Fig 4B). Increased mechanical allodynia was observed in both Sham and FMA implants group immediately following surgery. This hyperalgesia recovered by 4 weeks for the Sham cohort, but persisted through week 6 for the FMA implant cohort. Taken together, this suggests that surgery may cause initial allodynia, but persistent allodynia can be attributed to the presence of implant in the nerve. These data do not address the possibility for eventual recovery in the FMA cohort, although this may occur at a later time-point.

Tissue response to chronic array implantation

Nerve tissue was harvested upon electrode failure and the paraffin embedded in preparation for immunohistochemistry. Thin paraffin sections were stained to visualize axons, myelin and cellular nuclei. The response of nerve tissue to implanted electrodes was evaluated using immunohistochemical assessment of axons (β -Tubulin), myelin (P0) and cellular nuclei (DAPI). In Figure 5, uninjured sciatic nerve (left) was compared to the nerve harvested from FMA-3 (right), following 11 weeks of implantation. When compared to the uninjured nerve fascicle, axons in the implanted fascicle are of lower density and degree of organization. Myelination of axons is observable in the vicinity of the electrode shanks, although this does not preclude the possibility that the fibers are undergoing degeneration.

The electrode insertion technique employed renders minimal control of electrode shank placement with respect to fascicles inside the nerve, and in this instance, one electrode shank falls within the nerve fascicle, while the other falls outside (shanks indicated by white asterisk, Fig 5 right). Accumulation of cellular nuclei was observed surrounding the electrode shanks and immediately proximal to the fascicle, potentially indicating a foreign body response within the nerve resulting from array implantation.

Physical alterations of electrodes following implantation

Following termination of the recording sessions, electrodes were explanted and cleaned using a gentle enzymatic and detergent solution (1% Tergazyme and 0.5% Triton-X-100) for

three days and then imaged with SEM (Fig 6). As compared to the unimplanted electrode (Fig 6, bottom right), the implanted arrays show deformation of many electrode shanks, a possible consequence of implantation into the nerve (please note, this example array has slightly different shank length than the implanted electrodes, but is otherwise identical in all physical parameters). Higher magnification images reveal bending of electrode tips (Fig 6, yellow arrows), and missing or delaminated insulation gaps (Fig 6, white arrows). Changes to tip structure and dielectric adherence to the metal and integrity may contribute to differences in impedance measurements, due to changes to the conductive surface area.

Discussion

Injuries resulting in amputation are becoming increasingly common among members of the U.S. military service (Krueger et al., 2012). Amputations prevent a majority of soldiers from returning to active duty, and can result in detrimental physical and psychological effects on the individual service member (Stinner et al., 2010). For the civilian population in the US, traumatic injuries are a common cause of amputation (Pezzin et al., 2000). Although many otherwise healthy amputees wish to return to pre-injury daily activities, and initially embrace prosthetic technology to allow them to do so, prosthetic limbs are often abandoned by users. The reasons for prosthesis disuse are multifaceted, but a lack of intuitive, high-fidelity, reliable control of the prosthesis is often-cited (Biddiss and Chau, 2007a, Biddiss and Chau, 2007b, Kejlara, 1993, Millstein et al., 1986). Recording directly from the peripheral nervous system after amputation has the potential to provide low latency, high information content signals for the control of prosthesis (Grill et al., 2009). Residual peripheral nerves continue to relay efferent and afferent signals long after amputation (Horch et al., 2011, Jia et al., 2007, Raspopovic et al., 2014, Rossini et al., 2010, Tan et al., 2014, Tyler and Durand, 2002), providing opportunity to restore lost function using peripheral nerve interfaces. However, for this interface to be an effective solution for patients, the recording and stimulation capabilities of peripheral electrodes must be robust and stable over time. In this work, we sought to develop an animal model to study long term safety and performance of peripheral nerve interfaces and apply this model to evaluate an invasive peripheral interface, the FMA electrode array (Microprobes for Life Science).

To demonstrate electrode performance, we first designed a robust chronic recording connector system. Pilot experiments (data not shown) and prior studies have demonstrated the critical importance of a stable connector mount to allow for repeated electrical recording and stimulation (Branner et al., 2004, Clark et al., 2011, Garde et al., 2009, Kung et al., 2014). We created a customized connector mount, using 3D printed bronze infused stainless steel (Fig 1B), which integrated the connectors for both impedance and electrophysiology. The addition of Mersilene mesh to the bottom of the connector increased chronic stability by allowing adequate tissue re-growth around the connector mount. The length of the lead wire was chosen according to the size of the animal at the time of implantation and projected increase in weight during the experimental period. The EMG electrodes failed during the first several days following implantation through breakage at the connector mount interface, highlighting a need for wires better suited to the stress experienced at that juncture in future studies.

Immediately following implantation, electrode impedance increased 1.5 times pre-implantation values. Impedance increase has been reported previously for penetrating electrodes in PNS (Davis et al., 2016) and in cortex (Mercanzini et al., 2009, Simeral et al., 2011, Wang et al., 2013) and may be attributed to higher resistivity of tissue as compared to PBS, protein adsorption to the electrode (Grill and Mortimer, 1994, Malaga et al., 2016), or the well-documented fibrotic encapsulation of extraneural (Agnew et al., 1999, Christensen et al., 2014, Christensen et al., 2016, Lawrence et al., 2002, Malmstrom et al., 1998, Romero et al., 2001, Vince et al., 2004) and intraneural (Branner et al., 2004, Lawrence et al., 2002, Wark et al., 2014) array elements. We observed fluctuation in impedance post-implantation, which may be explained by both biotic and abiotic factors (Prasad et al., 2014). Biotic factors can include protein adsorption to the electrode, fibrosis, accumulation of blood-borne cells and tissue edema (Branner et al., 2004, Christensen et al., 2014, Christensen et al., 2016, Malaga et al., 2016, McConnell et al., 2009, Wark et al., 2014, Williams et al., 2007). We observed increased cellular accumulations surrounding the electrode shanks which may alter the resistivity of the local media and increase impedance. In addition, SEM analysis of explanted electrodes revealed abiotic factors such as bent electrode tips and shanks, insulation loss and delamination from the electrode (Fig 6), all of which can contribute to electrode impedance variability (Barrese et al., 2013, Prasad et al., 2014, Takmakov et al., 2015). We did not observe gradual impedance decline as others using penetrating arrays have reported (Branner et al., 2004, Rousche and Normann, 1998), perhaps due to differences in the electrode form factor and materials used in this study. Abrupt increases in electrical impedance recorded *in vivo* were highly predictive of device failure, as verified by visual inspection of breakage of the lead wire. Through post-hoc data analysis, the threshold for impedance change that signifies a nonfunctional array was 3x the average pre-implantation impedance. The vulnerability of lead wires to breakage has been reported both in animal models (Branner et al., 2004, Kung et al., 2014, Prasad et al., 2014), and in human experiments (Davis et al., 2016, Debnath et al., 2014), and should be a focus for improvements in electrode design.

Stable recording performance of the electrodes is crucial for obtaining high-fidelity control signals for neuroprosthetic devices. Previous studies of invasive recording electrodes report the ability to capture single action potentials, but are mixed with respect to the longitudinal stability of these signals (Davis et al., 2016, Rossini et al., 2010). We quantified the SNR of electrode channels using automated cluster cutting algorithms (KlustaKwik, SpikeSort 3D, Neuralynx) followed by manual refinement and report a yield of 9% during awake state and 6% during anesthetized state of functional channels over all recorded neural activity. In addition, the number and identity of electrodes with neural activity varied across sessions. The across-session variability can be explained in part by imprecision in the implantation technique, which does not allow for targeting of fascicles, and by relative motion between the electrodes and nerve due to strains produced by the array tether.

The implantation of penetrating electrodes necessarily leads to the severing of axons during the insertion process, which may result in loss of efferent or afferent signal transduction. Since the adult peripheral nerves have the ability to regenerate after injury (Faroni et al., 2015), restoration of function can occur unless impeded by the presence of the implanted array. We performed walking track analysis (Bain et al., 1989) to assess changes in the

sciatic function index (SFI) over time, as compared to the uninjured control animals (figure 4). The SFI was altered in the FMA implant cohort, but not the sham surgical cohort, in the week following implantation, but had recovered to control levels by week two. Similar trends in recovery of function after electrode implantation has been reported previously (Tyler and Durand, 2003, Wark et al., 2014). This indicates that implantation of the array damages motor axons, but does not impede their regeneration or restoration of motor function. In contrast, measurement of mechanical allodynia using the Von Frey test (Pitcher et al., 1999) revealed an unresolved hyperalgesia in the FMA implant cohort during the six week post-implantation testing period. Surgical sham animals also displayed hyperalgesia, with recovery by week six. Continued sensory deficits may be ascribed to chronic constriction of the nerve (Austin et al., 2012) or the preferential regeneration of smaller nerve fibers (Christensen et al., 2014), leading to hypersensitivity.

Histological evaluation of the nerve response to implantation revealed the presence of both myelinated and unmyelinated fibers in close proximity to the electrode shanks, but with lower density than in the control. Chronic implantation with a similar intraneural electrode, the Utah array, also resulted in reduced fiber packing but evidence for both myelinated and unmyelinated axons were observed (Christensen et al., 2014). Hypercellularity around the implant was also observed, and has been widely documented for both intraneural and cuff electrode arrays (Christensen et al., 2014, Grill and Mortimer, 2000, Leventhal et al., 2006, Romero et al., 2001, Vince et al., 2005), with cells identified primarily as macrophages.

Alterations to the electrode materials were assessed using SEM imaging post-harvest. The enzymatic decellularization process was effective in clearing cellular debris from the shanks, perhaps in part because these electrodes had not been exposed to fixative. The shanks in all electrodes were found to be deformed (figure 6) and damage to electrode tips and insulation is consistent with prior work evaluating aged electrodes (Prasad et al., 2014, Takmakov et al., 2015). These changes to the materials can alter the electrode surface area, which may change the electrical properties, perhaps resulting in poor recording performance.

Conclusion

We have developed a test platform to assess chronic performance for intraneural PNS interface electrodes used for recording. Our investigation of one commercially available research electrode array produced results consistent with past reports on intraneural array performance, and provides new insight into the signal quality and longevity for FMA electrode arrays in the PNS. FMA arrays are able to achieve low-yield neural recordings of action potentials, but chronic recording performance was limited by mechanical failure of the lead wire. Post-explantation analysis showed considerable damage to the electrode metal and insulation, likely due to the insertion process. Tissue analysis revealed a foreign-body response, but intact myelinated and unmyelinated axons near to the electrode shanks. Future applications of this testing platform will be used to assess the performance characteristics of other commercially available intraneural arrays, along with novel neural interface technology. Improvements in intraneural arrays may move technology closer to intuitive, neurally-driven prosthesis control for patients.

Acknowledgments

This research project was supported by the Defense Advanced Research Projects Agency (DARPA), Biotechnology Technology Office (BTO), Hand Proprioception & Touch Interfaces (HAPTIX) Program (Program Manager: Douglas J. Weber) through an Interagency Agreement with the U.S. Food and Drug Administration (DARPA-FDA IAA 224-14-6009).

The authors wish to thank Katherine I. Shea of the Center for Drug Evaluation and Research at the U.S. Food and Drug Administration for paraffin processing the nerve samples for immunohistochemistry.

References

- AGNEW WF, MCCREERY DB, YUEN TG, BULLARA LA. Evolution and resolution of stimulation-induced axonal injury in peripheral nerve. *Muscle Nerve*. 1999; 22:1393–402. [PubMed: 10487906]
- AUSTIN PJ, WU A, MOALEM-TAYLOR G. Chronic constriction of the sciatic nerve and pain hypersensitivity testing in rats. *J Vis Exp*. 2012
- BADIA J, BORETIUS T, ANDREU D, AZEVEDO-COSTE C, STIEGLITZ T, NAVARRO X. Comparative analysis of transverse intrafascicular multichannel, longitudinal intrafascicular and multipolar cuff electrodes for the selective stimulation of nerve fascicles. *J Neural Eng*. 2011; 8:036023. [PubMed: 21558601]
- BAIN JR, MACKINNON SE, HUNTER DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. *Plast Reconstr Surg*. 1989; 83:129–38. [PubMed: 2909054]
- BARRESE JC, RAO N, PAROO K, TRIEBWASSER C, VARGAS-IRWIN C, FRANQUEMONT L, DONOGHUE JP. Failure mode analysis of silicon-based intracortical microelectrode arrays in non-human primates. *J Neural Eng*. 2013; 10:066014. [PubMed: 24216311]
- BIDDISS E, CHAU T. Upper-limb prosthetics: critical factors in device abandonment. *Am J Phys Med Rehabil*. 2007a; 86:977–87. [PubMed: 18090439]
- BIDDISS EA, CHAU TT. Upper limb prosthesis use and abandonment: a survey of the last 25 years. *Prosthet Orthot Int*. 2007b; 31:236–57. [PubMed: 17979010]
- BORETIUS T, BADIA J, PASCUAL-FONT A, SCHUETTLER M, NAVARRO X, YOSHIDA K, STIEGLITZ T. A transverse intrafascicular multichannel electrode (TIME) to interface with the peripheral nerve. *Biosens Bioelectron*. 2010; 26:62–9. [PubMed: 20627510]
- BRANNER A, NORMANN RA. A multielectrode array for intrafascicular recording and stimulation in sciatic nerve of cats. *Brain Res Bull*. 2000; 51:293–306. [PubMed: 10704779]
- BRANNER A, STEIN RB, FERNANDEZ E, AOYAGI Y, NORMANN RA. Long-term stimulation and recording with a penetrating microelectrode array in cat sciatic nerve. *IEEE Trans Biomed Eng*. 2004; 51:146–57. [PubMed: 14723504]
- CHRISTENSEN MB, PEARCE SM, LEDBETTER NM, WARREN DJ, CLARK GA, TRESCO PA. The foreign body response to the Utah Slant Electrode Array in the cat sciatic nerve. *Acta Biomater*. 2014; 10:4650–60. [PubMed: 25042798]
- CHRISTENSEN MB, WARK HA, HUTCHINSON DT. A histological analysis of human median and ulnar nerves following implantation of Utah slanted electrode arrays. *Biomaterials*. 2016; 77:235–42. [PubMed: 26606449]
- CLARK GA, LEDBETTER NM, WARREN DJ, HARRISON RR. Recording sensory and motor information from peripheral nerves with Utah Slanted Electrode Arrays. *Conf Proc IEEE Eng Med Biol Soc*. 2011; 2011:4641–4. [PubMed: 22255372]
- COURTINE G, GERASIMENKO Y, VAN DEN BRAND R, YEW A, MUSIENKO P, ZHONG H, SONG B, AO Y, ICHIYAMA RM, LAVROV I, ROY RR, SOFRONIEW MV, EDGERTON VR. Transformation of nonfunctional spinal circuits into functional states after the loss of brain input. *Nat Neurosci*. 2009; 12:1333–42. [PubMed: 19767747]
- DAVIS TS, WARK HA, HUTCHINSON DT, WARREN DJ, O'NEILL K, SCHEINBLUM T, CLARK GA, NORMANN RA, GREGER B. Restoring motor control and sensory feedback in people with upper extremity amputations using arrays of 96 microelectrodes implanted in the median and ulnar nerves. *J Neural Eng*. 2016; 13:036001. [PubMed: 27001946]

- DEBNATH S, BAUMAN MJ, FISHER LE, WEBER DJ, GAUNT RA. Microelectrode array recordings from the ventral roots in chronically implanted cats. *Front Neurol.* 2014; 5:104. [PubMed: 25071697]
- DHILLON GS, LAWRENCE SM, HUTCHINSON DT, HORCH KW. Residual function in peripheral nerve stumps of amputees: implications for neural control of artificial limbs. *J Hand Surg Am.* 2004; 29:605–15. discussion 616–8. [PubMed: 15249083]
- DURAND DM, GHOVANLOO M, KRAMES E. Time to address the problems at the neural interface. *J Neural Eng.* 2014; 11:020201. [PubMed: 24503546]
- FARONI A, MOBASSERI SA, KINGHAM PJ, REID AJ. Peripheral nerve regeneration: experimental strategies and future perspectives. *Adv Drug Deliv Rev.* 2015; 82–83:160–7.
- FISHER LE, TYLER DJ, ANDERSON JS, TRIOLO RJ. Chronic stability and selectivity of four-contact spiral nerve-cuff electrodes in stimulating the human femoral nerve. *J Neural Eng.* 2009; 6:046010. [PubMed: 19602729]
- GARDE K, KEEFER E, BOTTERMAN B, GALVAN P, ROMERO MI. Early interfaced neural activity from chronic amputated nerves. *Front Neuroeng.* 2009; 2:5. [PubMed: 19506704]
- GRILL WM, MORTIMER JT. Electrical properties of implant encapsulation tissue. *Ann Biomed Eng.* 1994; 22:23–33. [PubMed: 8060024]
- GRILL WM, MORTIMER JT. Neural and connective tissue response to long-term implantation of multiple contact nerve cuff electrodes. *J Biomed Mater Res.* 2000; 50:215–26. [PubMed: 10679687]
- GRILL WM, NORMAN SE, BELLAMKONDA RV. Implanted neural interfaces: biochallenges and engineered solutions. *Annu Rev Biomed Eng.* 2009; 11:1–24. [PubMed: 19400710]
- HORCH K, MEEK S, TAYLOR TG, HUTCHINSON DT. Object discrimination with an artificial hand using electrical stimulation of peripheral tactile and proprioceptive pathways with intrafascicular electrodes. *IEEE Trans Neural Syst Rehabil Eng.* 2011; 19:483–9. [PubMed: 21859607]
- JIA X, KOENIG MA, ZHANG X, ZHANG J, CHEN T, CHEN Z. Residual motor signal in long-term human severed peripheral nerves and feasibility of neural signal-controlled artificial limb. *J Hand Surg Am.* 2007; 32:657–66. [PubMed: 17482005]
- KEJLAA GH. Consumer concerns and the functional value of prostheses to upper limb amputees. *Prosthet Orthot Int.* 1993; 17:157–63. [PubMed: 8134275]
- KRUEGER CA, WENKE JC, FICKE JR. Ten years at war: comprehensive analysis of amputation trends. *J Trauma Acute Care Surg.* 2012; 73:S438–44. [PubMed: 23192067]
- KUNG TA, LANGHALS NB, MARTIN DC, JOHNSON PJ, CEDERNA PS, URBANCHEK MG. Regenerative peripheral nerve interface viability and signal transduction with an implanted electrode. *Plast Reconstr Surg.* 2014; 133:1380–94. [PubMed: 24867721]
- LAWRENCE SM, DHILLON GS, JENSEN W, YOSHIDA K, HORCH KW. Acute peripheral nerve recording characteristics of polymer-based longitudinal intrafascicular electrodes. *IEEE Trans Neural Syst Rehabil Eng.* 2004; 12:345–8. [PubMed: 15473197]
- LAWRENCE SM, LARSEN JO, HORCH KW, RISO R, SINKJAER T. Long-term biocompatibility of implanted polymer-based intrafascicular electrodes. *J Biomed Mater Res.* 2002; 63:501–6. [PubMed: 12209893]
- LEVENTHAL DK, COHEN M, DURAND DM. Chronic histological effects of the flat interface nerve electrode. *J Neural Eng.* 2006; 3:102–13. [PubMed: 16705266]
- MALAGA KA, SCHROEDER KE, PATEL PR, IRWIN ZT, THOMPSON DE, NICOLE BENTLEY J, LEMPKA SF, CHESTEK CA, PATIL PG. Data-driven model comparing the effects of glial scarring and interface interactions on chronic neural recordings in non-human primates. *J Neural Eng.* 2016; 13:016010. [PubMed: 26655972]
- MALMSTROM JA, MCNAUGHTON TG, HORCH KW. Recording properties and biocompatibility of chronically implanted polymer-based intrafascicular electrodes. *Ann Biomed Eng.* 1998; 26:1055–64. [PubMed: 9846943]
- MCCONNELL GC, BUTERA RJ, BELLAMKONDA RV. Bioimpedance modeling to monitor astrocytic response to chronically implanted electrodes. *J Neural Eng.* 2009; 6:055005. [PubMed: 19721187]

- MERCANZINI A, COLIN P, BENSADOUN JC, BERTSCH A, RENAUD P. In vivo electrical impedance spectroscopy of tissue reaction to microelectrode arrays. *IEEE Trans Biomed Eng.* 2009; 56:1909–18. [PubMed: 19362904]
- MILLSTEIN SG, HEGER H, HUNTER GA. Prosthetic use in adult upper limb amputees: a comparison of the body powered and electrically powered prostheses. *Prosthet Orthot Int.* 1986; 10:27–34. [PubMed: 3725563]
- NAPLES GG, MORTIMER JT, SCHEINER A, SWEENEY JD. A spiral nerve cuff electrode for peripheral nerve stimulation. *IEEE Trans Biomed Eng.* 1988; 35:905–16. [PubMed: 3198136]
- NAVARRO X, KRUEGER TB, LAGO N, MICERA S, STIEGLITZ T, DARIO P. A critical review of interfaces with the peripheral nervous system for the control of neuroprostheses and hybrid bionic systems. *J Peripher Nerv Syst.* 2005; 10:229–58. [PubMed: 16221284]
- NGHIEM BT, SANDO IC, GILLESPIE RB, MCLAUGHLIN BL, GERLING GJ, LANGHALS NB, URBANCHEK MG, CEDERNA PS. Providing a sense of touch to prosthetic hands. *Plast Reconstr Surg.* 2015; 135:1652–63. [PubMed: 26017599]
- PEZZIN LE, DILLINGHAM TR, MACKENZIE EJ. Rehabilitation and the long-term outcomes of persons with trauma-related amputations. *Arch Phys Med Rehabil.* 2000; 81:292–300. [PubMed: 10724073]
- PITCHER GM, RITCHIE J, HENRY JL. Paw withdrawal threshold in the von Frey hair test is influenced by the surface on which the rat stands. *J Neurosci Methods.* 1999; 87:185–93. [PubMed: 11230815]
- POLASEK KH, HOYEN HA, KEITH MW, TYLER DJ. Human nerve stimulation thresholds and selectivity using a multi-contact nerve cuff electrode. *IEEE Trans Neural Syst Rehabil Eng.* 2007; 15:76–82. [PubMed: 17436879]
- PRASAD A, XUE QS, DIEME R, SANKAR V, MAYRAND RC, NISHIDA T, STREIT WJ, SANCHEZ JC. Abiotic-biotic characterization of Pt/Ir microelectrode arrays in chronic implants. *Front Neuroeng.* 2014; 7:2. [PubMed: 24550823]
- RASPOPOVIC S, CAPOGROSSO M, PETRINI FM, BONIZZATO M, RIGOSA J, DI PINO G, CARPANETO J, CONTROZZI M, BORETIUS T, FERNANDEZ E, GRANATA G, ODDO CM, CITI L, CIANCIO AL, CIPRIANI C, CARROZZA MC, JENSEN W, GUGLIELMELLI E, STIEGLITZ T, ROSSINI PM, MICERA S. Restoring natural sensory feedback in real-time bidirectional hand prostheses. *Sci Transl Med.* 2014; 6:222ra19.
- ROMERO E, DENEFF JF, DELBEKE J, ROBERT A, VERAART C. Neural morphological effects of long-term implantation of the self-sizing spiral cuff nerve electrode. *Med Biol Eng Comput.* 2001; 39:90–100. [PubMed: 11214279]
- ROSSINI PM, MICERA S, BENVENUTO A, CARPANETO J, CAVALLO G, CITI L, CIPRIANI C, DENARO L, DENARO V, DI PINO G, FERRERI F, GUGLIELMELLI E, HOFFMANN KP, RASPOPOVIC S, RIGOSA J, ROSSINI L, TOMBINI M, DARIO P. Double nerve intraneural interface implant on a human amputee for robotic hand control. *Clin Neurophysiol.* 2010; 121:777–83. [PubMed: 20110193]
- ROUSCHE PJ, NORMANN RA. Chronic recording capability of the Utah Intracortical Electrode Array in cat sensory cortex. *J Neurosci Methods.* 1998; 82:1–15. [PubMed: 10223510]
- SAAL HP, BENSMAIA SJ. Biomimetic approaches to bionic touch through a peripheral nerve interface. *Neuropsychologia.* 2015; 79:344–53. [PubMed: 26092769]
- SCHMALBRUCH H. Fiber composition of the rat sciatic nerve. *Anat Rec.* 1986; 215:71–81. [PubMed: 3706794]
- SIMERAL JD, KIM SP, BLACK MJ, DONOGHUE JP, HOCHBERG LR. Neural control of cursor trajectory and click by a human with tetraplegia 1000 days after implant of an intracortical microelectrode array. *J Neural Eng.* 2011; 8:025027. [PubMed: 21436513]
- STINNER DJ, BURNS TC, KIRK KL, FICKE JR. Return to duty rate of amputee soldiers in the current conflicts in Afghanistan and Iraq. *J Trauma.* 2010; 68:1476–9. [PubMed: 20068483]
- TAKMAKOV P, RUDA K, SCOTT PHILLIPS K, ISAYEVA IS, KRAUTHAMER V, WELLE CG. Rapid evaluation of the durability of cortical neural implants using accelerated aging with reactive oxygen species. *J Neural Eng.* 2015; 12:026003. [PubMed: 25627426]

- TAN DW, SCHIEFER MA, KEITH MW, ANDERSON JR, TYLER DJ. Stability and selectivity of a chronic, multi-contact cuff electrode for sensory stimulation in human amputees. *J Neural Eng.* 2015; 12:026002. [PubMed: 25627310]
- TAN DW, SCHIEFER MA, KEITH MW, ANDERSON JR, TYLER J, TYLER DJ. A neural interface provides long-term stable natural touch perception. *Sci Transl Med.* 2014; 6:257ra138.
- TYLER DJ, DURAND DM. Functionally selective peripheral nerve stimulation with a flat interface nerve electrode. *IEEE Trans Neural Syst Rehabil Eng.* 2002; 10:294–303. [PubMed: 12611367]
- TYLER DJ, DURAND DM. Chronic response of the rat sciatic nerve to the flat interface nerve electrode. *Ann Biomed Eng.* 2003; 31:633–42. [PubMed: 12797612]
- VASUDEVAN S, HUANG J, BOTTERMAN B, MATLOUB HS, KEEFER E, CHENG J. Detergent-free Decellularized Nerve Grafts for Long-gap Peripheral Nerve Reconstruction. *Plast Reconstr Surg Glob Open.* 2014; 2:e201. [PubMed: 25426384]
- VASUDEVAN S, YAN JG, ZHANG LL, MATLOUB HS, CHENG JJ. A rat model for long-gap peripheral nerve reconstruction. *Plast Reconstr Surg.* 2013; 132:871–6. [PubMed: 24076680]
- VINCE V, BRELEN ME, DELBEKE J, COLIN IM. Anti-TNF-alpha reduces the inflammatory reaction associated with cuff electrode implantation around the sciatic nerve. *J Neuroimmunol.* 2005; 165:121–8. [PubMed: 15951027]
- VINCE V, THIL MA, VERAART C, COLIN IM, DELBEKE J. Biocompatibility of platinum-metallized silicone rubber: in vivo and in vitro evaluation. *J Biomater Sci Polym Ed.* 2004; 15:173–88. [PubMed: 15109096]
- WANG C, BRUNTON E, HAGHGOOIE S, CASSELLS K, LOWERY A, RAJAN R. Characteristics of electrode impedance and stimulation efficacy of a chronic cortical implant using novel annulus electrodes in rat motor cortex. *J Neural Eng.* 2013; 10:046010. [PubMed: 23819958]
- WARK HA, MATHEWS KS, NORMANN RA, FERNANDEZ E. Behavioral and cellular consequences of high-electrode count Utah Arrays chronically implanted in rat sciatic nerve. *J Neural Eng.* 2014; 11:046027. [PubMed: 25031219]
- WILLIAMS JC, HIPPENSTEEL JA, DILGEN J, SHAIN W, KIPKE DR. Complex impedance spectroscopy for monitoring tissue responses to inserted neural implants. *J Neural Eng.* 2007; 4:410–23. [PubMed: 18057508]

Figure 1. Implant design and surgical procedure

(A) The connector mount was secured to the lumbar fascia and the FMA array and EMG wires were tunneled to the implant site. FMA electrode is shown implanted into the sciatic nerve and the ground wire is inserted into an adjacent muscle. (B) Custom designed connector mount designed to support two Omnetics connectors, for the FMA and for the EMG wires. (C) A schematic depicting the FMA array layout with 16 electrodes, a reference (R) and ground (G). (D) Implant assembly with blue arrows indicating observed breakage points for both EMG wires and FMA lead wire.

Figure 2. Electrochemical impedance spectroscopy of electrodes before and during implantation (A) Heat map reflecting impedance at 1 kHz of individual electrodes before implantation (PRE), one hour after implantation (POST), and weekly, starting two weeks post-implantation until device failure. (B) Average data plot showing impedance of functional channels in each electrode over time (The bars for each animal signify measurements taken PRE, POST, and weekly until device failure, as in part A). (C) The number of functional channels over time for all 5 implanted FMAs. Functional channels are defined as those with impedance between 10 k Ω and 2 M Ω .

Figure 3. Recorded action potential signal to noise ratio (SNR) and recording yield over time (A) Heat maps for individual electrodes showing SNR calculated from the peak-to-peak voltage of the average waveforms during awake state. Variability in SNR over time is seen for all channels. White areas indicate channels with impedance greater than 2 M Ω (non-functional). (B) Trace recorded in FMA-4, channel 16 at two weeks post-implantation and sorted waveform (below) showing neural activity (green) and noise (red). (C) Recording yield was calculated as the ratio of the number of channels with neural activity over the number functional channels for a given recording session. All arrays display session-to-session variation in recording yield, with an overall average of 9% yield across all arrays and recording sessions in the awake state.

Figure 4. Functional changes associated with implantation

(A) Walking track analysis was used to compute the sciatic function index (SFI). The FMA implant cohort shows deficits at 1 week with recovery at 2 weeks. (B) Von Frey test was employed to measure mechanical allodynia shows hypersensitivity in both Sham and FMA implant cohorts, as compared to Control. Hypersensitivity recovers in the Sham cohort by 6 weeks, while full recovery is not achieved for the FMA implant cohort in this time. Data on the graph represented as Mean $\pm$ S.E. Statistical differences represented as *P = 0.05, **P = 0.01 and ***P = 0.001.

Figure 5. Tissue response to chronic FMA implantation

Thin sections ($\sim 15 \mu\text{m}$) of nerve were stained for axons (β -Tubulin), myelin (P0) and cellular nuclei (DAPI). Uninjured nerve (left) provides a control for comparison with nerve from beneath the implanted array from animal FMA-3 (right). One electrode shank is visible inside a fascicle (right of dotted line) and the other one outside of it (left of dotted line) are indicated with a star. While both myelinated (yellow; green+red) and unmyelinated axons (green) surround the intrafascicular electrode shank, the overall axon density is lower than in the uninjured control. Multiple layers of cellular nuclei are accumulated around the shanks and nearby extrafascicular space, as indicated by DAPI staining.

Figure 6. Physical alterations of harvested electrodes with scanning electron microscopy
 In all five implanted electrodes (FMA-1 through FMA-5), gross deformation of the electrode shanks is visible. In addition, gaps between metal and insulation and damaged insulation are apparent (white arrows), as are bent electrode tips (yellow arrows). Ground and reference shanks within the electrode are indicated with blue arrows in FMA-5. An unimplanted electrode of slightly different shank length is shown to demonstrate that on receipt from the manufacturer, the electrode shanks are straight, insulation is intact and electrode tips are unbent.