33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Single- and Multi-Unit Activity Recorded from the Surface of the Dorsal Root Ganglia with Non-Penetrating Electrode Arrays

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***Abstract*—Non-penetrating surface electrode recording techniques are typically associated with field potential recordings, while extracellular recordings from single neurons are made using penetrating metal wire or microfabricated microelectrode arrays. Here, we report on single- and multi-unit neuronal recordings made using non-penetrating electrodes placed on the epineural surface of the dorsal root ganglia (DRG). Across four experiments in anesthetized cats, approximately 40% of the electrodes recorded single- and multi-unit spiking activity with spike-rates that covaried significantly with hindlimb movement. In two intraoperative experiments in humans, compound activity was recorded from the DRG surface in response to peripheral stimulation of the common peroneal nerve. This approach may have advantages over penetrating electrode arrays in terms of clinical acceptability and recording longevity.**

I. Introduction

NEURAL recordings using penetrating microelectrodes have been a mainstay of neurophysiology research for decades. In fact, developments in penetrating electrode technology including Hubel's tungsten microelectrode [1] and the silicon microelectrode array [2] have been primary enablers of advancements in neurophysiology and systems neuroscience. In the cerebral cortex, penetrating microelectrodes are required to record activity of single neurons as the cells of interest are generally located 1-2 mm below the cortical surface. Alternatively, electrodes placed on the surface of the cortex are typically used to record field potentials, which are associated with synaptic activity in large groups of neurons, rather than the spiking activity of single cells. However, in other regions of the nervous system, penetrating microelectrodes may not be necessary to detect the activity of single neurons. The cell bodies of primary afferent neurons are located in the dorsal root ganglia (DRG) and many tend to be located near the surface of the DRG, which is surrounded by a relatively thin epineurium. This presents the possibility that non-penetrating electrodes placed on the DRG surface could record action potentials associated with individual neurons in a similar manner to penetrating microelectrode arrays [3].

The primary aim of this this study was to determine if non-penetrating microelectrodes placed on the surface of the DRG could record spikes from individual primary afferents using commercially available, non-penetrating electrodes. Neural recordings were made from the surface of lumbar DRG of anesthetized cats during passive movement of the hindlimb. The results demonstrate that single- and multi-unit activity can be detected with good signal-to-noise ratios and that the spiking activity was strongly modulated by leg motion. Experiments in humans undergoing instrumented lumbar fusions demonstrated that evoked potentials could be recorded during peripheral nerve stimulation.

Manuscript received April 15, 2011. This work was supported in part by the NIBIB Grant 1R01-EB007749.

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II. Methods

Acute experiments were performed in four anesthetized cats in conjunction with other experiments occurring in the laboratory. All animal experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Two experiments were conducted in human subjects undergoing instrumented spinal fusions. Human experiments were approved by the University of Pittsburgh Institutional Review Board.

A. Animal Procedures

Adult cats (3–5 kg) were anesthetized with a ketamine-xylazine mixture and maintained on 1%-2% isoflurane for the duration of the experiment. Blood pressure, ECG, core body temperature, oxygen saturation and end-tidal CO_2 were monitored continuously throughout the experiment. Body temperate was maintained near 37 °C using warm water heating blankets. At the end of each experiment, the animals were euthanized with a 5 mg/kg dose of potassium chloride.

A laminectomy was performed to expose the spinal cord and the sixth and seventh lumbar (L6 and L7) DRG. LED markers were placed on the skin over the iliac crest and the hip, knee, ankle and metatarsal-phalangeal joints. Hindlimb kinematics were acquired using a 6-camera motion tracking system (PhaseSpace Inc., San Fransisco, CA) at 120 Hz. The left hindpaw was fixed to an industrial robot (VS-

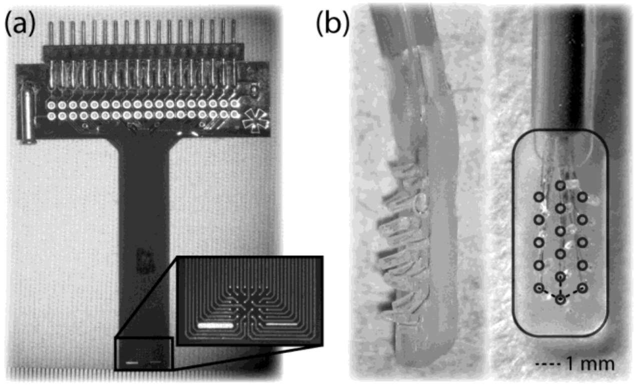

Fig. 1. Photographs of the two types of non-penetrating electrodes used in these experiments. (a) MCS electrode. (b) PMT electrode.

6556E/GM, DENSO Robotics, Long Beach, CA) that generated either center-out or ramp-and-hold movements. Neural data were acquired using either an RZ2 (Tucker-Davis Technologies, Alachua, FL) or Grapevine (Ripple LLC, Salt Lake City, UT) recording system.

B. Human Procedures

During instrumented spinal fusion surgeries to treat pain and instability, several DRG are frequently exposed. This provides an opportunity to briefly place non-penetrating surface electrodes on the DRG in human subjects. Micro Electrode Arrays (PMT Corp.) were placed between the lumbar DRG and the wall of the spinal canal. Neural data were recorded during 2.35 Hz electrical stimulation of the common peroneal and posterior tibia nerves using an XLTEK Protector (25-50 mA; percutaneous leads). Neural signals were acquired using the Grapevine system.

C. Electrodes

In the cat experiments, EcoFlexMEA36 (Multi Channel Systems Gmbh, Reutlinger, Germany) and Micro Electrode Array (PMT Corp., Chanhassen, MN) electrodes were used (referred to as the MCS and PMT electrodes hereafter). In the human procedures the FDA approved PMT electrodes were used. Fig. 1 shows photographs of each of the electrode arrays. The MCS array contains 32 contacts on a 300 μm grid as well as two ground and two reference electrodes. The recording sites are 50 μm in diameter and are patterned on a 50 μm thick polyimide substrate. PMT arrays were selected for use because these arrays are FDA approved for temporary recording of electrical signals on the surface of the brain. The device consisted of two leads, each terminating in an array of 16 electrodes with a 1 mm interelectrode spacing (see Fig. 1(b)). Individual electrodes were made from 50 μm diameter platinum wires bent at a right angle and embedded in a thin silicone substrate.

D. Data Analysis

For the data collected from the cat experiments, manual spike sorting was performed using Open Sorter (Tucker-Davis Technologies) or Offline Sorter (Plexon Inc.). For each identified unit, the signal-to-noise ratio (SNR) was calculated by dividing the averaged maximum spike amplitude by three times the standard deviation of the filtered data. Units were classified as exhibiting single-unit activity if no more than 1% of the interspike-intervals (ISI) were less than 2.5 ms. This prevented false rejections of single-units due to potential errors in spike sorting.

To determine whether movement of the hindlimb modulated the activity of an identified unit, multiple linear regression was used. Six kinematic parameters (angular position and velocity of the hip, knee and ankle) were used as the independent variables in the regression equation. Smoothed firing rates were calculated using a Gaussian kernel with a 100 ms bin width. The coefficient of determination (R2) was calculated from the regression between the smoothed firing rate and the kinematic variables and used as a metric to evaluate how much the firing rate of a given unit was modulated by hindlimb movement. In general, units with R2 values less than 0.1 are described as not being modulated by movement of the hindlimb. All data analysis was conducted using Matlab.

III. RESULTS

The primary result of this study is that modulated single- and multi-unit neural activity was recorded from the surface of DRG using non-penetrating electrodes in all four animals. In the human studies, single- and multi-unit activity were not detected, but compound action potentials arising from peripheral nerve stimulation were found.

A. Animal Experiments

Fig. 2 shows examples of the kinds of activity that were recorded in the animals during movement of the hindlimb imposed by the robot. In all cases, the regression analysis indicated significant modulation resulting from movement of the hindlimb. Fig. 2(a) and (c) show examples of single-unit recordings from the MCS and PMT electrodes as identified by the ISI histograms. Of all the units recorded, Fig. 2(c) had the highest amplitude (~ ±100 μV) which is typical of the types of signals recorded with penetrating electrodes. The responses in Fig. 2(b) and (d) are indicative of the types of multi-unit activity that were most frequently recorded.

On average, approximately 50% of the electrodes on the MCS array and 70% of the electrodes on the PMT arrays recorded neural activity that was not modulated by movement of the hindlimb (R2 < 0.1, see Fig. 3(a)). However, 25% of the MCS array electrodes and 10% of the PMT array electrodes recorded neural activity that was well modulated by movement of the hindlimb (R2 > 0.25, see Fig. 3(a)). In one particular experiment, more than half of the electrodes on an MCS array had R2 values > 0.5. This suggests that if the electrode array is well placed, the majority of electrodes can record neural activity that is highly modulated by hindlimb movement. Fig. 3(b) summarizes the number of units recorded per electrode in all of the trials. With both array types, there were approximately 1.1 identified units per electrode and in some cases this number was as high as 1.4 units per electrode.

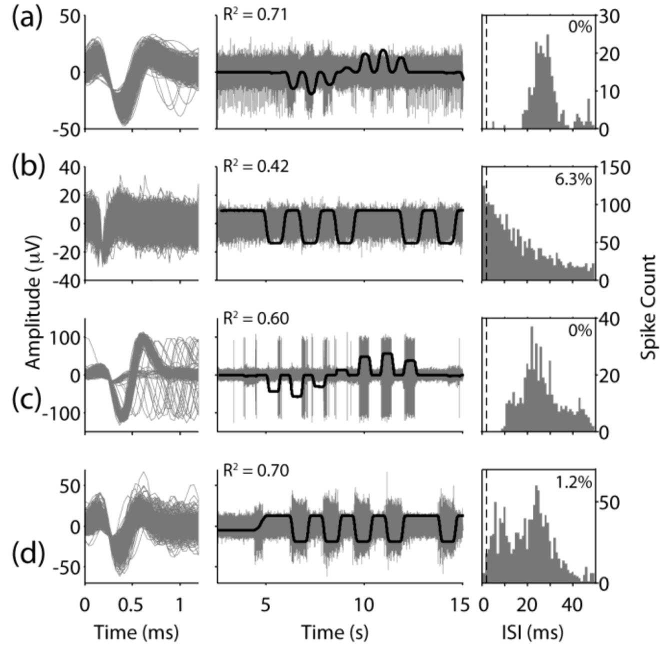

Fig. 2. Examples of neural recordings from the surface of the DRG using non-penetrating electrodes. (a) Well isolated single-unit activity. (b) Poorly isolated multi-unit activity that is still modulated by hindlimb movement. (c) The best example recorded of a single-unit response. (d) A multi-unit response that was highly modulated by hindlimb movement. (a) and (b) were recorded using the MCS array while (c) and (d) were recorded using PMT arrays. Individual spike waveforms are shown in the first column, raw data for a portion of the recording trial are shown in the second column, and the ISI histograms for the unit are shown in the last column. In the second column, the hip-to-foot distance is shown (thick black trace) as a representative kinematic parameter to demonstrate the correspondence between the observed modulation in the recordings and the hindlimb kinematics. The R2 value from the regression analysis is also shown. In the ISI histograms, the number in the upper right corner indicates the percentage of spikes with ISIs less than 2.5 ms (vertical dashed line).

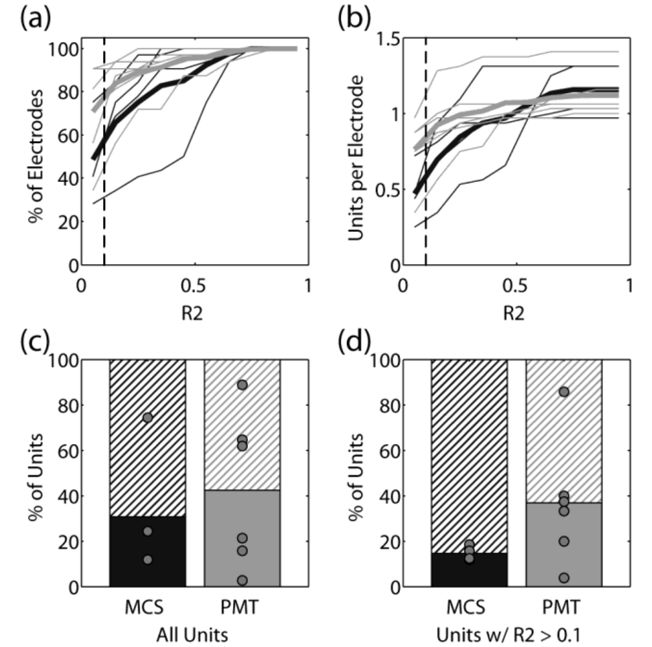

Fig. 3. Summary of the ability of non-penetrating surface arrays to record modulated neural activity. Black regions indicate data recorded using the MCS array while gray regions indicate data recorded using PMT arrays. In (a) and (b), thin lines indicate data from individual experiments while thick lines show the averaged result. (a) Cumulative histogram of the percentage of electrodes on a given array that recorded neural activity modulated by the kinematics with different R2 values. For example, 75% of the MCS array electrodes and 90% of the PMT array electrodes recorded activity with an R2 value less than 0.25. (b) Cumulative histogram of the average number of identified units per electrode with different R2 values. For example, on average, there were 0.84 units per electrode with R2 values less than 0.25 on the MCS array. (c) Percentage of all units that exhibited single-unit (solid) and multi-unit (hashed) activity. (d) Percentage of units with R2 > 0.1 that exhibited single-unit (solid) and multi-unit (hashed) activity. In (c) and (d), gray circles indicate individual trial results.

This indicates that in several experiments, up to 45 individual units were identified on a 32 channel array.

On average, 30% of the MCS array units and 40% of the PMT array units were identified as single-units by their ISI histograms (solid area of Fig. 3(c)). However, this percentage was highly variable (gray circles in Fig. 3(c)). Once modulated units are considered (R2 > 0.1), approximately 15% of the MCS array units and 35% of the PMT array units were identified as single-units (solid area of Fig. 3(d)) suggesting that at least some of these single-unit responses were being generated by afferents whose activity was not modulated by movement of the hindlimb.

Fig. 4(a) shows the SNR for every unit recorded across all experiments. The six data points on the right ordinate axes all had SNRs > 3 (max = 8.1). Fig. 4(b) shows that 75% of the MCS array units and 55% of the PMT array units had SNRs between 1 and 1.25. Despite these low SNRs, some units in this group still had high R2 values (up to 0.65). Alternatively, some units with high SNRs were poorly modulated by hindlimb movement (see Fig. 4(a)). Units in this first group (low SNR, high R2) suggest multi-unit recordings from groups of highly modulated afferents while units in the second group (high SNR, low R2) likely represents well isolated single-units from cutaneous afferents that were not modulated by hindlimb movement.

B. Human Experiments

PMT arrays were used to record neural activity from the DRG surface in two human subjects. In both experiments, evoked compound action potentials (CAPs) were recorded from the L4 DRG in response to stimulation of peripheral nerves using percutaneous needle electrodes. Fig. 5 shows examples of DRG recordings from one experiment. Stimulation of the common peroneal nerve at 35 and 50 mA (2.35 Hz) evoked L4 CAPs with increasing amplitudes while stimulation of the posterior tibia did not evoke CAPs, suggesting that the observed activity was not merely stimulus or muscle artifact.

IV. Discussion

This study demonstrates that non-penetrating surface electrodes can be used to record single- and multi-unit neural activity from primary afferent cell bodies located in the DRG. While the overall recording quality may not seem impressive compared to the typical responses achieved with modern penetrating electrode arrays, there is much to be

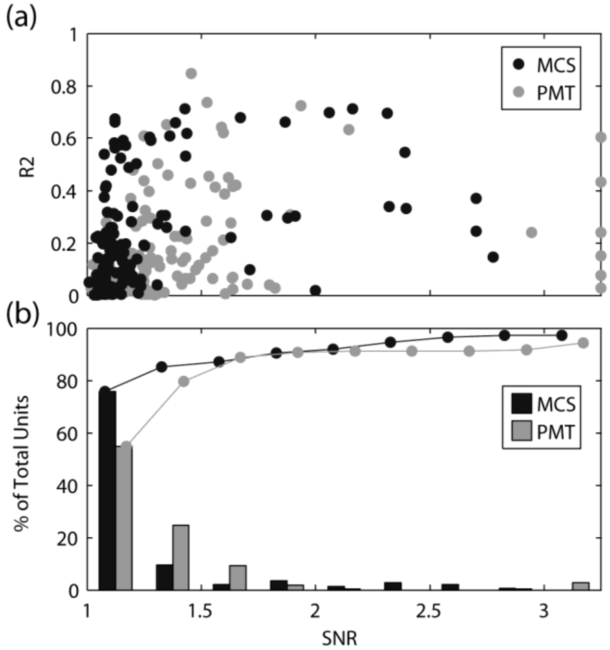

Fig. 4. Summary of the signal-to-noise ratios recorded using non-penetrating surface arrays. (a) For every unit identified, the SNR and R2 value are plotted. Units with SNR > 3 are indicated on the right ordinate axis. (b) Histogram of the SNR for all units from all experiments (bars). The cumulative histogram of the data is also shown (lines).

optimistic about. Initially, we expected to be able to record modulated signals using this approach, but were not expecting to be able to record clear single-unit activity. In contrast to our expectations, single-unit activity was frequently observed, and in some cases, the SNRs were within the range of what is typically recorded with penetrating electrode arrays in the DRG.

No quantitative comparative analysis of the signals recorded using the MCS and PMT arrays was performed. However, the MCS array tended to record modulated activity on more electrodes, while the SNR and percentage of single-units was higher when the PMT arrays were used. It remains to be determined what parameters of a non-penetrating interface should be modified to achieve optimal recording quality.

The investigational human experiments described represent an important step in the translation of this technology to the clinic. While the recordings themselves show little more than evoked activity, we believe that a more rigorous approach to activation of proprioceptive and cutaneous afferents while improving the placement of the electrode intraoperatively will lead to recordings of single- and multi-unit activity from human DRG.

When this set of experiments began, we selected an electrode that was already available in the lab (MCS array) to test feasibility. The selection of the FDA approved PMT electrode was made to provide the simplest path to human recordings. However, both of these electrodes are far from ideal to achieve optimal recordings. For the purposes of recording from the DRG, an electrode with a highly compliant substrate [4] would allow much greater contact between the array and the tissue. A reduction in the impedance of the electrode-tissue interface using surface modification techniques [5], [6] would also likely lead to an improvement in the yield of useful signals.

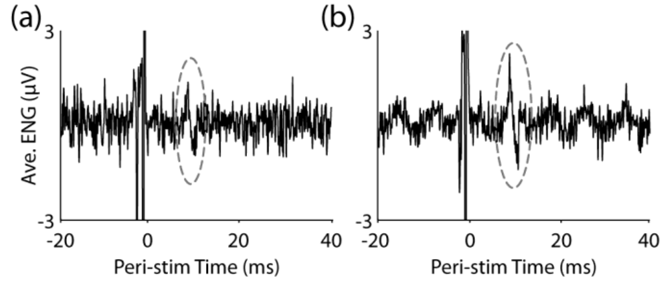

Fig. 5. Averaged electrical stimulation evoked compound action potentials (CAP) recorded from surface of human L4 DRG. (a) L4 CAP evoked by 35 mA stimulation of common peroneal nerve. (b) L4 CAP evoked by 50 mA stimulation of common peroneal nerve.

One of the primary reasons to pursue this approach is to improve the longevity of a neural interface at the DRG. Currently, recording quality in chronic animal experiments degrades over the course of several weeks [3]. This is likely due to electrode-tissue impedance mismatches and movement of the arrays within the DRG. If the recording yield and signal quality can be improved, a non-penetrating surface electrode might form the basis of a more effective neural interface at the DRG than more traditional penetrating microelectrode arrays.

Acknowledgment

The authors would like to thank Ingrid Albrecht, Chris Ayers, Jim Hokanson and Tyler Simpson for their assistance during the animal experiments and with data analysis, Tina Harrison for her assistance with development of the human study Review Board protocol, Cyndi Miklos for help with patient recruitment, and the patients who volunteered for the study.

References

[1] D. H. Hubel, "Tungsten Microelectrode for Recording from Single Units," *Science*, vol. 125, no. 3247, p. 549-550, Mar. 1957.

[2] P. Campbell, K. Jones, R. Huber, K. Horch, and R. Normann, "A silicon-based, three-dimensional neural interface: manufacturing processes for an intracortical electrode array," *IEEE Transactions on Biomedical Engineering*, vol. 38, no. 8, p. 758-768, 1991.

[3] D. J. Weber, R. B. Stein, D. G. Everaert, and A. Prochazka, "Limb-state feedback from ensembles of simultaneously recorded dorsal root ganglion neurons.," *Journal of Neural Engineering*, vol. 4, no. 3, p. S168-80, Sep. 2007.

[4] D. Kim et al., "Dissolvable films of silk fibroin for ultrathin conformal bio-integrated electronics.," *Nature Materials*, vol. 9, no. 6, p. 511-517, Jun. 2010.

[5] S. Venkatraman et al., "In vitro and in vivo evaluation of PEDOT microelectrodes for neural stimulation and recording.," *IEEE Transactions on Neural Systems and Rehabilitation Engineering* , vol. 19, no. 3, p. 307-316, June. 2011.

[6] Y. Xiao, D. C. Martin, X. Cui, and M. Shenai, "Surface modification of neural probes with conducting polymer poly(hydroxymethylated-3,4- ethylenedioxythiophene) and its biocompatibility.," *Applied Biochemistry and Biotechnology*, vol. 128, no. 2, p. 117-130, Feb. 2006.