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Localized Stimulation and Recording in the Spinal Cord with Microelectrode Arrays

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Abstract— **The use of microelectrodes for both recording and stimulation of cortical tissue is a well-established technique in neuroscience. We demonstrate that the use of existing microelectrode arrays and instrumentation can be extended to studying the spinal cord. We show that microelectrode arrays can be used to perform stimulation and recording in the corticospinal tract of an animal model commonly used in spinal cord injury (SCI) research. This technique could not only provide fundamental insights into the structure and function of the spinal cord, but also ultimately serve as the basis of a therapeutic treatment for severe spinal cord injuries.**

I. Introduction

Microelectrode arrays consist of miniaturized electrodes fabricated using semiconductor manufacturing technology [1]. They are well established as a neuroscience research tool for studying neural activity in the brain both *in vitro* and *in vivo*. Their use was pioneered in the United States by Ken Wise's group at the University of Michigan [2] and are now manufactured and sold by a variety of vendors and research centers around the world (University of Utah array, University of Michigan array, Aleva Inc., IMEC Inc., among others). Microelectrodes have become a standard tool for neural recording in neuroscience and neurotechnology. Initial work in this area involved electrodes made of silicon, the dominant material in traditional semiconductor manufacturing, although it has issues with flexibility and the potential not to contour to the structure being recorded or stimulated [3]. Other groups focused on using biologically compatible materials e.g. platinum and iridium-platinum electrodes, and also flexible substrates like polyimide [4]. Original electrode designs were 2-dimensional in nature, either a single column with multiple contacts [5] or a 2-D array of single contact electrodes [6]. Recently 3-D arrays have come to the market, for example, IMTEC led the European Union's Neuroprobes program that developed a novel three-dimensional microelectrode array for use in brain research and cortical stimulation. Unlike the original Utah arrays, which consist of a two dimensional array of stalks and each stalk with one electrode, each stalk on an IMEC array has multiple independent electrodes along its axis, thus providing true 3D coverage [7, 8].

Use of these micro-electrode arrays has been centered on cranial research. Scientific, translational, and clinical research involving the spinal cord is an important medical area, as no restorative therapies currently exist for rehabilitation of patients suffering from paraplegia and quadriplegia resulting from a severe spinal cord injury (SCI). Several groups have used microwires implanted into the spinal cord as a method to study the anatomy and function of the spinal cord. For example, previous research in animals has shown that microwires can be used to perform localized stimulation in the spinal cord to demonstrate independent activation of cat hind limb muscles and also functional combination activation, and has gone as far to demonstrate this intraspinal microstimulation (ISMS) technique to induce walking in cats and rats that have had their spinal cords transected [4, 9-16]. Others have demonstrated that microelectrode recording arrays can be implanted in the spinal cord of primates and used to resolve efferent signals in the cord [17-23].

Microwire-based stimulation and recording has been demonstrated to be a useful tool for spinal cord research, but the cumbersome nature of the implant procedure has limited its use outside of the few research groups that pioneered the work. Using a microelectrode array instead of a microwire for spinal cord recording and stimulation would offer considerable practical advantages. Most importantly, the electrode array would not need to be placed as precisely as a single microwire. Instead, the array could be inserted in the approximate area of interest, and then using the properties of field recording and/or stimulating to localize the area of interest. This would not just simplify the procedure but substantially reduce the risk of accidental damage to the cord caused by repositioning and/or reinsertion of a microwire. Another potential benefit of using an array is the expected robustness of the system to electrode dislocations caused by micromovements. The anatomy of the spinal cord (located behind vertebrae), the presence of the pia/arachnoid – a thin membrane outer layer around the cord - and the greater susceptibility of the spinal cord to serious damage, all make surgical implantations of neural recording and stimulation electrodes in the spinal cord a non-trivial but potentially achievable task.

II. Experimental Design

A. Overview

The goal of this study was to demonstrate the use of the IMEC multi-electrode array for stimulation and recording in a cat spinal cord. An experimental protocol was approved by the Lahey Clinic Institutional Animal Care and Use Committee (IACUC).

The specific aims were to demonstrate

1) Repeatable recording, from the microelectrode array, of near field potentials primarily from within or near the

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lateral corticospinal (CS) tract in response to rostral stimulation in the CS tract via micro-wires.

2) Activation of peripheral limb muscles both repeatably and independently through the stimulating microelectrode array in the conus region of the cat.

The first tests are to record from the microelectrode arrays implanted into the CS tract in the thoracic spinal cord of a cat. The stimuli were generated via microwires implanted in the cervical CS tract of the cat. The micro-wire placement was confirmed via EMG recordings in the hind limb muscles of the animal. The second tests were designed to stimulate an array placed in the conus medialus of the cat. This electrode was placed after sectioning the cord at the T5 level. Stimulation was then applied to each electrode independently. The animal was then sacrificed and the spinal record removed for gross pathological analysis of tissue injury.

The microelectrode arrays used in these studies, shown in Fig. 1, were provided by Interuniversity Microelectronics Centre (IMEC), Leuven, Belgium.

B. Surgical Protocol

At the start of the implant procedure each animal was placed under general anesthesia and put in the prone position. Electromyography (EMG) electrodes were placed in the following hind limb muscles: 1) Biceps Femoris; 2) Gastrocnemius; 3) Tibialis Anterior; 4) Sartoris; 5) Gracilis; 6) Extensor Digitorum Longus.

The animal's spine was exposed using standard surgical techniques with incisions and laminectomies performed at the C3 - C5, T3 – T5 level and the L4-L6 levels (lumbar enlargement area of the cat).

The dura overlying the spine at the thoracic T4 level was opened and secured using standard surgical techniques. The recording electrode was then inserted. The electrode array was inserted using moderate but steady rate (> 1 cm/sec) and relatively constant pressure. After electrode insertion the connector wire was secured using a strain relief made by putting a small smooth bend in the adapter wire which was then sutured to nearby tissue, and a second onto the skin for the duration of the experiment. There was minimal intraspinal bleeding during this procedure. The other end of the adapter wire was attached to the cable from the recording system.

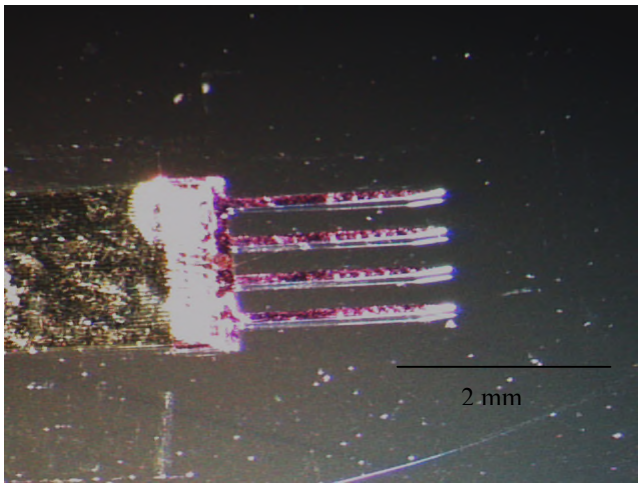

Fig. 1. Photograph of a 3D microelectrode array (MEA) of the type used in this study. This array was developed and validated by the European research group IMEC. Each vertical shaft has eight individual electrodes, for a total of thirty-two electrodes. Each vertical shaft is 2mm in length.

During surgical exposure, the electrodes were prepared per manufacturer guidelines. The impedance of each electrode on the implanted arrays was measured at 1000 Hz referenced to a stainless steel lead placed in the same normal saline bath. An array was considered acceptable as long as more than 90% of the electrodes on the array were within manufacturer specifications.

After all laminectomies and dural openings were performed, an array was placed in the corticospinal tract at the T5 level. The impedance of each electrode on the array was tested to insure it was not damaged during the implant procedure. The electrode was then connected to the recording system (Plexon, Omniplex). Microwires were then placed in the cervical CS region and stimulation was initiated.

At the point where EMG activity was noted in any EMG channel, recording from the array was begun. A series of 10 stimulation pulses were applied with a 5 second separation between each. After this test the polarity was reversed and a second set of 10 stimulation pulses were applied. All data was recorded and analyzed off-line. During these tests, array recording was only performed when an EMG response was noted, future studies will need to look at recordings when the stimulus is set below motor threshold.

A second array was implanted in the lumbar enlargement area. The impedance of each implant was also measured prior to implantation, similar to above. The array was implanted on one side of the conus in the area of Rexed laminae VIII and IX. Once the electrode arrays were determined to be acceptable, stimulation testing was performed. This consisted of sending a train of 5 stimuli through each contact using an amplitude from 1 μA to 150 μA. The threshold of any EMG activation was noted in identified muscle groups.

The arrays consisted of four 2 mm stalks separated by 0.5 mm with eight 35 μm IrO electrodes. The separation between each electrode was 250 μm (fig. 1).

Results and Discussion

The microwire placement in the corticospinal tract of the cervical spinal cord was able to elicit compound muscle action potentials (CMAP) in the Extensor Digitorum Longus at 20 μA for position 1 and the anterior Tibialis at 15 μA for position 2. Prior to placement of the first electrode, the impedance of each electrode was measured in saline. Of the thirty-two electrodes, three were found to have impedances greater than 5 MΩ and thus considered unusable. The mean impedance was 162.4 ± 18.0 kΩ. Small axonal responses were recorded at a latency of 816 μs (measured from the start of the first stimulus artifact to the start of the response) in the first array (figure 3). Given the 6 cm distance between

Fig. 2. Photo taken during a surgical implantation of a microelectrode array being inserted into the spinal cord of a cat. Center of photo shows the array, connected to flex cable, held by forceps under the operating microscope. Note that the array consists of four stalks, with eight individually addressable electrodes on each stalk.

the stimulation electrodes and the recording array and an estimated conduction velocity in the feline spinal cord of 69.61 m/sec, these responses correspond to the appropriate area. The peak-to-peak amplitudes varied from 87.5 µV to 423.8 µV with a mean amplitude of 130 µV. Upon removal of the electrode from the recording area the impedance was tested again. The mean impedance at this time was 131.1 ± 37.3 kΩ with five electrodes having impedances over 5 MΩ.

Stimulation in the conus region was able to elicit CMAPs in lower limb muscles in all working electrodes. Stimulation amplitudes varied from 5 µA to 90 µA. Responses varied from a single muscle activated to multiple muscles activated. It was difficult to determine if responses were directly from stimulation of nerve root or gray matter in these experiments.

The microelectrode array used in this study (Fig. 2) consisted of four stalks, each with eight individually addressable electrodes. The following patterns, indicating differential stimulation based on the location of the different microelectrodes, were observed:

- Stalk 1 – Primary extensor Digitorum Longus with Sartorius and Gracilis coming in as the stimulation moved more ventral eventually stimulating the root with the most peripheral electrode.
- Stalk 2 – Sartorius and Gracilis at the most distal electrodes with anterior Tibialis and gastrocnemius as the electrodes moved more ventral.
- Stalk 3 – The most distal electrodes activated the Gracilis muscles while the most peripheral electrodes activated the Extensor Digitorum Longus, and Gastrocnemius muscles.
- Stalk 4, which had all bad electrodes, stimulated the Biceps Femoris in the middle.

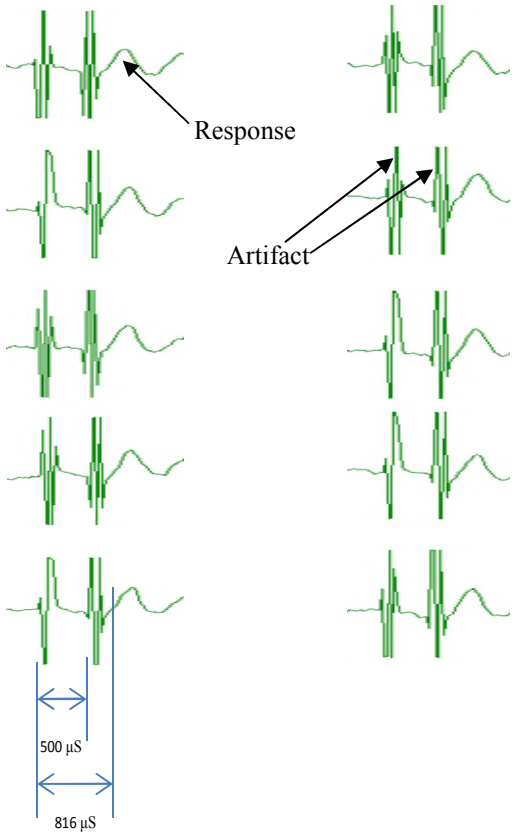

Fig. 3. The stimulation and response waveforms recorded from the array corresponding to 10 stimulations. Data shown is from a single channel, namely Channel 8. The initial two sharp pulses are the artifact from the 500 µs stimulation pulse. The response at around 815 µSec after the start of the initial stimulation artifact is the axonal response. The response time is the same in all cases demonstrating the lack synapses between the stimulation and response. For this particular animal the distance between the stimulation and the response was 6 cm giving a conduction velocity of 69.61 m/s.

In this study we were investigating the acute recording properties of the microarray in the spinal cord. Careful observation over a 30 min period demonstrated repeatable recording and stimulation properties. Since durability is also a key issue for these tests, we were able to demonstrate that the electrode could be explanted and re-implanted multiple times without much degradation. We believe that the two electrodes that failed the second impedance test may have become damaged from the electrode hitting the side of the saline container during testing rather than from forces imparted during implantation, implant, or explanation. The noise floor of the electrodes was on the order of 0.145 µV for the worst channel and 0.04 µV for the cleanest channel. All channels but two were in the 0.04 µV range. These values fall close to the recorded values, but the responses were easily distinguishable from the background and out of sync with the oscillating noise.

III. Conclusion

We have conducted an intra-spinal recording and stimulation experiment in a cat using commercially available microelectrode arrays. Our analysis has demonstrated that spinal micro-recording and micro-stimulation can be achieved with a high degree of spatial localization. This technique will make it possible both to record neural activity related to muscle movements and to stimulate those muscle groups. Our approach may facilitate an improved understanding of neuromuscular activity and lead to diagnostic and therapeutic applications.

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References

[1] J. Principe, J. C. Sanchez, and J. Enderle, *Brain-Machine Interface Engineering*: Morgan & Claypool Publishers, 2007.

[2] A. Starr, K. D. Wise, and J. Csongradi, "An evaluation of photoengraved microelectrodes for extracellular single-unit recording," *IEEE Trans Biomed Eng,* vol. BME-20, pp. 291-293, Mar. 2007.

[3] S. Takeuchi, T. Suzuki, K. Mabuchi, and H. Fujita, "3D flexible multichannel neural probe array," *J. Micromech. Microeng.,* vol. 14, pp. 104-107, Jan. 2004.

[4] V. K. Mushahwar and K. W. Horch, "Selective activation and graded recruitment of functional muscle groups through spinal cord stimulation," *Annals NY Acad Sci,* vol. 860, pp. 531-535, Nov. 1998.

[5] R. J. Vetter*, et al.*, "Chronic neural recording using silicon-substrate microelectrode arrays implanted in cerebral cortex," *IEEE Trans Biomed Eng,* vol. 51, pp. 896-904, Jun. 2004.

[6] E. M. Maynard, C. T. Nordhausen, and R. A. Normann, "The Utah intracortical Electrode Array: a recording structure for potential brain-computer interfaces," *Electroencephalogr Clin Neurophysiol,* vol. 102, pp. 228-239, Mar. 1997.

[7] H. P. Neves*, et al.*, "Development of modular multifunctional probe arrays for cerebral applications," in *IEEE/EMBS Conf. Neural Eng.*, Kohala Coast, HI, USA, 2007, pp. 104-109.

[8] A. A. A. Aarts*, et al.*, "A 3D slim-base probe array for in vivo recorded neuron activity," in *IEEE Eng. Med. Biol. Conf.*, Vancouver, BC, Canada, 2008, pp. 5798-5801.

[9] V. K. Mushahwar and K. W. Horch, "Muscle recruitment through electrical stimulation of the lumbo-sacral spinal cord," *IEEE Trans. Rehabil. Eng.,* vol. 8, pp. 22-29, Mar. 2000.

[10] V. K. Mushahwar and K. W. Horch, "Selective activation of muscle groups in the feline hindlimb through electrical microstimulation of the ventral lumbo-sacral spinal cord," *IEEE Trans. Rehabil. Eng.,* vol. 8, pp. 11-21, Mar. 2000.

[11] S. Yakovenko*, et al.*, "Spatiotemporal activation of lumbosacral motoneurons in the locomotor step cycle," *J Neurophysiol,* vol. 87, pp. 1542-1553, Mar. 2002.

[12] V. K. Mushahwar, D. M. Gillard, M. J. Gauthier, and A. Prochazka, "Intraspinal micro stimulation generates locomotor-like and feedback-controlled movements," *IEEE Trans Neural Syst Rehabil Eng,* vol. 10, pp. 68-81, Mar. 2002.

[13] S. Snow, K. W. Horch, and V. K. Mushahwar, "Intraspinal microstimulation using cylindrical multielectrodes," *IEEE Trans Biomed Eng,* vol. 53, pp. 311-319, Feb. 2006.

[14] R. Saigal, C. Renzi, and V. K. Mushahwar, "Intraspinal microstimulation generates functional movements after spinal-cord injury," *IEEE Trans Neural Syst Rehabil Eng,* vol. 12, pp. 430-440, Dec. 2004.

[15] L. Guevremont*, et al.*, "Locomotor-related networks in the lumbosacral enlargement of the adult spinal cat: activation through intraspinal microstimulation," *IEEE Trans Neural Syst Rehabil Eng,* vol. 14, pp. 266-272, Sep. 2006.

[16] V. K. Mushahwar, D. F. Collins, and A. Prochazka, "Spinal cord microstimulation generates functional limb movements in chronically implanted cats," *Exp. Neurol.,* vol. 163, pp. 422-429, Jun. 2000.

[17] E. V. Goodall and K. W. Horch, "Separation of action potentials in multiunit intrafascicular recordings," *IEEE Trans Biomed Eng,* vol. 39, pp. 289-295, Mar. 1992.

[18] K. Yoshida and K. Horch, "Selective stimulation of peripheral nerve fibers using dual intrafascicular electrodes," *IEEE Trans Biomed Eng,* vol. 40, pp. 492-494, May 1993.

[19] K. Mirfakhraei and K. Horch, "Classification of action potentials in multi-unit intrafascicular recordings using neural network pattern-recognition techniques," *IEEE Trans Biomed Eng,* vol. 41, pp. 89-91, Jan. 1994.

[20] T. G. McNaughton and K. W. Horch, "Action potential classification with dual channel intrafascicular electrodes," *IEEE Trans Biomed Eng,* vol. 41, pp. 609-616, Jul. 1994.

[21] J. W. Little, "Serial recording of reflexes after feline spinal cord transection," *Exp. Neurol.,* vol. 93, pp. 510-521, Sep. 1986.

[22] J. S. Riddell and M. Hadian, "Field potentials generated by group II muscle afferents in the lower-lumbar segments of the feline spinal cord," *J Physiol,* vol. 522, pp. 97-108, Ja. 2000.

[23] T. Yamamoto*, et al.*, "Spinal cord responses to feline transcranial brain stimulation: evidence for involvement of cerebellar pathways," *J Neurotrauma,* vol. 7, pp. 247-256, Winter 1990.