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14. ABSTRACT
Tissue inflammation due to chronic electrode implants has been reported as a dominant failure mechanism in most neural interfaces preventing acquisition of long term reliable neural information. The tissue reaction is thought to result from initial injury caused by the trauma of electrode implantation, as well as delayed damage due to chronic presence of electrode arrays. Evaluation of the foreign body response to neural interfaces have typically been limited to quantification of a small set of molecules. Thus, molecular components and pathways crucial for the tissue response specific to implanted neural interfaces remain largely unknown, especially in the peripheral nervous

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Report Title

Final Report: The molecular and electrophysiologic response to chronic intraneural silicone electrode implantation

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Tissue inflammation due to chronic electrode implants has been reported as a dominant failure mechanism in most neural interfaces preventing acquisition of long term reliable neural information. The tissue reaction is thought to result from initial injury caused by the trauma of electrode implantation, as well as delayed damage due to chronic presence of electrode arrays. Evaluation of the foreign body response to neural interfaces have typically been limited to quantification of a small set of molecules. Thus, molecular components and pathways crucial for the tissue response specific to implanted neural interfaces remain largely unknown, especially in the peripheral nervous system. In this study we use whole genome microarrays to understand local gene expression changes in response to silicone longitudinal intrafascicular multi electrode arrays (LIFEs) in rat sciatic nerve. In addition to the cellular and molecular response, we characterize neural signaling quality and histological changes to chronically implanted LIFEs. Understanding the changes induced by the chronic presence of implanted electrodes at the molecular level will speed development of neural prosthetic devices used to restore function in cases of paralysis, amputation, and degenerative disease.

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Scientific Progress

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Final Report- ARO contract #W911NF-16-1-0234

The molecular and electrophysiologic response to chronic intraneural silicone electrode implantation

Submitted by Nerves Incorporated
PI: Edward W. Keefer, PhD

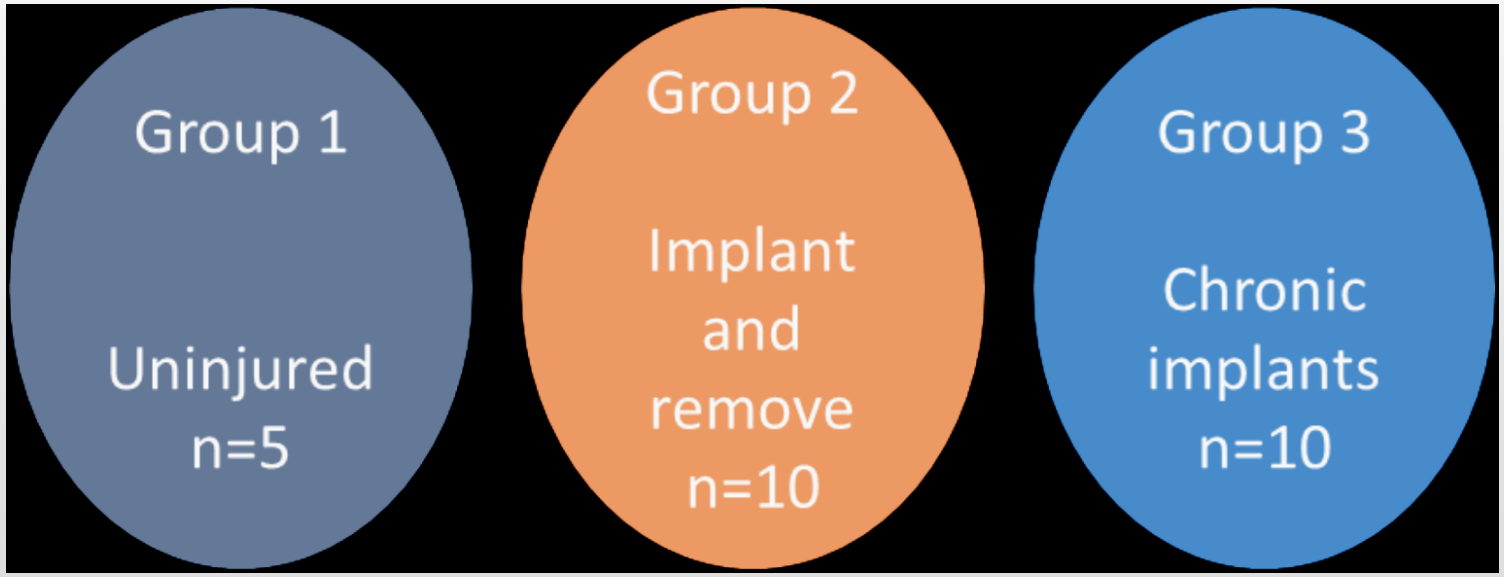
May 15, 2017

Background

Statement of Problem

Neural prosthetic devices such as computer cursors and robotic limbs offer an individual with disabilities greater interaction with world. These neural prosthetic devices rely on neural activity from brain or the peripheral nervous system for functional control. While recent progress in neural engineering has led to the clinical demonstration of thought-mediated control of computer interface devices by disabled subjects, current neuro-electrode interfaces lack the necessary reliability to ensure such control over a period of years. In the last 30 years considerable scientific and technological efforts has been devoted to develop and improve neural interfaces using different designs and materials. Limitations of functional prosthetic devices resulting from failure of the nerve-electrode interface vary depending on the type of electrode. Extra-neural approaches provide small signals from a very limited number of electrodes and are normally restricted to high amplitude units. While indwelling multi-electrode arrays appear to be promising for detection of smaller signals at early time points, the signal decay over time, possibly due to tissue response to implant has remained an insurmountable challenge. In some cases inserted electrodes lose function within a few months following device insertion. A leading hypothesis in the cause of failure of these chronic implants is the presence of persistent local inflammation. It is thought that initial insertion of electrodes causes an early foreign body response which results in tissue encapsulation of the electrodes and later extended tissue damage is caused by micro motion of the tethered electrodes relative to the soft tissue, which may lead to neuron cell death and extended gliosis. For this study, we undertook to measure the peripheral nerve response to chronically implanted electrode arrays using Affymetrix gene arrays in a rat sciatic nerve model.

Experimental Groups



Methodology

Multi-electrode Arrays

Silicone LIFE arrays were provided by Draper Labs, Boston MA with five intraneural electrodes, and a four-contact nerve cuff. The electrodes, leads, and contact pads are laser-cut platinum foil interleaved between two layers of silicone. The base layer of the silicone is reinforced with polypropylene surgical mesh. Electrode active sites and contact pads are laser deinsulated. All implanted components were sterilized using EtOH and UV radiation.

Animal Surgeries

2 surgical groups, 10 male Lewis rats (300 g) per group, 1 control group, 5 animals. Ketamine/medetomidine cocktail administered IP were used for anesthesia. The sciatic nerve was exposed through a muscle-sparing incision along the sciatic vein and muscles gently spread to expose the proximal part of the undivided nerve. For Group 2, Implant and Remove, the LIFE array was withdrawn, wound closed, and the animals recovered. For Group 3, Chronic Implants, after placing the LIFE arrays the suture needles were cut loose, and the distal end of the array secured using a microclip. The proximal end of the array was held in place by the cuff electrode flaps secured with a vascular clamp. The electrode arrays were connected to an 18-pin connector via subcutaneous gold micro wires. The connector was mounted to the pelvis using surgical mesh. The skin was closed around the pedestal using staples. Post-surgery, all animals received antibiotic (cephazolin; 5mg/kg, IM) and pain control (buprenorphine; 0.05–0.1 mg/kg, SC). All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center.

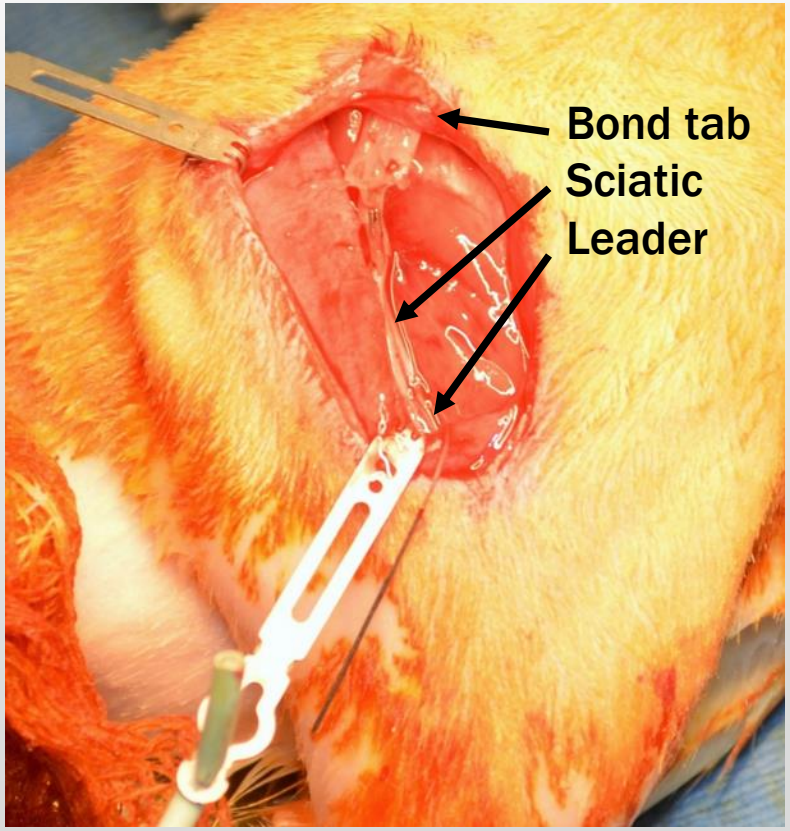
Total RNA isolation and Microarray Analysis

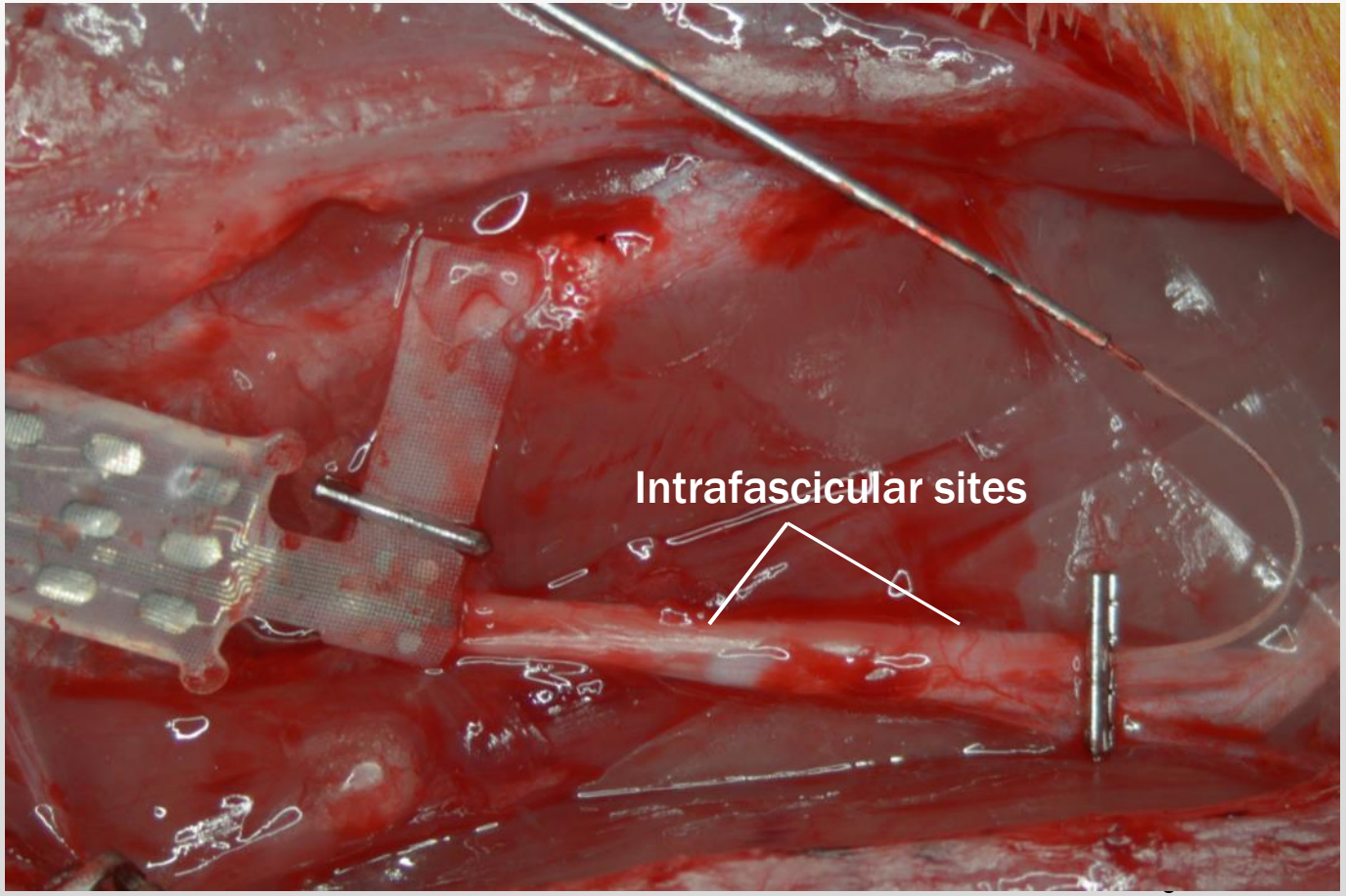
At 90 days, the animals were sacrificed (Euthasol) and the sciatic nerve portion containing the electrode arrays (Group 3), the portion of sciatic nerve that was injured by inserting and withdrawing the LIFE array (Group 2), and the uninjured control (Group 1) harvested and immediately preserved in RNA Later (Qiagen, Valencia, CA, USA). Total RNA from regenerated tissue was extracted with Qiazol and purified using RNeasy Micro kit (Qiagen, Valencia, CA, USA). RNA purity was verified by obtaining optical densitometry readings at 260/280 nm and 260/230 nm using Pico drop (Pico100). RNA quality was verified using an Agilent 2100 Bioanalyzer. Probe labeling, array hybridization, wash, stain, scanning and data collection was conducted at the UT Southwestern Medical Center Microarray Core Facility. All samples were run on the Affymetrix Rat Gene 2.0 ST Array.

Electrophysiology

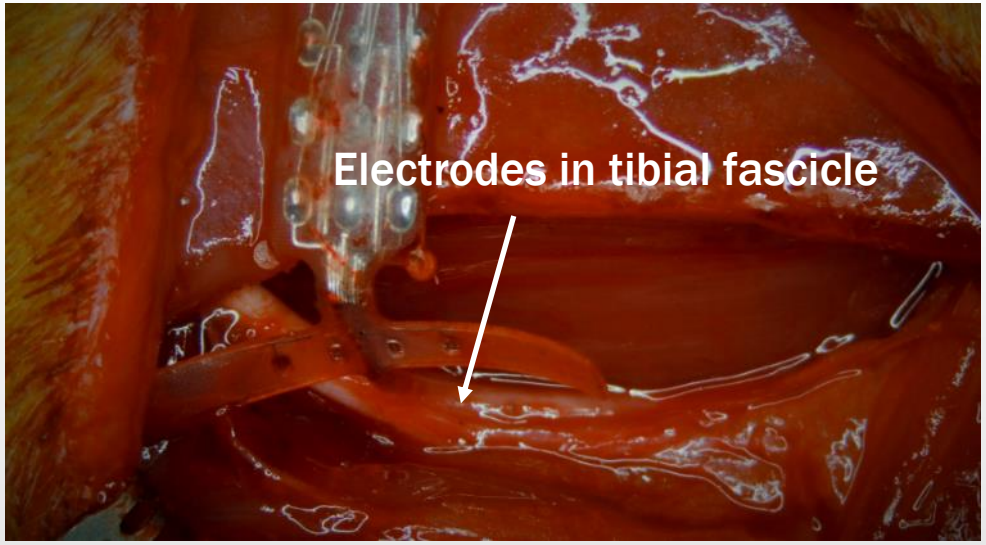
Electrophysiological signals were acquired with the Ripple Grapevine data acquisition system (Ripple Inc., Salt Lake City UT) beginning at 14 days post implantation, and repeated weekly during recording sessions lasting approximately one hour. Quantitative longitudinal analysis of the number of active electrodes, the number of discriminated (single units), the average firing frequency, the characteristic firing pattern (single spiking, tonic firing, phasic bursting), action potential amplitudes, and action potential widths were performed.

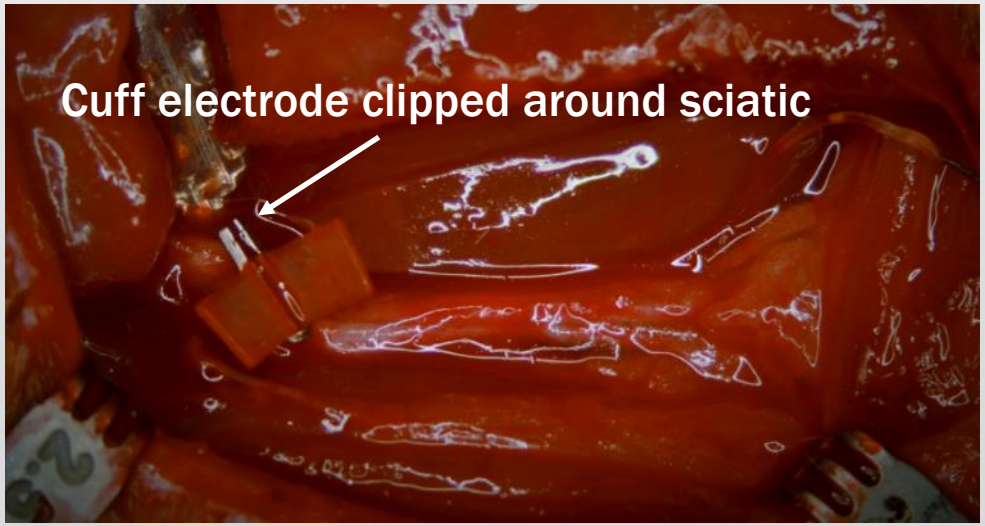
Electrode Implants

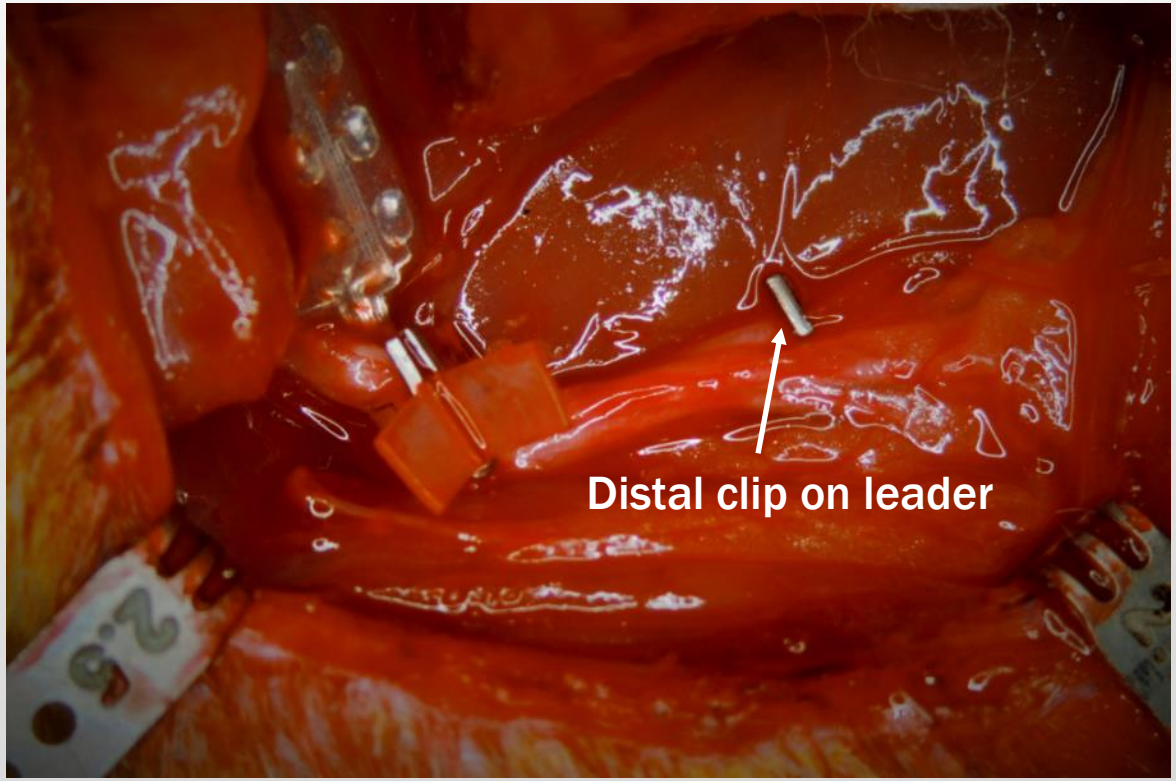




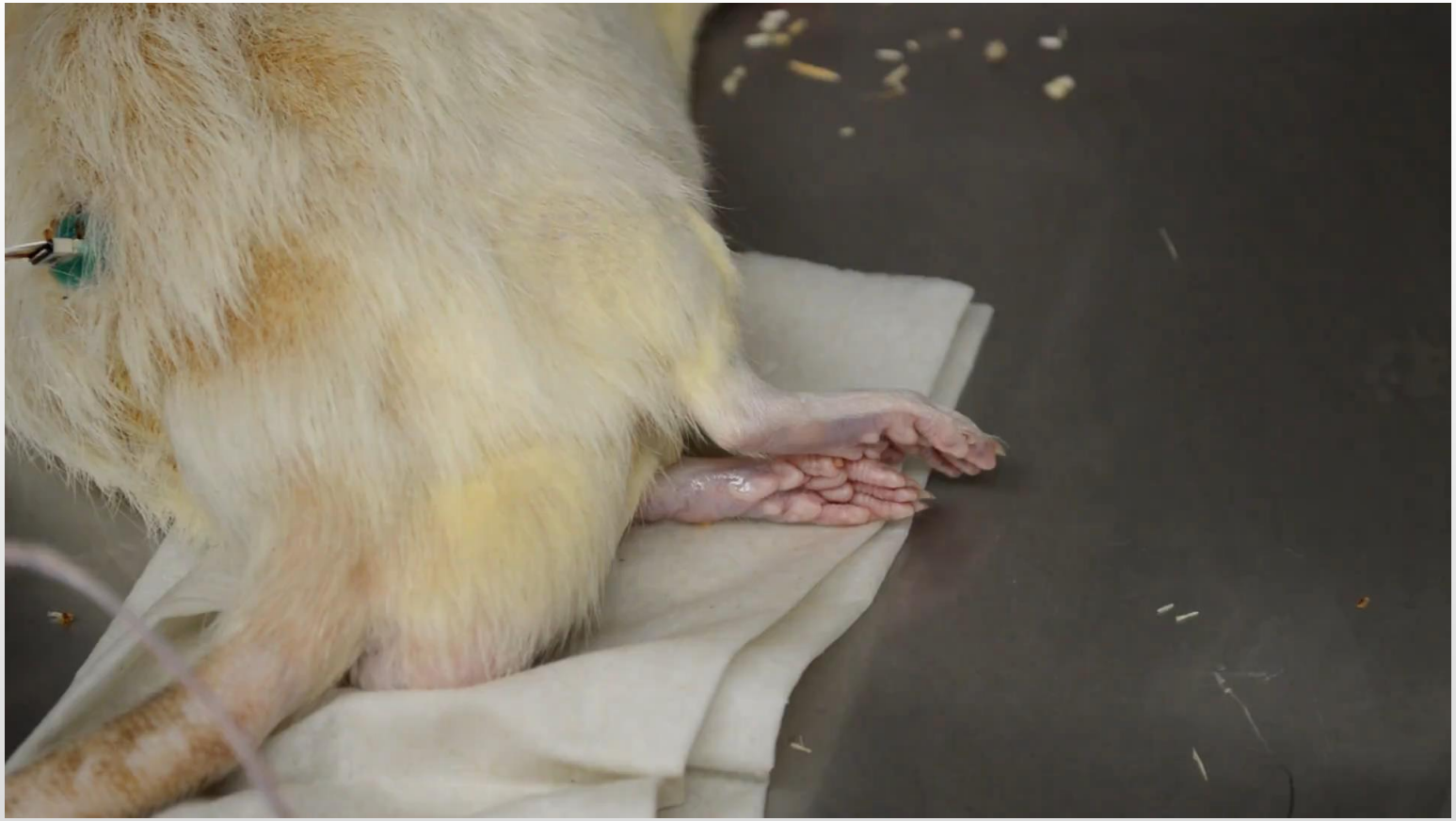
Implant and anchoring in nerve



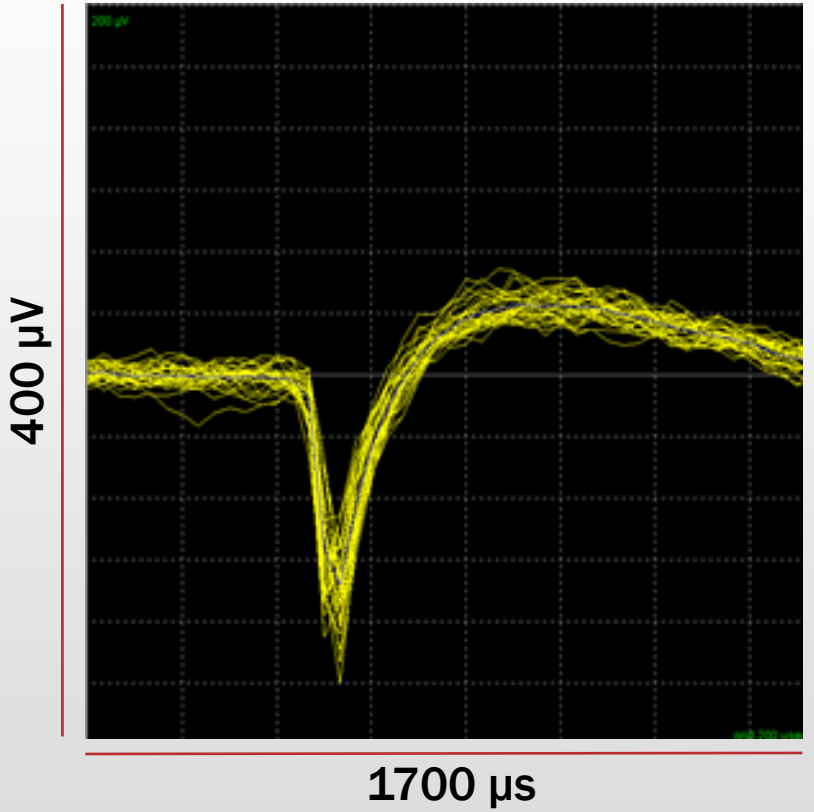




Rat recording and stimulation at 60 days



Stimulation- 9 µA, 2 Hz

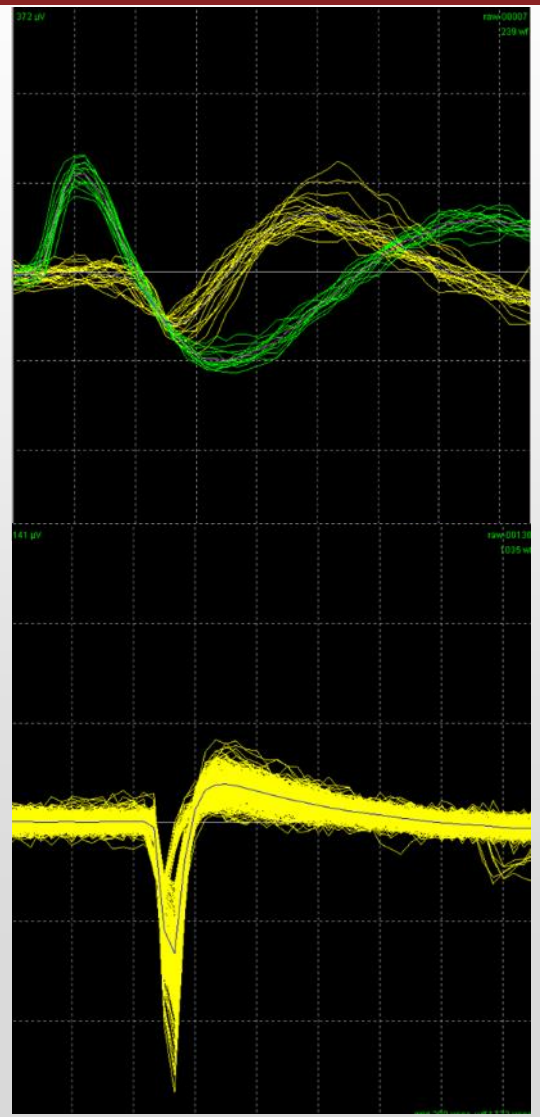


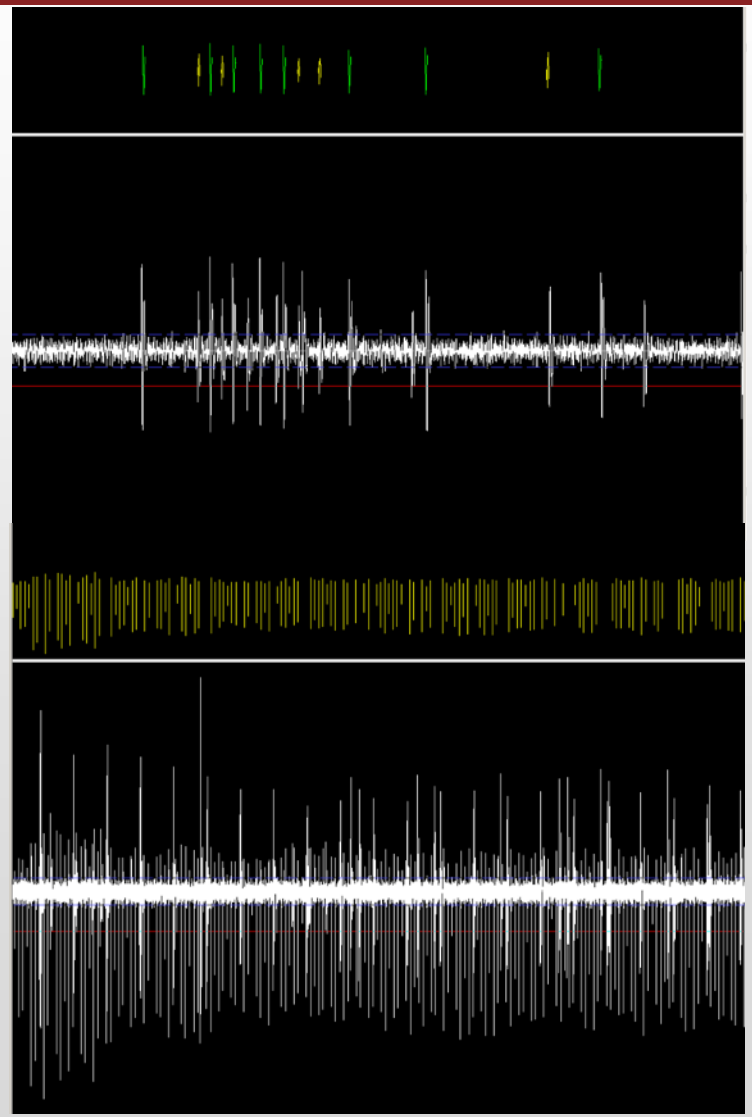
Single unit response to dorsiflexion, one spike per foot flex (gastroc stretch) specific to channel 1

Rat recording

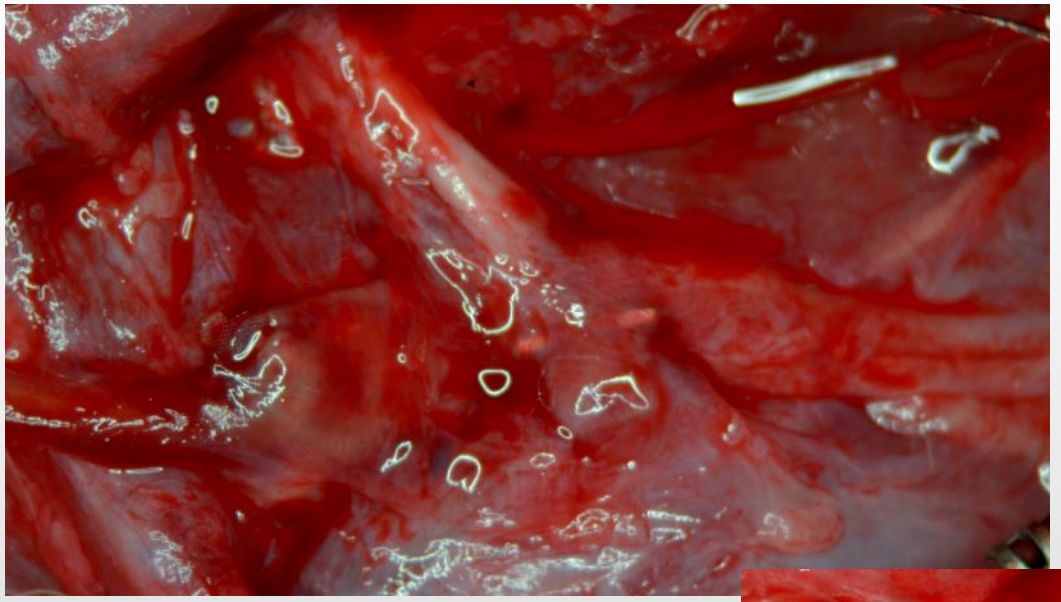
Ankle-knee manipulation 1 hr after implant

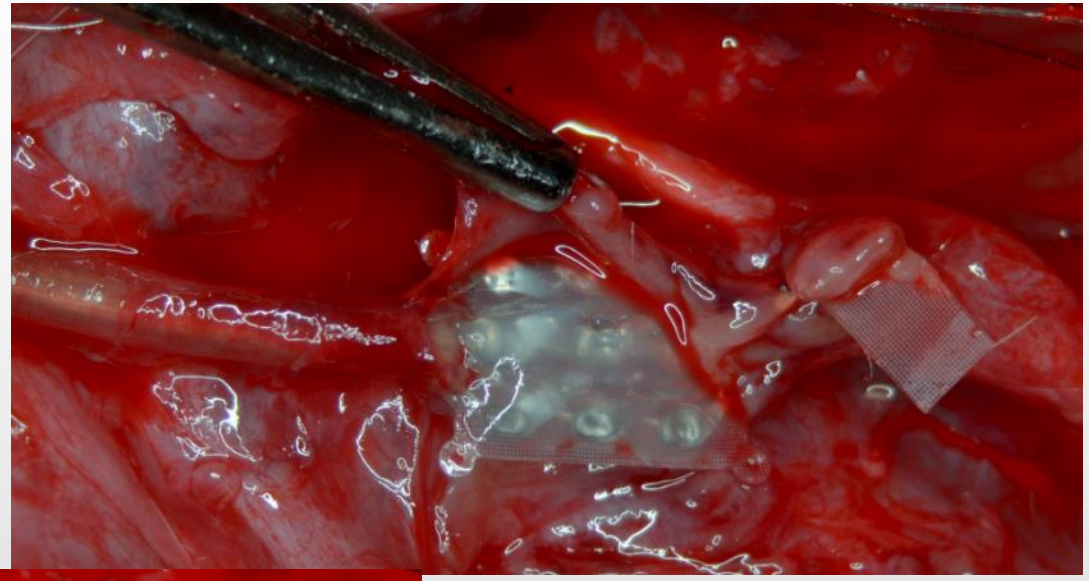
Spontaneous activity 30 days after implant

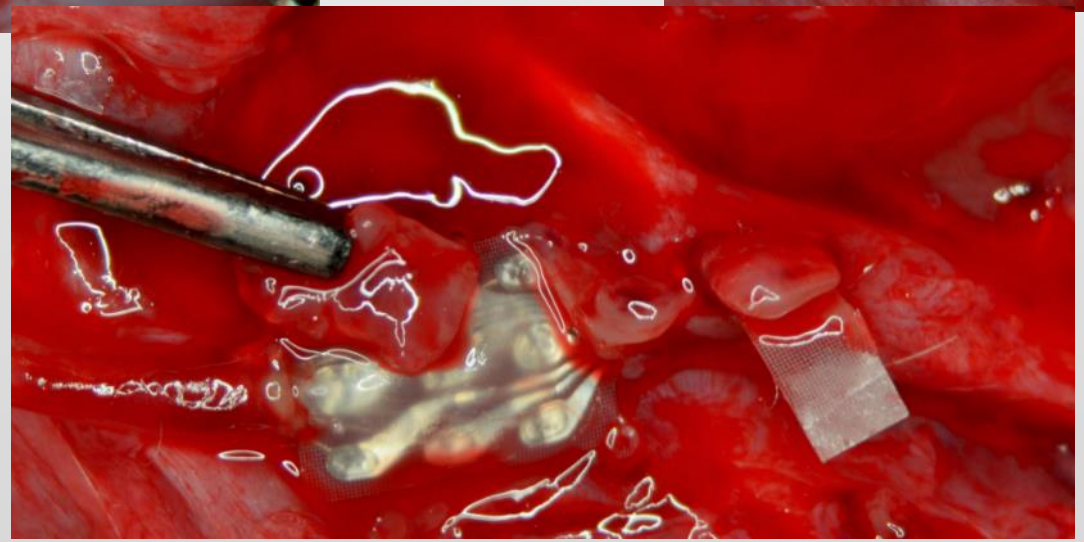




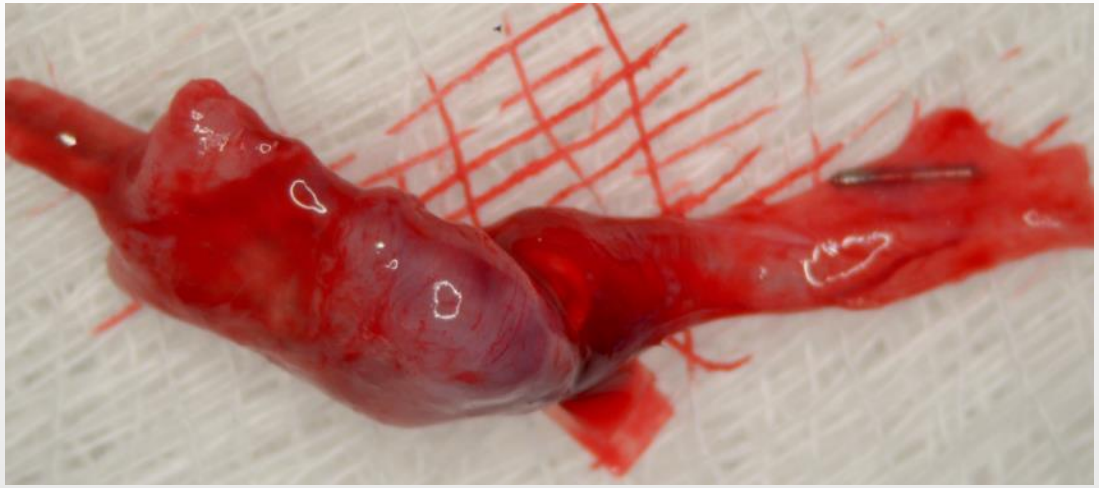
Tissue reaction to silicone based array-90 days

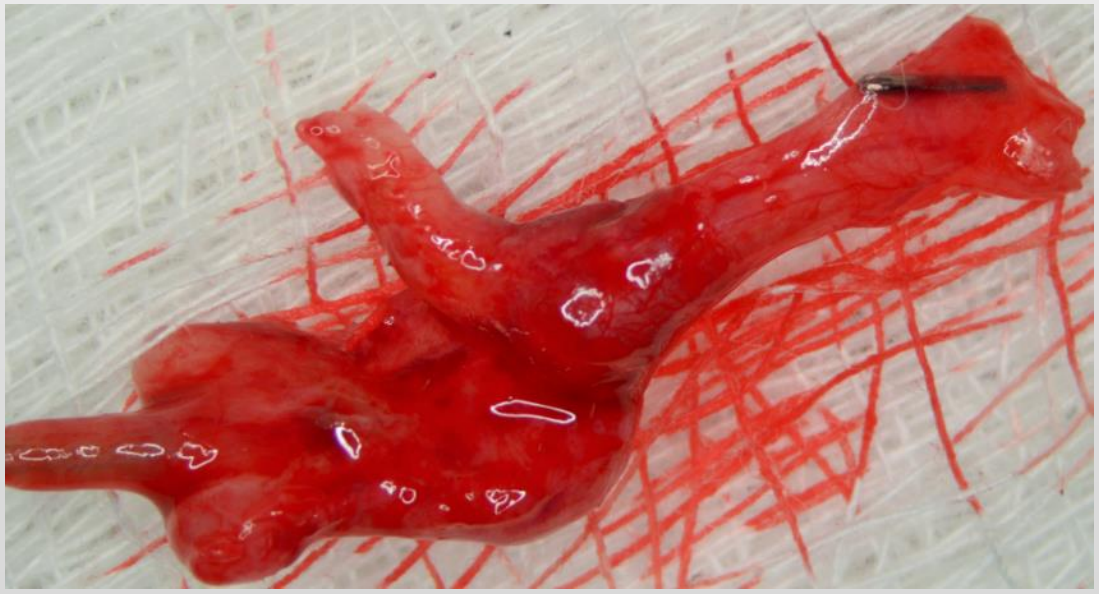


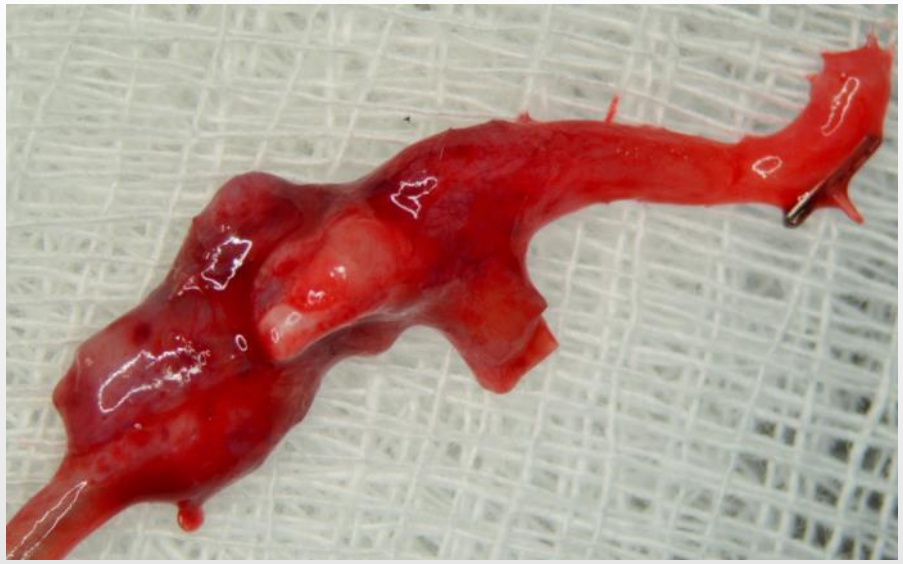


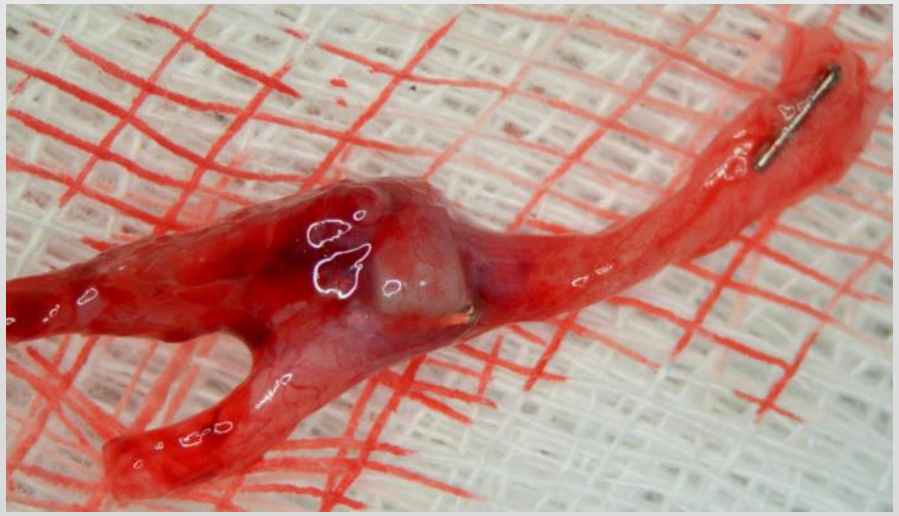


Harvesting of implanted arrays

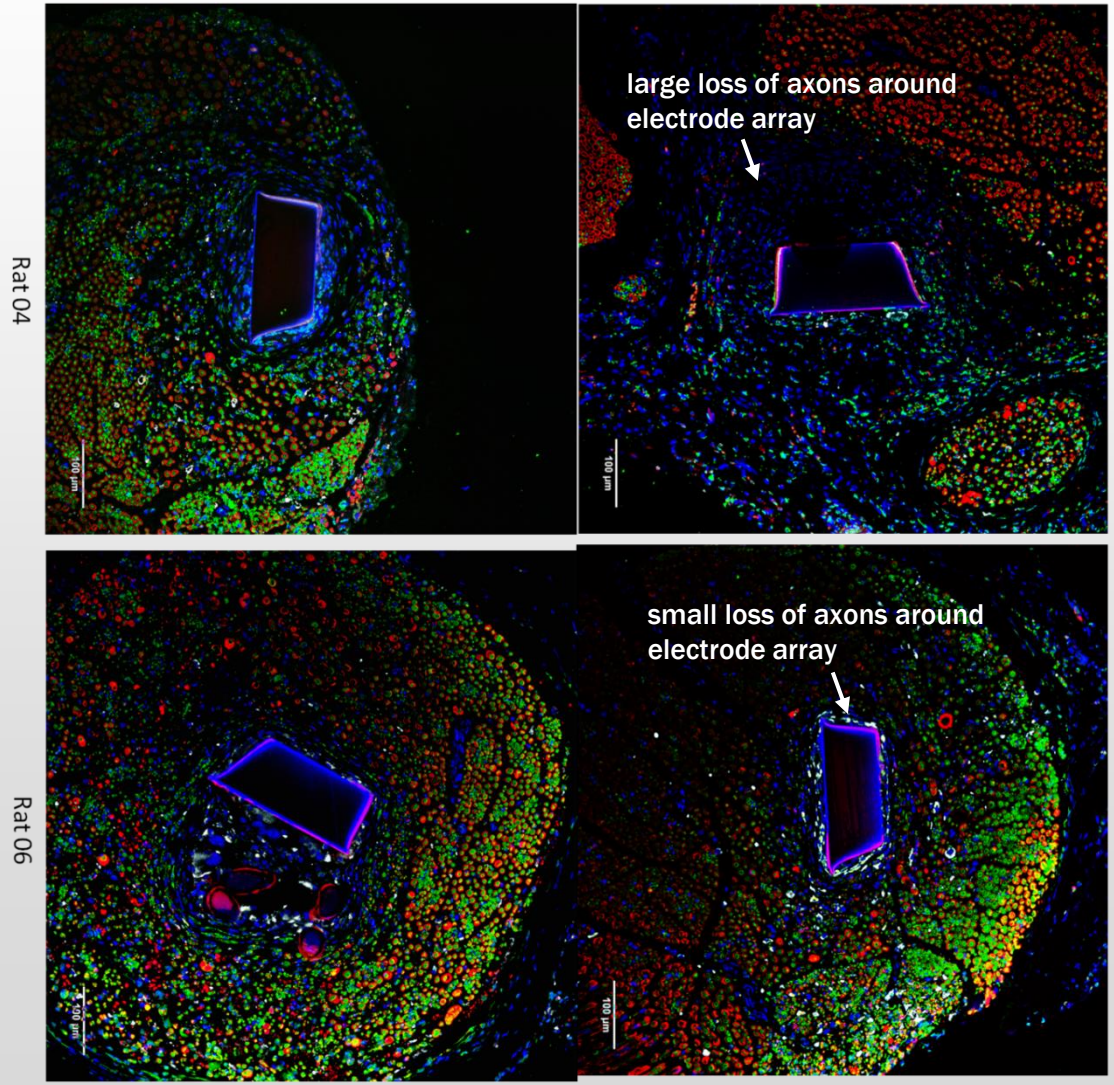








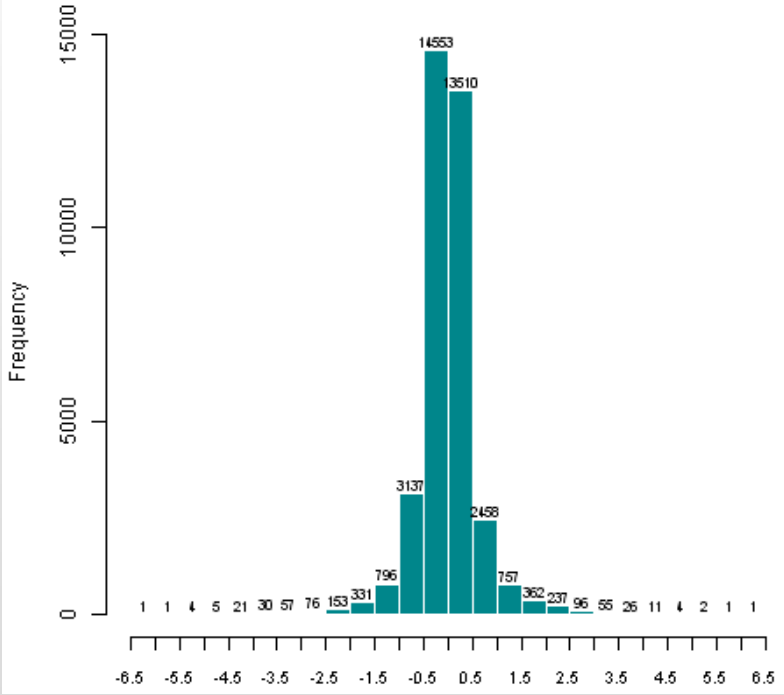
Histology of sciatic nerve surrounding chronic electrode array



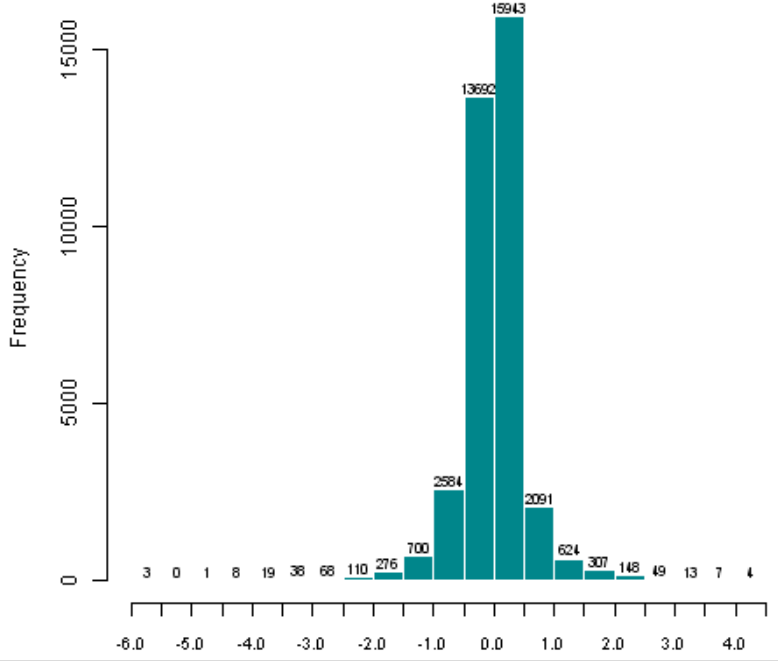
Blue- cell nuclei
Green- β-tubulin
Red- myelin
White- macrophages

Fold differences in gene expression

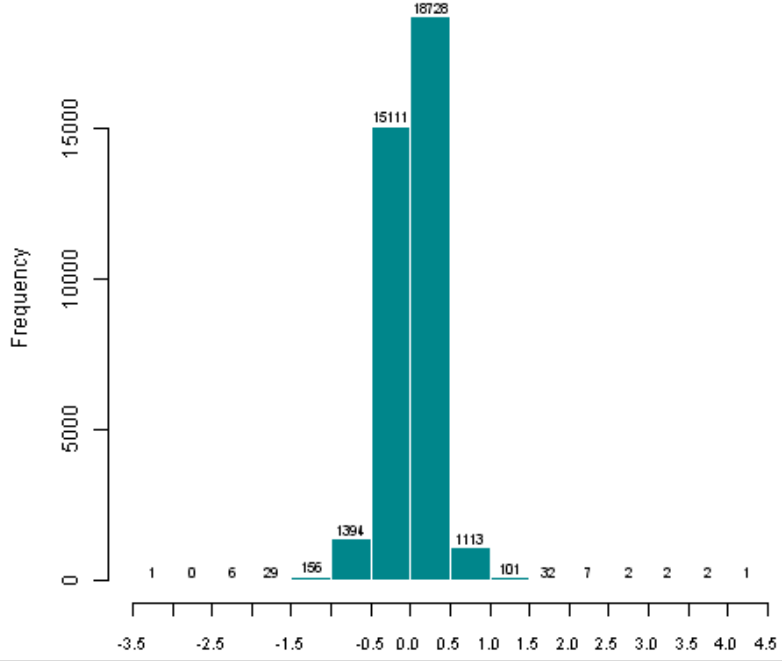
Uninjured control vs. electrode array



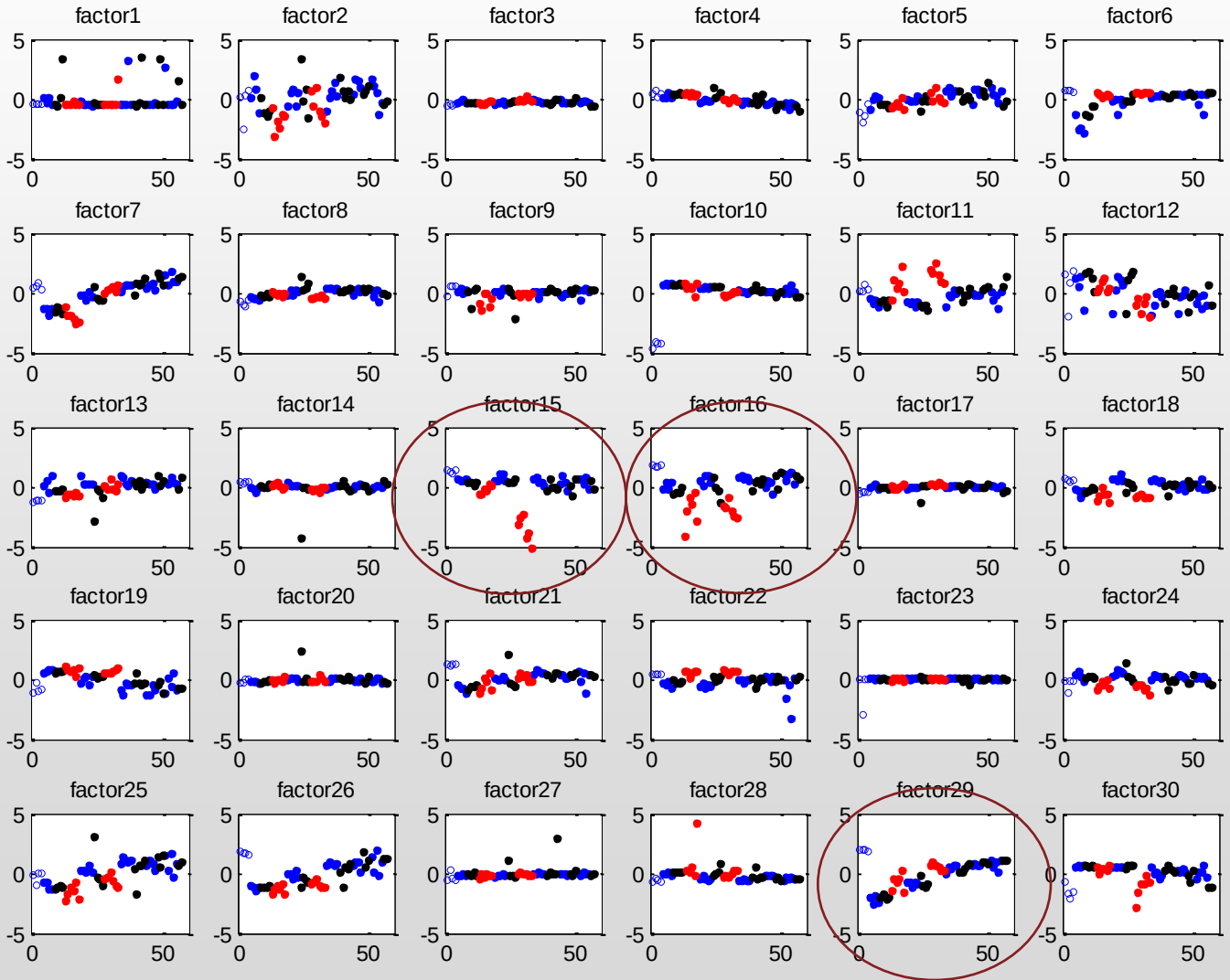
Uninjured control vs. implant/withdraw



Electrode array vs. implant/withdraw

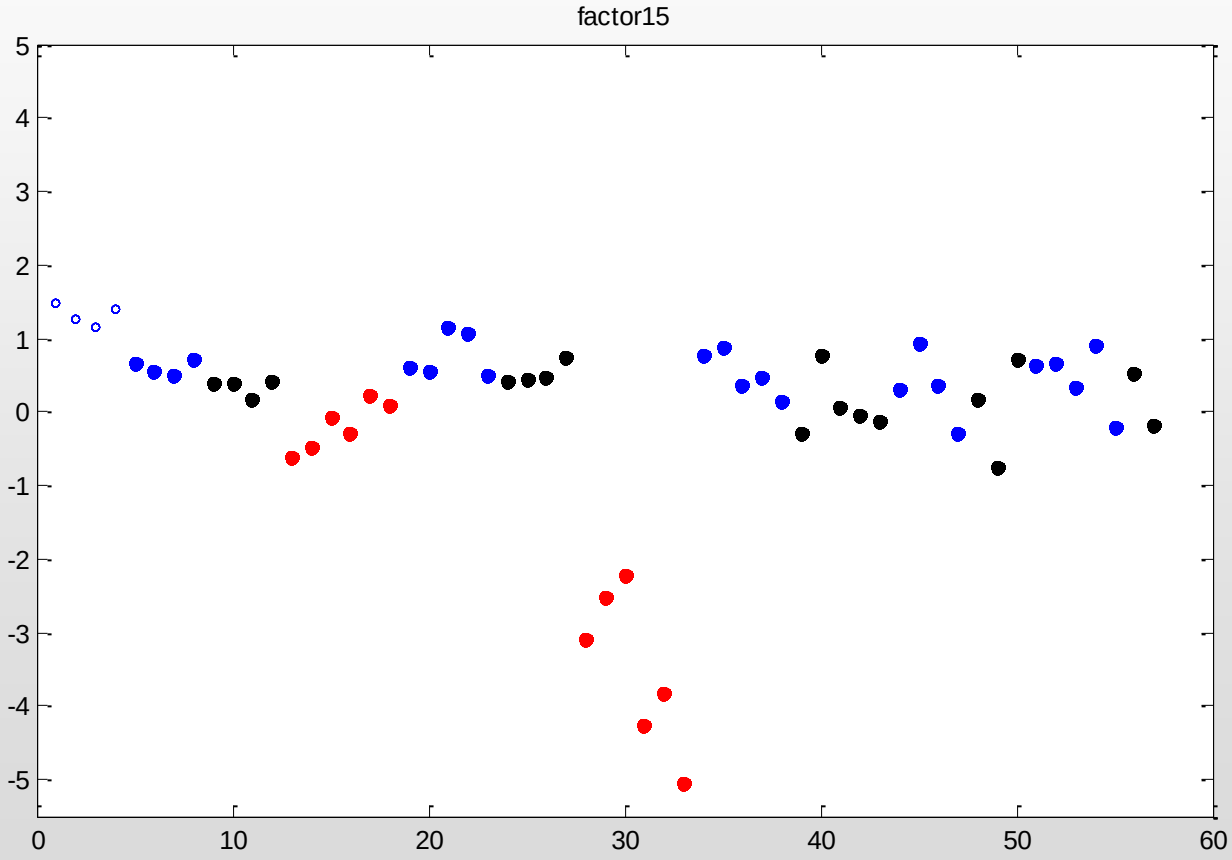


Factor analysis of gene array data



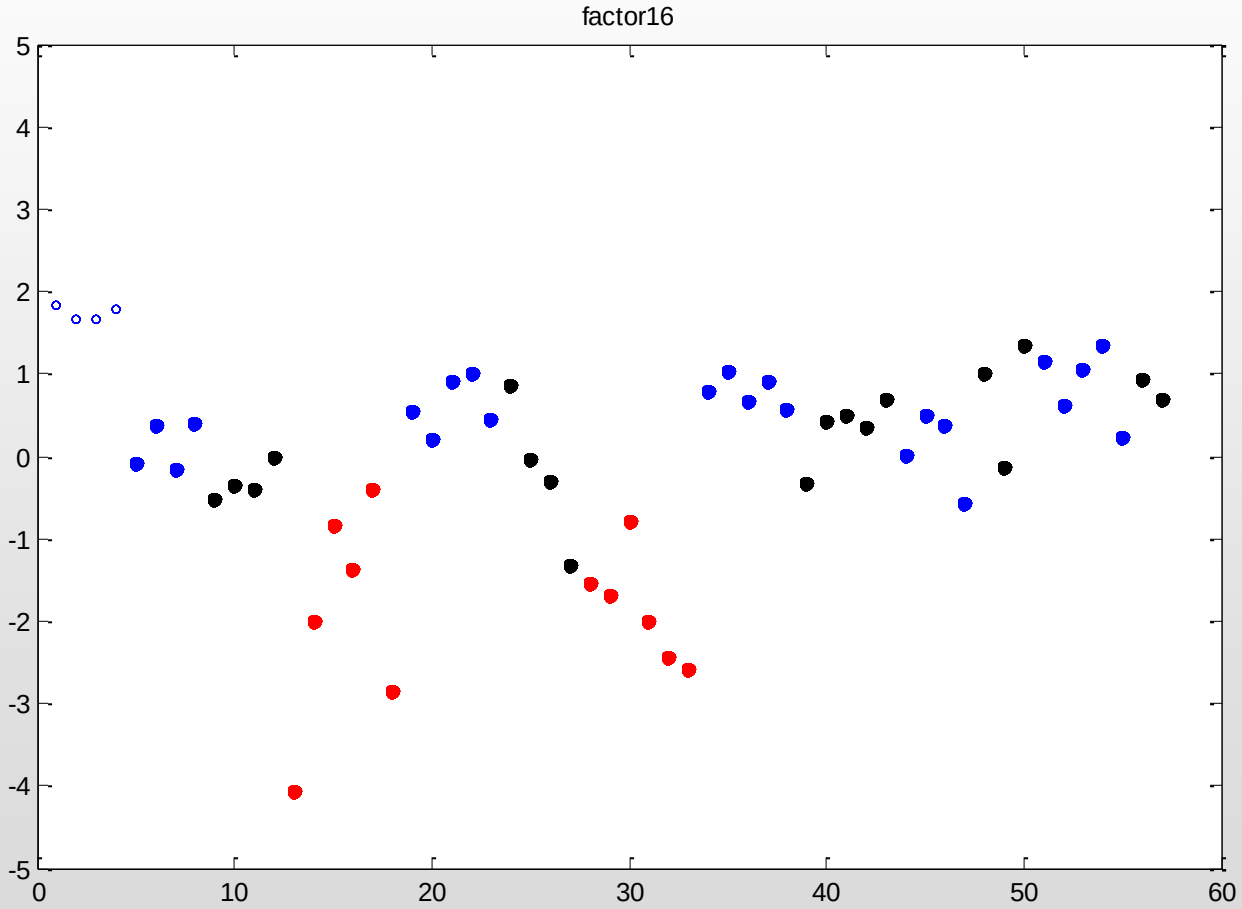
Top 20 genes explaining factor 15

'Ckb'
'St3gal5'
'Bhlhe41'
'Mmp9'
'Slco4a1'
'Mt3'
'Arsb'
'Plscr2'
'Meis3'
'Atp6v1c1'
'Esrra'
'Cln5'
'Rab11fip1'
'Frrs1'
'Col17a1'
'Snx8'
'Agap3'
'Gabbr2'
'Sctr'
'Cela1'



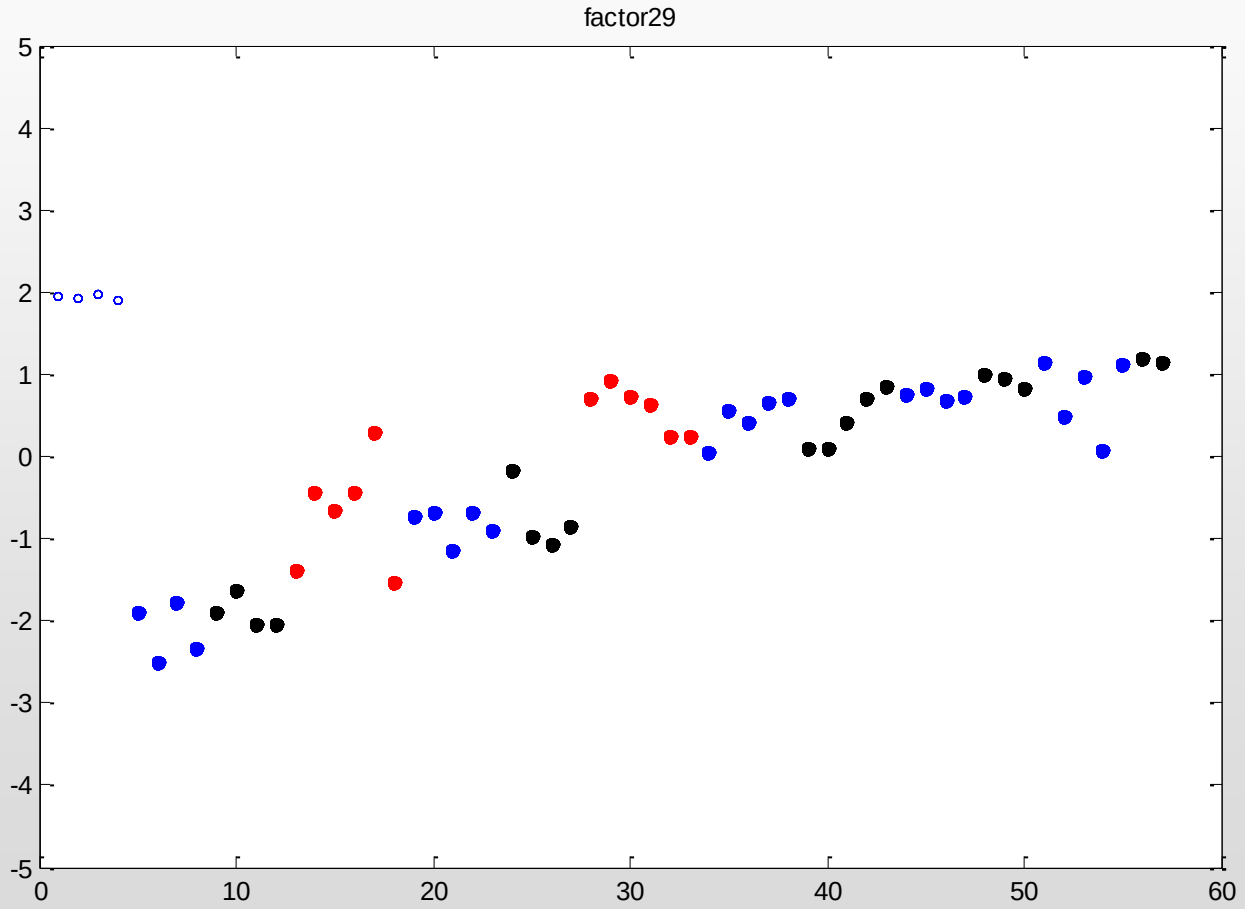
Top 20 genes explaining factor 16

'Clec10a'
'C3ar1'
'Cd4'
'C1qb'
'Cyp2d1 /// Cyp2d5'
'Prkcb'
'Scamp2'
'Fam49b'
'Pik3cd'
'C1qa'
'RGD1564450'
'Cd86'
'Lpxn'
'Ccl7'
'Tifa'
'Def8'
'Cklf'
'Pim2'
'MGC105649'
'LOC100362727'



Top 20 genes explaining factor 29

'Lphn1'
'Nov'
'Rarres2'
'Cdca8'
'Cdkn1c'
'Mat2b'
'Smtn'
'Espl1'
'Fam176a'
'Fam64a'
'Moxd1'
'Prss35'
'Steap4'
'Ramp3'
'Cxcl12'
'Fcer1a'
'Slc40a1'
'Igsf3'
'Bmper'
'Tmem47'



The 60 genes with maximum contribution to differences between groups

Factor 15		Factor 16		Factor 29	
Probe_ID	Gene_Symbol	Probe_ID	Gene_Symbol	Probe_ID	Gene_Symbol
'1367740_at'	'Ckb'	'1380822_at'	'Clec10a'	'1379747_at'	'Lphn1'
'1371021_at'	'St3gal5'	'1372097_at'	'C3ar1'	'1372684_at'	'Nov'
'1373398_at'	'Bhlhe41'	'1368464_at'	'Cd4'	'1392140_at'	'Rarres2'
'1382174_at'	'Mmp9'	'1392043_at'	'C1qb'	'1376084_a_at'	'Cdca8'
'1387383_at'	'Slco4a1'	'1376652_at'	'Cyp2d1 /// Cyp2d5'	'1391345_at'	'Cdkn1c'
'1376071_at'	'Mt3'	'1378244_at'	'Prkcb'	'1383999_at'	'Mat2b'
'1375934_at'	'Arsb'	'1379935_at'	'Scamp2'	'1390412_at'	'Smtn'
'1383834_at'	'Plscr2'	'1391227_at'	'Fam49b'	'1382995_at'	'Espl1'
'1387819_at'	'Meis3'	'1391269_at'	'Pik3cd'	'1371928_at'	'Fam176a'
'1385608_at'	'Atp6v1c1'	'1378633_at'	'C1qa'	'1387655_at'	'Fam64a'
'1369437_at'	'Esrra'	'1394122_at'	'RGD1564450'	'1372299_at'	'Moxd1'
'1372800_at'	'Cln5'	'1369483_at'	'Cd86'	'1387713_a_at'	'Prss35'
'1394407_at'	'Rab11fip1'	'1370377_at'	'Lpxn'	'1378171_at'	'Steap4'
'1378160_at'	'Frrs1'	'1392958_at'	'Ccl7'	'1390581_at'	'Ramp3'
'1374741_at'	'Col17a1'	'1388720_at'	'Tifa'	'1389039_at'	'Cxcl12'
'1377790_at'	'Snx8'	'1371090_at'	'Def8'	'1371691_at'	'Fcer1a'
'1374396_at'	'Agap3'	'1387956_s_at'	'Cklf'	'1376193_at'	'Slc40a1'
'1368109_at'	'Gabbr2'	'1377751_at'	'Pim2'	'1367996_a_at'	'Igsf3'
'1387671_at'	'Sctr'	'1369173_at'	'MGC105649'	'1395334_at'	'Bmper'
'1383751_at'	'Cela1'	'1392547_at'	'LOC100362727'	'1394472_at'	'Tmem47'

Conclusions

Implanted silicone/platinum foil electrode arrays elicited robust and persistent tissue encapsulation response at 90 days. Intraneural encapsulation of implanted electrodes was less severe than the extraneural response, but there were large differences in axon preservation seen between different animals, which correlated with the differences in electrophysiology results. Differences in gene expression between uninjured control and injured control were still present at 90 days, but most changes in gene expression was due to presence of chronic electrode arrays, not the implant/withdraw injury. Factor analysis of gene array data provided 60 genes that made the largest contribution to explaining the differences between uninjured and chronically implanted electrode arrays. Included in this gene list were factors involved in inflammation (CD86, Ccl7, CD4, CXCL12, etc.) and tissue matrix reorganization (MMP9, Col17a1, Cela1). Several genes with known functions seemingly unrelated to foreign body tissue response were included in the list (Bhlhe41, Fcer1a, Slc40a1). The experiments summarized here provide important insights into the chronic response of peripheral nerves to implanted electrodes, and suggest that changes to the design and materials of the chronic electrodes may be useful in minimizing tissue reaction and prolonging the useful lifetime of implanted arrays.