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UNIVERSITY OF CALIFORNIA SAN DIEGO

High-channel count, conformal microelectrode arrays for spinal cord recording and focal stimulation

A Dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Bioengineering

by

Samantha M. Russman

Committee in charge:

Professor Shadi Dayeh, Chair
Professor Gert Cauwenberghs, Co-Chair
Professor Sharona Ben-Haim
Professor Joseph Ciacchi
Professor Ester Kwon
Professor Mark Tuszynski

2024

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University of California San Diego

2024

Dedication

To my parents

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ABSTRACT OF THE DISSERTATION

High-channel count, conformal microelectrode arrays for spinal cord recording and focal stimulation

by

Samantha M. Russman

Doctor of Philosophy in Bioengineering

University of California San Diego, 2024

Professor Shadi Dayeh, Chair

Professor Gert Cauwenberghs, Co-Chair

Spinal cord neural interfaces are vital for treating neurological conditions, including chronic pain and spinal cord injury. Current spinal cord neural interfaces rely on bulky, low-channel count paddle arrays to interface with the spinal cord. In recent years, there has been growing interest to develop flexible, high-channel count microelectrode arrays for safe and highly

effective neural interfaces. These arrays provide high conformality and better contact to neural tissue, reducing the body's biofouling response, and offer more choice in stimulation and recording location due to their high channel count. However, few studies have directly evaluated the benefits of flexible, high-channel count microelectrode arrays for recording and stimulation of the spinal cord compared to existing technologies available clinically. Prior studies that have examined this relationship have primarily involved cortical electrodes. In this dissertation, we study the benefits of using flexible, high-channel count microelectrode arrays for spinal cord neural interfaces and provide evidence to support their further widespread adoption.

In Chapter 1, we introduce a conformal, high-channel count microelectrode array for intraoperative neuromonitoring during human spinal cord surgery. We show that this array can map spinal cord function at unprecedented spatial resolution, improving precision and responsiveness to potential spinal cord damage. In Chapter 2, we introduce a conformal, high-channel count microelectrode array for epidural electrical stimulation of the rat spinal cord for treatment of spinal cord injury, termed SpineWrap. We demonstrate that this electrode delivers ultra-focal stimulation, resulting in highly selective muscle recruitment. Thus, Chapters 1 and 2 emphasize the importance of achieving submillimeter resolution in spinal cord mapping and stimulation. In Chapter 3, we address the need for durable and conductive materials for chronic neural interfaces through the fabrication and characterization of a novel microelectrode array employing a more stable electrode coating material. We translate the use of this material from bulk film to microelectrodes and demonstrate its improved stability in benchtop and in vivo experiments. Together, the novel technologies established in this dissertation are important steps to drive the integration of flexible, high-channel count microelectrode arrays in the clinic for spinal cord applications.

Introduction

Primary objectives for spinal cord neural interfaces

In the broadest sense, there are two primary objectives for spinal cord neural interfaces: to record and to stimulate spinal cord neural activity. In a clinical setting, recording spinal cord neural activity can provide insight into the pathophysiology of neurological conditions, including spinal cord injury (SCI), tumors, and degenerative disorders (1, 2). Spinal cord recording also features prominently in spinal cord surgery as intraoperative neuromonitoring (IONM). IONM assists surgeons in planning and performing safe surgery by reducing the risk of spinal cord damage (3). Finally, spinal cord recording is used for monitoring neurological health in critical care, such as after experiencing spinal cord injury (SCI) (4, 5).

Spinal cord stimulation (SCS) also plays a vital role in clinic. Predominantly, SCS has been used for decades for treatment of chronic and neuropathic pain (6–9). SCS constitutes an alternative treatment to the traditional pharmacological approach of using strong drugs such as opioids, which are highly addictive. SCS can also manage pain in patients who are not responsive or have become tolerant to medication (10). Finally, SCS can enable real-time adjustment of stimulation parameters if previous parameters become ineffective, which is more convenient and straightforward to implement than changing pain medication.

Recently, there has been growing interest in using SCS for treating SCI, although spinal cord neural interfaces for SCI remain mostly unavailable clinically (11–17). There are compelling reasons to use spinal cord neural interfaces for SCI treatment. First, SCS can take advantage of preserved spinal cord circuitry below the point of injury, such as central pattern generators for walking, which can be activated with stimulation (18–22). Second, the tracts for sensory and motor information are spatially organized in the spinal cord into distinct areas, which facilitates

stimulating and recording targeted sites. Finally, undergoing surgery below or at the site of injury in patients with SCI carries less risk than alternative surgical approaches on non-damaged tissue, such as intracranial surgery to implant cortical grids to modulate the sensorimotor cortex.

In summary, spinal cord neural interfaces have already improved the quality of life for many patients due to the use of this technology in clinical settings. These technologies will only continue to impact patients more as the recording and stimulation capabilities continue to improve.

A case for high-channel, conformal count spinal cord microelectrode arrays

In recent years, there has been increasing interest to develop high-channel count, conformal microelectrode arrays for spinal cord neural interfaces (23–26). One reason for this is that such microelectrode arrays can bend along the curvature of the spinal cord and thus allow for a larger interface area for recording and stimulation. This results in greater spatial proximity between the individual electrode contacts and neurons, resulting in higher signal-to-noise ratio (SNR) over a larger volume of neural tissue. Another benefit of flexible microelectrode arrays is that they reduce foreign body response to the implant, which is a reaction that occurs when the immune response detects the presence of foreign material (27, 28). Flexible microelectrode arrays are based on substrate materials that are extremely thin, have a low Young's modulus, and can be designed to have new shapes and form factors, reducing this response. As a result of the weaker foreign body response, flexible microelectrode arrays are more stable chronically, improving long-term device performance by maintaining stimulation and recording capabilities (29). This reduces the need for implant replacement or adjustment, resulting in better patient outcomes.

While there have been many advancements in the field of flexible spinal cord microelectrode arrays, there have been few reports on flexible arrays with high-channel counts. The few studies that have explored the effect of channel count on the performance of neural

interfaces focused on cortical implants (30, 31). This dissertation addresses this knowledge gap. Chapter 1 reports on a novel, high-density microelectrode array with hundreds of channels to record neural activity from the exposed spinal cord during surgery. We reconstruct high-resolution, two-dimensional maps of neural activity with submillimeter precision. We show that flexible, high-channel count electrodes enhance the capabilities of IONM by detecting neural responses at lower thresholds and with fewer trials than existing clinical systems can. Chapter 2 evaluates how flexible, high-channel count arrays improve spinal cord stimulation. In this chapter, we develop a flexible, high-channel count microelectrode array for ultra-focal stimulation, termed SpineWrap. We compare the stimulation focality and muscle recruitment selectivity of both SpineWrap and a low-channel count array in rats and show that SpineWrap produces more selective muscle recruitment.

The need for low-impedance, stable electrode coating materials

Electrode material coating plays a crucial role in the development of flexible, high-channel count microelectronics. To create high-density microelectrode arrays, small contact sizes are necessary. However, standard electrode materials for neural interfaces, such as gold and platinum, cannot be reduced to such small contact sizes without experiencing a high impedance due to the reduced electrochemical interface area (32). Additionally, these materials have low charge injection capacity (CIC) (33). High impedance results in low SNR during recording and low CIC limits the stimulation current levels that can be safely delivered, reducing stimulation effectiveness.

A common strategy for increasing the electrochemical surface area is to coat the electrode contacts with a conductive polymer (34, 35). Conductive polymers must withstand damage caused by electrochemical reactions at the electrode-tissue interface during recording and stimulation,

tolerate the shear forces from tissue as the implant moves, and endure host biofouling responses (36). Thus, stability of the conductive polymer is critical to maintain the fidelity of the recorded signals and the delivery of effective stimulation. Most existing conductive polymers are not very stable so there continues to be a need for better stable materials for long-term neural interfaces (37). We address this need for highly stable neural interfaces in Chapter 3. Chapter 3 introduces a novel, stable PEDOT-based coating for neural interfaces developed in Prof. Darren Lipomi's laboratory, for which we evaluate the adhesion, mechanical, and electrical properties of this material in benchtop and *in vivo* experiments.

References

1. J. R. Wolpaw, A. M. Tennissen, "ACTIVITY-DEPENDENT SPINAL CORD PLASTICITY IN HEALTH AND DISEASE" (2001); www.annualreviews.org.
2. D. S. Yanni, S. Ulkatan, V. Deletis, I. J. Barrenechea, C. Sen, N. I. Perin, Utility of neurophysiological monitoring using dorsal column mapping in intramedullary spinal cord surgery. *J Neurosurg Spine* **12** (2010).
3. T. N. Pajewski, V. Arlet, L. H. Phillips, "Current approach on spinal cord monitoring: The point of view of the neurologist, the anesthesiologist and the spine surgeon" in *European Spine Journal* (2007)vol. 16.
4. C. M. Miller, M. T. Torbey, *Neurocritical Care Monitoring* (Demos Medical Publishing, 2014).
5. P. Costa, G. Faccani, F. Sala, E. Montalenti, M. L. Giobbe, V. Deletis, Neurophysiological assessment of the injured spinal cord: an intraoperative approach. *Spinal Cord* **52**, 749–757 (2014).
6. D. M. Long, N. Hagfors, Electrical stimulation in the nervous system: The current status of electrical stimulation of the nervous system for relief of pain. *Pain* **1** (1975).
7. M. Meglio, B. Cioni, A. Dal Lago, M. De Santis, P. Pola, M. Serricchio, "Pain control and improvement of peripheral blood flow following epidural spinal cord stimulation Case report" (1981).
8. C. N. SHEALY, N. TASLITZ, J. T. MORTIMER, D. P. BECKER, electrical inhibition of pain: experimental evaluation: *. *Anesth Analg* **46** (1967).
9. B. S. Nashold, H. Friedman, Dorsal column stimulation for control of pain: Preliminary report on 30 patients. *J Neurosurg* **36**, 590–597 (1972).
10. B. Linderroth, R. D. Foreman, Physiology of Spinal Cord Stimulation: Review and Update. *Neuromodulation: Technology at the Neural Interface* **2**, 150–164 (1999).
11. V. R. Edgerton, S. Harkema, Epidural stimulation of the spinal cord in spinal cord injury: Current status and future challenges. [Preprint] (2011). <https://doi.org/10.1586/ern.11.129>.
12. S. Harkema, Y. Gerasimenko, J. Hodes, J. Burdick, C. Angeli, Y. Chen, C. Ferreira, A. Willhite, E. Rejc, R. G. Grossman, V. R. Edgerton, Effect of epidural stimulation of the

- lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. *The Lancet* **377**, 1938–1947 (2011).
13. C. A. Angeli, M. Boakye, R. A. Morton, J. Vogt, K. Benton, Y. Chen, C. K. Ferreira, S. J. Harkema, Recovery of Over-Ground Walking after Chronic Motor Complete Spinal Cord Injury. *New England Journal of Medicine* **379**, 1244–1250 (2018).
 14. E. Choi, S. Gattas, N. Brown, J. Hong, J. Limbo, A. Chan, M. Oh, Epidural electrical stimulation for spinal cord injury. Wolters Kluwer Medknow Publications [Preprint] (2021). <https://doi.org/10.4103/1673-5374.313017>.
 15. N. Wenger, E. M. Moraud, J. Gandar, P. Musienko, M. Capogrosso, L. Baud, C. G. Le Goff, Q. Barraud, N. Pavlova, N. Dominici, I. R. Minev, L. Asboth, A. Hirsch, S. Duis, J. Kreider, A. Mortera, O. Haverbeck, S. Kraus, F. Schmitz, J. DiGiovanna, R. Van Den Brand, J. Bloch, P. Detemple, S. P. Lacour, E. Bézard, S. Micera, G. Courtine, Spatiotemporal neuromodulation therapies engaging muscle synergies improve motor control after spinal cord injury. *Nat Med* **22**, 138–145 (2016).
 16. F. B. Wagner, J. B. Mignardot, C. G. Le Goff-Mignardot, R. Demesmaeker, S. Komi, M. Capogrosso, A. Rowald, I. Seáñez, M. Caban, E. Pirondini, M. Vat, L. A. McCracken, R. Heimgartner, I. Fodor, A. Watrin, P. Seguin, E. Paoles, K. Van Den Keybus, G. Eberle, B. Schurch, E. Pralong, F. Becce, J. Prior, N. Buse, R. Buschman, E. Neufeld, N. Kuster, S. Carda, J. von Zitzewitz, V. Delattre, T. Denison, H. Lambert, K. Minassian, J. Bloch, G. Courtine, Targeted neurotechnology restores walking in humans with spinal cord injury. *Nature* **563**, 65–93 (2018).
 17. A. Rowald, S. Komi, R. Demesmaeker, E. Baaklini, S. D. Hernandez-Charpak, E. Paoles, H. Montanaro, A. Cassara, F. Becce, B. Lloyd, T. Newton, J. Ravier, N. Kinany, M. D’Ercole, A. Paley, N. Hankov, C. Varescon, L. McCracken, M. Vat, M. Caban, A. Watrin, C. Jacquet, L. Bole-Feysot, C. Harte, H. Lorach, A. Galvez, M. Tschopp, N. Herrmann, M. Wacker, L. Geernaert, I. Fodor, V. Radevich, K. Van Den Keybus, G. Eberle, E. Pralong, M. Roulet, J. B. Ledoux, E. Fornari, S. Mandija, L. Mattera, R. Martuzzi, B. Nazarian, S. Benkler, S. Callegari, N. Greiner, B. Fuhrer, M. Froeling, N. Buse, T. Denison, R. Buschman, C. Wende, D. Ganty, J. Bakker, V. Delattre, H. Lambert, K. Minassian, C. A. T. van den Berg, A. Kavounoudias, S. Micera, D. Van De Ville, Q. Barraud, E. Kurt, N. Kuster, E. Neufeld, M. Capogrosso, L. Asboth, F. B. Wagner, J. Bloch, G. Courtine, Activity-dependent spinal cord neuromodulation rapidly restores trunk and leg motor functions after complete paralysis. *Nat Med* **28**, 260–271 (2022).
 18. M. R. DIMITRIJEVIC, Y. GERASIMENKO, M. M. PINTER, Evidence for a Spinal Central Pattern Generator in Humansa. *Ann N Y Acad Sci* **860**, 360–376 (1998).
 19. J. T. Eisdorfer, R. D. Smit, K. M. Keefe, M. A. Lemay, G. M. Smith, A. J. Spence, Epidural Electrical Stimulation: A Review of Plasticity Mechanisms That Are Hypothesized to Underlie Enhanced Recovery From Spinal Cord Injury With Stimulation. *Front Mol Neurosci* **13** (2020).
 20. P. A. Guertin, Preclinical evidence supporting the clinical development of central pattern generator-modulating therapies for chronic spinal cord-injured patients. *Front Hum Neurosci* **8** (2014).
 21. G. Barrière, H. Leblond, J. Provencher, S. Rossignol, Prominent Role of the Spinal Central Pattern Generator in the Recovery of Locomotion after Partial Spinal Cord Injuries. *The Journal of Neuroscience* **28**, 3976 (2008).

22. K. Minassian, U. S. Hofstoetter, F. Dzeladini, P. A. Guertin, A. Ijspeert, The Human Central Pattern Generator for Locomotion: Does It Exist and Contribute to Walking? *The Neuroscientist* **23**, 649–663 (2017).
23. I. R. Mineev, P. Musienko, A. Hirsch, Q. Barraud, N. Wenger, E. M. Moraud, J. Gandar, M. Capogrosso, T. Milekovic, L. Asboth, R. F. Torres, N. Vachicouras, Q. Liu, N. Pavlova, S. Duis, A. Larmagnac, J. Vörös, S. Micera, Z. Suo, G. Courtine, S. P. Lacour, Electronic dura mater for long-term multimodal neural interfaces. *Science (1979)* **347**, 159–163 (2015).
24. Chang CW, Lo YK, Gad P, Edgerton R, Liu W, Design and fabrication of a multi-electrode array for spinal cord epidural stimulation. *36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 6834–6837 (2014).
25. P. Gad, J. Choe, M. S. Nandra, H. Zhong, R. R. Roy, Y. C. Tai, V. R. Edgerton, Development of a multi-electrode array for spinal cord epidural stimulation to facilitate stepping and standing after a complete spinal cord injury in adult rats. *J Neuroeng Rehabil* **10** (2013).
26. V. Giagka, A. Demosthenous, N. Donaldson, Flexible active electrode arrays with ASICs that fit inside the rat's spinal canal. *Biomed Microdevices* **17** (2015).
27. D. Bendersky, C. Yampolsky, Is spinal cord stimulation safe? A review of its complications. Elsevier Inc. [Preprint] (2014). <https://doi.org/10.1016/j.wneu.2013.06.012>.
28. G. Taccola, S. Barber, P. J. Horner, H. A. C. Bazo, D. Sayenko, Complications of epidural spinal stimulation: lessons from the past and alternatives for the future. Springer Nature [Preprint] (2020). <https://doi.org/10.1038/s41393-020-0505-8>.
29. J. Shi, Y. Fang, Flexible and Implantable Microelectrodes for Chronically Stable Neural Interfaces. *Advanced Materials* **31**, 1804895 (2019).
30. S. Duraivel, S. Rahimpour, C.-H. Chiang, M. Trumpis, C. Wang, K. Barth, S. C. Harward, S. P. Lad, A. H. Friedman, D. G. Southwell, S. R. Sinha, J. Viventi, G. B. Cogan, High-resolution neural recordings improve the accuracy of speech decoding. *Nat Commun* **14**, 6938 (2023).
31. T. Kaiju, K. Doi, M. Yokota, K. Watanabe, M. Inoue, H. Ando, K. Takahashi, F. Yoshida, M. Hirata, T. Suzuki, High Spatiotemporal Resolution ECoG Recording of Somatosensory Evoked Potentials with Flexible Micro-Electrode Arrays. *Front Neural Circuits* **11** (2017).
32. P. E. K. Donaldson, The Role of Platinum Metals in Neurological Pro sthe se s PROGRESS IN BIOMEDICAL APPLICATIONS. *Platin Met Rev* **31**, 2–7 (1987).
33. S. B. Brummer, L. S. Robblee, F. T. Hambrecht, CRITERIA FOR SELECTING ELECTRODES FOR ELECTRICAL STIMULATION: THEORETICAL AND PRACTICAL CONSIDERATIONS. *Ann N Y Acad Sci* **405**, 159–171 (1983).
34. R. Green, M. R. Abidian, Conducting Polymers for Neural Prosthetic and Neural Interface Applications. *Advanced Materials* **27**, 7620–7637 (2015).
35. K. M. Szostak, L. Grand, T. G. Constandinou, Neural Interfaces for Intracortical Recording: Requirements, Fabrication Methods, and Characteristics. *Front Neurosci* **11** (2017).
36. S. M. Wellman, J. R. Eles, K. A. Ludwig, J. P. Seymour, N. J. Michelson, W. E. McFadden, A. L. Vazquez, T. D. Y. Kozai, A Materials Roadmap to Functional Neural Interface Design. *Adv Funct Mater* **28**, 1701269 (2018).
37. S. M. Won, E. Song, J. Zhao, J. Li, J. Rivnay, J. A. Rogers, Recent Advances in Materials, Devices, and Systems for Neural Interfaces. *Advanced Materials* **30**, 1800534 (2018).

Chapter 1

Constructing 2D maps of human spinal cord activity and isolating the functional midline with high-density microelectrode arrays

Introduction

Spinal surgeries most often work around the spinal cord, nerve roots, and associated blood vessels, with some cases requiring entry into the neural elements. In either case, surgery has an inherent risk for debilitating or devastating postoperative neurological deficits (1–4). For surgery involving the spinal cord and nerves, this risk is particularly profound when a safe entry or dissection pathway cannot be readily identified, such as when resecting intradural spinal cord tumors (5). For surgeries with risk of neurological injury, the surgeons will often use intraoperative neurophysiological monitoring (IONM) of the spinal cord to indirectly assess the functional integrity of motor and sensory pathways when operating in or around spinal neural structures.

IONM has previously been shown to improve surgical outcomes (1–4, 6–10), but in its current form, it only provides an indirect evaluation of the structural and functional spinal pathways. During IONM, somatosensory evoked potentials (SSEPs) are recorded from the scalp in response to electrical current stimulation of a peripheral nerve, and motor evoked potentials (MEPs) (11–15) are recorded from the upper and lower limb muscles in response to transcranial electrical stimulation (TES) (16–19). In recording SSEPs from the brain, the scalp, cranium, and dura all act as insulators resulting in low amplitude responses. As a result, high-amplitude stimulation currents must be applied with high-count stimulation trains to improve signal-to-noise ratio of the averaged recordings and extract electrophysiological signals from the recording system's baseline noise (19–21). These stimulation trains also prevent real-time monitoring, where

neurological injuries may not be noticeable for many minutes after the triggering event (22). Finally, the physical separation of the recording contacts on the scalp from the surgical site on the spinal cord prevents recording of spatiotemporal, anatomical, and physiological features of responses.

IONM is also not without controversies. The sensitivity and specificity of SSEP and MEP are not perfect, where false positives may lead to cessation of surgery and incomplete resection, false negative may lead to undetected injury (23–25). Additionally, there is still the practice of wake-up testing in certain spine surgeries such as scoliosis. Additionally, SSEPs may not change if the injury is purely motor or vascular.

We hypothesized that direct recording of SSEPs by microelectrodes on the surface of the spinal cord could increase the fidelity of these recordings and reduce the number of required trials, allowing real-time spatiotemporal mapping. We also predicted that recordings with a high channel count microelectrode array would offer two dimensional (2D) SSEP responses, which would allow for more precise identification of the spinal cord functional midline at the point of resection. The functional midline is the only safe corridor to the inner structure of the spinal cord; therefore, its identification is of critical relevance. Unfortunately, there is no consistent or accurate anatomic landmark to the midline on the surface and moreover, it is often distorted with tumors/masses or rotated due to adhesions. Precise functional midline identification would be advantageous given that the position of the anatomical and functional midline has been found to be mismatched by up to 2 mm in 40% of patients (26, 27). Dorsal column (DC) mapping in spinal cord surgery was shown to decrease the occurrence of new postoperative sensorimotor dysfunction by 42% (28). There are limited studies that reported DC mapping by recording SSEPs directly from the surface of the spinal cord using linear 8-contact arrays (29, 30). In these studies, sparse mapping of the

midline was performed by direct electrical stimulation of the dorsal column using a linear array of 8 contacts made of Teflon-coated stainless-steel wires that were 76 μm in diameter and 1 mm apart. The midline was identified by phase reversal in the recorded SSEPs. In two other studies, SSEPs were stimulated from the spinal cord surface and recorded from the brain, again identifying a rudimentary functional midline (31, 32).

We fabricated high-channel number microelectrode arrays by leveraging low-impedance contact materials we have used before in cortical recordings such as poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) (33–36) and platinum nanorod (PtNR) (37) contacts. Our electrodes were fabricated on thin ($\sim 6.6 \mu\text{m}$) parylene C substrates (38, 39), which allowed for high conformability to the pial surface of the spinal cord during IONM. We observed dynamic patterns of ipsilateral and contralateral SSEPs with high-resolution that allowed us to identify a microscale midline boundary. This high-resolution boundary was identified at clinical stimulation currents and persisted even when scalp SSEPs could not be identified. Our microelectrode array also outperformed clinical equipment given that our recordings were directly on the spinal cord surface and thus did not require substantial trial averaging. In the future, this technology could provide a practically instantaneous IONM method once data analysis and plotting are incorporated in real-time. Taken together, our microelectrode grid can improve IONM and could have other applications, such as for evaluation or even treatment of spinal cord injury (SCI).

Results

Experiment design and surgical implantation

To assess the viability of recording spatiotemporal SSEP patterns with high quality and resolution from the surface of the spinal cord, we recorded SSEPs with a high-density

microelectrode array in subjects (N=6) undergoing intramedullary tumor or biopsy procedures in the cervical spinal cord (Fig. 1.1 A). The microelectrode array recordings were adjunct to the surgery, completed with minimal disruption to the surgical flow of the procedure time and performed with institutional review board (IRB) authorization at the University of California San Diego and at Oregon Health & Sciences University. The microelectrode array recordings were performed during three timepoints in the surgery: epidurally and subdurally before tumor resection or biopsy and subdurally post-resection or biopsy. The electrode was placed on the exposed surface spinal cord for tumor resection, covered the DCs and was centered over the anatomical midline of the spinal cord. That surface placement spanned an apparently normal areas of the spinal cord rostral and caudal to the lesion. The placement was documented with intraoperative photos from the surgical microscope. Suction and sponges were used to remove excess blood and cerebrospinal fluid (CSF) from the spinal cord surface to ensure good electrode-tissue contact during the recordings (Fig. 1.1 B). Suction and sponges near the electrode did not negatively impact recording quality or introduce substantiative noise into the data.

Electrode design

Multiple electrode designs were used in the recordings from the six subjects (Table 1.2), with results presented in this work from subjects 4-6. Recordings from these subjects used a single electrode design and leveraged a long, high-density connector that was not available for the earlier subjects (Fig. 1.1 C). Additionally, the overall width of the electrode was reduced from 1.6 cm to 6.4 mm in the final design to maintain stable contact with the dorsal column and account for variable width of the surgical area. These subjects were temporarily implanted with a microelectrode array with 372 PtNR contacts (diameter=30 μ m) arranged in a rectangular array of 12 $\times$ 31 with 350 μ m horizontal (circumferential) pitch and 400 μ m vertical (axial) pitch providing

a coverage area of $3.85 \times 12 \text{ mm}^2$. At the tip of the array, an additional 54 contacts of variable diameter in the range of $30 \text{ }\mu\text{m}$ – $480 \text{ }\mu\text{m}$ were arranged in clusters of three contacts per diameter on the left and right sides of the array. The goal of the multidiameter array was to validate the effectiveness of small contact sizes in recording high quality SSEPs.

The electrodes were designed to have a sterile/non-sterile interface junction along a long connector board (Fig. 1.1 C) to facilitate the use of these devices in the operating room (OR). Whereas the whole thin film device and extender board of Fig. 1.1 C were sterilized at the hospital before use, the extender board separated the recording electronics board from the sterile area, allowing it to be encased in a sterile bag, thus maintaining an overall sterile field in the OR. Additional details on packaging, sterilization, and OR use have been previously reported (40).

Our $6.6 \text{ }\mu\text{m}$ parylene-C microelectrode arrays were much thinner than current clinical spinal cord grids (Fig. 1.1 D, 2 mm thick silicone), therefore more conformal to the spinal surface and compliant to movement during recording. Additionally, the microelectrode arrays were transparent, which gave the surgeon an unobstructed view of the surface spinal anatomical features when the grid was in place. The 1kHz electrochemical impedance of the microelectrode array used in subject 5 measured in phosphate buffered saline post-fabrication was $22.77 \pm 15.11 \text{ k}\Omega$. The impedance values did not change significantly ($p=0.5926$) after Sterrad sterilization, a hydrogen peroxide gas plasma technology which is compatible with the PtNRGrids (40), and were $23.24 \pm 5.42 \text{ k}\Omega$ post-sterilization (Fig. 1.1 E). On the pial surface, the contacts exhibited slightly higher impedance values, with a mean of $35.82 \pm 14.21 \text{ k}\Omega$, an increase that is expected when measuring on tissue (41). These impedance values are representative of those measured across subjects 4-6 (Fig. 1.6). Our recording analysis focused on microelectrode contacts that exhibited a 1kHz impedance lower than $120 \text{ k}\Omega$ as recordings with contacts with higher impedances are susceptible

to noise. After fabrication, sterilization and throughout multiple surgical placements and recordings, our microelectrode arrays maintained a yield above 87% (Fig. 1.1 E, Fig. 1.6). These measurements show the stability of the microelectrode array throughout sterilization and all experimental procedures.

Stimulation paradigm

The IONM team delivered electrical stimulation to both upper limb (typically median) and lower limb (typically tibial) nerves in trains of 40 at 2.78 Hz. Stimulation current amplitudes were initially set to clinical amplitudes used by the IONM team between 30 mA – 50 mA depending on the subject. The stimulation amplitudes were then decreased to $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{8}$ of these clinical amplitudes. For subject 5, the current amplitudes used were therefore 30, 15, 7.5 and 4 mA, which was the lowest current amplitude used for stimulation across all patients.

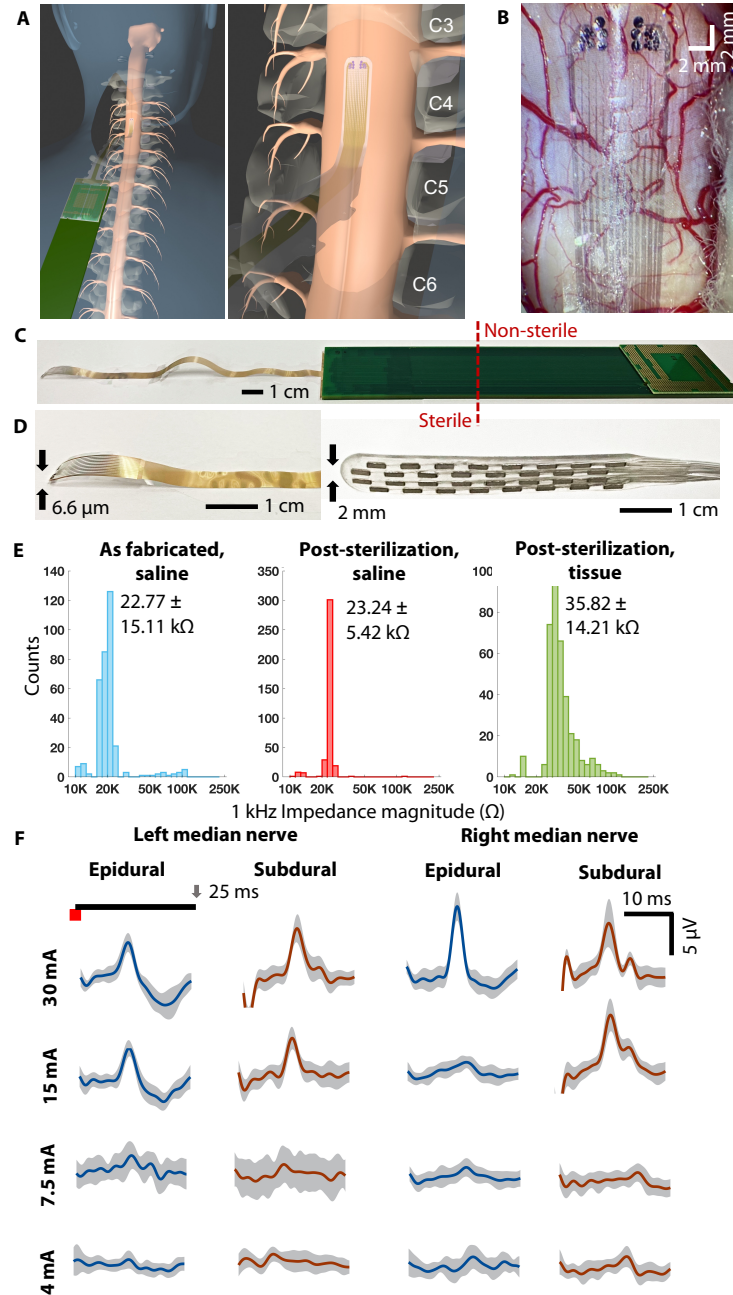


Figure 1.1 Experiment overview and electrode design. (A) schematic diagram of the placement of the research grid on the subject's spinal cord. (B) Photograph of the research grid placed on the subdural surface of spinal cord during recording. (C) Research microelectrode bonded to extender PCB with the sterile/non-sterile junction point highlighted. (D) Comparison of size and thickness of the research microelectrode versus a clinical grid (CoverEdge X 32, Boston Scientific). (E) 1-kHz impedance magnitude histograms measured as fabricated in saline, post-sterilization in saline, and on spinal cord tissue. (F) Example single-channel responses to different left and right median nerve stimulation current amplitudes recorded epidurally and subdurally pre-resection. The channel with the highest peak-to-peak amplitude response was chosen as the example channel for each condition.

Response amplitudes and thresholds

For both epidural and subdural placements, SSEPs had an onset latency of 10 ms and peak latency of ~ 12 ms (Fig. 1.1 F, Fig. 1.2 A-D). This is consistent with previous recordings with low-channel count microgrids from the surface of the spine (11, 42, 43). Averaged maximal peak-to-peak SSEP amplitudes ranged from 8-10 μ V for 30 mA stimulation to ~ 1 μ V for 4 mA stimulation for 30-40 trials. Representative channels with high response amplitudes are shown in Figure 1F. Statistical analysis was used to evaluate whether the peak-to-peak amplitudes were statistically significant compared to pre-stimulus baseline. This was in particular a question for low stimulation currents where the signal to noise ratio was low, which we classified as below $\text{SNR} = 6$ (Table 1.1). A Mann Whitney U test showed significance at almost all current amplitudes at $p < 0.001$ (Table 1.1). However, for 4 mA current amplitudes in both epidural and subdural placements, the effect size was equal to or less than 1, indicating that any differences may not be observable or clinically relevant. A similar low effect size of -1.15 was observed for 15 mA left subdural recording, which we suspect was caused by a fluid build-up on the spinal cord leading to poor electrode-tissue contact for this stimulation amplitude. Generally, for high current amplitudes, response amplitudes were significant (p-values can be found in Table 1.1) for both epidural and subdural placements. Overall, these results indicate that both placements are appropriate for detection of neural signals.

During each case, we also performed a control recording on skin to confirm that the changes in voltage we observed were due to neural activity and not the stimulation artifact. In this subject, the peak-to-peak amplitudes of skin recordings at the clinical stimulation amplitude were below 1 μ V (mean = 0.56 ± 0.18 μ V), which was lower compared to the baseline (mean = 0.75 ± 0.24 μ V) (Fig. 1.7). Similar patterns were observed among all subjects.

Table 1.1 Statistical analysis and significance of results from subject 5.

Current	Mean SNR	T-test	Effect size
Epidural Left			
30 mA	26.79 ± 0.93	2.45E-119	-1.73
15 mA	19.49 ± 1.29	2.45E-119	-1.73
7.5 mA	8.53 ± 2.05	1.24E-112	-1.67
4 mA	1.19 ± 0.12	4.32E-17	0.73
Epidural Right			
30 mA	14.25 ± 0.46	2.45E-119	-1.73
15 mA	18.94 ± 1.66	6.89E-119	-1.73
7.5 mA	5.11 ± 0.76	8.27E-78	-1.39
4 mA	3.00 ± 0.69	4.60E-5	0.29
Subdural Left			
30 mA	28.86 ± 2.27	7.50E-25	-1.72
15 mA	5.62 ± 1.02	7.45E-12	-1.15
7.5 mA	8.24 ± 1.71	2.53E-20	-1.54
4 mA	3.67 ± 0.82	0.0145	-1.00
Subdural Right			
30 mA	9.08 ± 0.45	7.50E-25	-1.72
15 mA	17.62 ± 3.15	5.29E-23	-1.79
7.5 mA	4.47 ± 1.05	0.275	-0.14
4 mA	4.69 ± 1.42	4.19E-4	-0.64

Spatiotemporal response patterns

Our microelectrode array was placed on the spinal cord allowing it to capture spatiotemporal features of the SSEPs, which current IONM clinical equipment cannot resolve because the recording electrodes are placed on the surface of the scalp. Whereas amplitudes of the responses were similar between the epidural and subdural placements, subdural recordings were more localized, especially for higher current amplitudes where response amplitude varied substantially across the array (Fig. 1.2 A-D). Epidural recordings showed more uniform responses across channels compared to subdural recordings, which manifested in a wider spread of responses across the array. Spatiotemporal profiles of subdural recordings showed similar patterns in response propagation for different stimulation currents. A plot of peak-to-peak responses for all current amplitudes calculated from these time evolution plots is shown in Figure 1.8. These

response patterns and dependencies on stimulation currents are consistent with recordings from other subjects (Figs. 2.9, 2.10). Higher response amplitudes were registered on the side of the body where the stimulation was delivered; left channels were more responsive for left median nerve stimulation and right channels for right median nerve stimulation. This is consistent with spinal cord anatomy as the DCs are largely ipsilateral to the peripheral nerves entering the spine.

The conduction velocity of the SSEPs was calculated from the approximate distance of the median nerve stimulating electrode to the recording microelectrode and the peak latency. This was found to be 50 ± 4.5 m/s assuming an arm length of 0.6 m. Previous reports provide conduction velocities in the range of 91.3 ± 7.2 m/s for A α nerve fibers (44) and 63.4 ± 4.5 m/s (45) and 69.3 ± 6.6 m/s for A β nerve fibers, which carry sensory information (46). Our lower estimated conduction velocity might be explained by errors in estimation of arm length and the synapsing of the nerve fibers onto interneurons in the spinal cord. Our calculated SSEP and the onset time of the response could be used to assess neurological damage since changes over 10% in onset latency in clinically recorded scalp SSEPs are considered a sign of spinal cord dysfunction (47).

Mapping of the functional midline

The anatomical spinal cord midline in normal tissue can be identified anatomically by the dorsal median sulcal vein as it enters the midline raphe or at the middle point between the root entry zones on either side of the cord (29). This anatomical midline can be distorted by tumors, edema, neovascularization, or scar formation, prohibiting its identification using anatomical landmarks. To prevent inadvertent dissection of the DCs, which can result in postoperative morbidity that can be disabling, functional mapping is used to locate the midline to inform the spinal incisions (myelotomy). Using the spatiotemporal features of the recorded SSEPs, we performed analysis to map the functional midline for epidural and subdural recordings pre-

resection and subdural recordings post-resection. The midline was identified with better precision in the subdural recording, which is consistent with the more localized response profiles for this placement. By tuning the thresholding method for positive responses and their amplitudes as described in the Methods section, we were able to isolate ipsilateral responses to stimulation and outline a midline spanning 2-5 channels equivalent to a distance of 700 μm to 1.75 mm (Fig. 1.2 F). This mapping is of higher resolution than reported previously with the use of 8-channel microgrids (29, 30), where their contact spacing were equal to or larger than 1 mm. Additionally, with our microelectrode, the midline can be traced in the rostrocaudal direction, which cannot be done with the linear microgrid. The epidural functional midline was difficult to define given the large spread of the responses (Fig. 1.2 E). These results were consistent in subject 4 (Fig. 1.11). We were not able to identify the midline in patient 6 given the positioning of the electrode and tumor effects (Fig. 1.12). Subdural post-resection placement also allowed for identification of the functional midline, which is described in a later section. These results show that subdural placement allows mapping of the functional midline.

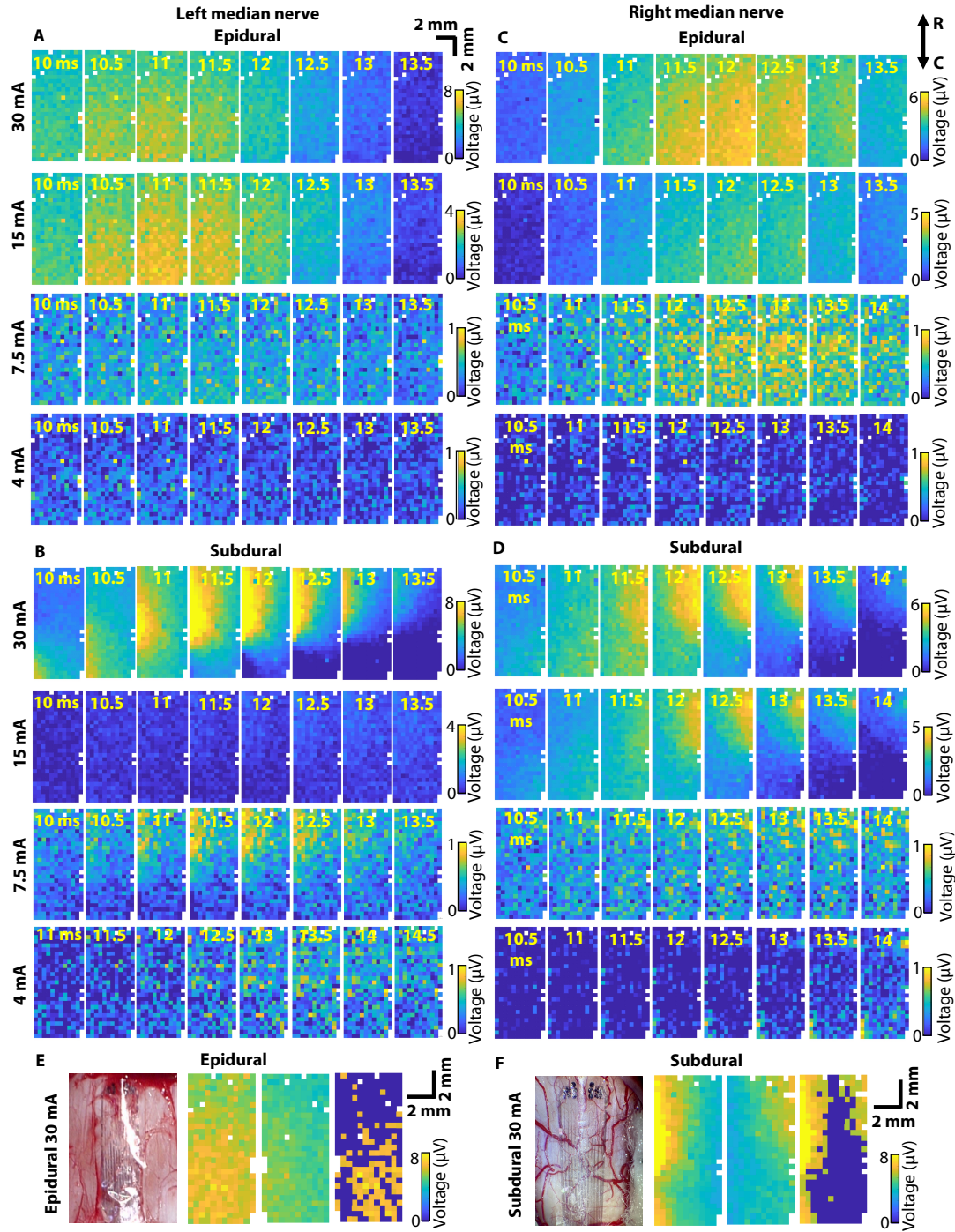


Figure 1.2 Time evolution plots of recorded SSEPs and functional midline mapping. (A) – (D) Spatial distribution of peak-to-peak amplitudes of responses to both left and right median nerve stimulation recorded epidurally and subdurally pre-resection. (E) Functional midline estimation from epidural recording of responses to 30 mA left and right median nerve stimulation. (F) Functional midline estimation from subdural recording of responses to 30 mA left and right median nerve stimulation. Peak-to-peak response amplitudes over the entire recording segment can be found in Fig. 1.8.

Spatial phase gradient analysis of response propagation

We performed an analysis of the spatial phase gradient of the response to better understand response propagation. We calculated the phase gradients and overlaid streamlines for better visualization of wave propagation dynamics. We hypothesized that the neural responses originate from a bundle of axons deep within the spinal cord, and therefore we would expect to see the electric field lines aligned parallel to the axons generating action potentials, dropping off in strength radially from the center of these axons (48). The microelectrode array captures the 2D projection of these electric field lines along the surface of the spinal cord. Furthermore, since the neural response is propagating vertically in a rostral direction, the electric field strength will also vary as a function of time. Thus, the spatial phase gradient should primarily point in the direction of the traveling action potentials, though due to the complex nature of the 2D projected electric field lines, will also spread outward from the center of the response in the direction of decreasing electric field strength. We observed these phase gradient features in the subdural pre-resection and to a lesser extent in the post-resection recordings (Fig. 1.3). Post-resection recordings will be discussed below. We did not see a strong directionality of the phase gradient in the epidural recordings, which can be explained by the wide spread of potential across the array resulting in small changes in phase. Phase gradient plots provide another means for SSEP monitoring and may inform of changes in SSEP propagation during surgical resection.

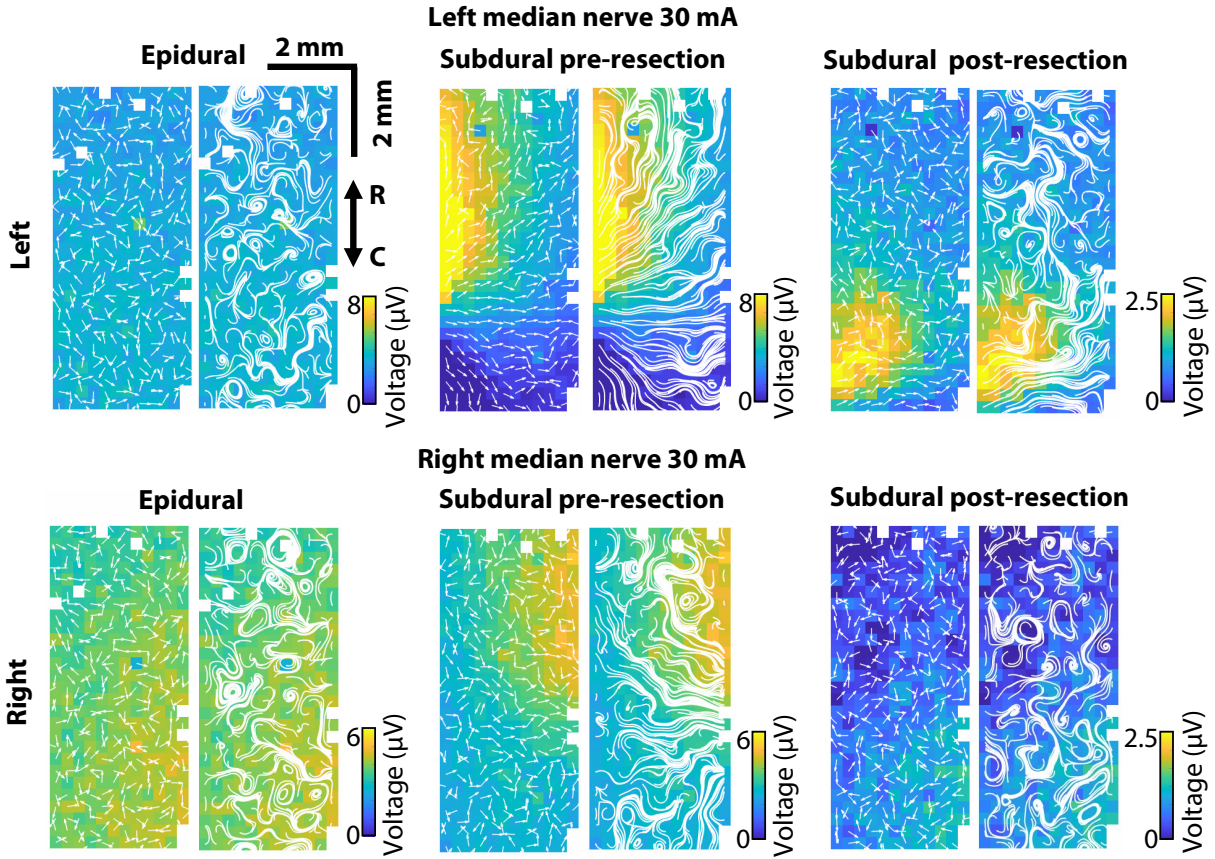


Figure 1.3 Spatial phase gradients and streamlines of SSEP responses. SSEP spatial phase gradients and streamlines in response to 30 mA left (top panels) and right (bottom panels) median nerve stimulation.

Validation of high-quality recordings with smaller PtNR contacts

Our microelectrode array included a multidiameter array at the tip to investigate any diameter-dependency of the recorded SSEPs and to confirm that smaller contact diameters, necessary for high-resolution mapping, do not lead to loss of information that is otherwise captured by larger contact diameter arrays. Larger contact diameters may have higher probability to overlap with fibers responsive to stimulation and may therefore potentially produce higher amplitude responses at lower stimulation currents. The diameters included were 30 μm , 60 μm , 80 μm , 110 μm , 140 μm , 170 μm , 210 μm , 240 μm and 480 μm . The contacts were organized in two mirrored clusters of 3 contacts per diameter (Fig. 1.4 A-B). Peak-to-peak amplitude responses from the left

and right-side clusters showed stronger variation in the subdural versus epidural placement (Fig. 1.4 C), with contacts positioned ipsilaterally to the stimulation side showing a stronger response compared to contacts positioned contralaterally. For example, epidurally, 30 mA left median nerve stimulation resulted in the left 140 μm cluster recording $4.85 \pm 0.09 \mu\text{V}$ amplitude versus the right cluster recording $5.07 \pm 0.06 \mu\text{V}$. Subdurally, those left and right clusters recorded amplitudes of $7.26 \pm 0.06 \mu\text{V}$ and $4.09 \pm 0.15 \mu\text{V}$, respectively (Fig. 1.4 D). Given that there was strong spatial variability across the array and the variable diameter electrodes were inherently in different locations, we could not directly compare the peak-to-peak amplitude responses. Therefore, we also evaluated the pre-stimulus baseline standard deviation for the contacts, with the assumption that this parameter should not be affected by the contact position. We found that the baseline standard deviation did not show a conclusive diameter dependency in either the epidural or subdural placement (Fig. 1.4 E,F). Epidural baseline standard deviation was consistently $\sim 0.38 \mu\text{V}$ whereas subdural baseline standard deviation was $\sim 0.18 \mu\text{V}$. Finally, we computed the power spectral density (PSD) for both the epidural and subdural recordings over a 300 ms time segment after stimulus (Fig. 1.4 G,H). We did not see a diameter effect on the PSD, with all channels exhibiting a $1/f$ noise relationship followed by white noise. These data suggest that recording with 30 μm diameter PtNR contacts is as effective as recording with larger diameters, which further substantiates their use for high-definition mapping from the surface of the spinal cord.

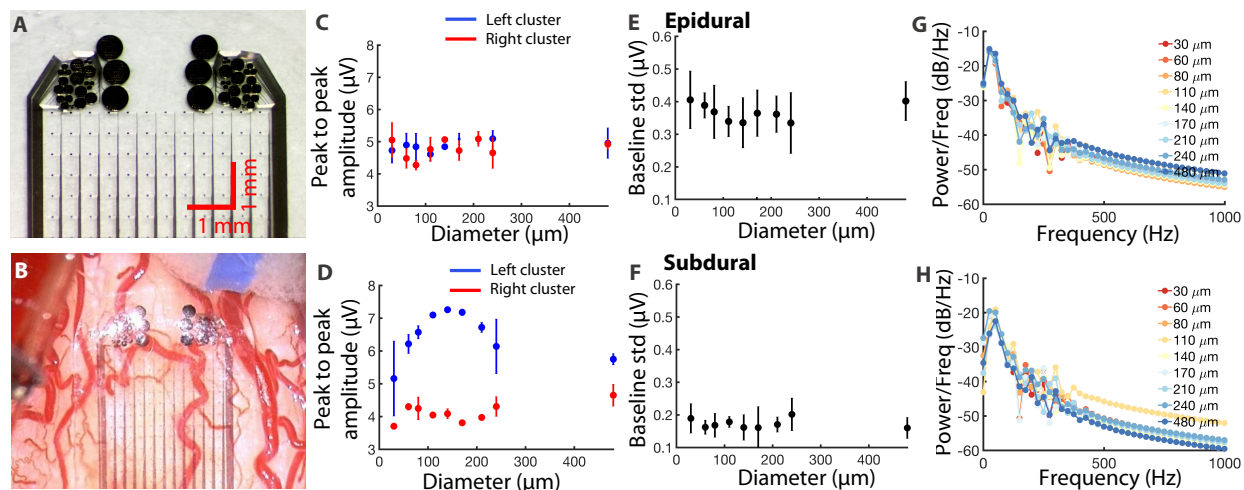


Figure 1.4 Multidiameter array response analysis. (A) Image of multidiameter array at tip of research microelectrode. (B) Image of electrode placed subdurally on spinal cord during recording. (C) – (D) Averaged peak-to-peak amplitude responses recorded epidurally and subdurally to 30 mA left median nerve stimulation. (E) – (F) Averaged baseline standard deviation recorded epidurally and subdurally. (G) – (H) Power spectrum density analysis on baseline recordings from (E) – (F).

Comparison to clinical IONM recordings

In subjects 5 and 6, we sought to understand how the research microelectrode recordings compared to clinical IONM recordings. Recordings from the microelectrode array could not be directly compared to those from the IONM equipment given the difference in trial numbers used to obtain the averaged traces for both. Only 30-40 trials were used in the averaging for the research grid at each current amplitude. The clinical IONM recordings needed longer stimulation trains given the lower amplitudes of responses at the scalp. As a result, the length of recording data segment that was averaged to achieve the mean traces differed between the research grid and clinical IONM data. However, a comparison could be made based on the general timeline of the surgery since any substantial changes in SSEPs were noted in the log sheet and typically lasted throughout the entire experiment recording session.

Epidural and subdural pre-resection and subdural post-resection recordings at 30 mA were compared to IONM recordings from similar timepoints in the surgery (Fig. 1.5). Research grid

recordings showed a higher response amplitude compared to IONM recordings. For high current amplitudes, the range of response amplitudes measured with the research grid was much higher than for clinical IONM recordings, where 30 mA right median nerve stimulation resulted in a maximal 3 μ V peak-to-peak amplitude response for the clinically recorded C3-C4 trace compared to microelectrode recordings of > 8 μ V and > 6 μ V responses for left and right 30 mA stimulation, respectively (Fig. 1.5 A,B). The remaining clinical traces were only $\sim$ 1 μ V.

Although there was a loss of response to median nerve stimulation post-resection in subject 5, affecting the right side more strongly, our microelectrodes still resolved responses; however, these responses were smaller than the pre-resection amplitudes. The maximum response amplitude decreased from 10 μ V to 2.5 μ V, whereas the clinical IONM equipment could not register a response (Fig.1.5 C,D). Furthermore, the spatial variation in the responses was preserved in the microelectrode recordings. Stimulation with 30 mA resulted in clear left and right activation patterns, similar to those seen pre-resection (Fig. 1.5 E,F). The ability to map the functional midline was also preserved (Fig. 1.5 G). Similarly, in subject 6, the research microelectrode recordings were of higher amplitude compared to the clinical recordings throughout the surgery (Fig. 1.13). These results highlight the potential of our microelectrode grids for IONM.

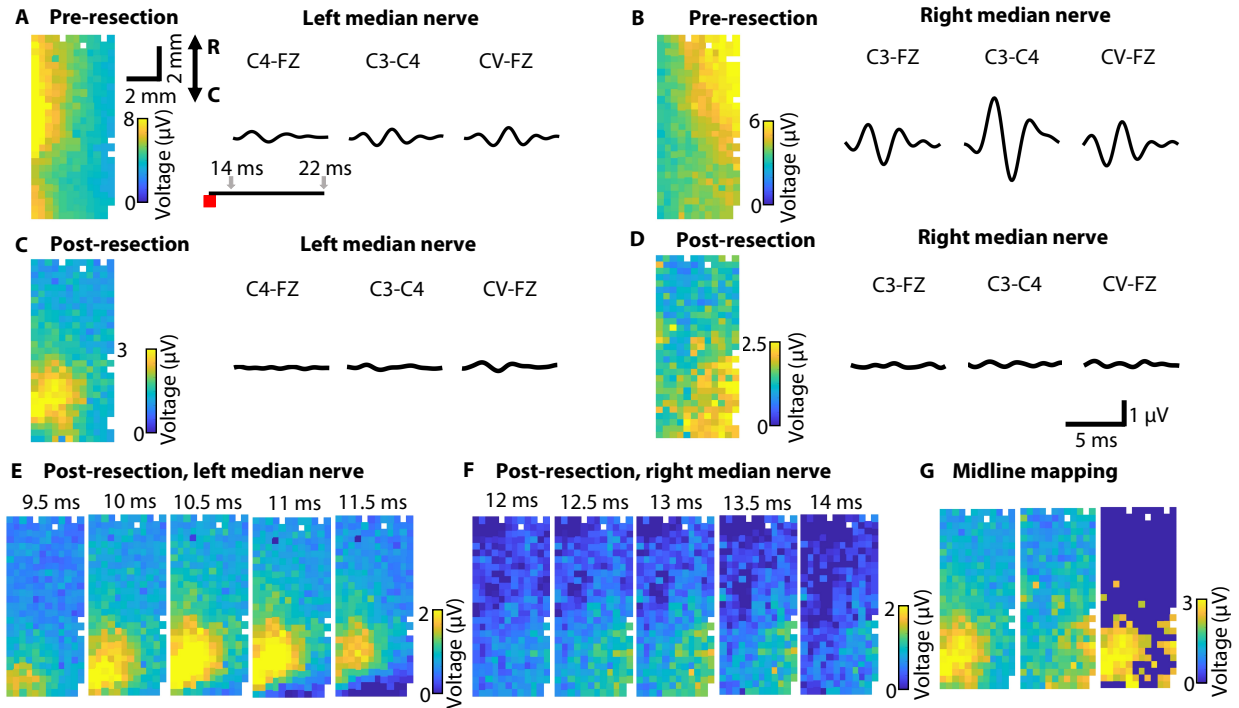


Figure 1.5 Comparison to clinical IONM recordings. (A) – (D) Subdural pre-resection and post-resection recordings to left and right 30 mA median nerve stimulation. (E) – (F) Time evolution of post-resection recorded responses to 30 mA left and right median nerve stimulation. (G) Functional midline estimation from post-resection recorded responses to 30 mA left and right median nerve stimulation.

Discussion

Currently deployed clinical spinal cord arrays are limited in usability by size. The thickness of these electrodes is at least on the scale of tens or hundreds of microns and often much thicker (29, 30, 32, 49, 50). As a result, these electrodes have poor contact with the spinal cord and record lower amplitude responses, requiring higher stimulation currents. Moreover, existing clinical microelectrodes typically have platinum contacts. Platinum has high impedance, requiring large contact diameters and resulting in low-resolution coverage. Large diameters limit the number of contacts across the circumference of the spinal cord, encumbering the isolation of the midline. The microelectrode arrays we developed address these limitations and could therefore be used for high

resolution functional mapping of the spinal cord. Because our grids are fabricated on parylene, they can be much thinner (here, 6.6 μm thick) than platinum electrodes. Electrode contacts are composed of PtNRs and have much lower impedances compared to standard planar platinum contacts. The lower impedance of PtNRs enables scaling of the contact diameter while preserving excellent impedance values ($\sim 36\text{ k}\Omega$ at 30 μm diameter) and recording capabilities, which offers the possibility of having higher density coverage.

In this study we transitioned from PEDOT:PSS to PtNR contacts, which have been shown to be more suitable for stimulation than other low-impedance microelectrode materials (38, 39). Although we do not perform stimulation in this study, having spinal cord electrodes that can both record and stimulate would be the goal for many applications outside of neuromonitoring. The current iteration was a recording electrode only, but a hybrid version of record and stimulate electrode is currently under development, where one or a subset of channels deliver the stimulation and the remaining channels record. This, combined with a more robust fabrication process (see Materials and Methods), suggests that PtNR microelectrodes can be a good choice for human neural devices. Our grid was 6 mm wide, as recommended by the surgical team that performed the research in the clinical setting on the first 3 subjects. For 2 out of the last 3 subjects, we were able to identify the midline and in the third, we could not. Increasing the width of the array and its axial coverage may aid isolation of the midline across diverse subjects.

The advantageous design of our research microelectrode allowed us to discriminate stimulation currents at and above 7.5 mA. This is important since 30 mA stimulation, as was used in subject 5, is considered high and may cause a pain response. In general, currents over 10 mA are considered potentially painful (51). Therefore, reducing IONM current below this amplitude may benefit the patient by reducing anesthesia interventions during a procedure. However,

systematic studies for correlating current amplitudes necessary for capturing when and where neural deficits occur in dorsal fibers can be formed are yet to be conducted.

Epidural and subdural recordings showed different spatiotemporal features, which means these placements could offer distinct applications. These differences are important given that epidural placement is easier to achieve and is less invasive. Epidural placement showed peak-to-peak response amplitudes generally on par with subdural placement. However, there was less localization of responses across the array, resulting in an incoherent phase gradient. Surgery that requires identification of the functional midline inherently involves exposure of the dorsal spinal cord and subdural clinical procedures. Therefore, the subdural microelectrode array placement can be a valuable asset for making clinical decisions in such applications because of the high-resolution identification of the functional midline and phase gradients in 2D. On the other hand, although epidural placements cannot resolve spatial variations in the response, they could be informative for evaluating the quality of the response (whether it is present and to what extent), even when IONM responses cannot be detected. This is particularly important for decompression and other surgeries that do not involve opening of the dura mater.

Our research grid was superior compared to the clinical equipment in terms of the sensitivity of the system. We averaged 30-40 trials to achieve the mean peak-to-peak amplitude responses at a stimulation rate of 2.78 Hz, therefore requiring 10-14 seconds of recorded data. The clinical equipment stimulates at 1.41 Hz, requires over 300 trials, and often throws out trials, resulting in a necessary data segment of 4 minutes or more. This is a large improvement in responsiveness of the system and could allow for quicker detection of neurological damage. In addition, at high current amplitudes, even fewer trials would be necessary with our microelectrode, resulting in practically real-time responsiveness to surgical intervention. In subject 5, we were also

able to capture SSEPs with our research microelectrode when the clinical IONM system could not. This subject showed temporary neurological deficit after surgery that quickly improved, which validates that the persistent responses seen by our microelectrode carry clinical value.

There were a few limitations to this study. First, the electrode location varied between subjects since surgery was performed at different heights of the cervical spinal cord. This made it impossible to directly compare recordings between subjects. The electrode was also placed in the vicinity of abnormal tumor tissue, which further increased the variability in recordings among patients. Additionally, there was slight variation in electrode impedances and the distribution of working channels between electrodes, which introduced more variability in the datasets. Nonetheless, the overall features of the responses were consistent, including response amplitudes, propagation velocity, midline mapping capability and epidural and subdural recording of spatiotemporal differences. Another limitation of this study was the inability to directly compare the clinical grid recordings and the microelectrode recordings due to different trial numbers used to produce the mean responses. Future studies will need to investigate and quantify these differences more thoroughly. Finally, this study included only six patients, with just one patient showing a decrease in SSEP amplitude during surgery. More patients need to be included in future studies to investigate the consistency in the research microelectrode performance and its ability to better detect neurological damage compared to existing IONM equipment.

This study was approved to be performed during operative pauses, and another limitation of this study was the inability to record during the surgical resection, which is a requirement for any IONM system. To address this limitation, we are developing microelectrodes that can surround but still leave access to the resection zone, creating a ‘window in a grid’ that will allow surgical procedures to proceed while providing real-time monitoring of spatiotemporal SSEP patterns from

the dorsal surface of the spinal cord. The ‘window in a grid’ electrode would provide coverage to the rostral and caudal segments of the spinal cord to the surgical plane, thus allowing direct comparison of SSEPs below and above the surgical site. With these improvements in place, our microelectrode system could become a complete and highly detailed IONM system. Furthermore, the PtNR microelectrode grid could be used for more traditional DC mapping given its stimulation capabilities (37).

In addition to IONM, we believe our PtNR microelectrodes have implications for the treatment of SCI by providing a high-resolution, high-coverage spinal cord grid. We speculate that our research grid could potentially be used to capture sensory information from the peripheral nerves below the point of injury to be relayed to the brain, which has been pursued recently (52–54), and provide the means for targeted sensory nerve stimulation. For this, further developments will need to be made to capture native sensory pathway neural activity as opposed to recording stimulated SSEPs and to perform stimulation with PtNR electrodes. Finally, we also believe our microelectrode array could mediate SCI treatment by identifying and stimulating the root entry zones in SCI patients, which has been shown recently to restore motor function (55).

In summary, we introduce a conformal, high-density microelectrode grid and demonstrate its ability to outperform standard clinical IONM during spinal cord surgery. For both subdural and epidural placements, we evaluated stimulation responses that were present even when IONM did not resolve any responses. Our microelectrode was able to capture detailed spatiotemporal patterns of responses that can define with high precision the 2D maps of the functional midline during surgery. Our use of this grid in high-resolution mapping from the surface of the spinal cord shows that this microelectrode holds the promise for applications that may help neurosurgical procedures and neuromodulation therapies in the spinal cord and beyond.

Materials and Methods

Study design

The research objectives of this study were to (1) evaluate whether direct recordings from the human spinal cord could reduce the stimulation thresholds required to detect neurological activity and quantify this difference, (2) quantify the spatial selectivity of our high-channel count grids and their ability to localize the spinal cord functional midline, (3) evaluate the differences in epidural versus subdural recordings to determine optimal grid placement and (4) assess whether the research grid can identify neurological signal when it is classified as absent by the standard intraoperative neuromonitoring (IONM) equipment. Randomization was not performed and did not apply to our study since this is a proof-of-concept technology study.

Research grid fabrication, characterization, and sterilization

Both PEDOT:PSS and PtNR research grids were fabricated on 7" × 7" × 0.06" photomask-grade soda lime glass plate (Nanofilm), which were cleaned via O₂ plasma at 200 W for 5 minutes. Diluted Micro 90 (0.1%) was spincoated onto the glass plate to act as a release layer, after which the plate was coated with 3.7-μm-thick-parylene C using a parylene deposition system (Specialty Coating Systems 2010 Labcoater). Next, two layers of gold traces (10 nm Cr, 250 nm Au) were deposited onto the plate using standard lithography techniques and AZ5214E-IR photoresist (MicroChemicals). This double layer of leads was found to improve device yield by reducing chance of disconnects due to particles during the photolithography process.

PEDOT:PSS grid fabrication

For PEDOT:PSS grids, the metal traces were then encapsulated in 2nd and 3rd parylene layers, each 3.1 μm thick. Concentrated Micro-90 (100μL in 50 mL DI) was spincoated before the 3rd parylene coating to allow for peel-off of this layer at a later step. Via (hole) through the parylene

was performed via oxygen etching at 200 W for 39 minutes. PEDOT:PSS solution was spincoated onto the exposed contacts, cured at 150 °C for 60 min, and the 3rd parylene layer was then peeled off. The electrodes were laser cut and lifted off with DI water. Preparation of the PEDOT:PSS solution and more fabrication details can be found in previous publications, including (39).

PtNR grid fabrication

Platinum nanorod (PtNR) contact formation on parylene C were prepared using a technique previously developed and described in our lab (40). Briefly, the metal traces and PtAg alloyed contacts were encapsulated in a second 3.1 μm parylene layer. After deposition of a Ti hard mask, the PtAg contacts and connector pads were exposed using a dry etch of the Ti layer with SF_6/Ar gas followed by oxygen dry etching of the parylene C. The samples were lifted off with 6:1 buffered oxide etchant (BOE) and the PtAg was dealloyed in 60°C nitric acid for 2 min, thereby forming the PtNRs with Pt/Ag composition of approximately 95%/5%. The microelectrodes were bonded to custom-made extender PCBs as described in a previous publication (40).

The quality of the fabricated devices and their PEDOT:PSS or PtNR contacts were first evaluated using optical microscopy. Impedance magnitude and phase at 1kHz was then measured to ensure device functionality. If the devices had high yield, they were sent for sterilization to UCSD's or OHSU's sterilization facility. The sterilization processes used were steam with gravity mode at 121°C for 30 min for PEDOT:PSS electrodes and Sterrad with the default sterilization mode for PtNR electrodes.

Subjects and research grid placement

The research subjects were patients undergoing an intramedullary spinal cord tumor resection or biopsy procedure at the cervical segment with IONM with both epidural and subdural spinal cord exposure. Subjects were recruited by the neurosurgery team involved in this study. All

patients voluntarily participated after informed consent in accordance with the University of California Institutional Review Board (IRB). Subjects were informed that participating in the study would not affect the treatment they received. Participants could withdraw at any time. Recordings were acquired from 6 participants.

Surgery proceeded as normal until epidural spinal cord exposure. During the surgical exposure, the recording equipment was set up on the non-sterile side of the operating room. The research grid was removed from the sterile tray by a scrubbed-in member of the neurosurgery team. A Situate Sterile Drape (Medtronic 01-0020) was used to interface between the sterile and non-sterile recording elements. The extender board of the research grid was inserted through an opening in the drape and touch-proof connectors of a sterile twisted pair of subdermal needle electrodes were inserted through another hole. Two Tegaderm films (3M) were taped together to seal the openings. A member of the research team then connected the extender board and the recording equipment. The research grid was then placed in sterile saline solution in a kidney dish until the recording started. Impedance was measured to ensure proper equipment connection and grid viability after sterilization. Further details are described in (40).

Data collection

Recordings were taken at three timepoints in the surgery: (1) epidurally after spinal cord exposure, (2) subdurally, on the pial surface, before tumor resection or biopsy, and (3) subdurally after tumor resection or biopsy. Impedances were measured and plotted before each recording in both sterile saline and on the spinal cord itself to check for electrode damage and equipment connectivity issues. The total recording time for all three timepoints did not exceed 30 minutes during downtime of the surgery, so as not to cause adverse effects to the patient and surgical outcome. Recordings were collected using Intan's RHD Recording Controller at 20 kHz sampling

rate. A member of the research team dictated stimulation amplitude and location to the neuromonitoring team member. Notes were taken during stimulation to note approximate stimulation time and amplitude in the recording. Reference and ground needles for the research microelectrode were placed in nearby tissue. The clinical IONM system used to capture data from subjects 5 and 6 was Cascade's IOMAX.

Data processing

Research grid data was processed in MATLAB. Data was filtered to remove 60 Hz noise and harmonics using a notch filter. Data was then bandpass filtered 30-300 Hz. The low frequency was chosen to be 30 Hz to remove changes in voltage due to DC voltage shifts and ECG artifact, which was below 30 Hz, while preserving the response frequency bandwidth (a spectrogram showed responses in the frequency range of 70-150 Hz). Stimulus artifacts from the non-filtered data were used to determine stimulation timepoints and these timepoints were compared to a custom-made stimulation artifact capture system for initial experiments, but this was found to be redundant for later experiments. Notes taken during surgery were used to classify each stimulus train based on the stimulation amplitude and location. Responses were computed by averaging a 20 ms time window after each stimulus artifact. Baseline data was computed by averaging a 20 ms time window within 20-80 ms before stimulus artifact. The onset of the time window varied between stimulations and was chosen to ensure no aberrant signals were present in the baseline recording. Baseline values were then subtracted from the averaged responses. Peak-to-peak amplitude was calculated by taking the difference between the highest and lowest data points in the time window 5-20 ms after stimulus and within the 20 ms time window for baseline. The clinical data was downloaded from the IONM software IOMAX (Cascade) in the form of a .json

file after the case. The data was bandpass filtered 30-750 Hz. The data was imported and plotted in Python along with corresponding timestamps and event notes from the IONM team.

Midline mapping

A signal-to-noise (SNR) thresholding technique was used to evaluate the functional midline. The mean and standard deviation of the baseline peak-to-peak amplitude was calculated. For both left- and right-side stimulation, peak-to-peak amplitudes of the response with root mean squared (RMS) numbers less than a certain value were zeroed (Equation 1), thus creating individual threshold maps for each side. For subject 5, the value of 13 was arbitrarily chosen for the subdural recording based on the number of channels that did not meet this criterium. The value of 18 was chosen for the epidural recording. The left- and right-side threshold maps were combined by taking the square root of the sum of squared thresholds (Equation 2).

$$find\left(\frac{intensities-m}{std}\right) < val = 0 \quad \text{Equation 1}$$

$$\sqrt{intensities_{right}^2 + intensities_{left}^2} = combined\ intensities \quad \text{Equation 2}$$

Phase gradient analysis

The spatial phase gradient of the responses was calculated in MATLAB based on previous methods described in literature (40, 56, 57). The streamlines were generated with the streamline MATLAB function to illustrate the general directional trends of the local spatial phase gradient.

Statistical analysis

We used the Welch's test to determine whether there was a significant change in impedance after Sterrad sterilization since we assumed unequal variance between samples.

We used the Mann Whitney U test to evaluate statistical significance of the peak-to-peak amplitude responses. This nonparametric test was chosen since the amplitude distributions for each current amplitude deviated slightly from a normal distribution, making a nonparametric test more

appropriate. Response peak-to-peak amplitudes were compared against baseline peak-to-peak amplitudes. In each statistical test, the channels in the top 20% based on SNR were used to ensure that the significance was not affected by non-responsive channels. The Z-score was used to evaluate effect size, which is appropriate for nonparametric data, and multiple test correction was applied to the p-values since multiple tests were performed to evaluate various current amplitudes and stimulation points. Effect size was calculated by dividing the Z-score by the square root of the number of pairs (Equation 3). The Z-score was calculated in Python from the U statistic (Equation 4). P-values were corrected using the Holm method.

$$effect\ size = Z / \sqrt{n} \text{ where } n = \text{number of pairs} \quad \text{Equation 3}$$

$$Z = \frac{U - n^2/2 + 0.5}{\sqrt{n^2 * (N+1) / 12}} \quad \text{Equation 4}$$

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density microelectrode arrays. *Sci. Transl. Med* **14**, 4744 (2022). The dissertation author was the primary investigator and author of this paper.

Supplementary Materials

Case details

Subject 1 was implanted with a 128-channel PEDOT:PSS microelectrode with 300 μm horizontal and 4 mm vertical pitch and with 30 μm diameter contacts. Subject 2 was implanted with a PEDOT:PSS microelectrode grid with 32x16 channels, 1 mm horizontal and 1 mm vertical pitch and 30 μm contact diameter. Subject 3 was implanted with a PEDOT:PSS microelectrode grid with 5x200 channels, 200 μm horizontal and vertical pitch and 90 μm contact diameter. A summary of participants and experimental details are shown in Table S1.

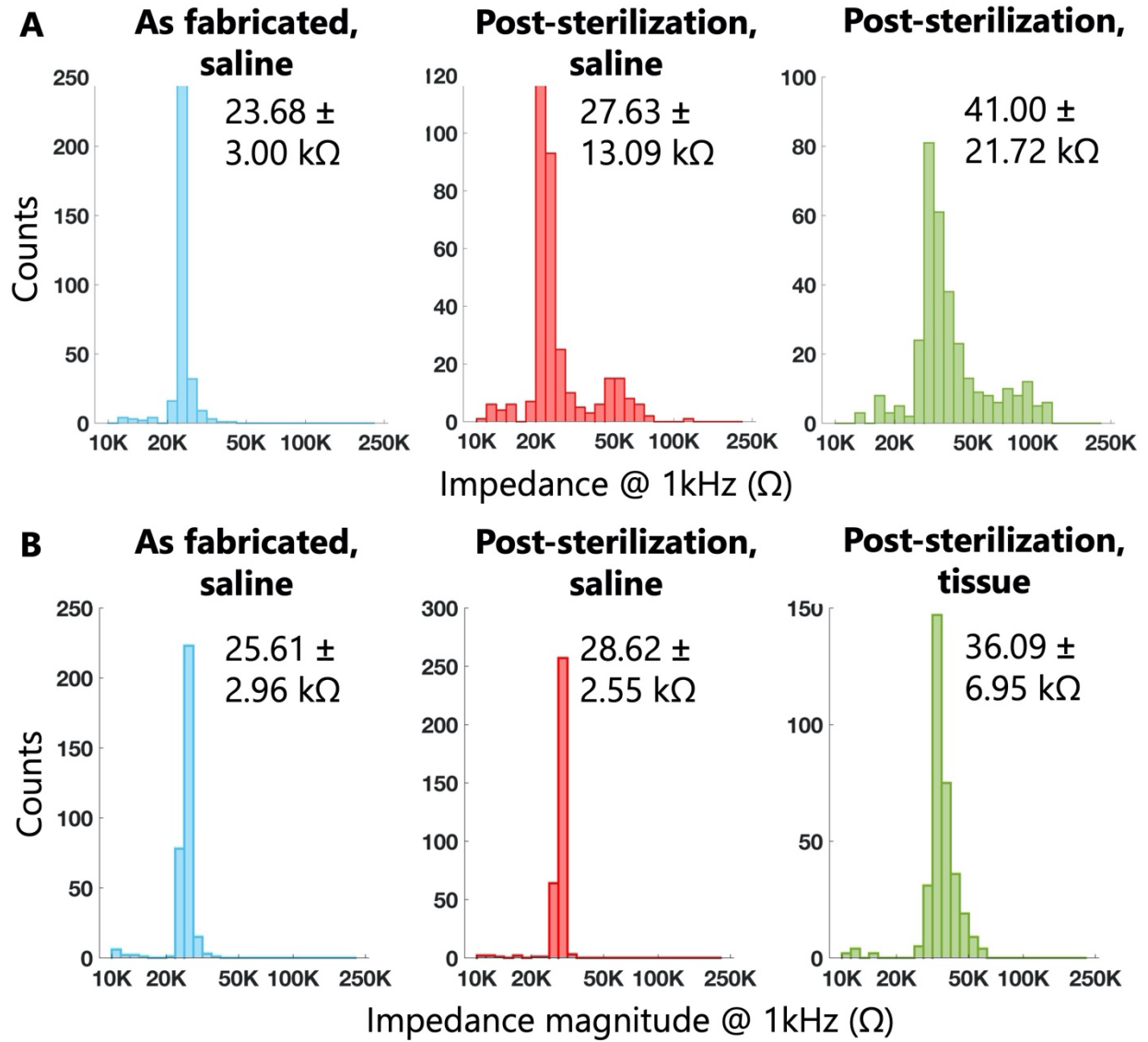


Figure 1.6 Microelectrode array impedance histograms. 1-kHz impedance magnitude histograms measured as fabricated in saline, post-sterilization in saline, and on spinal cord tissue for (A) subject 4 and (B) subject 6.

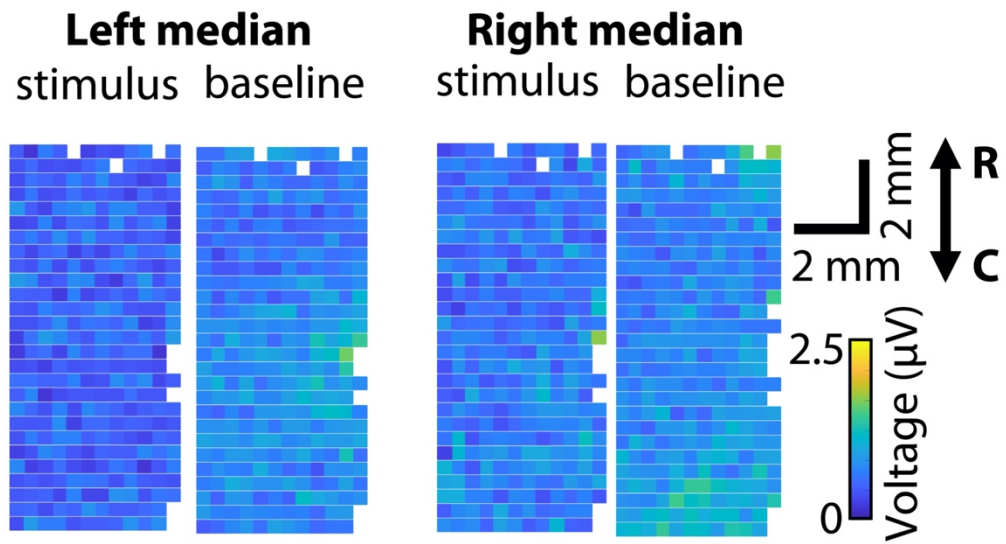


Figure 1.7 Skin control recordings during 30 mA left and right median nerve stimulation for subject 5.

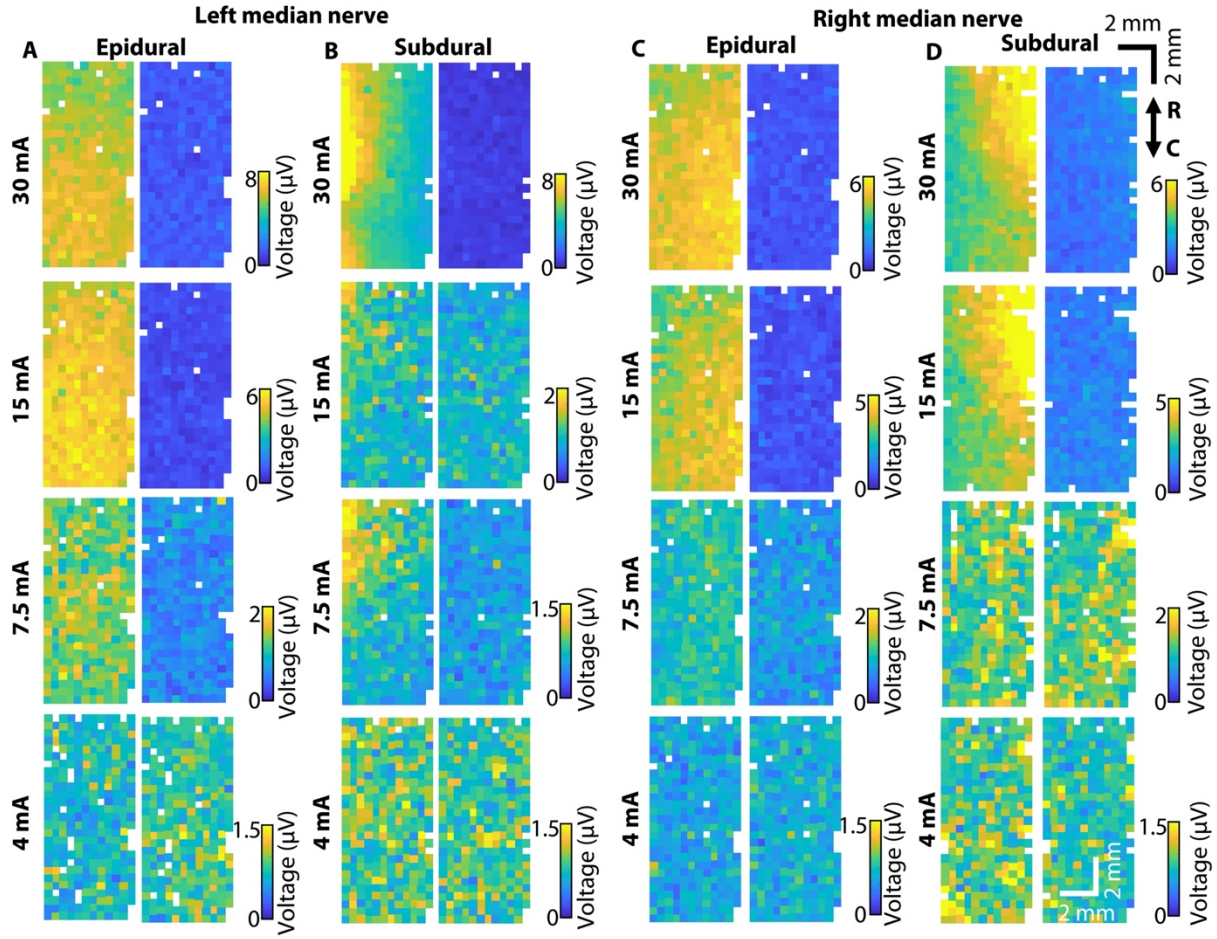


Figure 1.8 Peak-to-peak amplitude responses from subject 5. (A) – (D) Spatial distribution of peak-to-peak amplitudes of responses to both left and right median nerve stimulation recorded epidurally and subdurally pre-resection. Left panels show the response peak-to-peak amplitudes. Right panels show the baseline peak-to-peak amplitudes. Peak-to-peak amplitudes were calculated from the time evolution plots of Fig. 1.2 as described in Materials and Methods.

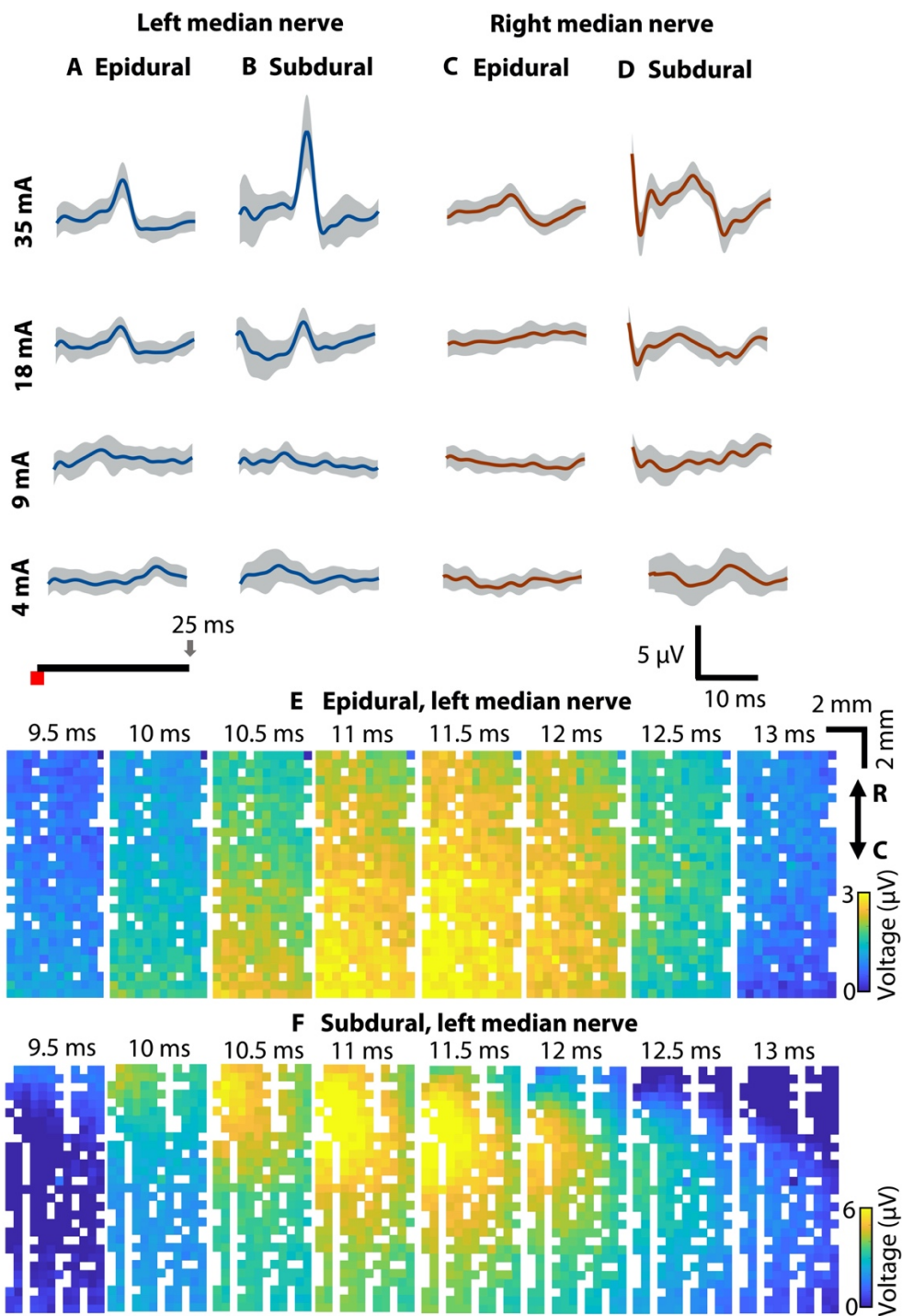


Figure 1.9 Single channel response and time evolution plots from subject 4. (A) – (D) Maximal single channel responses to left and right median nerve stimulation in both epidural and subdural placements. (E) Epidurally and (F) subdurally recorded time evolution of responses to left median nerve stimulation.

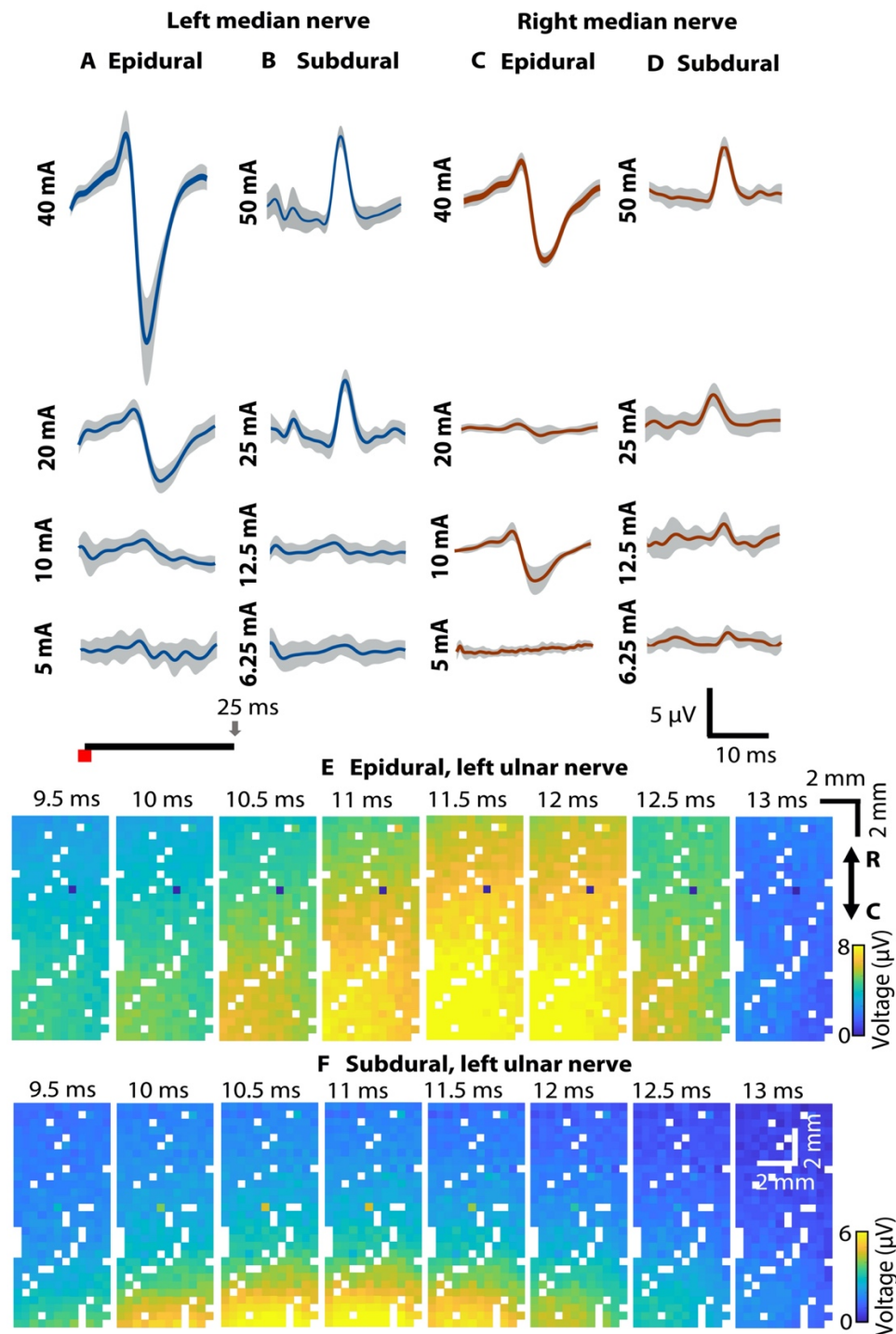


Figure 1.10 Single channel response and time evolution plots from subject 6. (A) – (D) Maximal single channel responses to left and right ulnar nerve stimulation in both epidural and subdural placements. In this subject, the spinal cord was heavily displaced to the right due to the tumor (see Table 1.2) and the localization of SSEPs on the electrode array was likely impacted by this displacement. (E) Epidurally and (F) subdurally recorded time evolution of responses to left ulnar nerve stimulation.

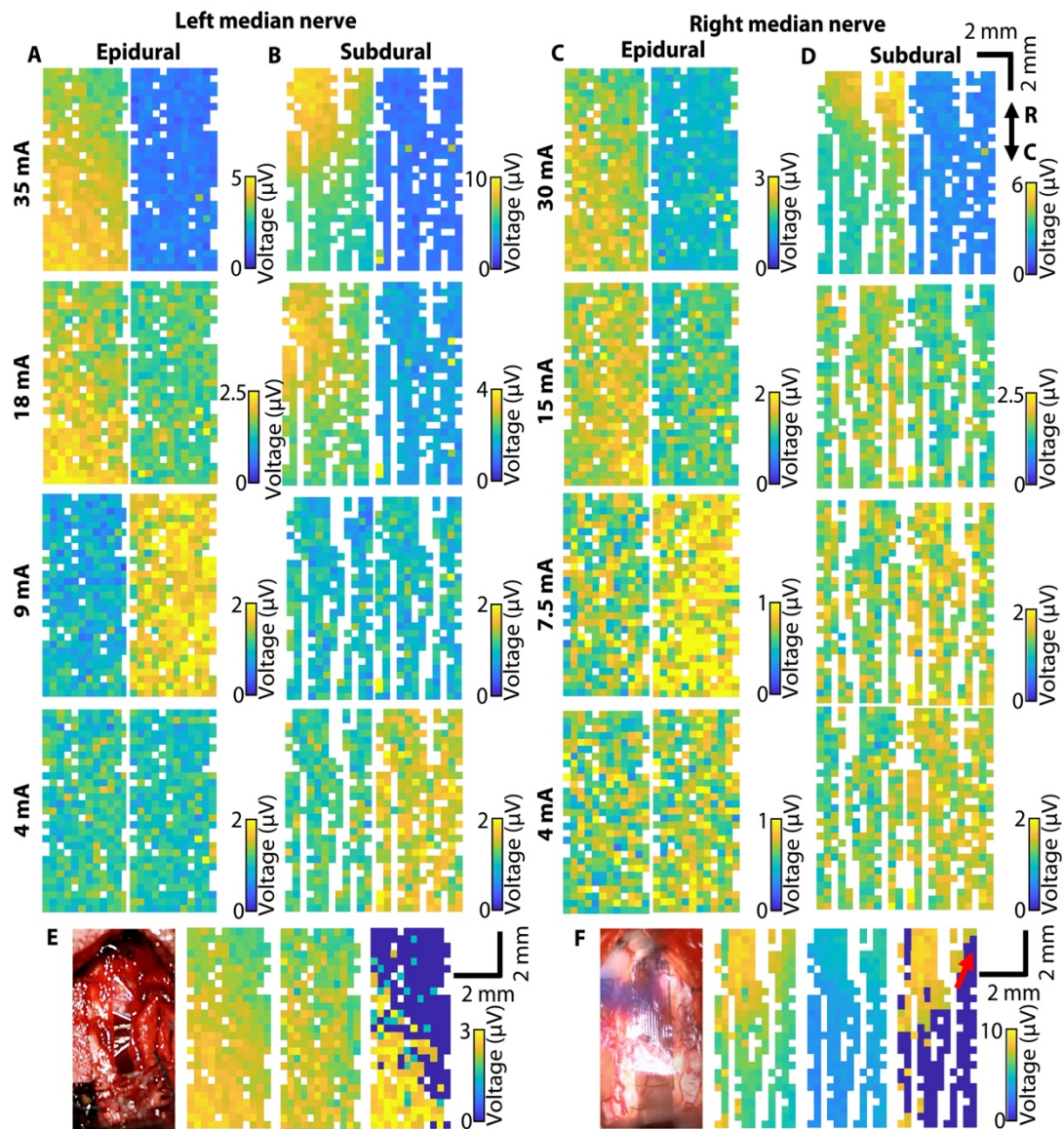


Figure 1.11 Peak-to-peak amplitude responses from subject 4. (A) – (D) Spatial distribution of peak-to-peak amplitudes of responses to both left and right median nerve stimulation recorded epidurally and subdurally pre-resection. Left panels show the response peak-to-peak amplitudes. Right panels show the baseline peak-to-peak amplitudes. (E) Functional midline estimation from epidural recording of responses to 30 mA left and right median nerve stimulation. (F) Functional midline estimation from subdural recording of responses to 30 mA left and right median nerve stimulation.

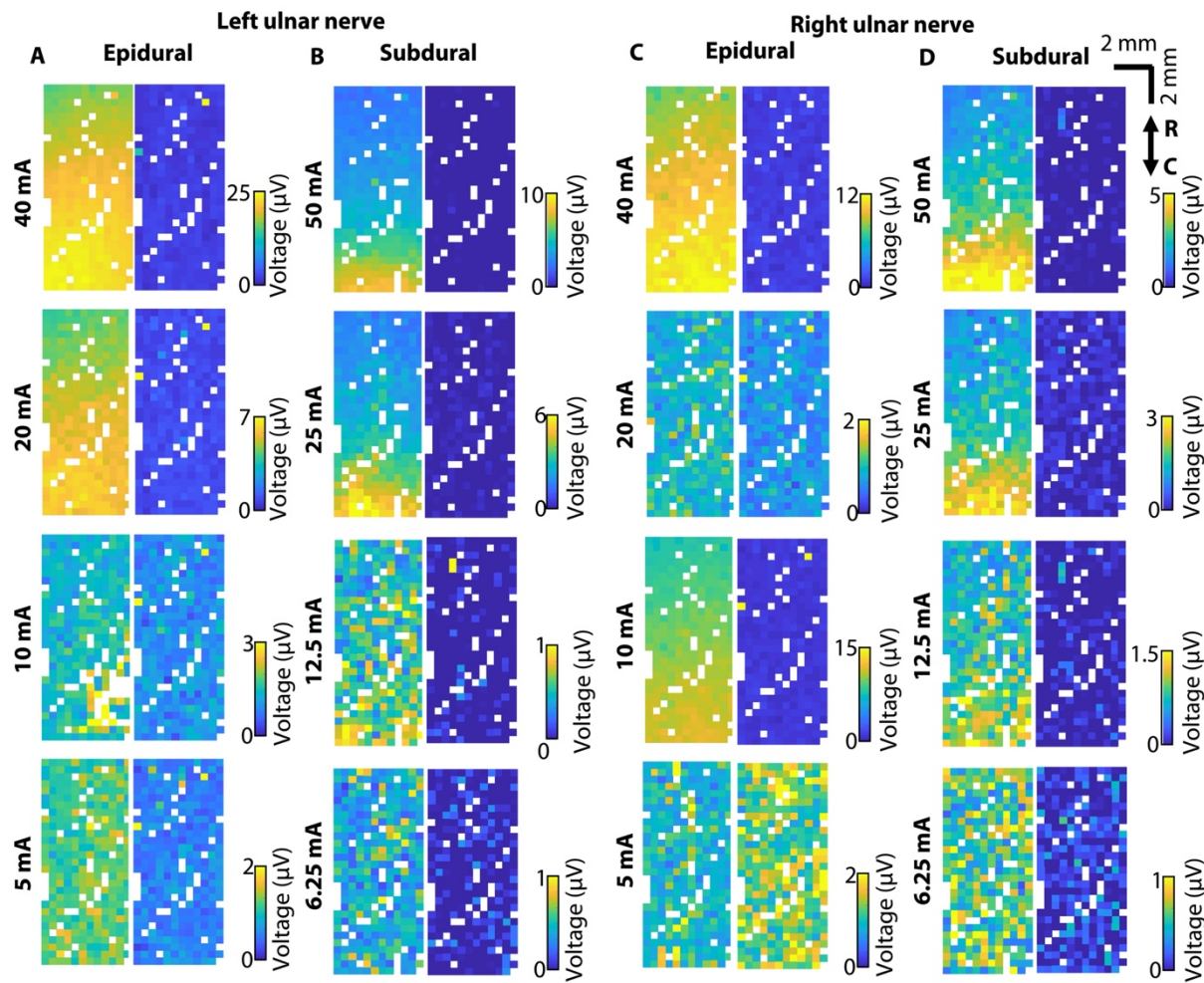


Figure 1.12 Peak-to-peak amplitude heatmaps from subject 6. (A) – (D) Spatial distribution of peak-to-peak amplitudes of responses to both left and right ulnar nerve stimulation recorded epidurally and subdurally post-resection. Left panels show the response peak-to-peak amplitudes. Right panels show the baseline peak-to-peak amplitudes. In this subject, the tumor displaced the spinal cord severely to the right and the electrode array was placed over the left dorsal column. Responses to left ulnar nerve stimulation were 2x greater in amplitude compared to right ulnar nerve stimulation.

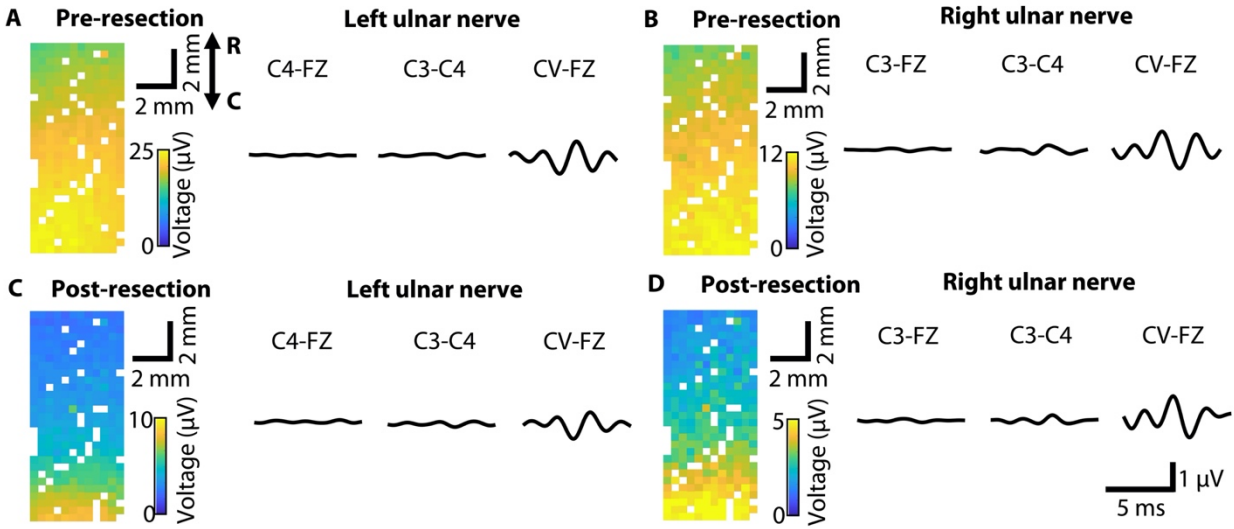


Figure 1.13 Comparison to clinical IONM recordings in subject 6. (A) – (D) Epidural pre-resection and subdural post-resection recordings to left and right 30 mA median nerve stimulation.

Table 1.2 Summary of performed cases.

Participant #	Spinal segment	Pre-op deficits	SSEP stimulation	Electrode
1	L1	No deficits	Median nerve	PEDOT:PSS, 4x32, d = 30 μm
2	C2-C5	Weakness in both arms and legs, worse on right side	Median nerve	PEDOT:PSS, 16x32, d=30 μm
3	C2-C5	No deficits	Median nerve	PEDOT:PSS, 5x200, d=30 μm
4	C5-C6	Weakness in left arm and leg	Median nerve	PtNR, 12x31, d=30 μm + multidiam array
5	C4-C5	Mild weakness on both sides of the body, upper SSEPs absent during resection	Median nerve	PtNR, 12x31, d=30 μm + multidiam array
6	C3-C6	Mild weakness in both hands, spinal cord pushed to right	Ulnar nerve	PtNR, 12x31, d=30 μm + multidiam array

References

1. P. R. Cooper, Outcome after Operative Treatment of Intramedullary Spinal Cord Tumors in Adults: Intermediate and Long-Term Results in 51 Patients. *Neurosurgery*. **25** (1989), doi:10.1227/00006123-198912000-00001.
2. S. H. Chang, Y. G. Park, D. H. Kim, S. Y. Yoon, Monitoring of motor and somatosensory evoked potentials during spine surgery: Intraoperative changes and postoperative outcomes. *Annals of Rehabilitation Medicine*. **40**, 470–480 (2016).
3. Z. Ng, S. Ng, V. Nga, K. Teo, S. Lwin, C. Ning, T. T. Yeo, Intradural Spinal Tumors—Review of Postoperative Outcomes Comparing Intramedullary and Extramedullary Tumors from a Single Institution’s Experience. *World Neurosurgery*. **109**, e229–e232 (2018).
4. A. Raco, V. Esposito, J. Lenzi, M. Piccirilli, R. Delfini, G. Cantore, Long-term follow-up of intramedullary spinal cord tumors: A series of 202 cases. *Neurosurgery*. **56**, 972–979 (2005).
5. M. K. Tobin, J. R. Geraghty, H. H. Engelhard, A. A. Linninger, A. I. Mehta, Intramedullary spinal cord tumors: A review of current and future treatment strategies. *Neurosurgical Focus*. **39**, E14 (2015).
6. T. Verla, J. S. Fridley, A. B. Khan, R. R. Mayer, I. Omeis, Neuromonitoring for Intramedullary Spinal Cord Tumor Surgery. *World Neurosurgery*. **95** (2016), pp. 108–116.
7. K. Rijs, M. Klimek, M. Scheltens-de Boer, K. Biesheuvel, B. S. Harhangi, Intraoperative Neuromonitoring in Patients with Intramedullary Spinal Cord Tumor: A Systematic Review, Meta-Analysis, and Case Series. *World Neurosurgery*. **125** (2019), pp. 498–510.e2.
8. M. R. Nuwer, E. G. Dawson, L. G. Carlson, L. E. A. Kanim, J. E. Sherman, “Somatosensory evoked potential spinal cord monitoring reduces neurologic deficits after scoliosis surgery: results of a large multicenter survey” (1995).
9. I. E. Sandalcioğlu, T. Gasser, S. Asgari, A. Lazorisak, T. Engelhorn, T. Egelhof, D. Stolke, H. Wiedemayer, Functional outcome after surgical treatment of intramedullary spinal cord tumors: Experience with 78 patients. *Spinal Cord*. **43**, 34–41 (2005).
10. S. H. Jin, C. K. Chung, C. H. Kim, Y. D. Choi, G. Kwak, B. E. Kim, Multimodal intraoperative monitoring during intramedullary spinal cord tumor surgery. *Acta Neurochirurgica*. **157**, 2149–2155 (2015).
11. V. Deletis, F. Sala, Intraoperative neurophysiological monitoring of the spinal cord during spinal cord and spine surgery: A review focus on the corticospinal tracts. *Clinical Neurophysiology*. **119** (2008), pp. 248–264.
12. A. A. Gonzalez, D. Jeyanandarajan, C. Hansen, G. Zada, P. C. Hsieh, Intraoperative neurophysiological monitoring during spine surgery: A review. *Neurosurgical Focus*. **27** (2009), , doi:10.3171/2009.8.FOCUS09150.
13. D. B. MacDonald, C. Dong, R. Quatrone, F. Sala, S. Skinner, F. Soto, A. Szelényi, Recommendations of the International Society of Intraoperative Neurophysiology for intraoperative somatosensory evoked potentials. *Clinical Neurophysiology*. **130** (2019), pp. 161–179.
14. A. R. Møller, S. Ansari, A. A. Cohen-Gadol, Techniques of intraoperative monitoring for spinal cord function: Their past, present, and future directions. *Neurological Research*. **33**, 363–370 (2011).

15. F. Sala, A. Bricolo, F. Faccioli, P. Lanteri, M. Gerosa, "Surgery for intramedullary spinal cord tumors: The role of intraoperative (neurophysiological) monitoring" in *European Spine Journal* (2007), vol. 16.
16. Weigang E, Hartert M, Sircar R, v Samson P, Pitzer K, Genstorfer J, Zentner J, Beyersdorf F, Setup of Neurophysiological Monitoring with tcMEP/SSEP During Thoracoabdominal Aneurysm Repair. *Thorac Cardiovasc Surg.* **Feb**, 28–32 (2005).
17. H. Singh, R. W. Vogel, R. M. Lober, A. T. Doan, C. I. Matsumoto, T. J. Kenning, J. J. Evans, Intraoperative Neurophysiological Monitoring for Endoscopic Endonasal Approaches to the Skull Base: A Technical Guide. *Scientifica (Cairo)*. **2016** (2016), , doi:10.1155/2016/1751245.
18. T. Yamada, Somatosensory Evoked Potentials. *Encyclopedia of the Neurological Sciences*, 230–238 (2014).
19. A. M. Padberg, K. H. Bridwell, SPINAL CORD MONITORING. *Orthopedic Clinics of North America*. **30** (1999), doi:10.1016/S0030-5898(05)70095-X.
20. E. (Enno) Freye, *Cerebral monitoring in the OR and ICU* (Springer, 2005).
21. Aage Moller, *Intraoperative Neurophysiological Monitoring* (Springer, ed. 3rd, 2010).
22. A. S. Hilibrand, D. M. Schwartz, V. Sethuraman, A. R. Vaccaro, T. J. Albert, "Comparison of Transcranial Electric Motor and Somatosensory Evoked Potential Monitoring During Cervical Spine Surgery" (2004), (available at <http://journals.lww.com/jbjsjournal>).
23. G. Kimchi, N. Knoller, A. Korn, Y. Eyal-Mazuz, Y. Sapir, A. Peled, R. Harel, Delayed variations in the diagnostic accuracy of intraoperative neuromonitoring in the resection of intramedullary spinal cord tumors. *Neurosurgical Focus*. **50**, 1–7 (2021).
24. R. P. Lesser, P. Raudzens, H. Liiders, " M R Nuwer, W. D. Goldie, H. H. Morris, " D S Dinner, " G Klem, J. F. Hahn, A. G. Shetter, H. H. Ginsburg, A. R. Gurd, Postoperative Neurological Deficits May Occur Despite Unchanged Somatosensory Evoked Potentials T Intraoperative. *Annals of Neurology*. **19**, 22–25 (1986).
25. M. O. Kelleher, G. Tan, R. Sarjeant, M. G. Fehlings, Predictive value of intraoperative neurophysiological monitoring during cervical spine surgery: A prospective analysis of 1055 consecutive patients. *Journal of Neurosurgery: Spine*. **8**, 215–221 (2008).
26. J. Holsheimer, G. Barolat, J. J. Struijk, J. He, Significance of the Spinal Cord Position in Spinal Cord Stimulation. *Advances in Stereotactic and Functional Neurosurgery 11. Acta Neurochirurgica Supplementum*. **64**, 119–124 (1995).
27. J. Holsheimer, G. Barolat, Spinal Geometry and Paresthesia Coverage in Spinal Cord Stimulation. *Neuromodulation: Technology at the Neural Interface*. **1**, 129–136 (1998).
28. A. I. Mehta, C. A. Mohrhaus, A. M. Husain, I. O. Karikari, B. Hughes, T. Hodges, O. Gottfried, C. A. Bagley, Dorsal Column Mapping for Intramedullary Spinal Cord Tumor Resection Decreases Dorsal Column Dysfunction. *Journal of Spinal Disorders & Techniques*. **25** (2012), doi:10.1097/BSD.0b013e318215953f.
29. D. S. Yanni, S. Ulkatan, V. Deletis, I. J. Barrenechea, C. Sen, N. I. Perin, Utility of neurophysiological monitoring using dorsal column mapping in intramedullary spinal cord surgery. *Journal of Neurosurgery: Spine*. **12** (2010), doi:10.3171/2010.1.SPINE09112.
30. V. Deletis, F. Sala, "The Role of Intraoperative Neurophysiology in the Protection or Documentation of Surgically Induced Injury to the Spinal Cord."
31. D. Nair, V. M. Kumaraswamy, D. Braver, R. D. Kilbride, L. F. Borges, M. v. Simon, Dorsal column mapping via phase reversal method: The refined technique and clinical applications. *Neurosurgery*. **74**, 437–446 (2014).

32. A. Quinones-Hinojosa, M. Gulati, R. Lyon, N. Gupta, C. Yingling, Spinal Cord Mapping as an Adjunct for Resection of Intramedullary Tumors: Surgical Technique with Case Illustrations. *Neurosurgery*. **51** (2002), doi:10.1097/00006123-200211000-00015.
33. J. Hermiz, L. Hossain, E. M. Arneodo, M. Ganji, N. Rogers, N. Vahidi, E. Halgren, T. Q. Gentner, S. A. Dayeh, V. Gilja, Stimulus Driven Single Unit Activity From Micro-Electrocorticography. *Frontiers in Neuroscience*. **14** (2020), doi:10.3389/fnins.2020.00055.
34. J. C. Yang, A. C. Paulk, P. Salami, S. H. Lee, M. Ganji, D. J. Soper, D. Cleary, M. Simon, D. Maus, J. W. Lee, B. v. Nahed, P. S. Jones, D. P. Cahill, G. R. Cosgrove, C. J. Chu, Z. Williams, E. Halgren, S. Dayeh, S. S. Cash, Microscale dynamics of electrophysiological markers of epilepsy. *Clinical Neurophysiology*. **132**, 2916–2931 (2021).
35. M. Ganji, E. Kaestner, J. Hermiz, N. Rogers, A. Tanaka, D. Cleary, S. H. Lee, J. Snider, M. Halgren, G. R. Cosgrove, B. S. Carter, D. Barba, I. Uguz, G. G. Malliaras, S. S. Cash, V. Gilja, E. Halgren, S. A. Dayeh, Development and Translation of PEDOT:PSS Microelectrodes for Intraoperative Monitoring. *Advanced Functional Materials*. **28** (2018), doi:10.1002/adfm.201700232.
36. A. C. Paulk, J. C. Yang, D. R. Cleary, D. J. Soper, M. Halgren, A. R. O'Donnell, S. H. Lee, M. Ganji, Y. G. Ro, H. Oh, L. Hossain, J. Lee, Y. Tchoe, N. Rogers, K. Kiliç, S. B. Ryu, S. W. Lee, J. Hermiz, V. Gilja, I. Ulbert, D. Fabó, T. Thesen, W. K. Doyle, O. Devinsky, J. R. Madsen, D. L. Schomer, E. N. Eskandar, J. W. Lee, D. Maus, A. Devor, S. I. Fried, P. S. Jones, B. v. Nahed, S. Ben-Haim, S. K. Bick, R. M. Richardson, A. M. Raslan, D. A. Siler, D. P. Cahill, Z. M. Williams, G. R. Cosgrove, S. A. Dayeh, S. S. Cash, Microscale Physiological Events on the Human Cortical Surface. *Cereb Cortex*. **31**, 3678–3700 (2021).
37. M. Ganji, A. C. Paulk, J. C. Yang, N. W. Vahidi, S. H. Lee, R. Liu, L. Hossain, E. M. Arneodo, M. Thunemann, M. Shigyo, A. Tanaka, S. B. Ryu, S. W. Lee, Y. Tchoe, M. Marsala, A. Devor, D. R. Cleary, J. R. Martin, H. Oh, V. Gilja, T. Q. Gentner, S. I. Fried, E. Halgren, S. S. Cash, S. A. Dayeh, Selective Formation of Porous Pt Nanorods for Highly Electrochemically Efficient Neural Electrode Interfaces. *Nano Letters*. **19**, 6244–6254 (2019).
38. M. Ganji, L. Hossain, A. Tanaka, M. Thunemann, E. Halgren, A. Devor, S. A. Dayeh, M. Ganji, S. A. Dayeh, L. Hossain, A. Tanaka, M. Thunemann, Monolithic and Scalable Au Nanorod Substrates Improve PEDOT-Metal Adhesion and Stability in Neural Electrodes, doi:10.1002/((please)).
39. M. Ganji, A. Tanaka, V. Gilja, E. Halgren, S. A. Dayeh, Scaling Effects on the Electrochemical Stimulation Performance of Au, Pt, and PEDOT:PSS Electrocorticography Arrays. *Advanced Functional Materials*. **27** (2017), doi:10.1002/adfm.201703019.
40. Y. Tchoe, A. M. Bourhis, D. R. Cleary, B. Stedelin, J. Lee, K. J. Tonsfeldt, E. C. Brown, D. Siler, A. C. Paulk, J. C. Yang, H. Oh, Y. Goo Ro, K. Lee, S. Russman, M. Ganji, I. Galton, S. Ben-Haim, A. M. Raslan, S. A. Dayeh, Human brain mapping with multithousand-channel PtNRGrids resolves spatiotemporal dynamics. *Science Translational Medicine*, 1441 (2022).
41. R. Vatsyayan, D. Cleary, J. R. Martin, E. Halgren, S. A. Dayeh, Electrochemical safety limits for clinical stimulation investigated using depth and strip electrodes in the pig brain. *Journal of Neural Engineering*. **18** (2021), doi:10.1088/1741-2552/ac038b.
42. D. B. MacDonald, S. Skinner, J. Shils, C. Yingling, Intraoperative motor evoked potential monitoring - A position statement by the American Society of Neurophysiological Monitoring. *Clinical Neurophysiology*. **124** (2013), pp. 2291–2316.

43. V. Deletis, K. Seidel, "Neurophysiological identification of long sensory and motor tracts within the spinal cord" in *Neurophysiology in Neurosurgery* (Elsevier, 2020), pp. 163–175.
44. J. G. McLeod, S. H. Wray, Conduction velocity and fibre diameter of the median and ulnar nerves of the baboon. *J Neurol Neurosurg Psychiatry*. **30**, 240–247 (1967).
45. A. Eisen, M. Hoirch, J. White, D. Calne, Sensory group Ia proximal conduction velocity. *Muscle & Nerve*. **7**, 636–641 (1984).
46. L. Pelosi, J. B. Cracco, R. Q. Cracco, "Conduction characteristics of somatosensory evoked potentials to peroneal, tibial and sural nerve stimulation in man" (1987).
47. Y. W. K. Sakatani, "Neurophysiological Mechanisms of Somatosensory-Evoked Potential Changes" in *Neural Monitoring* (Humana Press, Totowa, NJ, 1990), pp. 115–148.
48. J. Isakovic, I. Dobbs-Dixon, D. Chaudhury, D. Mitrecic, Modeling of inhomogeneous electromagnetic fields in the nervous system: a novel paradigm in understanding cell interactions, disease etiology and therapy. *Scientific Reports*. **8** (2018), doi:10.1038/s41598-018-31054-9.
49. M. J. Kržan, "Intraoperative Neurophysiological Mapping of the Spinal Cord's Dorsal Columns" in *Neurophysiology in Neurosurgery* (Elsevier, 2002).
50. D. Nair, V. M. Kumaraswamy, D. Braver, R. D. Kilbride, L. F. Borges, M. v. Simon, Dorsal column mapping via phase reversal method: The refined technique and clinical applications. *Neurosurgery*. **74**, 437–446 (2014).
51. The Fatal Current. *Physical Therapy*. **46** (1966), doi:10.1093/ptj/46.9.968.
52. K. L. Bunday, M. A. Urbin, M. A. Perez, Potentiating paired corticospinal-motoneuronal plasticity after spinal cord injury. *Brain Stimulation*. **11**, 1083–1092 (2018).
53. A. P. Yadav, D. Li, M. A. L. Nicolelis, A Brain to Spine Interface for Transferring Artificial Sensory Information. *Scientific Reports*. **10** (2020), doi:10.1038/s41598-020-57617-3.
54. A. P. Yadav, S. Li, M. O. Krucoff, M. A. Lebedev, M. M. Abd-El-Barr, M. A. L. Nicolelis, Generating artificial sensations with spinal cord stimulation in primates and rodents. *bioRxiv* (2020), doi:10.1101/2020.05.09.085647.
55. A. Rowald, S. Komi, R. Demesmaeker, E. Baaklini, S. D. Hernandez-Charpak, E. Paoles, H. Montanaro, A. Cassara, F. Becce, B. Lloyd, T. Newton, J. Ravier, N. Kinany, M. D'Ercole, A. Paley, N. Hankov, C. Varescon, L. McCracken, M. Vat, M. Caban, A. Watrin, C. Jacquet, L. Bole-Feysot, C. Harte, H. Lorach, A. Galvez, M. Tschopp, N. Herrmann, M. Wacker, L. Geernaert, I. Fodor, V. Radevich, K. van den Keybus, G. Eberle, E. Pralong, M. Roulet, J.-B. Ledoux, E. Fornari, S. Mandija, L. Mattera, R. Martuzzi, B. Nazarian, S. Benkler, S. Callegari, N. Greiner, B. Fuhrer, M. Froeling, N. Buse, T. Denison, R. Buschman, C. Wende, D. Ganty, J. Bakker, V. Delattre, H. Lambert, K. Minassian, C. A. T. van den Berg, A. Kavounoudias, S. Micera, D. van de Ville, Q. Barraud, E. Kurt, N. Kuster, E. Neufeld, M. Capogrosso, L. Asboth, F. B. Wagner, J. Bloch, G. Courtine, Activity-dependent spinal cord neuromodulation rapidly restores trunk and leg motor functions after complete paralysis. *Nature Medicine* (2022), doi:10.1038/s41591-021-01663-5.
56. D. Rubino, K. A. Robbins, N. G. Hatsopoulos, Propagating waves mediate information transfer in the motor cortex. *Nature Neuroscience*. **9**, 1549–1557 (2006).
57. L. Muller, G. Piantoni, D. Koller, S. S. Cash, E. Halgren, T. J. Sejnowski, Rotating waves during human sleep spindles organize global patterns of activity that repeat precisely through the night. *Elife*. **5** (2016), doi:10.7554/eLife.17267.001.

Chapter 2

The conformal, high-density SpineWrap microelectrode array delivers focal stimulation for selective muscle recruitment

Introduction

Spinal cord injury (SCI) can have a dramatic, lifechanging impact due to the debilitating loss of motor and sensory functions. Over recent decades, spinal cord stimulation (SCS) below the point of injury has been investigated as a mechanism for functional restoration (*1*). In particular, epidural electrical stimulation (EES) has emerged as a promising treatment strategy and has been applied to restore motor function in animals and humans with SCI (*2–8*). Computational and experimental studies have shown that EES targets the dorsal roots (DRs) and specifically the dorsal root entry zones (DREZs), which have a low threshold for EES activation (*9–12*). The DRs and DREZs carry type I and type II sensory afferents, which form direct and indirect connections to alpha motoneurons, which innervate the muscle fibers in the target muscles. Studies have shown that targeting these sensory afferents, as opposed to directly stimulating the motoneurons, is desirable since this results in less fatigue and allows EES to tap into inherent neural synaptic pathways in the spinal cord, resulting in naturalistic neural recruitment patterns (*13–18*).

When applied in humans for treatment of SCI, EES is currently delivered with clinical paddle arrays, which were originally developed to target the dorsal columns for management of chronic pain (*19, 20*), rather than specifically targeting the DREZs for muscle recruitment. As a result, these arrays do not deliver stimulation to the optimal location. Additionally, whereas the afferents innervating individual muscles are arranged segmentally and terminate in specific lamina in the dorsal horn based on their diameter, afferent organization is not so ordered as to allow for precise targeting of only those that activate single muscle (*21*). Given this lack of consistency in afferent organization, a unique challenge in applying EES for SCI is the significant overlap in

stimulation targets between muscles. Paddle arrays are therefore not optimized for this due to their low focality in stimulation.

There are several factors that limit the focality of stimulation that can be delivered by paddle arrays. First, paddle arrays in clinical use are made of ~ 2 mm thick silicone, making these devices non-conformal to the spinal cord. This lack of conformality leads to charge spreading in the space between the paddle array and the epidural surface, reducing stimulation focality (22, 23). Second, paddle arrays have low channel counts, making it difficult to deliver stimulation at dimensions that will target small populations of sensory afferents. The electrode contacts themselves are currently made of materials with relatively high impedance and low charge injection capacity (CIC), such as platinum (24–26). CIC is the charge per unit area that can be delivered from a given contact before the onset of irreversible electrochemical reactions at the electrode-tissue interface that can damage the contacts and surrounding tissue (27). Therefore, scaling these electrode contacts to smaller contact areas would significantly limit their CIC and introduce additional challenges due to the voltage compliance limits of the back-end electronics used for stimulation. Finally, the resulting low contact density of paddle arrays necessitates patient-specific designs to accommodate the varied neuroanatomy across subjects (28, 29), limiting the effectiveness and the suitability of this technology for broader populations.

Previous studies have focused largely on reducing the rigidity and thickness of EES arrays to minimize foreign body response and spinal cord compression that occurs during implantation of thick and rigid arrays (30, 31). Other studies have also developed new microelectrode arrays that are fabricated on either thin polymer substrates, such as polyimide or parylene-C (32–35), or elastomer substrates, such as polydimethylsiloxane (PDMS) (36–39). Although reducing substrate thickness and rigidity coincides with increased conformality, these arrays mostly possess channel

counts on par with clinical grids and the advantages of such arrays for delivering stimulation are not clearly illustrated.

The question remains whether the efficacy of EES in selectively recruiting target muscles can be further improved with more focal stimulation. The proximity of neurons to the current source makes them more susceptible to activation at lower stimulation current intensities. Therefore, we hypothesized that bipolar stimulation using a high-density, conformal microelectrode array would more selectively target afferents in a small volume directly under the stimulating electrode contact pair and identify submillimeter hotspots for optimal stimulation. Thus, such an array would allow for more precise and more complex stimulation patterns.

Here we introduce SpineWrap, a 6.6 μm thin film, 156-channel high-density microelectrode array for targeted EES. The thinness and unique design features of the parylene-C substrate allow SpineWrap to “wrap” around the spinal cord curvature, resulting in high conformality over a large area while ensuring each electrode contact is uniformly touching the dural surface (Fig. 2.1 A, B). The high density and small size of SpineWrap contacts is possible because of the material properties of the platinum nanorod (PtNR) contacts (Fig. 2.1 C). We demonstrate that SpineWrap can deliver focal EES to the rat lumbar spinal cord resulting in highly selective recruitment of six lower limb muscles and locate stimulation hotspots for highest selectivity. We quantify the focality of SpineWrap stimulation using a computational model of the lumbar spinal cord recreated from MRI scans and show that stimulation with SpineWrap can activate tissue at depth without compromising the focality of stimulation. Finally, we evaluate the impact of channel count on muscle recruitment selectivity with post hoc channel subsampling analysis and in vivo validation studies. We show that, in rats, high-density arrays require submillimeter pitches (median = 570 μm , IQR 461 – 799 μm) so as not to reduce maximum

achievable selectivity during bipolar stimulation across all muscles measured in this study. Our results suggest that SpineWrap can improve the efficacy of EES stimulation for treatment of SCI by delivering more focal stimulation compared to clinical paddle arrays and previously developed EES microelectrode arrays.

Results

SpineWrap design

The high contact density and small diameter of SpineWrap are enabled by the PtNR contact material. PtNRs have a very low impedance (40); at 1kHz the impedance of a 100 μm PtNR contact is 17 times lower than that of a planar platinum contact (Fig. 2.1 C). PtNRs also have high CIC; in saline, a 100 μm PtNR contact can inject up to 6 mA of current at 100 μs pulse width, which is 32 times greater than the 190 μA that can be safely injected by a planar platinum contact without causing water electrolysis (Fig. 2.1 C). *In vivo*, our 100 μm PtNR contacts permit the delivery of current levels up to $\sim 300 \mu\text{A}$, which is enough to elicit strong muscle activation. The difference between the saline and *in vivo* maximum currents is a result of the different conductivities and current spreading effects between the two media (41). PtNRs have been used previously for neural stimulation and recording in animals and patients in acute and chronic settings (40, 42–44). SpineWrap PtNR contacts were stable throughout the acute stimulation experiments performed in this study. The median impedance of the SpineWrap contacts was 5.12 k Ω when measured in saline. When measured on the spinal cord before stimulation, the median impedance increased to 16.3 k Ω and did not change significantly when measured again after the conclusion of the experiment, with a median impedance of 15.6 k Ω ($p=0.29$, two-sided Mann

Whitney U test, Fig. S1). Scanning electron microscopy (SEM) imaging of contacts before and after stimulation showed no morphological changes to the PtNRs (Fig. 2.6).

SpineWrap contact distribution can be customized and is designed here to target the dorsolateral L3-L5 spinal segments of the rat spinal cord. To design SpineWrap, we dissected the lumbar rat spinal cord to determine the average lengths of the lumbar DREZs ($n=5$, Fig. 2.1 D, Table 2.1). Based on these measurements, we determined the optimal electrode array dimensions to be 9 mm x 3 mm to provide adequate longitudinal and lateral coverage to the L3-L5 DREZs and account for small deviations in segmental length and electrode placement (Fig. 2.7). When determining the channel number and density of the array, we used COMSOL Multiphysics computational modeling based on an MRI reconstruction of the rat lumbar spinal cord (Fig. 2.1 D) to identify pitch ranges that resulted in focal and deep stimulation. We also considered the practical time constraint of acute studies, with each contact and current level requiring > 10 s of stimulation time. We optimized the contact layout with respect to these constraints and designed an electrode of 26 rows x 3 columns (78 contacts) on each side of the midline to arrive at a total of 156 contacts for the entire array. The horizontal (circumferential) pitch between the stimulating contacts was 500 μm and the vertical (axial) pitch was 360 μm . This array configuration allowed for complete longitudinal coverage of the L3-L5 DREZs while also extending laterally enough to cover the center points of the mediolaterally located DREZs along the circumference of the cord (Fig. 2.7), without so much lateral coverage as to compromise the conformality and uniform contact of the electrode on the entire surface of the cord. Assuming perfect placement, the L3 DREZ was covered by 66 contacts, the L4 DREZ was covered by 48 contacts and the L5 DREZ was covered by 42 contacts (Fig. 2.7). The lateral coverage of SpineWrap spanned 1250 μm from the midline, which

was sufficient to provide optimal stimulation to each DREZ based on our own modeling results presented below and previous literature (2).

To limit current spreading through highly conductive cerebrospinal fluid (CSF) that fills the space between the dura and the spinal cord surface, we delivered stimulation in a bipolar manner along the spinal cord surface between contacts in each column of SpineWrap, with the cathode position superior to the anode (Fig. 2.1 B). In this configuration, current flowed between the adjacent electrodes, which facilitated spatially selective stimulation. Bipolar stimulation is commonly used in the clinic setting (45), however for clinical grids the anode is set to ground. In our design, setting each anode to ground constituted a technical challenge and was impractical given the large number of contacts. Instead, the anode delivered the reverse polarity current to the cathode, thus driving current flow, and an on-device ground was placed separately on the array to sink any residual charge that is not extracted by the bipolar contacts.

In earlier studies the ground was not incorporated in the electrode design but placed separately in the paraspinal muscles (Fig. 2.8). Practical imperfection of charge balance in bipolar stimulation and the inability to fully extract injected charge led to current spreading resulting in the undesired direct activation of motoneurons, likely through the ventral nerve roots. Furthermore, while the bipolar stimulating contact can partially limit the spread of current along the axis of the spine, the lateral spread of current along the circumference of the spine is less controllable with a purely bipolar design. Direct motoneuron activation was identified by analysis of electromyography (EMG) latency (Fig. 2.9), with EMG responses appearing much sooner after stimulus in the case of direct motoneuron activation. In one of these earlier studies (Fig. 2.8, layout 3), bipolar stimulation resulted in early latency EMG responses in some muscles, such as the vastus lateralis (median onset time 2.45 ms after stimulus, Fig. 2.9), indicating direct motoneuron

activation. Since the EMG activation occurred primarily at the bottom of the electrode array at the L5 spinal level, we hypothesized that current spread to the L3 ventral nerve root, which was in a lateral position at the L5 spinal level and innervates the vastus lateralis. We confirmed that current spread was significant in the earlier designs by shifting the position of the ground needle in tissue (Fig. 2.10). Depending on the location of the ground needle, we observed a change in the contacts eliciting muscle activation.

We modeled the spread of current using COMSOL simulations for a reconstructed model of the rat spinal cord. To determine the L3-L5 nerve root organization and overlap and to preserve their *in situ* trajectory, we imaged the intrathecal lumbar and sacral nerve root organization using MRI (Fig. 2.1 D and Fig. 2.11). We then reconstructed a 3D anatomical model to serve as the basis for our finite element modeling to calculate field-distributions and imported the model into COMSOL. In COMSOL, we gave each tissue an electric conductivity based on previously reported values (10, 46) (Table 2.2). We modeled stimulation with and without charge mismatch to illustrate the advantage of electrode arrays with and without an on-device ground (Fig. 2.12). We found that charge mismatch significantly reduced stimulation focality without on-device ground.

These results led us to redesign the electrodes to incorporate the ground adjacent to the stimulating electrode contacts to create an on-device ground. An on-device ground was critical for the focal stimulation and selective activation of DREZs. This was included in the form of ground lines between columns of contacts (Fig. 2.1 B, middle). In subsequent experiments, the electrode with on-device ground lines did not show any current spreading, with significantly higher latency EMG responses recorded even at the highest current levels (median onset time 4.85 ms after stimulus, $p < 0.05$, two-sided Mann Whitney U test; Fig. 2.9). This indicates indirect

motoneuron activation through sensory afferents. Thus, with its on-device ground, SpineWrap provides focal stimulation without current spread.

In designing the on-device ground, we explored both a concentric ground ring around each contact and in-plane ground-lines in between rows of contacts. The concentric ground design was previously proposed as a method to deliver highly focal cortical stimulation (47). In our experiments, concentric ground did not result in EMG response amplitudes that were as high as for ground lines (Fig. 2.13). We concluded that the distance between the stimulating contact and surrounding ground was too small for even high stimulation currents to reach sufficient depths to recruit enough afferent fibers to produce large EMG responses. This resulted in a tradeoff between cathode-to-ground pitch and contact density. Therefore, we opted for vertical ground lines on SpineWrap to not compromise the high density we aimed to achieve.

For focal stimulation, it was critical that SpineWrap wraps conformally onto the spinal cord surface to ensure uniform contact of all electrode contacts and ground lines on the array and therefore generate consistent stimulation across all contacts. Uniform conformality across all contacts was a challenge since the array needed to capture enough longitudinal and lateral coverage of the DREZs while also allowing for longitudinal bending along the axial curvature present in rats at the L3-L5 spinal segments. We tested many iterations of the electrode array design to achieve a conformal device (Fig. 2.8). We ultimately opted for a device design that had two flaps in the upper and lower regions (Fig. 2.1 B, middle). These flaps allowed the electrode to latch onto the L3-L5 spinal levels while omitting the T13-L1 vertebral bone junction, which was left intact to not damage to the nerve roots exiting at this location. Between the flaps, teeth were etched into the lateral edge of the array to relieve buckling of the flat parylene C film. Ultimately, this design allowed for consistently conformal placement of SpineWrap across animals (Fig. 2.1 B, right).

During stimulation, we additionally placed gel foam onto the electrode surface and the surrounding tissues. This has kept the epidural surface moist and the electrode in place. We compared the placement of the electrode before and after the stimulation to confirm the electrode placement did not shift during stimulation (Fig. 2.14).

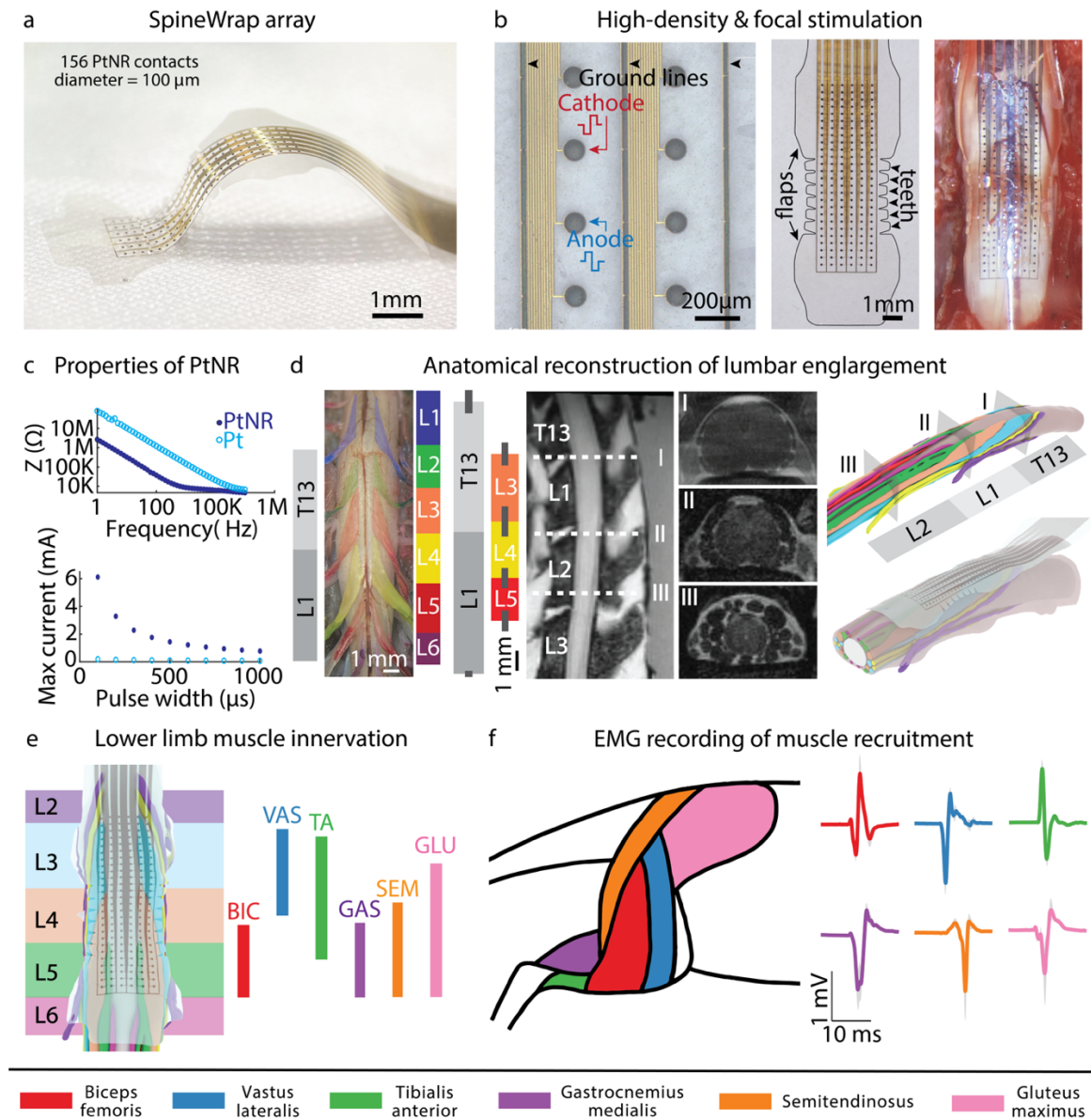


Figure 2.1 The SpineWrap microelectrode array for delivers focal spinal cord stimulation. (A) SpineWrap design features including the 156 channel PtNR contact array with 100 μm contact diameter and 6.6 μm thick PxC substrate. (B) Left: Close-up view of the microelectrode array. Stimulation was delivered in a bipolar configuration between neighboring contacts in each column. Ground lines limited current spreading. Middle: Flaps and teeth allowed for conformal placement over curved rat lumbar spinal cord. Right: Image of SpineWrap on spinal cord. (C) Low impedance and high CIC of PtNRs allowed for micro-scale contact size to deliver high stimulation amplitudes. (D) Dissections ($n=5$) and MRI ($n=2$) was used to identify the dimensions of the DREZs and guide SpineWrap design. (E) SpineWrap coverage of the rat lumbar spinal cord and corresponding muscle innervation levels based on previous work(2). (F) Muscles recorded during the experiment and example trial-averaged high-amplitude EMG responses ($n=6$, gray line represents ± 1 standard deviation).

Stimulation focality of SpineWrap

We used SpineWrap to recruit lower limb muscles in anesthetized intact rats. We stimulated at 0.5 Hz in a semi-random order and recorded muscle activity with bipolar EMG needles in six muscle groups innervated by the L3-L5 spinal segments (2): biceps femoris (BIC), vastus lateralis (VAS), tibialis anterior (TIB), gastrocnemius medialis (GAS), semitendinosus (SEM) and gluteus maximus (GLU) (Fig. 2.1 E,F). We computed the peak-to-peak (PTP) EMG amplitudes of the recorded response waveforms. PTP amplitudes were normalized (PTP_{norm}) by dividing them by the maximum peak-to-peak amplitude (PTP_{max}) across current levels (see Materials and Methods) to perform inter-muscular and inter-animal analysis. We performed stimulation on both left and right sides of the spinal cord in two rats (referred to as datasets Rat 1r/l and Rat 2r/l) and on the right side only in two rats (referred to as datasets Rat 3r and Rat 4r). Since in this study we investigated general trends in muscle segmental activation and selectivity without emphasis on precise mediolateral contact position, we grouped the left and the right datasets from different animals in our analysis to arrive at total of 6 datasets for this study.

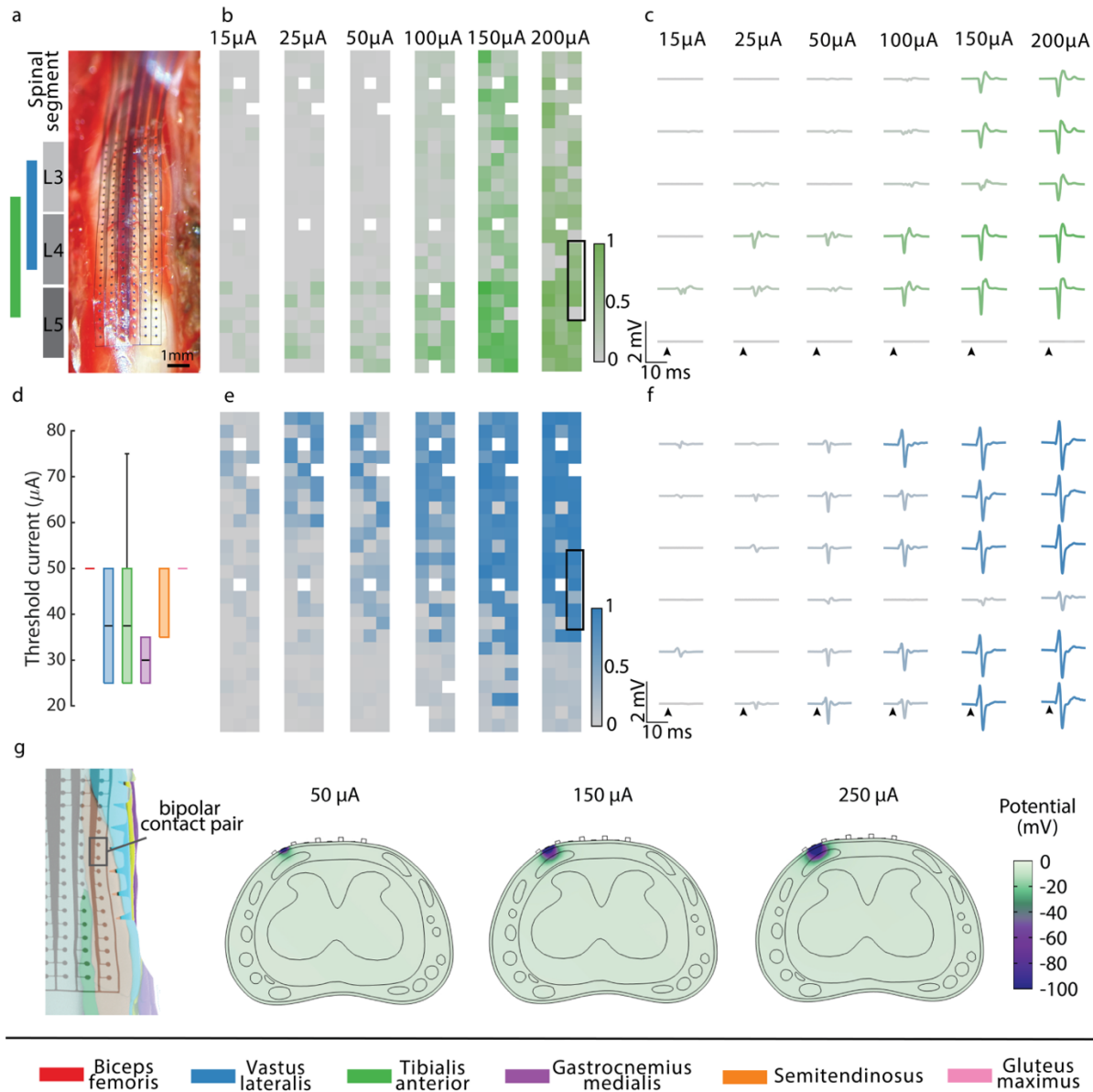


Figure 2.2 SpineWrap delivers focal spinal cord stimulation. (A) Placement of SpineWrap on spinal cord for Rat 5r and approximate locations of innervations for muscles tibialis anterior (green) and vastus lateralis (blue). (B) Increasing current amplitude results in widespread activation of TIB. (C) Close-up view of the black box in (B) with plotted trial-averaged EMG waveforms. Black arrows indicate stimulation time. (D) The stimulation current threshold necessary to produce a significant EMG response (≥ 0.1 PTP_{max}, n = 6) for each muscle. Each boxplot shows the distribution of the current threshold for a given dataset and muscle. The horizontal center lines and boxes indicate the median and the 25th/75th percentile and the vertical solid lines represent the full range of the distribution. (E) Increasing current amplitude results in widespread activation of VAS. (F) Close-up view of the black box in (E) with plotted trial-averaged EMG waveforms. Black arrows indicate stimulation time. (G) Modeling results from COMSOL quantifying the focality of stimulation delivered by a pair of electrodes on the L4 DREZ at increasing current levels.

We varied stimulation currents between 15 μA – 300 μA and measured the EMG responses. At low current levels, only a few pairs of bipolar contacts showed muscle activity at the corresponding spinal cord segmental level (Fig. 2.2 A-C & E-F). Increasing the stimulation current increased the number of bipolar pairs of contacts generating significant muscle response. As the current amplitude was increased, nearest neighboring bipolar contact pairs to those initially selective to specific muscles at lower amplitudes began to also elicit muscle responses, with highest current levels generating responses across many bipolar contact pairs and multiple segmental levels. We defined the current amplitude thresholds necessary to generate a significant muscle response as those resulting in an EMG response with $\geq 0.1 \text{ PTP}_{\text{max}}$ and identified the median current threshold to be 35 – 50 μA across muscles (Fig. 2.2 D).

We hypothesized that at low current levels there are few contacts eliciting muscle activation because the stimulation is very focal and only targets the sensory afferents directly under the bipolar contact pair. Increasing the stimulation current amplitude activates afferents further from the bipolar contact pair, thus many multiple pairs can activate the same afferents, resulting in widespread muscle activation. We used finite element modeling in COMSOL Multiphysics to illustrate this effect by increasing stimulation from a pair of contacts and the plotting resulting potential field (Fig. 2.2 E, G). To do this, we imported a 3D construction of the MRI imaged rat lumbar spinal cord into Blender, and individual spinal elements were then converted into .stl files and used in COMSOL Multiphysics (See Materials and Methods, Computational Modeling). Even at 250 μA current levels, there was no appreciable potential within the spinal cord white matter that would cause activation, with significant potential gradients only in the CSF.

Muscle recruitment selectivity

To assess the impact of SpineWrap's focal stimulation on selective muscle recruitment, we computed the selectivity index (SI) for each muscle and each contact using previously published equations for selectivity (9), where SI is the difference between PTP_{norm} for each current level, contact, and muscle and the mean PTP_{norm} of the remaining muscles (see Materials and Methods). Across datasets, we found that the SI distribution generally agreed with spinal innervation (Fig. 2.3 A, Fig. 2.15). The SI distribution per muscle skewed towards zero for most muscles and most datasets, with few channels per electrode eliciting high selectivity (Fig. 2.3 B, Fig. 2.16). Additionally, in some locations on the array, SI values varied significantly between neighboring contacts, indicating that stimulation from neighboring contacts activated distinct sets of muscles (Fig. 2.3 C).

We extracted the maximum SI (SI_{max}) per channel for each muscle and each dataset and overlaid the channel locations of SI_{max} (Fig. 2.3 D, Fig. 2.17). The locations of SI_{max} were closely distributed and corresponded to the expected muscle innervation. Exceptions were Rat 2r, where the SEM and GLU SI_{max} were positioned in the upper part of the array and Rat 4r, where the GAS SI_{max} was positioned in the upper part of the array (Fig. 2.17). To determine the average SI_{max} achievable with the SpineWrap array, we compared SI_{max} values across the datasets. We found that the average SI_{max} was ≥ 0.5 for all muscles (Fig. 2.3 E), indicating that the SpineWrap array could achieve moderate to high selectivity across all six muscle groups recorded in this study.

A motivator for this study was the hypothesis that the high-channel count of SpineWrap would result in more options for channel selection and point to hotspots of maximal selectivity, thus optimizing the stimulation locations compared to conventional, low-channel count paddle arrays. The SI distributions in Fig. 2.3 B indicate that SpineWrap can identify hotspots with very

high selectivity. To further evaluate this hypothesis, we investigated how selective the $SI_{\max}$ channels were compared to the remaining SIs. Across datasets and for each muscle, we identified contacts with comparable SI value to $SI_{\max}$ by calculating the number of contacts per dataset that had an $SI \geq 90\%$ of $SI_{\max}$. We found that the median number of bipolar contact pairs with a comparable SI value across datasets was between 1-3 per muscle (Fig. 2.3 F). Thus, we conclude that SpineWrap's high-density coverage can pinpoint to a few hotspot locations for highest muscle recruitment selectivity.

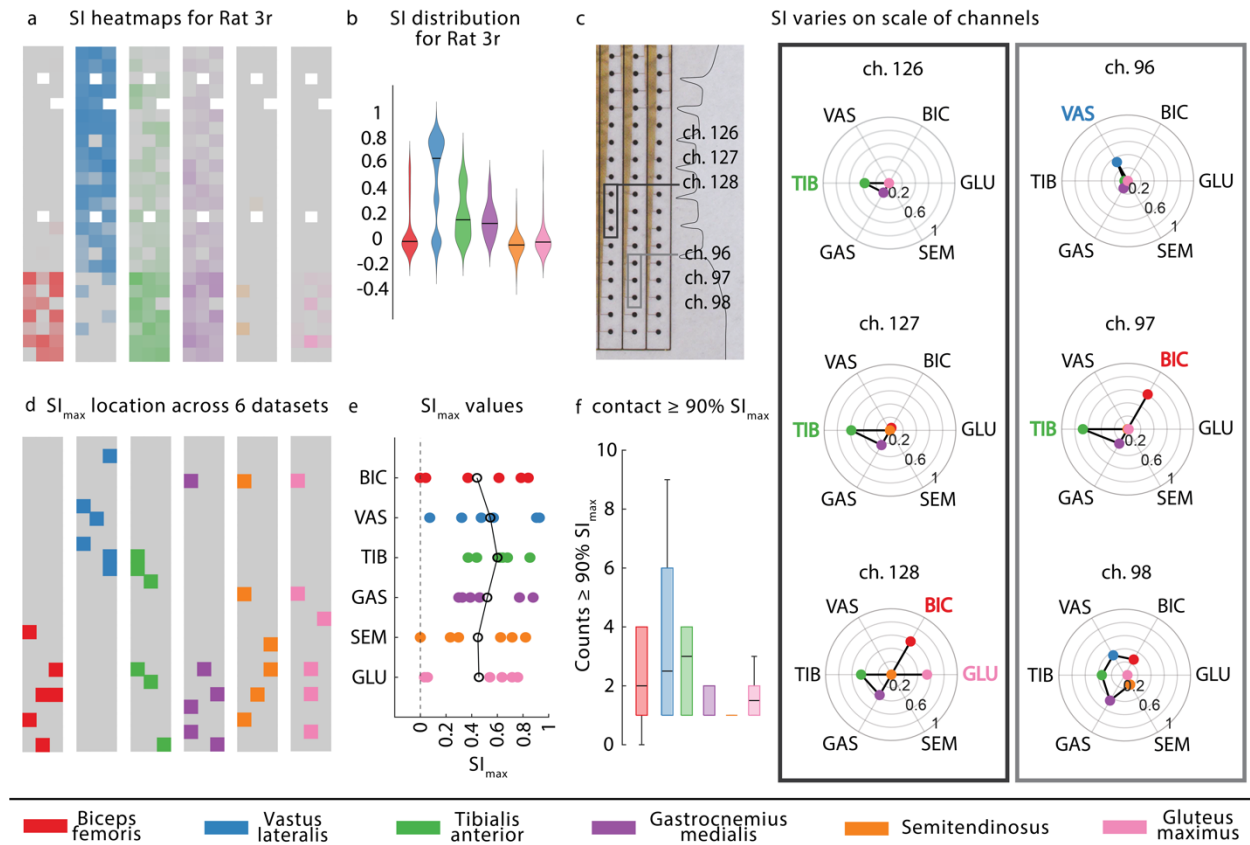


Figure 2.3 SpineWrap produces selective lower limb muscle activation. (A) Example SI distribution across electrode arrays for all muscles for Rat 3r. (B) The distribution of selectivity indices for Rat 3r. (C) Selectivity can vary on the scale of individual channels, with neighboring channels recruiting different muscles more selectively. For example, the dominant muscle shifts from TIB at channels 126 and 127 to BIC and GLU at channel 128. Similarly, the dominant muscle shifts from VAS for channel 96 to TIB and BIC for channel 97. At channel 98, no muscle is highly selective. (D) Comparison of cathode locations of bipolar contact pairs that produced SI_{max} for each muscle across all datasets. (E) SI_{max} values for each muscle across all datasets. The open black circles are the mean SI_{max} for each muscle, and the black line connects the mean SI_{max} from the different muscles. (F) The number of bipolar contact pairs that have SI values close to SI_{max} . Each boxplot shows the distribution of the number of pairs with SI values $\geq 90\%$ of SI_{max} for a given dataset and muscle. The horizontal center lines and boxes indicate the median and the 25th/75th percentile and the vertical solid lines represent the full range of the distribution.

Effect of array channel count and resolution on recruitment selectivity

Improving stimulation focality by increasing channel count and resolution has been a topic of interest in the neuromodulation community. However, to the best of our knowledge, this effect has never been quantified for spinal cord stimulation. Though our SI values indicate selective muscle recruitment, SI values are difficult to directly compare to previous studies using lower density arrays due to differences in experimental design and recorded muscle groups. We explored an alternative method for quantifying the impact of both channel count and resolution on stimulation selectivity.

We subsampled the SpineWrap array using Poisson Disk sampling and computed subsamples of 4-36 bipolar contact pairs ($n=1000$). The resultant range of pitches between the bipolar contact pairs (termed inter-cathode pitch) was 624.5 - 1873 μm (see Materials and Methods). We then evaluated the SI_{max} of these subsampled datasets (Fig. 2.4 A). Reducing the number of bipolar contact pairs available for stimulation reduces the median SI_{max} of the subsamples (Fig. 2.4 B, Fig. 2.18). We fit a 4th degree polynomial to the median SI for each pitch and each muscle and extrapolated the minimum inter-cathode pitch necessary to achieve $\geq 90\%$ SI_{max} of the original array. The minimum pitch to not reduce SI_{max} of the subsample was 570 μm , which is the median minimum pitch required for SEM (Fig. 4c, IQR 461 – 799 μm). Unlike previously developed low-density rat EES arrays (33, 35), SpineWrap possesses a pitch smaller than this minimum pitch. Therefore, we conclude that the electrode design does not contribute to reduction of SI.

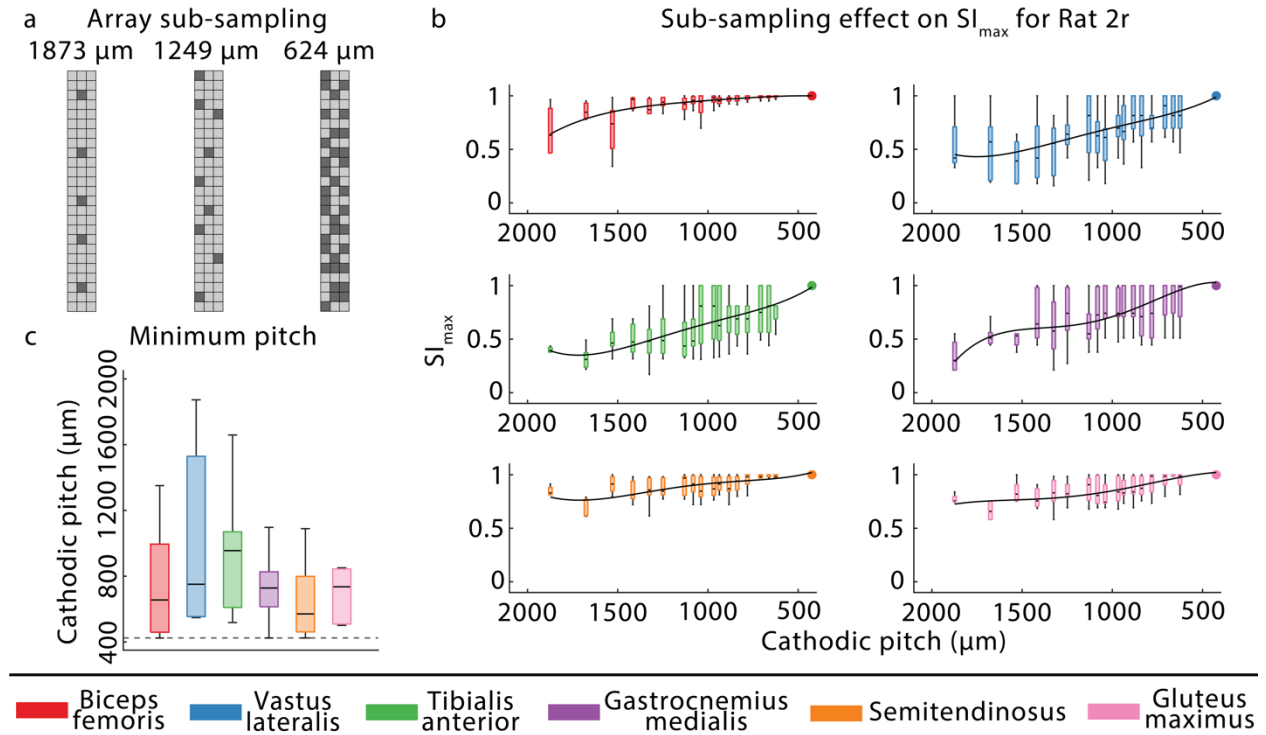


Figure 2.4 Muscle recruitment is more selective with high-spatial resolution and high-channel count spinal cord coverage. (A) Example subsamples of the array at various cathodic pitches ($n = 1000$ subsamples obtained using Poisson Disk sampling). (B) Median maximal recruitment selectivity, measured as the ratio of the subsample SI_{max} to the SI_{max} of the full array, increased with smaller decreasing inter-cathode pitch. The horizontal center lines and boxes indicate the median and the 25th/75th percentile and the vertical solid lines represent the full range of the distribution. Note that at the smallest pitch there is only one datapoint, which is the SI_{max} of the full array. Sub-sampling results are from Rat 2r. (C) The minimum inter-cathode pitch required to not reduce the median SI_{max} of subsamples across all muscles was 570 μm (minimum pitch for SEM). Dashed line represents the pitch of SpineWrap.

Validation of high-channel count array benefits with in vivo experiments

We validated the subsampling results with an in vivo study. We fabricated a similar array to the SpineWrap array, but we increased the horizontal and vertical pitch of the contacts from 500 μm and 360 μm to 1000 μm and 800 μm , respectively. The resulting channel count of this array was reduced from 156 contacts to 40 contacts and the resulting density dropped from 5.6 contacts/ mm^2 to 1.42 contacts/ mm^2 . The mean pitch (see Materials and Methods) increased from 424.3 μm to 837.9 μm , falling well outside of the 570 μm minimum pitch identified in the

subsampling analysis. We increased the contact diameter from 100 μm to 500 μm to match the contact area more closely in previously developed low-density rat EES arrays (33, 35). The array coverage, shape, thickness and ground lines design were the same as those of SpineWrap to limit variability in results due to conformality, placement, and grounding (Fig. 2.19).

We performed additional stimulation with the low-density array in Rats 2r/l and 4r. Current levels up to 1 mA were used while staying within the charge injection limit of the larger diameter. For analysis, we aligned the electrodes based on blood vessel patterns on the spinal cord surface (Fig. 2.5 A). We compared the SI value range of the two arrays and found that the distribution was narrower for the low-density array across all muscles (Fig. 2.5 B), highlighting that SpineWrap can identify stimulation hotspots to maximize SI. SI_{max} was larger for the high-density array compared to the low-density array, as indicated by the ratio between the low-density and high-density values (Fig. 2.5 C, see Materials and Methods). Notably, this empirical data shows a larger impact of channel count on selectivity than the subsampling analysis predicted, albeit for a particular choice of design parameters. At an inter-cathode pitch of 838 μm , the subsampling analysis predicted SI_{max} ratios ≥ 0.7 for all muscles whereas the *in vivo* data produced ratios less than that for most muscles. This may in part stem from non-linear effects of inter-cathode pitches on selectivity that are not captured in the post hoc subsampling analysis and in part from the larger contact diameter, resulting in stimulation delivered over a larger surface area. Moreover, the subsampling analysis did not account for changes in pitch between the cathode and anode (termed cathode-anode pitch) of each bipolar contact pair as the inter-cathode pitch increased; instead, the cathode-anode pitch remained 360 μm , whereas the cathode-anode pitch of the low-density array increased to 1000 μm . In conclusion, the *in vivo* results confirm that higher-channel count and

higher-density electrode arrays improve muscle recruitment and selectivity and reduce the necessary current amplitudes for stimulation.

Modeling the effect of contact diameter and spacing on potential gradients

Each design parameter for the electrode, including the contact-to-contact pitch (both circumferentially and axially) and the spacing between contact and ground lines, has a significant impact on the measured focality of stimulation. Practically, it is impossible to cycle through all possible design choices *in vivo*. Therefore, to predict the impact of the electrode design parameters on focality, we compared the spread of potentials and the width and the depth of afferent axon recruitment to various contact arrangements using computational modeling in COMSOL. First, we computed the potential profiles consistent with the stimulation paradigms that we employed *in vivo* (Fig. 2.5 D). We observed that with larger diameter contacts, the potential spreads more laterally, which effectively reduces the selectivity of stimulation, consistent with the experimentally observed results. Larger contacts have the capability of injecting significantly higher currents, but the selectivity of stimulation is greatly reduced at the same charge density ($318.3 \mu\text{C}/\text{cm}^2$). This highlights the benefit of using smaller diameter low-impedance materials for focal stimulation.

We evaluated the relationship between stimulation depth versus width. We used a previously published approximation of axon activation to unipolar stimulation to identify a threshold potential for afferent activation. This method relies only on the extracellular potentials of the node under the stimulating cathode and the neighboring nodes (48). The threshold potential was found to be 28 mV for a 10 μm diameter axon with internodal distance of 900 μm (Fig. 2.20, see Materials and Methods). This axon diameter and internodal distance correspond to the size of the group I fibers in the rat spinal cord, which travel through the DRs and DREZs (10). This

threshold potential was used to evaluate stimulation depth versus width of 100 μm and 200 μm bipolar contact pair arrangements (Table 2.3). Although changing the dimensions of the contacts and the contact spacing will affect potential gradient, which in turn affects the threshold potential, we chose 28 mV as the threshold across all bipolar contact pairs and currents tested as a reference for measuring stimulation depth and width consistently between different contact sizes without introducing additional dependent variables. Our results show that smaller diameter contacts with on-device ground lines stimulate deeper with lower lateral spread than larger diameter contacts (Fig. 2.5 E).

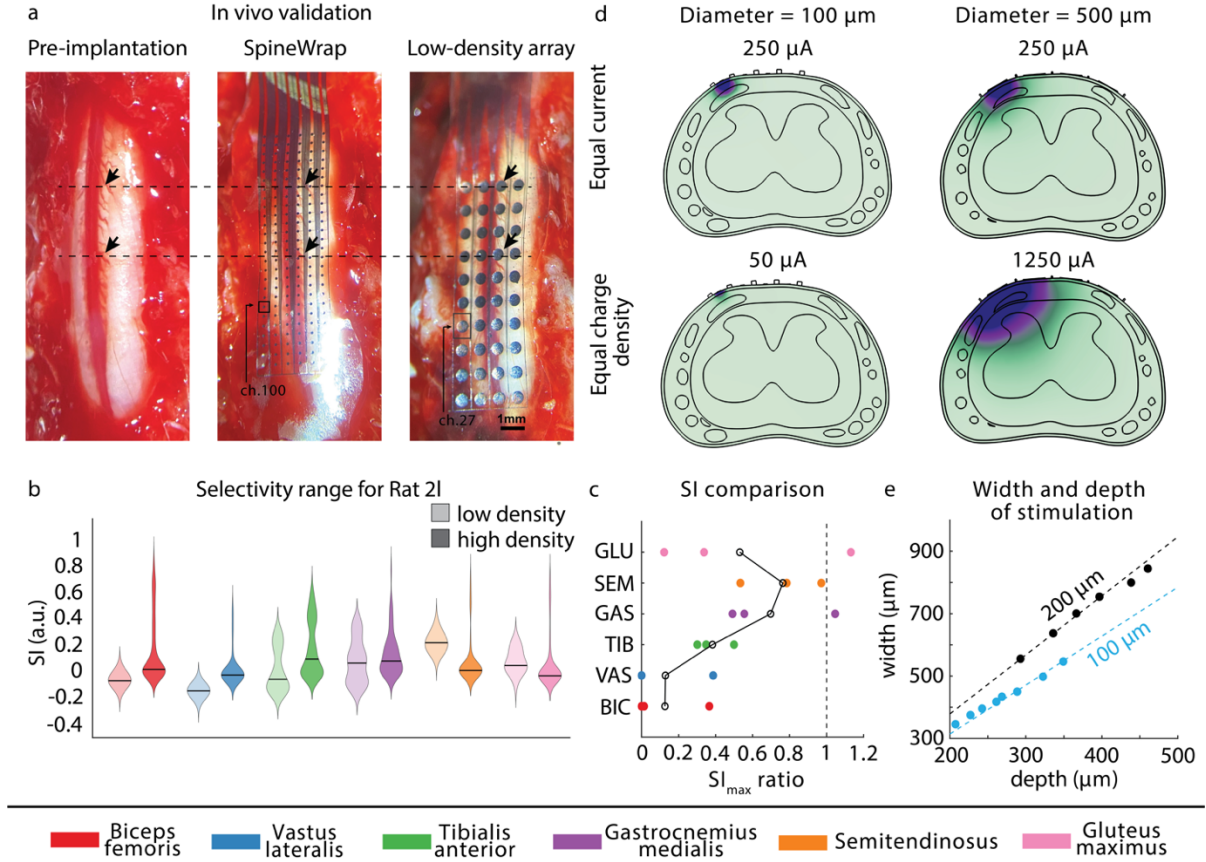


Figure 2.5 *In vivo* validation of high-channel count effect on muscle recruitment selectivity. (A) Placement of SpineWrap and the low-channel count array for Rat 2. Arrays were aligned in post-processing based on vasculature on the spinal cord surface highlighted with black arrows. (B) SI range for both arrays for Rat 2r. (C) Comparison of SI_{max} ratio between SpineWrap and the low-channel count array. Low-channel count array SI values are divided by SpineWrap SI values. The open black circles are the mean SI_{max} ratio for each muscle, and the black line connects the mean values from the different muscles. (D) Computational modeling of equal current and equal charge density delivered from 100 μm and 500 μm diameter bipolar contact pairs. (E) Stimulation depth versus stimulation width for 100 μm and 200 μm diameter bipolar contact pairs.

Discussion

SpineWrap is, to the best of our knowledge, the highest channel-count and highest density spinal cord microelectrode array for EES to date. SpineWrap was able to deliver focal stimulation to selectively activate muscles due to its unique design features, including its thin substrate, conformability, channel count, on-device ground, and the material properties of its PtNR contacts. We quantified the focality of the delivered stimulation through computational modeling. *In vivo*

recordings in rat spinal cord stimulations further supported these results. We showed that SpineWrap selectively recruited all six lower limb muscles recorded in this study, with median SIs ≥ 0.5 . We showed that SpineWrap can elucidate hotspots for optimal stimulation at submillimeter resolution, on the scale of individual 100 μm contacts. Finally, we performed post hoc analysis and additional *in vivo* studies to determine that high-channel count improves selectivity.

In our subsampling analysis, the minimum inter-cathode pitch before significant loss in selectivity ($\text{SI} \leq 90\% \text{SI}_{\text{max}}$) was observed was 570 μm . Given that the L3-L5 DREZ lengths range from 3.28 ± 0.67 mm for L3 to 2.40 ± 0.40 mm for L5, this indicates that at minimum 5-7 contacts must cover each DREZ longitudinally to achieve the same SI results. One limitation in this analysis is that the inter-cathode pitch did not reflect the cathode-anode pitch as these were determined physically by the electrode design. As a result, the delivered stimulation was more focal than would be the case with a lower channel count array and larger cathode-anode pitch than the subsampling analysis predicted. We accounted for this limitation in the *in vivo* validation studies. We confirmed selectivity is reduced in lower channel count arrays with *in vivo* studies, where we compared SpineWrap stimulation capabilities to a lower density array with only ~ 3 contacts in the longitudinal direction per DREZ. We showed that the high-density SpineWrap indeed outperformed the low-density array in stimulation focality and selective muscle recruitment.

The results we report here on contact density and selectivity are proof-of-concept and should not be generalized. In particular, the low-density array we used to compare to the high-density SpineWrap was thin film, thus it likely delivered stimulation in a different manner than thicker paddle arrays. Further studies investigating the impact of contact density on selectivity will be performed to fully explore this relationship. Additionally, since the low- and high-density arrays could not be placed simultaneously on the spinal cord, the stimulation delivered from the low-

density and high-density arrays could not be randomized. Therefore, there could be unaccounted for effects of time on the results we report here. However, across studies, we did not observe an effect of time on EMG amplitude, therefore we expect this effect to be minimal. Additionally, for the width versus depth analysis, we used a threshold of 28 mV. The threshold for activation is dependent on the spatial distribution of the potential field, therefore this value is not the de facto threshold and is used only to standardize the potential gradient analysis.

One limitation in the development of high-channel count electrode arrays for SCS is the lack of implantable pulse generators (IPGs) that can control a large number of channels and the resulting increase in energy consumption. In our study, we used the benchtop RHS Stim/Controller, which can stimulate up to 128 channels. Since our electrode contained more than 128 channels, we only stimulated one half of the array at a time. Commercially available implantable IPGs can only stimulate up to 16 channels. Therefore, further development of more advanced IPGs will be necessary to fully leverage the benefits of SpineWrap's high-channel count in a clinical setting.

This was a mechanistic study of stimulation focality and contact density effects on muscle recruitment selectivity, and thus, the experiments were performed in anesthetized animals. Future work will use SpineWrap in awake rats with SCI. Literature has shown that the co-activation of muscles can differ between anesthetized and awake animals and this co-activation can also be affected by SCI(18, 49). Thus, some results in our study, such as the specific SI values, may not directly translate to awake animals and human subjects with SCI. Although in this study we did not validate the functionality of SpineWrap chronically, previous studies have successfully deployed thin film parylene-C electrode arrays in the spinal cord with minimal spinal compression and foreign body response, and the arrays remained functional after many weeks (33, 34).

Therefore, we believe SpineWrap will be stable long-term and lead to minimal damage to the spinal cord.

Another future direction is to tailor SpineWrap's form factor to larger animal models and humans. Modifications to shape and contact arrangement will be necessary to ensure enough coverage while maintaining high conformality and muscle selectivity. However, PtNR arrays with hundreds and thousands of contacts have been previously used for brain and spinal cord recordings (42, 43). Therefore, creating a larger coverage array with similar density for human use should be possible. The implications of high-channel count microelectrodes for EES in humans need to be additionally explored in future studies since the contact density in humans can be even greater than in rats given that the human spinal cord is much larger in cross-sectional area and the DREZs are larger as well (50). The axon diameters of human afferents are also larger (51), and the human dura is thicker (52, 53), which may affect the minimum contact pitch required to maximize muscle recruitment selectivity in humans.

In this study, we did not investigate the effect of stimulating multiple channels in spatial or temporal configurations. Prior work has shown that spatial and temporal summation of neural recruitment due to stimulation is nonlinear (54–58), thus there could be beneficial, compounding effects of highly focal EES. Spatiotemporal stimulation with paddle arrays mimicking natural spinal cord activation patterns during locomotion has resulted in functional restoration of movement in animals and patients with SCI (2, 17, 59). The use of a high-channel count microelectrode array such as SpineWrap could provide a more sophisticated recording of intact spatiotemporal spinal cord activity. Higher density grids could also reproduce these sophisticated patterns with fine-tuned stimulation to produce more refined movements. In this study, the time to perform stimulation and data analysis was limited due to the acute nature of the experimental setup.

This prevented the exploration of multichannel stimulation effects. Additionally, the benefits of spatiotemporal stimulation paradigms are most pronounced in awake subjects who can volitionally produce movements (18, 60), therefore its benefits would not have been clear in our studies in anesthetized rats. Future studies will investigate the potential compounding effects of multichannel, spatiotemporal stimulation in awake rats with SCI. However, even with single channel stimulation, the improvement in stimulation focality and muscle recruitment selectivity with high-channel count and high-density microelectrode arrays was still apparent in our results.

In summary, our findings indicate that SpineWrap greatly improves stimulation focality and muscle recruitment selectivity compared to the conventional clinical paddle arrays currently employed to deliver EES for treatment of SCI. Consequently, the adoption of SpineWrap in EES treatment has the potential to push the boundaries of current EES technology and offer more efficient therapies.

Materials and Methods

Vertebrate animal subjects

Adult (> 5 months old, weight range 500-650g) male Sprague-Dawley rats (Charles River Laboratories) were used as vertebrate animal subjects in this study. All animal experiments were approved by the UC San Diego Institution Animal Care & Use Committee (protocol S16020).

Fabrication of SpineWrap

SpineWraps were fabricated in small batches on a 7" × 7" × 0.06" photomask-grade soda lime glass plate (Nanofilm), which were cleaned via O₂ plasma at 200 W for 5 minutes. The plate was coated with 3.7-μm-thick-parylene C using a parylene deposition system (Specialty Coating Systems 2010 Labcoater). Next, two layers of gold traces (10 nm Cr, 150 nm Au) were deposited onto the plate using standard lithography techniques and AZ5214E-IR photoresist

(MicroChemicals). This double layer of leads was found to improve device yield by reducing chance of disconnects due to particles during the photolithography process.

PtNR contact formation on parylene-C was done using a technique previously developed and described in our previous publications (42, 43). Briefly, the metal traces and PtAg alloyed contacts were encapsulated in a second 3.1 μm parylene layer. After deposition of a Ti hard mask, the PtAg contacts and connector pads were exposed by dry etching the Ti layer in the exposed regions with SF_6/Ar gas, followed by oxygen dry etching of the parylene C. The entirety of the Ti layer etching as well as the sample release from the substrate was achieved with dip in 6:1 buffered oxide etchant (BOE) and the PtAg was dealloyed in 60°C heated nitric acid for 2 min, thereby forming the PtNRs with Pt/Ag composition of approximately 95%/5%.

Evaluation of electrochemical properties of SpineWrap

Impedance magnitude and phase at 1kHz was measured in saline and in tissue before and after stimulation using the RHS Stim/Recording System (Intan Technologies). The EIS spectrum and CIC of the PtNR contacts was measured in 3-point configuration with a Pt counter and Ag/AgCl reference using the Reference 620 potentiostat (Gamry Instruments).

Scanning electron microscopy imaging

The Apreo HiVac SEM was used to image the PtNRs on the electrode surface before and after stimulation in vivo. Samples were sputtered coated with iridium before imaging. Images were taken at x10,000 magnification, a working distance of 10.5 mm, and an accelerating voltage of 3kV.

Spinal cord dissections

Male Sprague-Dawley rat spinal cords ($n = 5$), including the T11-L6 vertebral segments, were extracted after euthanasia and placed in 4% phosphate buffered paraformaldehyde (PFA).

After one week of fixation, spinal cords were dissected to identify spinal segments and DREZ locations. The vertebral bone was removed to reveal the entire dorsal aspect of the spinal cord and access nerve roots at their exit point from the vertebral column. The dura mater was cut along the rostrocaudal axis to access the nerve roots. Nerve roots were identified by their exit point from the vertebral canal and traced rostrally to the entrance into their spinal segment. DREZs and vertebral segments were labeled by color, measured along their rostrocaudal length, and optically imaged.

MRI reconstruction

A male Sprague-Dawley rat weighing 650 grams was perfused transcardially with phosphate buffered saline (PBS) followed by 4% phosphate buffered paraformaldehyde (PFA) to achieve tissue fixation. The spinal cord, from the T12 to S1 vertebral segment, was extracted and placed in 4% PFA for one week and subsequently transferred to PBS. The MRI scan was performed using a Bruker 3 Tesla scanner with a 40mm volume coil and an axial resolution of at least 0.08 x 0.08 x 0.3mm. Nerve roots were identified in 3D Slicer at their exit point from the vertebral canal and traced rostrally to their entry point into the spinal cord. 3D reconstructions of intrathecal nerve root trajectories were built using MRI segmentation tools in 3D Slicer.

Computational modeling

The 3-D reconstruction of the rat lumbar spinal cord was imported into Blender and individual spinal elements were converted into .stl files to be imported into COMSOL Multiphysics. To manage the complexity of simulations, only the T13-L1 section of the spinal cord was simulated. The electrode contact material was assigned to be platinum, and the electrode-tissue interface was not modeled. For consistency, the stimulating electrode injecting the cathodic-first current was always in the same longitudinal and lateral location on the spine. The stimulating contacts and the ground lines were assumed to be in direct contact with the dura mater. Time

domain current analyses were performed using the Electric Currents (ec) physics module. The simulation results were exported into MATLAB for post-processing and visualization.

Determining activation threshold for potential gradient analysis

The width and depth of the stimulation area was calculated by thresholding the resulting potential gradients by 28 mV. Axon activation was estimated using the second spatial difference $\Delta^2 V_e(n)$ approximation at node n from Peterson et al. (48) as follows:

$$\Delta^2 V_e(n) = V_e(n - 1) - 2V_e(n) + V_e(n + 1) \quad \text{Equation 1}$$

Where $V_e(n - 1)$, $V_e(n)$, and $V_e(n + 1)$ are the extracellular potentials at three neighboring nodes $n-1$, n , and $n+1$.

An exponential decay curve was fitted to the 10 μm diameter data from Peterson et al. to identify the threshold relationship between the extracellular potential and the second spatial difference (Fig. 2.20 B). Next, the potential gradients from 100 μA unipolar, cathodic stimulation were computed at three node locations: in the x,y plane of the electrode contact (the center node was located below the electrode), and 900 μm in either z -direction, where the closest neighboring nodes were located. The internode distance for a group I sensory rat afferent has previously been estimated to be 900 μm (10). In this analysis, we assumed the center node was directly below the electrode contact, which should have limited effect on the activation threshold since previous analysis showed that significant errors in threshold occurred only at large alignment offsets. The resulting distribution of extracellular potentials and corresponding second spatial differences were plotted and 2nd degree polynomial was fit to the data. The intersection point between the threshold curve and the 2nd degree polynomial was regarded as the threshold potential (Fig. 2.20 F).

Surgical procedures

Rats were sedated with 4% isoflurane and fixed in a stereotaxic frame (Kopf Instruments). Once stable, anesthesia was reduced to 1.5-2.5% during the surgery. A laminectomy was performed on the T12-L2 vertebral segments, exposing the dorsolateral surface of the spinal cord without breaching the dura. The SpineWrap array was implanted over the T13-L1 vertebral segments, corresponding to the L3-L5 spinal segments. Gel foam was used to cover the microelectrode array and the surrounding tissues to secure it in place and maintain moisture on the cord. Bipolar needle EMGs were placed in both left and right muscle groups of the lower limbs.

Once the surgery was completed, rats were transitioned from isoflurane to ketamine/xylazine (90 and 10 mg/kg⁻¹, respectively; MWI) and re-dosed 20-30 minutes for the duration of the experiment. Heart rate, body temperature, and blood oxygenation were continuously monitored throughout the experiment. A heating pad maintained body temperature between 34-36°C throughout the experiment. At the end of the study, animals were deeply euthanized with 120 mg/kg⁻¹ sodium pentobarbital (MWI). Spinal cords were extracted from the T12 through L6 vertebral segments and placed in 4% PFA at 4°C overnight. Spinal cords were then stored in saline at 4°C.

Data collection

Stimulation with the microelectrode array was performed using the RHS Stim/Recording controller (Intan Technologies). EMG recording was performed with the RHD Recording controller (Intan Technologies). For each experiment, stimulation was delivered to pairs of contacts in a semi-random order at a single current level. Each stimulation consisted of five pulses delivered at 0.5Hz. The order of current levels was randomized. Data was captured on the RHS

and RHD controllers at a sampling rate of 30 kHz/s and 20 kHz/s, respectively. During recording, data was bandpass filtered 0.09 Hz to 7.6 kHz.

Data processing

EMG recordings were first notch filtered at 60 Hz, then bandpass filtered 10-1000 Hz using a 2nd order Butterworth filter. After filtering, data was segmented into trials (30 ms window of data) and trial averaged. Peak-to-peak amplitudes were calculated from the maximum and minimum potentials within 1.5-15 ms after stimulus as follows:

$$PTP_{norm}^M(amp) = \frac{PTP(amp)}{PTP_{max}^M(amp \in Amps)} \quad \text{Equation 2}$$

where PTP_{norm} is the normalized peak-to-peak amplitude of the EMG response for a given muscle M , a given electrode contact, and a given current level amp , PTP is the peak-to-peak amplitude of the EMG response for a given muscle M , a given electrode contact, and a given current level amp , and PTP_{max}^M is the maximum peak-to-peak amplitude for muscle M across all stimulation contacts and all current levels $Amps$.

Selectivity index calculation

Selectivity indices were calculated based on previously published literature (9). For a given muscle M and a given set of current amplitudes $amps$ in range $Amps$, the selectivity index for a given electrode contact was calculated as follows:

$$SI_M = \max_{amp \in Amps} \left\{ PTP_{norm}^M(amp) - \frac{1}{N_M - 1} \sum_{M' \neq M} PTP_{norm}^{M'}(amp) \right\} \quad \text{Equation 3}$$

Where SI_M is the selectivity of a given muscle and a given electrode contact, $PTP_{norm}^M(amp)$ is the normalized peak-to-peak amplitude of the EMG response for muscle M and current amplitude amp , and N_M is the number of muscles recorded.

Statistical analysis

MATLAB was used for all statistical analyses. For each p-value, the statistical test used is specified in the text.

Inter-cathode pitch calculation from Poisson Disk sampling

The resultant inter-cathode pitch from each subsample in the Poisson Disk sampling was calculated as follows:

$$pitch = \sqrt{\frac{x \times y}{n}} \quad \text{Equation 4}$$

where x and y are the length and width of the SpineWrap array and n is the number of bipolar contact pairs in the subsample. Note that the pitch between the cathode and anode in each pair remained constant at 360 μm .

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Chapter 2 is currently being prepared for submission for publication of the material. S. M. Russman, R. Montgomery-Walsh, R. Vatsyayan, H. S. U, L. D. Diaz-Aguilar, E. Y. Chang, Q. Tang, K. Lee, T. L. Yaksh, S. Ben-Haim, J. Ciacchi, S. A. Dayeh, The conformal, high-density

SpineWrap microelectrode array delivers focal stimulation for selective muscle recruitment. The dissertation author was the primary investigator and author of this material.

Supplementary Materials

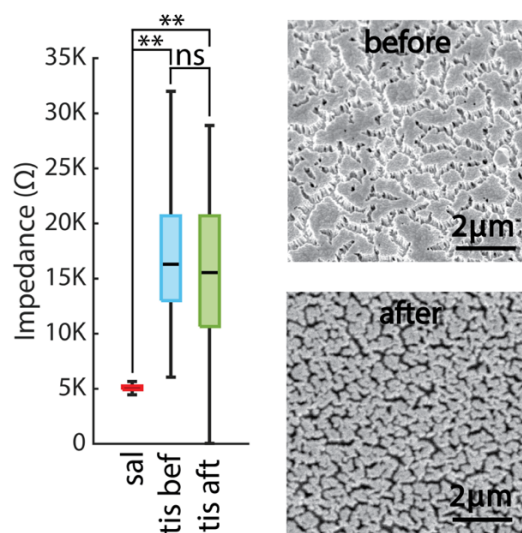


Figure 2.6 PtNRs were stable during the acute stimulation study. Left: SpineWrap average impedances measured at three timepoints during experiment: in saline before implantation, *in vivo* before stimulation, and *in vivo* after experiment completion. Right: SEM images before and after stimulation showed no morphological changes to PtNR structure.

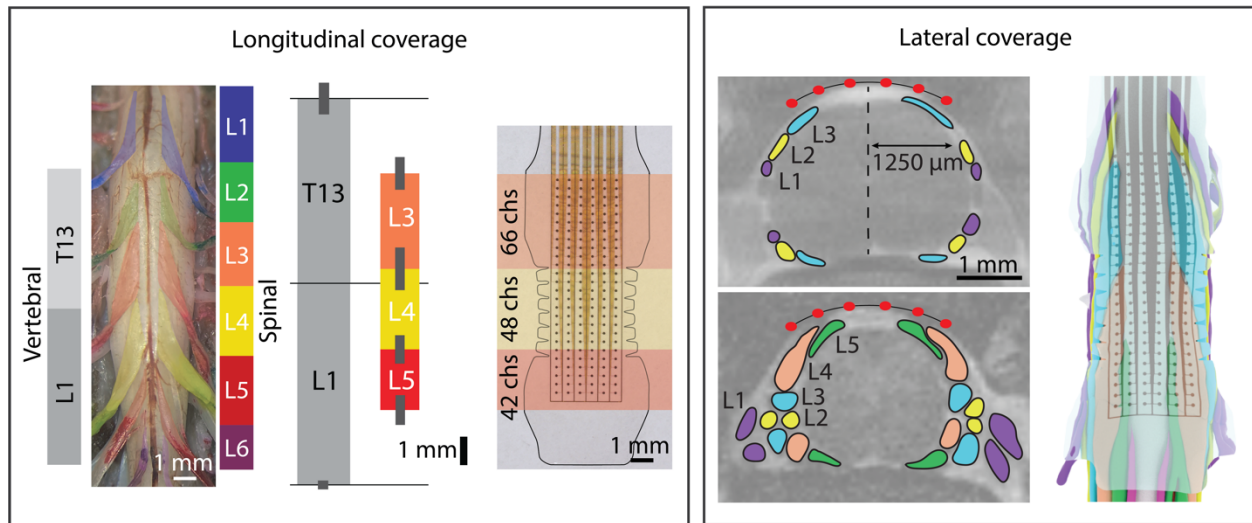


Figure 2.7 SpineWrap dimensions are tailored to the L3-L5 DREZs. Longitudinal coverage: Dissection of L1 through L6 DRs and their DREZs allowed for determination of optimal longitudinal contact pitch and electrode length. The mean T13-L1 vertebral and L3-L5 spinal segment lengths are shown ($n = 5$). Gray vertical bars indicate ± 1 standard deviation. Lateral coverage: Cross-sectional MRI scans identified the lateral spread of the DREZs and allowed for determination of optimal lateral contact pitch and electrode width.

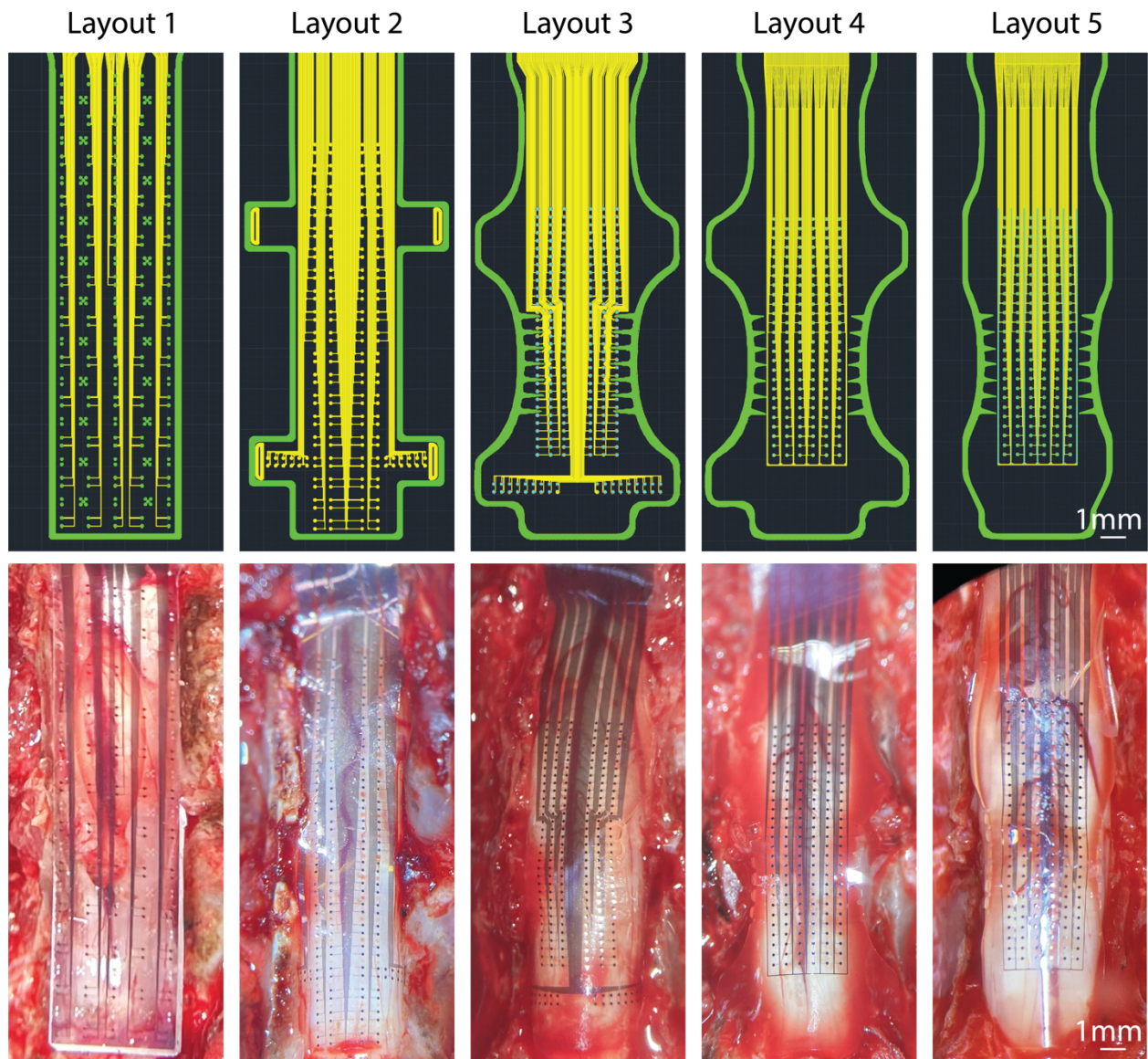


Figure 2.8 Electrode design iterations. Each of the five electrode iterations are shown. Top: Electrode layout in AutoCAD. Metal leads are in yellow and vias for PtNR contacts and for perfusion (only left most) are in green. Bottom: Images of the corresponding electrodes on the spinal cord.

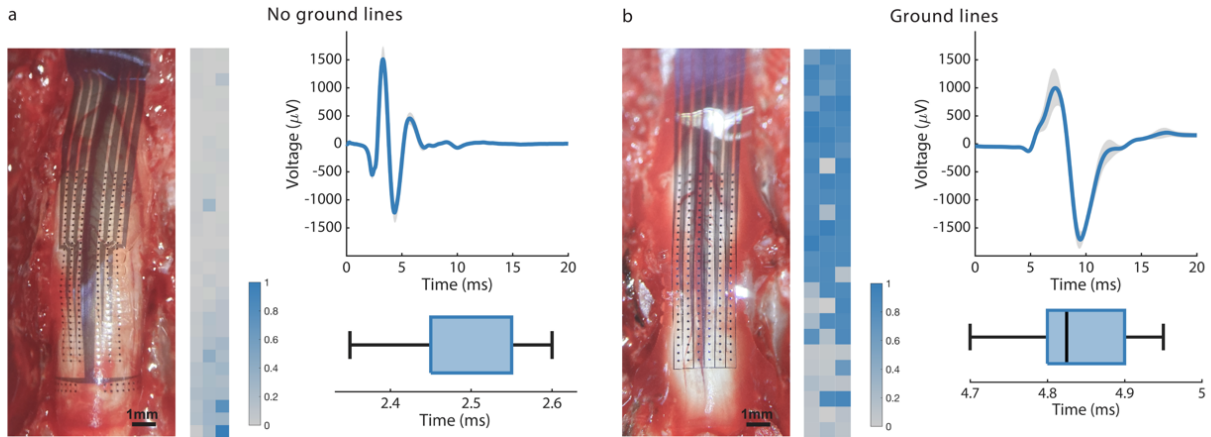


Figure 2.9 On-device ground limits current spread during stimulation. (A) Left: Electrode design without on-device ground and example peak-to-peak amplitude response heatmap for 150 μA stimulation current. Right: An example response and the median response latency. The boxplot shows the distribution of the current threshold for a given dataset and muscle. The horizontal center lines and boxes indicate the median and the 25th/75th percentile and the vertical solid lines represent the full range of the distribution. (B) Left: Electrode design with on-device ground and example peak-to-peak amplitude response heatmap for 150 μA stimulation current. Right: An example response and the median response latency. The boxplot shows the distribution of the current threshold for a given dataset and muscle. The horizontal center lines and boxes indicate the median and the 25th/75th percentile and the vertical solid lines represent the full range of the distribution.

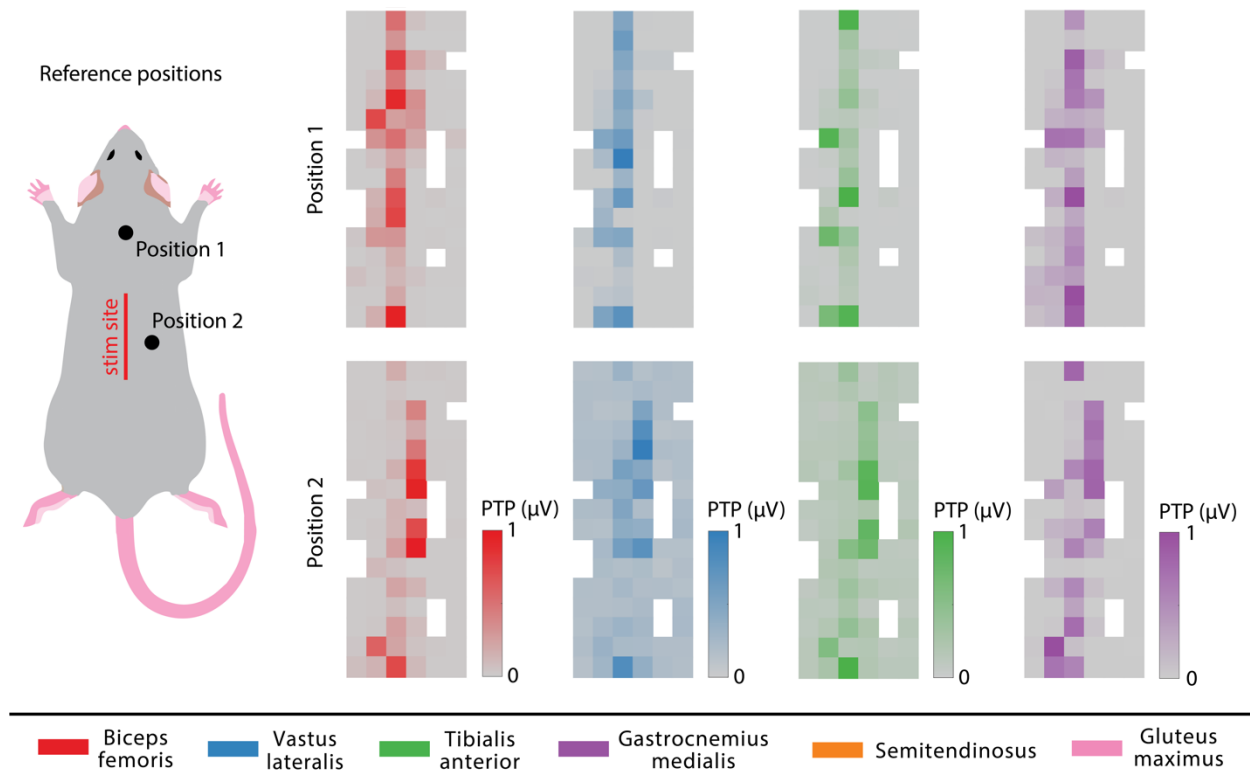


Figure 2.10 Shift in stimulation hotspots with ground placement in electrode without on-device ground in a single electrode placement on one rat's spinal cord. When ground needle was moved from position 1 to position 2, the locations where stimulation resulted in muscle activity shifted towards position 2.

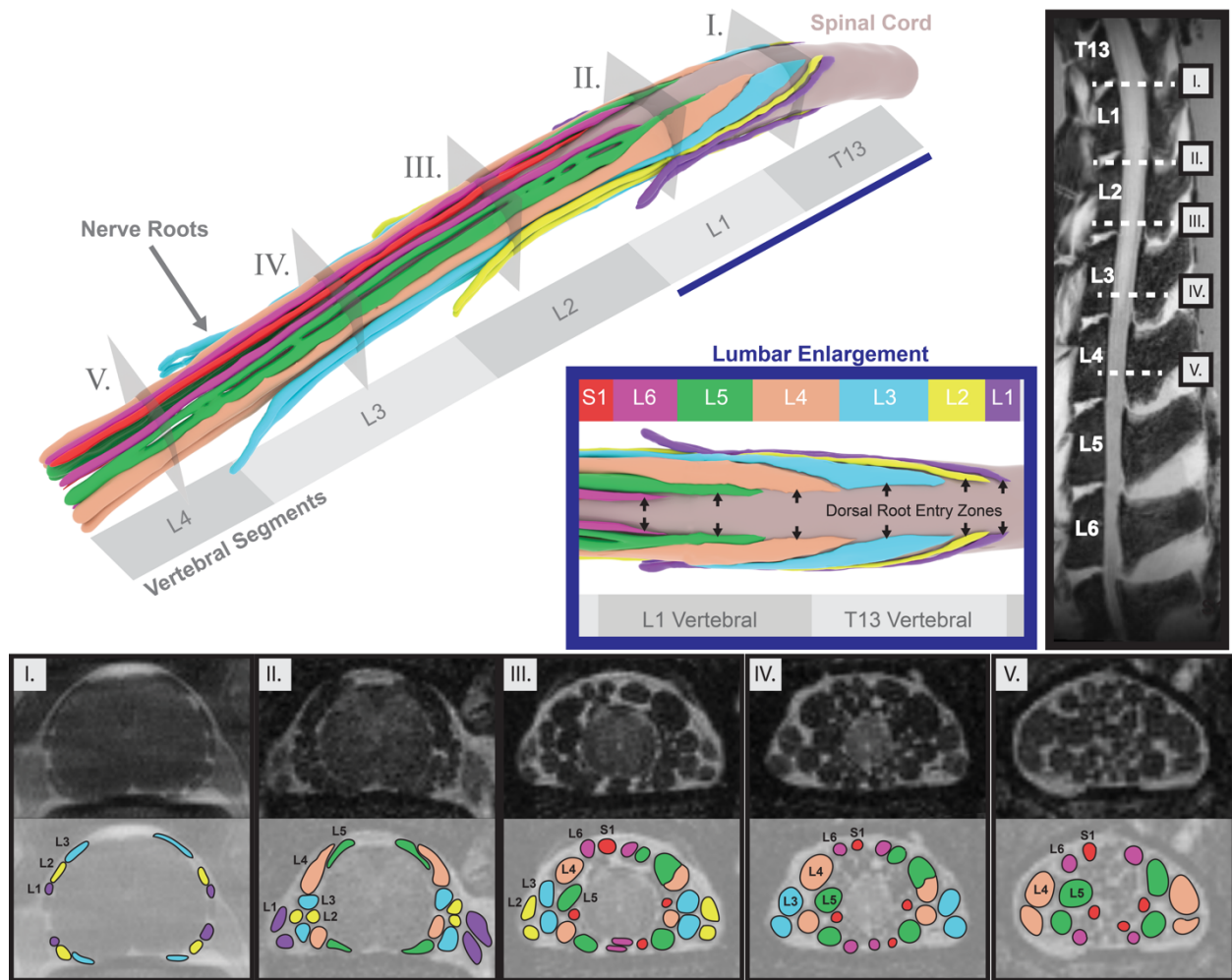


Figure 2.11 3D reconstruction of nerve roots in the lumbosacral rat spinal cord. The spinal cord and nerve roots were reconstructed between the T13 - L4 vertebral segments using coronal views of the lumbar enlargement. The resulting reconstruction was used for electrode layout considerations and computational modeling.

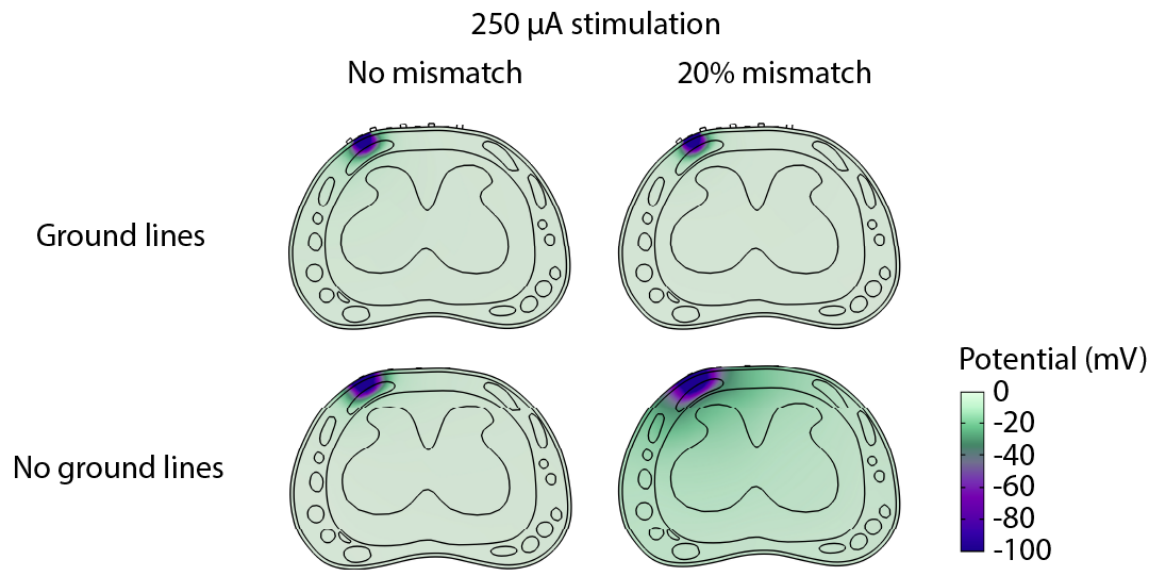


Figure 2.12 Modeling stimulation focality during charge mismatch. The resulting potential gradients during 250 μ A bipolar stimulation were evaluated for electrode arrays with (top) and without (bottom) on-device ground. Potential gradients were computed for a bipolar stimulation that is perfectly matched (left) and with 20% charge mismatch (right).

Rat 2

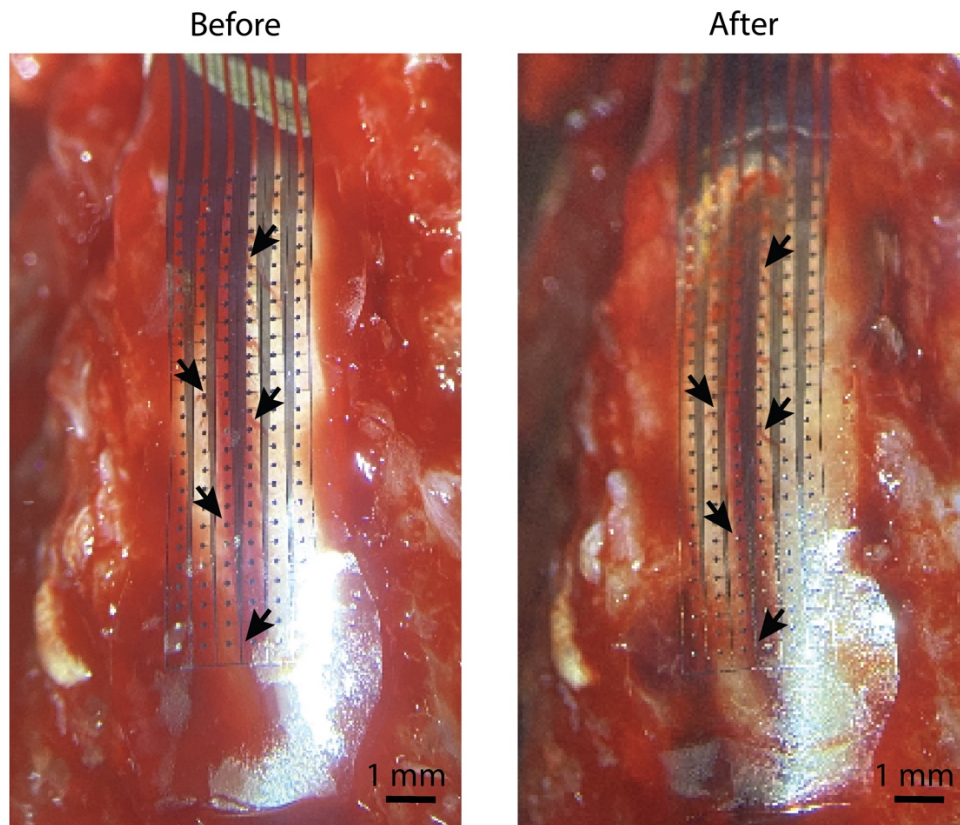


Figure 2.14 Electrode placement remained consistent during stimulation. Black arrows highlight vasculature used to determine relative placement before and after stimulation.

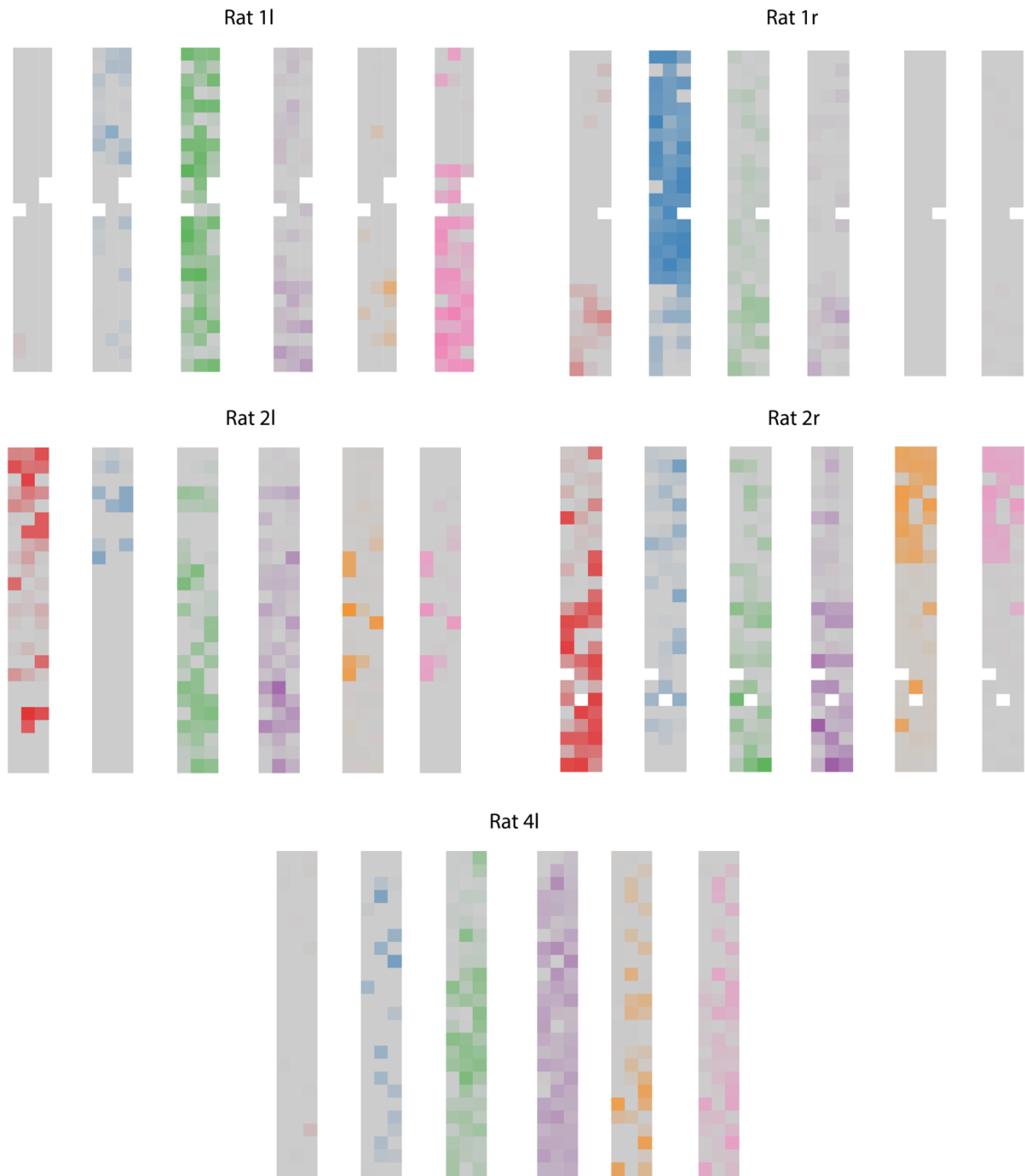


Figure 2.15 Selectivity index distribution across all datasets. The segmental locations of highly selective channels were consistent across animals with a few exceptions, such as the BIC in Rat 2l and the SEM and GLU in rat 2r, which were selective in the upper part of the array.

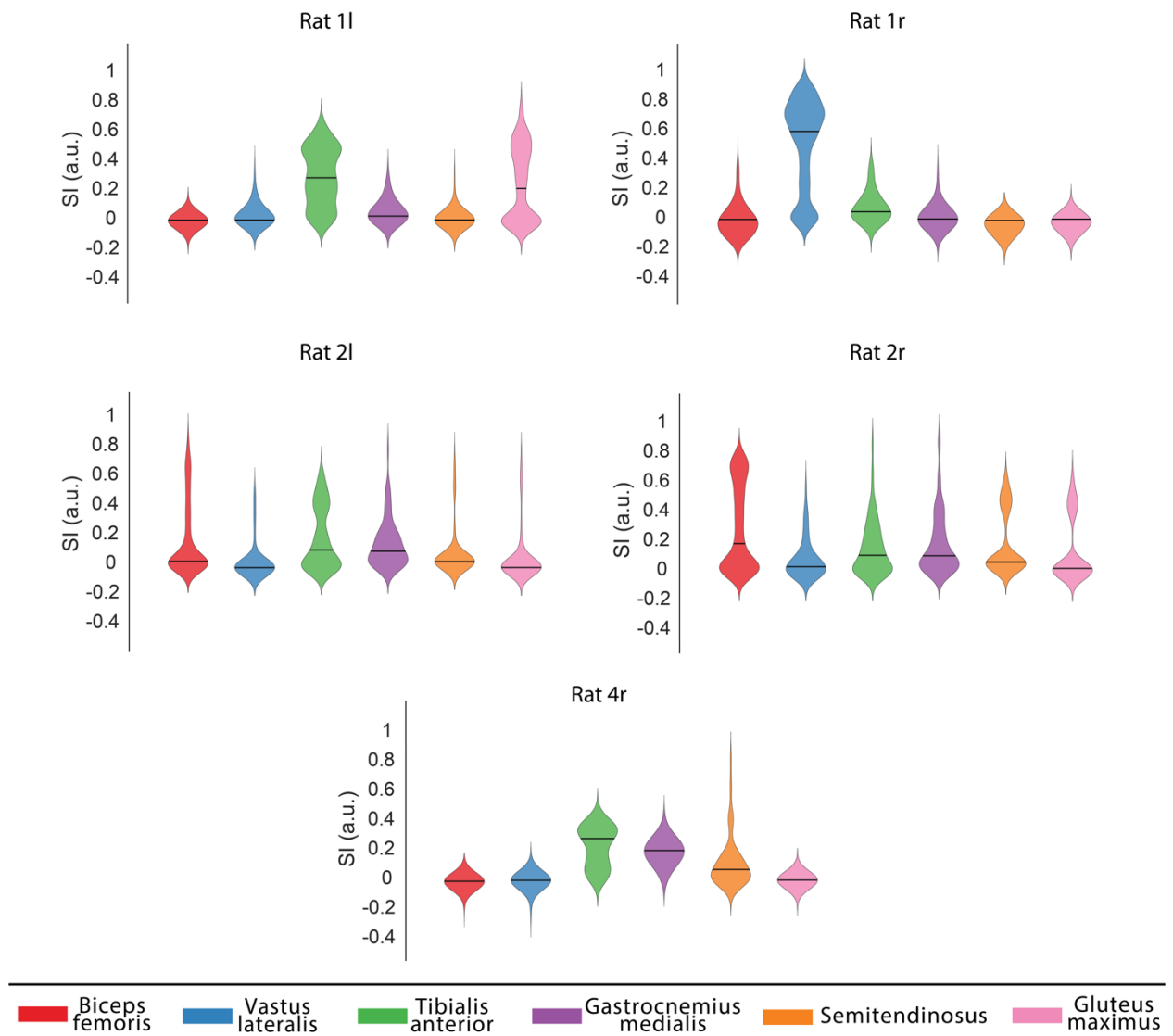


Figure 2.16 Selectivity index distribution per dataset. The SI distribution per muscle skewed towards low SI values.

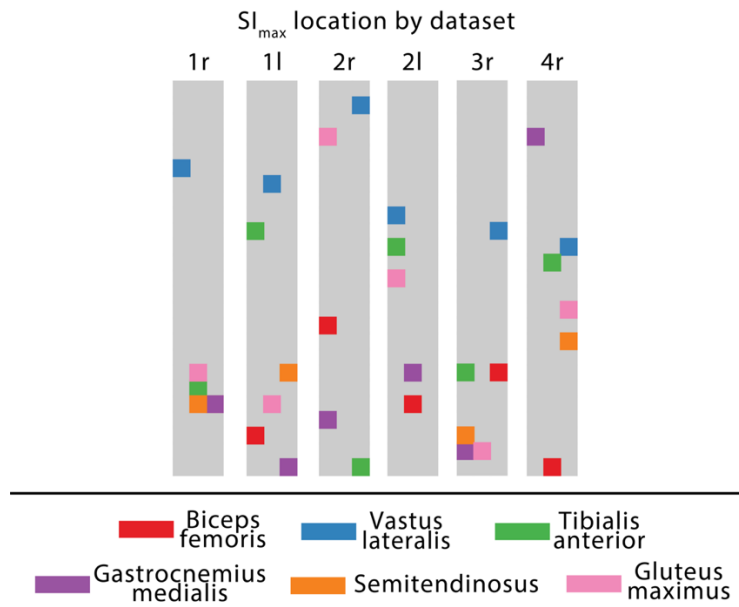


Figure 2.17 Locations of most selective channels by muscle for each dataset.

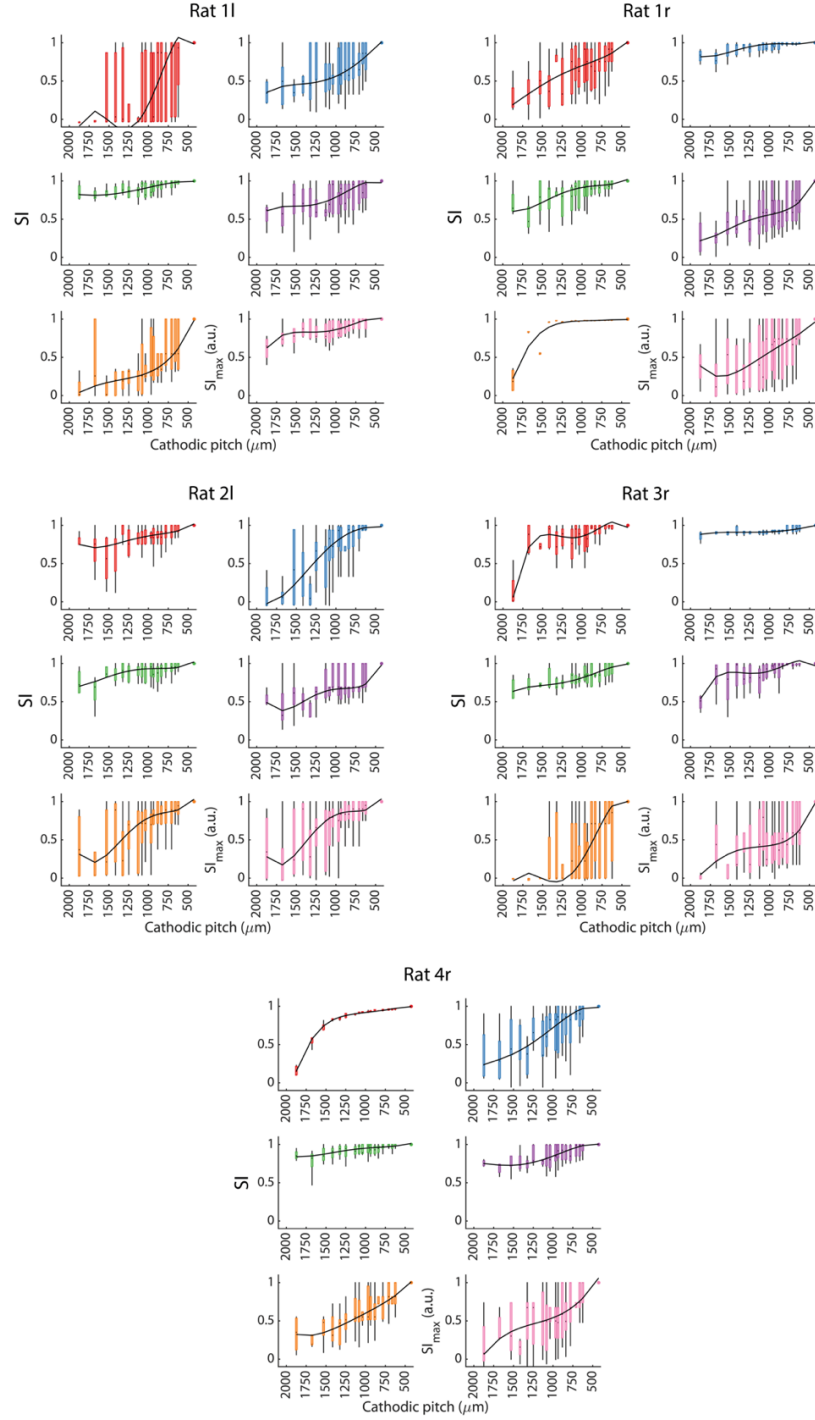


Figure 2.18 Maximum selectivity for subsamples across datasets. The maximum selectivity increases with decreasing pitch (increasing density) of the array. The horizontal center lines and boxes indicate the median and the 25th/75th percentile and the vertical solid lines represent the full range of the distribution. Note that at 100% there is only one datapoint, which is the SI_{max} of the full array.

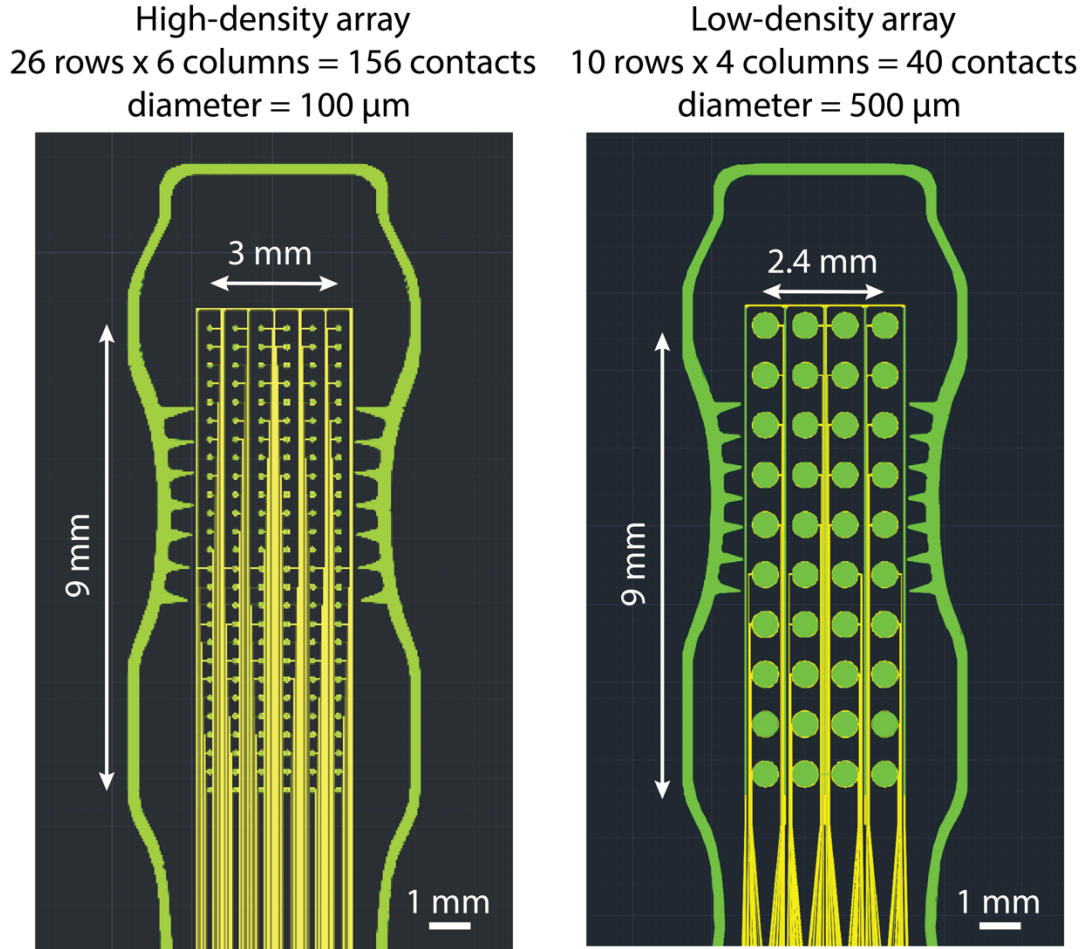


Figure 2.19 Comparison of design of high-density SpineWrap array and the low-density array. The horizontal and vertical pitch of the contacts increased from 500 μm and 360 μm to 1000 μm and 800 μm , respectively. The resulting channel count of the low-density array was reduced from 156 contacts to 40 contacts and the resulting density dropped from 5.6 contacts/ mm^2 to 1.42 contacts/ mm^2 .

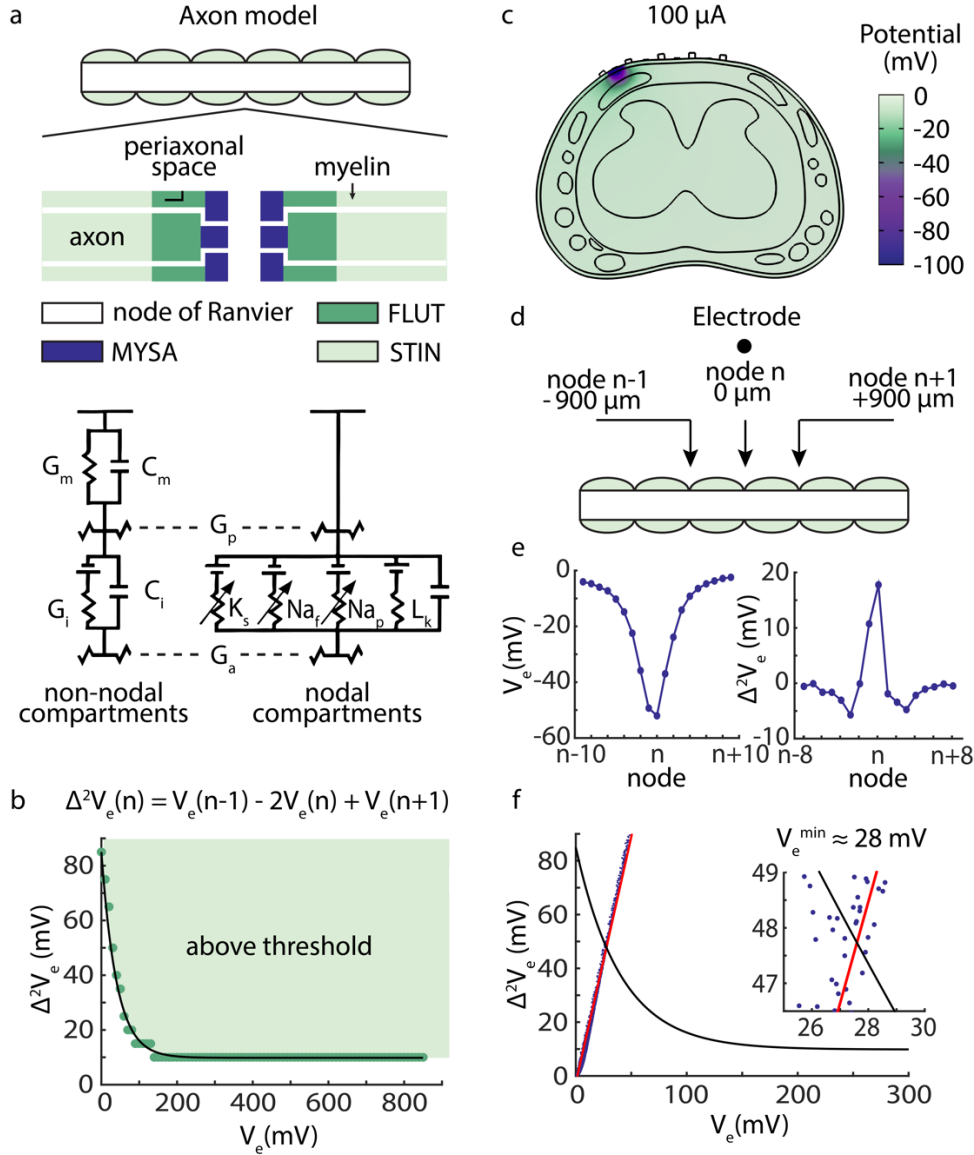


Figure 2.20 Determination of activation threshold. (A) The axon model used by Peterson et al. for evaluating the effect of the extracellular potential field on neural activation. (B) The single node approximation evaluated by Peterson et al. The green points are taken from Petersen et al. and represent the threshold data. The black line is an exponential fit to the data. V_e is the extracellular potential at the node below the electrode and $\Delta^2 V_e$ is the second spatial difference of the extracellular potential. (C) The potential gradient during 100 μ A unipolar, cathodic epidural stimulation with a 100 μ m diameter contact. (D) The central node was assumed to be in the x,y plane of the stimulation electrode. The neighboring nodes were located -900 μ m and +900 μ m from the central node in the z-direction. (E) The extracellular potential and the second spatial difference of the extracellular potential due to the stimulation. Node n is the node below the electrode. (F) Determination of the threshold potential $V_e^{\min}$. The blue points are all the extracellular potentials in the x,y plane and the resulting $\Delta^2 V_e$. The red line is the 2nd degree polynomial fit. The threshold was calculated as the intersection point between the threshold curve and the polynomial fit.

Table 2.1 Vertebral and spinal segment lengths determined through dissection.

	Segment Name	Mean ($\bar{X}$)	Standard Deviation (s)
Vertebral Segment Length	T13	6.60mm	0.57mm
	L1	7.30mm	0.14mm
Spinal Segment Length	L3	3.28mm	0.67mm
	L4	2.73mm	0.44mm
	L5	2.40mm	0.40mm

Table 2.2 Volume conductor model parameters. Conductivity values were taken from Capogrosso et al.

Tissue	Conductivity (S/m)
White matter	0.6 longitudinal, 0.083 axial
Gray matter	0.23
Epidural fat	0.04
CSF	1.7
Saline	2

Table 2.3 Vertebral and spinal segment lengths determined through dissection.

Radius (μm)	Cathode-anode pitch (μm) 10 x Radius	Cathode-ground pitch (μm) 5 x Radius	Current amplitude (μA)
50	500	250	50
50	500	250	60
50	500	250	70
50	500	250	80
50	500	250	90
50	500	250	100
50	500	250	125
50	500	250	150
100	1000	500	30
100	1000	500	50
100	1000	500	60
100	1000	500	70
100	1000	500	80
100	1000	500	90
100	1000	500	100

References

1. N. D. James, S. B. McMahon, C. Field-Fote, E. J. Bradbury, “Review Neuromodulation in the restoration of function after spinal cord injury” (2018); www.thelancet.com/neurology.
2. N. Wenger, E. M. Moraud, J. Gandar, P. Musienko, M. Capogrosso, L. Baud, C. G. Le Goff, Q. Barraud, N. Pavlova, N. Dominici, I. R. Minev, L. Asboth, A. Hirsch, S. Duis, J. Kreider, A. Mortera, O. Haverbeck, S. Kraus, F. Schmitz, J. DiGiovanna, R. Van Den Brand, J. Bloch, P. Detemple, S. P. Lacour, E. Bézard, S. Micera, G. Courtine, Spatiotemporal neuromodulation therapies engaging muscle synergies improve motor control after spinal cord injury. *Nat Med* **22**, 138–145 (2016).
3. F. B. Wagner, J. B. Mignardot, C. G. Le Goff-Mignardot, R. Demesmaeker, S. Komi, M. Capogrosso, A. Rowald, I. Seáñez, M. Caban, E. Pirondini, M. Vat, L. A. McCracken, R. Heimgartner, I. Fodor, A. Watrin, P. Seguin, E. Paoles, K. Van Den Keybus, G. Eberle, B. Schurch, E. Pralong, F. Becce, J. Prior, N. Buse, R. Buschman, E. Neufeld, N. Kuster, S. Carda, J. von Zitzewitz, V. Delattre, T. Denison, H. Lambert, K. Minassian, J. Bloch, G. Courtine, Targeted neurotechnology restores walking in humans with spinal cord injury. *Nature* **563**, 65–93 (2018).
4. M. Capogrosso, T. Milekovic, D. Borton, F. Wagner, E. M. Moraud, J. B. Mignardot, N. Buse, J. Gandar, Q. Barraud, D. Xing, E. Rey, S. Duis, Y. Jianzhong, W. K. D. Ko, Q. Li, P. Detemple, T. Denison, S. Micera, E. Bezard, J. Bloch, G. Courtine, A brain-spine interface alleviating gait deficits after spinal cord injury in primates. *Nature* **539**, 284–288 (2016).
5. S. Harkema, Y. Gerasimenko, J. Hodes, J. Burdick, C. Angeli, Y. Chen, C. Ferreira, A. Willhite, E. Rejc, R. G. Grossman, V. R. Edgerton, Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. *The Lancet* **377**, 1938–1947 (2011).
6. R. M. Ichiyama, Y. P. Gerasimenko, H. Zhong, R. R. Roy, V. R. Edgerton, Hindlimb stepping movements in complete spinal rats induced by epidural spinal cord stimulation. *Neurosci Lett* **383**, 339–344 (2005).
7. M. L. Gill, P. J. Grahn, J. S. Calvert, M. B. Linde, I. A. Lavrov, J. A. Strommen, L. A. Beck, D. G. Sayenko, M. G. Van Straaten, D. I. Drubach, D. D. Veith, A. R. Thoreson, C. Lopez, Y. P. Gerasimenko, V. R. Edgerton, K. H. Lee, K. D. Zhao, Neuromodulation of lumbosacral spinal networks enables independent stepping after complete paraplegia. *Nat Med* **24**, 1677–1682 (2018).
8. C. A. Angeli, M. Boakye, R. A. Morton, J. Vogt, K. Benton, Y. Chen, C. K. Ferreira, S. J. Harkema, Recovery of Over-Ground Walking after Chronic Motor Complete Spinal Cord Injury. *New England Journal of Medicine* **379**, 1244–1250 (2018).
9. N. Greiner, B. Barra, G. Schiavone, N. James, F. Fallegger, S. Borgognon, S. Lacour, J. Bloch, G. Courtine, M. Capogrosso, Recruitment of Upper-Limb Motoneurons with Epidural Electrical Stimulation of the Primate Cervical Spinal Cord. doi: 10.1101/2020.02.17.952796 (2020).
10. M. Capogrosso, N. Wenger, S. Raspopovic, P. Musienko, J. Beauparlant, L. B. Luciani, G. Courtine, S. Micera, A computational model for epidural electrical stimulation of spinal sensorimotor circuits. *Journal of Neuroscience* **33**, 19326–19340 (2013).

11. M. Murg, H. Binder, M. R. Dimitrijevic, “Epidural electric stimulation of posterior structures of the human lumbar spinal cord: 1. muscle twitches $\pm$ a functional method to define the site of stimulation;” www.nature.com/sc.
12. J. Ladenbauer, K. Minassian, U. S. Hofstoetter, M. R. Dimitrijevic, F. Rattay, Stimulation of the human lumbar spinal cord with implanted and surface electrodes: A computer simulation study. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* **18**, 637–645 (2010).
13. E. M. Moraud, M. Capogrosso, E. Formento, N. Wenger, J. DiGiovanna, G. Courtine, S. Micera, Mechanisms Underlying the Neuromodulation of Spinal Circuits for Correcting Gait and Balance Deficits after Spinal Cord Injury. *Neuron* **89**, 814–828 (2016).
14. B. Barra, S. Conti, M. G. Perich, K. Zhuang, G. Schiavone, F. Fallegger, K. Galan, N. D. James, Q. Barraud, M. Delacombaz, M. Kaeser, E. M. Rouiller, T. Milekovic, S. Lacour, J. Bloch, G. Courtine, M. Capogrosso, Epidural electrical stimulation of the cervical dorsal roots restores voluntary upper limb control in paralyzed monkeys. *Nat Neurosci* **25**, 924–934 (2022).
15. I. A. Rybak, N. A. Shevtsova, M. Lafreniere-Roula, D. A. McCrea, Modelling spinal circuitry involved in locomotor pattern generation: Insights from deletions during fictive locomotion. *Journal of Physiology* **577**, 617–639 (2006).
16. B. Jilge, K. Minassian, F. Rattay, M. M. Pinter, F. Gerstenbrand, H. Binder, M. R. Dimitrijevic, Initiating extension of the lower limbs in subjects with complete spinal cord injury by epidural lumbar cord stimulation. *Exp Brain Res* **154**, 308–326 (2004).
17. E. Formento, K. Minassian, F. Wagner, J. B. Mignardot, C. G. Le Goff-Mignardot, A. Rowald, J. Bloch, S. Micera, M. Capogrosso, G. Courtine, Electrical spinal cord stimulation must preserve proprioception to enable locomotion in humans with spinal cord injury. *Nat Neurosci* **21**, 1728–1741 (2018).
18. V. R. Edgerton, G. Courtine, Y. P. Gerasimenko, I. Lavrov, R. M. Ichiyama, A. J. Fong, L. L. Cai, C. K. Otsu, N. J. K. Tillakaratne, J. W. Burdick, R. R. Roy, Training locomotor networks. [Preprint] (2008). <https://doi.org/10.1016/j.brainresrev.2007.09.002>.
19. C. N. Shealy, J. T. Mortimer, J. B. Reswick, “Electrical Inhibition of Pain by Stimulation of the Dorsal Columns: Preliminary Clinical Report;” <http://journals.lww.com/anesthesia-analgesia>.
20. G. Molnar, G. Barolat, Principles of cord activation during spinal cord stimulation. *Neuromodulation* **17**, 12–21 (2014).
21. A. G. Brown, REVIEW ARTICLE THE DORSAL HORN OF THE SPINAL CORD. *Quarterly Journal of Experimental Physiology* **67**, 193–212 (1982).
22. K. Kumar, D. L. Caraway, S. Rizvi, S. Bishop, Current challenges in spinal cord stimulation. *Neuromodulation* **17**, 22–35 (2014).
23. S. F. Lempka, P. G. Patil, Innovations in spinal cord stimulation for pain. Elsevier B.V. [Preprint] (2018). <https://doi.org/10.1016/j.cobme.2018.10.005>.
24. P. E. K. Donaldson, “The Role of Platinum Metals in Neurological Prostheses” PROGRESS IN BIOMEDICAL APPLICATIONS” (1987).
25. S. B. Brummer, L. S. Robblee, F. T. Hambrecht, CRITERIA FOR SELECTING ELECTRODES FOR ELECTRICAL STIMULATION: THEORETICAL AND PRACTICAL CONSIDERATIONS. *Ann N Y Acad Sci* **405**, 159–171 (1983).

26. A. Petrossians, J. J. Whalen, J. D. Weiland, F. Mansfeld, Electrodeposition and Characterization of Thin-Film Platinum-Iridium Alloys for Biological Interfaces. *J Electrochem Soc* **158**, D269 (2011).
27. D. R. Merrill, M. Bikson, J. G. R. Jefferys, Electrical stimulation of excitable tissue: Design of efficacious and safe protocols. [Preprint] (2005). <https://doi.org/10.1016/j.jneumeth.2004.10.020>.
28. H. Lorach, A. Galvez, V. Spagnolo, F. Martel, S. Karakas, N. Interling, M. Vat, O. Faivre, C. Harte, S. Komi, J. Ravier, T. Collin, L. Coquoz, I. Sakr, E. Baaklini, S. D. Hernandez-Charpak, G. Dumont, R. Buschman, N. Buse, T. Denison, I. van Nes, L. Asboth, A. Watrin, L. Struber, F. Sauter-Starace, L. Langar, V. Auboiroux, S. Carda, S. Chabardes, T. Aksenova, R. Demesmaeker, G. Charvet, J. Bloch, G. Courtine, Walking naturally after spinal cord injury using a brain–spine interface. *Nature* **618**, 126–133 (2023).
29. A. Rowald, S. Komi, R. Demesmaeker, E. Baaklini, S. D. Hernandez-Charpak, E. Paoles, H. Montanaro, A. Cassara, F. Becce, B. Lloyd, T. Newton, J. Ravier, N. Kinany, M. D’Ercole, A. Paley, N. Hankov, C. Varescon, L. McCracken, M. Vat, M. Caban, A. Watrin, C. Jacquet, L. Bole-Feysot, C. Harte, H. Lorach, A. Galvez, M. Tschopp, N. Herrmann, M. Wacker, L. Geernaert, I. Fodor, V. Radevich, K. Van Den Keybus, G. Eberle, E. Pralong, M. Roulet, J. B. Ledoux, E. Fornari, S. Mandija, L. Mattera, R. Martuzzi, B. Nazarian, S. Benkler, S. Callegari, N. Greiner, B. Fuhrer, M. Froeling, N. Buse, T. Denison, R. Buschman, C. Wende, D. Ganty, J. Bakker, V. Delattre, H. Lambert, K. Minassian, C. A. T. van den Berg, A. Kavounoudias, S. Micera, D. Van De Ville, Q. Barraud, E. Kurt, N. Kuster, E. Neufeld, M. Capogrosso, L. Asboth, F. B. Wagner, J. Bloch, G. Courtine, Activity-dependent spinal cord neuromodulation rapidly restores trunk and leg motor functions after complete paralysis. *Nat Med* **28**, 260–271 (2022).
30. D. Bendersky, C. Yampolsky, Is spinal cord stimulation safe? A review of its complications. Elsevier Inc. [Preprint] (2014). <https://doi.org/10.1016/j.wneu.2013.06.012>.
31. G. Taccola, S. Barber, P. J. Horner, H. A. C. Bazo, D. Sayenko, Complications of epidural spinal stimulation: lessons from the past and alternatives for the future. Springer Nature [Preprint] (2020). <https://doi.org/10.1038/s41393-020-0505-8>.
32. B. Harland, Z. Aqrave, M. Vomero, C. Boehler, E. Cheah, B. Raos, M. Asplund, S. J. O’Carroll, D. Svirskis, A Subdural Bioelectronic Implant to Record Electrical Activity from the Spinal Cord in Freely Moving Rats. *Advanced Science* **9** (2022).
33. P. Gad, J. Choe, M. S. Nandra, H. Zhong, R. R. Roy, Y. C. Tai, V. R. Edgerton, Development of a multi-electrode array for spinal cord epidural stimulation to facilitate stepping and standing after a complete spinal cord injury in adult rats. *J Neuroeng Rehabil* **10** (2013).
34. Mandheerej. S. Nandra, I. A. Lavrov, V. R. Edgerton, Y.-C. Tai, “A parylene-based microelectrode array implant for spinal cord stimulation in rats” in *2011 IEEE 24th International Conference on Micro Electro Mechanical Systems* (2011), pp. 1007–1010.
35. Chang CW, Lo YK, Gad P, Edgerton R, Liu W, Design and fabrication of a multi-electrode array for spinal cord epidural stimulation. *36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 6834–6837 (2014).
36. K. W. Meacham, R. J. Giuly, L. Guo, S. Hochman, S. P. DeWeerth, A lithographically-patterned, elastic multi-electrode array for surface stimulation of the spinal cord. *Biomed Microdevices* **10**, 259–269 (2008).

37. V. Giagka, A. Demosthenous, N. Donaldson, Flexible active electrode arrays with ASICs that fit inside the rat's spinal canal. *Biomed Microdevices* **17** (2015).
38. A. Garcia-Sandoval, A. Pal, A. M. Mishra, S. Sherman, A. R. Parikh, A. Joshi-Imre, D. Arreaga-Salas, G. Gutierrez-Heredia, A. C. Duran-Martinez, J. Nathan, S. M. Hosseini, J. B. Carmel, W. Voit, Chronic softening spinal cord stimulation arrays. *J Neural Eng* **15** (2018).
39. I. R. Mineev, P. Musienko, A. Hirsch, Q. Barraud, N. Wenger, E. M. Moraud, J. Gandar, M. Capogrosso, T. Milekovic, L. Asboth, R. F. Torres, N. Vachicouras, Q. Liu, N. Pavlova, S. Duis, A. Larmagnac, J. Vörös, S. Micera, Z. Suo, G. Courtine, S. P. Lacour, Electronic dura mater for long-term multimodal neural interfaces. *Science (1979)* **347**, 159–163 (2015).
40. M. Ganji, A. C. Paulk, J. C. Yang, N. W. Vahidi, S. H. Lee, R. Liu, L. Hossain, E. M. Arneodo, M. Thunemann, M. Shigyo, A. Tanaka, S. B. Ryu, S. W. Lee, Y. Tchoe, M. Marsala, A. Devor, D. R. Cleary, J. R. Martin, H. Oh, V. Gilja, T. Q. Gentner, S. I. Fried, E. Halgren, S. S. Cash, S. A. Dayeh, Selective Formation of Porous Pt Nanorods for Highly Electrochemically Efficient Neural Electrode Interfaces. *Nano Lett* **19**, 6244–6254 (2019).
41. R. Vatsyayan, D. Cleary, J. R. Martin, E. Halgren, S. A. Dayeh, Electrochemical safety limits for clinical stimulation investigated using depth and strip electrodes in the pig brain. *J Neural Eng* **18** (2021).
42. Y. Tchoe, A. M. Bourhis, D. R. Cleary, B. Stedelin, J. Lee, K. J. Tonsfeldt, E. C. Brown, D. Siler, A. C. Paulk, J. C. Yang, H. Oh, Y. Goo Ro, K. Lee, S. Russman, M. Ganji, I. Galton, S. Ben-Haim, A. M. Raslan, S. A. Dayeh, Human brain mapping with multithousand-channel PtNRGrids resolves spatiotemporal dynamics. *Sci Transl Med*, 1441 (2022).
43. S. M. Russman, D. R. Cleary, Y. Tchoe, A. M. Bourhis, B. Stedelin, J. Martin, E. C. Brown, X. Zhang, A. Kawamoto, H. A. Ryu, A. M. Raslan, J. D. Ciacchi, S. A. Dayeh, Constructing 2D maps of human spinal cord activity and isolating the functional midline with high-density microelectrode arrays. *Sci. Transl. Med* **14**, 4744 (2022).
44. K. Lee, A. C. Paulk, Y. G. Ro, D. R. Cleary, K. J. Tonsfeldt, Y. Kfir, J. S. Pezaris, Y. Tchoe, J. Lee, A. M. Bourhis, R. Vatsyayan, J. R. Martin, S. M. Russman, J. C. Yang, A. Baohan, R. M. Richardson, Z. M. Williams, S. I. Fried, U. Hoi Sang, A. M. Raslan, S. Ben-Haim, E. Halgren, S. S. Cash, S. A. Dayeh, Flexible, scalable, high channel count stereo-electrode for recording in the human brain. *Nat Commun* **15** (2024).
45. J. Holsheimer, W. A. Wesselink, "Optimum electrode geometry for spinal cord stimulation: the narrow bipole and tripole" (1997).
46. C. C. McIntyre, W. M. Grill, Extracellular Stimulation of Central Neurons: Influence of Stimulus Waveform and Frequency on Neuronal Output. doi: 10.1152/jn.00147.2002 (2002).
47. I. Uguz, K. L. Shepard, "Spatially controlled, bipolar, cortical stimulation with high-capacitance, mechanically flexible subdural surface microelectrode arrays" (2022); <https://www.science.org>.
48. E. J. Peterson, O. Izad, D. J. Tyler, "Predicting myelinated axon activation using spatial characteristics of the extracellular field" in *Journal of Neural Engineering* (2011)vol. 8.
49. Y. P. Gerasimenko, I. A. Lavrov, G. Courtine, R. M. Ichiyama, C. J. Dy, H. Zhong, R. R. Roy, V. R. Edgerton, Spinal cord reflexes induced by epidural spinal cord stimulation in normal awake rats. *J Neurosci Methods* **157**, 253–263 (2006).

50. A. Toossi, B. Bergin, M. Marefatallah, B. Parhizi, N. Tyreman, D. G. Everaert, S. Rezaei, P. Seres, J. C. Gatenby, S. I. Perlmutter, V. K. Mushahwar, Comparative neuroanatomy of the lumbosacral spinal cord of the rat, cat, pig, monkey, and human. *Sci Rep* **11** (2021).
51. R. V. Haberberger, C. Barry, N. Dominguez, D. Matusica, Human dorsal root ganglia. Frontiers Media S.A. [Preprint] (2019). <https://doi.org/10.3389/fncel.2019.00271>.
52. J.-Y. Hong, S.-W. Suh, S.-Y. Park, H. N. Modi, I. J. Rhyu, S. Kwon, H. Yu, J. Byun, Analysis of dural sac thickness in human spine—cadaver study with confocal infrared laser microscope. *The Spine Journal* **11**, 1121–1127 (2011).
53. J. T. Maikos, Z. Qian, D. Metaxas, D. I. Shreiber, Finite Element Analysis of Spinal Cord Injury in the Rat. *J Neurotrauma* **25**, 795–816 (2008).
54. J. A. Hokanson, R. A. Gaunt, D. J. Weber, Effects of Synchronous Electrode Pulses on Neural Recruitment During Multichannel Microstimulation. *Sci Rep* **8** (2018).
55. D. G. Sayenko, C. Angeli, S. J. Harkema, V. Reggie Edgerton, Y. P. Gerasimenko, Neuromodulation of evoked muscle potentials induced by epidural spinal-cordstimulation in paralyzed individuals. *J Neuro-physiol* **111**, 1088–1099 (2014).
56. P. K. Shah, S. Sureddi, M. Alam, H. Zhong, R. R. Roy, V. R. Edgerton, Y. Gerasimenko, Unique spatiotemporal neuromodulation of the lumbosacral circuitry shapes locomotor success after spinal cord injury. *J Neurotrauma* **33**, 1709–1723 (2016).
57. F. Dose, R. Deumens, P. Forget, G. Taccola, Staggered multi-site low-frequency electrostimulation effectively induces locomotor patterns in the isolated rat spinal cord. *Spinal Cord* **54**, 93–101 (2016).
58. D. G. Sayenko, D. A. Atkinson, T. C. Floyd, R. M. Gorodnichev, T. R. Moshonkina, S. J. Harkema, V. R. Edgerton, Y. P. Gerasimenko, Effects of paired transcutaneous electrical stimulation delivered at single and dual sites over lumbosacral spinal cord. *Neurosci Lett* **609**, 229–234 (2015).
59. P. K. Shah, S. Sureddi, M. Alam, H. Zhong, R. R. Roy, V. R. Edgerton, Y. Gerasimenko, Unique spatiotemporal neuromodulation of the lumbosacral circuitry shapes locomotor success after spinal cord injury. *J Neurotrauma* **33**, 1709–1723 (2016).
60. M. P. Côté, M. Murray, M. A. Lemay, Rehabilitation Strategies after Spinal Cord Injury: Inquiry into the Mechanisms of Success and Failure. Mary Ann Liebert Inc. [Preprint] (2017). <https://doi.org/10.1089/neu.2016.4577>.

Chapter 3

Surface-grafted biocompatible polymer conductors for stable and compliant electrodes for brain interfaces

Introduction

Understanding and treating neurological disorders—originating from injury, aging, and genetics—depends on the ability to record neural activity with high resolution and over the long term (1, 2). Among different technologies that either physically penetrate the brain tissue or interrogate brain activity without penetration, electrocorticography (ECoG) is a technique that measures brain activity from the cortical surface or dura mater without penetration and allows both recording and stimulation with high-resolution (3, 4). The basis of this interaction is the detection and manipulation of ionic currents resulting from the neuronal activity at the interface between the electrode and the tissue electrolytes (5). To achieve high-resolution recording of the neural activity there is a need for microscale electrodes. However, electrodes with small areas of contact have decreased charge injection capacity for stimulation, and less efficient charge exchange at the interfacial contact between the tissue and the electrode which results in increased impedance and compromised recordings (6).

Currently, electrodes for neural interfaces are made of inert metals such as gold and platinum, or metal oxides (e.g., iridium oxide). However, films of these inorganic materials are two-dimensional and thus have reduced area for electrochemical interfacing. A common strategy to increase the electrochemical surface area is to coat the metal with a conductive polymer (7). In particular, polymers with mixed ionic and electronic charge transport and a large, three-dimensional electrochemically active volume as opposed to surface area (Fig. 3.1 A). These characteristics lead to significantly reduced electrochemical impedance, and thus a higher signal-

to-noise ratio (SNR) and charge injection capacity (CIC), and reduced irreversible reactions and heating of the metal during its operation (8). The biocompatible and commercially available poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) is a prominent electronic-ionic mixed-conductive material for coating neural interface electrodes (9). PEDOT groups carry a partial positive charge, enabling them to adsorb negatively charged ions. In contrast, the negatively charged PSS can adsorb positive ions, resulting in the formation of an electric double layer (EDL). The EDL and capacitance increase as the coating becomes thicker or rougher. Increased surface area correlates with the increased charge storage capacity (CSC) of the electrode (10) and may facilitate greater CIC than conventional metallic films (11).

In addition to maximizing the capacitance and charge injection, it is also critical to reduce the mechanical mismatch between the polymer and soft biological tissue. This mismatch between conventional metal-based rigid bioelectronic devices and soft brain tissue can reach several orders of magnitude (hundreds of GPa *versus* several kPa, respectively) (12). This asymmetry introduces a mechanical barrier at the interface with the tissue which limits the current injection and may also induce neural damage and glial scar formation (8). PEDOT:PSS coating can narrow this mechanical mismatch, yet, conventional formulations of PEDOT:PSS have moduli of several hundred Mpa to a few Gpa (13–15). Hence, numerous attempts are aiming to further decrease Young's modulus of the PEDOT-based conductive coatings, using polymer blends (16), hydrogels (17), and PSS chain engineering (18).

The durability of conventional formulations of this polymeric coating and strategies to attach these coatings to the metal electrodes (i.e., by van der Waals forces alone) are, however, critical concerns for implants intended for long-term use. Damage caused by electrochemical reactions at the interface may change the composition and the integrity of the coating over time.

In addition, the polymer interface must withstand the corrosive environment of the brain, shear forces with the tissue, and immune responses of the host (19). Moreover, the interactions between the polymer coating and the underlying noble metal electrodes are often weak (20). Therefore, there is a significant incentive to improve the adhesion between the film and the metal, to avoid possible swelling, delamination, and even detachment from the metal surface (21).

Robust interfaces require either strong bonds between the polymer and the metal, high interfacial areas, or both (22). Enhancing adhesion can be achieved by increasing the surface area via etching to create pores (23) or incorporating a nano-structured rod layer (21) to assist in mechanical locking of PEDOT on the electrode surface. Alternatively, an adhesion layer can be spun-coated on a surface that is pre-functionalized with amines (24) or grown electrochemically directly on the metal (25). Another strategy to enhance the thin film stability is cross-linking the PEDOT:PSS with (3-glycidyloxypropyl)trimethoxysilane (GOPS). For example, Dijk et al. demonstrated that PEDOT:PSS cross-linked with GOPS, spin-coated on gold electrodes, remained stable during 4 months of incubation in culture media conditions at 37 °C (26). Despite exhibiting prolonged stability in vitro, the cross-linking of the PEDOT:PSS film with GOPS reduces its electrical conductivity (27). Therefore, additives such as ethylene glycol (EG) and dodecylbenzenesulfonic acid (DBSA) have been used to improve electrical and mechanical properties (28, 29). Other attempts to increase the adhesion include chemical approaches, such as covalent tethering the polymer to the surface. These approaches include either grafting the styrenesulfonate monomers from the surface (30) or growing the PEDOT from the surface in a ladder-like polymer brush (31). These attempts showed a potential for improved stability against sonication and irradiation, respectively. However, the conductivity of the PEDOT-based brushes was inferior to that of the commercially available material, and the mechanical properties were not

evaluated(30, 31). Our laboratory recently demonstrated surface-initiated atom transfer radical polymerization (SI-ATRP) of PSS, emphasizing the importance of grafting density to create film-like polymer brushes. Importantly, the PEDOT:PSS polymer brushes showed enhanced stability in comparison with a spin-coated film (32).

Here, we aimed to address three aspects of polymer brush coatings which may limit their widespread adoption in neural recordings: (1) the molecular structure has been limited to homopolymers such as PSS; (2) as such, mechanical mismatch and conformability at the molecular scale may lead to delamination; and (3) the surface hydrophilicity can be tuned by the choice of the second block copolymer for more efficient ion transport.

Results

Synthesis of conformal PEDOT-based polymer brush on gold surfaces

Current strategies for surface grafting employ non-living free radical polymerization (LRP), which leads to a random assembly of monomers (33). SI-ATRP is a living free radical polymerization (LRP) which allows creating of a well-defined block copolymer brush with high grafting density (Fig. 3.1 B). Thus, we can add functionalities to the polymer using suitable monomers in a bottom-up approach, avoiding blending with additives that leach out over time. Additives leaching might result in toxic molecules released around the tissue and loss of functionality. Moreover, to allow better conformability with the brain (18) we designed and synthesized a second, soft, block copolymer composed of poly(poly(ethylene glycol) methyl ether methacrylate (PPEGMEMA) i.e., PSS-*b*-PPEGMEMA (Fig. 3.1 C). Moreover, to improve the polymer brush stability against hydrolysis, here we created an amide bond between the gold bound thiol SAM and the ATRP initiator.

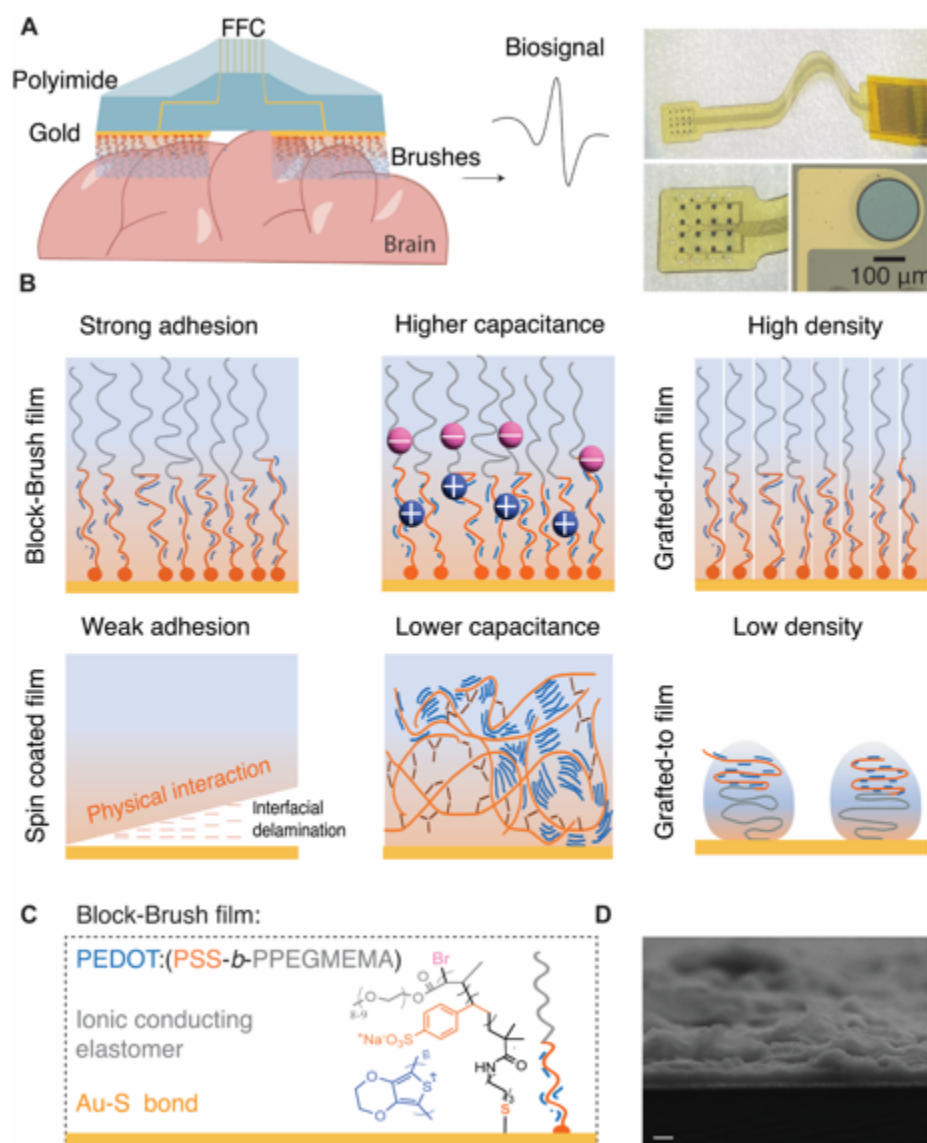


Figure 3.1 PSS-*b*-PPEGMEMA brushes tethered to gold surface as a backbone for PEDOT polymerization enable stable, conductive, conformal, and uniform coverage of the surface. (A) Schematic Illustration showing the ECoG microelectrode placed on the brain tissue. Right: the flexible electrode with zoom-in of the Block-Brush tethered to the gold contact's surface. (B) The desired properties for the interface between the metal electrode and the brain tissue for long-term and efficient charge transport during recording or stimulating brain activity, which is facilitated by strong adhesion, electronic and ionic transport, and high density of the brushes. (C) Schematic representation and the molecular structure of the PEDOT complexed with PSS-*b*-PPEGMEMA brushes, composed of the polyelectrolyte PSS and the ionic conducting elastomer PPEGMEMA. The adhesion to the surface is enabled by the Au-S bond. (D) SEM images of the cross-section of the gold surfaces. The cross-section verifies dense, film-like brushes on the gold surface. The scale bar is 200 nm.

Using SI-ATRP (“grafting-from”), we demonstrate high grafting densities with a mechanically stable film of PEDOT:PSS-*b*-PPEGMEMA. PEDOT was dispersed in this block copolymer brush via oxidative polymerization in an aqueous phase, resulting in an adhesive and conductive PEDOT:(PSS-*b*-PPEGMEMA brushes) polyelectrolyte complex, i.e. Block-Brush film (Fig. 3.6). Moreover, SEM imaging verified that the polymer brushes created a dense coating due to their extended conformation (Fig. 3.1 D). Importantly, the presence of the hydrophilic PPEGMEMA block led to a decrease in the water contact angle from 80° to 63°. (Fig. 3.2 A and Fig. 3.7). Increased hydrophilicity of the conductive polymeric film might increase capillary adhesion to biological tissue and facilitate ion injection into the film (10). To our knowledge, this is the first report of PEDOT:(PSS-*b*-PPEGMEMA) polymer brushes grown from a gold surface of a flexible electrode for long-term ECoG recording of brain activity, with improved charge storage capacity and lower mismatch with the brain (Fig. 3.1).

Electrochemical characterization

To elucidate the role of the brush morphology on the conductivity and charge storage capacity of the film, we evaluated its electronic and electrochemical properties. As a control, we selected a formulation composed of the commercial PEDOT:PSS cross-linked with GOPS and mixed with EG and DBSA additives, which is widely used for neural recording (26), and also contains polyethylene glycol units (though in the control film, they are not bound covalently to the polymer). The brush-based PEDOT films had a very similar electrical conductivity to pristine PEDOT:PSS, but were about three times higher than the spin-coated formulations, spin-coated PEDOT:PSS with 1% GOPS (SpinG), spin-coated PEDOT:PSS with additives (SpinGA, 1% GOPS, 5% EG, 0.002% DBSA) (Fig. 3.2 A and Fig. 3.7). Next, we characterized the impedance and capacitive properties of high- and low-density brushes (Fig. 3.8). These quantities are

important predictors of the quality of electrophysiological recordings achievable (Figure 3.2 B,C). The high-density brushes that were grafted from the surface, resulted in the lowest impedance below 1 kHz (Fig. 3.2 B-D and Fig. 3.8). This might be attributed to a larger interfacial capacitance that is also responsible for a higher CSC (Fig. 3.2 E), and a lower voltage build-up (Fig. 3.9) at the electrode-PBS interface. The capacitance per unit area calculated by the standard equivalent circuit was found to be $2200 \mu\text{F cm}^{-2}$. The thickness of the film directly influences the volumetric capacitance, which is correlated with the EDL, and the amount of charge that can be injected from the electrode to the tissue (5, 10). The Block-Brush film had a thickness of about 180 nm which is much thinner than other reported PEDOT-based films such as 3 – 180 μm (34) or 700 nm (35). The latter had a comparable capacitance for a film that is 3.8 thicker (700 vs. 180 nm). Normalizing the areal capacitance with the thickness resulted in a volumetric capacitance of 122 F cm^{-3} . The volumetric EDLC for the Block-Brush had a similar value of 107 F cm^{-3} which was 6 times higher than that of the control SpinGA formulation (Fig. 3.10) (36). We further evaluated the electrochemical stability of the Block-Brush PEDOT coating during multiple CV cycles and an accelerated aging test. Importantly, potential sweeps can also harm the film electrochemically, by the charge build-up and potential damage/modification to the covalent bonding and electronic structure of PEDOT induced by excessive charging?. The Block-Brush films showed higher electrochemical stability for up to 3500 cycles, compared to the cross-linked film that started to delaminate after 2500 cycles (Fig. 3.11). This is higher than other reported covalently bound dopants with up to 50% loss of the original CSC over 800 CV cycles only (37). The impedance spectra and the CV AUC of the Block-Brush presented negligible changes over the repeating cycles of CV, demonstrating a very high retention of its original CSC (Fig. 3.11).

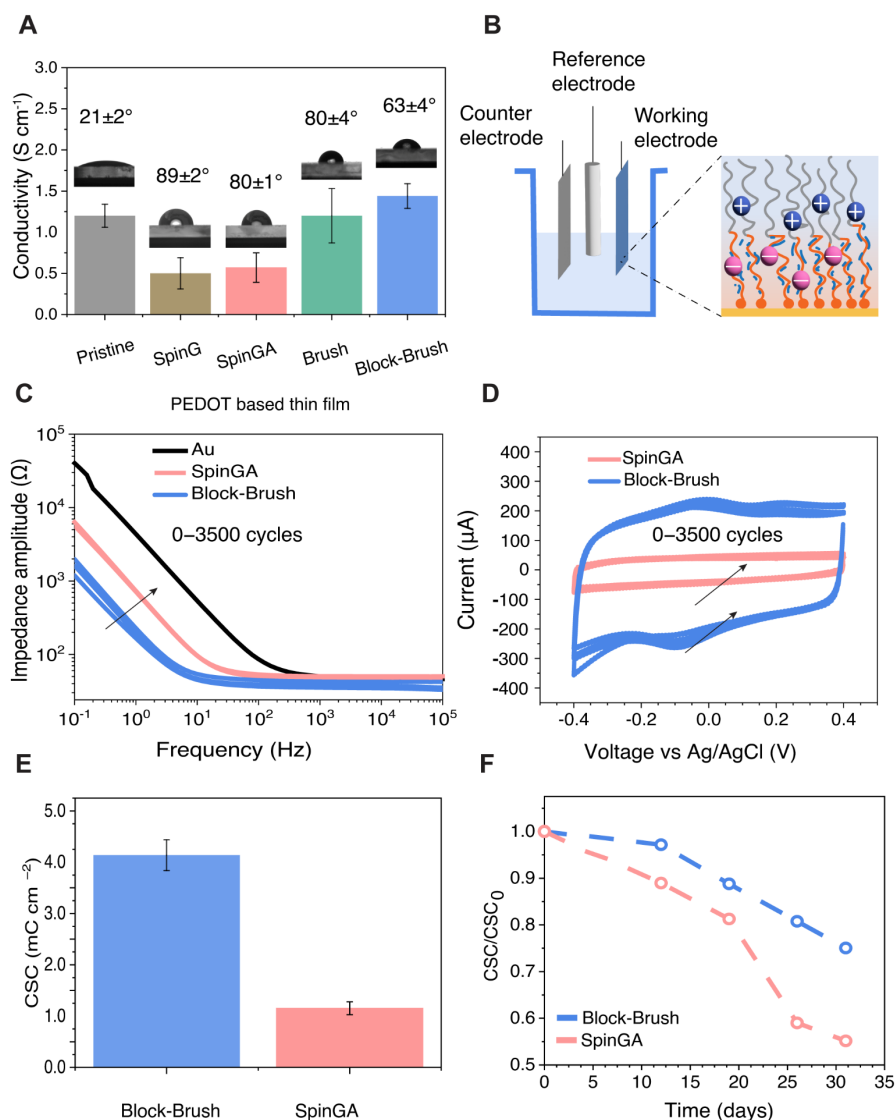


Figure 3.2 Electrical properties and stability tests of the adhesive films. (A) The conductivity of the films including, pristine, SpinG, SpingGA, PEDOT:PSS (Brush), and Block-Brush ($n=3$). The insets indicate the water contact angle measurements of the different PEDOT-based films on gold substrates. The Block-Brush PEDOT demonstrated the lowest water contact angle among the stable films. (B) Schematic illustration of EIS/CV three-electrode setup. The inset shows EDL in the Block-Brush polymer film. EIS (C) and CV (D) curves of bare gold, and PEDOT-based films on gold before and during multiple CV cycling. The Block-Brush films are stable during 3500 cycles of CV stressing (0.4 to -0.4 V) (E) Characterization of the CSC of the Block-Brush film versus the SpinGA film before incubation in PBS ($n=3$, 0.8 to -0.4 V). (F) The Block-Brush films show a slower decrease in CSC over 31 days of incubation in PBS at 50° . The SpinGA films show a faster decrease to 80% of the initial CSC (CSC_0) only 12 days after incubation and to 55% after 31 days of incubation.

The optical microscopy inspection revealed that the Block-Brush films did not show any damage during 3500 CV cycles, nor for the whole incubation time of 31 days at 50 °C (Fig. 3.2 C-F and Fig. 3.12 B,F) while the SpinGA films showed damage starting from day 12 of incubation at 50 °C (Fig. 3.12 C,G). This correlates to 75 days and 35 days for Block-Brush *versus* SpinGA films, respectively at a body temperature of 37 °C. Importantly, the CSC of the brushes was more stable with a slower decrease in comparison with the control SpinGA formulation (Fig. 3.2 E,F and Figs. 3.12, 3.13).

Mechanical stability of the bulk films

To test the adhesion between the gold surface and the brushes, we challenged the films with ultrasonication (100W at 40 kHz) (Fig. 3.3 A,B). The Block-Brush films remained stable against ultrasonication for up to 4 h, with no detectable damage by optical microscopy (Fig. 3.3 B) or Raman spectroscopy (Fig. 3.3 C). The low-density Block-Brush film remained on the gold surface; however, they developed cracks within 30 min of ultrasonication, emphasizing the importance of the high density of grafting for a stable film. The pristine PEDOT:PSS spin-coated film was fully delaminated after only 2 min of ultrasonication, showing the weakest adhesion to the gold surface. To the best of our knowledge, a stability duration of 4 h represents the longest period ever reported for PEDOT film on metal subjected to ultrasonication. It indicates that the covalent bond of the brushes with the gold surface provides strong adhesion and stability. The stronger adhesion of the brush-based versus spin-coated films to the gold surface was also demonstrated by the higher force needed for a 90° peel-off test (Fig. 3.3 D). Furthermore, we postulated that the rougher surface of the brush-based films would translate to a higher adhesion against soft surfaces compared to the spin-coated films. Indeed, the lap-joint shear strength against

soft PDMS (with a ratio of 1:50) (38) for the brush-based films was higher compared to the spin-coated PEDOT films (Fig. 3.3 E).

Nanomechanical characterization

Elevated levels of PEDOT relative to PSS are linked with enhanced conductivity. However, this advantage is counterbalanced by an accompanying increase in modulus, leading to a material with greater stiffness (39). Hence, we investigated the nanomechanical properties of Block-Brush film in liquid, to mimic the wet environment of the brain. Using nanoindentation of the nanometer-thick film, we quantified the mechanical response of the polymer to the force applied by the AFM tip (Fig. 3.3 F, Figure 3.6). The resulting Young's modulus for the soft stretchable Block-Brush film was 4 MPa (Fig. 3.3 G). This Young's modulus is lower than other reported electrodes for neural recording materials or PEDOT formulations (14, 15, 40, 41), approaching that of the brain (dura mater) tissue (42) (Fig. 3.3 H).

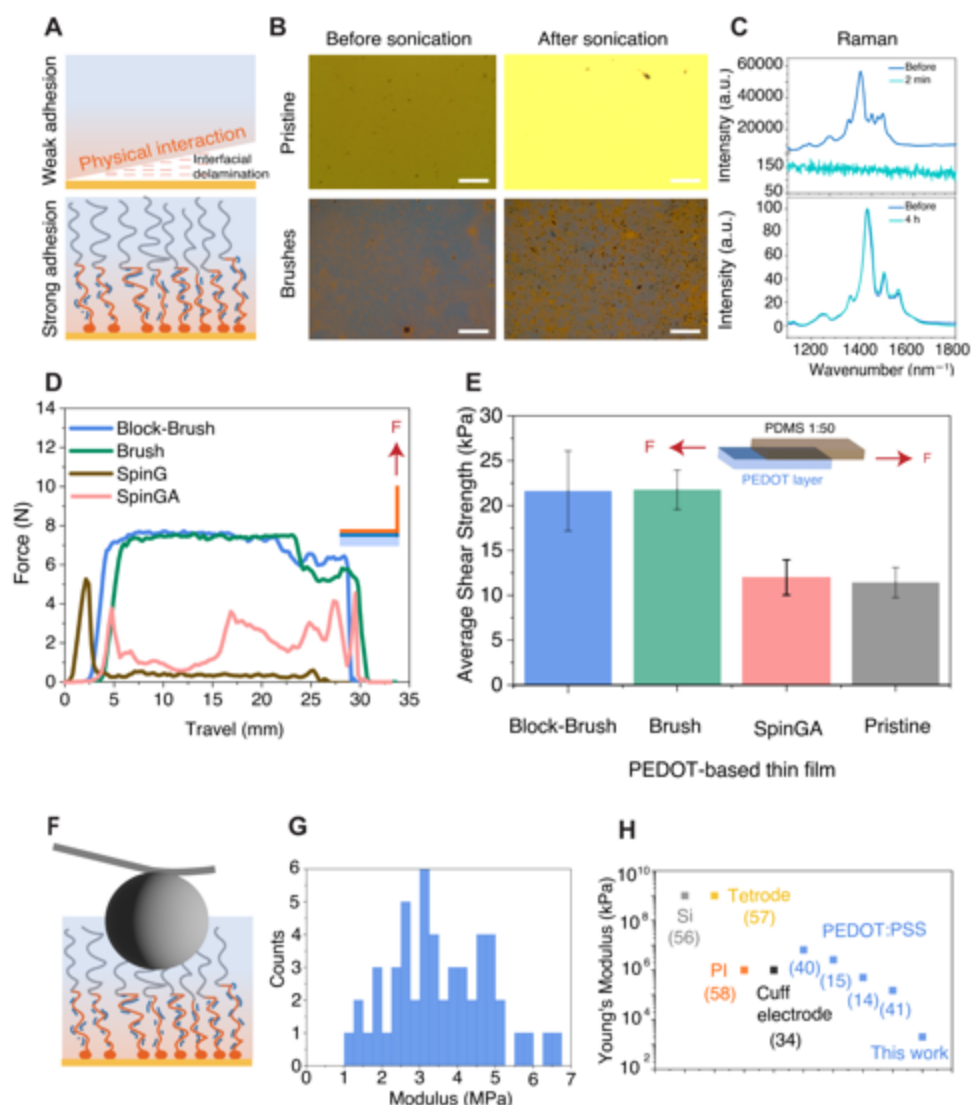


Figure 3.3 Mechanical stability of the bulk films. (A) Schematic illustration of the weak adhesion of spin-coated pristine PEDOT:PSS versus the strong adhesion of Block-Brush PEDOT. (B) Corresponding optical microscope images of the films before (left) and after (right) ultrasonication tests. The scale bars are 100 μm . (C) Corresponding Raman spectra before (blue) and after (cyan) ultrasonication, show complete PEDOT removal for the pristine sample after 2 min ultrasonication, versus the negligible difference in PEDOT spectrum for the brushes after 4h sonication. (D) 90° Peel (glass/Cr/Au/PEDOT-based film/PI tape) test for the PEDOT-based films. (E) Average shear strength between PDMS (1:50) and PEDOT-based films on Au/Cr/glass. Data is shown for pristine (92 ± 3 nm), SpinGA (167 ± 20 nm), Brush (115 ± 7 nm), and Block-Brush (180 ± 46 nm), ($n=3$). (F) Schematic Illustration of the AFM tip indenting Block-Brush film for nanomechanical characterization. (G) The Young's modulus in water was calculated via the Dimitriadis model, using force deformation curves and a deformation map (Fig. S21). (H) Comparison of previously reported conductive materials-based films with our work in terms of Young's modulus. Such conventional implantable electrical probes include silicon electrodes (56), tetrode (57), planar polyimide probes(58) and flexible Au–PET cuff electrodes (34) or PEDOT formulations (14, 15, 40, 41).

PEDOT electrodeposited on PSS-*b*-PPEGMEMA polymer brushes on thin-film microelectrode arrays

To evaluate the Block-Brush film as an efficient and stable interface for high-resolution neural interfaces, we fabricated a polyimide-based thin-film electrode with micro-sized gold contacts of varying diameters from 100 μm to 1 mm (Fig. 3.4 A and Figs. 3.14-3.17). This range spans the contact sizes typically used in microelectrode arrays for neural applications (43). To demonstrate the versatility of our strategy, the PEDOT was electrodeposited on the PSS-*b*-PPEGMEMA polymer brushes. The Block-Brush PEDOT electrodes showed a similar EIS spectrum and water window to the control SpinGA and electrodeposited samples (Fig. 3.4 B and Figs. 3.18-3.20). Importantly, CIC was highest for the brush-based coating across all pulse widths and all diameters (Fig. 4D and Fig. 3.21). This difference in CIC is significant and is higher than previously reported PEDOT-based films (44). Moreover, the CSC was highest for the Block-Brush film in comparison with control formulations (Fig. 3.22), which was consistent with the bulk film results. These results suggested that the brush-based PEDOT:PSS can deliver higher stimulation amplitudes and may be more stable during long-term, repeated current injection. To validate this, we stressed the Block-Brush film and the SpinGA formulation with biphasic current pulsing. We delivered 500 μA amplitude and 100 μs duration cathodic-first biphasic pulses at 50 Hz to a 400 μm contact. These parameters are within the current amplitude and frequency range of typical neural stimulation (45, 46). The contact with the SpinGA control formulation delaminated after 764,000 pulses, while the contact with the Block-Brush delaminated after 1,031,000 pulses, constituting a 34% improvement in stability (Fig. 3.4 E). These results highlight the improved long-term stability during stimulation of the brush-based PEDOT films. Next, we evaluated the long-term stability of the PEDOT-based coatings using CV cycle stressing. We found that the

Block-Brush coating was highly stable against 5,000 voltage sweeps, while the SpinGA formulation showed multiple regions of delamination under optical microscopy (Fig. 3.4 F). Moreover, all materials showed stable impedance spectra even up to 10,000 cycles (Fig. 3.23), which stands in line with former reports of IrOx (47), Au nanorods (21) and GOPS (48) adhesion promoters.

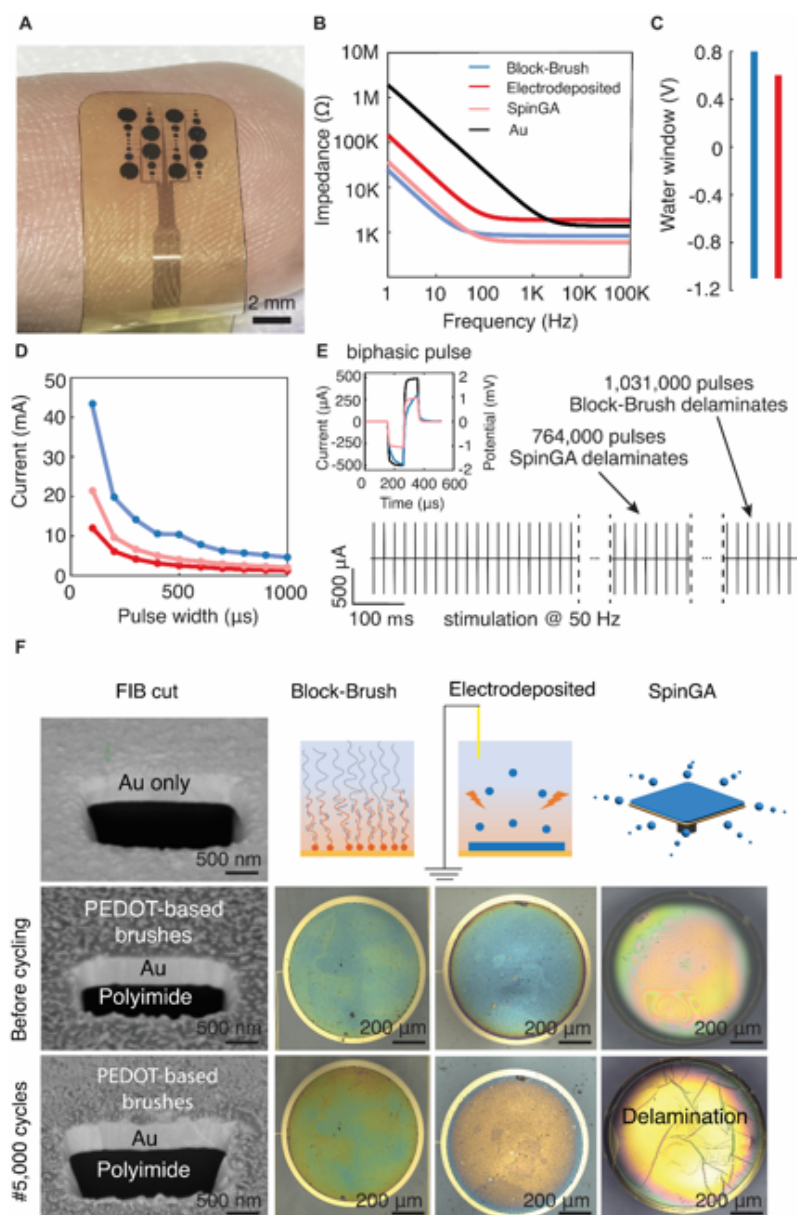


Figure 3.4 Electrochemical characterization of multidiameter microelectrode array and films long-term stability. (A) Image of the 32-channel electrode array containing 100 μm, 200 μm, 400 μm and 1000 μm diameter contacts. (B) Impedance spectrum and (C) water window comparison between the three PEDOT-based thin films for the 1000 μm diameter contact. (D) CIC for the three PEDOT-based thin films for the 400 μm diameter contact. (E) Breakdown of the 400 μm electrode contacts during biphasic current pulse stressing. Pulses were delivered at 50 Hz. Top left inset: Example biphasic pulse delivered during current stressing. (F) Left: Focused ion beam (FIB) image of the Block-Brush contact before and after 5000 CV cycles, showing no change to the film morphology. Right: Microscope images of the 1000 μm diameter contacts before (top) and after (bottom) 5000 CV cycles.

In vivo implantation and neural recording

To validate the neural recording capabilities of the Block-Brush coating, we recorded the somatosensory evoked responses activity in anesthetized rats subjected to repeated air-puff stimulation that caused mechanical deflection of the whiskers (Fig. 3.5 A). The rat barrel cortex exhibits a well-defined organization of somatosensory cortical structures that map one-to-one with the whiskers in the whisker somatosensory cortex (wS1) (49). We designed a 16-channel array with 200 μm contact diameters coated with Block-Brush and control formulations (Fig. 3.5 C). We first measured baseline noise recorded by the microelectrode arrays and found the root mean square (RMS) noise to be similar between the three formulations (Fig. 3.5 B). The neural activity recorded with the Block-Brush electrode showed a similar spectral response, with activity recorded at a range of frequencies up to high gamma ($<190\text{Hz}$) (Fig. 3.5 D and Fig. 3.24). The raw waveforms exhibited a similar shape across materials with peak responses observed ~ 25 ms after the onset of the air puff (Fig. 3.5 E). The Block-Brush electrode exhibited a similar peak-to-peak amplitude response and SNR compared to the SpinGA and the electrodeposited controls (Fig. 3.25). In summary, these results confirm that the brush-based PEDOT film can capture all relevant local field potentials and is an essential coating for neural interfaces.

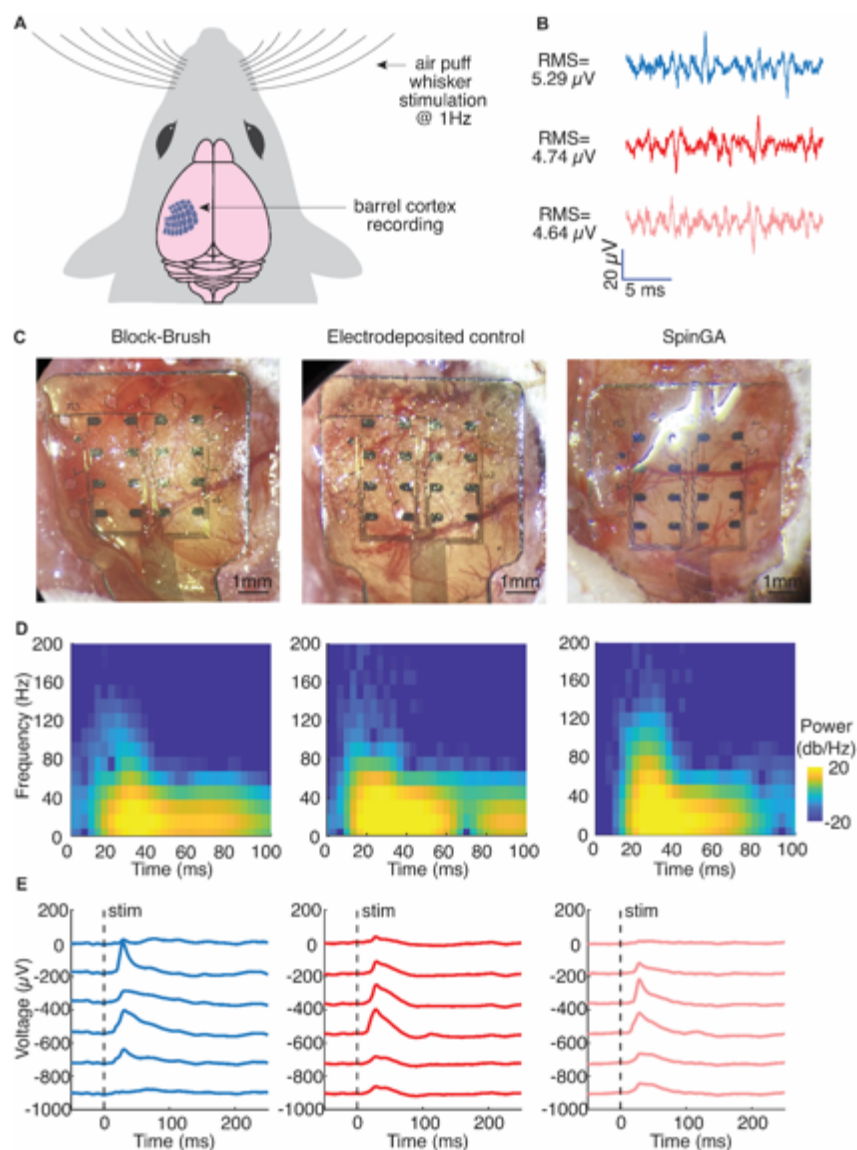


Figure 3.5 Block-Brush PEDOT film on microelectrode array record somatotopic functional cortical columns. (A) Schematic of the rat brain implanted with a 16-channel, 4.8 mm-by-4.8 mm array, and the air puff stimulation of individual whiskers. (B) Baseline recording of the brain activity, with baseline RMS values for the three materials. (C) Magnified microscope image of the electrode on the rat barrel cortex. (D) Spectral analysis of the mean trial-averaged response across low-impedance channels to whisker air puff stimulation for the Block-Brush (left), electrodeposited control (middle), and SpinGA control (right) samples. Responses from the three materials showed similar spectral profiles, with onset time ~ 20 ms post-stimulus and high power in frequency range < 80 Hz. (E) Trial-averaged responses from six individual low-impedance channels from each array. The dashed line indicates the time of air puff stimulation. Responses from the three materials are similar in shape and amplitude, indicating the Block-Brush film can capture neural activity.

Discussion

The generation of stable interfaces for recording and stimulation of neural activity holds promise for research and translational clinical applications requiring chronic implantations. Here, we demonstrate for the first time the SI-ATRP of Block-Brush PEDOT with a full characterization of nanomechanical, electrical, electrochemical, and long-term stability properties together with a successful recording of neural activity. This chemical pathway is compatible with a polyimide-based thin and flexible electrode, resulting in a uniform, conductive, and stable film. Moreover, the SI-ATRP living polymerization nature enables us to design and synthesize block copolymers with combined functionalities in a film that is composed of a one-component. Other common strategies such as blending two different polymers are prone to phase segregation and loss of function over time.

Some studies have demonstrated a lower CSC and CIC for PEDOT:PSS coatings in comparison with other coating materials, such as PtNR (50). This study can open new directions for exploring novel avenues to enhance the electrochemical properties of PEDOT:PSS coating through the modulation of polymer brush segments, thickness, density, or composition. Our results showed that the brush-based film had superior electrical properties in comparison with the commonly used approach in which a spin-coated PEDOT:PSS film is cross-linked and was on par with coating materials with high CIC, such as PtNR. The improvement in electrochemical properties can be attributed to the open pathways within the extended polymer brushes network, and the greater level of ordering at the molecular level (51). This morphology might facilitate greater charge movement than the crosslinked formulation (52, 53). Moreover, the enhancement in electrochemical properties, achieved without the need for additional dopants or treatments, hints at the potential for implementing the Block-Brush in various applications requiring both

biocompatibility and good conductivity. This potential extends to areas such as the development of conductive scaffolds, particularly in situations where high density of PEDOT is crucial. Other relevant areas necessitate avoiding stringent conditions such as acid treatment (54) or thermal processes (e.g. annealing) (55), which are incompatible with living cells or tissues.

Materials and Methods

Synthesis of PEDOT:PSS brushes on gold

We aimed to compare two strategies of binding the polymers to the gold surface, grafting-to, and grafting-from. We hypothesized that the grafting from approach would provide a more uniform coverage of the gold surface, with a stretched regime of the polymer brushes.

Grafting from of poly(sodium 4-styrenesulfonate) (PSS) by SI-ATRP

The polymerization of sodium styrene sulfonated was carried out as previously described (2). Gold-coated Si wafers (100 nm Au, 10 nm Cr adhesion layer, Si) were cleaned by sonicating in Alconox, acetone, and 2-propanol for 10 min each. The sample was dried with compressed air and then oxygen plasma treated for 10 min before soaking in 1 mM 6-Amino-1-hexanethiol hydrochloride in ethanol for 24 h. The resulting 6-Amino-1-hexanethiol hydrochloride-coated gold was rinsed with ethanol and then transferred to a new flask. The flask was purged and refilled with nitrogen (x3) before adding anhydrous tetrahydrofuran (THF) and triethylamine (1.1 eqv). α -Bromoisobutyryl bromide (0.1M, 1 eqv) was added and the solution was gently stirred under nitrogen for 3 min before the samples were removed and rinsed with ethanol and DI water. The sample was dried under compressed air and used immediately for the polymerization of PSS.

The initiator-coated gold surface was added to a clean vial. A stock solution of the polymerization reactants was prepared in a separate flask under nitrogen. Sodium styrene sulfonate (NaSS) (4.12 g, 1000 eq), copper (I) bromide (0.0029 g, 1 eq), copper (II) bromide (0.0015 g, 0.33

eq), 2,2'-bipyridine (0.0050 g, 1.6 eq) were added to a round bottom flask and purge and refilled (x3). NaCl (0.2338g, 200 eq) was added before purging. A 3:2 mixture of MilliQ water and methanol was sparged with nitrogen for at least 2 hours to remove all oxygen. 31 mL of sparged DI/methanol was added to the stock solution (0.65M NaSS). The solution was stirred vigorously to dissolve all reagents and turned a light tan. Once all reagents were solubilized, ethyl α -bromoisobutyrate (eBiB) (2.9 μ L, 1 eq) was added to initiate the synthesis of free polymer. Immediately after adding eBiB, 5-8 mL of the stock solution was transferred into the flask containing the gold sample under nitrogen to begin SI-ATRP. The reaction was terminated by opening the flask to air. The free polymer was collected for GPC by precipitation in room temperature acetone and filtered. The gold surfaces were rinsed with DI water.

Grafting from of poly(sodium 4-styrenesulfonate-block-poly(poly) (PSS-*b*-PPEGMEMA) by SI-ATRP

Due to the living nature of radical polymerization, we can tailor the molecular structure by adding a second, soft, block copolymer to decrease the mechanical mismatch with the tissue and the hydrophobicity of the PEDOT film. The polymerization of the second block of PPEGMEMA was modified from Robinson et al. (3). The PSS-modified gold surface was added to a clean vial. A stock solution of the polymerization reactants was prepared in a separate flask under nitrogen. Poly(ethylene glycol) methyl ether methacrylate (PPEGMEMA) (9.663 g, 1000 eq), copper (I) bromide (0.0029 g, 1 eq), copper (II) bromide (0.0015 g, 0.33 eq), 2,2'-bipyridine (0.0050 g, 1.6 eq) were added to a round bottom flask and purged and refilled (x3). A 3:2 mixture of MilliQ water and methanol was sparged with nitrogen for at least 2 hours to remove all oxygen. 31 mL of sparged DI/methanol was added to the stock solution (0.65M NaSS). The solution was stirred vigorously to dissolve all reagents and turned a light tan. Once all reagents were solubilized, ethyl

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Oxidative polymerization of EDOT on PSS and/or PSS-*b*-PPEGMEMMA brushes.

PSS brushes on gold were used as a scaffold for the polymerization of EDOT following literature procedures (4). Briefly, the PSS or PSS-*b*-PPEGMEMMA brushes were immersed in 0.1 M aqueous solution of EDOT and stirred vigorously in a sealed vial for at least 15 h before adding $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.75M). Blue particles formed over the reaction time (12-48 h). Once the PEDOT polymerization was complete, the sample had a bluish tint and was rinsed with DI water and dried in a desiccator overnight.

Chemical characterization

Further analysis of chemical composition was done using a Nicolet iS50 Fourier transform infrared (FTIR) spectrometer. Raman spectroscopy was performed on Renishaw inVia upright microscope using a 532 nm source. SEM micrographs were captured on a Zeiss Sigma 500 SEM with an accelerating voltage of 3.00 kV and an InLens detector. Chemical composition was confirmed via X-ray Photoelectron Spectroscopy (XPS) performed at UC Irvine Center for Complex and Active Materials on a Kratos AXIS-Supra. A survey was acquired at low x-ray intensity (5mA) and detailed elemental spectra were acquired at 20 eV and 40 eV at 20 mA. The thickness of PEDOT:PSS and PSS films was measured using SEM and ellipsometry on a J.A. Wollam M-2000D spectroscopic ellipsometer (Fig. 3.6) with a beam size of 3 mm. Surface profilometry measurements were also conducted on the DektakXT Stylus Profiler to obtain the

film thicknesses. All the measurements were taken with a vertical range of 6.5 μm and a Stylus force of 3 mg. Five measurements were taken of each sample to obtain the average thickness. Film thickness was estimated assuming a 5 nm chromium layer and a 100 nm gold layer on silicon. Water contact angle images were obtained with a ramé hart Model 200 goniometer (Fig. 3.7).

PEDOT film deposition

The working electrodes were deposited/grafted on glass or Si substrates. The glass/Si substrates (2.5 cm $\times$ 2.5 cm) were cleaned sequentially by 10 minutes of sonication cycles in soap water, DI water, acetone, and isopropanol. Before use, the cleaned glass substrates were treated by a UV Ozone reactor for 10 minutes at 30 W and 450 mTorr. 10 nm Cr adhesion layer and 100 nm Au layer were then deposited by thermal evaporation using Orion System, AJA International. Next, the slide was cleaned sequentially by 10 min sonication with Ethanol and DI water. The thin films grafted-TO or grafted-FROM the surface were created as described above. The spin-coated films were fabricated as follows. Before spin coating of PEDOT ink, the Au-coated slides were treated by a UV Ozone reactor for 10 minutes at 30 W and 450 mTorr. Spin-coated films were prepared as follows; PEDOT:PSS (Clevios) was mixed with 1% GOPS (SpinG). 20 mL aqueous dispersion of PEDOT:PSS was mixed with 5mL EG, 50 μL of DBSA, and 1% wt% GOPS (SpinGA). PEDOT:PSS without additional materials is referred to as Pristine PEDOT:PSS. The different solutions were spun-coated on top of Glass/Cr/Au or Si/Cr/Au substrates at 500 rpm (250 rpm s^{-1}) for 120 s, followed by 2000 rpm (1000 rpm s^{-1}) for 10 s. Following deposition, films were annealed at 120 $^{\circ}\text{C}$ for 15 min on a hot plate in atmosphere before being allowed to slowly cool down to room temperature by removing them from the hot plate.

Measuring electrical conductivity

The resistances of the films were measured using a four-point probe wired to a Keithley Standard Series 2400 Source Measure Unit (SMU), with a probe spacing of 2 mm and a sample size of 25 × 25 mm. The thickness of the films was measured using a Dektak XT profilometer or by ellipsometry, and the cross-sectional area was used to convert resistance to conductivity. The conductivity, σ , was calculated from an average of three samples using the following equations:

$$R_s = \frac{\pi}{\ln(2)} R \quad \text{Equation 1}$$

$$\sigma = \frac{1}{t \times R_s} \quad (2) \quad \text{Equation 2}$$

Where R is the resistance measured by the four-point probe, R_s is the sheet resistance, and t is the thin film thickness. A correction factor of 0.9497 was applied according to the geometry of the measurement.

Electrochemical impedance spectroscopy and cyclic voltammetry measurements

The EIS and CV measurements were used to evaluate the electrochemical activity of our different PEDOT-containing films. The Gamry Interface 1000E was used to perform EIS in 0.01 M 1 × PBS solution (consisting of 0.022M Na₂HPO₄ (pH = 7.2 ± 0.2), using a three-electrode configuration, i.e., PEDOT electrodes as the working electrodes, Ag/AgCl electrode as a reference electrode, a platinum rod electrode as a counter electrode. 10 mV root-mean-square (RMS) sinusoidal signal with zero DC bias was applied and the frequency was swept from 0.1 Hz to 1 × 10⁶ Hz. The capacitance and electrical double layer testing were measured by cyclic voltammetry under low current density, near equilibrium conditions in (1×) PBS solution, with the tested electrode potential swept cyclically within the potential windows of −0.4–0.8 V or −0.4–0.4 V relative to the Ag/AgCl electrode at a constant scan rate of 100 mV s^{−1} with 5 mV potential steps. The current was injected in biphasic, anodic, and cathodic stimulation, from 1 mA up to 36 mA,

with a voltage limit of ± 10 V (Fig. 3.9). All voltametric measurements were conducted in an ambient atmosphere.

The specific capacitance of the film was obtained according to Equation 3:

$$C^* = \frac{\oint j dV}{2v(V_{max} - V_{min}) * A} \quad \text{Equation 3}$$

Where integration is performed over the area of the voltammogram, j is the current density, V is the voltage, v is the scan rate, and A is the area of the film.

The volumetric capacitance of the film was calculated according to Equation 4:

$$C^* = \frac{\oint j dV}{2v(V_{max} - V_{min}) * v} \quad \text{Equation 4}$$

Where integration is performed over the area of the voltammogram, j is the current density, V is the voltage, v is the scan rate, and v is the volume of the film. Calculations are summarized in Fig. 3.10.

Accelerated ageing tests

PEDOT-based films, including the Block-Brush, and SpinGA were challenged by an accelerated aging test in PBS, pH 7.4, at 50 °C for 35 days. EIS and CV measurements and optical microscope inspections were taken every several days as described above. The size of the exposed areas was 6 mm $\times$ 8 mm (Fig. 3.11).

Oxidative reactive accelerated aging test

PEDOT-based films, including the Brush, SpinG (0.2% wt% GOPS), and the brushes grafted to the gold surface, were incubated in 20mM H₂O₂ in PBS at 50 °C for 56 days (Fig. 3.12). This procedure was modified from literature.

Ultrasonication stability test

The PEOBT-based film, including the Block-Brush, grafted from and to the gold surface, and Pristine PEDOT:PSS were challenged by ultrasonication (100 W at 42 kHz) for different time points (Fig. 3.13).

Peel test

To conduct a 90° peel test, a Mark-10 linear actuator equipped with the peel test accessory kit was operated in the upright position. Samples were taped with Kapton tape for 12 h before the measurements. The glass/Cr/Au/PEDOT films were fixed to the sliding plate using double-sided tape. The edge of the Kapton tape was attached to a grip connected to a 0.5 N force gauge. The tapes were removed at a rate of 330 mm min⁻¹ to obtain a plot of force relative to displacement (travel). The adhesion force between the surface of the films and the PDMS (1:50), is calculated from the max force achieved during the shear test divided by the joint area of the films.

Adhesion force between the PEDOT films and soft PDMS surface

Polydimethylsiloxane (PDMS, Sylgard 184 Silicone) was mixed in a 50:1 base-to-curing agent ratio and degassed under vacuum to remove air bubbles. PDMS was then deposited using a plastic cup on a glass slide and spun at 1000 rpm (500 rpm s⁻¹) for 60 s. The substrate was cured at 70 °C in an oven for 3 h. lap-joint shear tests (shear properties) were performed against the soft 50:1 PDMS.

Elastic modulus characterization of the Block-Brush film

The elastic deformation was obtained by analysis of AFM-tip-polymer brush interactions by nanoindentation, according to the dimitriadis model, using Equation 5.

$$F = \frac{4E}{3(1-\nu^2)} R^{1/2} \delta^{3/2} \left[1 - \frac{2\alpha_0}{\pi} \chi + \frac{4\alpha_0^2}{\pi^2} \chi^2 - \frac{8}{\pi^3} \left(\alpha_0^3 + \frac{4\pi^2}{15} \beta_0 \right) \chi^3 + \frac{16\alpha_0}{\pi^4} \left(\alpha_0^3 + \frac{3\pi^2}{5} \beta_0 \right) \chi^4 \right] \quad \text{Equation 5}$$

$$\chi = \frac{\sqrt{R\delta}}{h_{compressible}}$$

$$\alpha_0 = -\frac{1.2876 - 1.4678\nu + 1.3442\nu^2}{1 - \nu}$$

$$\beta_0 = -\frac{0.6387 - 1.0277\nu + 1.5164\nu^2}{1 - \nu}$$

Where δ is the deformation due to the force imposed by a spherical indenter, R is the tip radius (500 nm), E^* is the composite modulus, ν_s is Poisson's ratio of the substrate (Au, 0.42), ν_f is Poisson's ratio of the brushes' "film" (PEG, 0.3).

Microelectrode array fabrication

The microelectrode arrays were fabricated on 75x25 mm glass slides (Corning), which were cleaned with ethanol and IPA and baked at 180°C for 10 minutes before use. The glass slide was coated with 10- μ m-thick-polyimide (PI) 2611 and baked in a carbolite oven (Carbolite Gero) at 350°C for 1 hour. Next, the metal lead traces (10 nm Cr, 150 nm Au) were deposited onto the glass slide using standard lithography techniques and AZ5214E-IR photoresist (MicroChemicals). A 3 μ m PI encapsulation layer was spin-coated onto the surface and baked at 350°C for 1 hour. Finally, the via holes were patterned with AZ12XT-20-10 photoresist (MicroChemicals) and oxygen plasma etched for 30 minutes. The samples were then soaked in Remover PG at 80°C for 1 hour to remove photoresist residues. PSS and PSS-*b*-PPEGMEMA brushes were then grafted from the exposed micro-scale Au contact surface.

Electrodeposition of PEDOT on the microelectrode array

Once PSS brush synthesis was completed, PEDOT was electrodeposited onto the surface of the micro-scale contacts. The electrodeposition solution was prepared by dissolving 50 μ L of 3

4-ethylenedioxythiophene (EDOT) in 47 mL of DI water. The solution was vortexed until EDOT dissolved completely. Potentiostatic electrodeposition was performed using the Reference 6000 (Gamry Instruments) at 1.1 V for 80s.

Charge storage capacity (CSC) calculation

All reported charge storage capacities were calculated from cyclic voltammograms between -0.6 V and 0.6 V. CSC was calculated using Equation 6.

$$CSC = \sum_{\Delta t \in T} I(t) \times \Delta t / A \quad \text{Equation 6}$$

Where Δt is a single timestep in the total duration of one CV cycle T , $I(t)$ is the current measured at time t in the CV cycle, and A is the area of the sample.

Charge injection capacity (CIC) calculation

The charge injection capacity for a given contact was calculated with the Reference 6000 (Gamry Instruments) from the water window and the maximum negative polarization potential (E_{mc}) computed across a range of pulse widths between 100-1000 μ s and current amplitudes between 3 μ A – 10 mA, depending on the contact size and impedance. The CIC was identified as the point of intersection between E_{mc} and the negative water window limit.

Biphasic pulse stimulation

Repeated current pulsing was performed by delivering a train of biphasic, cathodic-first current pulses with 100 μ m pulse width and 500 μ A current amplitude. Pulses were delivered at 50 Hz using the RHS Stim/Recording System (Intan Technologies). The contact was considered delaminated when it could no longer deliver the desired current amplitude, which was identified by a voltage compliance flag on the RHS System.

Vertebrate animal subjects

An adult (> 5 months old, weight 450 g) male Sprague-Dawley rat (Charles River Laboratories) was used as the vertebrate animal subject in this study. All animal experiments were approved by the UC San Diego Institution Animal Care & Use Committee (protocol S16020).

Surgical procedures

Rats were sedated with 4% isoflurane and fixed in a stereotaxic frame (Kopf Instruments). Once stable, anesthesia was reduced to 1.5-2.5% during the surgery. A craniotomy was performed, exposing the whisker barrel cortex without breaching the dura. The electrode array was placed onto the whisker barrel cortex. Gel foam (SURGIFOAM Absorbable Gelatin Sponge) was used to cover the microelectrode array to secure it in place and maintain moisture on the brain.

Once the surgery was completed, rats were transitioned from isoflurane to ketamine/xylazine (90 and 10 mg/kg⁻¹, respectively; MWI) and re-dosed 20-30 minutes for the duration of the experiment. Heart rate, body temperature, and blood oxygenation were continuously monitored throughout the experiment. A heating pad maintained body temperature between 34-36°C throughout the experiment. At the end of the study, animals were euthanized with 120 mg/kg⁻¹ sodium pentobarbital (MWI).

Data collection

Once the electrode was in place, the reference needle electrode was placed near the neck of the rat, and ground was connected to the stereotaxic frame. An individual whisker was stimulated with the air puff using the Pneumatic PicoPump (WPI, PV830). Neural activity was recorded with the RHS Stim/Recording System (Intan Technologies). After a 1 min baseline recording, each whisker was stimulated for 2 minutes at 1Hz for a total of 120 trials. The air puff

stimulation was time locked to the recording system by sending TTL signals to both the air puff stimulator and the RHS.

Data analysis

A 1-minute fragment of baseline recording (without air puff stimulation) was high pass filtered >300 Hz with a 2nd order Butterworth filter. The root mean square (RMS) of the baseline noise was calculated in MATLAB. To perform spectral analysis of the whisker barrel cortex activity in response to stimulation, neural recordings were first notch filtered at 60 Hz, then bandpass filtered 0.1-300 Hz using a 2nd order Butterworth filter. The spectral analysis of the trial averaged data (120 trials) was performed in MATLAB using the spectrogram function. The peak-to-peak amplitude of the response was calculated by subtracting the trial averaged minimum potential from the maximum potential over the period between 1-200 ms after stimulation. The signal to noise ratio was calculated by dividing the peak-to-peak amplitude by the RMS of the baseline noise.

Acknowledgements

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Chapter 3 has been submitted for publication of the material as it may appear in Science Advances, 2024. R. Blau†, S. M. Russman†, Y. Qie, W. Shipley, A. Lim, A. X. Chen, L. Ah, A. Abdal, G. L. Esparza, S. Edmunds, R. Vatsyayan, S. P. Dunfield, D. P. Fenning, A. R. Tao, S. A. Dayeh, D. J. Lipomi, Surface-grafted biocompatible polymer conductors for stable and compliant electrodes for brain interfaces. The dissertation author was one of the primary investigators and co-first authors of this material. The dissertation author fabricated and characterized the microelectrode arrays and performed the in vivo study.

Supplementary Materials

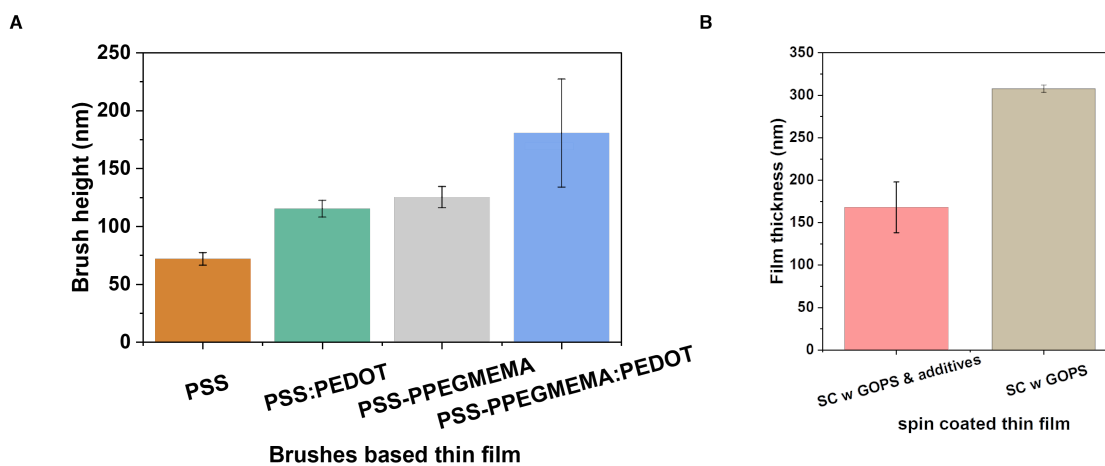


Figure 3.6 Polymer brush film thickness. The films made of polymer brushes were evaluated by ellipsometry for each step of the synthesis, including PSS, PSS-*b*-PPEGMEMA, PEDOT:PSS (Brush) and PEDOT:PSS-*b*-PPEGMEMA (block-Brush).

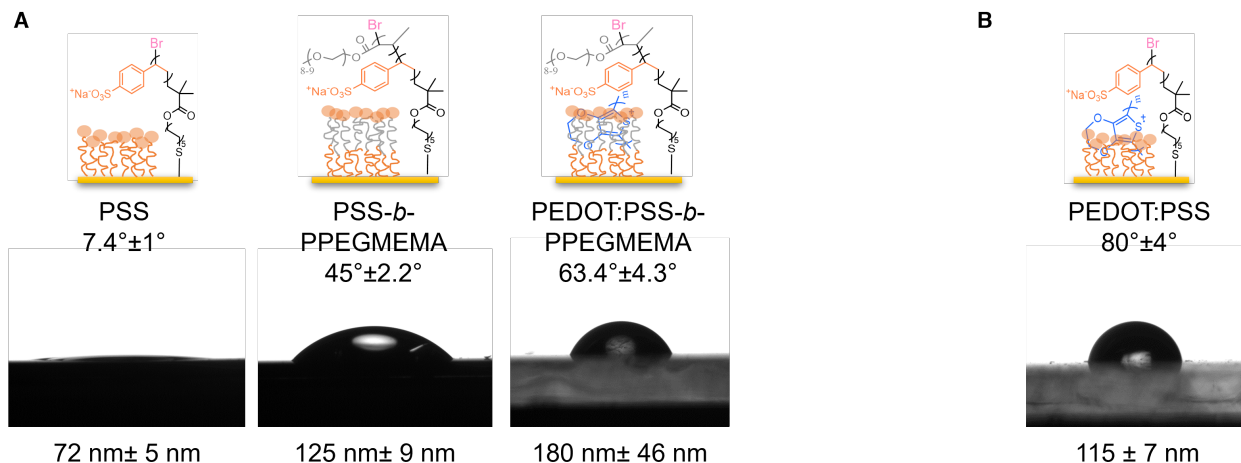


Figure 3.7 Water contact angles measurements. (A) PSS brushes, PSS-*b*-PPEGMEMA brushes, and Block-Brush. (B) Brush. The number below indicates the thickness of the films, measured by ellipsometry. The below 10° water contact angle of PSS indicated a highly hydrophilic surface post PSS SI-ATRP modification. Importantly, the water contact angle of Block-Brush was lower than that of the Brush films. This suggests that the modification with PPEGMEMA renders the surface less hydrophobic, which is beneficial for ion penetration during operation.

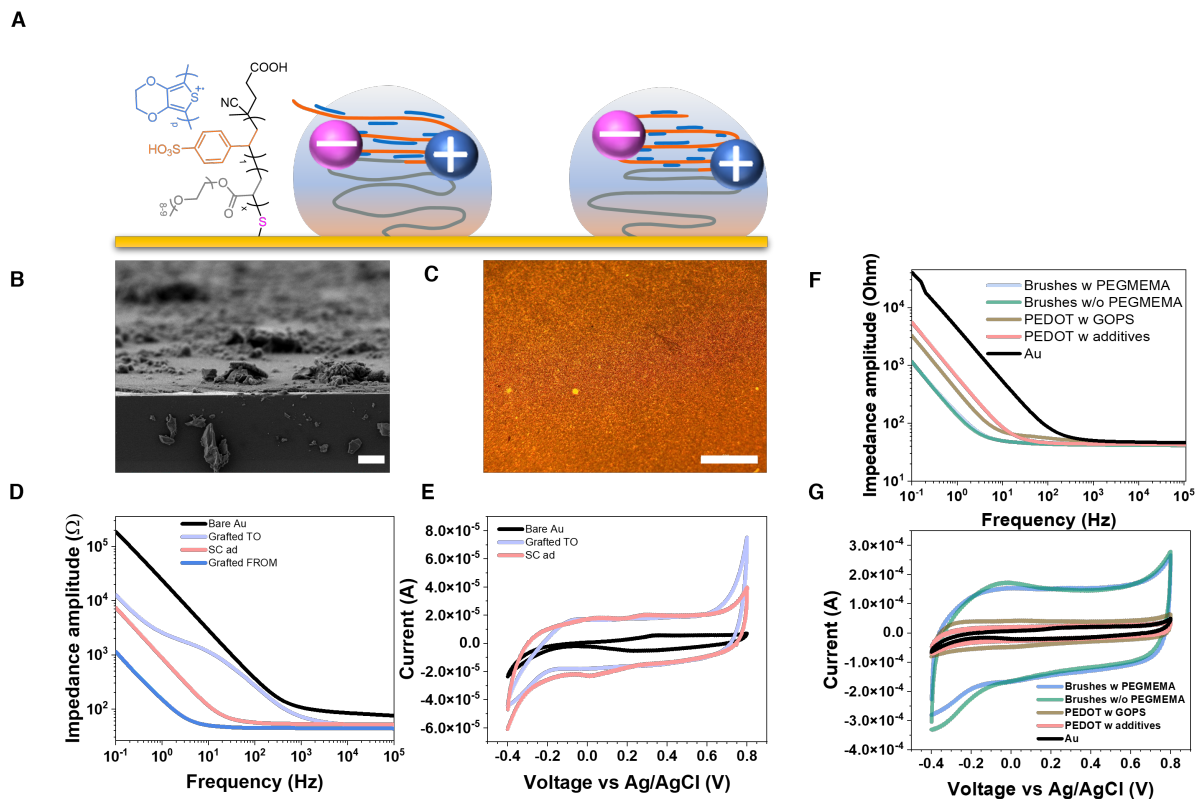


Figure 3.8 Electrochemical characterization of the PEDOT-based film. (A) Schematic illustration of the EDL in low-density film grafted to the gold surface. (B) SEM and (C) optical microscope images of Block-Brush grafted to the gold surface. The scale bars are 2 μm and 500 μm , respectively. (D) EIS and (E) CV curves of the Block-Brush grafted-TO the gold surface in comparison with the bare gold, SpinGA sample, and Block-Brush grafted from the gold surface. (F) The Block-Brush film that was grafted from the surface shows the lowest impedance EIS, and (G) highest AUC of the CV of compared to bare gold, and spin-coated films on gold.

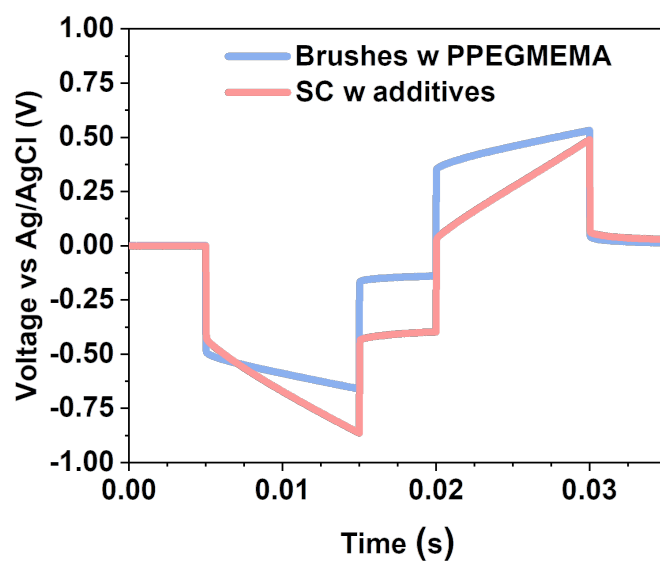


Figure 3.9 Voltage transient of injected current pulses. The current was injected with a 10 ms pulse width, measured at anodal/cathodal limits for SpinGA and the Block-Brush.

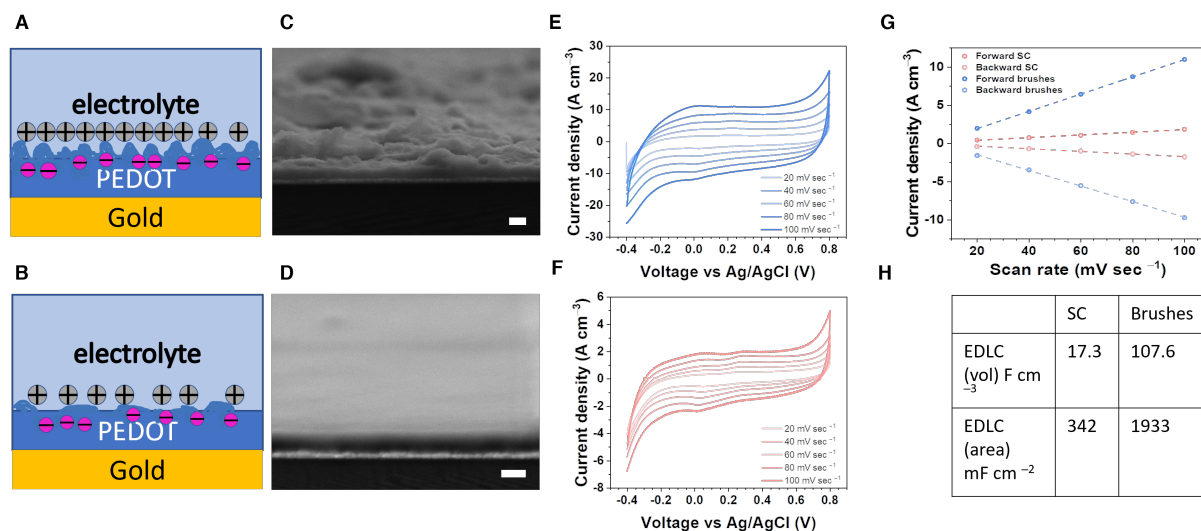


Figure 3.10 Block-Brush film exhibits a higher volumetric electrical double layer (EDLC) in comparison with SC formulation. (A, B) Schematic illustration of the rougher surface of the Block-Brush film (A) and SpinGA film (B). (C) Representative SEM image of the Block-Brush film, that demonstrates its rough surface in comparison with SpinGA (D). The scale bars are 200 μm. (E) Cyclic voltammetry scans at low and different scan rates, showing greater charge storage capacity for the Block-Brush film and in comparison with the SpinGA film (F). (G) Current density comparison shows a linear increase in current density for increasing scan rate. The average of the slopes of the forward and background scan reveal the electric double layer (EDL) capacitance of the Block-Brush and the SpinGA that is summarized in (H).

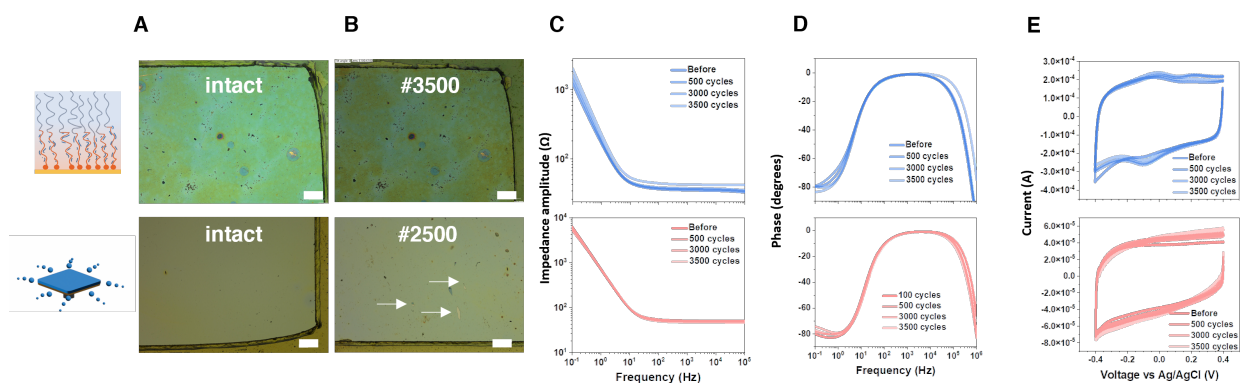


Figure 3.11 Block-Brush film shows longer durability versus spin-coated PEDOT:PSS under CV cycling stress. (A) Optical microscope images taken during stability tests under cycling stress show the Block-Brush (upper panel) and SpinGA (lower panel) before (A) and (B) after CV cycles. No damage was detectable up to 3500 CV cycles for the Block-Brush film versus the damages that can be observed for the SpinGA film after 2500 CV cycles (lower panel, white arrows). (C to E) Corresponding Impedance magnitude (C) phase (D) spectra and (E) CV cycles. Scale bars are 250 μm .

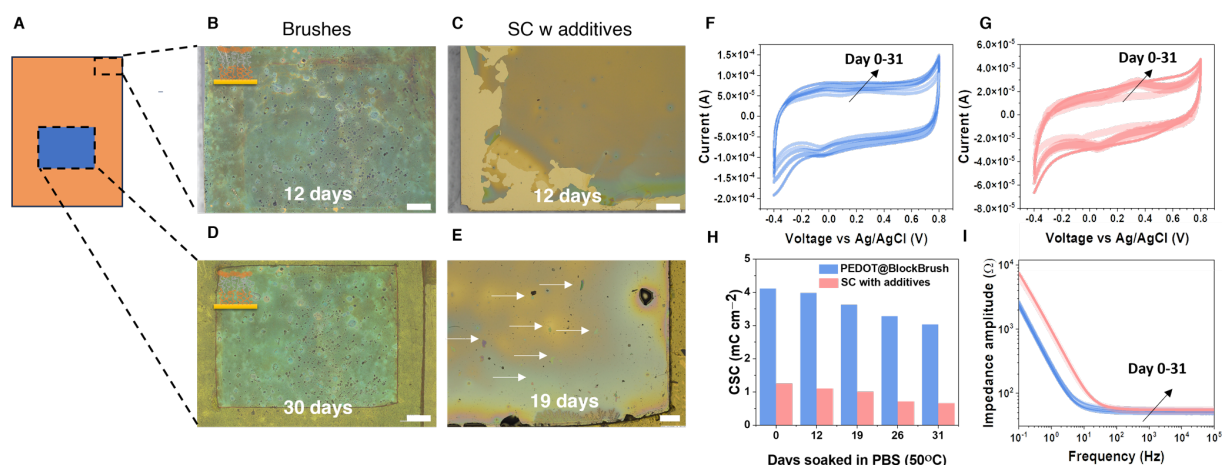


Figure 3.12 Block-Brush films show increased stability on Au surfaces during accelerated aging test, in PBS, 50°C. (A) Schematic illustration of the Au surface covered with Kapton tape, exposing film area of 6 mm × 8 mm during an accelerated aging test (PBS, 50°C). (B) Optical microscope images of the edges of the coated surfaces that were covered by Kapton tape. PEDOT Block-Brush films are fully covering the area under the Kapton tape and the exposed rectangular (B), while the spin-coated film, SpinGA, was delaminated under the Kapton tape 12 days post incubation in PBS (50°C) (C). (D). The Block-Brush film is intact showing no damage under an optical microscope 30 days post incubation. The scale bars for B to D, are 1 mm. (E) The exposed area of the spin-coated PEDOT:PSS film had multiple delamination (white arrows) spots 19 days post-incubation. The scale bar is 250 μm . Accordingly, the CV cycling of the brushes (F, blue) showed a lower change in the CSC compared with the spin-coated films (G, pink) and (H). (I) The impedance stability of the Block-Brush and the SpinGA films soaked in PBS solution at 50°C for 26 days. The geometric surface area of the surfaces is 0.48 cm^2 .

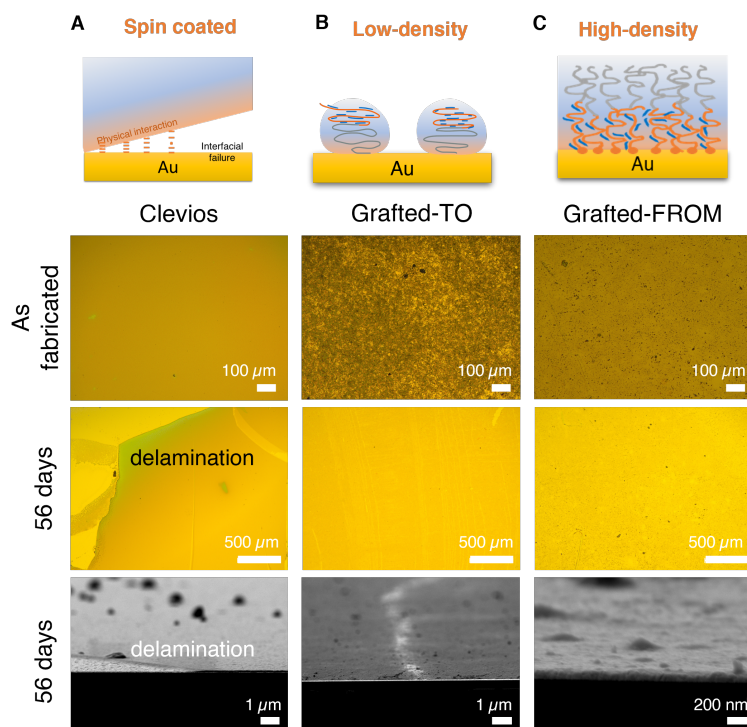


Figure 3.13 High-density Block-Brush PEDOT films show increased stability against accelerated aging test. Oxidative reactive accelerated aging test was done in 20mM H_2O_2 in PBS at 50°C for 56 days. (A) Spin-coated samples were delaminated from the surface. (B) Low-density brushes, grafted-TO the gold surface developed cracks in the film. (C) High-density Block-Brush films show an intact film with no observed damage through an optical microscope (two upper panels) or SEM (lower panel).

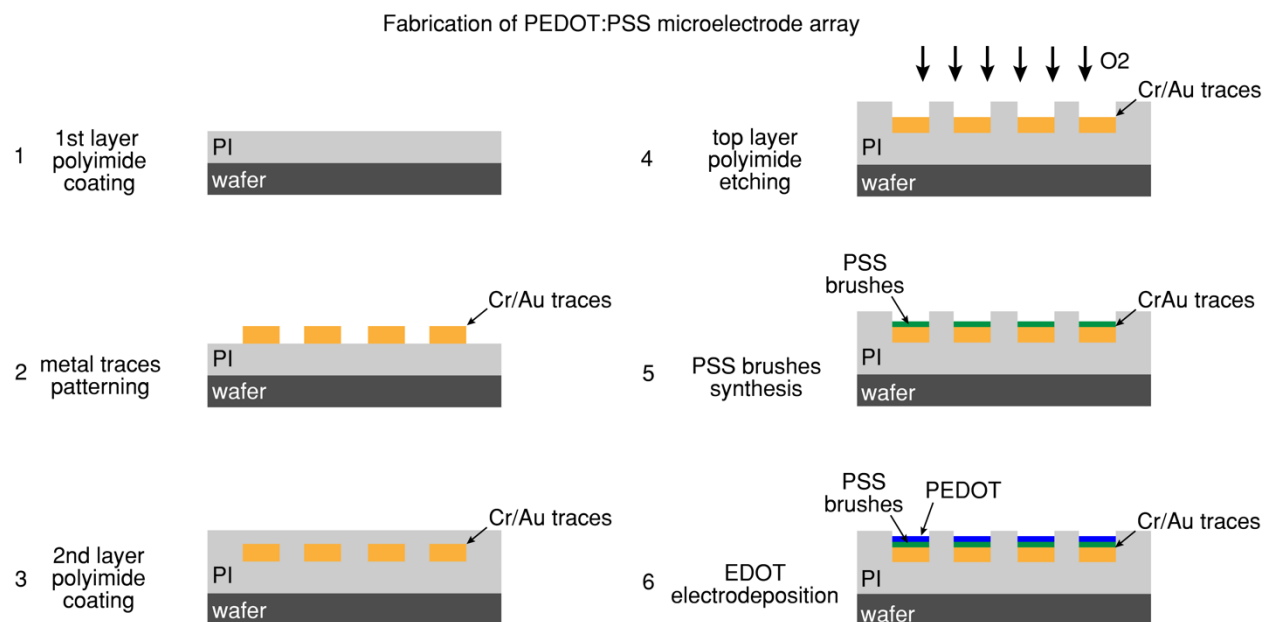


Figure 3.14 Fabrication process of microelectrode array coated with Block-Brush. (1) The first layer of PI was spin coated onto wafer surface. (2) Metal traces consisting of 10 nm Cr and 150 nm of Au were subsequently deposited by e-beam evaporation. (3) The second layer of PI was spin coated onto the surface of the sample for encapsulation. (4) The via holes were etched using O₂ plasma etching. (5) PSS-*b*-PPEGMEMA brushes were grafted from the Au surface. (6) PEDOT was electrodeposited on the polymer brush film as described in section VI.

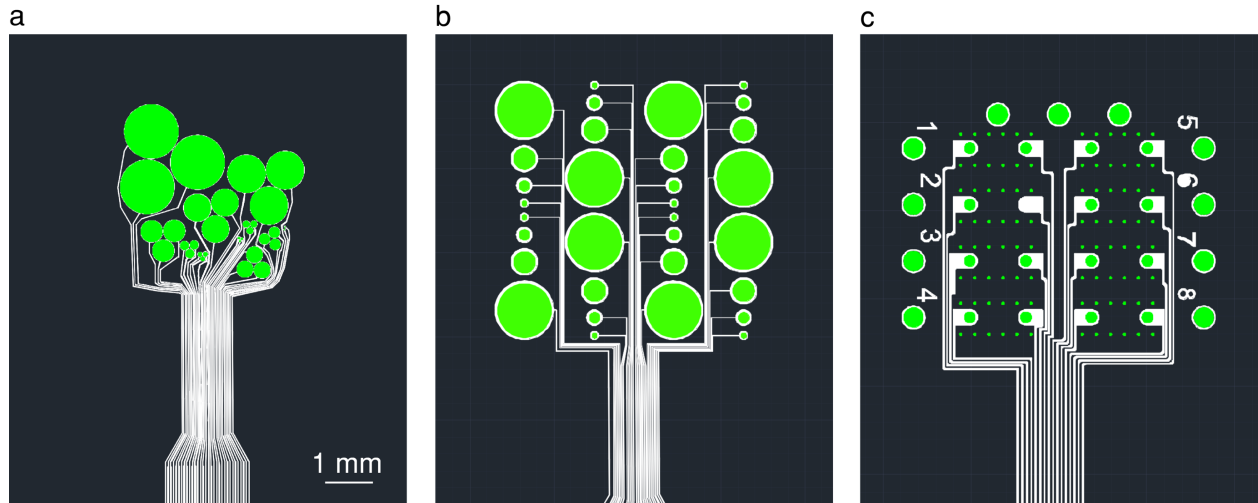


Figure 3.15 Multidiameter microelectrode array design. (a) Version 1 of the benchtop multidiameter array. (b) Version 2 of the benchtop multidiameter array with 8 x 1000 μm , 8 x 400 μm , 8 x 200 μm and 8 x 100 μm diameter contacts. (c) The rat microelectrode array with 16 x 200 μm diameter contacts.

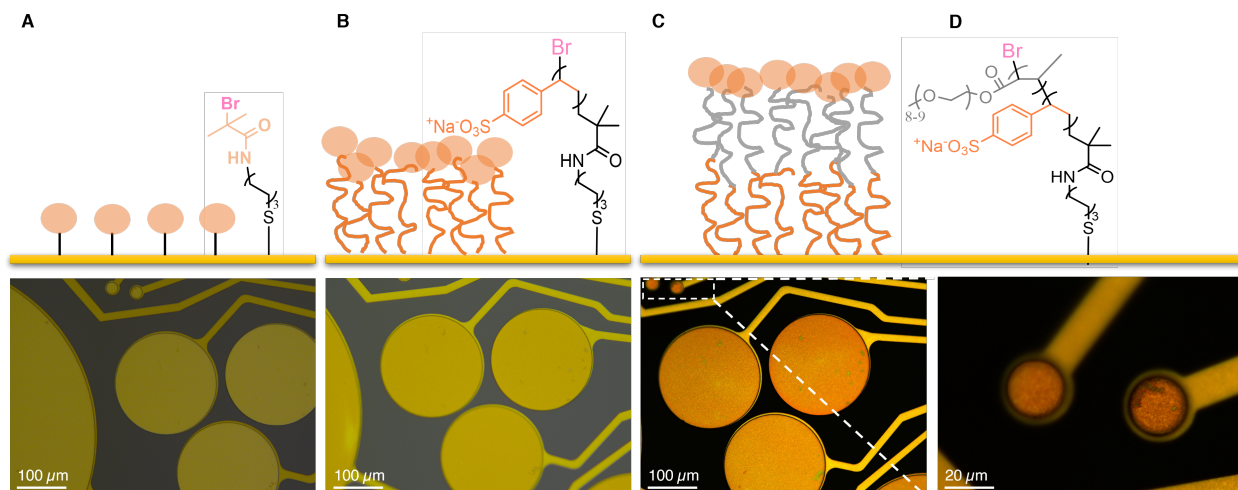


Figure 3.16 Polymer brushes grafting to the Au surfaces on the multi-diameter electrode. Optical microscope image of the Au surface after the activation with ATRP initiator (A), modification with PSS brushes (B), and further modification with the second block copolymer PPEGMEMA (C). D. The inset shows the uniformity on the 20 μm diameter electrode.

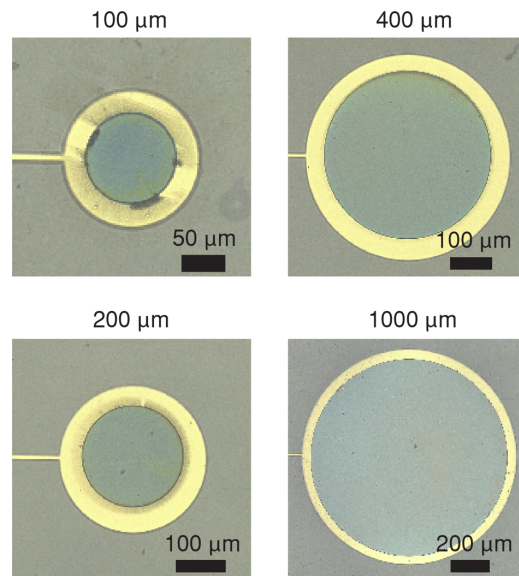


Figure 3.17 Example of Block-Brush coated contacts of the multidiameter array.

Fabrication of PEDOT:PSS microelectrode array

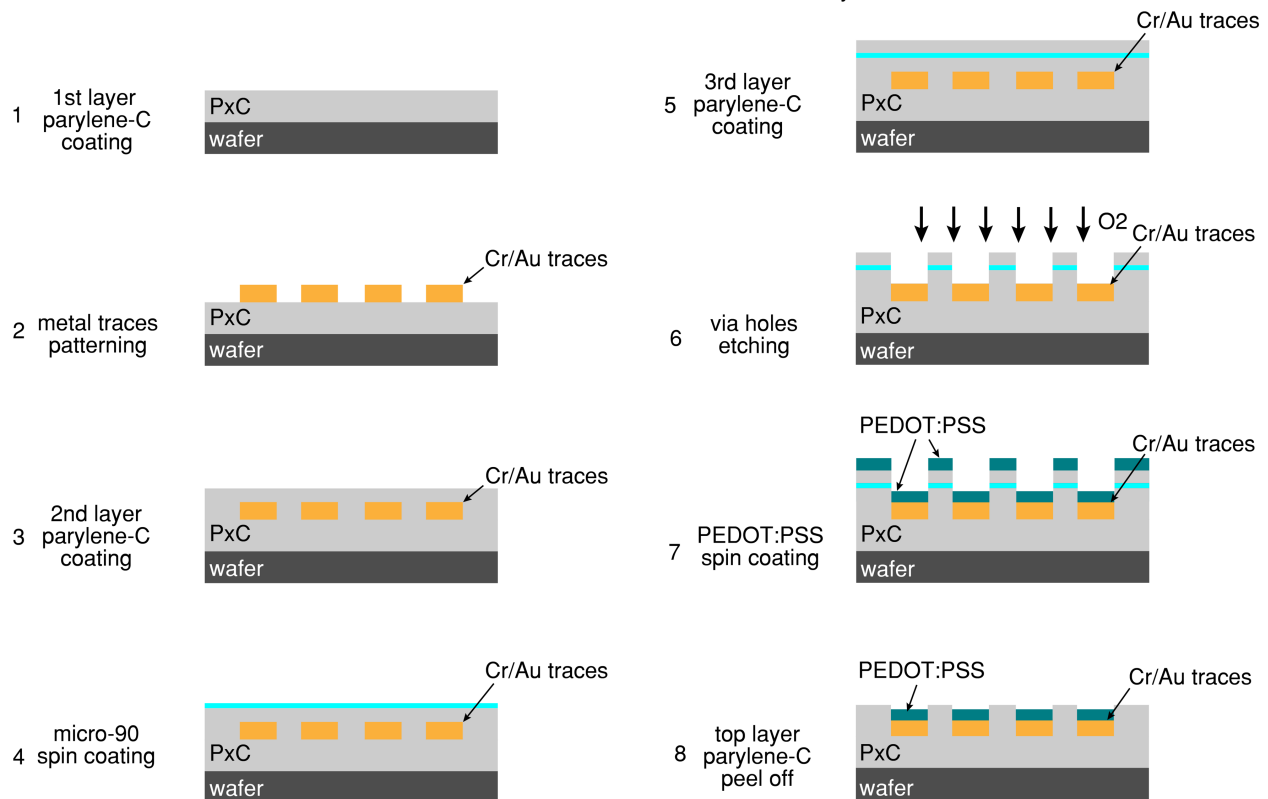


Figure 3.18 Fabrication process of spin coated PEDOT:PSS control microelectrode array. The process is similar to the preparation of Block-Brush coating, but requires additional steps needed for the spin coated sample preparation. These include the addition of a third sacrificial layer of parylene C and then peel it off of to create the PEDOT:PSS pattern on the gold circular contacts only.

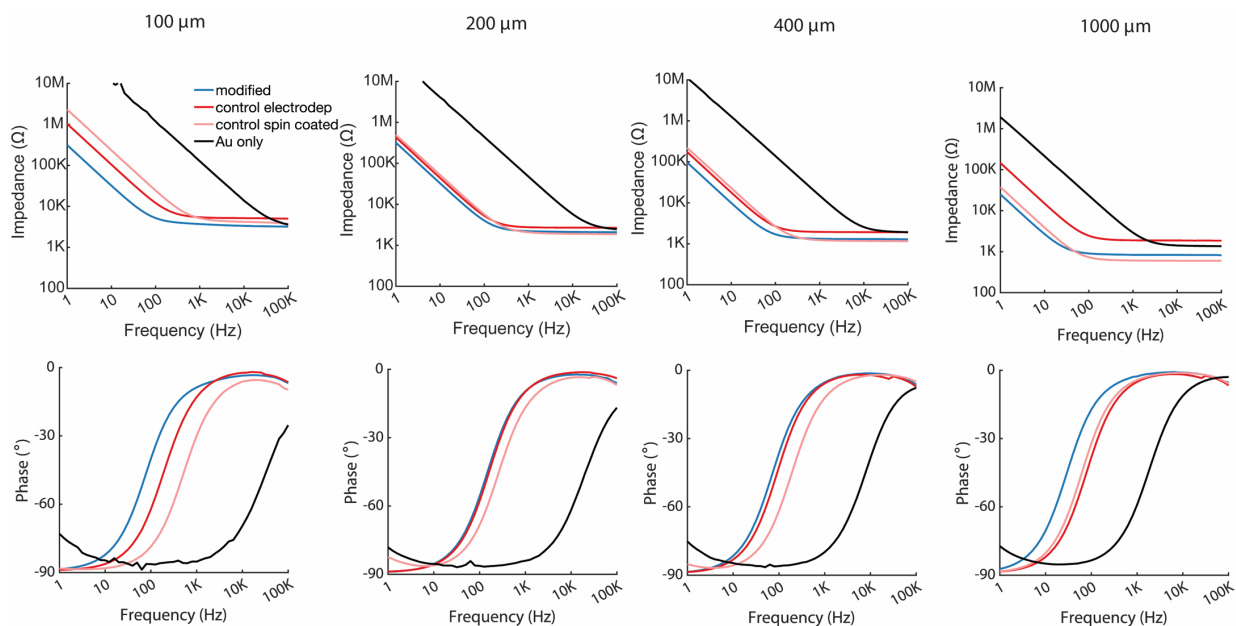


Figure 3.19 Impedance spectra of different contacts diameters after electrodeposition. The Block-Brush films (blue) show the lowest impedance under 100Hz for all diameters, in comparison with the spin coated formulation and the electrodeposited control.

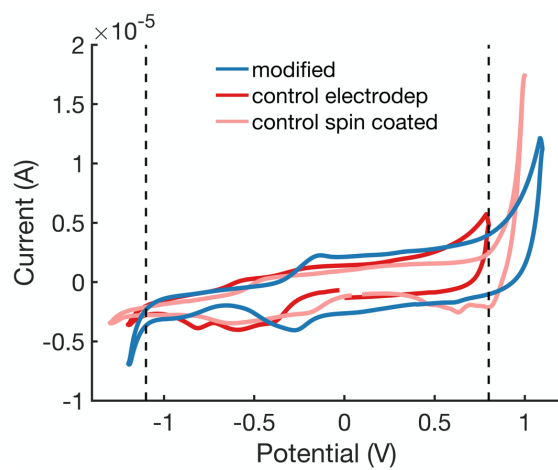


Figure 3.20 Water window of the 1000 μm diameter array for the three PEDOT formulations. The water window of the Block-Brush between -1.1V and 0.8V is marked by the dashed lines.

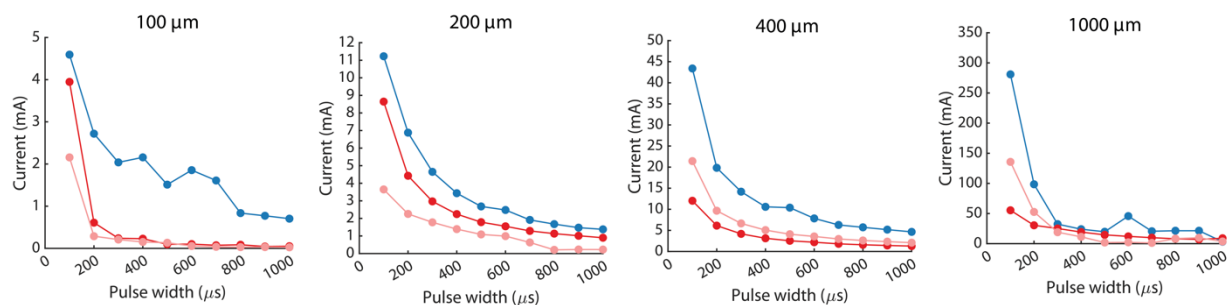


Figure 3.21 CIC for the three PEDOT:PSS materials different contacts diameters.

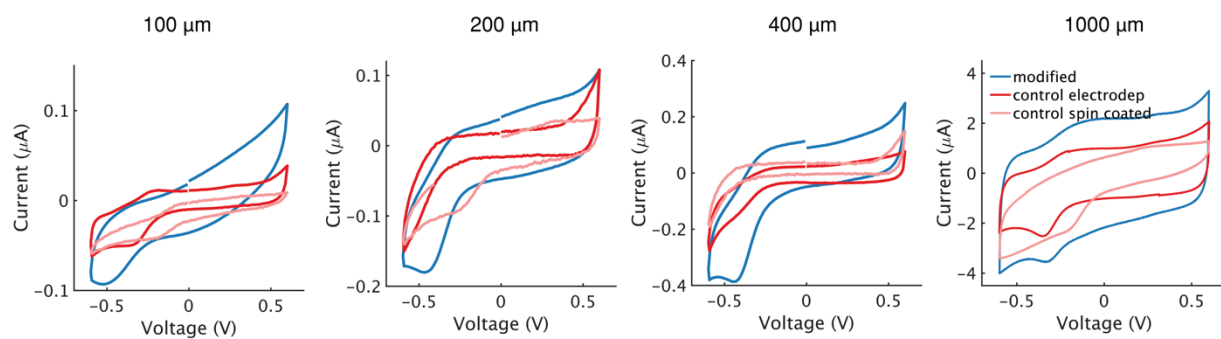


Figure 3.22 CV of different contacts diameters after electrodeposition. The Block-Brush films (blue) demonstrate the largest AUC, hence the highest CSC in comparison with the spin coated formulation and the electrodeposited control.

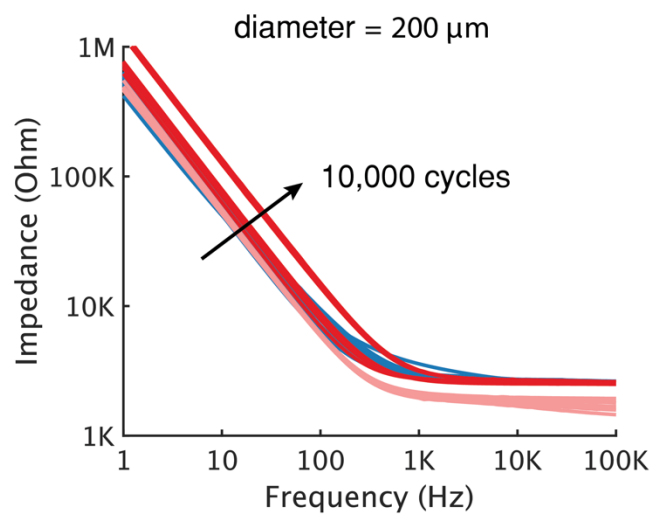


Figure 3.23 Stability of spin coated, electrodeposited and Block-Brush film under electrochemical cycling. EIS measurements show the stability of the films over 10,000 CV cycles.

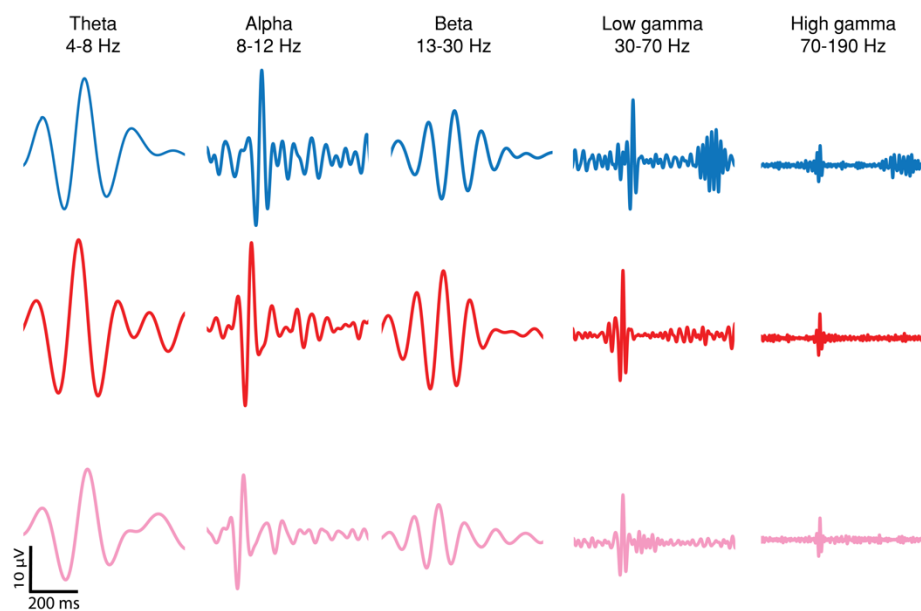


Figure 3.24 Spectral analysis of the mean trial-averaged response across channels to whisker air puff stimulation. Across frequency bands, responses from the three materials showed similar spectral profiles. mean trial-averaged response across channels filtered by frequency band for the Block-Brush (top, blue), electrodeposited control (middle, red), and spin coated control (bottom, pink) samples. This indicates that the modified PEDOT:PSS material can capture all frequency components of neural activity.

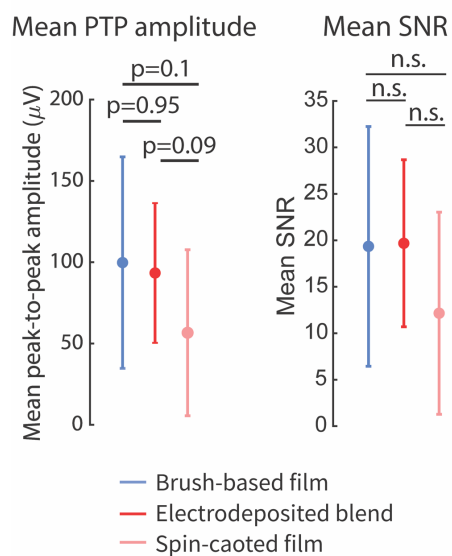


Figure 3.25 Mean peak-to-peak (PTP) amplitude and SNR of the different PEDOT based films. The Block-Brush film on the gold contact of the multi array electrode showed the highest PTP amplitude.

References

1. R. P. Lesser, H. Lueders, D. S. Dinner, J. Hahn, L. Cohen, THE LOCATION OF SPEECH AND WRITING FUNCTIONS IN THE FRONTAL LANGUAGE AREA RESULTS OF EXTRAOPERATIVE CORTICAL STIMULATION. *Brain* **107**, 275–291 (1984).
2. E. R. Dorsey, R. Constantinescu, J. P. Thompson, K. M. Biglan, R. G. Holloway, K. Kiebertz, F. J. Marshall, B. M. Ravina, G. Schifitto, A. Siderowf, C. M. Tanner, Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology* **68**, 384–386 (2007).
3. J. J. Williams, J. J. Wheeler, A brain-computer interface using electrocorticographic signals in humans * You may also like Spatial co-adaptation of cortical control columns in a micro-ECOG brain-computer interface A G Rouse. doi: 10.1088/1741-2560/1/2/001 (2004).
4. M. P. Branco, S. H. Geukes, E. J. Aarnoutse, N. F. Ramsey, M. J. Vansteensel, Nine decades of electrocorticography: A comparison between epidural and subdural recordings. *European Journal of Neuroscience*, doi: 10.1111/EJN.15941 (2023).
5. H. Yuk, B. Lu, X. Zhao, Hydrogel bioelectronics. *Chem. Soc. Rev* **48**, 1642 (2019).
6. G. Hong, C. M. Lieber, Novel electrode technologies for neural recordings. doi: 10.1038/s41583-019-0140-6.
7. K. M. Szostak, L. Grand, T. G. Constandinou, Neural interfaces for intracortical recording: Requirements, fabrication methods, and characteristics. *Front Neurosci* **11**, 240291 (2017).
8. R. Green, M. R. Abidian, R. Green, M. R. Abidian, Conducting Polymers for Neural Prosthetic and Neural Interface Applications. *Advanced Materials* **27**, 7620–7637 (2015).
9. Y. Jiang, Z. Zhang, Y.-X. Wang, D. Li, C.-T. Coen, E. Hwaun, G. Chen, H.-C. Wu, D. Zhong, S. Niu, W. Wang, A. Saberi, J.-C. Lai, Y. Wu, Y. Wang, A. A. Trotsyuk, K. Yong Loh, C.-C. Shih, W. Xu, K. Liang, K. Zhang, Y. Bai, G. Gurusankar, W. Hu, W. Jia, Z. Cheng, R. H. Dauskardt, G. C. Gurtner, J. B-H Tok, K. Deisseroth, I. Soltesz, Z. Bao, Topological supramolecular network enabled high-conductivity, stretchable organic bioelectronics. *Science (1979)* **375**, 1411–1417 (2022).
10. A. V Volkov, K. Wijeratne, E. Mitra, U. Ail, D. Zhao, K. Tybrandt, J. W. Andreasen, M. Berggren, X. Crispin, I. V Zozoulenko, Understanding the Capacitance of PEDOT:PSS. *Adv Funct Mater* **27**, 1700329–1700329 (2017).
11. M. D. Ferro, N. A. Melosh, Electronic and Ionic Materials for Neurointerfaces. *Adv Funct Mater* **28** (2018).
12. S. H. Sunwoo, S. I. Han, H. Joo, G. D. Cha, D. Kim, S. H. Choi, T. Hyeon, D. H. Kim, Advances in Soft Bioelectronics for Brain Research and Clinical Neuroengineering. *Matter* **3**, 1923–1947 (2020).
13. U. Lang, N. Naujoks, J. Dual, Mechanical characterization of PEDOT:PSS thin films. *Synth Met* **159**, 473–479 (2009).
14. S. Benaglia, S. Drakopoulou, F. Biscarini, R. Garcia, In operando nanomechanical mapping of PEDOT:PSS thin films in electrolyte solutions with bimodal AFM. *Nanoscale* **14**, 14146–14154 (2022).
15. J. Qu, L. Ouyang, C. C. Kuo, D. C. Martin, Stiffness, strength and adhesion characterization of electrochemically deposited conjugated polymer films. *Acta Biomater* **31**, 114–121 (2016).
16. Y. Jiang, Z. Zhang, Y.-X. Wang, D. Li, C.-T. Coen, E. Hwaun, G. Chen, H.-C. Wu, D. Zhong, S. Niu, W. Wang, A. Saberi, J.-C. Lai, Y. Wu, Y. Wang, A. A. Trotsyuk, K. Yong

- Loh, C.-C. Shih, W. Xu, K. Liang, K. Zhang, Y. Bai, G. Gurusankar, W. Hu, W. Jia, Z. Cheng, R. H. Dauskardt, G. C. Gurtner, J. B-H Tok, K. Deisseroth, I. Soltesz, Z. Bao, Topological supramolecular network enabled high-conductivity, stretchable organic bioelectronics.
17. N. Lopez-Larrea, M. Criado-Gonzalez, A. Dominguez-Alfaro, N. Alegret, I. Del Agua, B. Marchiori, D. Mecerreyes, Digital Light 3D Printing of PEDOT-Based Photopolymerizable Inks for Biosensing. *Cite This: ACS Appl. Polym. Mater* **2022**, 6749–6759 (2022).
 18. R. Blau, A. X. Chen, B. Polat, L. L. Becerra, R. Runser, B. Zamanimeymian, K. Choudhary, D. J. Lipomi, Intrinsically Stretchable Block Copolymer Based on PEDOT:PSS for Improved Performance in Bioelectronic Applications. *ACS Appl Mater Interfaces* **14**, 4823–4835 (2022).
 19. S. M. Wellman, J. R. Eles, K. A. Ludwig, J. P. Seymour, N. J. Michelson, W. E. Mcfadden, A. L. Vazquez, T. D. Y. Kozai, A Materials Roadmap to Functional Neural Interface Design. doi: 10.1002/adfm.201701269 (2017).
 20. S. M. Won, E. Song, J. Zhao, J. Li, J. Rivnay, J. A. Rogers, Recent Advances in Materials, Devices, and Systems for Neural Interfaces. doi: 10.1002/adma.201800534 (2018).
 21. M. Ganji, L. Hossain, A. Tanaka, M. Thunemann, E. Halgren, V. Gilja, A. Devor, S. A. Dayeh, Monolithic and Scalable Au Nanorod Substrates Improve PEDOT–Metal Adhesion and Stability in Neural Electrodes. *Adv Healthc Mater* **7**, 1800923 (2018).
 22. G. Raos, B. Zappone, Polymer Adhesion: Seeking New Solutions for an Old Problem. *Macromolecules* **54**, 10617–10644 (2021).
 23. A. S. Pranti, A. Schander, A. Bödecker, W. Lang, PEDOT: PSS coating on gold microelectrodes with excellent stability and high charge injection capacity for chronic neural interfaces. *Sens Actuators B Chem* **275**, 382–393 (2018).
 24. A. Inoue, H. Yuk, B. Lu, X. Zhao, Strong adhesion of wet conducting polymers on diverse substrates. *Sci Adv* **6** (2020).
 25. L. Ouyang, B. Wei, C. chen Kuo, S. Pathak, B. Farrell, D. C. Martin, Enhanced PEDOT adhesion on solid substrates with electrografted P(EDOT-NH₂). *Sci Adv* **3** (2017).
 26. G. Dijk, A. L. Rutz, G. G. Malliaras, Stability of PEDOT:PSS-Coated Gold Electrodes in Cell Culture Conditions. *Adv Mater Technol* **5**, 1900662 (2020).
 27. A. Håkansson, S. Han, S. Wang, J. Lu, S. Braun, M. Fahlman, M. Berggren, X. Crispin, S. Fabiano, Effect of (3-glycidyloxypropyl)trimethoxysilane (GOPS) on the electrical properties of PEDOT:PSS films. *J Polym Sci B Polym Phys* **55**, 814–820 (2017).
 28. P. D’Angelo, N. Coppedè, G. Tarabella, A. Romeo, F. Gentile, S. Iannotta, E. Di Fabrizio, R. Mosca, Liquid electrolyte positioning along the device channel influences the operation of Organic Electro-Chemical Transistors. doi: 10.1016/j.orgel.2014.08.029 (2014).
 29. P. D’angelo, G. Tarabella, A. Romeo, S. L. Marasso, A. Verna, M. Cocuzza, C. Peruzzi, D. Vurro, S. Iannotta, PEDOT:PSS Morphostructure and Ion-To-Electron Transduction and Amplification Mechanisms in Organic Electrochemical Transistors. *Materials* **12** (2018).
 30. K. L. Mulfort, J. Ryu, Q. Zhou, Preparation of surface initiated polystyrenesulfonate films and PEDOT doped by the films. *Polymer (Guildf)* **44**, 3185–3192 (2003).
 31. G. Grześ, K. Wolski, T. Uchacz, J. Bała, B. Louis, I. G. Scheblykin, S. Zapotoczny, Ladder-like Polymer Brushes Containing Conjugated Poly(Propylenedioxythiophene) Chains. *Int J Mol Sci* **23**, 5886 (2022).
 32. J. X. Tuermer-Lee, A. Lim, L. Ah, R. Blau, Y. Qie, W. Shipley, L. V Kayser, S. M. Russman, A. R. Tao, S. A. Dayeh, D. J. Lipomi, Synthesis of PEDOT:PSS Brushes Grafted

- from Gold Using ATRP for Increased Electrochemical and Mechanical Stability. *ACS Macro Lett* **12**, 1718–1726 (2023).
33. K. C. Spencer, J. C. Sy, K. B. Ramadi, A. M. Graybiel, R. Langer, M. J. Cima, Characterization of Mechanically Matched Hydrogel Coatings to Improve the Biocompatibility of Neural Implants. *Sci Rep* **7**, 1–16 (2017).
 34. Y. Liu, J. Liu, S. Chen, T. Lei, Y. Kim, S. Niu, H. Wang, X. Wang, A. M. Foudeh, J. B. H. Tok, Z. Bao, Soft and elastic hydrogel-based microelectronics for localized low-voltage neuromodulation. *Nature Biomedical Engineering* **2019 3:1** **3**, 58–68 (2019).
 35. A. Williamson, M. Ferro, P. Leleux, E. Ismailova, A. Kaszas, T. Doublet, P. Quilichini, J. Rivnay, B. Rózsa, G. Katona, C. Bernard, G. G. Malliaras, A. Williamson, A. Kaszas, T. Doublet, P. Quilichini, C. Bernard, M. Ferro, P. Leleux, E. Ismailova, J. Rivnay, G. G. Malliaras, B. Rózsa, G. Katona, Localized Neuron Stimulation with Organic Electrochemical Transistors on Delaminating Depth Probes. doi: 10.1002/adma.201500218 (2015).
 36. J. Rivnay, P. Leleux, M. Ferro, M. Sessolo, A. Williamson, D. A. Koutsouras, D. Khodagholy, M. Ramuz, X. Strakosas, R. M. Owens, C. Benar, J. M. Badier, C. Bernard, G. G. Malliaras, High-performance transistors for bioelectronics through tuning of channel thickness. *Sci Adv* **1** (2015).
 37. J. Goding, A. Gilmour, P. Martens, L. Poole-Warren, R. Green, J. Goding, A. Gilmour, P. Martens, L. Poole-Warren, R. Green, Interpenetrating Conducting Hydrogel Materials for Neural Interfacing Electrodes. doi: 10.1002/adhm.201601177 (2017).
 38. M. Liu, Q. Chen, Characterization study of bonded and unbonded polydimethylsiloxane aimed for bio-micro-electromechanical systems-related applications. <https://doi.org/10.1117/1.2731381> **6**, 023008 (2007).
 39. A. M. Díez-Pascual, Environmentally Friendly Synthesis of Poly(3,4-Ethylenedioxythiophene): Poly(Styrene Sulfonate)/SnO₂ Nanocomposites. *Polymers* **2021, Vol. 13, Page 2445** **13**, 2445 (2021).
 40. B. Yao, H. Wang, Q. Zhou, M. Wu, M. Zhang, C. Li, G. Shi, B. W. Yao, H. Y. Wang, Q. Q. Zhou, M. M. Wu, M. Zhang, C. Li, G. Q. Shi, Ultrahigh-Conductivity Polymer Hydrogels with Arbitrary Structures. *Advanced Materials* **29**, 1700974 (2017).
 41. M. ElMahmoudy, S. Inal, A. Charrier, I. Uguz, G. G. Malliaras, S. Sanaur, Tailoring the Electrochemical and Mechanical Properties of PEDOT:PSS Films for Bioelectronics. *Macromol Mater Eng* **302** (2017).
 42. R. J. J van Daal, J.-J. Sun, F. Ceyssens, al -, N. V Apollo, B. Murphy, B. Thielen, E. Meng, A comparison of insertion methods for surgical placement of penetrating neural interfaces. *J Neural Eng* **18**, 041003 (2021).
 43. R. Vatsyayan, J. Lee, A. M. Bourhis, Y. Tchoue, D. R. Cleary, K. J. Tonsfeldt, K. Lee, R. Montgomery-Walsh, A. C. Paulk, H. Sang, S. S. Cash, S. A. Dayeh, Electrochemical and electrophysiological considerations for clinical high channel count neural interfaces. *MRS BULLETIN • VOLUME* **48** (2023).
 44. M. Ganji, A. Tanaka, V. Gilja, E. Halgren, S. A. Dayeh, Scaling Effects on the Electrochemical Stimulation Performance of Au, Pt, and PEDOT:PSS Electroecorticography Arrays. *Adv Funct Mater* **27** (2017).
 45. S. F. Lempka, P. G. Patil, Innovations in spinal cord stimulation for pain. *Curr Opin Biomed Eng* **8**, 51–60 (2018).

46. T. Koeglsperger, C. Palleis, F. Hell, J. H. Mehrkens, K. Bötzel, Deep brain stimulation programming for movement disorders: Current concepts and evidence-based strategies. *Front Neurol* **10**, 442748 (2019).
47. C. Bohler, F. Oberueber, S. Schlabach, T. Stieglitz, M. Asplund, Long-Term Stable Adhesion for Conducting Polymers in Biomedical Applications: IrOx and Nanostructured Platinum Solve the Chronic Challenge. *ACS Appl Mater Interfaces* **9**, 189–197 (2017).
48. P. O. Osazuwa, C.-Y. Lo, X. Feng, A. Nolin, C. Dhong, L. V. Kayser, Surface Functionalization with (3-Glycidyloxypropyl)trimethoxysilane (GOPS) as an Alternative to Blending for Enhancing the Aqueous Stability and Electronic Performance of PEDOT:PSS Thin Films. *ACS Appl Mater Interfaces* **15** (2023).
49. C. C. H. Petersen, The Functional Organization of the Barrel Cortex. *Neuron* **56**, 339–355 (2007).
50. M. Ganji, A. C. Paulk, J. C. Yang, N. W. Vahidi, S. H. Lee, R. Liu, L. Hossain, E. M. Arneodo, M. Thunemann, M. Shigyo, A. Tanaka, S. B. Ryu, ▽ Seung, W. Lee, Y. Tchoe, M. Marsala, A. Devor, D. R. Cleary, J. R. Martin, H. Oh, V. Gilja, T. Q. Gentner, S. I. Fried, E. Halgren, S. S. Cash, S. A. Dayeh, Selective Formation of Porous Pt Nanorods for Highly Electrochemically Efficient Neural Electrode Interfaces. *Nano Lett* **19**, 6244–6254 (2019).
51. C. M. Proctor, J. Rivnay, G. G. Malliaras, Understanding volumetric capacitance in conducting polymers. *J Polym Sci B Polym Phys* **54**, 1433–1436 (2016).
52. A. Hå, S. Han, S. Wang, J. Lu, S. Braun, M. Fahlman, M. Berggren, X. Crispin, S. Fabiano, Effect of (3-Glycidyloxypropyl)Trimethoxysilane (GOPS) on the Electrical Properties of PEDOT:PSS Films. *J. Polym. Sci., Part B: Polym. Phys* **55**, 814–820 (2017).
53. G. L. Whiting, H. J. Snaith, S. Khodabakhsh, J. W. Andreasen, D. W. Breiby, M. M. Nielsen, N. C. Greenham, R. H. Friend, W. T. S. Huck, Enhancement of Charge-Transport Characteristics in Polymeric Films Using Polymer Brushes. doi: 10.1021/nl051803t (2006).
54. N. Kim, S. Kee, H. Lee, B. H. Lee, Y. H. Kahng, Y.-R. Jo, B.-J. Kim, K. Lee, Highly Conductive PEDOT:PSS Nanofibrils Induced by Solution-Processed Crystallization. doi: 10.1002/adma.201304611 (2013).
55. J. Huang, P. F. Miller, J. C. De Mello, A. J. De Mello, D. D. C. Bradley, Influence of thermal treatment on the conductivity and morphology of PEDOT/PSS films. *Synth Met* **139**, 569–572 (2003).
56. D. A. Schwarz, M. A. Lebedev, T. L. Hanson, D. F. Dimitrov, G. Lehew, J. Meloy, S. Rajangam, V. Subramanian, P. J. Ifft, Z. Li, A. Ramakrishnan, A. Tate, K. Z. Zhuang, M. A. L. Nicolelis, Chronic, wireless recordings of large-scale brain activity in freely moving rhesus monkeys. *Nature Methods* **2014 11:6 11**, 670–676 (2014).
57. H. Lee, R. V. Bellamkonda, W. Sun, M. E. Levenston, Biomechanical analysis of silicon microelectrode-induced strain in the brain. *J Neural Eng* **2**, 81 (2005).
58. P. J. Rousche, D. S. Pellinen, D. P. Pivin, J. C. Williams, R. J. Vetter, D. R. Kipke, Flexible Polyimide-Based Intracortical Electrode Arrays with Bioactive Capability. *IEEE Trans Biomed Eng* **48**, 361 (2001).

Conclusion

We are entering what can be considered the golden age of neural interfaces as more interest and effort is put into delivering neuromodulation therapies to the clinic and everyday consumers. SCS for treatment of chronic and neuropathic pain, the first major application of a spinal cord neural interface, is already available clinically, and future advances will continue to improve the efficacy of this technology. The treatment of SCI with SCS is anticipated to evolve rapidly in the coming years as more studies report some degree of functional restoration in humans and companies form to leverage this technology. This progress takes place against the backdrop of numerous emerging therapies and applications for neuromodulation and neuromonitoring. I believe that adopting flexible, high-channel count microelectrode arrays is necessary to maintain this momentum and will ultimately be the norm in clinical settings. In this dissertation, we develop high-channel count microelectrodes for various spinal cord applications and evaluate the advantages of these devices over existing clinical neural interfaces.

In Chapter 1, we develop a high-density, thin film microelectrode array for high-resolution neuromonitoring of the human spinal cord and evaluate its performance in a clinical setting. We demonstrate that our microelectrode captures detailed spatiotemporal patterns of responses and is more sensitive to neural activity compared to the clinical IONM system. Our microelectrode array captures SSEPs at lower stimulation currents and requires only a few seconds of recorded data to generate reliable, high-quality spatiotemporal maps, while current clinical equipment requires data segments of multiple minutes and does not offer spatiotemporal features. Our implementation is a large improvement in responsiveness of the clinical system that could allow for quicker detection of neurological damage. Overall, this study shows that microelectrodes may improve the safety and effectiveness of neurosurgical procedures.

In Chapter 2, we introduce SpineWrap, a 6.6 μm thin film, 156-channel high-density microelectrode array for targeted EES. We demonstrate that SpineWrap can deliver ultra-focal EES to the rat lumbar spinal cord resulting in highly selective recruitment of limb muscles and enabling the location of stimulation hotspots for highest selectivity. We evaluate the impact of channel count on muscle recruitment selectivity and show that, in rats, high-density arrays require submillimeter pitches to achieve maximum achievable selectivity. This study suggests that SpineWrap can improve the efficacy of EES stimulation for treatment of SCI by delivering more focal stimulation compared to clinical paddle arrays and previously developed EES microelectrode arrays. High-channel count microelectrode arrays such as SpineWrap could reproduce sophisticated patterns of spatiotemporal spinal cord activity with fine-tuned stimulation and generate highly refined muscle movements. Consequently, the adoption of SpineWrap in EES treatment has the potential to push the boundaries of current EES technology and offer more efficient therapies.

In Chapter 3, we develop a novel PEDOT:PSS coating, termed block-brush PEDOT, synthesized on gold with surface-initiated atom transfer radical polymerization of PSS copolymer brushes. We apply this PEDOT coating to flexible, polyimide-based microelectrode arrays and consistently produce uniform, conductive, and stable films. Results from bulk film and microelectrode array characterization show that the block-brush film has superior electrical properties compared to the commonly used spin-coated film and is on par with coating materials with high CIC, such as PtNR. We perform neural recording with these microelectrode arrays and show the block-brush PEDOT can capture multi-frequency neural signals. The synthesis method of block-brush PEDOT allows for the modulation of polymer brush segments, density, or

composition as one chooses, thus creating possibilities for a microelectrode array coating with customizable chemical modifications.

In summary, this dissertation introduces novel high-density spinal cord microelectrode arrays for stimulation and recording and showcases their ability to perform high-resolution functional mapping and targeted EES. This dissertation also presents the development and application of an enhanced, stable PEDOT electrode coating for microelectrode arrays. The work presented in this dissertation brings flexible, high-channel count microelectronics a step closer to becoming the standard for spinal cord neural interfaces.