VALIDATION AND APPLICATION OF A NOVEL HIGH DENSITY ELECTRODE ARRAY FOR USE IN SMALL DIAMETER PERIPHERAL NERVES

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Several neural interfaces have been designed in order to improve access to peripheral nerve fibers and more accurately monitor or evoke various functions. Currently, such devices are more successful in larger nerves, but are limited in potential applications involving smaller target nerves. For example, the restoration of micturition (urination) to spinal cord injured patients requires the ability to monitor the changes in bladder pressure, to gauge bladder fullness as well as detect hyper-reflexive contractions. The pudendal nerve has been shown to be an appropriate site for monitoring bladder status. However, its small diameter, particularly in animal models, and the need for multiple access points required the development and fabrication of a novel high density electrode array. The research presented here describes the validation of such a device in small diameter nerve applications, as well as its use in the pudendal nerve to provide information about bladder pressure, showing the potential expansion of neural interfaces to new applications in both research and clinical fields.

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CHAPTER 1

INTRODUCTION

We often take for granted our ability to perform seemingly trivial tasks in our daily lives. It is not until we lose this ability that we realize its value. In the United States, 1 in 50 people suffer from some form of paralysis, due to stroke, brain injury, spinal cord injury (SCI), or neurological disorder [1]. In addition to the loss of controlled movement, paralysis results in many lesser known challenges, such as the loss of bladder, bowel, or sexual function, or secondary issues, such as cardiovascular complications, mental health problems and difficulties with social integration [2–5]. Additionally, recent surveys have found that the majority of spinal cord injured patients rank bladder and bowel control, as well as sexual function, among the top three priorities to improve quality of life [2]. Research in the field of neural interfaces is dedicated to interacting with the nervous system in hopes of interpreting neural signals and reestablishing control of lost functions. One of the challenges of expanding the range of applications of neural engineering is finding new architectures for better interfacing with the nervous system. The research presented here describes the validation of one new architecture, the High Density Utah Slanted Electrode Array (HD-USEA), in small diameter nerves and its preliminary application in the restoration of bladder control, expanding the range of potential applications of neural engineering.

1.1 Nervous System Physiology

The nervous system consists of the peripheral and central nervous systems. The peripheral nervous system, which consists of nerves innervating the entire body, has both sensory and motor components. Sensory nerve fibers sense changes in the body and send that information to the central nervous system, which consists of the spinal cord and brain,

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via discrete electrical signals, or action potentials. The information is processed in the central nervous system, and control signals are sent back through motor fibers to evoke an appropriate response. When the nervous system sustains damage, such as in a spinal cord injury, the line of communication between the central and peripheral nervous systems can be broken, resulting in a loss of sensory information and motor control of body functions. With the appropriate neural interfaces, however, peripheral nerves can still be used to interpret sensory information and send commands to various organs, potentially restoring functional control.

1.1.1 Peripheral Nerve Anatomy

Peripheral nerves are bundles of nerve fibers, or axons, that each innervates a specific target area. Fibers are organized into smaller bundles, or fascicles, often by their target site of innervation. A cross-section of a peripheral nerve, showing separation of fibers into different fascicles, is shown in Figure 1.1 [6]. Axons can either be motor fibers, sending control signals to an organ, or sensory fibers, sending information back to the central nervous system. A single nerve can contain a combination of such fibers. For example, the pudendal nerve contains both sensory and motor fibers that innervate the external urethral sphincter, the external anal sphincter, and the sexual organs [7, 8]. Therefore, an ideal neural interface would be able to interact independently with different fascicles, if not fibers, so as not to misinterpret information or send signals to the wrong target areas.

1.2 Current Neural Interfaces

Because communication in the nervous system is based on electrical activity, various electrode architectures can be used to record and send signals in peripheral nerves. The design of such electrodes is based on minimizing the invasiveness of electrode implantation while maximizing the selectivity with which signals can be recorded and sent [9]. Electrode architectures can be categorized as either extraneural electrodes, which sit outside the nerve, or intraneural electrodes, which penetrate the nerve and sit closer to the nerve fibers.

1.2.1 Extraneural Interfaces

The simplest form of neural interfaces is a wire with a deinsulated section that is secured next to the nerve. This can be accomplished by attaching such a wire to the

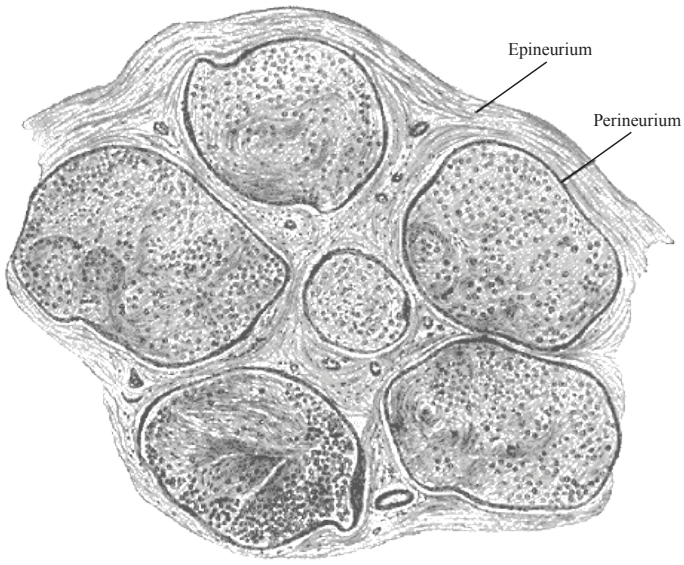

Figure 1.1. Cross-section of a human tibial nerve. Axons are organized into bundles, or fascicles, and separated by connective tissue called the perineurium. The entire nerve is then surrounded by the epineurium [6], public domain.

inside of a flexible cuff. These "cuff electrodes" can then be wrapped around the nerve, enabling the nonselective recording of neural signals. This can be sufficient for detection of simple changes in neural activity, but is limited in accuracy, particularly in nerves that have various types of neurons or multiple sites of innervation. For example, cuff electrodes implanted around the pudendal nerve have been shown to detect similar increases in nerve activity in response to both bladder pressure changes [10] and genital stimulation [11]. To improve selectivity, particularly in terms of delivering stimulation, cuff electrodes have been adapted in various ways. For example, cuff electrodes have been designed to include multiple contacts [12, 13], or to hold the nerve such that fascicles are arranged into a flatter cross-section and are therefore more accessible (Flat Interface Nerve Electrodes, FINEs) [14–16].

1.2.2 Intraneural Interfaces

To further improve the selectivity of neural interfaces, a class of electrodes has been created to penetrate the nerve and reside in much closer proximity to nerve fibers. At the simplest level, single microelectrodes can be implanted inside a nerve fascicle to record action potentials from individual nerve fibers. These electrodes have evolved into longitudi-

nal intrafascicular electrodes (LIFEs), which have been used to successfully record signals from individual fibers, when threaded along a nerve, in both acute [17, 18] and chronic applications [19]. Similarly, transverse intrafascicular multichannel electrodes (TIMEs) were adapted from LIFEs to allow for multiple recording sites across the cross-section of a nerve [20] . Because of the limited number of contacts and single point of penetration, a high degree of accuracy in implantation of each electrode is required to yield useful information [9].

Another approach to providing multiple recording sites within a nerve is to implant an array of electrodes that penetrate into the nerve tissue. One such device, the Utah Slanted Electrode Array (USEA), was developed at the University of Utah to accomplish this [21]. This array consists of 100 electrodes arranged in a 10 x 10 grid on a 4 x 4 mm base, resulting in a spatial density of 6.25 electrodes/mm^2. The length of the electrodes vary from 0.5-1.5 mm in height and allow for a large number of recording sites across the cross-section of a nerve, reducing the need for precision of device implantation. In order to implant this array, a high-velocity pneumatic insertion method was developed, in which arrays are inserted at speeds greater than 8 m/s [22]. Research has shown that this is a useful device for selectively monitoring and sending signals in larger diameter nerves (2-4 mm), such as the feline sciatic and femoral nerves [21, 23–25] in the lower limbs for the control of stance or gait, or the median, ulnar, and radial nerves of monkeys to control coordinated grasp movements [26] . Because of the spatial density of the electrodes, however, the device is limited in its application to target nerves of larger diameters. To address the need for a neural interface in small diameter nerves while maintaining the advantages provided by the USEA, an electrode array of similar architecture, but higher spatial electrode density (25 electrodes/mm^2) was developed (High Density Utah Slanted Electrode Array, HD-USEA, shown with a traditional USEA in Figure 1.2) [27].

1.3 Outline of Research

This thesis presents work used to validate the HD-USEA as a viable device for recording neural activity in small diameter nerves (<1 mm). **Chapter 2** describes the recording of unique neural signals from individual HD-USEA electrodes implanted in a rat sciatic nerve, verifying that viable nerve fibers remain after a high-velocity array implantation.

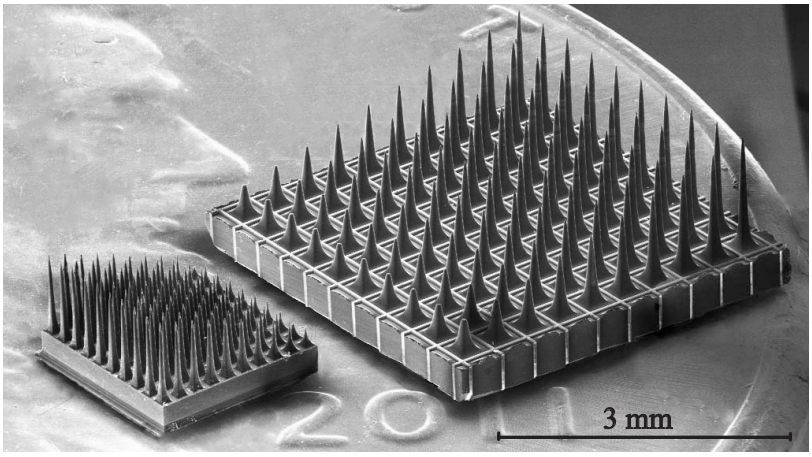

Figure 1.2. Traditional USEA(right) and HD-USEA on top of US penny [27].

In addition, compound action potentials and muscle responses were recorded before and after array implantation, where maintenance of these waveforms was observed after implantation. The results presented support the hypothesis that a high-velocity implantation of HD-USEAs in small diameter nerves does not cause excessive damage to the nerve and enables the monitoring of neural activity in acute experimental settings. This work has in part been reported in an article accepted for publication into the Journal of Neural Engineering [27], and the remaining results have been reported in a paper that has been accepted by Muscle & Nerve for publication [28].

Chapter 3 discusses preliminary research in the expansion of applications of the HD-USEA, in which arrays were implanted in the feline pudendal nerve in order to record neural activity related to changes in bladder pressure. Results show the preliminary success in recording not only neural signals that were relevant to bladder pressure, but also those that were driven by sensory stimulation of the anal sphincter and the genital region. The successful recording of such neural activity from individual HD-USEA electrodes provides preliminary evidence suggesting the potential of the HD-USEA as a device well suited to the access of fibers in the pudendal nerve and for accurately monitoring bladder activity as well as sensory information from the anal sphincter and the sexual organs, ultimately to provide a means of restoring bladder, bowel, and sexual function. These preliminary results have been reported in a paper that was co-written with Heather Wark and has been accepted by Urology for publication [29].

CHAPTER 2

ASSESSMENT OF RAT SCIATIC NERVE FUNCTION FOLLOWING ACUTE IMPLANTATION OF HIGH DENSITY UTAH SLANTED ELECTRODE ARRAYS

High Density Utah Slanted Electrode Arrays (HD-USEAs) have been developed recently for intrafascicular access to submillimeter neural structures. Insertion of such high electrode density devices into small diameter nerves may cause nerve crush injury, counteracting the intended improved selective nerve fiber access. HD-USEAs were implanted into the sciatic nerves of anesthetized rats. Nerve function was assessed before and after HD-USEA implantation by measuring changes in evoked muscle and nerve compound action potentials and single unit neuronal recordings. Neural activity was recorded with over half of all wired, implanted electrodes. Following array implantation, a 38%, and 36%, and 13% decreases in nerve, medial gastrocnemius, and tibialis anterior compound action potential amplitudes, respectively, were observed following array implantation. Only 1 out of 9 implantations resulted in loss of all signals. These studies demonstrate that HD-USEAs provide a useful neural interface without causing a nerve crush injury that would otherwise negate their use in acute preparations (<12 h).

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2.1 Introduction

In the field of neural interfaces, the use of intrafascicular electrodes has allowed for increasingly selective access to peripheral nerve fibers, resulting in lower stimulus intensities and higher signal-to-noise ratios for recording of neural activity [9]. One such device, the Utah Slanted Electrode Array (USEA), contains large numbers of penetrating microelectrodes of varying lengths [21]. The architecture of the USEA provides many access points distributed across a nerve. However, the spatial density of electrodes (6.25 electrodes/mm^2) in the USEA has limited its use to large diameter nerves. To provide comparable nerve fiber access in smaller neural structures, a high density electrode array (HD-USEA) architecture was developed that has 4 times the spatial density of electrodes (25 electrodes/mm^2) as the USEA [27]. Preliminary data have shown that the HD-USEA can provide selective intrafascicular access for both stimulation and recording in small nerves [27], showing unique activations patterns or neural recordings from electrodes only 200 μm apart. However, further studies were warranted to show that the high-velocity pneumatic implantation of such a high electrode density array does not cause excessive nerve crush injury.

We hypothesize that HD-USEA implantation does not cause such damage in small diameter nerves during acute experiments (<12 h). Two paradigms were used to assess changes in nerve function following implantation of the HD-USEA in the rat sciatic nerve (~1 mm diameter). First, afferent neural activity evoked by physical stimulation of the lower limb was recorded from individual electrodes of an implanted array to verify that sensory nerve fibers were intact and functional at the site of array implantation. In the second study, evoked compound action potentials (CAPs) of the nerve and relevant compound muscle action potentials (EMGs) were recorded before and after implantation of the array. Changes in these signals following array implantation were used to quantify nerve damage. These paradigms are comparable to those used to validate the traditional USEA and the nonslanted Utah Electrode Array (UEA) as useful neural tools for intrafascicular stimulation and recording in large diameter nerve preparations, such as the ~4 mm diameter feline sciatic nerve [21, 30, 31]. The results reported here demonstrate that the HD-USEA is a useful neural interface for acute preparations in small diameter peripheral nerves.

2.2 Methods

All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah. Experiments were performed on a total of 9 Sprague-Dawley rats (250-500 g). Anesthesia was induced and maintained using isoflurane (5% and 0.5-2% gas, respectively). Heart rate, respiratory rate, oxygen saturation level, and body temperature were monitored and maintained within normal ranges throughout the experiments.

In all animals, the sciatic nerve was exposed via separation and retraction of the biceps femoris, as reported previously [27]. In 3 animals, an HD-USEA was implanted in the exposed nerve, distal to the muscular branch of the sciatic nerve, using previously reported pneumatic insertion methods [22]. The HD-USEA used in these studies was a 12x12 grid of electrodes with 48 electrodes wired for recording and 4 electrodes wired for use as references (7x10 grid containing wired electrodes). In these animals, sensory fibers were stimulated, either by brushing hairs or skin on the lower limb, or by flexing the foot around the ankle joint. Nerve activity was recorded from wired electrodes on the array for up to 10 hours after implantation.

In the other 6 animals, 2 cuff electrodes were sutured around the nerve: one cuff distal and one proximal to the array implantation site, with 0.5 - 1 cm between the cuffs, as shown in Figure 2.1. Each cuff electrode was constructed from silicone tubing (1.57 mm ID x 2.41 mm OD) (A-M Systems, Inc., Carlsborg, WA). Three stainless steel wires (Model #M146240, California Fine Wire Co., Grover Beach, CA) were sutured into the tubing with approximately 1 or 5 mm spacing for the proximal and distal cuff, respectively. To prevent nerve dehydration, a lubricant (Altaire Pharmaceuticals, Inc., Aquebogue, NY or Novartis Pharmaceuticals Co., East Hanover, NJ) was used to cover the exposed areas of the nerve. CAPs were evoked by delivering cathodic monophasic pulses of varying voltage (0.5 - 10 V) and pulsewidths (20 - 50 μs) through the middle electrode of the proximal cuff using a constant voltage SD-9 stimulator (Grass Technologies, West Warwick, RI), with the return path through the proximal electrode on the same cuff. The responses evoked with varying stimulus parameters were used to determine a supramaximal stimulus intensity. The distal electrode on the proximal cuff was used as a ground. Pairs of stainless steel electrodes (Model #M146240, California Fine Wire Co., Grover Beach, CA)

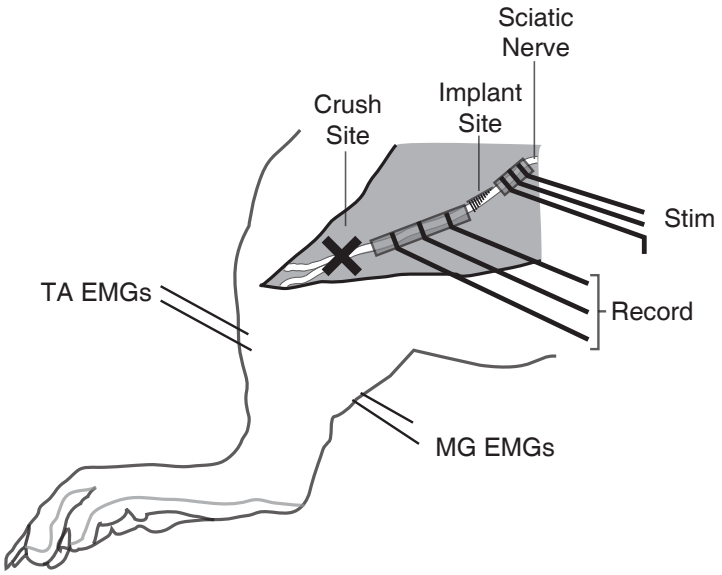

Figure 2.1. Schematic showing experimental set-up for evoking and recording CAPs. Cuff electrodes were used to evoke and record CAPs before and after an HD-USEA was implanted between the cuffs. In two preparations, the nerve was crushed distal to all cuff electrodes (marked with an X). EMGs were also recorded from the TA and MG muscles.

were implanted in the medial gastrocnemius muscle (MG) and the tibialis anterior (TA) muscles. Differential recordings of the CAPs were taken from each electrode on the distal cuff with respect to an Ag/AgCl wire sutured under the animals skin near the surgical site. Ten to fifteen supramaximally-evoked CAPs and differential EMGs were recorded and averaged every 5 to 10 minutes for up to 70 minutes. If these evoked CAP signals did not maintain consistent peak amplitudes for at least 30 minutes, the experiment was aborted. Otherwise, supramaximally-evoked CAP signals and EMGs were recorded for up to 180 minutes after implantation of a nonwired HD-USEA between the two cuffs. Because of the limited available length of the nerve and the size of the cuff electrodes, the placement of the HD-USEA was fairly consistent (within ˜2 mm) over all experiments. The procedure was then repeated on the contralateral nerve. In 1 animal, nerves were crushed distal to the distal cuff after recording evoked CAPs for 30 minutes to determine how much of the observed CAP signal was directly evoked and not due to sensory neural or muscle activity in response to stimulation. The CAP waveform that included both directly

evoked neural activity and activity corresponding to muscle and sensory responses was considered the complex CAP waveform. Three metrics were used to quantify changes in signal transmission following array implantation. First, the amplitude of the CAP waveform was measured as the difference between the peak of the directly evoked CAP and the average of the waveform. Second, the complex waveform was quantified by calculating the root mean square (RMS) of the complex CAP signal. Lastly, peak-to-peak amplitudes were measured from supramaximally-evoked EMG signals. Values for each metric were determined from pre-implantation recordings and averaged to create a pre-implantation baseline; these baseline values were then used as a control and compared to postimplantation values of CAP amplitude, RMS, and EMG amplitudes over time. All data was collected using a Cerebus data acquisition system (Blackrock Microsystems, Inc., Salt Lake City, UT). Analysis was performed using MATLAB (The MathWorks, Inc., Natick, MA), Offline Sorter (Plexon, Inc., Dallas, TX), and Microsoft Excel (Microsoft, Redmond, WA).

2.3 Results

2.3.1 HD-USEA Recordings

For up to 6 hours after implantation of wired HD-USEAs, 119 of the 144 total implanted electrodes (48 electrode per implanted array, n = 3) were able to record either multiunit or single unit activity that increased in response to specific types of physical stimulation. Figure 2.2 shows two neural units recorded by different electrodes on one implanted device that was driven by dorsiflexion (a) and plantarflexion (b), respectively. Recordings could be used to map receptive fields to different electrodes on the implanted array. Figure 2.3b shows the regions on one animal's lower limb that, when stimulated, evoked single-unit activity, and the corresponding electrodes on the implanted array that recorded each unit are shown in Figure 2.3a. In 1 animal, only single units that were recorded immediately after implantation were monitored over the duration of the experiment. In that experiment, 4 of the 8 units originally observed remained over 6 hours. In the other 2 experiments, 61 of the 96 implanted electrodes continued to record neural activity more than 6 hours after implantation. Of the 26 total electrodes originally showing single neural units in these 2 experiments, 7 electrodes showed neural events driven by the same stimulus throughout

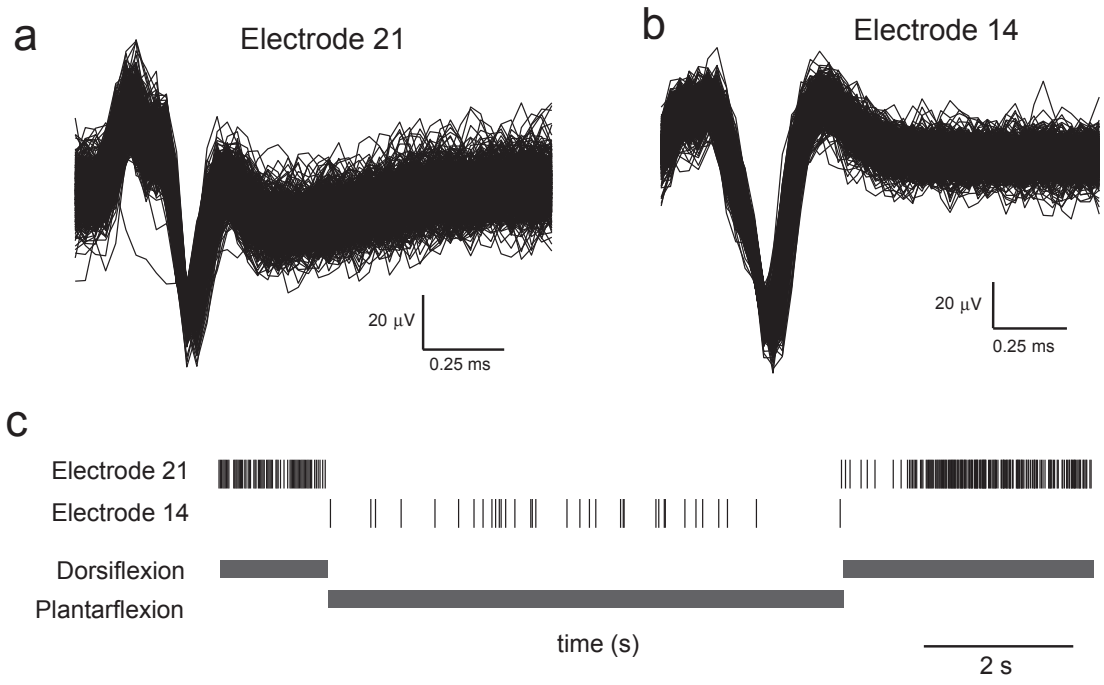

Figure 2.2. Neural units recorded in rat sciatic nerve from 2 HD-USEA electrodes on a single implanted array. (a) 968 waveforms show the summation of the responses from two units to dorsiflexion of the foot around the ankle joint recorded by electrode 21 (SNR=5.9). (b) 1416 waveforms show a unit recorded on electrode 14 that was driven by plantarflexion (SNR=10.8). (c) The neural events recorded by electrodes 21 (top) and 14 (bottom) during both dorsiflexion and plantarflexion (shown below raster plot). Note that electrode 21 recorded neural responses only during dorsiflexion, while electrode 14 recorded responses only during plantarflexion [27].

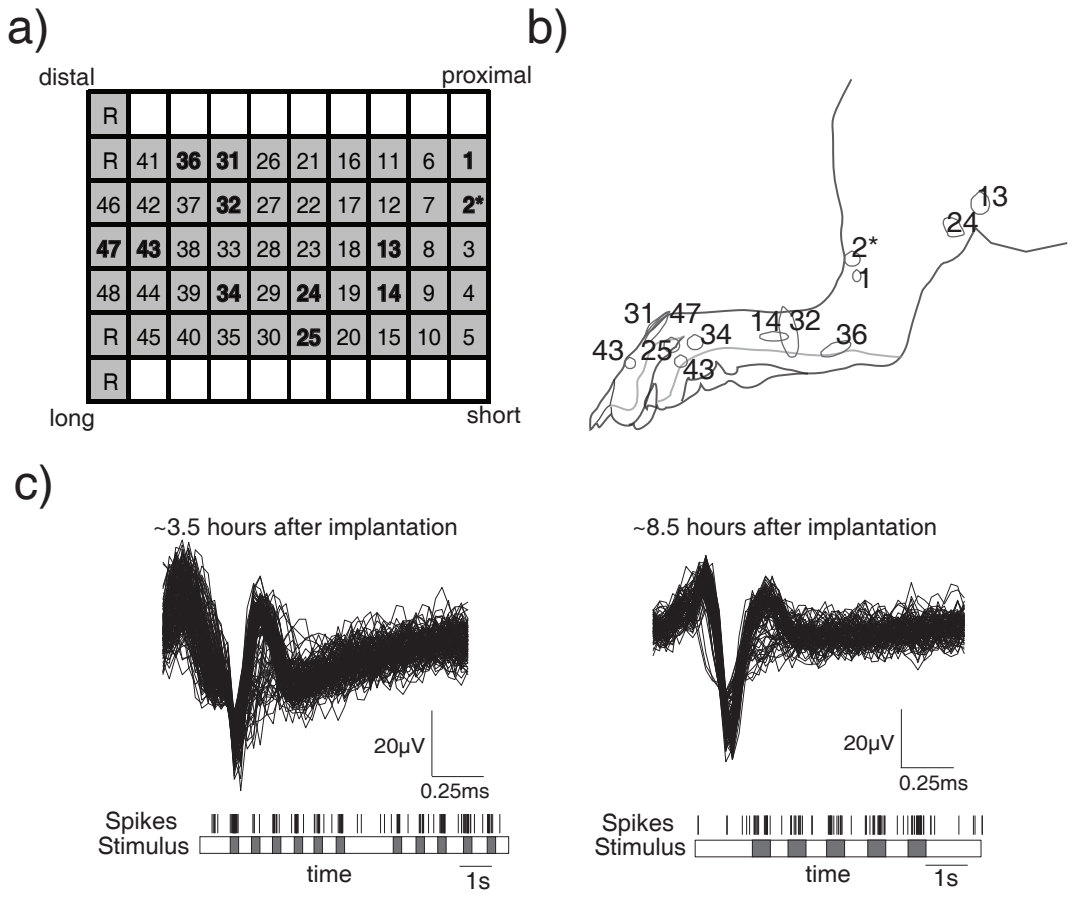

Figure 2.3. Somatotopic map of rat sciatic nerve using HD-USEA. (a) Spatial map of the HD-USEA used in this study. The array was implanted such that the row of longest electrodes was distally located in the nerve. Blank tiles on the map show unwired electrodes. Tiles marked R were reference electrodes; however, a separate reference wire was used in these studies. (b) Somatotopic map of the lower limb in one animal. Numbers represent individual electrodes on an implanted array (bold numbers in (a)) that recorded neural activity corresponding to physical stimulation of different areas of the foot and leg. (c) Waveforms recorded by electrode 2, driven specifically by brushing the skin proximal to the ankle (marked with * in (a) and (b)). A total of 133 waveforms over 9 seconds were recorded 3.5 hours after HD-USEA implantation (waveforms on the left) and were superimposed to show similarity in kinetics, suggesting that they were recorded from a single unit. The 99 waveforms shown on the right were recorded 8.5 hours after implantation, and were driven by the same stimulus for 7 seconds.

the experiment, suggesting that signals were likely recorded from the same unit. Figure 2.3c shows one such unit recorded from one electrode on the array 3.5 hours and 8.5 hours after implantation. In contrast, 10 electrodes showed different or new single units in response to stimuli given at least 6 hours after array implantation. Neural activity was recorded for up to 10.5 hours after implantation, the duration of the experiment. Tables 2.1 and 2.2 show the number of electrodes that were able to record single or multiunit activity less than 6 hours after implantation, and more than 6 hours after implantation. Average signal-to-noise ratios (SNRs), which were calculated by dividing the amplitude of the average waveform by 2 times the standard deviation of the waveforms [32], are also shown for each experiment (mean$\pm$standard deviation).

2.3.2 CAP Waveforms

In each experiment, 10 or 15 supramaximally-evoked CAPs were averaged to reduce noise. Complex waveforms were observed in these supramaximally stimulated CAPs. In

Table 2.1. Number of HD-USEA electrodes recording single or multiunit activity less than 6 hours after array implantation.

Less than 6 hours after implantation			
Animal	# Elec. w/ Single Unit Activity	# Elec. w/ Multiunit Activity	SNR
1	8	28	5.11$\pm$3.14
2	12	26	4.84$\pm$2.07
3	14	31	4.04$\pm$1.77

Table 2.2. Number of HD-USEA electrodes recording single or multiunit activity more than 6 hours after array implantation.

More than 6 hours after implantation				
Animal	# Elec. w/ Same Single Unit Activity	# Elec. w/ New Single Unit Activity	# Elec. w/ Multiunit Activity	SNR
1	4	N/A	N/A	6.18$\pm$2.41
2	4	4	19	4.52$\pm$1.60
3	3	6	25	4.14$\pm$1.89

2 nerve preparations, the nerve was crushed distally to all cuffs, after which only the first trough remained. Figure 2.4a and b show the evoked waveform before (a) and after (b) crushing the distal portion of the nerve (Figure 2.4c shows a magnified view of the directly evoked CAP shown in (b)). All but one of the local extrema (marked with a circled 1) were lost after crushing the nerve. The remaining peak was defined as the directly evoked CAP signal. Stimulation was delivered at time 0, and the corresponding stimulus artifact varied in duration based on stimulus intensity and pulsewidth. To verify that the recorded signals were in fact neural activity, CAPs were evoked using stimulation with normal and reversed polarity. Because the CAP waveform, including the latter peaks (marked with circled numbers 2, 3, and 4) did not invert when polarity was reversed, it is unlikely that the latter portion of the CAP waveform is caused by contamination due to instrumentation, and the entirety of the waveform was considered the "complex" CAP signal. Four prominent peaks or troughs were found to be consistent over pre-implantation CAPs recorded from 6 nerves. The amplitudes and latencies of the observed peaks from stimulus onset (normalized with respect to the voltage difference between the absolute maximum and minimum values of the CAP) were used to construct a representative CAP, shown in Figure 2.4d. The dashed line represents the portion of the signal that was lost after crushing the distal nerve. CAP waveforms from 4 nerves did not resemble the others, and were not used to build the representative CAP signal.

2.3.3 CAP Waveform Following Array Implantation

The amplitudes of the directly evoked CAPs with respect to the average of the waveforms were measured over the duration of each experiment. Additionally, the complex CAP waveform was quantified by calculating the RMS value of each average signal. The peak amplitude and RMS values before array implantation were averaged to describe the baseline activity, and the postimplantation values of each of the recordings were compared to these baseline values. One experiment was carried out to identify the directly evoked CAP and did not involve array implantation. In 3 experiments, the amplitude of the CAP waveform either decreased to less than 10% of its original amplitude within 30 minutes or could not be evoked consistently with large voltages (>10 V), and an array was not implanted. The results of the remaining 8 experiments are summarized in Table 2.3. For 1

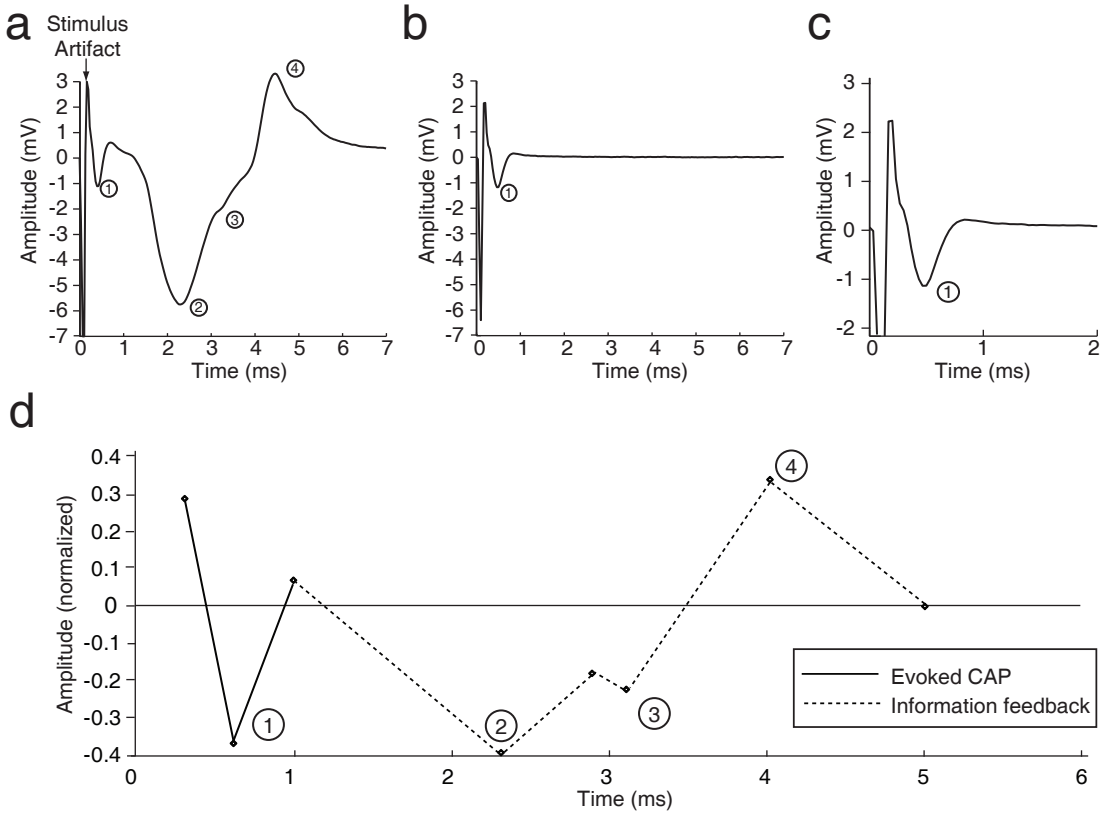

Figure 2.4. Rat sciatic nerve compound action potentials. (a) Experimental CAP signals from 1 experiment (Nerve #8), before crushing the nerve distal to all cuff electrodes. To reduce noise, CAPs were averaged over 10-15 supramaximally-evoked signals. Points corresponding to those chosen to construct the representative CAP (d) are marked with circled numbers. Stimulation was delivered at time 0, and stimulus artifact was observed immediately after stimulation (marked with labeled arrow). (b) Average CAP from same experiment after crushing the distal nerve. Note the loss of the latter portion of the signal previously seen in (a). (c) Magnified view of evoked CAP waveform, shown in (b). (d) Representative rat sciatic CAP, constructed by averaging the amplitudes and times of the characteristic points. Amplitudes were normalized with respect to the peak-to-peak amplitude of the signal. The peaks and troughs used to construct the representative waveform are marked with circled numbers. The dashed line corresponds to the portion of the signal that was lost after the nerve distal to the cuff electrodes was crushed, suggesting that it was not directly evoked by stimulation, but was instead information feedback from muscles or sensory neurons.

Table 2.3. Summary of CAP and EMG metrics following HD-USEA implantation

	Minutes after Implant	CAP Peak Amplitude (% of baseline)	MG (% of baseline)	TA (% of baseline)	RMS (% of baseline)
Nerve #1	0 60 120	77 84 76	84 84 78	112 100 93	87 85 78
Nerve #2	0 60 120	88 71 62	28 39 46	28 91 92	58 56 53
Nerve #3	0 60 120	53 50 49	53 65 66	99 92 84	99 94 90
Nerve #4	0 60 120	65 88 83	43 44 50	87 81 82	74 105 100
Nerve #5	0 60 120	104 16 N/A	63 19 N/A	87 11 N/A	59 36 N/A
Nerve #6	0 60 120	59 70 69	57 60 78	91 82 83	54 60 68
Nerve #7	0 60 120	N/A N/A N/A	104 90 94	90 90 94	108 111 111
Nerve #8	0 60 120	24 44 32	N/A N/A N/A	N/A N/A N/A	N/A N/A N/A
Average Values (mean±std. dev.)	120	62±19	64±15	87±5	78±18

experiment (Nerve #5), all signals were lost less than 2 hours after implantation. In another experiment (Nerve #7), the stimulus artifact was too large, and the directly evoked CAP could not be observed. Over the remaining experiments, the directly evoked CAP peak amplitude decreased to 62$\pm$19% (mean$\pm$std. dev.) of the pre-implantation baseline. Figure 2.5a shows CAP and EMG recordings at various times before and after array implantation from one of these experiments (Nerve #1). Figure 2.5b shows the minimal decrease of the amplitudes of the peaks over the same experiment, in which reported values are normalized to the pre-implantation baseline amplitude. Two hours after implantation, the RMS values of the complex CAP waveform from each experiment reduced to 78$\pm$18% of their respective pre-implantation baseline values. Figure 2.6a shows a second set of CAP recordings from a different experiment (Nerve #4). Figure 2.6b shows maintenance of the RMS value of the CAP waveform over the duration of the experiment, despite observable changes in the CAP waveform. Stimulus artifacts (marked in Figures 2.4 and 2.5 with an arrow) were excluded when locating peaks or calculating RMS values. In 3 experiments (Nerves #4, 6, and 8), there was an initial reduction in CAP peak amplitudes and RMS values immediately after implantation, followed by increases in the values over the duration of the experiment. Note that the last experiment shown in Table 2.3 only includes the evoked CAP signal: this experiment included the distal crush of the nerve, eliminating the complex waveform and EMG signals.

2.3.4 EMG Signals Following Array Implantation

In 6 out of the 7 experiments that included evoked EMG signals, the amplitude of EMGs corresponding to the TA muscle twitches decreased by an average of 13$\pm$5% over the 2 hours after implantation. EMG signals corresponding to MG contractions were not as consistent and decreased by an average of 36$\pm$15% over the 2 hours following array implantation. Three out of these 6 experiments showed a recovery of the MG EMG signal over the 2 hours following array implantation. These changes in the EMG signal amplitudes corresponded to similar changes in the amplitudes of the directly evoked CAP waveforms.

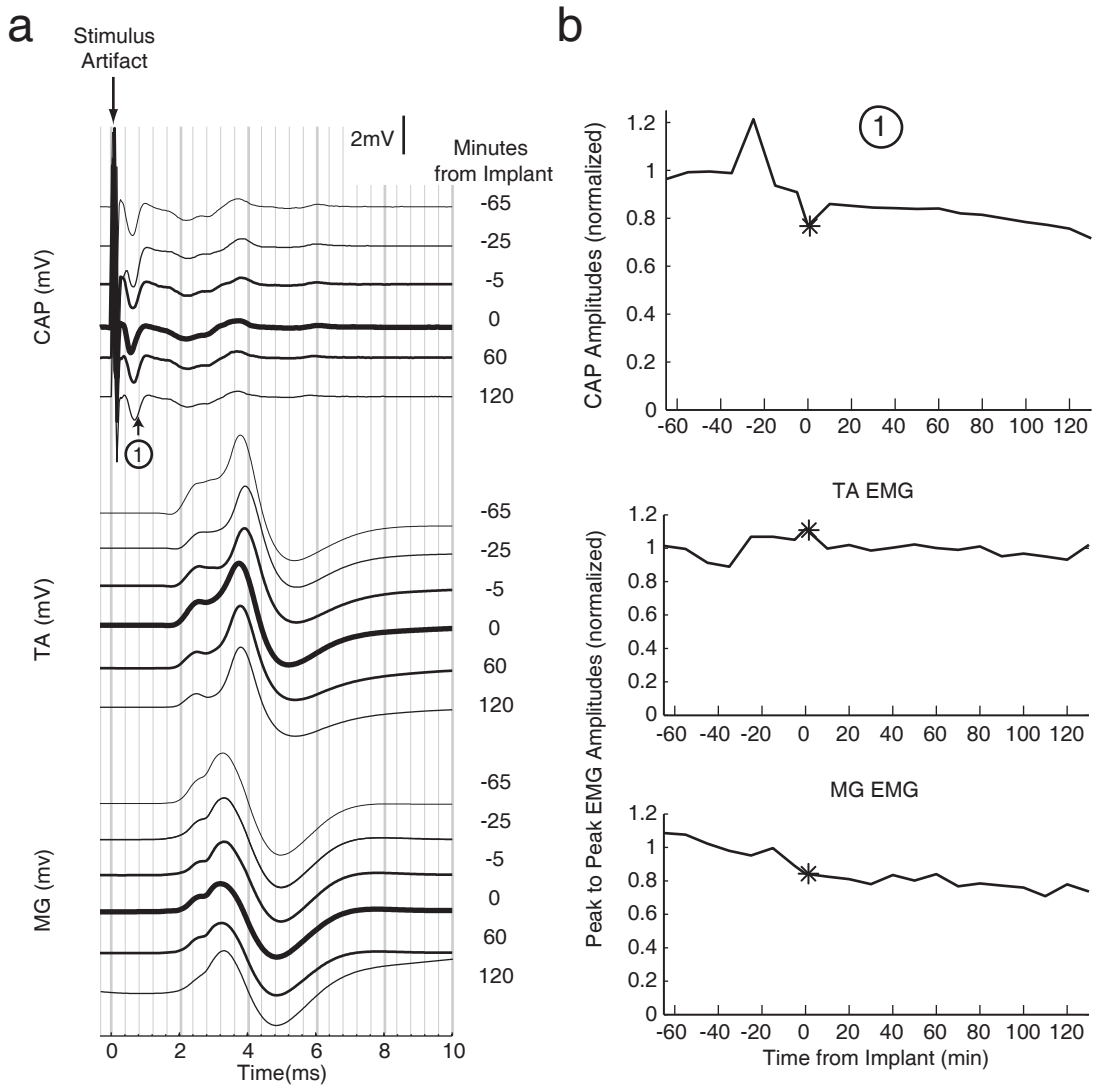

Figure 2.5. Evoked CAP and EMG signal amplitudes. (a) CAP and EMG signals in response to supramaximal stimulation at various times before and after implantation in 1 experiment (Nerve #1). While some changes in amplitude were observed, the general kinetics of the waveform were maintained. (b) Peak amplitudes of the directly evoked CAP (marked with a circled 1 on waveform) and EMG signals shown in (a) over the duration of the experiment. Implantation occurred at time 0 (marked on trace with asterisk). Values were normalized with respect to the average of peak values before implantation. Some reduction of the CAP and EMG amplitudes was observed. Such reduction may have been in part due to a loss in the amplitudes before array implantation.

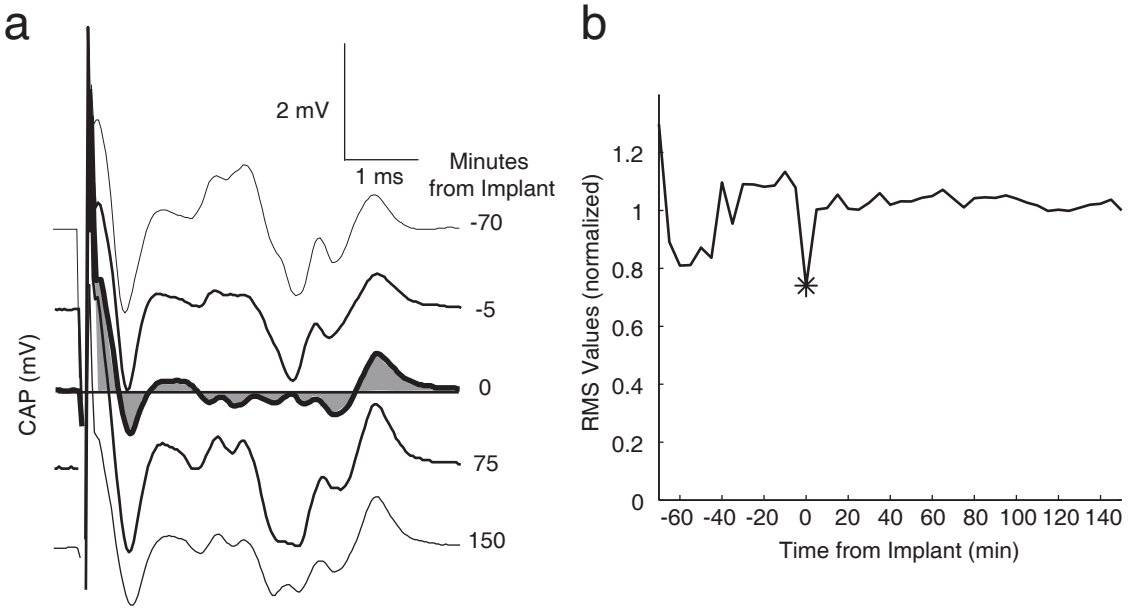

Figure 2.6. RMS values of complex CAP waveform. (a) CAP signals at various times before and after implantation during 1 experiment (Nerve #4). Note that peaks and waveforms change over the course of the experiment. The shaded areas on the bold trace represent the portion of the waveform that was used to calculate the RMS value of the signal (note that this excludes the stimulus artifact). (b) RMS values over the duration of experiment. Implantation occurred at time zero (marked on trace with an asterisk). As with amplitudes shown in Figure 2.5b, values were normalized with respect to the pre-implantation average. Despite the changes in the waveform after implantation, both the kinetics of the waveform and the RMS values show recovery over time.

2.4 Discussion

2.4.1 Single and Multiunit Recordings

We have not only demonstrated that the HD-USEA is a useful device for acute monitoring of neural signals in small diameter nerves, but also that HD-USEA implantation does not cause excessive damage to the nerve during such acute experiments (<12 h). The successful recordings of single and multiunit activity that was driven by specific stimuli to the lower limb suggest that nerve fibers can survive the impact of a high-velocity insertion of such a dense spatial arrangement of electrodes. Changes in recordings over time, as shown in Figure 2.2c, may be due to movement of fibers with respect to implanted electrodes. These results described here, in which 83% of all implanted electrodes recorded neural activity, are comparable to previous recording experiments with the UEA, in which neural activity was recorded from 29% of all implanted electrodes [30]. The higher percentage of recording electrodes on the HD-USEA compared to the UEA may be in part due to the varying electrode lengths, allowing more access to nerve fibers. While effort was made to keep the general placement of the HD-USEA consistent, it was impossible to know to relation of electrodes to nerve fiber across all animals. Therefore, calibrations and somatotopic mapping were necessary for every implantation.

2.4.2 CAP and EMG Recordings

Current literature [33–40] lacks consistency and reports a large variance when describing the kinetics and amplitudes of the components of the rat sciatic nerve CAP signal. While this is in part dependent on whether the nerve preparation is *in vivo* or *in vitro*, it may also be due to the use of different recording techniques in various experimental procedures, variance in orientation of both stimulating and recording electrodes with respect to the nerve and to each other, or external factors, such as the level of nerve hydration and temperature [41, 42]. These factors could contribute to the variability in the CAP waveforms described here. Despite variations between experiments, similar peaks in the CAP waveform were found over most experiments. Variations in the latencies of the CAP peaks are likely due to the small differences in the placement of recording electrodes along the nerve. This justifies the averaging of time markers in addition to the normalized amplitudes in our construction of the representative signal shown in Figure 2.4d.

Because the CAP is a weighted summation of single fiber action potentials [43, 44], there is a relationship between the CAP waveform and the fiber distributions in the nerve. In addition to the directly evoked CAP, a more complex CAP was observed, corresponding to either signals from the sensory afferent fibers that were likely activated by the evoked muscle twitches and movement of the foot or the electrical activity of the muscles themselves in response to delivered stimulation. Therefore, observing changes in the RMS value of the complex CAP waveform may also provide additional qualitative information about damage in the rat sciatic nerve. Reduction of RMS values of the complex CAP waveforms was generally less than the reduction of CAP peak amplitudes, suggesting that the magnitude of the physical response to stimulation was maintained after array implantation. A dispersion of the waveform was also observed over time, rather than a loss of the signal, as shown in Figure 2.6. This may reflect changes in the limb movement in response to stimulation, and thus a change in sensory information traveling along the nerve.

In 4 experiments, the CAP amplitude was inconsistent before an array could be implanted or did not show any signs of recovery following implantation. This indicates that damage may also be due to factors that are unrelated to array implantation. For example, in order to implant 2 cuffs and an array in the rat sciatic nerve, the entire length of the nerve above the knee must be exposed. This long exposure length, combined with the small nerve diameter, leaves the nerve more vulnerable to dehydration than larger nerves, such as the feline sciatic nerve, where such challenges have not been reported [30]. These factors, along with the possible crush injury sustained by the nerve, may also account for the loss of all signals in 1 experiment. However, successful CAP experiments yielded results that were comparable to similar tests performed with the UEA, in which a loss of up to 30% of CAP amplitudes following UEA implantation was reported [30]. Furthermore, the recovery of CAP amplitudes, as seen in 3 experiments, suggests that the loss of neural signals due to penetration of the electrodes may be a transient phenomenon. It is also possible that irreversible damage seen during experiments may not be due to electrode penetration into the nerve, but rather to poor implantation technique (for example, using excessive pressure when pneumatically inserting the array, so that the nerve was crushed by the base of the array rather than the penetrating electrodes). This emphasizes that

the successful application of the HD-USEA as a neural interface depends on qualified surgical technique during implantation. In order to address the long-term effects of array implantation, chronic behavioral and histological studies are being investigated, and the results will be reported in a separate publication.

In order to more thoroughly assess damage to the nerve, EMG signals were recorded from the muscles innervated by the sciatic nerve. EMGs were recorded from MG and TA muscles, which have antagonistic functions (plantarflexion versus dorsiflexion) and are innervated by the tibial and peroneal branches of the sciatic nerve, respectively [45, 46]. In each experiment, changes to EMG signals were similar to changes observed in the CAP waveform, suggesting that the directly evoked CAP signal includes fast motor fibers as well, and was not masked by the large stimulus artifact in most experiments. EMG signals from TA showed less reduction following implantation than MG. This may be because the tibial fascicle, which innervates the gastrocnemius muscles, is larger [46] and may have been located more directly under the implanted array than the peroneal fascicle, and thus, prone to more damage.

These results suggest not only that HD-USEAs may provide selective intrafascicular nerve fiber access in small diameter nerves that is comparable to access provided by the less dense USEA in large diameter nerves, but also that the damage caused by pneumatic insertion of an array with such a high density of electrodes may not be greater than that caused by the implantation of the USEA. The results further indicate that with proper implantation technique, the HD-USEA provides a valuable tool for the study of acute physiology in small diameter nerves, and that it could form the basis for new therapeutic approaches to nervous system disorders.

CHAPTER 3

ACUTE MONITORING OF GENITOURINARY FUNCTION USING INTRAFASCICULAR ELECTRODES: SELECTIVE PUDENDAL NERVE ACTIVITY CORRESPONDING TO BLADDER FILLING, BLADDER FULLNESS, AND GENITAL SIMULATION

The objective of these studies was to investigate the use of a microelectrode array with a high spatial density of penetrating intrafascicular electrodes for selective recording of pudendal nerve activity evoked by a variety of genitourinary stimuli. Felines were anesthetized with alpha-chloralose and High Density Utah Slanted Electrode Arrays (HD-USEAs; 48 microelectrodes, 200 μm spacing) were implanted into the pudendal nerve for acute experimentation. Neural activity was recorded during bladder filling, spontaneous reflexive distention-evoked bladder contractions (DECs), and tactile somatosensory stimulation. The intrafascicularly implanted pudendal nerve electrodes were able to selectively record neural activity that corresponded to various genitourinary stimuli. Across all 7 experimental animals, a total of 10 microelectrodes recorded neural units that were selectively driven by bladder filling or DECs. Twenty-two electrodes were selectively driven by tactile stimulation. These results suggest that microelectrode arrays implanted intrafascicularly into the pudendal nerve can be used to selectively record the neural responses that reflect bladder status and urogenital tactile stimulation. This work sets the stage for developing future implantable closed-loop neuroprosthetic devices for restoration of bladder function.

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3.1 Introduction

The work described herein is focused on investigating a critical element in achieving closed-loop control of genitourinary function: the selective monitoring of urogenital status via pudendal nerve single-unit recordings. Previous research has shown that whole-nerve cuff electrodes can record pudendal nerve activity that corresponds to genital stimulation [11] or bladder contractions [10], but this method records the overall evoked nerve activity (and not the selective activity from individual axons) and, thus, suffers from false-positive detection of bladder fullness produced by tactile stimulation. Therefore, a neural interface that can selectively detect and differentiate different genitourinary functions could improve the accuracy of bladder monitoring and the effectiveness of such a closed-loop system.

Here we investigated the use of penetrating microelectrode arrays implanted intrafascicularly into the pudendal nerve of anesthetized felines as a means for selectively monitoring various genitourinary states. The array [27] contained 48 wired microelectrodes of varying lengths and was used to record neuronal activity at multiple sites across the cross-section of the pudendal nerve evoked by bladder filling, distension-evoked bladder contractions (DECs), and by tactile stimulation. Further, because the tips of the implanted electrodes sample neural activity at discrete loci across the cross-section of the nerve, we also show that such recordings can be used to determine the distribution of afferent and efferent fibers within the pudendal nerve. This study demonstrates that an intrafascicularly-based pudendal neural interface could provide an important component of a closed-loop approach for accurately controlling genitourinary function.

3.2 Methods

All acute (<30 h) experimental procedures were approved by the University of Utah's Institutional Animal Care and Use Committee. Male felines (n = 7 animals; 3-5 kg; intact sexual organs) were induced with Telazol (9-12 mg/kg; IM) and surgical anesthesia was maintained with isoflurane (0.25-2.0% gas). Alpha-chloralose (5-30 mg/kg IV; prn) anesthesia was used during experimental procedures. Vital signs were monitored every 15 minutes throughout the experimentation to ensure maintenance of a stable anesthetic plane.

A dual-lumen suprapubic catheter (12 French, Cook Medical, Inc., Bloomington, IN, USA) was purse-string sutured into the bladder for filling and measuring bladder pressure. A syringe infusion pump was used to fill the bladder at constant rates (2-8 ml/min) [47–49]. In 2 animals, the ureters were ligated, transected proximal to the ligation, and drained internally. DECs were stimulated by filling the bladder with room temperature sterile saline to resting pressures of 5-15 cm H_2O (infusion volumes ranged from approximately 5-20 ml) [48, 49]. Felines were positioned either prone or laterally.

The pudendal nerve was exposed by dissection of the ischioanal fossa and the pudendal nerve trunk was identified via anatomical branching patterns. The nerve trunk and branches were further verified via whole nerve stimulation while resulting muscle contractions were located visually (penile, anal or both). In most animals, the gluteal muscle groups were reflected in order to gain access to the pudendal nerve trunk for the implantation of High Density Utah Slanted Electrode Arrays (HD-USEAs). A nerve platform was placed under the nerve trunk and then an HD-USEA was implanted using a hand-held pneumatic insertion technique [22]. After implantation, the nerve and HD-USEA were wrapped in low-density polyethylene wrap to keep the nerve hydrated and contain the implanted electrode array. To investigate possible reflexive pathways in the origin of the recorded responses, the pudendal nerve in 1 animal was crushed proximal to the implant site. The HD-USEAs had 48, 300-800 μm long, wired electrodes arranged in a 5x10 grid (200 μm spacing, with 4 neighboring electrodes used as reference electrodes) [27]. The ˜50 μm long deinsulated and metalized tips formed the active electrode surfaces. This architecture was chosen to custom-fit the anatomy of the feline pudendal nerve [50].

Recordings were taken from all wired electrodes while filling and emptying the bladder or brushing the skin near the anus, scrotum, or penis. Neural units were detected using a threshold initially set at six times the root mean square (RMS) of the continuously recorded signal and thresholds were adjusted during the experiments. Units were then screened subjectively and categorized as either: 1) multimodal - defined as a unit that was driven by multiple forms of stimulation or a unit that fired spontaneously, or 2) selective - defined as units that were driven only by tactile stimulation, or by changes in bladder pressure produced either via filling or by bladder DECs. For units with activity that correlated with DECs, unit firing rates (calculated as the number of neuronal events in 100 ms bins) were

smoothed with a 0.5 Hz fourth-order low-pass Butterworth filter. Recorded suprapubic pressure was regressed against the firing rates for one trial, and the regression coefficients were used in subsequent trials to predict the bladder pressure. Finally, the coefficient of determination (R^2) of the regression of the recorded and predicted bladder pressures and the root mean square errors (RMSE) of the estimated bladder pressures were calculated with respect to the recorded pressures.

All data were collected using a Cerebus Data Acquisition System (Blackrock Microsystems, Salt Lake City, UT, USA). Neural data were bandpass filtered (750-7,500 Hz) and sampled at 30 kHz. Bladder pressure recordings were sampled either at 10 kHz or 30 kHz. Data analysis was performed using Offline Sorter (Plexon, Inc., Dallas, TX, USA), MATLAB (The MathWorks, Inc., Natick, MA, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA).

After 1 animal was sacrificed, the exposed nerve containing the HD-USEA implant site was soaked in 4% paraformaldehyde phosphate buffer solution (PBS) for 30 minutes. The array was explanted and the nerve was excised and stored in 4% paraformaldehyde PBS for 24 h, then rinsed and stored in 1% PBS with 0.01% sodium azide. Tissue was then stained with 1-2% OsO_4 in a 0.1M sodium cacodylate buffer, dehydrated in graded concentrations of ethanol, cleared with propylene oxide, and embedded in Embed 812 (Epon, Electron Microscopy Science, Hatfield, PA, USA). Serial 0.5-0.6 μm semithin sections were stained and examined using a color CCD camera attached to a Nikon E600 microscope. Serial overlapping images were taken over the entire nerve (at 60x magnification) and then reconstructed to form a mosaic image (Adobe Photoshop CS, Adobe Systems Inc., San Jose, CA, USA).

3.3 Results

The mean electrode impedance of the 4 HD-USEAs used in this study was 130 kΩ. Electrode impedances above 500 kΩ were considered nonfunctional and not included in the mean impedance calculation. The mean number of functional electrodes was 45 $\pm$ 2 (mean $\pm$ standard deviation) in each of the 7 experiments.

The drawings of Figure 3.1a and b illustrate that a 5x10 HD-USEA implanted into the feline pudendal nerve provides uniform electrical access across its cross-section. A cross-

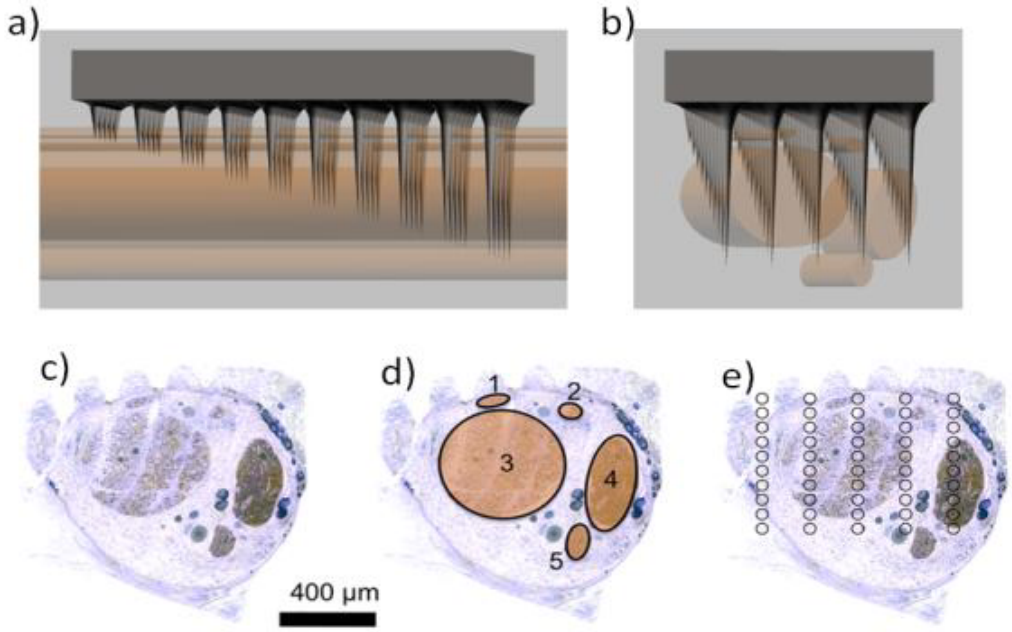

Figure 3.1. Pudendal nerve and HD-USEA histology. (a) and (b). Side and end-on perspective diagrams of a High Density Utah Slanted Electrode Array (HD-USEA) implanted into the feline pudendal nerve (fascicles indicated with shaded cylinders). The 200 μm spacing of the HD-USEA electrodes was based upon feline pudendal nerve dimensions. The location and sizes of the pudendal nerve fascicles were estimated from the cross–sectional image shown in (c). The diagrams show the tips of electrodes accessing fibers distributed throughout the nerve. (c) Cross-section of the pudendal nerve after an acute (10 h) HD-USEA implantation. The base of the array was located on the top surface of the imaged nerve and evidence of the electrode tracks penetrating into the nerve can be seen (histological processing produced curvature in the electrode tracts in this image). (d) Same cross-section as in (c) but with the fascicles outlined and numbered. At the site of this implantation, the fascicular area made up 39% of the total cross-sectional area of the pudendal nerve. (e) Distribution of electrode tips (represented as circles) throughout cross-section of nerve. In this image, the electrode tips of 18-24 of the 48 implanted electrodes appear to be located within fascicles #1-4, such that the axons inside these fascicles are within ˜100 μm of an electrode tip. Scale bar is for (c-e).

section of the feline pudendal nerve that was implanted with an HD-USEA for 10 h is shown in Figure 3.1c, d, and e. The base of the array was located at the top portion of the nerve and the tracks made by the extracted electrodes are evident. At the implant site shown in this figure, the feline pudendal nerve trunk is 39% neuronal tissue (Figure 3.1d with fascicular areas filled in) and 61% non-neuronal tissue (connective, vascular, adipose, etc).

In the 7 feline experiments performed, 50 of the 336 wired electrodes recorded single unit activity. In 2 of the experiments, 25% of the implanted electrodes recorded single unit activity. In 4 of the 7 preparations, we recorded at least one neuronal unit that selectively responded only to bladder pressure changes due to bladder filling or DECs. In 2 of these 4 preparations, we additionally recorded at least one additional neuronal unit that selectively responded to tactile stimulation only. In each animal, there was at least one neuronal unit recorded that was exclusively driven by tactile stimulation, by bladder filling, or by DECs. In 1 animal, we recorded a nonselective, multimodal unit that was driven by both tactile stimulation and bladder filling. In 3 animals, we recorded spontaneous neural activity that could not be associated with either tactile stimulation or any bladder states. Across all preparations, 22 electrodes recorded single unit activity that was driven by genital tactile stimulation alone and 10 electrodes recorded single unit activity that was driven by changing the bladder pressure due to filling or due to DECs. The temporal profiles of the action potentials of these 10 units varied: bladder filling produced a monotonic increase in firing over the duration of filling, while DECs produced bursts of firing.

Figure 3.2a and b show examples of single unit waveforms that were evoked in one animal by stroking the scrotum. A raster of spiking events recorded by each electrode evoked by periodic tactile stimulation was plotted (Figure 3.2c), and 12 electrodes recorded neural activity driven by the stimulus (scrotal stroking). The map of electrodes that recorded single unit activity (Figure 3.2d) indicates that the afferent fibers responding to scrotal stimulation were distributed across the entire cross-section of the nerve.

Figure 3.3 shows an example of bladder filling along with the spiking data recorded on 2 HD-USEA electrodes. These electrodes recorded increases in the firing rates of nearby axons as the bladder began to fill. For example, in 10 second windows before and after

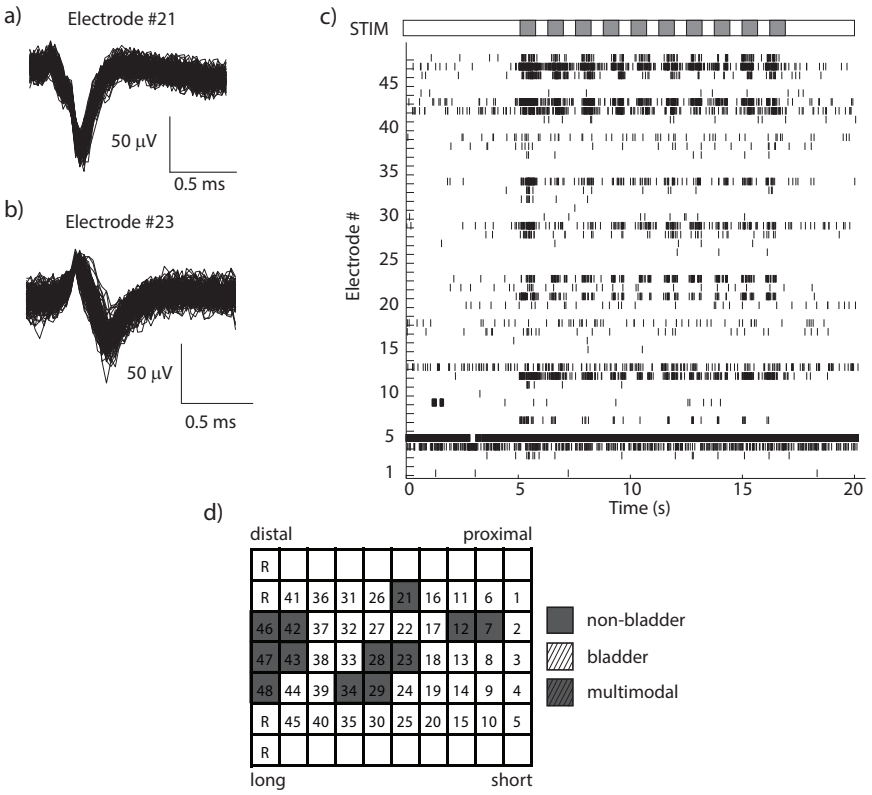

Figure 3.2. Selective detection of genital tactile stimulation. Tactile stimulation evoked neural activity that could be recorded via individual microelectrodes on an HD-USEA implanted into the feline pudendal nerve (data shown from cat #3). Neural units could be recorded from different electrodes in response to stroking of the scrotum such as (a) electrode 21 (SNR = 6.5) and (b) electrode 23 (SNR = 4.9). (c) Map of array showing electrodes that recorded neural activity over the full experiment (d) Raster plots of the spikes recorded on each microelectrode were plotted during ten scrotal stroking events (top bars, marked STIM) showing the selectivity of detecting tactile stimulation activity and redundancy of information afforded by the intrafascicular microelectrodes.

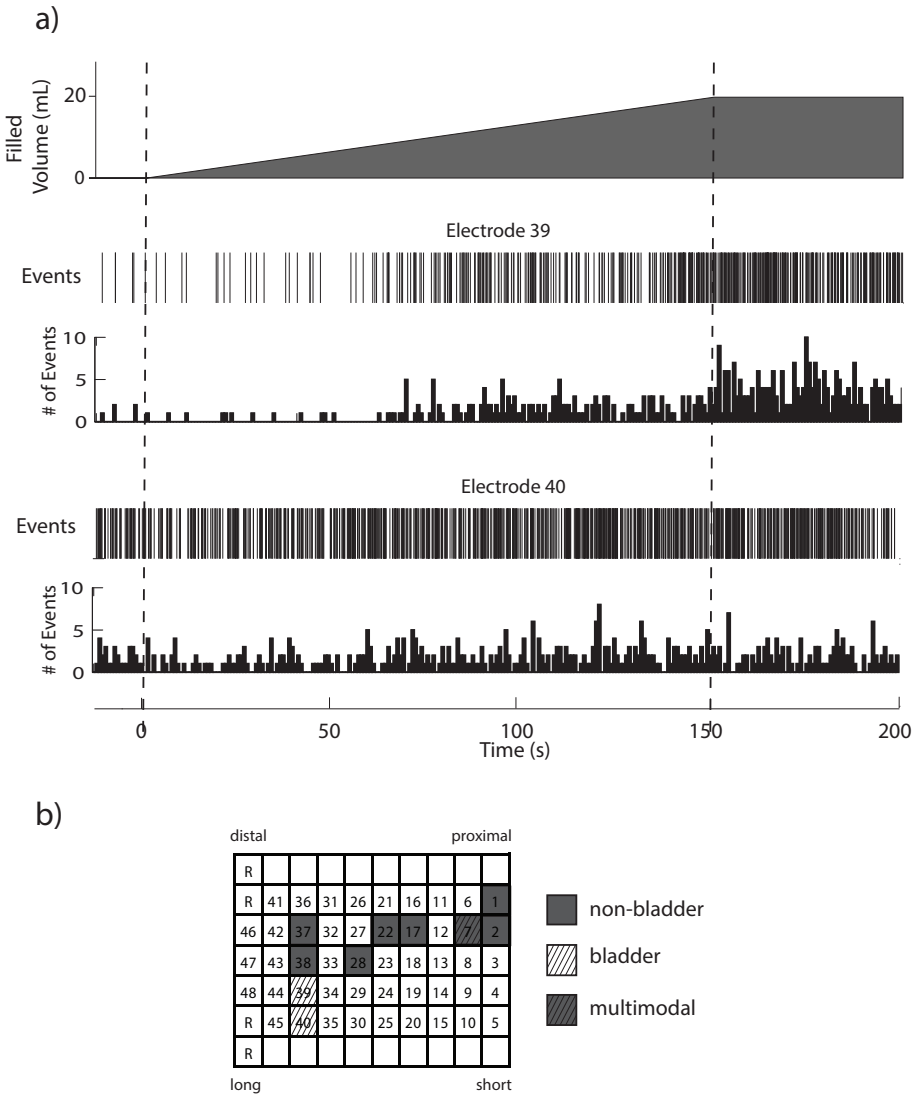

Figure 3.3. Selective monitoring of bladder fullness. (a) The top trace shows the volume infused into the bladder (20 mL) over 150 seconds (cat #4). The dashed lines mark the beginning and end of filling. The two raster plots show the rate of neural events recorded with two different electrodes during filling. Note the increase in spike firing for each unit over the course of filling, emphasized by the histogram showing number of spike events over 750 ms time bins. (b) Array map showing electrodes that recorded neural activity over the full experiment.

filling, the number of spikes recorded by electrode 39 increased from 5 to 47 spikes. These firing rates either remained or decreased with constant volume (20 mL) in the bladder or upon emptying of the bladder. The neural activity map (Figure 3.3b) suggests that the afferent fibers encoding tactile or bladder fullness may be somewhat segregated in the pudendal nerve. The 3 electrodes recording activity that correlated with bladder fullness were located to one side of the nerve and the electrodes recording activity that correlated with tactile stimulation were localized on the opposite side of the nerve.

Filling the bladder to 5-15 cmH_2O resting pressure evoked reflexive DECs (Figure 3.4a, top trace). This figure also shows the spiking data from 4 electrodes during this experiment. Electrodes 6-8 recorded transiently increased firing from neurons in response to bladder contractions, while electrode 5 recorded only spontaneous activity. The histogram (Figure 3.4a, bottom trace) shows the summed spiking events recorded across electrodes 6-8 and these events were correlated with bladder contractions (R^2 = 0.60; normalized RMSE = 0.42 cmH_2O). The neural activity from these 4 electrodes was also recorded during ten periods of tactile genital stimulation (Figure 3.4b, upper trace). Neural activity recorded on electrode 5 was driven by genital stimulation (the binned spiking events on electrode 5 are shown in the bottom histogram), but the neural activity recorded on electrodes 6-8 was not driven by such stimuli. In order to identify if this recorded activity arose from afferent or efferent pathways, the pudendal nerve of this animal was crushed proximal to the implanted HD-USEA, resulting in the loss of activity selectively driven by bladder filling and DECs. The neural activity map (Figure 3.4c) supports the fiber segregation seen in Figure 3.4b, but in this experiment, the fibers with activity that correlated with bladder fullness were localized to the upper region of the nerve, and the electrodes responding to tactile stimulation were localized to one side of the nerve.

3.4 Discussion

Neurogenic bladder can result from many different central or peripheral nerve injuries or diseases, such as spinal cord injury or spina bifida. Patients for whom pharmacological or surgical interventions are not effective are being considered for various implantable neuroprosthetic interventions [51]. The pudendal nerve is one of the locations that is being investigated for the development of a neuromodulation system to restore genitourinary

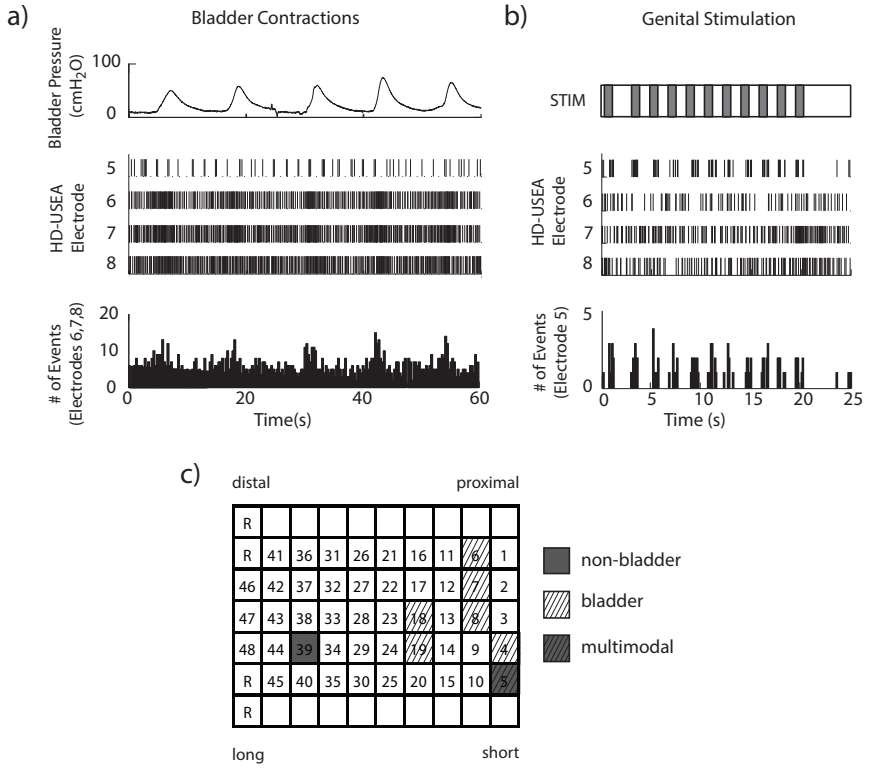

Figure 3.4. Selective activation of different neural units. (a) The bladder was filled with 5 mL (cat #5), a volume that evoked reflexive bladder contractions (top trace). Spike data recorded with 4 HD-USEA microelectrodes are shown in the middle traces. Electrodes 6-8 showed increased firing activity during bladder contractions while electrode 5 showed no consistent change in neural activity. The bottom trace shows the summed spike counts (100 ms bins) for spike events recorded on electrodes 6-8. Increased neural activity in electrodes 6-8 correlated with increases in bladder pressure (i.e., bladder contractions) (R^2 = 0.60). (b) Neural activity recorded from the same 4 electrodes had different patterns of evoked activity during scrotal stimulation (top bars, marked STIM). Electrode 5 was specifically driven by tactile stimulation (summed spike count in 100 ms bins at bottom), whereas electrodes 6-8 were not modulated.(c) Array map showing electrodes that recorded neural activity over the full experiment.

function in people with problems such as neurogenic bladder [52]. A pudendal nerve closed-loop control system would presumably achieve improved control of urogenital function, but such an approach would require the monitoring of detrusor muscle status (via reflexive circuitry reflecting different bladder states) and the use of this information to provide electrical stimulation to subsets of nerve fibers that modulate detrusor and/or external urethral sphincter (EUS) activity.

Animal studies have shown that whole-nerve (cuff electrode) stimulation of pudendal afferent fibers can be used to drive the micturition reflex (20-40 Hz) or inhibit bladder contractions (10-20 Hz) [53], while very high-frequency stimulation (1-20 kHz) [11, 54] can be used to block efferent EUS motor fibers and produce sphincter relaxation. Whole-nerve cuff electrodes have also been used to record pudendal nerve activity corresponding to genital stimulation [10, 11] or bladder contractions [10]; however, such recordings are unable to differentiate activity evoked by these different stimuli. Thus, a closed-loop neuroprosthetic device based on whole-nerve cuff electrodes for the treatment of bladder dysfunction would likely suffer from false-positive detection of bladder fullness due to unrelated genital tactile stimulation [10].

Intrafascicular penetrating peripheral nerve electrodes have been developed for large (3-4mm) [21] and small ($\leq$1mm) [27] diameter neuronal structures. Using the nonslanted version of these microelectrode arrays, Bruns et al. have achieved selective detection of bladder-driven units by recording neurons in sacral dorsal root ganglia, another potential site for a closed-loop neuroprosthesis for bladder control [47]. We previously demonstrated that USEAs can be implanted into the canine pudendal nerve to electrically stimulate small subsets of nerve fibers, allowing for the selective activation of the detrusor and EUS muscles [55]. Here we have expanded upon that work and investigated whether recording of pudendal nerve neural activity could be used to selectively detect various genitourinary stimuli, and thus, provide an important component in the future development of a single implant site for a closed-loop neuroprosthetic device for the control of human urinary function.

HD-USEAs have a slanted architecture, allowing the tips of the electrodes to penetrate into various depths of the nerve, thereby providing access to nerve fibers in different regions of most of the nerve fascicles. However, as shown in the pudendal nerve

cross-section (Figure 3.1d), much of the nerve is nonfascicular tissue (61%), causing many electrodes to reside in non-neural regions of the nerve. Many of these electrodes could be used for stimulation of the nerve fibers, but with less selectivity than electrodes located within fascicles. We have also used HD-USEAs to investigate the distribution of afferent fibers within this small diameter nerve (electrode activity maps of Figures 3.2, 3.3, and 3.4). Despite observed differences, likely due to variations in nerve orientation, these activity maps support previously published histology work describing the fascicular organization of the pudendal nerve in felines [50]. The value of the use of HD-USEAs would be greater when used in larger diameter pudendal nerves where more electrodes could be implanted, giving a more complete description of its fascicuclar organization.

Bladder filling, reflexive DECs, and tactile stimulation evoked neural activity that was selectively and nonselectively recorded via different HD-USEA microelectrodes. Of all successfully recording electrodes across all animals, one electrode was found to record multi-modal activity that could not distinguish between tactile stimulation and different bladder states. Thus, microelectrode arrays may be expected to have few electrodes, if any, that provide false-positive detection of bladder contractions during tactile stimulation.

The temporal profile of firing evoked by bladder filling or DECs differed; contractions evoked transient activity and filling evoked monotonic increases in firing. Therefore, a closed-loop system for monitoring bladder status will likely need to incorporate an algorithm that detects different bladder states based on the temporal signature of neuronal firing. In 1 animal, the pudendal nerve was crushed proximally to the implanted HD-USEA in order to investigate whether signals in the pudendal nerve corresponding to bladder filling/DECs were being recorded from afferent or efferent pudendal fibers. The loss of all neural signals post-neuronal crushing suggests that these units were reflexively activated, and therefore, a pudendal neural interface for detection of bladder status may necessitate intact reflexive arcs.

Over the course of an experiment, many of the electrodes drifted in and out of their ability to record neural activity. This may be a result of micromovements at the device-nerve interface causing the electrode tips to move in and out of the listening radius of individual nerve fibers, a problem that may be mitigated in chronically implanted HD-USEAs where tissue encapsulation would tend to stabilize the electrode-nerve interface.

It is likely that implantation of the HD-USEA also causes minor nerve damage that leads to a loss in neuronal signals over time. However, previous research has shown that these devices can be implanted without major nerve damage for acute electrophysiology studies [27, 28]. Additionally, if nerve damage were causing the loss of these signals, we would not expect to have regained the ability to record the signals that were lost at earlier times. Future chronic studies in feline pudendal nerves are warranted to investigate the long-term recording capabilities and anatomical consequences of HD-USEA implantations, and various containment systems that may better serve to decrease possible movement at the device-nerve interface.

The experiments described in this report were performed in the small diameter feline pudendal nerve, and it is expected that this approach may be more effective in the larger human pudendal nerve [8] where implantation of larger electrode arrays with more electrodes could allow even greater numbers of single unit recordings of neural activity that correlate with bladder fullness. Further, pairs of electrode arrays could be implanted bilaterally in both pudendal nerves, enabling even greater selective monitoring of bladder status.

3.5 Conclusion

In order to develop a clinically useful neuroprosthesis for the restoration of genitourinary functions-such as inhibition of overactive bladder or the restoration of controlled micturition-a fully implantable system must be able to both detect various genitourinary states and then send the appropriate electrical stimulation to drive the desired physiological response (e.g., control the states of the external urethral sphincter and the detrusor muscle). The results presented herein provide evidence that an array of microelectrodes inserted intrafascicularly into the pudendal nerve could be used as a neural interface for the selective monitoring of various genitourinary stimuli, such as bladder filling, DECs, and tactile stimulation of the genital region. Such a neural interface could form the basis for an implantable system that provides closed-loop control of genitourinary functions without suffering from false-positive detection of different bladder states due to unrelated genital tactile stimulation.

CHAPTER 4

CONCLUSIONS

There exists a need for neural interface architectures that provide selective access to fibers in small diameter nerves. The research discussed in this thesis provides evidence that the HD-USEA is a potential solution to this challenge, showing that such an array can be implanted with high-velocity pneumatic insertion methods into small diameter nerves to successfully monitor neural activity in acute experimental settings (12-30 h). Additionally, it has been shown that the HD-USEA may provide a novel means for the research on and ultimate restoration of bladder function, not to mention bowel and sexual function. While the results shown are encouraging, there are some challenges that must be considered and explored with further research.

4.1 Summary of Research Findings

In Chapter 2, the maintenance of the compound action potential and evoked muscle responses following HD-USEA implantation show that the high-velocity implantation of an array with such a high spatial electrode density does not result in excessive nerve crush injury, but instead leaves nerve fibers intact. The successful recording of single neural units not only supports this conclusion, but also emphasizes the capability of selective access to nerve fibers provided by the HD-USEA. Therefore, the HD-USEA has been shown to be a valid neural interface for acute research settings. In Chapter 3, novel applications of the HD-USEA with respect to bladder function are explored. Preliminary success in recording bladder relevant neural activity in the pudendal nerve suggests that HD-USEA electrodes provide selective access to pudendal nerve fibers, potentially improving accuracy of monitoring changes in bladder pressure. HD-USEA electrodes could also be used to record neural activity relevant to sensory stimulation of genitals and the anal sphincter, suggesting that electrodes were in the appropriate location to access fibers involved in sexual function and fecal continence as well.

4.2 Future Work

The HD-USEA provides a novel architecture for small diameter nerves, where selective access to nerve fibers has previously been limited. This is particularly relevant in expanding neural engineering research not only to target nerves that were previously inaccessible, but also to the study of various applications in small animal models. Such an expansion of neural interfaces to small diameter nerves presents a number of new challenges. While the research presented here suggests that the HD-USEA is one appropriate device for such applications, such small nerves are more vulnerable to damage and environmental factors, requiring even more skill and caution during nerve exposure and array implantation. This is emphasized in the loss of compound action potential signals, as discussed in Chapter 2, likely due to poor surgical technique and nerve dehydration. Additionally, proper positioning and complete implantation of the array is required to ensure maximum probability of accessing the appropriate fibers. This is likely the cause of the small number of neural units recorded from the pudendal nerve, as discussed in Chapter 3.

These challenges emphasize the need for further examination of the optimal conditions to effective use of such a device. This becomes an even more prevalent concern when considering the HD-USEA for chronic applications. While the research discussed here has shown the viability of the HD-USEA as an acute neural interface, chronic applications require further consideration of foreign body response to implanted device, the need for recalibration of monitoring and stimulation parameters, and deterioration of the electrodes over time. To partially address this, behavioral and histological studies of rats with non-wired arrays implanted in their sciatic nerves have been conducted, and the results have been reported in an article submitted to the Journal of Neural Engineering for publication [56]. Previous work with the traditionally sized USEA has shown some successful chronic recording and stimulation in larger diameter nerves over several months [25, 31]. This suggests that a similar architecture such as the HD-USEA may have comparable success in chronic applications, and further studies of its recording and stimulation capabilities over long time periods are warranted to confirm this hypothesis, validating the HD-USEA not only for acute applications, but also as a viable chronic neural interface.

In addition to testing its general capabilities in acute and chronic research settings, the utility of the HD-USEA in monitoring and restoring bladder control must also further

explored. To this end, continued research is needed to improve the monitoring of bladder relevant activity, so that such activity can be used to develop a means for real-time monitoring of bladder pressure. Doing so would provide a patient with self-knowledge of when voiding was necessary, preventing potential over-distension and damage to the bladder, and providing greater independence to the patient. Because of its large number of penetrating electrodes, the HD-USEA presents a unique opportunity for simultaneous and selective monitoring and controlling of bladder function. Previous work has shown the ability to use cuff electrodes to detect hyper-reflexive bladder contractions [10], which can result in incontinence or, in cases of bladder-sphincter dyssynergia, pressure-related damage to the bladder wall [57]. Through this detection method, closed-loop control algorithms have been developed to stimulate the EUS during such contractions and prevent incontinence [48]. Once stimulation and recording parameters have been optimized, such closed-loop control methods can be developed for HD-USEAs as well. Because of its selective access to nerve fibers, the HD-USEA has the potential to improve accuracy of activity detection and to reduce the amount of stimulation delivered to the nerve, resulting in a more effective and a likely longer lasting solution to bladder control. High frequency stimulation has been shown to selectively block neural activity and muscle responses when delivered via USEA electrodes (2 kHz) [58], and has been explored in nonselective blocking of EUS activity (10 kHz) [59]. Therefore, high frequency stimulation delivered to pudendal nerve fibers via HD-USEA electrodes may also provide a means to block EUS activity while evoking bladder contractions within one implanted device, preventing bladder-sphincter dyssynergia and producing efficient voiding.

The combination of research into the chronic capabilities of the HD-USEA and its applications for restoring bladder function is expected to pave the way for the development of a clinical solution for bladder control in spinal cord injured patients. Because this research has shown some selective access to other organs innervated by the pudendal nerve, such as the genitals and anal sphincter, research should be expanded to investigate the use of intrafascicular electrodes in the pudendal nerve to restore sexual function and fecal continence as well. Ultimately, these applications, made possible by the development of the HD-USEA as a novel architecture, show the increased capacity for research in neural

engineering and the potential range of application of neural interfaces to restore function and improve quality of life for those suffering from paralysis.

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