UNIT RECORDING AND MUSCLE ACTIVATION IN PERIPHERAL NERVES FOR IMPROVED NEUROPROSTHESES

by

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STATEMENT OF DISSERTATION APPROVAL

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ABSTRACT

High-count microelectrode arrays implanted in peripheral nerves could restore motor function after spinal cord injury or sensory function after limb loss via electrical stimulation. The same device could also help restore volitional control to a prosthesis-using amputee, or sensation to a Spinal cord Injury (SCI) patient, via recordings from the still-viable peripheral nerves.

The overall objective of these dissertations studies is to improve the usefulness of intrafascicular electrodes, such as the Utah Slanted Electrode Array (USEA), for neuroprosthetic devices for limb loss or spinal cord injury patients. Previous work in cat sciatic nerve has shown that stimulation through the USEA can remain viable for months after implant. However, stimulation parameters were not stable, and recordings were lost rapidly and were subject to strong contamination by myoelectrical activity from adjacent muscles.

Recent research has shown that even when mobility is restored to a patient, either through prosthesis or functional electrical stimulation, difficulties in using the affected limbs arise from the lack of sensory input. In the absence of the usual proprioceptive and cutaneous inputs from the limb, planning and executing motions can be challenging and sometimes lead to the user's abandonment of prostheses. To begin to address this need, I examined the ability of USEAs in cat hindlimb nerves to activate primary sensory fibers by monitoring evoked potentials in somatosensory cortex via skull-screw electrodes. I

also monitored evoked EMG responses, and determined that it is possible to recruit sensory or motor responses independently of one another.

In the second study of this dissertation, I sought to improve the long-term stability of USEAs in the PNS by physically and electrically stabilizing and protecting the array. To demonstrate the efficacy of the stabilization and shielding technique, I examined the recording capabilities of USEA electrodes and their selectivity of muscle activation over the long term in cat sciatic nerve.

In addition to long-term viability, clinically useful neuroprosthetic devices will have to be capable of interfacing with complex motor systems such as the human hand. To extend previous results of USEAs in cat hindlimb nerves and to examine selectivity when interfacing with a complex sensorimotor system, I characterized EMG and cortical somatosensory responses to acute USEA stimulation in monkey arm nerves. Then, to demonstrate the functional usefulness of stimulation through the USEA. I used multi-array, multi-electrode stimulation to generate a natural, coordinated grasp.

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CHAPTER 1

INTRODUCTION

The use of the hand and arm are integral to the way most people interact with the world. The loss of the use of any limb, but particularly the hand, is an immense psychological and physical challenge. Though recording and stimulating from neural tissue holds great promise for creating a two-way man-machine interface, current technologies offer far less than the natural intuitive movement achievable by the intact limb. Natural physiological movement, which involves the response of thousands of independent motor units in each muscle, is impossible to mimic perfectly without an electrode system with a similar number of independent channels. Though no such system exists, greater selectivity allows muscle contractions to be more coordinated, smooth, and fatigue resistant, and enables sensory stimulation to be focal and uni-modal. In this dissertation, I explore the use of selective intrafascicular electrode stimulation and recording through USEA implants in the Perepherial Nervous System (PNS) as a potential component of a bi-directional man-machine interface. The introduction begins with an examination of the motivation behind the use of electrical stimulation. Next, the motor and sensory systems that are disrupted by limb loss or Spinal Cord Injury (SCI) will be described, followed by a review of current and emergent treatment technologies for patients with those conditions. Finally, I will describe the research plan of this dissertation.

Sources and Impact of Loss of Arm Function

Paralysis, including Spinal Cord Injury (SCI) and limb loss, though seemingly quite different, are both disruptions of neural tissue that leave nerves in the body still in contact with either the brain or body, but not both. Because of this similarity, recording and stimulating microelectrodes in conjunction with software and robotics can be used, in both conditions, to bridge the neural block between the user's desire and the execution of an action (Moritz et al. 2008). Electrical stimulation could conceivably be used for restoring function to any limb affected by limb loss or paralysis. This work will pay special attention to the challenges of the loss of hand function, due to the enormous role hand function plays in the everyday life of normal individuals.

Spinal Cord Injury

Some form of paralysis affects between four and five million people in the United States. Of the many different causes of paralysis, SCI receives the most attention due to its disproportionately high cost of treatment and adverse affects on quality of life. The population of 250,000 SCI patients in the United States consists mainly of young men (mean age of 33 years) and grows by approximately 11,000 patients a year (NASS; BRPF 2009). The relative youth of SCI patients contributes to the astronomical cost of treating them; the lifetime treatment cost of a 25-year-old tetraplegic, for example, approaches $2.4 million. Total annual cost estimates for treating SCI range from $4 billion to $10 billion. Much of the cost of treatment for SCI comes not from the initial injury, but from the many subsequent complications that result from lack of mobility, including pressure sores, cardiovascular compromise, and reduction of bone and muscle mass. In addition to

the monetary costs of treatment, many patients suffer emotional problems related to their lack of environmental access and impaired ability to interact with others.

Limb Loss

Approximately 1.7 million Americans have lost a limb; over half a million have lost a hand or arm. As medical technology and emergency care standards advance, many patients with traumatic limb injuries are able to survive as a result of careful surgical amputation. The population of amputees is expected to double by 2050 (Ziegler-Graham et al. 2008). Limb loss, especially loss of a hand or arm, can greatly compromise a patient's ability to interact with the environment. For an arm amputee, everyday tasks like eating and driving can become difficult or impossible without some sort of prosthetic. Eighty percent of limb loss patients also suffer from phantom pain syndrome, a condition which causes the patient to experience painful sensations from the missing limb (Flor 2008) (Kern et al. 2009). Phantom pain does not typically respond to treatments for pain, but ongoing research suggests the possibility of using somatosensory or motor stimulation to prevent this condition from developing (Roux et al. 2001; Saitoh and Yoshimine 2007; Ray et al. 2009).

The Motor and Sensory Structures of the Hand and Foot

Motor and sensory neural systems are linked by a host of complex feedback and feed-forward mechanisms. The interplay between sensory information to inform the brain's decision making and correct errors, and motor commands to execute and refine movements, is an integral part of coordinated movement (Kandel et al. 2000). Though the

same nerve may carry the sensory and motor neurons from a limb, the sensory and motor nerve fibers do not interface until they reach the spinal cord. The information density in the nerve is thus very high. Almost all the information the somatosensory cortex can ever receive about a limb must come through the nerves of the limb or an artificial source. Likewise, any muscle movement in the limb must be evoked by an action potential through the nerves or an artificial source. Conversely, in the brain's cortex, the motor response of even a single muscle is represented in a widely spread network of nerve cells that are distributed throughout the frontal lobe. At the cortex, sensory information, which started out as the firing of only a few cells, can cause widely divergent activity in the frontal and parietal areas. The two reversed information processing schemes (motor cortex converging to motoneurons, and sensory neurons diverging to sensory cortex) both result in a widespread representation involving many neurons at the CNS, and a more focused representation involving relatively few neurons in the PNS.

Motor and sensory systems cannot fully be discussed in isolation. Therefore, this section will begin with a review of the motor pathway descending from the brain, the mechanisms of muscle activation and movement, and associated physiology. The next section will examine the generation and propagation of a sensory signal from the distal sensors to the brain. Finally, the relationship between sensory and motor systems will be more fully described.

Motor System of the Distal Limbs

Motor planning begins with complex processes involving the prefrontal association areas in the frontal lobe of the brain, areas that have been shown to be

involved in decision making, prediction of outcomes, and other executive functions. Because of the complexity of such a decision making process, it would be outside the scope of this dissertation to review the process in its entirety (see (Goldman-Rakic 1996; Anderson et al. 1999; Fuster 2000; Miller et al. 2002; Yang and Raine 2009). Local regions of the CNS, including the motor cortex, basil ganglia, thalamus, midbrain, spinal cord and cerebellum, are all specialized for motor and association functions, and are more directly involved with the production of movement. Neurons in the premotor area, Brodmann area 6, fire strongly before a movement in a specific direction, sometimes as long as one to two seconds before a movement, indicating the intent to make a particular movement (Kandel et al. 2000). This area receives many inputs that will be discussed later during the review of motor and sensory integration. Input sources to premotor cortex include sensory inputs, feedback connections from the cerebellum, and thalamic nuclei. The premotor neurons involved in planning a movement project to neurons in primary motor cortex as well as directly to the corticospinal tract, indicating that they play a direct role in generating and modulating movement as well as planning. Interestingly, the premotor areas that project directly to the corticospinal tract also project primarily to the arm area of the primary motor cortex (Dum and Strick 1991).

Most neurons from the premotor area project to primary motor cortex (M1), Brodmann area 4 (the precentral gyrus), where, since the mid 1900s, it has been known that there is a gross map of the body where body parts are more-or-less grouped as they are on the body, a mapping often referred to as a motor homunculus (Penfield and Welch 1951). Many of the neurons in primary motor cortex project directly down the spinal cord

(corticospinal neurons), though some make additional connections in brainstem and midbrain nucleui (vestibulospinal, oliviospinal, rubrospinal, reticulospinal, or colliculospinal neurons, Figure 1.1) before continuing on to the cerebellum or spinal cord. However, research has also shown that the comparatively straightforward organization of the primary of movement in motor cortex is far more complex than originally thought (Meyer 1987; Porter and Izraeli 1993; Schieber 2001). Axons of corticomotor cells are known to branch out and innervate multiple muscles through different spinal motoneuron pools (Fetz et al. 1980; Shinoda et al. 1981; Cheney and Fetz 1985; Fetz et al. 1989). Corticomotor cells associated with a single muscle can be widely distributed throughout primary motor cortex (Rathelot and Strick 2006). Movement of a single finger muscle may be controlled by cells spread across multiple regions, each of which is three square mm or larger (Rathelot and Strick 2006). Further complicating the mapping of the motor cortex is the fact that cortical representation of different muscles overlaps extensively (Schieber and Hibbard 1993; Schieber 1999; Beisteiner et al. 2001). Axons exiting the motor cortex, the projections of cells known as upper motor neurons or Betz cells, travel to either brain nuclei, which give rise to the extrapyramidal tracts, or to the corticospinal tract. The corticospinal tract proceeds down the spinal cord in the lateral or ventral columns and ultimately converges on either interneurons or motoneurons in the ventral horn that communicate with the rest of the body's muscles. The extrapyramidal tracts are mostly associated with modulation and integration of sensory and motor function and will be returned to in the section of motor-sensory integration. As corticomotor axons descend the spinal cord, neurons controlling progressively more

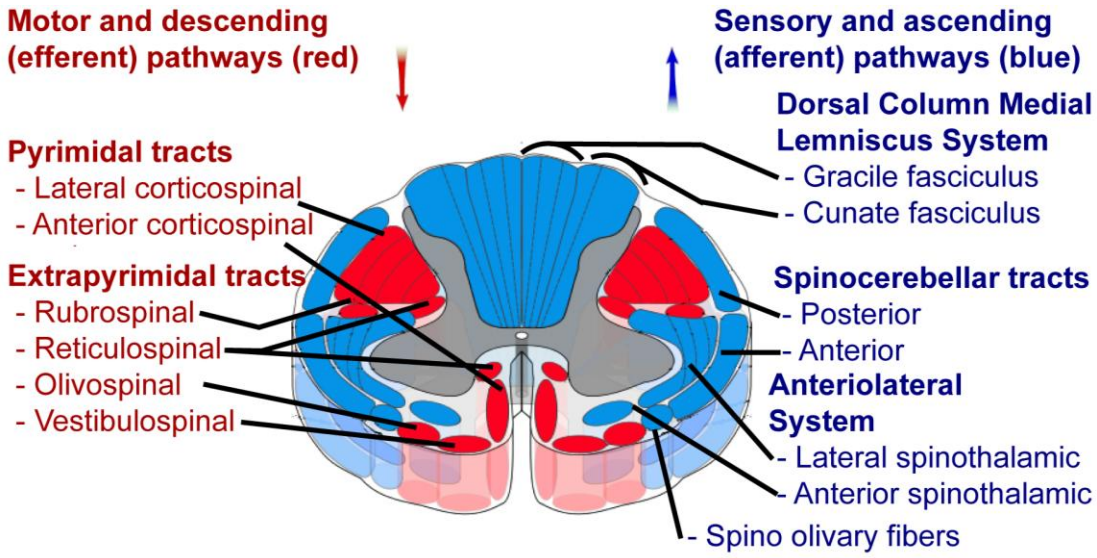

Figure 1.1: Descending and ascending spinal tracts.

Descending spinal tracts are largely associated with different classes, or kinds, of movement.

1. Corticospinal tracts arise from pyramidal neurons in the premotor and motor cortices.
    a. Lateral corticospinal neurons control voluntary motor impulses, particularlly ipsilateral limb muscles
    b. Anterior corticospinal neurons control voluntary motor impulses, particularly central axial and girdle muscles
2. Vestibulospinal neurons modulate movement through interneurons by conducting information from the inner ear regarding balance and body position.
3. Olivospinal neurons integrate proprioceptive input to influence muscle activity.
4. Rubrospinal neurons mediatie arm and leg movement.
5. Reticulospinal neurons coordinate stereotypical movements of locomotion and posture, as well as modulate voluntary and invoulntary muscle tone
6. Colliculospinal/ tectospinal neurons mediate reflex movements of the head in response to visual or auditory sensory information.

distal muscles synapse with ventral horn cells, also called α-motoneurons, exit the spinal cord to form a nerve innervating one or more muscles. The second type of motoneuron, the γ-motoneuron, is also controlled in this fashion, and will be discussed in the section on sensory-motor cortical connections. The α-motoneurons are further modulated by interneuron feedback and reflex connections, such as the negative feedback that arises from Renshaw cells (Renshaw and Rosenbaum 1948; Eccles et al. 1954; Hultborn 2006). As the axons of the α-motoneurons exit the spinal cord and pass through ventral spinal roots, they become nerves. Many nerves, particularly those associated with limbs, are reorganized through splitting and recombination of the spinal nerves into plexuses and, subsequently, into true peripheral nerves. For instance, the median nerve is comprised of fibers that exit the spinal cord at spinal roots C6-C8 and T1. It is important to note the implication such convergence has on somatotopy; the spatial organization of nerve fibers going to locally adjacent body parts becomes more and more pronounced as the neurons travel distally. The information to produce a movement is repeatedly converging to fewer and fewer neurons. Furthermore, those neurons that will innervate the same muscles get closer and closer to one another through organization into fascicles as the motorneuron courses distally (Sunderland 1945).

Ultimately, individual α-motoneurons synapse with a group of muscle fibers, collectively known as a motor unit (Mines 1913; Eccles and Sherrington 1930). Each motor unit is composed of one type of muscle fiber (Burke and Tsairis 1973), either Type I, Type IIA or IIB (Figure 1.2). Type I motor units are small, aerobic, slow twitch fibers capable of maintaining a force over a long period, innervated by small diameter axons.

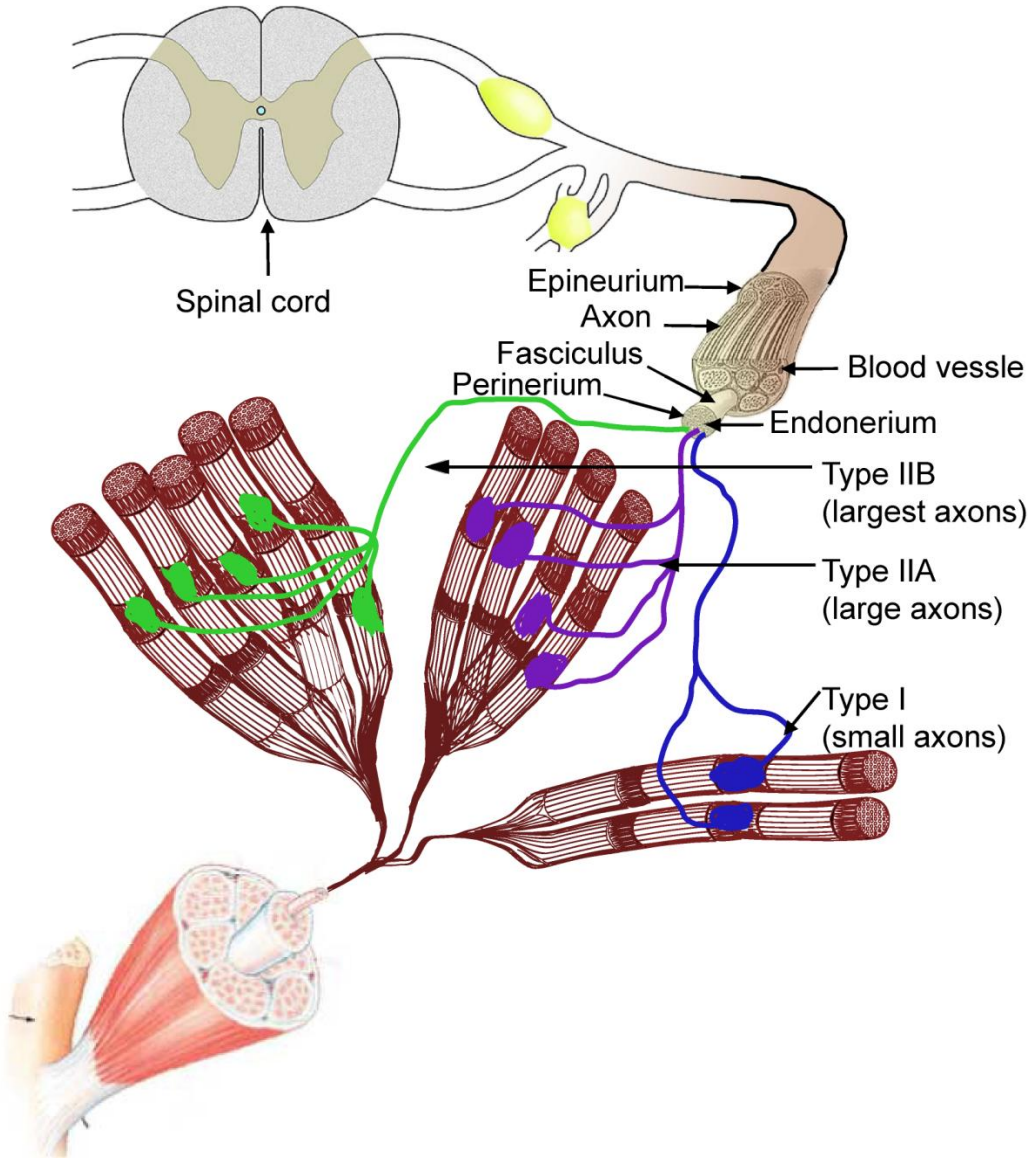

Figure 1.2: The path from spinal cord to skeletal muscle.

A motoneuron and all the muscle fibers it innervates comprise one motor unit. The axons innervating a single muscle at a neuromuscular junction are bundled together as the nerve root reaches the muscle. Muscle fibers of a single motor unit are always of the same type of the three subtypes of motor unit. Adapted from work under Creative Commons 3.0 unported (EUSKALANTO).

Type II fibers are larger, faster, stronger fibers that maintain force for much shorter periods of time innervated by large axons; Type IIA motor units are aerobic, somewhat fatigue-resistant fibers, whereas Type IIB motor units are glycolic, rapidly-fatiguing muscle fibers (Eccles and Sherrington 1930; Burke et al. 1973; Buchthal and Schmalbruch 1980; Bodine-Fowler et al. 1990; Ounjian et al.). The work of Henneman (Henneman and Olson 1965; Henneman et al. 1965; Somjen et al. 1965) shows that the motor units are physiologically recruited on the basis of motorneuron diameter, smaller neurons first, largest last. Simply put, this means that the smallest diameter nerves that control the most fatigue-resistant fibers are recruited first and the faster, stronger type-II fibers are recruited later, which minimizes fatigue (Monster and Chan 1977). This has important implications for artificial stimulation, which we will return to in the later section on electrical stimulation. The final synapse of the α-motoneuron is the neuromuscular junction where the release of acetylcholine begins the complex cascade of events in the muscle cell, resulting in the cycling of actin-myosin cross-bridges that lead to muscle contraction (Kandel et al. 2000) (Huxley and Niedergerke 1954; Huxley and Hanson 1954). The contraction of the muscle represents the end of the neural pathway from the motor cortex; however, a neural signal returning to the brain associated with the movement in progress has already begun in the sensory system.

Sensory System of the Distal Limbs

Sensory signals start in the distal limb in specialized neural cells and accessory structures that are designed to detect one specific type of stimuli. For instance, a tactile

stimulus begins as mechanoreceptors in the skin fire in response to movement. The specialized structures around the neurons alter what mechanical stimulus activates them, such as Merkel disks that enhance response to pressure, or Pacinian corpuscles, which detect vibration and texture but not pressure (Kandel et al. 2000; Bensmaia and Hollins 2005). These and other submodalities of touch are carried in parallel to the central nervous system where they are hierarchically processed, involving an ever-increasing number of neurons; this is the inverse path of the information descending in the motor system.

Sensory nerve cell bodies lie in the dorsal root ganglia near the spinal cord. Cells in a dorsal ganglion, also known as pseudounipolar cells, have axons that extend both centrally and peripherally. Peripherally, many of these cells are surrounded by accessory cells that shape the response of the neuron (Table 1.1). Each neuron encodes a specific stimulus which is transduced into neural action potentials through either the accessory structures in Table 1.1 or transient receptor protein channels **(**ion channels specialized to detect one of a variety of touch, pain, or temperature modalities).

As the axons in the nerve follow their course toward the cell bodies in a dorsal root ganglion, neurons with sensory structures in the same area remain more-or-less in close proximity, giving the nerve as a whole a somatotopic structure (Jabaley et al. 1980; Schady et al. 1983). There is evidence that as axons travel centrally they split and recombine, rearranging themselves to bring neurons encoding the same modality in neighboring regions together to traverse the nerve in close proximity (Dykes et al. 1982; Hallin et al. 1991; Wu et al. 1998; Hallin and Wu 2001).

Table 1.1: Sensory accessory structures

Name of structure	Structure	Modality	Fiber type
Ruffini corpuscle	Nerve endings in connective sheath	Tension in skin	Aβ
Meissener corpuscle	Neuron coiled within laminar structures	Mechanical deflection	Aβ
Pacinian corpuscle	Nerve ending in a large corpuscle	Vibration (250 Hz)	Aβ
Merkel disk	Specialized disk cells abutting nerve endings	Pressure and texture	Aβ
Bulboid	Nerve endings in connective sheath	Low-frequency vibration	Aβ
Golgi tendon organ	Nerve endings spiral around strands of collagen in a tendon	Stretch, muscle force	Aα
Muscle spindle	Muscle spindle	Stretch	Aα
Chemceptors Nociceptor Thermoceptors	Distinct repertoires of ion channels and receptors	Pain Temperature	Aδ / C
No accessory structure	None	Polymodal Touch/pain	Aβ / Aδ

Combined, these findings indicate the beginning of the change from the map of the body formed by the physical positions of sensory structures to the more complex map, based only partially on modality (Bensmaia 2008), that exists in the cortex, begins in the nerve even before the neurons reach the dorsal ganglia. The central processes of the dorsal horn cells enter the spinal cord and synapse with either local interneurons (to form reflexes) or those ascending to the medulla.

It is important to note the continuing alteration of somatotopy forced by the arrangement of neural fibers and how they enter the spinal cord. The neurons that join the spinal cord most distally ascend in the middle of the spinal cord, near the midline. The closer to the brain that the sensory neurons enter the spinal cord, the more lateral they are while coursing to toward the brain. The spinal cord is not a direct routing system, but is a complex, intertwined, multi-level neural processing system that can function independently of the brain (i.e., postural reflexes) (Sherrington 1898, 1910); this will be discussed further in the section on motor and sensory integration. In the spinal cord, neurons separate into tracts primarily based on their sense modality (Figure 1.1), sometimes making synapses on the dorsal horn cells in the spinal cord (crude touch, pain and temperature) or with second-order neurons in the medulla in the gracile (dorsal) and cuneate (posterior) nuclei. Somatotopy is largely maintained for each separate touch modality in these nuclei with neurons separated into those from the lower body and upper body in distinct nuclei, the gracile and cuneate respectively. These second-order neurons then cross the midline and synapse in the ventral nuclei group in the thalamus, again maintaining some degree of somatotopy, mapping the lower body to the lateral thalamus and the upper body to the medial thalamus. Connections in the thalamus are subjected to substantial excitatory and inhibitory inputs from both local connections (intrathalamic) and feedback from the brain's distant structures. From the thalamus, tertiary neurons route sensory signals to the somatosensory cortex. Most neurons relaying somatic information terminate in the primary somatosensory cortex (S1) in a fairly somatotopic manner, originally described as a second homunculus (Penfield and Welch 1948; Penfield

1972; Metman et al. 1993). However, there are at least four representations of the body in primary somatosensory cortex, roughly corresponding to Brodmann areas 3a, 3b, 1, & 2 (Penfield and Welch 1948, 1951; Kaas et al. 1979). Neurons in S1 have more complex encoding of sensory stimulus than the periphery, combining the responses of slowly adapting and rapidly adapting neurons of different sensory modalities into a unified percept (Pei et al. 2009). Most tertiary sensory neurons synapse to area 3b (Iwamura 1998) before being routed to the other, secondary areas mentioned, but there is also evidence for direct connections to the secondary areas (Edell et al. 1992; Iwamura et al. 1993; Ploner et al. 1999), particularly for nociception (area 1) and proprioception (Brodmann area 3a). As the information received by the cortex diverges to additional areas of cortex, the encoding of sensory information grows more complex. The primary and secondary somatosensory areas make synapses to many other areas of the brain, including multi-modal sensory areas, the hippocampus, and, most importantly, motor and premotor cortex. The close linkage through a large number of neurons in sensory and motor cortex indicates the important role sensory information plays in shaping movement (Oscarsson and Uddenberg 1965; Kandel et al. 2000).

Integration of Sensory and Motor Systems

At every level of the nervous system, from muscle to the cortex, the motor and sensory systems are integrated neutrally (Sherrington 1910; Strick and Preston 1978; Kakei et al. 2003). Motor commands are constantly modulated by sensory information, some from primary afferents which have not even passed information onto the brain. Likewise, sensory signals are modulated by motor commands, such that sensation may be

depressed in one phase of a cyclic movement, such as stepping, and facilitated in another (Clarac et al. 1992). The integration of the motor and sensory systems is fairly easily understood at the level of the spinal cord, through monosynaptic reflexes that connect sensory afferents directly to motor efferents (Eccles and Sherrington 1930) or feed-forward connections from the brain. Within the brain however, there is a great deal of distributed connectivity linking the motor and sensory systems to each other as well as many other common brain areas. These areas, in turn, project back to the somatosensory and motor cortices, as well as many other areas, creating a complex web of neural pathways that is difficult to unweave (Iuppino et al. 1986; Matelli et al. 1986; Barbas and Pandya 1987; Dancause et al. 2006).

Many of the connections between tertiary structures, such as the basil ganglia and the cerebellum, to somatosensory or motor cortex are well understood (Delong et al. 1984; Schell and Strick 1984) (Malis et al. 1953; Kornhuber 1971; Blakemore et al. 1998b), and a significant amount of research has been done on those that involve decision making, consciousness, and memory (Deiber et al. 1991; Shibasaki et al. 1993; Hikosaka et al. 1996; Middleton and Strick 1996; Humberstone et al. 1997; Ikeda et al. 1999). Although a complete review of the many networks that contribute to both the generation of motion and the interpretation of somatic sensation is outside the scope of this work, I will briefly address the most direct connections between motor and sensory systems (Mountcastle and Powell 1959).

Motor commands are principally modulated by 1) cerebellar feedback, 2) basal ganglia projections, 3) corticocortal connections, and 4) spinal synapses and interneurons

(Asanuma 1981; Asanuma and Arissian 1984; Kandel et al. 2000). The cerebellum and basil ganglia are involved with extensive error correction and moto-sensory modulation; they receive sensory inputs and send outputs to motor structures, but do not involve any direct, monosynaptic connections. Connections between the cerebellum and the motor and sensory systems have been studied for years (Allen et al. 1974; Ito 1984; Gao et al. 1996; Ito 2006), and are primarily implicated in timing and motor learning, specifically supervised learning (Ito 1984; Fine et al. 2002; Ito 2002, 2008). Basil ganglia connections have also been investigated extensively and are implicated in reinforcement learning (Montague et al. 1996; Houk 1997; Brown et al. 1999; Houk et al. 2007). Recently a number of researchers have suggested that these structures are more appropriately considered learning structures and that their large number of motor and sensory connections are essential to cognitive processing and memory (Leiner et al. 1993; Kim et al. 1994; Middleton and Strick 1994; Raymond et al. 1996; Allen et al. 1997; Parsons et al. 2000) (Hikosaka et al. 1999).

Many corticocortical connections directly link sensory cortex outputs to motor and premotor cortex (Porter and Sakamoto 1988; Tokuno and Tanji 1993) and other elements of the motor system (Yumiya and Ghez 1984; Ikeda et al. 2000; Tsujimoto et al. 2009). Direct connections between S1 and M1 have been shown in monkeys (Boudreau et al. 2001) (Rizzolatti et al. 1981; Tanji and Wise 1981; Strick and Preston 1982b, 1982a; Picard and Smith 1992) and humans (Moore et al. 2000). Thus, the first brain area associated with processing sensory information (where the crudest information is received) and the last motor area of the brain (where the finalized motor plan is sent to

the periphery) are linked monosynapticly, bypassing much of the brain's processing. Interestingly, the corticocortical connections from S1 to M1 maintain the separation of modalities, with proprioceptive inputs synapsing rostrally but cutaneous inputs synapsing caudally, and are especially associated with the hand and limb representations (Stepniewska et al. 1993; Schieber and Poliakov 1998; Schieber 1999; Kim and Cruse 2001; Stepniewska et al. 2009). Lesions in the caudal area of M1 result in very similar deficits in precision movement to those caused by S1 (3b) lesions (Xerri et al. 1998; Friel et al. 2005), indicating that these areas play similar roles in forming motor plans. The direct connections between the primary cortices are important for motor skill learning (Pavlides et al. 1993), and are still under wide investigation (Witham et al.; Widener and Cheney 1997; Friel et al. 2005; Dancause et al. 2008).

Spinal motoneurons, i.e. α-motoneurons, are activated by the upper motor neurons of the brain. These spinal motoneurons are all parts of spinal reflex circuits that contain several linked circuits including negative feedback from the motor system itself, inhibition of antagonist muscles, and sometimes even monosynaptic reflexes directly linking a sensory output to a motor input. There are several well-studied reflex circuits that depend upon direct sensory modulation of the motor system through only a few neurons (Eccles and Sherrington; Eccles et al. 1957; Lundberg et al. 1962; Fetz and Cheney 1979; Lundberg et al. 1987; Kandel et al. 2000). In the simplest reflex, a monosynaptic reflex, stretch receptors coming from a tendon activate muscles resisting the stretch. Although this reflex appears simple, it is subject to further modulation through a network of spinal interneurons that receives connections from upper

motoneurons and primary sensory afferents (Eccles and Lundberg 1959; Lundberg and Voorhoeve 1962; Kandel et al. 2000). A more typical arrangement of a spinal reflex circuit (Figure 1.3) includes interneurons, either excitatory or inhibitory, that receive synapses from primary sensory afferents, feed-forward connections from the brain, and other spinal circuits (Grillner et al. 1969; Baldissera et al. 1971; Rudomin et al. 1975; Shik and Orlovsky 1976; Grillner and Jessell 2009). These reflex circuits are integral components of both the path from motor cortex to muscle and the pathway from sensory structure to the brain, and as such they must be taken into account by the motor cortex when generating a motor plan. However, these reflex circuits can simplify the motor plan, because the cortex can use the reflexes as components of any given motion. for example, in the reflex shown in Figure 1.3, antagonist muscles will be inhibited automatically by descending motor commands that activate the agonist muscle, freeing the motor cortex from having to send descending commands to do the same (Illert et al. 1976). By the same token, the motor system must also use these circuits to generate all muscle states, such as static limb position, through activation of both the agonist and antagonist. This requires additional motor and sensory inputs to the spinal interneurons, creating yet more overlapping reflex networks.

The sensory system, like the motor system, receives modulation at every level from the brain to the spinal cord. In the brain, the sensory system receives modulatory inputs from the motor system through cortical inputs to tertiary sensory neurons in the thalamus (Palmeri et al.; Lee et al. 2008), cerebellar connections to S1 (Blakemore et al. 1998a; Bays et al. 2005; Bays et al. 2006), or corticocortical connections (Blakemore et

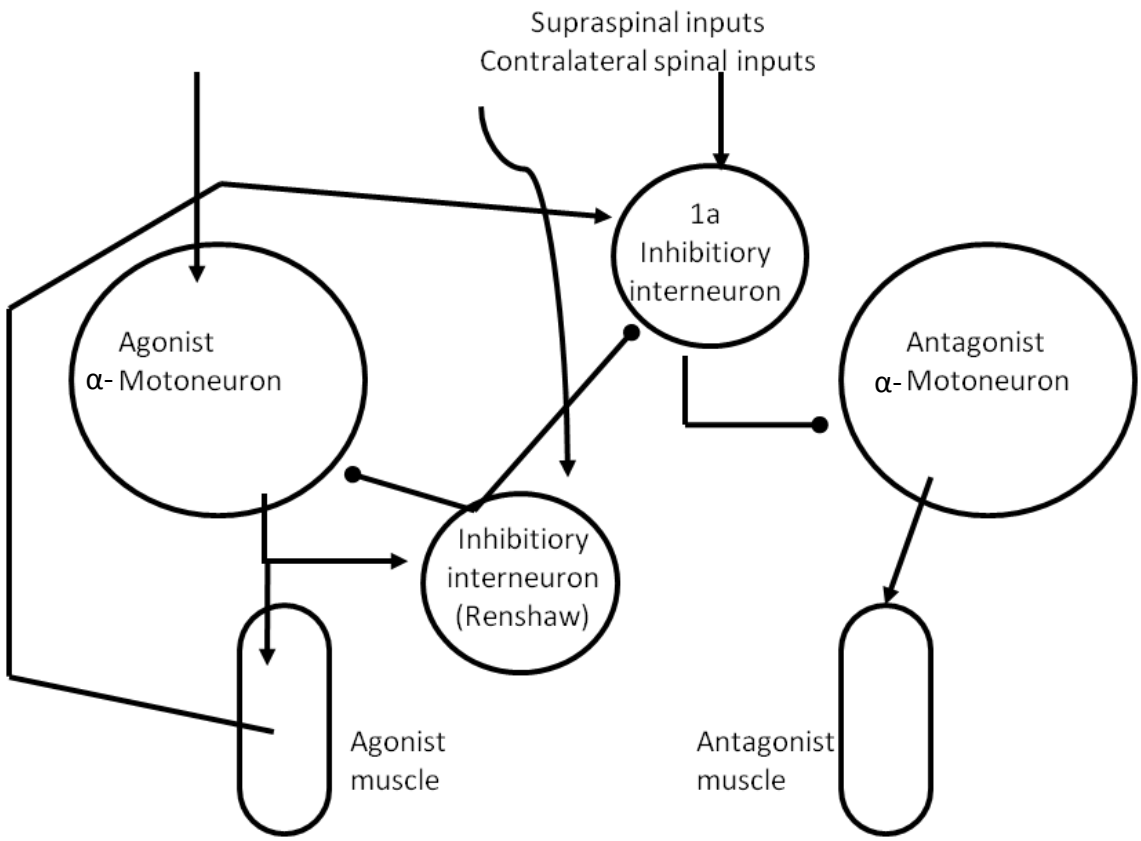

Figure 1.3: A spinal reflex circuit.

In this example of a spinal reflex circuit, α-motoneuron activation excites a Renshaw cell. The Renshaw cell then inhibits the same α-motoneuron that initially fired, as well as disinhibits an antagonist α-motoneuron. In an intact spinal cord both of these effects are modulated by descending control from the cortex.

al. 1998a; Doya 2000; Kandel et al. 2000; Witham et al. 2007; Legon et al. 2008). As in the motor system, the complex network of modulatory connections in the cerebellum, frontal or parietal cortex, and thalamus preclude a full examination. However, we must note that there are reciprocal modulatory inputs at nearly every synapse of the motor and sensory systems. In the sensory system these connections are involved with the sensorimotor integration of movement for posture and error correction (Lee et al. 2008),

suppressing expected responses, and with enhancing sensory input during exploratory movement (Kandel et al. 2000).

Primary motor cortex plays a direct role in modulating sensory information through the activation of γ-motoneurons (Kandel et al. 2000). Some neurons from M1 travel down the spinal cord to the ventral horn and connect with this second type of motor fiber, which is not used to generate movement. The γ-motoneurons activate muscle spindle intrafusal muscle fibers, adjusting the tension in the muscle spindle (see Table 1.1). This allows the muscle spindle to detect stretch in a muscle across a wide range of lengths (Boyd et al. 1979). The muscle spindle afferent is involved in the stretch reflex, a well studied monosynaptic reflex that flexes the muscle that contains the muscle spindle (Clarac et al. 1992).

Upper motor neurons in the spinal cord can also enhance or inhibit other sensory responses by supplying feed-forward information about a planned movement (Seki et al. 2003). This inhibition happens in most, but not all, of primary sensory afferents (Carpenter et al. 1963b; Carpenter et al. 1963a; Eguibar et al. 1994; Seki et al. 2003). Because this inhibition happens at the presynaptic terminal of the first synapse in the sensory system, the CNS, by way of motor cortex, has very specific control over what information it receives about planned motions. Suppressing primary afferents has the functional consequence of eliminating inappropriate reflex actions that would act against a planned movement (Ranck 1975; McNeal 1976; Fretz and Fravel 1985; Fang and Mortimer 1991b; Durand et al. 2004; Haensel et al. 2004).

Neuroprosthetic Motor Replacement or Restoration Systems

Because of the voltage sensitive nature of the neuron, specifically the ion channels that initiate or propagate the action potential, electrodes of all kinds can be used to artificially generate neural signals. Any method which transfers charge across a neural membrane applies a voltage across that membrane, and can cause an action potential (Burke et al. 1973; Monster and Chan 1977; Fang and Mortimer 1991b, 1991a; Kilgore et al. 1997; Fisher et al. 2008). Electrodes in appropriate locations can also detect the changes in the flow of ions caused by natural action potentials, both from muscles and neurons. Muscle action potentials are large potentials that can be detected at some distance; however, neural events are not detectable at great distances. Many different electrodes types and shapes have been used to stimulate or record from neural or muscular tissue in a wide variety of locations. All electrodes have advantages and disadvantages for different applications but can be analyzed in terms of their: 1) safety, 2) invasiveness, 3) size (proportional to the number of neurons the electrode will interact with), and 4) long-term stability.

Mechanisms of Neural Stimulation

During cathodic stimulation, the negative charge of an electrode causes redistribution of charge on and around the cell membrane. The positive charge in the cell is drawn to the membrane near the cathodic electrode, causing a membrane depolarization, and possibly an action potential. Although many factors contribute to the effectiveness and safety of electrical stimulation of neurons, including stimulation waveform used, stimulation history, current paths, and electrode material, all electrodes

induce action potentials in a similar manner by forcing a current across the cell membrane. This mechanism has the disadvantage of reliably recruiting larger diameter neurons before smaller neurons due to the interaction of Ohm's law and the extracellular current source. Ohms law states that the voltage drop across the membrane (which will trigger the cascade of ion channel openings that will create an action potential) is proportional to the resistance of the membrane for any given current. Because large and small axons are made of the same materials (myelin and cell membrane), their electrical resistance is inversely related to their surface area. To stimulate smaller neurons requires greater charge injection, which will in turn recruit more large-diameter neurons.

Many different electrode types and shapes have been used to stimulate neural tissue in a wide variety of applications, from cochlear electrodes to restore hearing (Brown and Buchwald 1973; Novak and Wheeler 1988; Hallin 1990) to peripheral nerve electrodes to restore movement in the limbs (Kuiken et al. 2004; Kilgore et al. 2006; Kilgore et al. 2008). Early studies involved large electrodes that stimulated many neurons as group. As electrode design and manufacturing technologies advance, the focus of stimulation gets ever smaller, allowing newer microelectrodes to interact with fewer neurons in a smaller area. It is important to note that all of these strategies to restore function, frequently referred to as Functional Electrical Stimulation (FES) strategies, face some similar problems matching physiological muscle activation. The smooth and powerful movements generated by the motor system are the result of many independent motor units activating asynchronously, the slow twitch muscles, with the smallest associated neural fibers being recruited first, followed by fast twitch muscles as force is

required (Kuiken et al. 2009). Square wave stimulation applied across any extraneural electrode recruits nearby neurons in the reverse of physiological order, making it difficult to simulate natural force recruitment in a muscle (Popovic 2003). By manipulating stimulation waveforms and electrode position, Fang and Mortimer (Saxena et al. 1995; Popovic 2003; Fisher et al. 2009) show that a tripolar cuff can stimulate smaller neurons while blocking stimulation in larger neurons (associated with faster fatiguing motor units); however, this technique has not been tested in a chronic situation.

Principles of Neural Recording

Using recordings of action potentials from either single cells (single unit) or many adjacent cells (multi-unit or local field potentials), researchers have been able to determine the function and connectivity of neurons *in vivo* for many years (Peckham et al. 2001). Surface electrodes, large electrodes placed on the outside of the brain or around a nerve, can detect the synchronous activity of millions of cells. Smaller electrodes are sensitive to the activity of fewer cells; however, the higher resolving power to detect individual neurons comes at the cost of detecting activity across a smaller distance, requiring the electrodes to be very close to a cell. Research has also shown that micro-electrodes in proximity to certain cell structures, specifically the cell body's axon hillock or the nodes of Ranvier in the axon (Peckham et al. 2001; Kilgore et al. 2008), can detect the small extracellular component of action potentials of single neurons (<1 mV). Because smaller microelectrodes must be close to the cells they record from (less than 250 um), they are often more invasive than the larger electrodes that record activity from a group of neurons.

Limb Loss Prostheses

Limb replacement prosthesis technology available today takes electromyographic (EMG) signals from either the remnant limb's muscles or muscles innervated with remnant limb nerves surgically redirected to still-existent muscles (Davis et al. 2001). These EMG-based solutions all suffer from the same drawback: the remnant limb muscles being recorded from need to be remapped into control signals for the prosthetic device. The new operations the muscles have to perform need to be learned by the user. This process is often complicated by the possibility of there being few muscles to use, such as in the case of a high arm amputation. Targeted reinnervation, wherein remnant nerves are surgically attached to separated muscles, takes advantage of the fact that the PNS is still carrying signals of intent to the missing limb by remapping it to a new muscle (Veraart et al.; Grill and Mortimer 1996; Lago et al. 2005; Brill et al. 2009). When attached to a new muscle, the nerve grows synapses and integrates itself; to the PNS the new muscle appears as part of the original nerve location due to the labeled-line principle. EMG signals from the newly-innervated muscle are mapped to the motions of the prosthetic, allowing for more natural control over the prosthetic device (Fang and Mortimer 1991a; Durand et al. 2004; Polasek et al. 2009). However, each new connection between the remnant limb's nerve and the patient's muscle requires a surgeon to attach a portion of the nerve to a suitable muscle that the patient is not already using for each desired control signal.

Electrode Technologies for SCI

Many electrode types have been used to elicit muscle movement in clinical FES systems. In general, electrodes trade invasiveness for specificity. Initial systems used electrodes on the surface of the skin to elicit functional muscle movement for standing and walking (Polasek et al. 2009). Though they are minimally invasive, these systems have low selectivity, reliability, and acceptance by patients (Branner and Normann 2000; McDonnall et al. 2004a; Normann et al. 2005; Zheng et al.). With similar electrodes implanted under the skin, on the surface of the muscles to be stimulated, the amount of current needed to stimulate the muscle is reduced and the between muscle specificity is increased. These electrodes, called epimysial electrodes, require minimal surgery but have specificity only at the muscle level (i.e., they recruit an entire muscle) and are hard to place in some deep muscles (Wilder et al. 2009). Intramuscular electrodes inserted into the belly of a muscle provide greater specificity and control over muscles than epimysial electrodes, as in the second-generation Freehand system; however, the electrodes are subject to high forces and large motions in the muscle and their long-term stability.

Neural electrodes are more invasive than any of the muscle stimulation technologies but provide better selectivity, lower effective stimulus currents, and offer the potential of recording naturally occurring action potentials through the same kind of device that can be used for stimulation. The least invasive neural electrode is an epineural electrode, an electrode that lies on a neural surface but does not cause any damage to the nervous system. Epineural electrodes can take the form of simple single contact electrodes to complex spiral or helix cuffs that wrap around peripheral nerves with many

individual electrodes (Thoma et al. 1989). Stimulation through multicontact epineural electrodes has been shown to selectively activate portions of nerve, most likely entire fascicles. When placed appropriately, fascicle stimulation can be selective for a single muscle; however, larger, highly fasciculated nerves (such as the hand and arm nerves) present problems for epineural electrodes. Fascicles in the center of these nerves are difficult to selectively stimulate. To counter this limitation, the flat interface nerve electrode (FINE) has been developed. This electrode system alters the physical organization of the nerve, making individual fascicles easier to stimulate by moving them closer to the surface (Tyler and Durand 2002). Using this system with up to eight electrodes, researchers have been able to show selective activation of multiple muscles in animal (Brill et al. 2009) and human experiments (Fisher et al. 2009). Although this electrode system can recruit muscle activity in a graded fashion from few muscles to whole nerve activation, it has limited subfascicular specificity (Leventhal and Durand 2003) which would be required to emulate a natural muscle recruitment strategy.

Intrafascicular Electrodes

Intrafascicular stimulation may overcome the problems of current treatment technologies for SCI or limb loss by providing highly selective stimulation and recording capabilities (Nannini and Horch 1991; Zheng et al. 2008; Rossini et al. 2010). The USEA (Figure 1.4), for example, has been used to selectively activate muscles to produce stance in felines (McDonnall et al. 2004a). More selective stimulation could benefit both SCI and limb loss patients by increasing the patient's control and the prosthetic's range of abilities. In addition to the stimulation capabilities offered by an intrafascicular electrode,

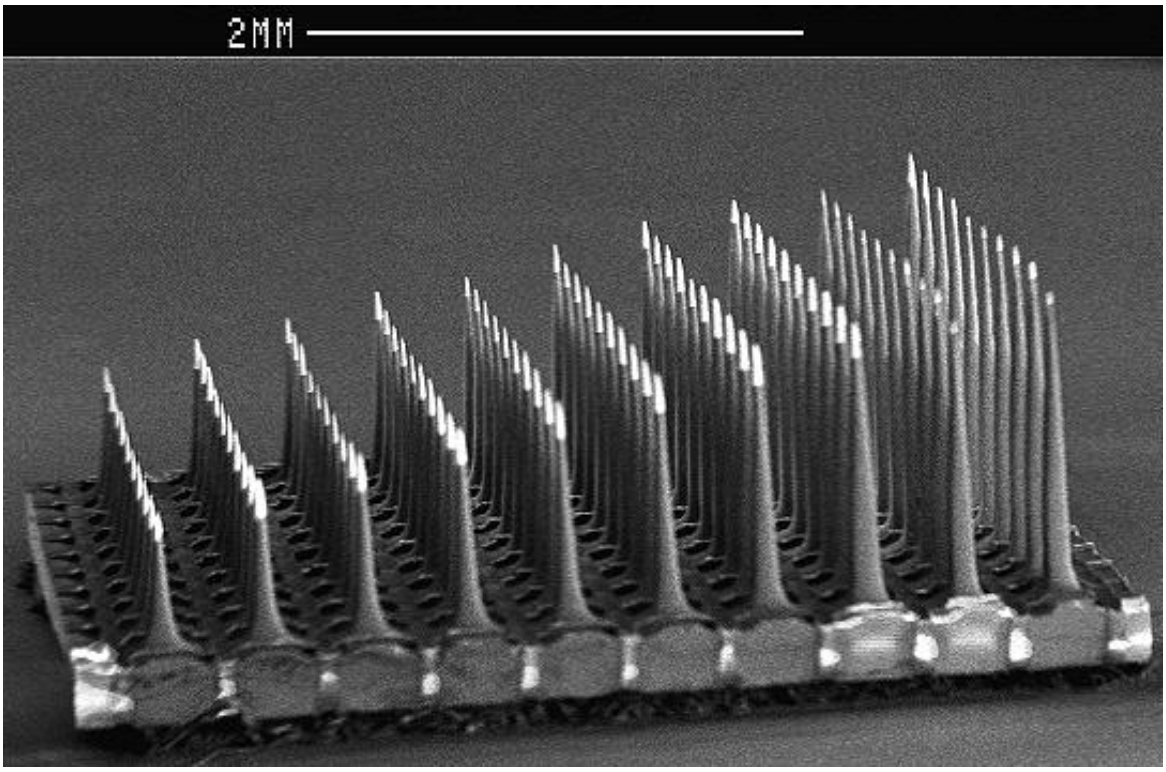

Figure 1.4: A Utah Slanted Electrode Array (USEA).

Each USEA consists of a 10 x 10 grid of electrodes with 0.4 mm spacing and shaft lengths varying between 1.5 mm and 0.5 mm. Each electrode has a metalized iridium oxide tip and is isolated from all other electrodes. When implanted, the electrodes cover the width and breadth of the nerve such that most nerve fibers will be less than 200 μm away from an active electrode tip. Copyright © 2001, The American Physiological Society (Branner et al., 2001.

such a device could also allow for recording of neural signals, thereby opening the door for two-way communication. This ability to record could allow SCI prostheses to return sensation from a reanimated limb or read intention directly from the remnant limb of an amputee without the need for a prosthetic in the motor cortex to detect the user's intent. The advantages that intrafascicular electrodes have over other prosthetics come with accompanying challenges. Electrodes arrays may require specialized software and

hardware to take maximum advantage of the microelectrode array architecture. for complete control over stimulation of one-hundred electrodes, including intrastimulation timing and pulse delivery, a multichannel amplifier is required. A quick and effective assay of all electrode responses is also necessary if we plan to build our prosthesis on the interactions and responses of array stimulation.

Research Outline

In this work I will suggest solutions to the complex problems of long-term stimulation, recording, and device stabilization. Chapter 2 will investigate our ability to stimulate sensory fibers to give modern prosthetics the ability to provide proprioceptive and tactile feedback to users of artificial limbs. The long-term stability of USEA implants for stimulation and recording will be investigated in Chapter 3. The UINTA stimulator has been used to selectively stimulate nerve fascicles with very fine control that allows for multi-muscle-group control. Although these experiments have had success generating stance maneuvers via hind limb nerve stimulation cats, Chapter 4 aims to determine whether this technology can maintain high selectivity in the more complex motor system of the hand.

This work will enhance the abilities and techniques used in neuroprosthetic applications by stabilizing selectivity and long-term performance and providing the potential for real-time feedback from prosthetics or paralyzed limbs.

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CHAPTER 2

DIRECT STIMULATION OF PRIMARY SENSORY AFFERENTS VIA THE USEA IN A FELINE NERVE INJURY OR LIMB LOSS MODEL

Abstract

Restoring lost sensory function in limbs through artificial sensory input for limb loss patients may be possible with the Utah Slanted Electrode Array (USEA) implanted in peripheral nerves. As proof of concept, we implanted 100-electrode USEAs in the sciatic nerve of anesthetized felines (n = 6). We assessed the ability of electrical stimulation through USEAs to elicit sensory responses by monitoring the cortex for Somatosensory Evoked Potentials (SSEPs) recorded via skull screws placed over somatosensory cortices. Muscle activation was monitored via evoked myoelectric responses recorded from wire pairs implanted in four muscles in the lower leg. To confirm direct activation of primary sensory afferents, the sciatic nerve was crushed and cut distal to the implanted array; electrical stimulation was then performed again to evaluate the SSEP. Comparisons of the pre-cut and post-cut cortical potentials confirm that we can activate primary sensory fibers directly in both circumstances with similar latency and amplitude. Additionally, at lower intensities, most electrodes were selective for either sensory or motor responses, implying that USEA stimulation could be used to

evoke motor or sensory responses without engaging undesired modalities. These results indicate that stimulation via USEAs implanted in peripheral nerves could be used to restore sensation analogous to cutaneous and proprioceptive sensation in individuals with a prosthesis.

Introduction

Although there have been recent promising advances in prosthetics and intuitive prosthetic control, including targeted reinnervation or Functional Electrical Stimulation (FES), users often find it difficult to use their prosthetic limb without any form of sensory feedback (Ziegler-Graham et al. 2008). Electrical stimulation in the Perpherial Nervous System (PNS) could enhance the usability of prosthetic devices and increase user satisfaction by providing proprioceptive feedback and tactile sensation. Because the USEA has been used previously to activate motor units selectively in a Spinal Cord Injury (SCI) model (Branner et al. 2001), the USEA can also likely be used to stimulate individual sensory fibers, or small groups of sensory fibers, given that the sensory and motor neurons are not fundamentally different in terms of activation mechanisms and properties.

The loss of a limb affects 1.7 million Americans, with one-third of them having lost a hand or arm. These numbers are expected to double by 2050 despite advances in vascular repair and other emergency care (Ziegler-Graham et al. 2008). Limb loss is also an increasingly common injury for soldiers facing modern explosive weapons; soldiers' lives are often saved with body armor and rapid medical care, but saving limbs damaged by explosives is quite difficult (Fox et al. 2005).

Prosthesis abandonment has been reported as high as 20%, with as many as one third of prosthesis users describing themselves as unsatisfied (Pezzin et al. 2004). One of the abandoners' primary complaints was that lack of tactile and proprioceptive information makes manipulating objects or responding to changes during movement difficult (Biddiss and Chau 2007). Because stimulation of micro-wire electrode arrays implanted in the PNS with as few as 25 electrodes have successfully conveyed sensory information to the human nervous system (Warwick 2005), the USEA, a 100 channel intrafascicular electrode, should be capable of doing so as well. By combining an advanced prosthesis that has tactile sensors with electrodes to deliver sensation, one could close the loop within the body and allow for a more natural and intuitive control of the limb.

Limb-loss patients could benefit from the restoration of sensation even if they choose not to use a prosthesis, because stimulation might also reduce the incidence of phantom pain syndrome. Phantom pain syndrome, the chronic feeling of pain from missing or paralyzed limbs, has been closely linked to the remapping of somatosensory cortex that results from long-term cessation of sensation from the injured area and, specifically, lack of action potentials from the damaged nerves. This remapping and the referred pain sensation have been shown in some studies to be reduced by stimulation of the remnant limb muscles or nerves (Flor et al. 1995; Lotze et al. 1999; Moore et al. 2000; Flor 2008). USEA stimulation of sensory fibers could likewise be used to prevent or reverse the cortical remapping and the associated pain.

Even a neuroprosthetic device to be used for restoring sensation to limb-loss

patients will need to avoid unintentional activation of lower motor axons, through unspecific stimulation of the nerve. Lower motor axons in an amputee may terminate in intact muscles that have limited or lost functionality, such as the extrinsic hand muscles, which are located in the forearm, in a hand-loss patient. Alternatively, the lower motor axons could no longer be associated with muscles, as would be the case in an elbow-level amputee. In either case, the lower motor neurons may still be associated with reflex circuits that could induce unwanted, unplanned, corrective movements from the body in response to movements the body is not actually making. When the muscles are intact in these patients, contractions will induce sensory responses from stretch receptors that could engage reflexes despite the lack of an actual limb movement. In patients where the α-motoneurons terminate without connecting to muscle, there is still a possibility of a reflexive action through the central synapse of the α-motoneurons with Renshaw cells that may disinhibit antagonist muscles through Ia interneurons. Although these reflexes may be functionally harmless, especially if the muscles involved are missing or impaired in function, they are still undesirable because they may complicate the use of a neurally-controlled prosthesis or cause discomfort.

In SCI patients, where the same types of electrodes can be used to stimulate motor fibers to generate movements, this problem will be exacerbated by a total lack of feed-forward control from the brain that results in intact reflex circuits that are hypersensitive. Additionally, in cases of partial paralysis, the activation of intact sensory afferents would cause sensation in the still-functional portions of the limb.

Thus, an additional aim of this study was to examine the relationship between

muscle twitch thresholds and sensory response thresholds on each electrode by monitoring EMG responses in the muscles innervated by the implanted nerve. Although it is not strictly necessary to have exclusive access to one modality or another to create viable prosthetics, such selectivity would facilitate the functionality of the device.

Methods

Because previous work has been successful in selective muscle stimulation in feline sciatic nerve, the same model was chosen to test the selectivity of sensory stimulation. Each feline in the study (females, $n = 6$) was examined per University of Utah Institutional Animal Care And Use Committee protocols. Anesthesia was initialized with an intramuscular injection of 10 mg/kg Telazol (fort Dodge Animal Health) and maintained through a respirator mixture of 1-3% by volume isoflurane. To maintain a stable depth of anesthesia, vital signs including heart rate, rectal temperature, expired CO_2 partial pressure, and blood oxygen saturation were monitored and recorded at regular intervals. The respiration rate, tadal volume, and isoflurane percentage were adjusted to maintain vital signs within normal surgical ranges.

Surgery

The sciatic nerve was exposed at mid-thigh via an incision parallel to the femur at the fascicle connection of the biceps femoris. The biceps femoris was then reflected and the sciatic nerve was exposed and separated from surrounding tissues through blunt dissection. We then implanted a 100-electrode USEA (10 x 10 electrodes) in the sciatic nerve using a high-speed insertion system as described in previous work (Rousche and

Normann 1992; McDonnall et al. 2004a). The implant was then protected with a silicone cuff, and the leg was closed for the duration of the experiment (Figure 2.1 A).

Sensory Cortex Monitoring

To monitor Somatosensory Evoked Potentials (SSEPs), the skull was exposed by an incision along the midline of the skull, and the temporalis muscle was reflected to allow skull screws (Veterinary Orthopedic Implants 10 mm 316L Cx screws) to be placed over primary sensory cortex (S1) based on skull landmarks (Figure 2.1 B) (Dykes et al. 1980). Each skull screw was monitored against an occipital reference and recorded and filtered at (10-7500 Hz) on a Cerebus data acquisition system (Blackrock Microsystems, Salt Lake City, UT). Each screw's location relative to the specific animal's cortex was confirmed posthumously through dissection.

Muscle Response Monitoring

Muscle activity was monitored on the Cerebus data acquisition system through pairs of fine-wire EMG electrodes (California Fine Wire Company) inserted into the bellies of the Medial and Lateral Gastrocnemius (MG & LG), Tibialis Anterior (TA), and Soleus (Sol). To ensure appropriate placement, we stimulated through EMG wires directly; the motions generated were used to confirm activity in the expected muscle through palpitation and action of the limb. The differential signal in each EMG pair was used to quantify muscle response.

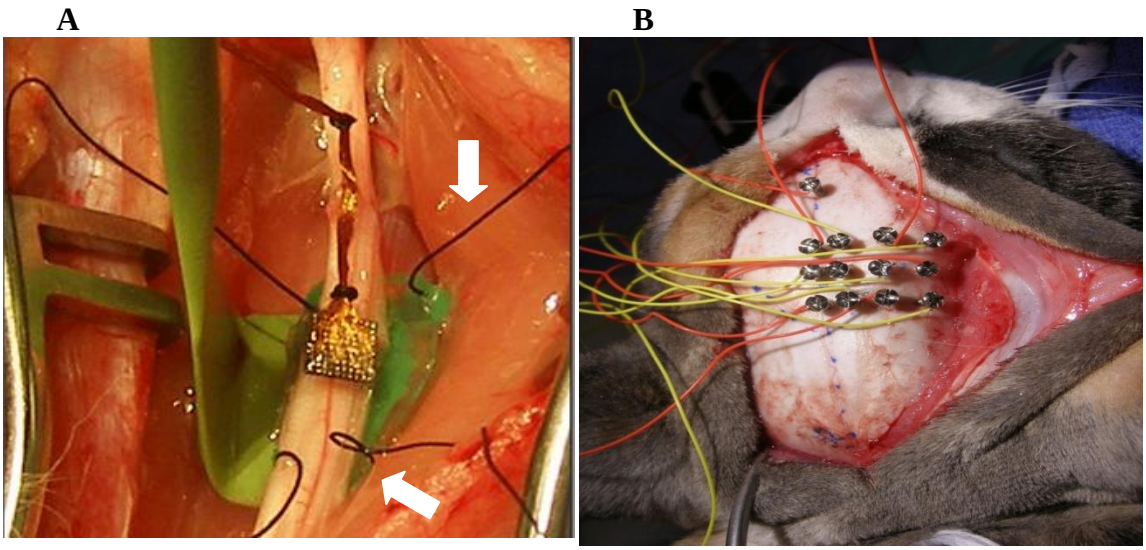


Figure 2.1: Neural and cortical electrodes implanted in an animal.

A. The USEA implanted in the sciatic nerve. The array was implanted pneumatically while being supported from below by a Teflon platform. Sutures, indicated with white arrows, were used to tie the cuff closed after array implantation.

B. Skull screw electrodes placed over S1 cortex. Stainless steel screws were advanced through the skull to make contact with the cortex; recording leads were soldered directly to the skull screws.

Electrophysiology

After implantation of all electrodes and monitoring equipment, we stimulated through USEA electrodes using either the UINTA stimulation system or a GRASS SD-9, using pulse-width modulated (between 0.1 μs and 1024 μs), constant-voltage waveforms at 1–7 Volts. The UINTA system delivered stimulation pulses to every electrode individually according to a binary search routine controlled by the UINTA system. The maximal EMG response and the maximal cortical response were calculated by the UINTA system as part of its automated recruitment curve routine and were used to determine the pulse width of the next pulse delivered. In this way, every electrode was stimulated through at a variety of pulse widths such that the EMG of the maximally

responsive muscle was saturated and the EMG recruitment of that muscle was well characterized. Stimulation trains were also delivered through the SD-9 at either high (50-Hz) frequency, to generate fused muscle trains, or low (4-Hz) frequency to collect SSEP data. Between 16 and 64 stimuli were delivered to each tested USEA electrode, and cortical responses were averaged, similar to other SSEP studies (Lesnick et al. 1986; Highland et al.; Shokunbi and Gelb 1990). Averaged cortical recordings were examined for short-latency responses likely to be caused by action potentials in primary afferents. Due to the time required to collect SSEP data, only some electrodes were selected to run the low-frequency Input Output (IO) curves that are necessary for SSEP averaging. Electrodes were chosen for collection of SSEP IO curves on the basis of their response to a test train of low-frequency stimulation of 500-μs pulses (half of the maximum pulse width used in the experiment) at 5 volts, or their ability to stimulate either muscle or cortex at the lowest pulse widths, typically less than 10 μs at 5 volts, as determined by the UINTA stimulator. Pulse widths were adjusted by 10% for each step of the SSEP IO curves.

After the responses of the array electrodes to stimulation were characterized, the nerve was crushed and transected distal to the implant, severing the connection between the nerve and lower leg. Stimulation was then repeated on electrodes that evoked SSEPS before the severing of the nerve. With the nerve severed, stimulation cannot cause muscle twitching, eliminating the possibility that SSEPs are generated by normal somatosensory pathways through movement of the leg.

Results

Somatosensory Evoked Potentials

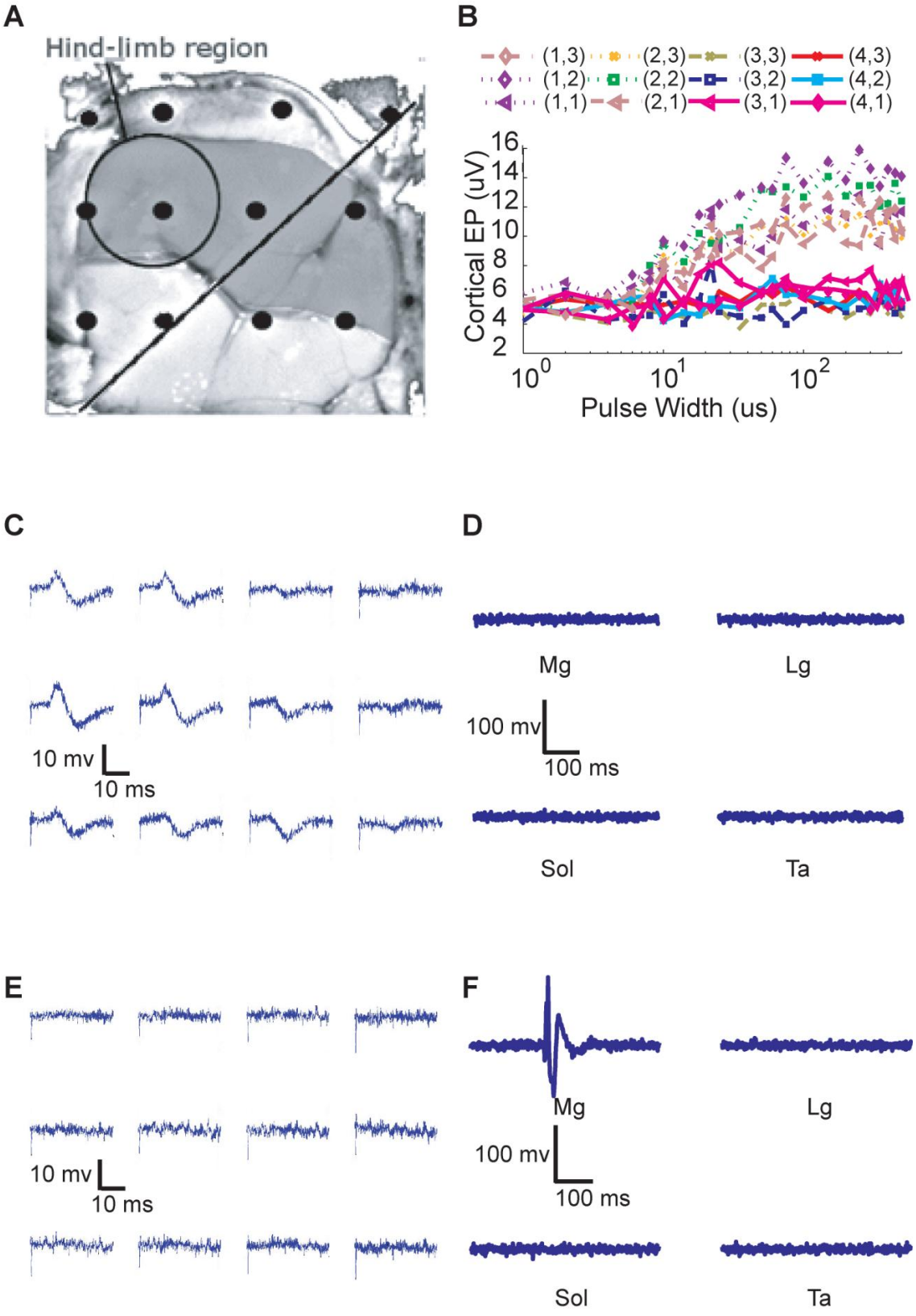
On average, across all animals (n = 6), SSEPs with latencies of 10 ms and 20 ms, known as the P10 and N20 components, were reliably evoked by low-frequency test trains on approximately 53% of USEA electrodes tested (84 electrodes, 6 animals). The full array was not characterized in every preparation. Short-latency SSEP waves were largest in medial cortex, anterior of the ansate sulcus and posterior of the cruciate sulcus, consistent with studies that have used whole-nerve stimulation to generate SSEPs from the hindlimb nerves (Davenport et al.) (Figure 2.2). The magnitude of the response fell off in the lateral and posterior directions, and as distance from the ansate and cruciate sulci increased, in a fashion consistent with electrotonic spread from a localized source. Thus, EPs exhibited appropriate location and spatial selectivity.

Cortical responses varied with stimulus intensity for all USEA electrodes that evoked a potential. Skull-screw electrodes consistently showed a sigmoid response to pulse-width modulated stimuli (Figure 2.2 B) delivered through USEA electrodes, indicating stronger stimuli recruited the activity of more sensory neurons. Additionally, on electrodes that caused a cortical response prior to nerve transaction, SSEPs persisted with little change in the shape of short latency waveforms when the nerve was severed distal to the implant, indicating that primary afferent activity was evoked in either case.

Muscle Responses

Muscle responses to USEA stimulation were similar to those in previous studies in that more than 50% of USEA electrodes evoked muscle responses (72.3%, n = 6

Figure 2.2: Cortical responses evoked by USEA stimulation.
A. Skull screws' positions shown in relation to the cortex as determined by posthumous examination. **B.** Recordings from 12 skull screw electrodes, positions as in A. Medial electrodes anterior of the ansate sulcus showed the largest response, with response magnitude increasing as pulse width increased. **C.** Cortical responses were detected without EMG responses on some electrodes. **D.** EMG responses from the same stimulation event as in C. **E.** Cortical recordings showed no response during an evoked muscle twitch (shown in F). **F.** EMG responses from the same event as shown in E.


A
Hind-limb region
B
(1,3) (2,3) (3,3) (4,3)
(1,2) (2,2) (3,2) (4,2)
(1,1) (2,1) (3,1) (4,1)
Cortical EP (uV)
16
14
12
10
8
6
4
2
10^0
10^1
10^2
Pulse Width (us)
C
10 mv
10 ms
D
Mg
Lg
100 mv
100 ms
Sol
Ta
E
10 mv
10 ms
F
Mg
Lg
100 mv
100 ms
Sol
Ta

animals, 84 electrodes). As in previous studies, electrodes that activated the same muscle were somatotopically organized, meaning they were usually near one another physically (Branner et al. 2001; McDonnall et al. 2004a; Dowden et al. 2009). Stimulation was selective for each of the muscles monitored on some electrodes, as in previous studies (Dowden et al. 2009).

Motor and Sensory Relative Thresholds

On 29 USEA electrodes that could evoke an SSEP, extensive IO curves of low-frequency stimulation were recorded to determine the relative thresholds of motor and sensory stimulation. Not all electrodes could be characterized in this fashion due to the time involved in generating an IO curve for SSEPs with low-frequency stimulation.

Of those 29 USEA electrodes capable of evoking potentials in S1, 41% evoked SSEPs at pulse widths that did not cause muscle twitches while the nerve was intact (mean SSEP threshold of 62.5 μs ± 23.3 μs, Figure 2.2 C, D); whereas 47% recruited muscle twitches before sensory responses (mean twitch threshold 68.5 μs ± 37.4 μs, Figure 2.2 E, F). Thus, importantly, it was possible to activate either sensory or motor fibers selectively, at least with low stimulus strengths. For most USEA electrodes, the motor and sensory thresholds were very similar, varying by only a few μs, and were not statistically different.

Discussion

Depending on the circumstances, it may be desirable to activate sensory fibers without activating motor fibers (e.g., to provide cutaneous and proprioceptive input after

limb loss), or to activate motor fibers without activating sensory fibers (e.g., to reanimate paralyzed limbs after SCI). Because motor fibers are often large-diameter fibers, they may be likely to be activated by extracellular electrical stimulation (Szlavik and de Bruin 1999). However, some sensory fibers associated with muscle and tendon stretch or tactile sensation are as large or larger, and hence may be as likely to be activated as are motor fibers. Furthermore, there is a wide range of fiber sizes associated with each modality, meaning that any given stimulation could stimulate either motor or sensory fibers, and possibly even both simultaneously. With intrafascicular stimulation, the proximity of the electrode tip to the given fibers is also an important determinant of which fibers are activated.

In an intact nerve, stimulation of motor fibers could affect the sensory cortex through secondary mechanisms caused by foot movement, principally the stretching or force of contraction of the muscles involved. Even in an amputee there will likely be residual muscles that could be activated in this fashion, though they may not cause any movement about a joint. The simplest way to avoid this problemin our animal model is to sever the nerve distal to the implant. This solution not only eliminates the possibility of secondary effects being measured, but also replicates more accurately the circumstances in an amputee. However, in the intact nerve there also exists an opportunity to determine the relative thresholds of neurons of different modalities. Motor fiber activation is fairly easily detectable through EMG electrodes. Even single motor units, which represent the activity of a single neural fiber, can be detected with an appropriately placed EMG electrode pair due to the large potentials produced by muscle cells. However, it is

possible that muscle activity in a muscle that was distant from any EMG electrode would go undetected.

Somatosensory potentials are not amplified in a similar fashion, and thus are harder to detect. By using SSEPs, which require the averaging of many responses, as an assay of sensory stimulation, we were probably limited to detecting the concurrent activity of many sensory neurons. A second difficulty with using SSEPs to detect sensory activation is that SSEP amplitude and latency can change due to anesthesia, nerve damage, and even animal temperature. Because these factors were relatively stable during recording sessions, there is little chance that they were significant contributors to the changes seen in SSEPs during the generation of an IO curve. Recordings of the SSEPs were also collected multiple times on some individual electrodes to determine that the responses were consistent.

Selective recruitment of both sensory and motor fibers in this experiment indicate that microelectrodes *in vivo* can elicit potentials in a small enough area to interact with either a uni-modal region of a fascicle or a purely sensory or purely motor fascicle. As fasciculation of the nerve changes along its length (Stewart 2003), the ability of penetrating microelectrodes to activate solely sensory or motor responses may change with implantation site. Some studies have shown that nerves begin clustering by sensory modality very early in the nerve (Ekedahl et al. 1997; Wu et al. 1998; Hallin and Wu 2001). The implants in this study were placed in the sciatic nerve just proximal to the knee; it is possible that more proximal implants, closer to the separation of the dorsal and ventral roots, could have greater specificity for sensory/motor modality. There is

evidence that the changes in fasciculation trend to fewer fascicles at the proximal end of the nerve, which could reduce specificity in terms of the number of neurons; however, not all nerves have significant fascicular joining (Stewart 2003; Gustafson et al. 2009). The clustering of similar modality sensory fibers seen in the work of Hallin et al., combined with the ability of the USEA to elicit SSEPs without eliciting muscle twitches strongly implies that there are microzones within the nerve where there is a dense clustering of fibers associated with a either sensory or motor function. This organization of nerve fibers within the nerve is consistent with the plexiform model of nerve organization.

The clustering of nerve fibers by modality and proximity of receptive field has implications for both stimulation and recording through intrafascicular electrodes. Fiber type clustering creates microzones where nearby neurons encode information addressing similar stimulus features. Stimulation of such a microzone could produce a response that is highly selective in terms of muscle/sensory response. Individual electrodes may only be able to elicit small percepts due to the close thresholds of the sensory and motor responses seen in this study. However, stimulation through multiple electrodes capable of selective sensory activation could be combined to create a bigger percept without engaging muscles simultaneously. Additionally, recordings made from an electrode situated in such a microzone would be useful for decoding sensation (or motor command) even if the recordings have a low signal-to-noise ratio or are multiunit recordings because the neighboring neurons would convey similar information.

Hence, we propose that single unit recordings are not strictly necessary for use in

PNS prosthesis development, because the multiunit activity in such a microzone could be used to accurately decode what region and modality were being activated. Threshold crossings of multiple unit recordings and local field potentials in the CNS have already been used by several researchers to accurately decode data in the cortex (Carmena et al.; Chestek et al. 2009; Fraser et al. 2009).

Motoneurons may cluster in a similar fashion, as evidenced by the musculotopic organization of stimulation maps in this and previous experiments (McDonnall et al. 2004a) (Dowden et al. 2009). Similar to the argument for intrafascicular stimulation and recording in the sensory system, motoneuron clustering would be advantageous selectively stimulating motor fibers in a SCI patient, or for decoding intent in a limb-loss patient.

In this study, we were able to determine only the areas of cortex activated, but not the sensory modality, or modalities, being engaged. Sensory modalities such as touch and proprioception are useful in prosthetics; in contrast, we desire to avoid recruiting pain fibers. Although it is not possible to determine the modalities engaged from an SSEP, it is unlikely that pain fibers will be common responders because they are, on average, much smaller than cutaneous mechanoreceptors and stretch receptors.

The SSEP response onset latencies in this study were short, from 5–10 ms, indicating primary afferent activation. Relative thresholds of motor and sensory fibers vary by electrode. The close thresholds of motor and sensory responses on many electrodes likely relate to similar fiber sizes for several sensory modalities and motor unit types. This implies that the electrode position is most important in determining the

specific fibers accessed. Both muscle and cortex could be recruited on some electrodes which could not recruit the other modality, indicating high specificity for USEA stimulation.

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CHAPTER 3

LONG-TERM EMG-FREE RECORDING AND SELECTIVE MUSCLE ACTIVATION WITH UTAH SLANTED ELECTRODE ARRAYS IN A FELINE MODEL[1]

Abstract

Recording and stimulation via high-count penetrating microelectrode arrays implanted in peripheral nerves may help restore precise motor and sensory function after nervous system damage or disease. Here we report successful long-term *in-vivo* recording and muscle activation through the Utah Slanted Electrode Arrays (USEAs) implanted in cat sciatic nerve. Although previous work had demonstrated safety and the ability to activate muscles with similar long-term implants (Branner et al., 2004), several major challenges remained: 1) to maintain stable electrode impedances; 2) to maintain viable recordings of nerve action potentials; 3) to overcome contamination of unit recordings by myoelectric (EMG) activity in awake, moving animals; and 4) to maintain selectivity and functionally useful long-term muscle activation. In conjunction with improvements to USEAs themselves, we redesigned our USEA containment and connector systems to minimize relative motion between the array and connector, avoid wires' crossing of joints, include an on-array reference, and provide electrical isolation and physical protection for the array. In contrast to earlier work, electrode impedances remained

[1] Portions of this chapter reprinted with permission from IEEE, Copyright © 2011, IEEE (Clark et al., 2011)

relatively stable for periods up to 4 months (the terminal experimental time point). Further, we successfully recorded unit activity from USEAs and effectively eliminated EMG contamination of unit recordings in the moving animal via our containment system. As an example of functional usefulness, broadband or spike-threshold data were acquired during imposed limb movements in an anesthetized animal using a wireless recording integrated circuit attached to implanted USEAs. These data were then decoded blindly to drive a virtual prosthetic limb in real time up to 127 days after implantation of the USEA (r = 0.91 in cross validation tests). Chronic stimulation indicated that selectivity of muscle activation (the ability to recruit one particular muscle without other muscles) was also maintained. Demonstrating functionality, multi-electrode, multi-USEA interleaved stimulation through USEAs chronically implanted in femoral, sciatic, and muscular sciatic nerves produced fatigue-resistant sit-to-stand behavior up to 2 months after implantation (ongoing experiments). These results support the possibility of using USEAs in peripheral nerves to provide motor control and cutaneous or proprioceptive sensory feedback in individuals after limb loss or spinal cord injury.

Introduction

The upper and lower limbs and digits exhibit high innervation densities, thereby allowing fine motor control and high-resolution, multi-modal sensory input. Consequently, to restore motor and sensory function effectively after limb loss or spinal cord injury, peripheral nerve interfaces will need to record from and stimulate a large number of different sites in a highly selective manner. for example, because residual nerves remain viable after limb amputation, recordings obtained from motor fibers could

provide natural, intuitive control signals for a highly dexterous prosthetic arm, and stimulation of sensory nerves could evoke cutaneous and proprioceptive percepts. Similarly, after spinal cord injury (SCI), recordings from sensory fibers could provide cutaneous and proprioceptive information that could be used to evoke percepts (e.g., via stimulation of somatosensory cortex), and stimulation of the intact motor nerve could evoke muscle contractions. These first-order requirements strongly imply the need for multiple intrafascicular electrodes whose active tips closely abut small subsets of motor or sensory nerve fibers.

Current Technologies

Of the many different causes of paralysis, SCI receives the most research attention due to its disproportionately high cost of treatment and adverse affects on quality of life. Clinically usable systems, such as Parastep, can restore stance to paralyzed individuals, but they rapidly fatigue the muscles involved and have limited acceptance due to the high physical effort required by the patient (Brissot et al. 2000; Spadone et al. 2003). The strong benefit from exercise of paralyzed limbs gives us additional reasons to explore better stimulation paradigms that could extend the time a patient could exercise. With relatively nonselective stimulation of paralyzed limbs, current clinical systems also face the possibility of stimulating antagonist muscles that work against the planned movement.

Limb loss, especially the loss of a hand or arm, can greatly compromise a patient's ability to interact with the environment. Eighty percent of limb loss patients also suffer from phantom pain syndrome, a condition that causes the patient to experience

painful sensations from the missing limb (Flor 2008). Phantom pain does not typically respond to treatments for pain, but ongoing research suggests the possibility of using somatosensory or motor stimulation to prevent this condition from developing (Roux et al. 2001; Saitoh and Yoshimine 2007; Ray et al. 2009).

Prosthesis technology available today takes electromyographic (EMG) signals from either the remnant limb's muscles or muscles innervated with remnant limb nerves surgically redirected to still-existent muscles (Kilgore et al. 2006; Kilgore et al. 2008). Targeted reinnervation, wherein remnant nerves are surgically attached to separated muscles, takes advantage of the fact that the Peripheral Nervous System (PNS) is still carrying signals of intent to the missing limb by remapping it to a new muscle (Kuiken et al. 2004); (Kuiken et al. 2009).

Intrafascicular stimulation could build upon the advantages of current treatment technologies for SCI or limb loss by providing highly selective stimulation and recording capabilities in the PNS. The USEA (Figure 1.4), for example, has been used to selectively stimulate muscles to produce stance in felines (McDonnall et al. 2004a; Normann et al. 2005). In addition to the stimulation capabilities offered by an intrafascicular electrode, such a device could also allow for recording of neural signals, thereby allowing for two-way communication (Branner and Normann 2000 Branner, 2004). This ability to record could allow an SCI prosthesis to return sensation from a reanimated limb, or infer intention directly from the remnant limb of an amputee without the need for a prosthetic in the motor cortex.

Proposed Advantages of the USEA

Among its advantages, the USEA provides ~100 independent sites of stimulation and recording a high degree of selectivity and ease of implantation (Rousche and Normann 1992; Nordhausen et al. 1996; Branner et al. 2004; McDonnall et al. 2004a, 2004b; Clark et al. 2008; Dowden et al. 2009). The 100 microelectrodes are spaced 400 μm apart on a 10 x 10 grid, with lengths from 0.5 to 1.5 mm. A single USEA thus provides almost complete coverage of both the width and depth of the cat sciatic nerve. The USEA provides highly selective stimulation and recording for multiple different motor and sensory fibers in cat hind limb nerves (Branner et al. 2001; Branner et al. 2004; McDonnall et al. 2004a, 2004b; Clark et al. 2008; Wilder et al. 2009), and, more recently, in monkey arm nerves (Chapter 4). A similar Utah Electrode Array (UEA) with equal-length electrodes has been used successfully for years in motor cortex of paralyzed humans (Simeral et al.) and has been implanted chronically in the median nerve of one individual without pain or loss of hand function (Warwick et al. 2003).

The work of Branner (Branner et al. 2004) first examined chronic USEAs and containment systems to protect and stabilize the array in cat sciatic nerve. These important initial studies demonstrate that long-term USEA implants are relatively benign and cause little or no behavioral locomotor deficits. The ability to evoke motor responses remained for the life of the implant. However, in general it was not possible to record single units long term, and recordings were contaminated by myoelectric (EMG) activity during movement. Additional challenges included large, rapid drops in electrode impedances, increasing stimulation thresholds, and failure of connectors and electrodes

(perhaps due to broken lead wires), particularly in early implant systems.

Here we report substantial progress toward addressing these remaining challenges, including in particular the ability to obtain long-term, EMG-free unit recordings from USEAs implanted in cat sciatic nerve. Further, to demonstrate their functional utility, we recorded neural signals via a 100-channel wireless integrated circuit (Harrison et al. 2009) and used the wirelessly transmitted spike-threshold data to drive a virtual prosthetic limb in real time.

Materials and Methods

Six purpose-bred adult female cats were used in this study. All experimental procedures were approved by the University of Utah Institutional Animal Care and Use Committee. All animals were chronically implanted with a Utah Slanted Electrode Array (USEA) in the sciatic nerve in the left leg. Five USEAs were implanted proximal to the nerve's bifurcation into the tibial and fibular nerves, and, in one case, USEAs were chronically implanted in both the femoral nerve and the high sciatic, proximal to the bifurcation of the muscular branch of the sciatic nerve. The data provided herein will relate only to implants in the sciatic nerve. The six implants are further distinguished by whether the transcutaneous connector system was implanted in a single stage (one-stage implant) or in two stages (two-stage implant). Details of the implants are provided on an individual-animal basis in Table 3.1.

Table 3.1: Feline connector systems

Cat Number	Connector Type	2 stage set-in time (days)	Duration of implant (days)
F08-029	TDT Cat Bone Mount in 2 stages	69	70
F08-048	TDT, bone mount in 1 stage	0	33
F08-059	TDT, bone mount in 1 stage, bone cement assist	0	127
F08-062	TDT, bone mount in 2-stage	167	127
F09-029*	TDT, bone mount in 2-stage	190	>365
F09-063**	TDT, bone mount in 2-stage	96	65

* additional implants in the femoral nerve and muscular branch of the sciatic
**50 electrode USEA, implanted in high sciatic, additional implant in the femoral nerve

Implantation Surgery

Two to 6 hours prior to induction, analgesia was initiated with buprenorphine (Buprenex®, 5 µg/kg, IM). Anesthesia was induced with a cocktail of Tiletamine and Zolazepam (Telazol®, 6 to 13 mg/kg IM) and maintained with isoflurane (0.5 to 3%). While under the induction anesthesia, the animal was intravenously cannulated and tracheally intubated. Following cannulation, a single injectable dose (2.5 to 5 mg/kg, IM injection) of the antibiotic enroploxacin (Baytril®) and atropine (0.02 to 0.074 mg/kg, IV), to control tracheal secretions, were administered. Lactated Ringer's solution was

administered intravenously at a rate of 8-12 mL/kg/hr. The animal was artificially ventilated (20 to 40 breathes per minute, 75 to 150mL tidal volume) with oxygen-augmented air. The heart rate, SAO_2, $ETCO_2$, and rectal body temperature were monitored.

The minute volume, proportion of oxygen augmentation, and isoflurane levels were adjusted to maintain a heart rate of 120 to 200 beats per minute, a SAO_2 percentage above 90%, and $ETCO_2$ of between 20 and 40 mm-Mg. A warmed water blanket and a heat lamp were used to maintain a core body temperature between 37 and 39 °C. After a stable level of anesthesia was established under isoflurane, the connector system and/or the USEA were implanted.

For one-stage surgeries, the array and entire connector system was implanted during a single surgery. For two-stage surgeries, a mounting plate was implanted during a first surgery, and the array and the remainder of the connector system were implanted during a second surgery. The second surgery followed the first surgery by at least 2 months.

For all implants, a long, thin Titanium plate (50 mm by 5 mm by 1.5 mm thick) was attached to the femur via Titanium self-tapping bone screws (VOI). For one-stage surgeries, the remainder of the connector system was immediately attached to the implanted plate, and the USEA was implanted. For two-stage surgeries, the incisions were closed with suture, and the animal was recovered (as described below). During a second surgery, the remainder of the connector system was attached to the implanted plate ,and the USEA implanted.

To expose the sciatic nerve for USEA implantation (and to expose the femur for the femur connector system), a 5-6 cm long incision was made from the knee towards the iliac crest, and the biceps femoris muscles were separated and retracted. After freeing the sciatic nerve from the surrounding connective tissue, a rigid plate, with the gold mesh screen of the containment system (described below), was slid under nerve at the site of USEA implantation. The USEA was positioned on the sciatic nerve 1-3 cm proximal to the nerve's branching into the tibial and fibular nerves and implanted using the pneumatic impulse inserter technique (Rousche and Normann 1992). After USEA implantation, a containment system was wrapped around the nerve and array to help keep the array in place. All Pt/Ir reference wires (20IR2T, Medwire®) were placed in the tissue adjacent to the nerve.

After implantation of the connector system and/or the USEA, the incisions were closed with suture, and the animal was recovered by termination of the isoflurane anesthesia. Following recovery from the survival surgery, oral doses of enroploxacin were administered (one 22.7 mg Baytril ® tablet administered once a day) for the three days following the surgery. Continual analgesia was provided by an adhesive, transdermal Fentanyl patch (25 µg/hour) attached to the animal in the area overlying the shoulder blades. Prior to attaching the patch, skin was closely clipped, and this region was cleaned with a damp cloth and allowed to air dry. The patch was attached approximately 2 to 3 hours before the end of the surgery and removed when depleted (approximately 3 days). Between 7 and 14 days following the surgery, all skin closure sutures were removed.

USEA Manufacturing

To address the previously observed drops in impedance, electrode or wire shunting, and de-insulation, changes in the processes of array manufacturing were made that included improved precision in electrode geometry, SIRIOF tip metallization, and encapsulation of electrode shanks and wires with Parylene C (Bhandari et al.; Hsu et al. 2009), and wire bonding, instead of soldering, of the wires from the array to the electrical connector. In vitro testing of these improvements indicates that the electrodes will be better insulated against saline leakage and tip degradation. (Negi et al.; Sharma et al.). Before implantation of the USEA, an Automatic Impedance Tester (AIT) (Gunalan et al. 2009) measured the individual electrode impedances, both conventionally and at the electrode tip (in the absence of shunting on any electrode). Four near-corner long electrodes with large exposed tips and low impedances converged to common bus and served as an on-array electrical reference, in addition to conventional Pt/Ir wire references. Electrodes measuring over 2 MΩ were assumed to be broken, and rejected from analysis.

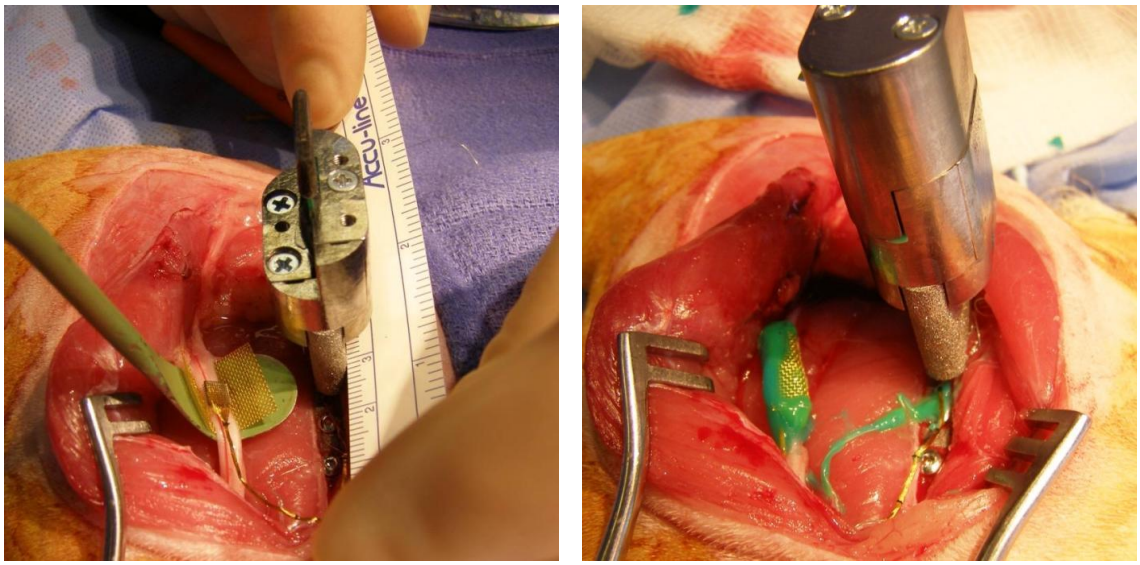
Transcutaneous Connector

Connector and containment systems were modified from previous designs to improve skin closure around transcutaneous portions of the system and to increase array stability. All versions of the connector were made from medical grade titanium and designed to make the transcutaneous portion of the connector as small as possible to limit tissue insult. Additionally, the transcutaneous portions of the connectors were surface treated to increase the surface area and roughness of the skin-interface surfaces. The connector systems were surface treated by Orchid Coating (Southfield, MI).

Initial implant systems, for which data are not reported here, were back mount systems based on previously reported designs (Branner et al. 2004). Because of previous history with similar designs our pilot back mount system was designed to protect wires inside of the transcutaneous connector and routed wires from the transcutaneous connector on the animal's back to the implant site in the distal sciatic nerve. Because of wire breakage at the hip, most likely due to repetitive stress where the wires crossed the hip joint, the connector system was redesigned to be placed on the femur, which reduced the relative motion between the transcutaneous connector system and the implanted array. This also was anticipated to provide a more stable base for a two-stage implant due to the bone integrating with the connector's base-plate (Figures 3.1, 3.2).). Both one-stage (n = 2) and two-stage (n = 4) implants of the femur-mounted connector system were performed. The electrical connector for the femur mount connector system was designed to attach to a ZIF-Clip 96 (TDT) active headstage. The transcutaneous portion of the connector was a rounded 5 mm by 20.5 mm rectangle, and the extracorporeal portion was a rounded 16-mm by-28.5 mm rectangle that was 35.25 mm tall. The height from the bone to the extracorporeal portion was 18 mm, which was sufficient to traverse intervening muscle and to clear the outer surface of the skin. Two cats in the study also received additional USEA implants in the femoral nerve or muscular branch of the sciatic nerve (Table 3.1).

Containment System

To protect the array after implantation, we enclosed the array in a custom-made containment system composed of gold and silicone. A 13-mm by 19-mm gold mesh screen (#52 gold mesh, Alfa Aesar, Ward Hill, MA) whose edges had been coated with Parylene-



A **B**

Figure 3.1: The connector and containment system around an *in vivo* implant.

A. The gold mesh containment system in place under the nerve
B. Containment and connector system with silicone encapsulation
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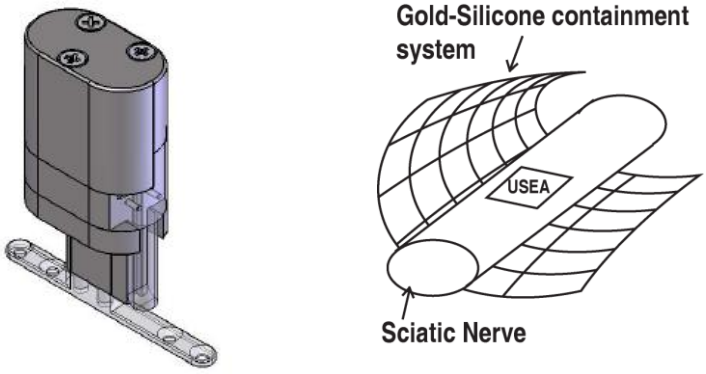

Figure 3.2: Connector and containment system for femur-mounted implants.

A. The transcutaneous connector system designed for mounting on the femur consists of a bone plate and a separate transcutaneous portion holding the PCB of the array that can be implanted in a subsequent surgery. **B.** A schematic of the containment system components shown before it is closed around the array and nerve. (Clark et al., 2011) Copyright © 2011, IEEE

C to prevent unraveling of the screen and to minimize snagging on tissue was initially placed under the portion of the sciatic nerve exposed, prior to implantation of the USEA (Rousche and Normann 1992). After implantation of the USEA, the gold mesh was wrapped around the array and nerve. To create as close a fit as possible, a fast-curing silicone (Kwik-Cast) was used to fill gaps under the mesh and to join the edges of the gold mesh to one another (Figure 3.1 B). A wire connected the mesh to the titanium connector to provide a path to ground during recording sessions.

Postsurgical Physiological and Behavioral Testing

Chronically implanted animals were followed for a period of one to five months (Table 3.1), at which time all but one animal was sacrificed for quantitative histological analyses (data to be reported separately). Each animal's locomotive behavior was assessed after surgery and subsequently throughout the study to confirm normal leg function.

We obtained four different physiological measures in postsurgical tests at frequent intervals immediately following USEA implantation, and subsequently at successively longer intervals. The four measures are: (1) electrode impedance (both conventional and tip) with the AIT under anesthesia, (2) sensory unit recordings when the animal was anesthetized while the experimenter manually manipulated the limb, (3) sensory and motor unit recordings when the animal was awake while the animal was stationary and moving, and (4) the ability of electrical stimulation of electrodes to evoke motor responses under anesthesia. Neural signals and EMG were captured on a Cerebus

recording system. During each recording session, the thresholds for unit detection were set to 6 times each channel's root mean square.

Individual electrode impedances were measured by the AIT using a 1-kHz, 10-mV sine wave in the traditional fashion as well as in the absence of shunting to other electrodes (which presumably represents the impedance at the tip of a single electrode) as described in Gunalan et al 2009 (Gunalan et al. 2009). During electrical stimulation, motor responses were sensed with either EMG signals recorded with fine-wire electrodes acutely implanted in the Medial and Lateral Gastrocnemius (MG & LG), Tibialis Anterior (TA), Soleus (Sol), and the Peronial muscles (Per) or ground reaction force measured with a 6-axis force plate. The motor response signal was used as feedback to generate automated input/output curves between the stimulus duration, of a constant voltage stimulus, and the motor response. All anesthetized recordings were performed after induction with Telazol® (6 to 13 mg/kg IM) and maintenance with isoflurane (0.5% to 1%).

Results

General

As in previous work (Branner et al. 2004), USEA implants appeared behaviorally benign. Cats showed little or no signs of locomotor deficits shortly after recovery from surgery. They used the implanted hindlimb fully in a weight-bearing manner. Animals were allowed to move freely around their enclosures and would bump into objects with the connector during locomotion, subjecting it to mechanical stress. Although the small sample sizes preclude definitive conclusions, the two-stage implants (n = 4), in which the

bone plate was first implanted separately to allow time for osseointegration, were mechanically more stable than the one-stage implants, both of which loosened at the bone attachment site. In one case, we repaired the loosened connector with bone cement, and it remained stable for 3 weeks before ultimately failing. The implant in the second one-stage cat was strengthened with bone cement at the time of implantation. Even with this added initial support, after 127 days it ultimately loosened as well. To the contrary, none of the two stage implants came loose from the femur.

Impedances Stable

Conventionally measured electrode impedance and tip impedance remained relatively stable across the lifetime of the implant. There was a small rise in impedance immediately after implantation (Mean increase of 26 KΩ ± 11.4 KΩ, for tip impedance, 14 KΩ ± 6.7 KΩ for conventional impedance), presumably because tissue impedances were higher than impedances of saline test solutions. Impedances remained relatively constant for several weeks or months thereafter (Figure 3.3). Additionally, shunting of electrodes, as measured by the AIT did not increase over the lifetime of the implant. In most cases there was little apparent lead-wire or electrode breakage evidenced by impedances (> 2 Mohm).

Unit Recordings

Multiple electrodes recorded either single or multiple units in each long-term animal. Unit activity from anesthetized animals often showed clear units that strongly responded to specific experimenter stimuli. Stimuli delivered included toe dorsiflexion

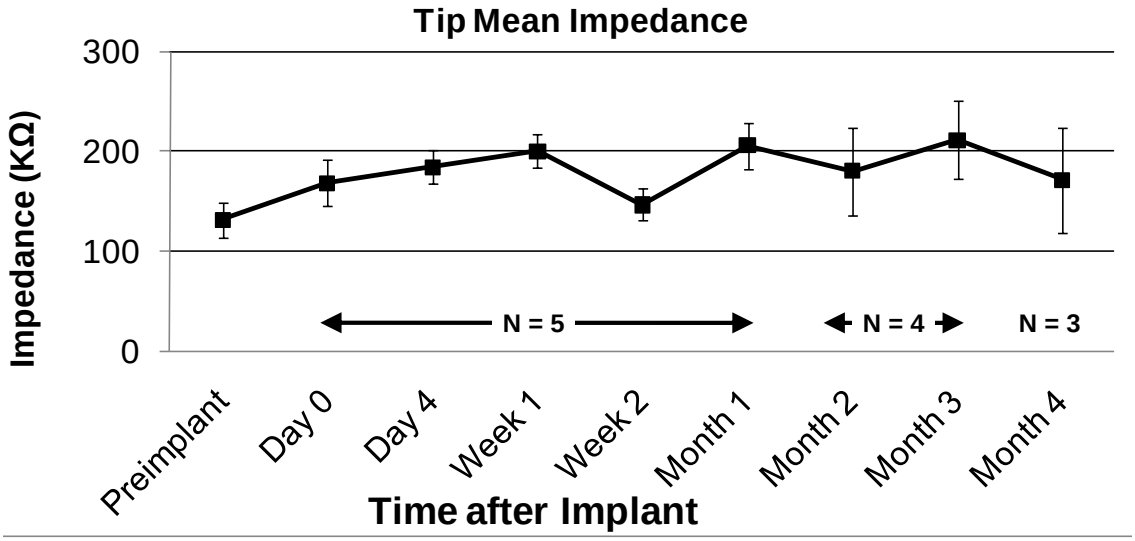

Figure 3.3: *In vivo* impedances over time.

USEA electrode impedances (mean ± SEM) remained relatively stable across time. Day 0 to 3 weeks, $n = 6$. At 2 months, $n = 4$. At 3 and 4 months, $n = 3$. (Clark et al., 2011) Copyright © 2011, IEEE

and plantarflexion, ankle dorsiflexion and plantarflexion, ankle abduction and adduction, and hair brushing of the hindlimb (Figure 3.4). The functional usefulness of these types of recordings was tested by performing a real-time decode of limb position from the recordings of USEA electrodes with an accuracy of R = 0.91.

Shortly after implantation, across all animals 40 ± 7 (mean ± SEM$n = 6$) units were recorded in these anesthetized sessions. There was a drop in unit activity over the course of the first month. Thereafter, the number of units recorded stabilized or increased (depending on the animal) for a period of weeks or months, until animal sacrifice (Figure

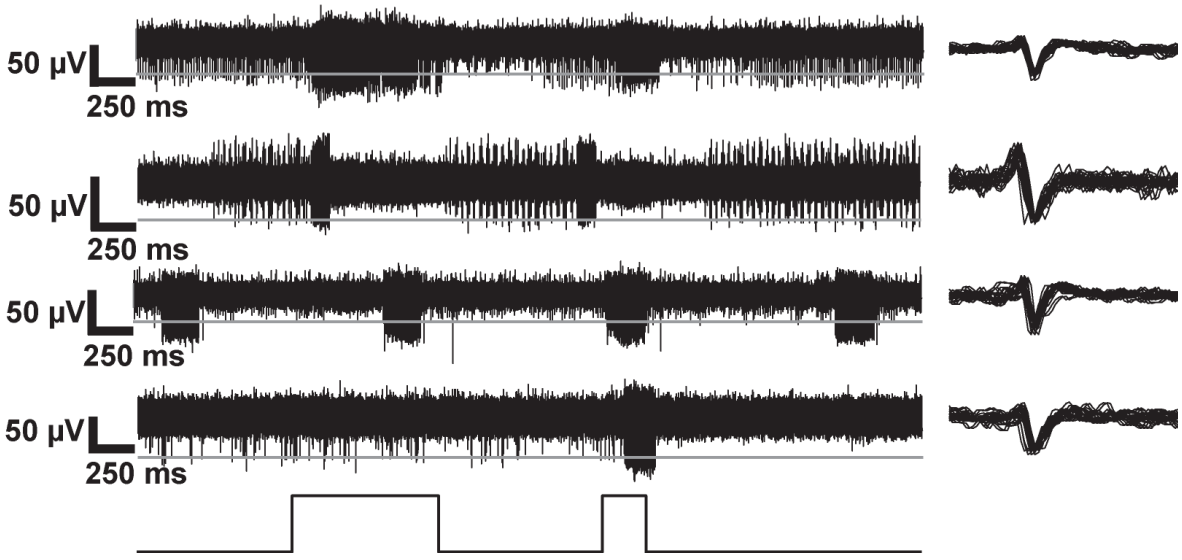

Figure 3.4: Anesthetized recordings.

Stimulus driven (channels 3, 13, and 15), spontaneously active (channel 11 units from a single session are shown. Stimulus marker (in red) indicates toe dorsiflexion. Isolated unit traces (which passed the threshold indicated in red on the left trace) shown to the right for each channel. (Clark et al., 2011) Copyright © 2011, IEEE

3.5, at 4 months, 33 $\pm$ 9 units recorded per USEA, n = 3). Histological examinations of cats with similar sciatic implants (Christensen 2011) indicate that active neuron growth & repair occurs as late as 160 days after implantationaround the electrode shanks, implying that it may be possible for the number of axons near an electrode to increase over time.

Containment Effectiveness

To examine the ability of the containment system to shield neural recordings from contamination by myoelectric activity, we also recorded from awake, moving animals. In

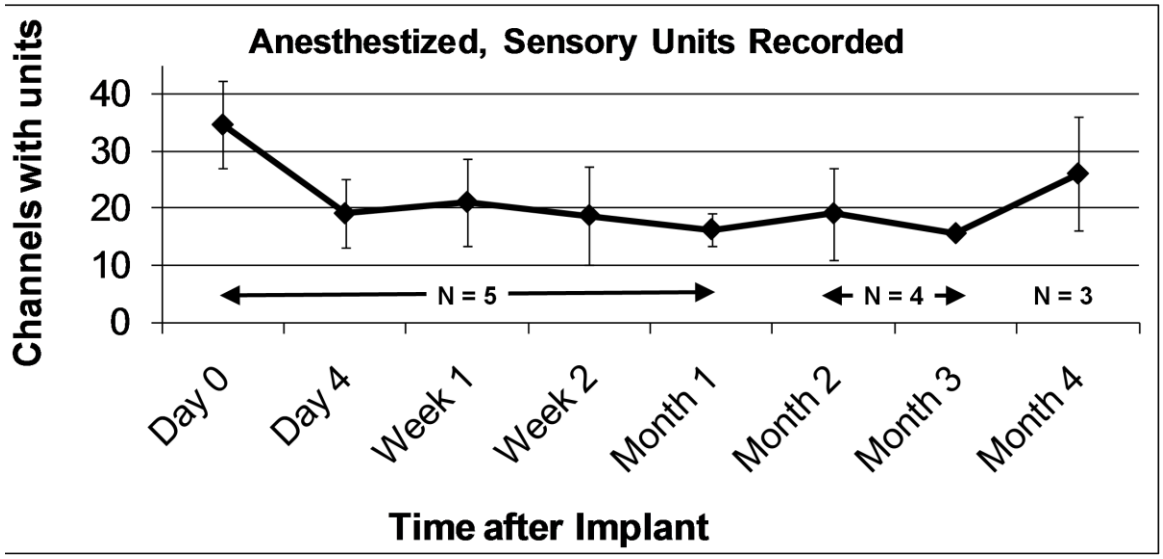

Figure 3.5: Mean number of electrodes recording neural activity per array.

The number of single/multi-unit channels recorded through USEAs chronically implanted in sciatic nerve remained relatively stable for several months (the terminal experimental time point). Number of cats as indicated in Figure 3.3. (Clark et al., 2011) Copyright © 2011, IEEE

conjunction with use of on-array references, the shield dramatically reduced contamination by EMG (Figure 3.6).

Recordings in awake animals showed a greater number of electrodes with units, both multiple and single units, than did recording in anesthetized animals, suggesting that USEAs recorded motor as well as sensory discharges. USEA electrodes, using the shielded reference, were thus able to record unit activity for up to 4 months (Figure 3.7),

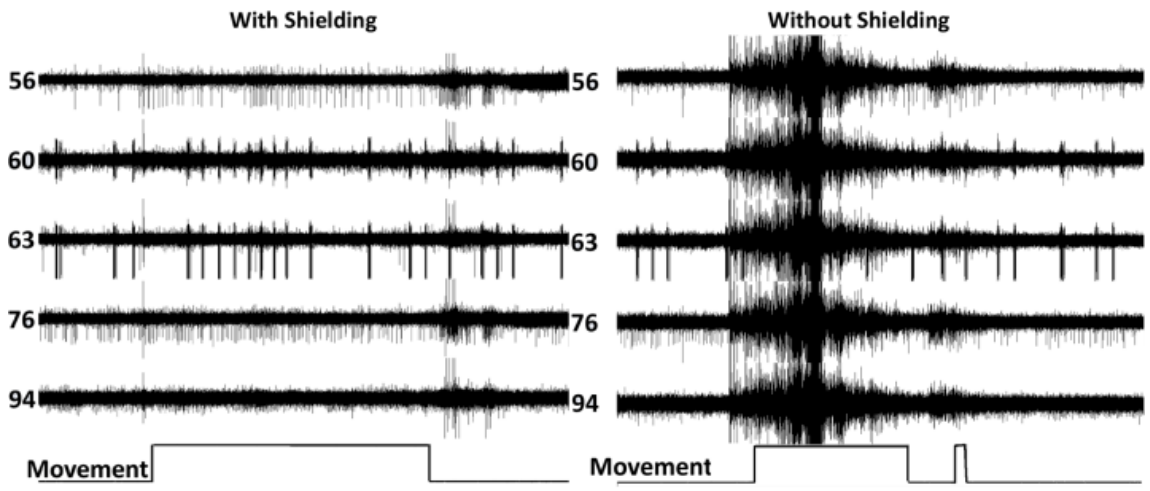

Figure 3.6: EMG-free chronic recordings from sciatic nerve in an awake cat.

Left: Recordings obtained using the on-array reference within the containment-system shield were nearly entirely free of EMG contamination even during movement (bottom marker trace). **Right:** Recordings obtained with reference wires outside the containment-system shield showed large myoelectric contamination on all channels. (Clark et al., 2011) Copyright © 2011, IEEE

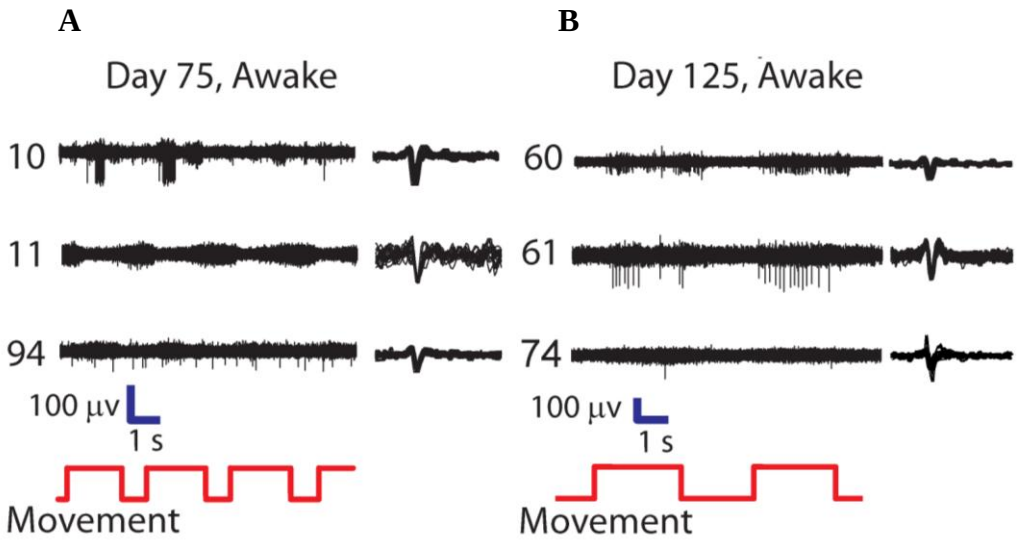

Figure 3.7: Long-term single unit recordings in an awake animal.

A. Single units on channels 10 and 94 (on-array reference). Multi-unit activity is visible on channel 11. Red line indicates cat transitioning from sit to stand. **B**. Single units on channels 60 and 61 (on-array reference). (Clark et al., 2011) Copyright © 2011, IEEE

the terminal experimental time point for histological purposes. Even at the late time points of the experiment, the units recorded in awake animals were often correlated with specific animal movements, such as sit to stand.

Muscle Activation

Stimulation through individual USEA electrodes evoked contractions in the muscles necessary for stance and gait in sessions throughout the study. The number of effective stimulating electrodes reduced in number at the outset, before returning to near-initial levels, similar to the trend observed with unit recordings (Figure 3.8). Similar to results in previous experiments (Branner et al.), absolute threshold to evoke a motor response increased over time for most electrodes. However, even at the longer pulse-widths of voltage pulses delivered in later sessions, single-electrode selectivity for single muscles remained on many electrodes (Figure 3.8, dashed line).

Selectivity Index (SI) of the motor responses was quantified using the formula:

$$\frac{LargestEMG - 2ndLargestEMG}{LargestEMG}$$

(Dowden et al. 2009). Electrodes showed some similarity in the muscles they could recruit from session to session but were not reliably the same across sessions. Musculotopy was observed in every implant at the time of implantation, as in previous studies (Branner et al.; McDonnall et al.); however, the musculotopy of the nerve changed over time (Figure 3.9). The previously mentioned histological results (Christensen 2011) indicating axons continue to grow in and around the electrodes could effect selectivity as well as recording.

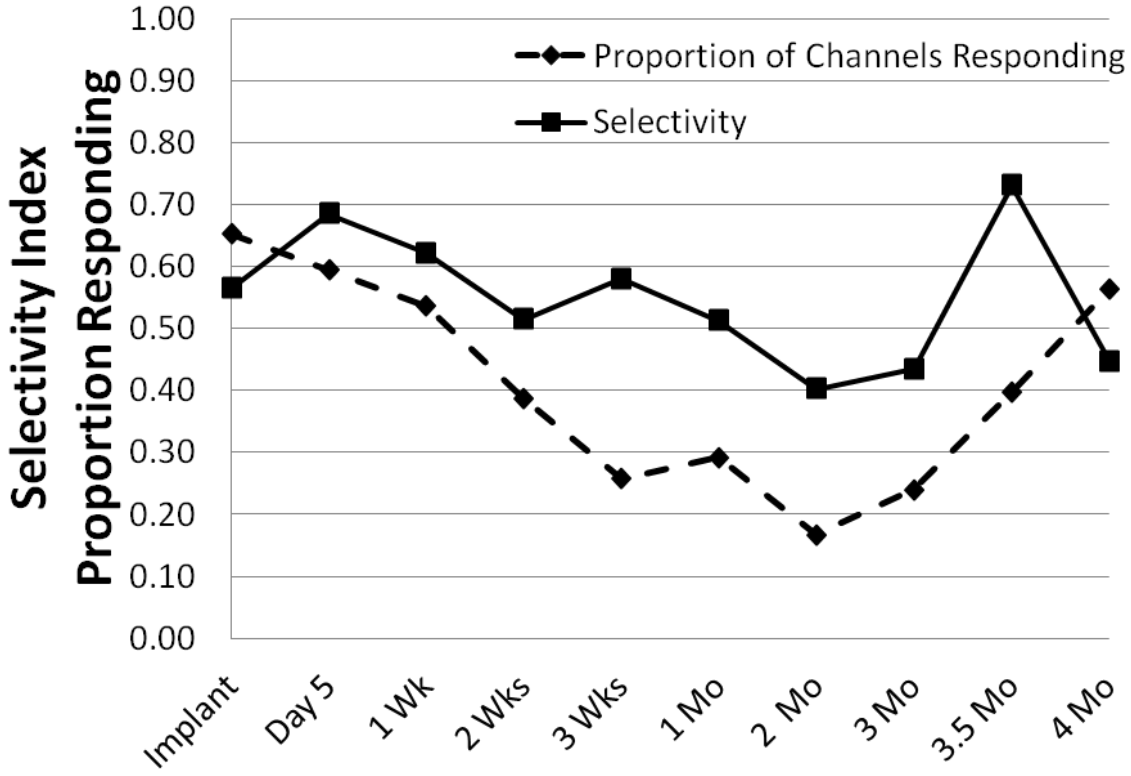

Figure 3.8: *In vivo* stimulation and selectivity over time.

SEM bars shown. The solid line indicates the mean SI of all electrodes, across all implants for each time point. An SI of one would indicate recruitment of a single muscle with no recruitment of other muscles. The dashed line indicates the proportion of electrodes on the array capable of evoking any motor response using a maximum pulse-width of 512 µs at 5 V. (Clark et al., 2011) Copyright © 2011, IEEE

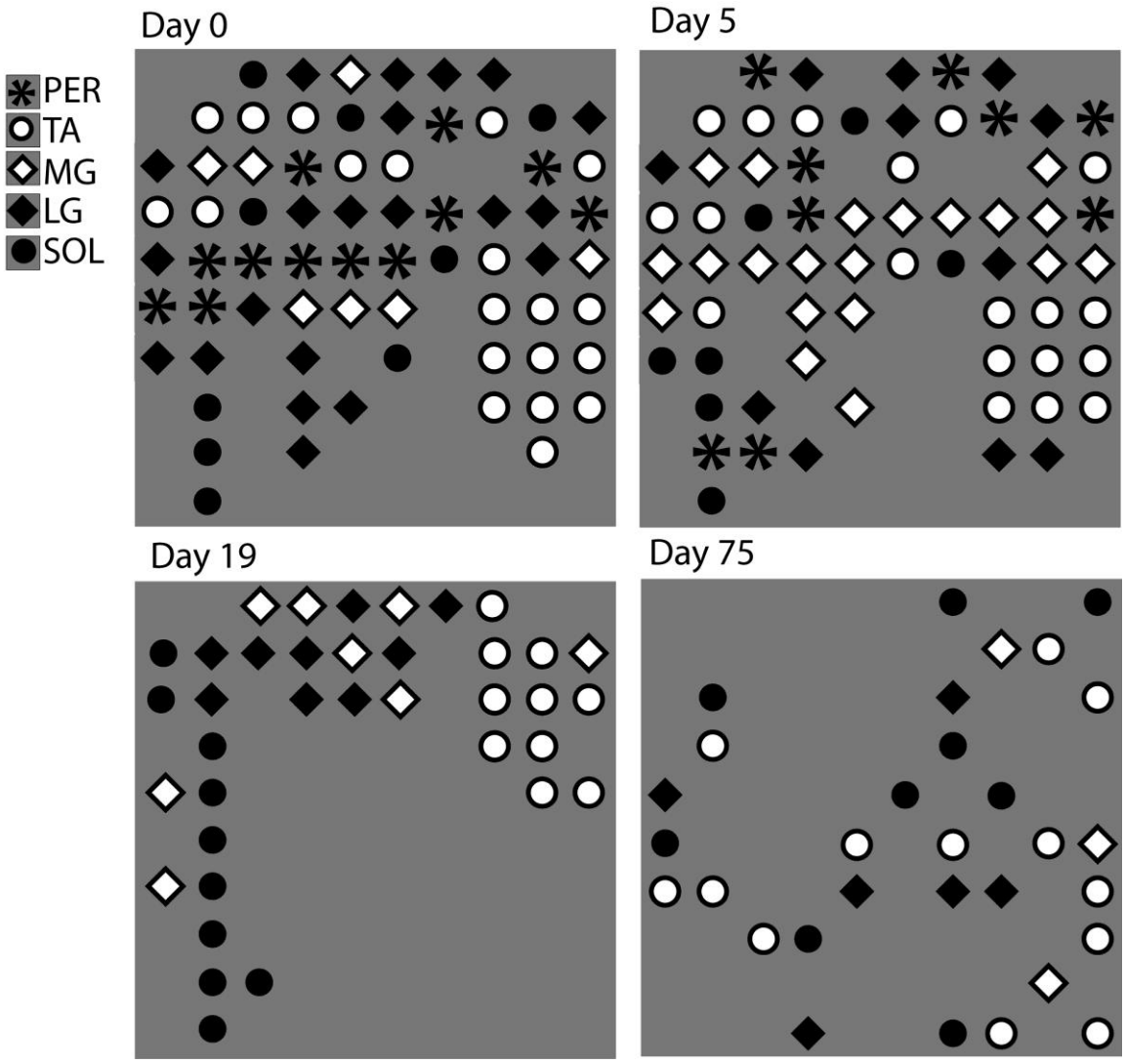

Figure 3.9: USEA motor response maps across time for one animal.

Each square in a grid represents the response of a muscle to stimulation of an electrode. The responding muscle is represented by the symbol in the square. Muscle responses for individual electrodes changed over time. (Clark et al., 2011) Copyright © 2011, IEEE

In two animals, the motor responses were also used to generate sit-to-stand behavior while the animal was under anesthesia, further validating the functional usefulness of the long-term motor responses. The stance generated by stimulation through the USEA was made fatigue resistant as in (Frankel et al.) by interleaving multiple electrodes activating the same muscles. By using this technique of stimulating multiple electrodes capable of activating the same muscle in a non-synchronous fashion, the motor units associated with a specific electrode were not rapidly fatigued.

Discussion

These data show the successful long-term recording of multiple and single unit activity from the PNS in an awake animal with a high-channel count penetrating electrode in the challenging environment of the PNS. The persistent recordings in this study show the effectiveness of stabilizing the implant physically with some kind of protective system. Physically coupling the array and the nerve into which it is implanted minimized the array backing out of the nerve, as seen in Branner (2004) and minimized movement of the electrodes within the nerve (Christensen 2011). Late recording sessions showed some single units that strongly encoded specific stimuli even in an awake and moving animal. Additionally, we could record a number of units with threshold crossing data sufficient to accurately decode limb position (using only threshold crossings in cross-validation, $r = 0.91$) as late as 4 months. Both of these results indicate that long-term PNS implants can record activity that will be useful for the control of prosthetic devices.

By attaching the containment system to earth ground during recordings and using

a reference electrode within the containment system, we were able to eliminate much of the contamination of non-neural signals. This strategy may be complicated in a clinical prosthesis for a mobile patient, where an earth ground is not available. Even in this case, a circuit ground should be effective in removing electrical interference introduced from the outside of the recording system. Recordings taken with the grounded containment show that extra-neural noise can be removed by simple noncomputational methods. Such considerations are important as scientists try to reduce the size and power consumption of all elements of a prosthesis in their attempts to make such a device wearable and long-lasting.

Neural interfaces may be implanted for decades in clinical use. Therefore, it is important to demonstrate viability lasting many years. Although the work herein follows intraneural implants only over the course of a few months, it represents important progress toward that goal. Unit counts increasing at later time-points most likely indicates the recording of new fibers growing close to the electrode tips (Christensen 2011). If this process continues (Ceballos et al. 2002; Lago et al. 2007), we can expect performance of the array to continue to change over the life of the implant.

Rising stimulation threshold levels were seen in this study as in previous studies on the USEA (Branner et al. 2004). Threshold increases and reductions in the number of responsive electrodes could be due to tissue encapsulation of electrodes, or neurons near the electrodes dying or changing positions. Although thresholds increased, selectivity largely remained in all muscles necessary for coordinated stance. The long-term selective muscle activation achieved in this study was successfully used to generate fatigue-

resistant muscle contractions, confirming that the previous results of McDonnall et al extend into the chronic time frame (McDonnall et al. 2004b; Dowden et al. 2009). The generation of coordinated multijoint stance in these and related experiments is an important step toward natural motor restoration prostheses. These initial results achieving selective stimulation are good, but improvements to the selectivity of stimulation are still possible. Current steering through the USEA is a promising possibility that may give experimenters even more focal stimulation.

The changes in musculotopy observed in this study have important implications for the long-term implantation of USEAs for FES. The timescale of the changes in musculotopy will determine how useful such a device can be for creating a prosthesis designed for daily use. Rapid changes in the electrode-muscle relationships, such as that caused by movement of the array relative to the nerve, would complicate the development and use of a neural interface by requiring frequent recalibration. Slower changes in the electrode-muscle relationships, such as that caused by neural growth near the electrodes, may not have a major impact on the day-to-day operation of a neural interface, because the device would need less frequent recalibration.

The nature of the day-to-day changes in musculotopy could not be examined with this experimental design because repeated anesthetization of an experimental animal can cause health problems. Thus, to acquire more regular information about the success and selectivity of muscle activation through the USEA will require a stimulation paradigm that can be used in an awake animal. Chronically implanted EMG electrodes would make day-to-day EMG comparisons possible and allow for stimulation to be easily quantified

in an awake animal. In an awake animal, it will be difficult to determine evoked EMG activity from volitional EMG activity. However, a nerve block proximal to the array implantation, achieved by injection of Lidocane or a similar drug, would eliminate the generation of volitional EMG activity, and allow researchers to assess EMG function in an awake animal at more frequent timepoints than could be done with a repeatedly anesthetized animal.

Multiple improvements to the array manufacturing and the design of the transcutaneous connector have contributed to improved electrode stability. New USEA and lead wire insulation may have reduced previously seen drops in impedance, and together with SIROF tips, improved unit recording capabilities. Stable impedances, particularly tip impedances, indicate that electrode tips are not changing over time. Stable shunting values indicate that no fluid leakage occurred in either the array or the wire bundle.

The femur-mount connector allowed lead wires to avoid crossing moving joints, thereby reducing wire bending and potential breakage. Further, the bone mount and the two-stage surgical approach allowed for osseointegration and good connector stability. High-density 96-pin connectors provided access to almost all 100 USEA electrodes and allowed for a reduction in overall connector size. Nonetheless, the skin never fully closed around the transcutaneous post, most likely due to the large movements of the biceps femoris very near the surface of the transcutaneous post. Though the skin continued to separate from the implant, the wound remained small and did not become infected with preventative topical antibiotics.

Because wound closure remained one of the most obvious problems to a comfortable long-term implant, we additionally investigated wireless technology and decoding via only threshold crossings. The recent, successful development and implementation of wireless technologies presages further enhancements of long-term reliability and performance (Harrison et al. 2008). Enhancements to overall array stability and electrode functionality are largely independent of the connector system used and will benefit either wired or wireless recordings. A fully wireless device may require a different containment system, to accommodate signal transmission.

Future improvements to the design of the connector could include alternative surface treatment to enhance skin adhesion, smaller connectors using either higher-density plugs, on-board ICs to digitize signals, or further wireless technology to replace the connector entirely.

Although further increases in unit yield and long-term stability remain desirable, the present results support the possibility of using USEAs in peripheral nerves to provide motor control and cutaneous or proprioceptive sensory feedback in individuals after limb loss or spinal cord injury.

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CHAPTER 4

INTRAFASCICULAR STIMULATION OF MONKEY ARM NERVES EVOKES COORDINATED GRASP AND SENSORY RESPONSES[2]

Abstract

High-count microelectrode arrays implanted in peripheral nerves could restore motor function after spinal cord injury or sensory function after limb loss. In this study, we implanted Utah Slanted Electrode Arrays (USEAs) intrafascicularly in arm nerves of anesthetized rhesus monkeys (n = 4) at the elbow or shoulder. Input-output curves indicated that pulse-width-modulated single-electrode stimulation in each arm nerve could recruit single muscles with little or no recruitment of other muscles. Stimulus trains evoked specific, natural, hand movements, which could be combined via multielectrode stimulation to elicit coordinated power or pinch grasp. Stimulation also elicited short-latency evoked potentials (EPs) in primary somatosensory cortex, which might be used to provide sensory feedback from a prosthetic limb. Recordings from sensory afferents showed units that responded to changes in finger and wrist position, which might be used to provide sensory feedback from paralyzed limbs for SCI patients. These results demonstrate a high-resolution, high-channel-count, bidirectional interface to the peripheral nervous system for restoring hand function after neural injury or disruption.

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Introduction

Disruptions of neural transmission resulting in paralysis—primarily from spinal cord injury (SCI), but also from lesions, stroke, head injuries and acute nerve injury—leave the patients' limbs and other affected body parts intact, but partially or totally unable to move. One emerging treatment for paralyzed individuals is Functional Electrical Stimulation (FES) (e.g., ParaStep I, Freehand, Vocare, and IST-12) (Martens et al.; Brissot et al. 2000; Fromm et al. 2001; Kilgore et al. 2008). FES-based prostheses can enable paralyzed individuals to grasp objects with a few simple grips, or even enable paraplegics to walk a short distance in conjunction with external support. However, FES systems can be fatiguing and relatively difficult to use because they typically activate near-maximal contractions, preferentially activate fatigable motor units, and provide no somatosensory or proprioceptive sensory feedback (Popovic et al. 1993; Spadone et al. 2003).

The 100-electrode Utah Slanted Electrode Array (USEA) provides a prime candidate for restoring hand function in paralyzed patients by activating motor fibers, and may ameliorate some of the challenges associated with full-muscle FES or extraneural stimulation. The USEA electrodes are arranged in a 10 x 10 configuration, spaced at 400-μm intervals, with electrode lengths ranging from 0.5 mm to 1.5 mm (Branner and Normann 2000), thereby providing relatively complete coverage of a nerve. Because the electrodes penetrate directly into the nerve fascicles, their tips closely abut different populations of motor or sensory axons, allowing multiple, selective sites for stimulation or recording. The USEA has been used previously to activate cat hindlimb muscles selectively and independently, and in a fatigue-resistant manner via interleaved activation

of multiple different motor units for a single muscle, each at a relatively low frequency (McDonnall et al. 2004b; Frankel et al.). Thus, intrafascicular nerve stimulation with USEAs may also provide an improved level of hand movements, compared with conventional FES. Among other advantages, a USEA may access multiple muscles with a single implant site and independent access to multiple different motor units within the same muscle, thereby also allowing more graded force control, and more fatigue-resistant movements via interleaved stimulation (Normann et al. 2012). It may also allow access to intrinsic hand muscles, which is difficult to achieve with conventional extraneural nerve stimulation. Finally, intrafascicular electrodes, such as those of the USEA, can also record single-unit action potentials, opening the possibility of detecting afferent signals from sensory receptors in intact limbs distal to the neural disruption (Branner et al. 2004).

Similarly, amputees also could benefit from the selective stimulation and recording capabilities of intrafascicular electrodes which would allow the patients' nervous system to communicate with computer-controlled prostheses, such as robotic hands or knees. In this instance, implanted electrodes would be used to record from efferent motor fibers to obtain motor command signals, and to activate small populations of sensory afferents in order to restore discrete sensations. However, the electrodes' functionality with respect to selective stimulation and recording would remain the same.

Previous studies have shown, at a gross level, motor fibers do cluster according to their function (Gustafson et al. 2009), and some motor fibers may be part of more than one nerve (Badia et al.). However, these studies do not address the relationship between the sensory and motor fibers within a single fascicle, and it remains unclear whether

fibers innervating a given body region tend to cluster together, or if the nerve fibers organize separately into sensory and motor bundles within the fascicle.

The human hand is a complex mechanical system with 27 degrees of freedom that is difficult to emulate. Monkeys have opposable thumbs, independent finger control (Schieber 1991), and intrinsic and extrinsic muscles controlling the hand and arm similar in number to that in humans (Liu et al. 1996). Monkeys thus provide an attractive model for testing the ability of the USEA to restore human hand function. The muscles used for generating power grip and precision grip are innervated by the median, ulnar, and radial nerves in both humans and monkeys. Selective activation of monkey hand muscles has also been reported with the use of flat interface nerve electrodes (FINEs) (Brill et al. 2009).

In the present study, we examined the feasibility and potential advantages of USEAs for activation of motor and sensory fibers in the median, radial, and ulnar nerves of nonhuman primates, using acute, anesthetized preparations. Although the commercial version of a single Utah Electrode Array (with equal-length electrodes) has been previously implanted in the median nerve of one human subject with success (Warwick et al. 2003), the data set from that study was limited. Aside from that somewhat anecdotal report, there have been no previous investigations of USEAs in nonhuman primates, or in any of the forelimb nerves of any species. Here we examined the ability of different USEA electrodes to provide access to different extrinsic and intrinsic hand muscles and the selectivity of that activation. We also examined the ability to activate multiple motor groups via multiple nerves so as to achieve coordinated gripping sequences that could restore clinically useful hand movements after paralysis. In addition to motor responses,

we examined stimulation-evoked responses centered around primary somatosensory cortex that could be useful for restoration of cutaneous and proprioceptive sensation in amputees. The combination of sensory and motor responses was also examined to determine whether fibers from a single body region lie together, or if the nerve fibers organize separately into sensory and motor regions within the fascicle. Finally, we also recorded discharges of afferent sensory fibers in response to cutaneous stimulation or experimenter-imposed manipulation of joint angles.

Materials and Methods

Surgery

These experiments were performed in nonrecovery surgical procedures on four monkeys that were being euthanized following a series of unrelated studies. All procedures were performed under deep surgical levels of anesthesia, using isoflurane gas anesthetic following premedication with Buprenorphine as approved by the Institutional Animal Care and Use Committee of Northwestern University. Experiments lasted approximately 30 hours. Differences in procedures across animals are summarized in Table 4.1.

Table 4.1: Procedures performed on each monkey

Name	**I/O curves**	**Pulse-Train**	**ECoG**	**Skull-Screw**
NHP1	x	x		x (Lesion)
NHP2		x		x
NHP3	x	x		x
NHP4	x	x	x	

ECoG Electrode Grid and Skull Screws

The anesthetized monkey was placed in a stereotaxic frame. In three monkeys, skull screws were placed according to steretotaxic coordinates and skull landmarks so as to lie primarily over postcentral cortex for cortical monitoring. The skull screws' positions in relation to the cortex were confirmed posthumously. In the fourth monkey, a craniotomy was performed, and an ECoG grid was placed over somatosensory cortex and adjacent cortices.

EMG Wires

Fine-wire EMG electrodes were placed in forearm, finger, and wrist muscles, and electrical potentials were recorded on a Cerebus recording system (Blackrock) at 10,000 samples per second with a low-pass filter at 7.5 KHz. Bipolar recordings were made with intramuscular electrodes inserted into each muscle, including, in some cases, separate compartments in a single muscle. In all experiments, the main muscles used in grasp were monitored, including flexor carpi radialis (FCR), flexor digitorum superficialis (FDS), flexor carpi ulnaris (FCU), medial head of flexor digitorum profundus (FDPm), ulnar head of flexor digitorum profundus (FDPu), flexor pollicis brevis (FPB), brachioradialis, extensor carpi radialis (ECR), extensor digitorum communis (EDC), extensor carpi ulnaris (ECU), pronator teres (PrT), flexor digitorum profundus (FDP), the dorsal interossicles, and lumbricals. In some monkeys additional electrodes were inserted in triceps lateralis, triceps longus, abductor pollicis brevis (AdP), and palmaris longus. Additionally, separate compartments in EDC and ECR were monitored in two monkeys.

Nerve Exposure

Nerves in the arm were then exposed at the elbow and shoulder for subsequent implantation of USEAs. First, a longitudinal incision was made just posterior to the location of the brachial artery in the proximal arm at the mid-humeral level and continued distally beyond the antecubital fossa. Hemostasis was achieved using Bovie electrocautery.

The median nerve lies adjacent to the brachial vessels and was dissected distally toward the elbow. The fibers of the bicipital aponeurosis were cut in order to gain better access to the median nerve just distal to the elbow crease. The pronator teres muscle was reflected medially, and the brachioradialis muscle was reflected laterally in order to dissect the median nerve free just proximal to its branch point in the proximal forearm.

In order to gain access to the ulnar nerve, the medial antebrachial cutaneous nerve was severed at the elbow, and the ulnar nerve was dissected free just proximal to the elbow. To expose the radial nerve, the arm was turned over, exposing the dorsal aspect of the forearm. A longitudinal incision was made between the brachioradialis and extensor carpi radialis longus (ECRL) muscles, exposing the radial nerve just proximal to its branch points to the brachioradialis and forearm extensor muscles. Alternatively, the radial nerve was exposed from the volar side of the arm by continuing the dissection of the muscles deep to median and ulnar nerves.

All three nerves were also exposed at the plexus to allow implantation of USEAs in multiple locations in each nerve and to examine the effectiveness of different implant locations. The incision in the arm was extended proximally, and in order to fully expose the nerves of the brachial plexus, the pectoralis minor and pectoralis major muscles were

incised and retracted out of the way. The median and ulnar nerves were followed proximally from the mid-humeral level to their origins from the medial and lateral cords of the brachial plexus. Care was taken to not disrupt the axillary artery and vein at this level, which can be difficult because of their intimate anatomic relationship with the brachial plexus. At this level, the musculocutaneous nerve was dissected out anterior to the median nerve. The radial nerve could then be dissected out as it branched off of the posterior cord of the brachial plexus.

USEA Implantation

USEAs were implanted in nerves just distal to the brachial plexus (Figure. 4.1A) and near the elbow (Figure. 4.1B) by means of a high-speed insertion system (Rousche and Normann 1992). Arrays were connected to stimulation and recording systems via a modified ICS or TDT96-pin connector and adapter board.

USEA-Evoked Motor Responses

Electrical stimulation was delivered through the USEA electrode tips via either a Grass SD-88 stimulator or a custom-built, 300-channel "UINTA" stimulation system (Wilder et al. 2009). We generated EMG stimulus-response curves individually for all 96 electrodes on each of 11 USEAs using pulse-width-modulated (0.1 μs -1026 μs), single-pulse, constant-voltage (3V ± 2 V) stimuli controlled by custom software. Stimulation thresholds, plateaus, and intermediate stimulus-response functions were determined through a closed-loop binary search using the evoked EMG signals for feedback.

Individual muscle responses were analyzed to determine which electrodes provided access to appropriate hand muscles. After muscle access had been determined

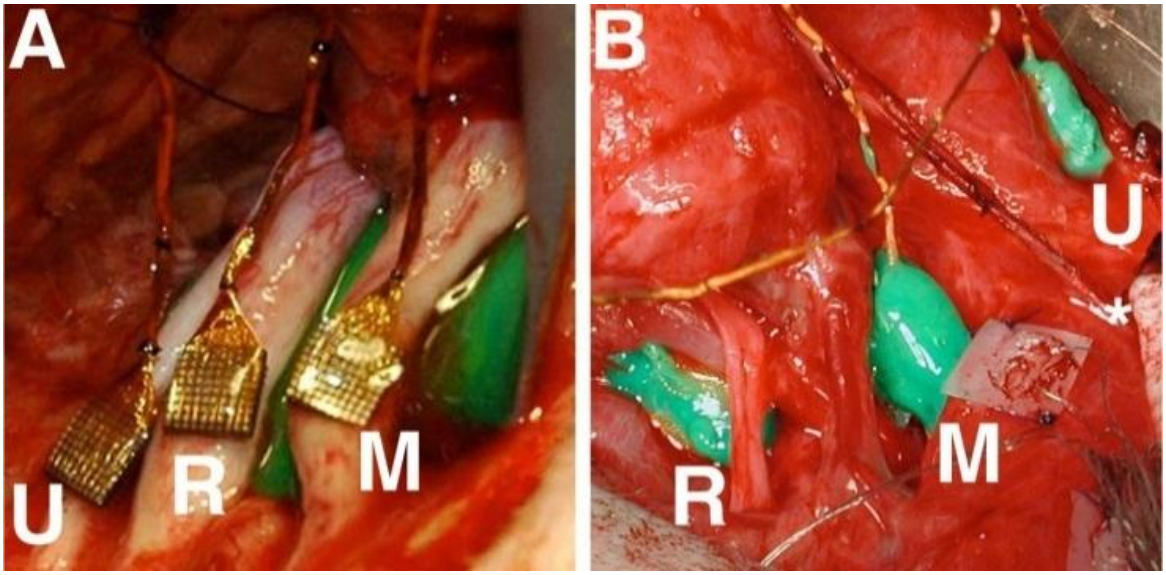

Figure 4.1. USEAs implanted in arm nerves.

Surgical access to all three target nerves was achieved through a single surgical site at either the elbow or shoulder. In both images, the more proximal limb is at the top, the more distal limb at the bottom, and the volar (palm-side) surface of the arm is depicted. R, Radial nerve; M, Median nerve; U, Ulnar nerve; *, olecranon process at the elbow. A, Left shoulder-level radial, median, and ulnar nerves, each shown implanted with a 100-electrode USEA. Insertion support (subsequently removed) seen below the median nerve. B, Right elbow-level arm nerves, just proximal to the elbow. USEA implants are shown protected by a custom containment system composed of metal mesh and Kwik-cast silicone (World Precision Instruments, Inc.). (Ledbetter et al., 2013), © The American Physiological Society.

by the delivery of single-pulse stimulation, pulse trains were delivered in an attempt to generate prolonged, useful, movements of the hand and wrist. Frequency of stimulation for pulse trains was between 30 Hz and 50 Hz. Cortical activation was monitored during all nerve stimulation. Somatosensory evoked potentials were computed using 64 averaged trials for each pulse on each electrode.

Before inferential statistical analyses of evoked EMG activity were conducted, EMG values were normalized to the largest response from the maximum of either bipolar stimulation through nerve cuffs, or single-, or multi-electrode stimulation through the

USEA. The EMG values for each run were divided by the maximum evoked EMG to produce a normalized EMG value (nEMG).

A muscle stimulation selectivity index (SI) was calculated for each electrode at specific normalized electromyographic (nEMG) value, by use of the following formula (Dowden et al. 2009):

$$\frac{LargestEMG - 2ndLargestEMG}{LargestEMG}$$

We analyzed SI values statistically with an overall analysis of variance (ANOVA) with monkey number, nerve implanted (median, radial, or ulnar), and level of implant (elbow or shoulder) as factors using a hierarchical sum of squares, followed by multiple-comparison tests with a Scheffe correction as appropriate. Unequal group sizes were adjusted via weighted means. Multiple-factor interactions with incomplete terms were not analyzed.

Recording of Cortical Somatosensory Evoked Potentials (SSEPs)

Electrical potentials from each screw or grid electrode were recorded in relation to a distal reference by a Cerebus recording system (Blackrock) at 10,000 samples per second with a low-pass filter at 10 KHz.

We also compared selectivity of cortical activation for USEAs implants at the elbow and shoulder. Biologically, it is unknown whether the degree of musculotopic organization of motor nerve fibers (i.e., their anatomical arrangement, corresponding to their target muscles) remains constant throughout the nerve length. Thus, from a practical perspective, it was unclear whether both implant sites would work equally well, which

was particularly important given that only relatively proximal nerve sites would be available after high-level transhumeral amputations. To address the relationship of motor and sensory fibers within the nerve, we investigated whether different USEA electrodes that activated a given muscle also would evoke responses on a given ECoG electrode, which would imply that sensory and motor fibers travel in the same fascicle in a mixed nerve. We first examined whether the amplitude of the SSEP recorded on a given ECoG electrode was statistically correlated with the pulse width of the stimuli delivered through a given USEA electrode during the recruitment curve that had also been used for muscle activation. For USEA electrodes that could drive cortical activity, we then determined which muscle responded most strongly to that electrode. Finally, for each ECoG electrode, we averaged the correlations across different USEA electrodes that had activated each muscle to determine the mean correlation between muscles activated by USEA electrodes and somatosensory cortical response location.

Results

Implants in all nerves, across all implant levels were capable of evoking muscle contractions in nerve-appropriate muscles that were detectable through EMG or visual inspection. Currents to evoke these contractions were not directly measured (given the use of constant-voltage stimulation at 3V), but lie below levels that could damage tissue with short-term stimulation sessions, between 5 and 50 uA, as documented in cat, including for short-term stimulation across multiple sessions (Branner et al. 2004; Frankel et al.; Normann et al. 2012).

Single-Pulse, Single-Electrode Stimulation: Muscle Activation and Selectivity

Recruitment curves. We first examined the ability to recruit responses in individual muscles by delivering single-pulse stimulation through individual USEA electrodes (typically using a series of varying stimulus-pulse durations) while measuring the evoked EMG responses. As in previous work, the muscle responses to USEA stimulation were graded across the range of pulse widths, peri-threshold pulse widths had a mean of 15.4 ± 0.5. Calculated SI values indicated that single-electrode, single-pulse intrafascicular nerve stimulation could often activate individual extrinsic muscles to functionally useful levels without activating other muscles (Figure. 4.2*A*), and that different muscles could be recruited selectively by different USEA electrodes (Figure

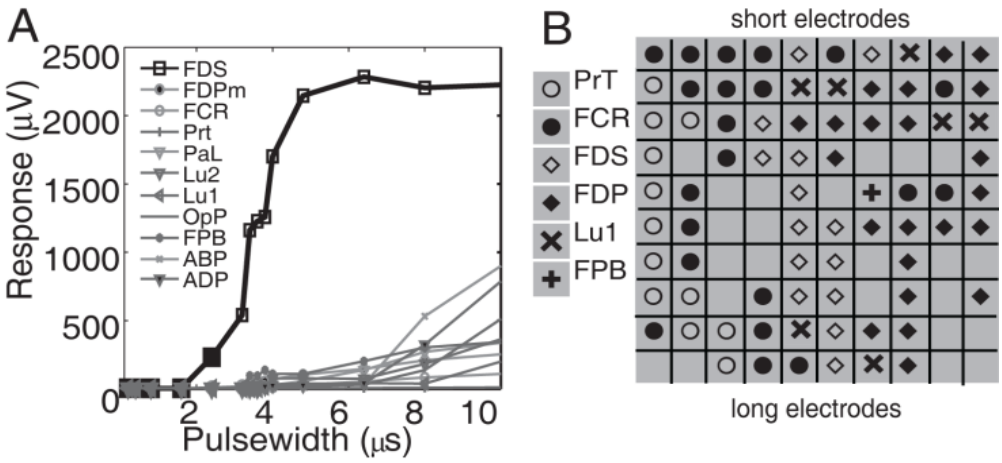

Figure 4.2: Muscle activation shows selectivity and musculotopy.
A. *Selectivity*. Stimuli of increasing pulse width evoked successively larger responses in FDS with little or no activation of other muscles. **B.** *Musculotopy*. Each tile in the 10-by-10 grid represents an electrode on the USEA, the symbol indicating the activated muscle. Electrodes are shown as in a cross-section of the nerve with the most superficial aspect of the nerve at the top of the picture. Responses in a given muscle tend to be recruited by adjacent USEA electrodes, whereas responses in other muscles are recruited by other USEA electrodes, indicating a musculotopic arrangement of nerve fibers. FDS, flexor digitorum superficialis; ABP, abductor pollicis brevis; FDPm, flexor digitorum profundus; FCR, flexor carpi radialis; Pal, palmaris longus; PrT, pronator teres; Lu1, 1st lumbrical; Lu2, 2nd lumbrical; OpP, opponens pollicis; FPB, flexor pollicis brevis; ADP, abductor pollicis brevis. (Ledbetter et al., 2013), © The American Physiological Society.

4.2*B*). Intrinsic muscles could also be activated by USEA stimulation, although they were usually co-activated with other intrinsic muscles.

Of a possible 1056 electrodes across 11 implants, 462 (43%) evoked at least low-level responses (defined as 0.2 nEMG) at pulse widths less than 512 μs. Many electrodes presumably ended in extrafascicular, non-neuronal tissue, and hence would not have evoked responses except at very strong stimulus levels. In the three monkeys in which input-output curves were generated, the mean SI across all implants at 0.2 nEMG was 0.44 ± 0.01 (mean ± standard error of the mean reported for all selectivity measures). The mean number of electrodes per array that activated muscles at 0.2 nEMG was 42, and it dropped to 34 at 0.5 nEMG and 18 at 0.9 nEMG. However, some selectivity was maintained at the stronger activation values, 0.5 nEMG (0.43 ± 0.01) and 0.9 nEMG (0.31 ± 0.02). A single USEA thus provided selective activation of multiple muscles innervated by a single nerve, at a variety of activation levels. At the elbow (672 total electrodes, 7 implants) and the shoulder (384 total electrodes, 4 implants) in all three nerves, 382 of the electrodes (36% of all electrodes) elicited strong EMG responses (defined as 0.5 nEMG) in the same muscles in which they elicited weaker responses. At the elbow, all implants could reach 0.9 nEMG in some muscles (178 electrodes, 26.5% of elbow electrodes), whereas at the shoulder only the median nerve implants were capable of evoking contractions at 0.9 nEMG (23 electrodes, 5.9%). Because data for values above 0.2 nEMG are incomplete, the selectivity analysis was confined to 0.2 nEMG (Figure 4.3), data are summarized for selectivity at higher nEMG values in Table 4.2.

Figure 4.3. Selectivity of muscle activation for all USEA electrodes and implant sites.

The number of electrodes that recruited responses at a given level of selectivity is depicted across all levels and nerves. Left column: results for USEA implants near the elbow for median nerve (top row), radial nerve (second row), ulnar nerve (third row), and across all nerves (bottom row). Middle column: results for USEA implants in nerves near the shoulder. Right column: results summated for USEAs at both the elbow and shoulder. The bottom right panel indicates group results across all nerves at both levels. For each panel, the large number in the top right indicates how many different muscles could be preferentially activated at that particular level-implant combination across all SIs. The smaller numbers in parentheses below the number of muscles indicate the number of implants and number of electrodes used in the analyses, respectively. (Ledbetter et al., 2013),

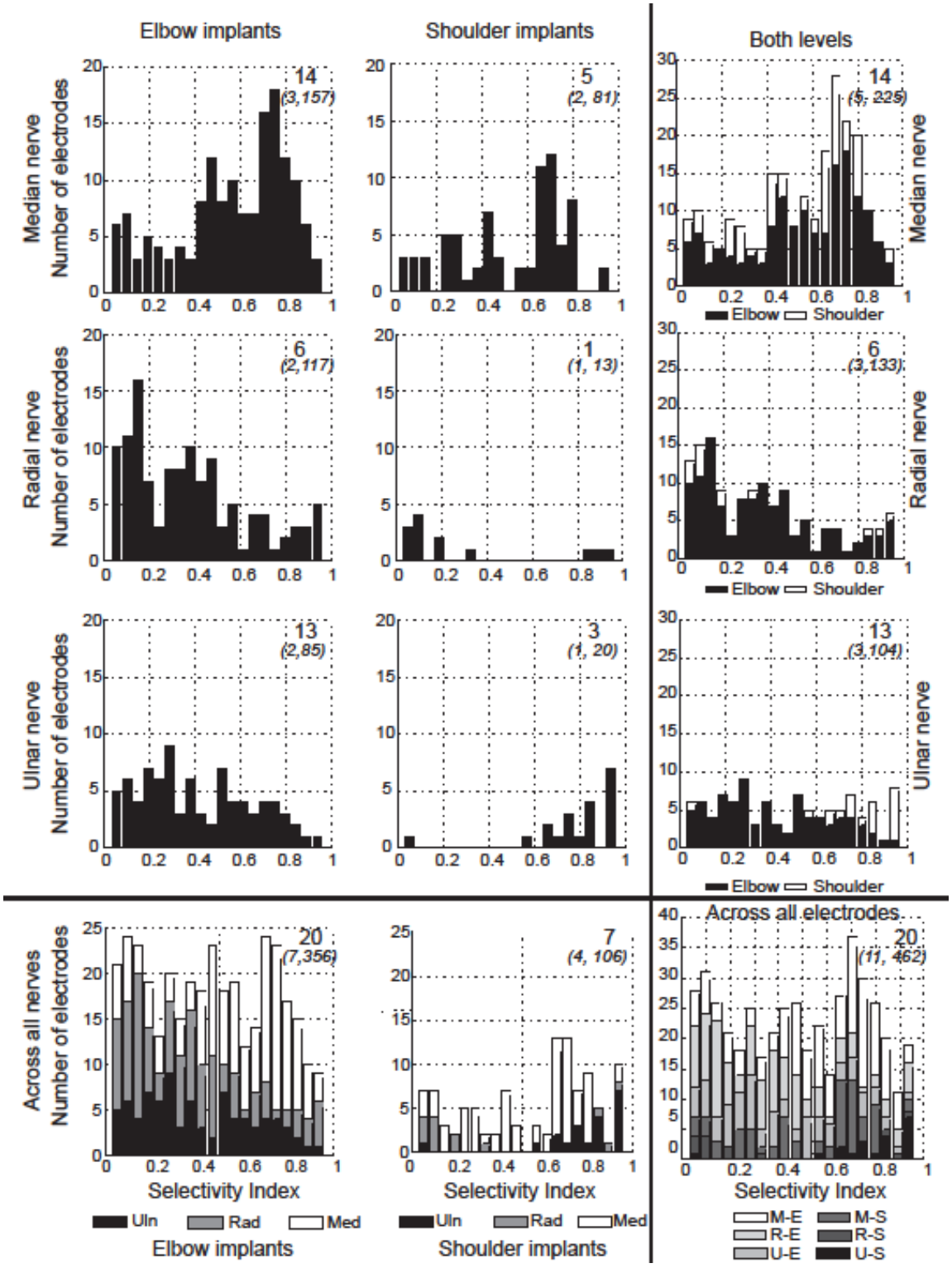
Elbow implants
Shoulder implants
Both levels
Median nerve
Number of electrodes
14
(3,157)
5
(2, 81)
14
(5, 225)
Elbow
Shoulder
Radial nerve
6
(2,117)
1
(1, 13)
6
(3,133)
Ulnar nerve
13
(2,85)
3
(1, 20)
13
(3,104)
Across all nerves
20
(7,356)
7
(4, 106)
Across all electrodes
20
(11, 462)
Selectivity Index
Uln
Rad
Med
M-E
M-S
R-E
R-S
U-E
U-S

Table 4.2: Selectivity of muscle responses at multiple strength levels.

Selectivity at different stimulus strengths						
nEMG	**Mean SI**	**SEM**	**Arrays (of 11)**	**Elbow electrodes**	**Shoulder Electrodes**	**Total (of 1056)**
0.2	0.44	0.01	11	356	106	462
0.5	0.43	0.01	11	296	86	382
0.9	0.31	0.02	6	178	23*	201
* median nerve only						

Muscle Selectivity at the Elbow and Shoulder

An ANOVA of the SI calculated at 0.2 nEMG for the factors of nerve, primate, and implant level indicated that the implant level (elbow or shoulder) was not a significant factor, whereas the individual animal, and nerve implanted were significant factors (Table 4.3). The mean SI calculated at 0.2 nEMG of all elbow implants tended to be lower than the mean of all shoulder implants (0.42 ± 0.01 vs. 0.52 ± 0.03 (elbow: 356 electrodes, 7 arrays, shoulder: 106 electrodes, 4 arrays), due primarily to results from the ulnar nerve; however, in the median and radial nerves, this trend was reversed. Specific comparisons regarding implant level for the different nerves were not analyzed for statistical significance, because there was only a single shoulder-level implant done in the radial and ulnar nerves, and because the implant level was not a statistically significant factor. Descriptively, however, within-nerve comparisons of elbow- and shoulder-level SIs in the median nerve (0.54 ± 0.02 vs. 0.47 ± 0.03; 153 and 73 electrodes, 3 and 2 arrays, respectively), and radial nerve (0.32 ± 0.02 vs. 0.26 ± 0.06; 120 and 13 electrodes, 2 and 1 arrays) showed that selectivity tended to be higher at the elbow than at the shoulder, whereas in the ulnar nerve at the elbow, selectivity tended to be lower than at the shoulder (0.26 ± 0.02 vs. 0.78 ± 0.05; 84 and 20 electrodes, 2 and 1 arrays). Multiple-

Table 4.3: Statistical analysis of selectivity index .

ANOVA of SI					
Factor	**Sum Sq.**	**d.f.**	**Mean Sq.**	**F**	**Prob>F**
Implant level	0.04	1	0.04	0.76	3.83E-01
Animal#	2.02	2	1.01	17.30	5.71E-08
Nerve	3.15	2	1.58	27.07	7.79E-12
Error	26.57	456	0.06		
Total	32.17	461			

comparison tests with a Scheffe correction indicated that SI was statistically different across all nerve pairings (P's < 0.05), with population-normalized-mean-values as follows: median, 0.56 ± 0.02; ulnar 0.44 ± 0.03; radial 0.36 ± 0.02.

Musculotopic arrangement of nerve fibers. To evaluate the musculotopic arrangement of fibers within a nerve, we examined the extent to which neighboring USEA electrodes evoked responses in the same muscle. For all implants, electrode sites that recruited the same muscle or close synergist muscles were usually in close proximity to one another, suggesting a musculotopic arrangement (Figure. 4.2B). To quantify musculotopy, for each USEA electrode we first calculated the expected number of neighboring (adjacent) electrodes that would activate the same muscle if nerve fibers were randomly distributed, based on the number of responses evoked in each muscle for each given USEA. We then compared the number expected from chance with the number of neighboring electrodes that had actually recruited the same response as the given test electrode. Significantly more neighboring electrodes recruited the same motor response than expected from chance alone ($\bar{x}$ = 0.98± 0.07 electrodes, P<0.05) (Figure 4.4), indicating that motor fibers were organized musculotopically within the nerve.

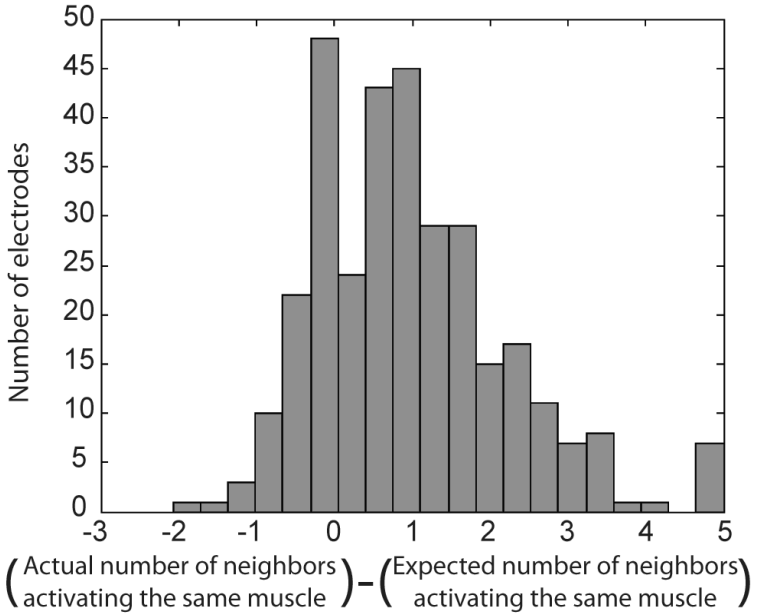

Figure 4.4: Quantification of musculotopic arrangement of motor fibers.

We assessed the musculotopic organization of nerve fibers by comparing the muscle activated by each USEA electrode with the muscles activated by neighboring USEA electrodes. For each electrode capable of activating a muscle, we calculated the probability that a neighboring electrode would activate the same muscle from chance alone. The actual number of neighboring electrodes that preferentially activated the same muscle was consistently higher than the number expected from chance (i.e., the actual – expected difference was greater than zero), indicating a musculotopic arrangement in which motor fibers to a given muscle were close together within the nerve. This pattern held for muscles of all types, and each nerve individually. (Ledbetter et al., 2013), © The American Physiological Society.

Single-Electrode Pulse Trains Also Recruited Selective Movements

Functionally useful movements require stimulus trains, rather than single-pulse activation of motor nerve fibers. To test our ability to generate individuated and coordinated movements using the USEA, we applied pulse trains (30-50 Hz, 1.8-3 V) to particular electrodes. Pulse widths used in the functional muscle contraction sequences were higher than peri-threshold values. We monitored movements at the hand, elbow and shoulder, as well as rotation of the forearm. Motions were observed and categorized in terms of the joint at which the movement occurred and its direction, together with the

muscles that showed EMG activity. Across all subjects, median nerve stimulation generated 6-9 visually different movements across different combinations of joints (Figure. 4.5). These movements approximately corresponded to the activation of different individual muscles associated with each movement in various combinations (e.g., flexor carpi radialis (FCR) for wrist flexion; FDS and FDP for finger flexion; the intrinsic muscles and FPB for small finger and thumb movements; and pronator teres for arm pronation). The ability of the different USEA electrodes to elicit distinct movements and different EMG responses indicates that selective stimulation was partially maintained during pulse-train delivery, such that even with the low-level activation of additional

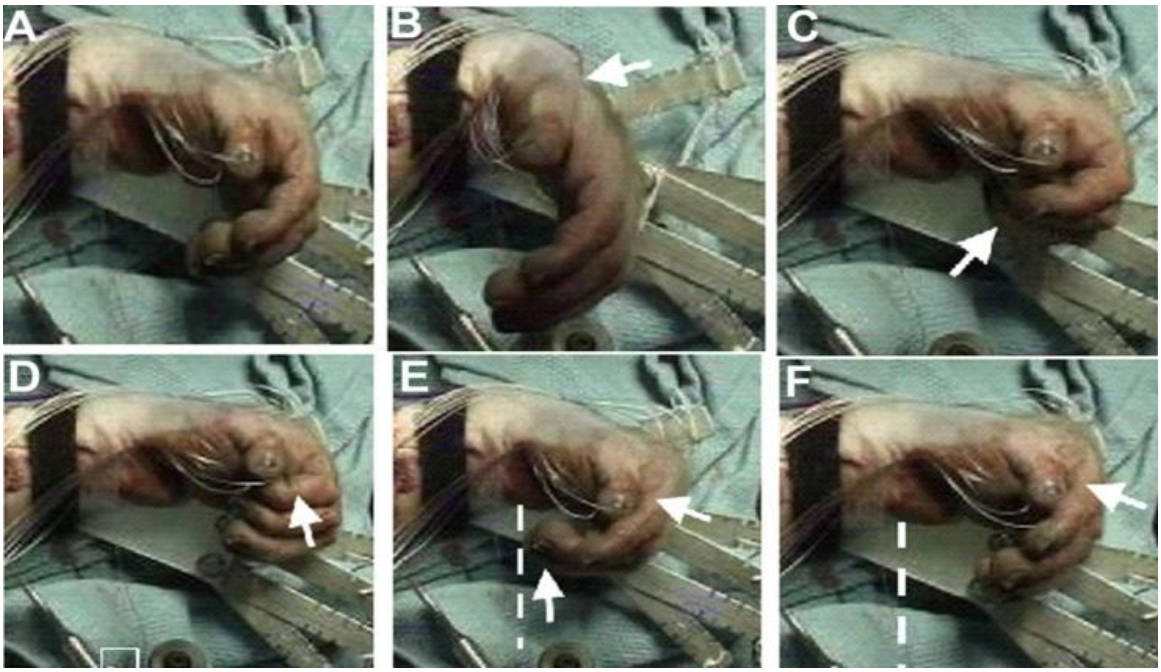

Figure 4.5: Single channel stimulation elicits multiple motions.
USEA single-electrode pulse-train stimulation of median nerve recruits specific digit and wrist movements (pronation not shown). White arrows indicate fingers/joints in motion. Different USEA electrodes evoked different movements. A. Rest. B. Wrist flexion. C. Digits 3-5 flexion (in shadow). D. Digit 2 tip flexion; notice the different fingers engaged in C and D. E. Digits 2-5, flexion at metacarpophallangeal joints. F. Digits 2-5 tip extension, with flexion at MCP joints. Note the relative straightening of the finger tips in F compared with the extent of finger flexion in E, demarcated by white lines in E and F. (Ledbetter et al., 2013), © The American Physiological Society.

muscles the motions evoked were clearly related to the muscle which was selectively activated through single-pulse stimulation.

Multielectrode, Multi-USEA Pulse Trains Evoked Coordinated Grasp

In order to produce a coordinated grasp, muscles must not only be selectively activated, but must also contract and relax in specific patterns (Long et al. 1970; Maier and Hepp-Reymond 1995b). To test the ability to evoke these more complex types of movements, between three and nine electrodes were selected that activated the muscles necessary for power grip through the UINTA stimulation system custom software. The monkey's hand was unconstrained during all stimulation. A two-second movement sequence was programmed consisting of finger extension to open the hand; finger flexion to grasp an object; and, finally, finger extension to release the object. Activation of extrinsic finger flexors that span the wrist typically caused undesired secondary wrist flexion along with flexion of the fingers. In these cases, wrist extensors were also activated to counteract the undesirable flexion forces, a combination that is necessary under normal conditions as well. A 50g ball was placed in the animal's palm as it was initially opened. When the hand closed, it held the ball until the program instructed the fingers to extend (Figure 4.6). The shown movement was evoked with 6 electrodes with pulse-widths of 10, 100, 10, 50, 100, 500 µs (average 128 µs). Once programmed, the control sequence reliably produced the desired movement sequence for the duration of the experiment. Via this technique, the anesthetized monkey's hand also engaged a variation of power grip sometimes called bucket grip. In addition, electrodes associated

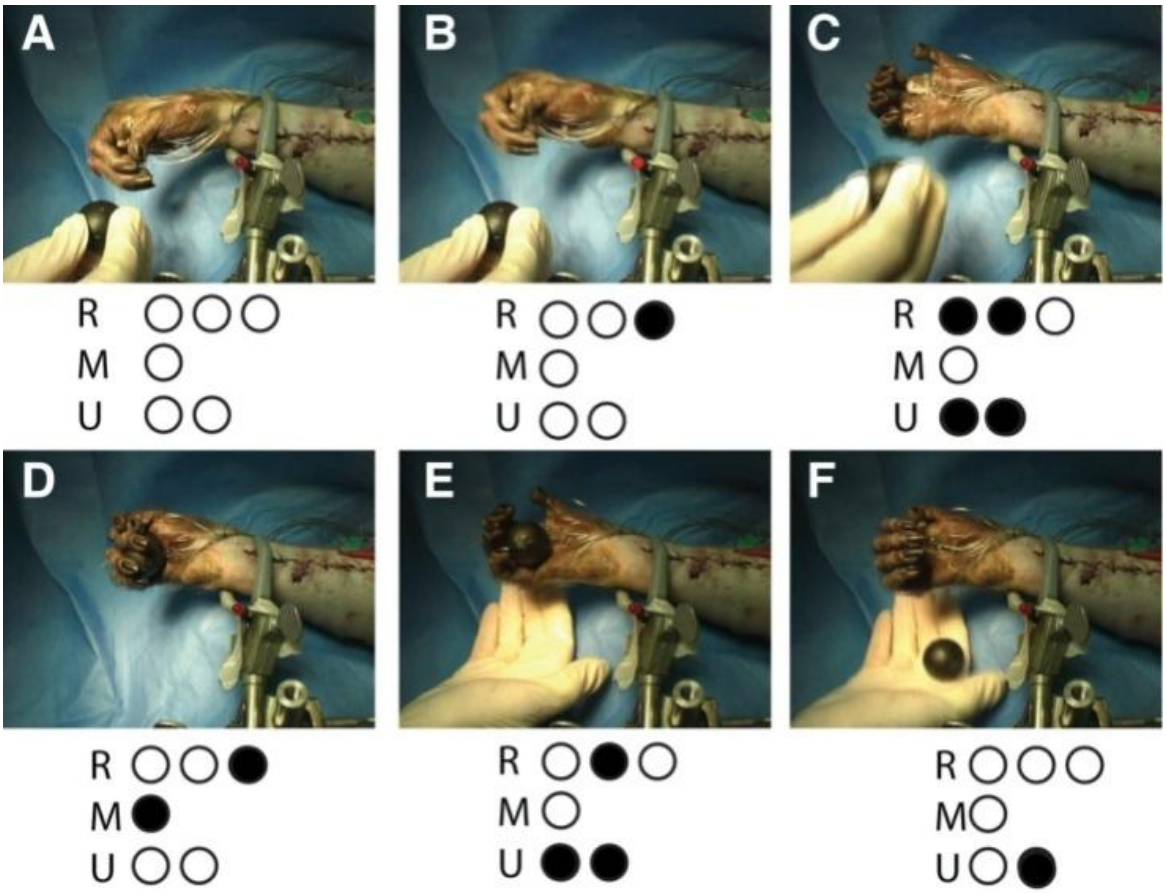

Figure 4.6: Coordinated, sequential grasp-and-release movements produced by multielectrode, multi-USEA stimulation.

USEA stimulation generated grip sufficient to hold a ball. Under each panel, the electrodes used in the grip sequence are shown for the three implanted nerves; filled dots indicate electrodes active at the time of the picture. **A.** Rest position. **B**, Wrist extension. **C.** One-second hand opening and forearm supination to accept the ball. The experimenter introduces the ball to the anesthetized primate's hand. **D.** One-second power grip. **E.** The wrist and fingers extend again, releasing the ball. **F.** The wrist flexes and forearm pronates to drop the ball. (Ledbetter et al., 2013), © The American Physiological Society.

with intrinsic hand muscles were combined with the extrinsic muscles to generate a pinch grip between the thumb and forefinger .

USEA Recordings of Sensory Fiber Discharges

To test the USEA's suitability for providing sensory feedback to an SCI patient, we recorded sensory afferent signals from the median nerve in two monkeys. Single-unit activity was recorded on several electrodes while experimenters manipulated the monkey's hand (Figure 4.7). Thus, intrafascicular USEAs also potentially provide the ability to obtain cutaneous and proprioceptive sensory information that could be incorporated into a more complete closed-loop control system.

USEA Activation of Sensory Fibers

To examine our ability to evoke sensory signals in an anesthetized monkey model, as would be necessary in a limb-loss prosthesis that restores sensation, we monitored (SSEPs) using either skull screws (n = 3) or an electrocorticography (ECoG) grid (n = 1) during USEA stimulation (Allison et al. 1991). Stimulation produced short-latency (~5 ms to onset) SSEPs in and around primary somatosensory cortex on 52% of

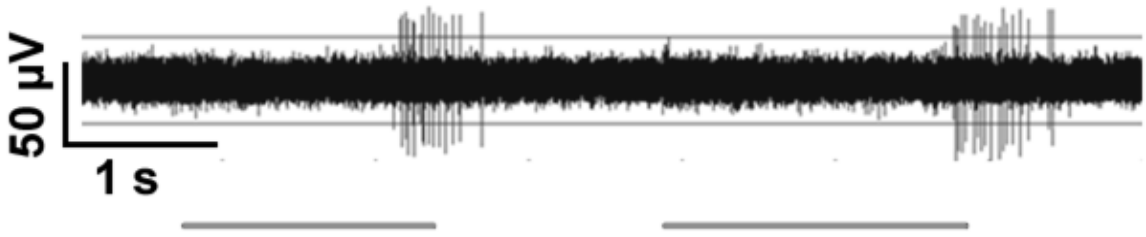

Figure 4.7: Recording from a USEA electrode implanted in the median nerve at the elbow.

The grey line indicates the approximate time the thumb was extended. The neural response occurred at the end of digit extension, suggesting that it was driven by thumb flexion. (Ledbetter et al., 2013), © The American Physiological Society.

tested stimulating electrodes. To avoid the possibility of indirect sensory activation (e.g., H or F reflexes), the analysis of SSEP data was limited to the first 20 ms after stimulation (Figure 4.8). The short latency of these responses indicates that they are likely due to direct afferent fiber activation, not indirect sensory responses due to movement caused by concurrent muscle activation (Cheron and Borenstein 1987; Halonen et al. 1988). Low-level stimulation applied to USEAs in the monkey with the ECoG grid (n = 3 USEAs) recruited cortical responses at a pulse duration that did not activate muscles in 32% of electrodes, providing further evidence that direct sensory fiber activation was achieved.

Relationship Between Somatotopic and Musculotopic Organizations

We next examined whether afferent nerve fibers were organized somatotopically and the relationship between somatotopic and musculotopic organizations.

Different USEA electrodes evoked different cortical responses. Consistent with a somatotopic organization, different electrodes on the same USEA, or on different

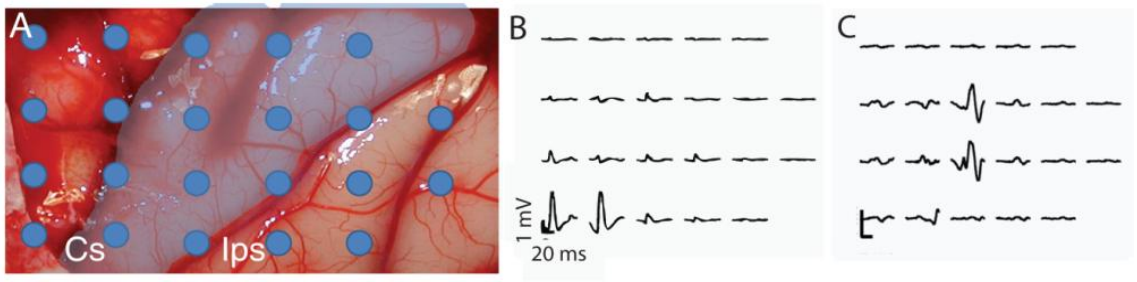

Figure 4.8: Primary somatosensory cortex (blue shading) was activated through USEA peripheral nerve stimulation of sensory nerve fibers.

Anterior to the left, medial on the top in all panels. **A.** ECoG electrode positions shown in relation to the cortex. **B-C.** Cortical recording pattern associated with **B.** electrodes in the median nerve that activated thumb and index finger intrinsic muscles, or **C.** electrodes in the radial nerve that activated brachioradialis, an elbow flexor. Cs, central sulcus; Ips intraparietal sulcus. (Ledbetter et al., 2013), © The American Physiological Society.

USEAs, evoked responses in different cortical regions in three monkeys. (Upon post-mortem dissection, NHP1 was found to have an S1 lesion from previous work that precluded cortical analyses for the present work.) for monkeys with skull screws (n = 2) rather than the ECoG electrode grid, different patterns of cortical activation were discernable only with stimulation via USEAs on different nerves, presumably because of the relatively coarse resolution provided by skull-screw recordings. For example, the cortical responses to median nerve stimulation were different from responses for radial nerve stimulation. Additionally, for the one monkey with the ECoG grid, different USEA electrodes on a single USEA in a given nerve evoked responses in discernibly different cortical regions.

Somatotopic and musculotopic maps covary. Results showed that the amplitude of the SSEP on some cortical electrodes was significantly correlated with stimulation strength on USEA electrodes that activated muscles with similar function (Figure 4.9). In addition, adjacent cortical electrodes showed similar correlations, whereas cortical electrodes distant to one another did not. Instead, responses on distal cortical electrodes were correlated with stimulus strength on USEA electrodes that activated other muscles. For example, stimulation strengths on USEA electrodes implanted in the median nerve that activated wrist flexor muscles were correlated (0.45 r or greater, $P < 0.05$) with response magnitudes on ECoG electrode 18, whereas stimulus strengths on USEA electrodes that activated finger flexor muscles were correlated (0.45 or greater) with response magnitudes on ECoG electrodes 1 and 2 (Figure 4.9).

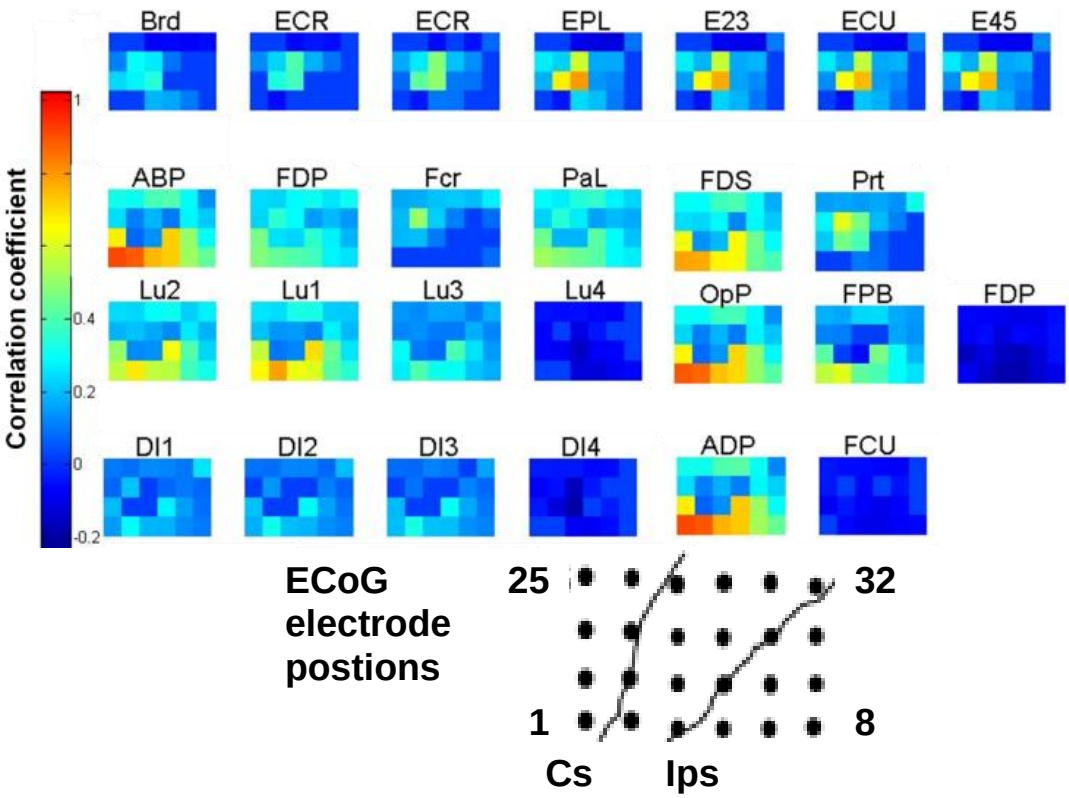

Figure 4.9: Co-registration of musculotopic and somatotopic maps.

Different USEA electrodes that evoked responses in a given muscle, via activation of motor nerve fibers, also evoked responses on the same cortical ECoG electrodes, via activation of sensory nerve fibers. Each grid displays a color map for the 32 ECoG electrodes for a given muscle, indicated by the label above the grid (e.g, Brd, ECR, etc.). Colors correspond to the correlation between the variation in stimulus pulse width and the variation in the amplitude of the evoked cortical response. ECoG electrodes within each grid are numbered from 1 to 8 from left to right on the bottom row, extending through 25 to 32 on the top row. USEA electrodes that evoked responses in a given muscle or similar muscles, e.g., wrist extensors, also evoked responses in a similar set of cortical electrodes, for example, USEA electrodes that activated extensor muscles ECU and ECR also evoked responses on ECoG electrodes 10 and 11, as indicated by the high correlation between stimulus pulse width and the amplitude of the evoked SSEP on those ECoG electrodes. In contrast, USEA electrodes that activated the flexor muscle FDS evoked responses in more anterior-lateral cortical regions (ECoG electrodes 1 and 2). Muscles are grouped according to their dominant innervation, e.g., radial nerve (top group), median nerve (middle group), and ulnar nerve (bottom group).
(Ledbetter et al., 2013), © The American Physiological Society.

These results imply that somatosensory fibers and motor fibers for a given body region travel closely together within the nerve. Given that USEA-evoked motor selectivity appears to hold even at the subfascicular level, it is plausible that the motor-sensory co-organization occurs at the subfascicular level as well. These findings complement earlier work demonstrating that somatosensory fibers of the same submodality and receptive field region cluster together within the nerve (Hallin 1990; Ekedahl et al. 1997).

Discussion

Here we report the first USEA implantation in the peripheral nerves of a nonhuman primate, the first attempt to quantify the efficacy and selectivity of the USEA in activating extrinsic and intrinsic hand muscles, and the first recordings of cortical sensory responses evoked through USEA stimulation of arm nerves. The results here demonstrate that intrafascicular electrodes can provide excellent access to multiple muscles, including intrinsic hand muscles not typically accessed in conventional FES. The different electrodes of a single USEA could activate multiple different muscles, and the combination of just three USEAs in the median, radial, and ulnar nerves could access nearly all forearm and hand muscles. Although the procedure to implant USEAs for clinical applications would be invasive, it is less invasive and would require less recovery time than, for example, targeted reinnervation approaches presently used successfully for control of prosthetic limbs (Kuiken et al. 2009).

Recruitment of Motor Responses via USEA Stimulation of Motor Fibers

Activation of motor fibers provided fine-resolution control of forearm movements. Selective activation of the muscles used to grip objects was achieved with both the elbow-level and shoulder-level implants, indicating that both locations have potential uses for PNS-based prostheses. Although shoulder-level implants had a comparable mean SI to elbow-level implants, the low sample size makes determining the strength of that trend difficult. However, the greater number of usable electrodes suggest that the elbow may be a more desirable implant location when available. Nonetheless, shoulder-level implants would be useful in cases of high-humeral amputation, or for recruiting muscles of the upper arm after SCI, given that some electrodes at the shoulder level were selective (40 electrodes with an SI > 0.5).

Single-pulse activation of individual muscles was often selective, particularly for extrinsic hand muscles. Although the intrinsic muscles with similar functions (such as the lumbricals) were usually recruited together, the intrinsic muscles in different groups (thenar, interossi and lumbrical) were often recruited separately. On some electrodes (in elbow-level implants in the median nerve), the index lumbrical was recruited alone, without activity on the other lumbricals, further indicating the specificity of muscle stimulation possible through intrafascicular electrodes. From previous work with intrafascicular electrodes, it is known that it is possible to evoke a response from only a portion of a fascicle. In the present study, it was not directly demonstrated whether the selectivity seen is principally due to a similar level of subfascicular selectivity, or a more segregated set of fascicular bundles; however, the high impedance of the endoneurium surrounding each fascicle substantially limits current spread from one fascicle to another.

In either case, under the assumption the nerve is musculotopically and somatotopically organized, current spread would cause physically close muscles and sensory areas to be activated together. Moreover, current spread cannot fully account for the musculotopy (or somatopy) observed here. Current spread from a given electrode to the neural tissue at an adjacent electrode might indeed active some fibers there; but such current spread could not fully explain why the dominant normalized EMG response at the given electrode was the same as that at the adjacent electrode. The strongest activation at the given site will reflect activation of the greatest number of nerve fibers, which probabilistically would occur in close proximity to the given electrode tip. Our data indicate that the selectivity of muscle activation was highly variable among different nerves and individuals. However, the overall musculotopic arrangement of fibers across the broad distribution of SIs likely indicates that, independent of the degree to which the selectivity seen in this study is due to fasciculation or instead to subfascicular organization, there is a strong tendency for axons to particular muscles to group together, in agreement with other recent studies of nerve organization (Badia et al. 2010; Brill, Polasek et al. 2009).

Pulse-train stimulation of selective electrodes generated smooth and distinct movements. Furthermore, different movements evoked by pulse-train stimulation were combined into functional grip-and-release sequences by activating several electrodes simultaneously or in sequence, and multiple types of grip (power, bucket, and pinch) could be reliably generated. These results all indicate the feasibility of using a penetrating electrode in the PNS as a prosthesis for limb reanimation in paralyzed patients. In the cat hindlimb, contractions produced by stimulation through multiple USEA electrodes that activate different motor units of the same muscles can be combined and interleaved to

produce fatigue-resistant movements and stable static positions (Normann et al. 2005). So long as stimulation through the USEA electrodes can evoke responses in independent, non-overlapping motor units, the same approach may work for monkey arm nerves, and presumably for human nerves as well. However, the time constraints of the present acute studies precluded systematic investigations of the overlap of USEA electrode responses, and the effects of interleaved stimulation on fatigue resistance (see Normann, Dowden et al. 2012, for details of the overlap and fatigability tests).

Studies of precision grip indicate that the intrinsic hand muscles, particularly the 1st dorsal interosseous and the muscles in the thenar group, are important for stabilizing the thumb and finger metacarpophalangeal (MCP) joints (Maier and Hepp-Reymond 1995b). Unfortunately, present FES-based solutions do not fully access the hand muscles required for grasp, particularly the intrinsic hand muscles. Although direct stimulation of extrinsic hand muscles does provide functional power grip, the same intramuscular electrodes cannot easily be used for control of intrinsic hand muscles, largely due to their small size and the difficulty of surgical access. Because of these limitations, additional surgeries such as tendon transfers are sometimes necessary to achieve strong, stable grip force (Kilgore et al. 2008). In contrast, our three implanted USEAs allowed access to all the instrumented hand muscles, including all extrinsic and intrinsic muscles implicated in grip (Maier and Hepp-Reymond 1995a; Schieber 1995). The activation of intrinsic and extrinsic hand muscles in a coordinated fashion allows for versatile hand posturing and gripping. Thus, for example, we were able to encode a stimulation sequence with four electrodes that brought the thumb and forefinger together.

Recordings from Sensory Fibers

Additionally, we have demonstrated here that intrafascicular USEAs might also provide sensory feedback for the upper limb after either SCI or limb loss, via recording from or stimulating sensory fibers, respectively.

In the absence of sensory feedback, gripping objects with appropriate force levels is sometimes difficult (Warwick 2005). Therefore, restoring full arm function in an SCI patient will require the ability to use sensory information recorded from the paralyzed limb. To this end, stimulation of the patient's intact sensory cortex, based on the recordings from afferent nerve fibers, could be used to evoke cutaneous and proprioceptive percepts. Alternately or additionally, sensory information recorded from electrodes in the PNS could be used in conjunction with algorithms to control the limb directly (Pezzin et al. 2004; Biddiss and Chau 2007).

Here, we recorded action potentials evoked by cutaneous and proprioceptive stimuli generated by manipulation of the hand, indicating that the implanted USEAs can record neural signals useful for detection and decoding of sensory afferent information. These recordings represent the first use of USEAs for obtaining somatosensory and proprioceptive information from monkey arm nerves. Ultimately, combining sensory afferent recording with muscle stimulation might provide closed-loop, neuroprosthetic control for SCI patients.

Stimulation of Sensory Fibers

Lack of sensory feedback is also a major challenge for users of a limb-replacement prosthesis. Without normal somatosensory feedback, many patients complain that their prosthetic limb is unwieldy and difficult to use (Anani et al. 1977;

Dhillon et al. 2004; Dhillon and Horch 2005; Warwick 2005; Rossini et al.). Intrafascicular electrode arrays, such as the USEA, should be capable of selectively activating multiple, independent subsets of sensory fibers, just as they can for motor fibers. Motor and sensory nerves remain functional long after limb amputation, and stimulation of sensory fibers can elicit sensation (Schady et al. 1983; Chaudhuri et al.). Hence, it may be possible to stimulate sensory fibers through USEAs and thereby evoke graded and varied sensory responses, including proprioception and pressure, to aid in gripping and reaching tasks.

Here, stimulation through individual USEA electrodes generated a variety of patterns of somatosensory cortical activation. In principle, such differentiable sensory signals could be used to provide cutaneous and proprioceptive sensory feedback from a neuroprosthetic artificial limb. Further, the responses on a given cortical electrode were associated with stimulation on USEA electrodes that were also associated with specific muscles or classes of muscles (e.g., finger flexors). Because motor axons are organized musculotopically, and USEA electrodes that stimulate muscles with similar function are often near one another (e.g., Fig. 2B, FDP and FDS, or FCR and PrT), we can conclude that the somatotopic and musculotopic maps in the nerve are in approximate register with one another. Because muscle activity could often be evoked on an electrode that also evoked sensory responses, it is likely that individual fascicles are mixed sensory-motor, consistent with previous studies (Brill et al. 2009).

The modality of sensory responses is difficult to determine from recordings from the cortical surface with the electrodes used in this study, especially given that there is some overlap in the representation of body space in the cortex. However, activity from

stretch receptors in a given muscle would be expected to lie in close proximity to motor fibers associated with the same muscle, indicating a high likelihood that the evoked potentials could convey some proprioceptive feedback for use in a prosthetic application. Such feedback might provide both intuitive, closed-loop prosthetic control and enhanced integration of the artificial limb with the user's own internal body image.

Considerations for Long-Term Intrafascicular Electrode Implants

In SCI patients, the lower motoneurons remain mostly intact within the spinal cord. However, their chronic deinnervation can cause secondary degeneration, disassembly or disorganization of the neuromuscular junction, changes in muscle excitability, and muscle atrophy. Thus, in a chronic implantation in a paralyzed individual, the initial conditions of the muscle and neuromuscular junction might be quite different from those in the intact animals in the present study. However, the initial peripheral changes that occur after SCI are largely reversible through FES, which, over time, can restore the neuromuscular junction's natural arborization and can improve the efficacy of muscle activation (Biran et al. 2005). Indeed, the ability to return the neuromuscular system toward its normal preinjury conditions may constitute an additional benefit of the intrafascicular electrode technology. However, without early intervention SCI-induced hypertonia and spasticity can cause permanent changes to the functionality of muscles. All potential therapies, including the proposed USEA, PNS-based prosthesis, thus give the most benefit when provided immediately after injury.

Neurons may undergo important changes at the sites of chronic electrode implants that could affect electrode functionality. Fibrosis around electrodes and a continuing foreign body response can push axons away from the electrode tips, hampering their

ability to record and stimulate neurons selectively (Simeral et al. 2011). Although all neural implants face the problems associated with tissue response, CNS implants of Utah Electrode Arrays (UEAs) are subjected to less motion than nerve implants, and traditionally have been more reliable (Branner et al. 2004) than long-term USEA implants in initial studies (Clark 2011; Clark et al. 2011a; Frankel et al. 2011; Normann et al. 2012). However, recent and ongoing research has demonstrated substantive improvements in both long-term recording and stimulating capabilities of USEAs in cat sciatic nerve (Pohlmeyer et al. 2007), which may translate to comparable success for USEAs in monkey arm nerves, and ultimately for clinical applications.

Issues of Muscle Control for the Design of the Motor Program

Strategies for motor restoration that are based on nerve stimulation explicitly involve the activation of lower motor neurons, which can engage spinal reflexes that can operate independently of the brain. For example, Renshaw reflexes involve negative feedback circuits in which a motoneuron inhibits itself (among other neurons). However, synaptic inhibition that occurs at the motoneuron soma many space constants away will have almost no effect on the direct activation of motor fiber axons at the USEA stimulation site.

Because sensory and motor fibers are mixed within the nerve, activation of proprioceptive, cutaneous, or even nociceptive reflex pathways might be engaged coincidently with motor fiber stimulation in the awake animal. In principle, effects of these might need to be incorporated into our artificial motor program. However, such considerations have not proven to be overly problematic in other clinical applications of FES with extraneural stimulation. Given the high selectivity and relatively low currents

associated with intrafascicular stimulation, these considerations also seem unlikely to be overly problematic for USEAs. Further, because the Aδ fibers involved in the withdrawal reflexes are smaller than α-motor fibers, they require higher extracellular stimulation levels to activate, and thus would be some of the last fibers to be activated by any given USEA electrode.

Brain-Controlled Activation of Motor Nerve Fibers and Behavior

In a closely related project, we have demonstrated that recordings from similar Utah electrode arrays implanted in the primary motor cortex of monkeys can provide accurate information about muscle activity during normal or intended movement (Moritz et al. 2008; Pohlmeyer et al. 2009; Ethier et al. 2012). The information can be used to restore simple voluntary movement to monkeys during peripheral nerve block, used as a temporary paralysis model of spinal cord injury.

During this nerve-block paralysis, stimulation through intramuscular electrodes is used to evoke the intended movement, as inferred from the cortical recordings in real-time (Popovic et al. 1993; Lee et al. 2008; Legon et al. 2008). Potentially, in future work, USEA-based stimulation of motor fibers could be controlled in a similar manner, providing the monkey—and ultimately, a paralyzed person—volitional control of more dexterous and coordinated hand movements than can be achieved with intramuscular or extraneural electrodes.

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CHAPTER 5

DISCUSSION

Summary of Major Findings from Chapters 2, 3, and 4

Chapter 2

In Chapter 2, this work showed that an intrafascicular electrode can be used to selectively stimulate sensory fibers by using constant voltage, monophasic, square-wave stimulation to evoke SSEPs with low latency in and around S1, sometimes in the absence of any muscle response. The demonstrated activation of sensory responses without the activation of muscle responses, and vice-versa, are an important capability for electrodes to be used for neuroprostheses (Branner et al. 2001; McDonnall et al. 2004a). The relatively close stimulation thresholds of the two different modalities are likely due to the mixing of the two types of fibers within a fascicle. Purely sensory fascicles and purely muscular fascicles would likely cause electrodes within the fascicle to have very high thresholds for the out-of-fascicle modality.

Chapter 3

In Chapter 3, I presented advances in the electrical isolation and physical protection of electrode arrays implanted in the PNS for enhancing the functional lifetime of a long-term implant (Clark et al. 2011b). This work shows that by stabilizing and protecting the array within the nerve from movement and exogenous signals, long-term implants can function in the challenging environment of the PNS. Intrafascicular

electrodes implanted for long periods of time retain the muscle stimulation selectivity that has been demonstrated in acute studies; intrafascicular electrodes also maintain the ability to record multi-unit activity and single unit activity across chronic timeframes of up to 4 months. Improvements to the connector system, array electrodes, and the containment system around the implant were essential to this success. The long-term implants in this study were ultimately used to decode limb position form neural signals and generate stance in an anesthetized animal, demonstrating the functional usefulness of such a long-term implant system.

Chapter 4

In Chapter 4, this work showed that intrafascicular electrodes provide sufficient selectivity to evoke a coordinated grasp in the complex system of the hand. Although previous work shows selective activation of large muscles in individual nerves with relatively few fascicles (Ledbetter et al. 2013), the present work extended those findings to the more complicated motor system of the hand where the co-activation of muscles is common and nerve fasciculation is more complex (Burns et al. 2007). This chapter also extended the previous work of primate researchers who have used relatively low-selectivity electrodes to generate grasping motions. Additionally, this chapter demonstrated that individual electrodes can evoke different cortical responses, implying that the percepts evoked through different electrodes could be qualitatively different.

Limitations of Results

Sensory Specificity

Precise measures of specificity for sensory stimulation are difficult in a non-verbal animal. Although short-latency SSEP generation does indicate sensory fiber activation, the specificity for different sensory modalities as well as motor-sensory specificity is difficult to determine. The generation of SSEPs without the generation of muscle twitches (as determined by an absence of EMG activity) strongly indicates selective stimulation of sensory fibers, though it is impossible to eliminate entirely the possibility of low-level twitches in muscles that were not monitored. Likewise, the generation of twitches without the generation of SSEPs is an indicator of selective activation of motor fibers, though sensory stimulation could easily occur without generating cortical potentials that are detectable by our skull screw or ECoG electrodes. The lack of certainty regarding the motor-sensory selectivity, particularly the lack of a direct assay of sensory stimulation in the nerve, will always remain without a verbal experimental subject. A well-trained animal does provide the possibility of having a more direct assay of sensory stimulation; however, determining the specificity and modality of sensory stimulation would require an extremely complex training paradigm.

Challenges of Long-Term Work

The work in Chapter 3 represents some of the first successful long-term peripheral nerve implants with a penetrating electrode array. Although this work was successful in recording and selectively stimulating over the period of many months, many improvements in performance and stability remain necessary for neural prosthetic devices to be clinically useful. Although the containment system described in chapter three did

protect and immobilize the array, further improvements to the fit of the containment around the nerve would further enhance nerve-array coupling. Additionally, material changes in the components of the containment system may reduce the tissue response and enhance the stability and efficacy of the implant and its ability to function in a noisy environment.

Coordinated Grasping

The work on hand function presented herein involves short-term studies in a single anesthetized session. Though this work provided strong evidence that the muscle selectivity and specificity via USEA stimulation is sufficient to evoke coordinated grasp in an intact limb, the translation of this work to long-term studies, or studies of an actual paralyzed limb, involve many complications not present in our model system. Specifically, motoneuron degeneration and deefferentation of muscles will change the usability of any nerve implant. In SCI patients, lower motoneurons remain intact; however, they have greatly reduced excitability due to the degeneration of upper motoneurons (Taylor et al. 2002; Kirshblum 2004; Zinck and Downie 2006). In turn, the low, essentially zero, firing rate of these deinnervated lower motoneurons leads some (though not all) NMJs to atrophy or sprout in a disorganized manner. Although the altered innervations of the muscles will change the ability of nerve stimulation to produce forceful contractions and may alter selectivity, there is strong evidence that stimulation of the nerve through any means will reverse the process of degeneration caused by chronic removal of the efferent excitation (Maier and Hepp-Reymond 1995b, 1995a; Winges et al. 2008).

The success this work showed in generating power grip is only the beginning of functionality necessary for the natural, intuitive use of the hand. A myriad of different hand postures are used by most people every day to engage a wide variety of power and precision grips. Though power grip is important in many ways, its usefulness pales in comparison to the precision grips, which are used for all fine manipulations. We succeeded only in generating a partial precision grip, without any extension of the thumb and forefinger to release the grip. Additionally, the thumb and forefinger did not align reliably across multiple attempts, indicating the importance of replicating the function and activation patterns of intrinsic hand muscles necessary for holding the hand in a posture useful for a grasp (Kozin et al. 1999).

Future Work

Sensory Assays

A better test for sensory stimulation is necessary to determine how effective USEA stimulation is at activating sensory fibers. Evoked potentials, such as the SSEPs used in Chapter 2, represent the activation of many neurons near-simultaneously. It is likely that the activation of a single primary afferent fiber would not generate an SSEP detectable through skull screws. Smaller electrodes in closer proximity to the brain, such as ECoG or microECoG, could be used to detect smaller numbers of concurrently activated neurons, however only small penetrating electrodes have been shown to record from individual neurons.

Connector Design

The wound closure around the transcutaneous portion of the implant requires further improvement to be useful for long-term use. The skin around the transcutaneous, bone-mounted implants in Chapter 3 eventually separated from the implant. The surface treatments applied to the titanium did enhance the connection of the skin to the device, but the relative movement of the skin of the leg above eventually caused abrasions and separation of the skin and implant. Alterations in the geometry of the connector, combined with a surface treatment, could prevent skin separation. An alternative connector shape could distribute the forces acting on the skin-connector interface by increasing the surface area and changing the angle at which that force is applied. Specifically, by changing the connector to have a surface parallel to the skin via a flange on the connector, the skin would contact the connector over a much larger area. Preliminary work (not reported here) supports this possibility.

Containment Redesign

By stabilizing the array physically, the containment system used in Chapter 3 was able to record signals over the period of many months in the nerve. The design of the containment, and the necessity to build it *in vivo*, resulted in it being approximately 0.5 cm wider than the nerve. Reducing the size of the containment around the nerve, as well as making it more conformal to the implant and nerve, could further improve the stability of the array. Material changes to the containment system could also enhance the physical protection provided or improve shunting of contaminant signals to ground. Silicone-based compounds are obvious materials to explore as an alternative to the Kwik-Cast used in this study. Silicone has the advantage of being a common biocompatible implant

material that can be mixed with other materials, such as an electroconductive material, to combine the properties of the two materials. Material combinations such as this could eliminate the need for the gold mesh in the current design of the containment system. The silicone in this containment design was used primarily to fill gaps and create a close fit around the array and nerve to limit relative array-nerve movement, while the gold mesh provided a semi-rigid protective sheath around the nerve. If a single material could be used for both purposes, this would simplify the creation of the containment *in-vivo*.

Chronic Studies for Grasping

Monkeys can be trained to perform complex tasks that give researchers insight into what sensations are evoked by electrical stimulation. Although there are some aspects of complex, multi-modal, sensation that are difficult to determine from a training paradigm, simple responses to specific physical stimuli can be learned by most primates. Replacing the physical stimuli with electrical stimuli then allows researchers to determine if they can deliver an analogous sensation to the initial, physical, sensation. Future long-term stimulation studies could be done on animals trained to respond to specific somatic stimulation. A primate could be trained to respond differently to proprioceptive stimulation, tactile stimulation, and other touch modalities in a specific region. Though this technique is promising, the combinations of different stimuli in different regions quickly add up to a large list of complex responses to be made by an animal, which would require extensive and complex training. For this reason it is important that any future sensory stimulation work should also include the implantation of an ECoG grid, or other cortex monitoring technology. By mapping the animal's cortical responses to a variety of delivered sensations and electrical stimulation during behavioral training and

testing, researchers will have a better sense of how closely matched the physical and electrical stimuli are matched.

The power grip achieved in this study is only one of many desired grip styles desired for use in a clinically useful prosthesis. One of the hand's most useful features is its ability to perform a huge variety of grips anywhere in a person's peripersonal space regardless of body position or posture. To create a useful reanimation prosthesis for paralysis patients, future work must focus on generating additional grasps, such as the support grips used for bearing weight (i.e. bucket grip), or the pinch grips (lateral pinch, or pulp-to-pulp). Generating either appropriate power grip or pinch grips requires excitation of intrinsic and extrinsic hand muscles in specific patterns. In natural power grip, intrinsic and extrinsic muscles are synchronously activated, whereas in pinch grip the intrinsic muscles position the fingers and thumb before the extrinsic muscles are activated . Replicating the complex timing and synergy of these muscles during a natural grip will require high specificity of stimulation and precise timing of stimulus delivery. Although the USEA showed specificity for some muscles, future work could improve upon the specificity achievable through the array by using current steering between electrodes. The use of local return electrodes, within the nerve, would cause a much smaller excitation volume, potentially giving greater control to experimenters.

Conclusion

A neurally-integrated prosthesis for limb replacement or restoration should allow a user intuitive, natural control that is as close to a normal intact limb as possible. This dissertation focuses on the use of micro-electrode technology to decode the intention or experience of a human subject by recording the electrical discharges of the nervous

system or inducing discharges in the neurons. Current technologies for sensing the activity of neurons or activating them cannot rival the density of the cells in the nervous system, and therefore density of information being conveyed within the neurons. Many researchers have looked to the brain, where all intentions and sensations are known to be experienced, as the location to record or deliver this information. This dissertation discusses an alternative approach wherein the computer-nervous system interface is as distal as possible in the nervous system. This approach takes advantage of the simple representation of the information within the nervous system in the nerve and spinal cord when compared with the representation of the same information in the brain.

High-channel-count intraneural electrode arrays, such as the USEA, can provide the access to many neural channels necessary to approximate natural limb function. To be useful in a clinical context for patients who have lost the use of their limbs, such an electrode array will have to be stable and safe for decades, sensitive enough to detect small numbers of neurons, non-invasive enough to be accepted by users, and effective enough to outperform traditional prosthetics. This dissertation shows the development of systems designed to make USEA electrode technology stable for the long term, as well as showing what selectivity such an electrode array can provide. Although the work herein shows some success in achieving functional electrostimulation and creating a long-term implant, there are many remaining challenges to overcome before a neuroprosthetic system can be put into practice. The synergistic effects of foreign body response, physical motion, electrode material and design, as well as other factors make the contribution of each factor to device failure difficult to determine. For this reason, the continued

improvement of all aspects of a neuroprosthetic system are necessary to advance the field of creating a man-machine interface.

Although the task of creating a device capable of replicating the functionality of an intact limb is daunting, the development of multiple electrode technologies that provide ever-more refined access to the nervous system gives us hope that it is possible. Innovations from all corners of the field of bioengineering, from biomaterial design to the modeling of complex neural networks, come together to create devices that minimize the body's foreign body response, read information from the nervous system with ever-increasing accuracy, and deliver stimuli that effect more and more precise targets. The USEA is an electrode that can be refined and modified to take advantage of advances in materials science, information processing, and wireless technology to provide the kind of access necessary to create a useful, intuitive interface between a patient and a computerized assistive prosthetic. The experiments presented in this dissertation show that the basis for this technology can work, and continue to work, over a chronic timeframe.

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