Validation of Neurotrophic Electrode Long-Term Recordings in Human Cortex

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Published online on: 10 Jan 2018

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Validation of Neurotrophic Electrode Long-Term Recordings in Human Cortex

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15.1 INTRODUCTION

For the development of long-term neural prosthetics that require cortical control signals, it is essential to develop an electrode that can continuously record these cortical signals over the lifetime of the subject. Over the past several decades, different types of long-term electrodes have been developed. Tine-type electrodes (Rousche and Normann 1998), wire electrodes (Presacco et al. 2011),
and many other electrode types (Ward et al. 2009) have successfully recorded signals for months or years. However, signal quality declines gradually, with only 43% of electrodes producing usable single-unit recordings at 3 years with the Utah array (Simeral et al. 2011) and with only 15% of signals remaining in humans (from 96 original signals) (Hochberg et al. 2011). These signal losses did result in serious degradation of function (Perge et al. 2013) though some function remained (Hochberg et al. 2011). Over the past decade, major efforts have been undertaken to understand the factors that lead to loss of signal. These factors include micro-movements that result in fluctuating signal amplitudes and glial scars that separate the recording tip from the recorded neurons resulting in loss or destructive degradation of the signal. Despite extensive efforts, satisfactory solutions for these problems remain elusive (Ward et al. 2009).

A different approach to chronic recordings of brain signals began in 1986. Instead of inserting an electrode into the brain’s neuropil, the neuropil is grown into the hollow, cone-shaped, glass tip of a coiled wire electrode (Kennedy 1989). This tip is impregnated with trophic factors to induce growth into the tip. In the weeks and months following insertion, the neural tissue grows into and through the 2-mm glass tip forming a bridge of neuropil. This anchors the electrode within the cortex (Bartels et al. 2008). This chapter focuses on the longevity of this type of electrode in humans. It describes and references how it is constructed and provides tests of impedance, quality of signals, and function of signal over the 10 years in the, so far, longest surviving implant.

15.2 METHODS

Assembly of the electrode has been described in great detail previously (Bartels et al. 2008). Briefly, the 2-mm glass conical-shaped tip contains two to eight 2-mil Teflon-insulated gold wires that record the axonal firings of the ingrown neurites. As shown in Figure 15.1a, the wires are first coiled for strain relief and then bent appropriately to produce a shelf that lies along the surface of the cortex to prevent too deep a penetration (Bartels et al. 2008). The tip consists of a glass pipette drawn to produce a tip diameter of 25 to 50 μm, and an upper opening several hundred microns in diameter that provides space for placement of the recording wires. As many as seven wires have been placed within the glass cone secured with methyl methacrylate glue (Figure 15.1b) (Bartels et al. 2008). The wire tips are spaced several hundred microns apart. During human surgery, after placement of the (proprietary) growth factors, the 5-mm-long tip (measured from the angle of the wire) is inserted at a 45° angle to reach the layer 5 corticospinal tract neurons in motor cortex (Figure 15.1c). The trophic factors are drawn into the glass tip by capillary action. Then, the tip is left to dry so the factors are mainly on the inside of the glass tip. After insertion, the factors diffuse into the neuropil.

For recordings in humans, a power-induced pair of amplifiers (typical gain ×800, band pass 5 to 5000 Hz) are implanted above the skull under the scalp, connected to the electrode, and secured to the skull with acrylic cement. The amplified neural signals are transmitted through the scalp using a frequency-modulated transmitter operating in the 42 ± 8 MHz range for external processing (Kennedy et al. 1992b). During the recording sessions, the power induction coil and the FM receiving coils are secured to the scalp, using EEG paste to provide stability (Figure 15.1g). The demodulated signals are passed through CWE amplifiers (Ardmore, PA; 20× gain, band pass filtered 3–10 kHz). After A/D conversion, the digital filters are set at 300 Hz to 6 KHz for single units and 1 Hz to 6 KHz for continuous recordings. Trauma to the electronics due to handling of the head during hygiene has led to replacement of the units. The electrodes have never needed replacement. The electronic units are protected by Elvax and Silastic for insulation and trauma protection. The recording electronics are totally passive, contain no batteries, and are not used for stimulation, so there is so danger of electrical discharge.

15.3 RESULTS

Following implantation under full sterile protocol, neurites are induced to grow into the tip from layers 5 and upper layers 2 and 3 where interneurons reside. Growth into the tip begins within a
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FIGURE 15.1 Neurotrophic electrode design and characteristics. (a) Two-mil, Teflon-insulated coiled gold wires are shown on the left of the figure with the 5-mm shelf of wire that limits implantation depth, and to the right, the glass tip. (b) The glass tip contains four gold wires whose black shadowed tips are visible. The calibration bar is 500 μm. (c) The 5- to 6-mm-long tip is inserted into the cortex at a 45° angle to reach the corticospinal tract layer. Trophic factors encourage neurites to grow into and through the tip, thus anchoring it within the neuropil. (d) Impedances remain stable over months as measured in early monkey recordings. e1 and e2 refer to the wires inside the glass cone with recordings referred to a ground electrode on the rat’s skull. e1–e2 refers to impedance measurements between e1 and e2. 

(Continued)
FIGURE 15.1 (CONTINUED)  Neurotrophic electrode design and characteristics. (e) Impedances in saline remain stable over 4 years so far. Note the initial drop in impedance for both in vivo and in vitro measurements. (f) Electron-microscopic images of the Teflon coating near the tips demonstrating the peeling back of the Teflon that may explain the drop in impedances. (g) Recordings are obtained from implanted wireless systems transmitted using FM carriers. The receiving coils are temporarily fixed to the scalp using water-soluble EEG paste. Power is provided by induction via a coil on the opposite side of the head (not seen).
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week or two (Kennedy 1989) and it takes as long as 3 months before signals stabilize. Control placements of electrodes without the trophic factors in rats resulted in no recordings (Kennedy 1989).

Histological analysis of the tissue inside the recording tip has shown that there is normal neuropil except for the lack of neurons. Non-myelinated axons appear during the first few weeks and myelinated axons are abundant after 3 weeks. Blood vessels and axo-dendritic synapses are seen, but no microglial cells (that would indicate gliosis are found) and no gliosis is seen. These results have been described and illustrated in detail (Kennedy et al. 1992b). Stable impedances seen after several months coincide with signal stability as shown in Figure 15.1d for recordings in primates. After an initial drop in impedance, signals from this animal and others were stable and functional, being related to arm movements throughout the nearly 6 months of study (Kennedy et al. 1992a; Kennedy and Bakay 1997). We have seen an initial similar gradual drop in impedance with the four-wire electrode tested in saline over 4 years as shown in Figure 15.1e (still stable after 5.5 years). The initial reduced impedance is attributed to retraction of the Teflon insulation of the recording wires as shown (Figure 15.1f). Impedance measurements in humans during follow-on surgery for replacing the implanted electronics revealed impedances of 70 to 100 kΩ similar to non-human primate measurements A photo of ER’s two recording coils are shown in Figure 15.1g. The white substance is water-soluble EEG paste used to attach and stabilize the coils.

15.4 FOUR YEARS AFTER IMPLANTATION

Recording stability over long time periods is addressed by the following data. The two channels of continuous recordings shown in Figure 15.2a are from the same subject (ER). This subject is “locked-in” following a brainstem stroke at age 16. He was implanted in December 2004. His electrode has three wires inside the cone, the center wire acting as reference for the other two, thus creating two bipolar recording channels. The figure illustrates labeled multi-units, some with matching peak amplitudes. Since these are recorded from myelinated axons, these are really action potentials and not somatic recordings, and thus there may be some differences between these axonal recordings and somatic single-unit recordings. Somatic recordings have a deeper after-hyperpolarization, whereas the axonal spikes do not and hence appear sharper. Furthermore, the neuron is refractory to firing during this time for about 1 ms, so with the axonal spikes, this refractory period is shorter (between 0.5 and 1 ms). These continuously recorded multi-units are cluster cut into single units by using Neuralynx Inc.’s (Bozeman MT) convex hull technique (see examples in Figure 15.2b). The technique uses simple parameters such as total height, peak, valley, spike width, and energy. These are used to cut the continuous signals into single units.

To assess whether the separated wave shapes reflect the activity of single units, interspike interval distributions are constructed as shown under each single unit in Figure 15.2b from post-implant day 1582. These histograms demonstrate single peaks as expected for single units. It is observed that units of different amplitudes have vastly different firing rates as shown in Figure 15.2c. The larger units have the slowest modulating (or firing) rates and are likely related to corticospinal tract neurons, whereas the rapidly modulating small-amplitude units likely originate from interneurons. These data were recorded and archived in 2009, 4.4 years after implantation. The units shown here have been used in functional studies involving the development of the speech prosthesis (Brumberg et al. 2011; Guenther et al. 2009).

To further assess for single units, interspike interval histograms are constructed as shown in Figure 15.2d for data recorded on post-implant day 1556 (2009). If the interspike interval histogram is constructed by using data from individual neurons, it ought not to contain firing activity at very short interspike intervals, because action potentials during these times would fall into the cell’s refractory period. (Note the software retrigger time after a threshold crossing is a quarter millisecond so it will not blank out this time.) If such short intervals of no firings (minus the quarter millisecond) are identified, there is a high probability that the firings came from single units. A similar analysis of data containing multiple cells did contain data at short intervals so there was no “gap” near time zero because firings of multiple cells overlapped as shown previously [Kennedy et al. 2011 (fig 5b)].
FIGURE 15.2 Multi-unit data are received and analyzed in real time. (a) Two channels of data from the three wires within the electrode demonstrate units of similar amplitude as labeled by letters. Voltage levels (not shown) above and below the data stream separate the single units from the multi-units. (b) Examples of units cluster cut from the multi-unit data are shown along with interspike interval distributions that demonstrate a single peak, strongly suggesting a single unit (June 22, 2009, post-implant day 1582). (Continued)
FIGURE 15.2 (CONTINUED) Multi-unit data are received and analyzed in real time. (c) Unit firing rates shown over a 15-s range from less than 1 Hz to 100 Hz or more. The largest units have the slowest rates, suggesting that they originate from corticospinal tract neurons. The smallest units have the fastest rates, suggesting that they originate from interneurons (April 6, 2009, post-implant day 1556). Units are labeled ch1_SE_0* meaning they originate from channel 1, single electrode, and number 0*. (d) Interspike interval histograms demonstrate no firing at less than 1 ms, strongly suggesting that these are single units. Multi-units would fill in the 1-ms gap. The non-trigger time applied to the voltage threshold is 250 μs, so only a quarter of the gap could be caused by non-triggering.
15.5 NINE YEARS AFTER IMPLANTATION

Data recorded in October 2013, *nine years after implantation*, are shown in Figure 15.3a. Examples in the top row demonstrate single wave shapes, and interspike interval distributions are shown in the next row, indicating a high probability of single units. Further examples are shown in the next two rows. Interspike interval histograms are shown in Figure 15.3b, strongly suggesting the presence of single units. To provide further evidence, cross-correlation analyses were performed with the aim of determining if units were related to each other during rest and during task performance. An example is shown in Figure 15.3c during rest and in Figure 15.3d during activity. During rest, all units indicated little if any correlation with the index unit (SE1-se-01: abbreviated to 1-1 in the text). However, during activity, increased correlation was evident between unit 1-1 and units 1-5.
FIGURE 15.3 (CONTINUED) Ensemble activity almost nine years post implant. (b) Interspike interval histograms are shown for data recorded October 2, 2013 (post-implant day 3081). (c) During quiet resting, minimal if any cross-correlation of units is demonstrated using unit 1-1 as the index unit (also from October 2, 2013). (Continued)
FIGURE 15.3 (CONTINUED)  Ensemble activity almost nine years post implant. (d) However, during activity, using the same 1-1 index unit, cross-correlations appear only on channel (wire) 1, such as SE1_se_02, SE1_se_03, SE1_se_05, SE1_se_08, and SE1_se_09. (e) Some units during activity such as SE2_se_05, on the other hand, display no cross-correlations with other units.

(Continued)
1-8, and 1-9, and weak correlations with units 1-2 and 1-3. It is noteworthy that only some units demonstrate cross-correlations, while others do not. An example of noncorrelation is shown in Figure 15.3e where unit 2-5 does not cross-correlate with other units during task performance (task described below). In contrast, Figure 15.3f illustrates the correlations between the index unit 1-9 and units 1-1, 1-2, 1-3, 1-5, 1-8, and inverse correlations with units 1-4 and 1-7. Thus, these units are active as an ensemble after almost 9 years of implantation.

15.6 FUNCTIONAL STUDIES AT YEAR 9

To further examine the question of functionality after almost 9 years of implantation, we used these units in conditioning experiments as part of a speech prosthesis development project (Brumberg et al. 2011; Guenther et al. 2009). An audible guitar chord, “D7,” was tagged to unit 2-7 so that every time unit 2-7 fired, the guitar chord sounded through the computer speakers. The subject was asked to sing the guitar sound in his head by firing the unit, a task he performed many years before (Kennedy 2011). Average unit activities 10 s before and after the request to sing were compared. This comparison revealed a ratio of firing rates for each unit: when the rates before and after the “go” signals were equal, the ratio was 1, whereas an increase in the firing rate increases the ratio during singing. The ratio for recording day 3135 is illustrated in Figure 15.4a. Two epochs of conditioning during the same session (gray and dark gray bars) demonstrated little effect on the ratio for most of the units with a few exceptions such as number 18 (unit 2-7), which is the unit being conditioned. Four days later, this study is repeated as shown in Figure 15.4b. An effect on most ratios was now evident with a larger ratio increase during the second (dark gray) and third (white) epochs.
FIGURE 15.4 Examples of conditioning of units. (a) Normalized firing rates of unit SE2_se_07 (#18 in the figure) on day 3135 after implantation (October 25, 2013). All 21 units are shown. To normalize the different firing rates, the ratio of task-related firings (averaged over 10 s) after the “go” signal are compared with 10-s averages before the signal. The ratio does not increase for most units. (b) Four days later, the same paradigm suggested that unit SE2_se_07 (the conditioned unit, #18) did dramatically increase its ratio on the second set (dark gray bar) and maintained this increase on the third set (white bar). Most other units also increased their ratios, at least by the third set. (c) All units are shown as time stamps over 30 s of data. The lower trace shows the wave files of the words “I,” “Love,” and “Sing.”
indicating a learning effect in some of the units. Of most importance was the large effect seen with unit 2-7 (#18 in Figure 15.4), which was the unit being conditioned. Thus, these data suggested that functional conditioning of units was possible even after 9 years of implantation.

The above data illustrate increases in averaged firing rates after the “go” signal compared with before the signal. Sometimes, however, the rate decreased. To determine if increases and/or decreases of unit activity were important in the development of a speech prosthetic, a different paradigm was examined. Figure 15.4c illustrates that audible wave files containing the words “I,” “Love,” and “Sing” are illustrated by their spectrograms and tagged to single units. Above the spectrograms for “Love,” “I,” and “Sing,” the firing frequencies of all the units are illustrated as time stamps. Units were chosen based on previously determined strong modulation during time periods when the subject attempted to speak these three words in his head (inaudible as the subject is mute).
During the present task shown in Figure 15.4c, production of the word from the speakers was triggered by unit firings in a “winner-take-all” paradigm. The successfully emitted word was contained in a wave file so that it could be identified by its frequency spectrogram illustrated along the bottom of the 30-s data segment in the figure. The illustrated task required the subject to produce the word “Sing,” and not “I” or “Love.” The “go” signal is marked at the approximate time the word was requested (time “0”). The spectrogram for the requested word “Sing” is rarely seen before the “go” signal. After the “go” signal, he did not initially produce the word “Sing” more frequently than the other words, as illustrated. However, after about 5 s, the word “Sing” appeared more frequently.

To understand why and how the word “Sing” appeared when it did, the specific single units attached to the three wave files are shown over a ±10-s time period in Figure 15.4d centered on the “go” signal. Only unit 2-5 is attached to the word “Sing.” It appeared to increase its firing rate near time zero but the word “Sing” was not produced. The explanation is likely seen in the suppression of firing of the other units attached to the other two words. Units 1-1 and 2-1 (attached to “I”) and unit 2-2 (attached to “Love”) decreased their firing rates, thus allowing unit 2-5 to be the “winner” and produce the word “Sing.”

To illustrate this point further over a complete session, the conditioning of the word “Sing” is illustrated in Figure 15.4e. During requests to produce “Sing,” the subject could produce “Sing” significantly above baseline (14 compared to 4 words per 20-s bin). Even more telling is that the production of “Sing” was restrained during requests to produce “I” and “Love.” The chi-square test values 0.44 for “I” and 0.25 for “Love” were not significant, but when asked to say “Sing,” the number of “Sing” words above baseline was statistically significant with a chi-square test value of 7.14. Note the expected shifting baseline (going from 7 to 4 to 3) previously documented (Kennedy 2011).

15.7 DISCUSSION

Recording functionally active neural signals after almost a decade of implantation in the human cortex strongly suggests that the neurotrophic electrode fulfills the requirement for a reliable neural interface. The examples of cross-correlation data and the conditioning data strongly suggest that units remain individual and functional after almost 9 years. In fact, the electrode and electronics were implanted in 2004 with signals recorded in 2015, producing 11 years of recording. The subject is too ill to record functional activity. Attempts to record on January 1, 2016, resulted in no evidence of the FM transmitter signal. The electrode is likely intact, but the electronics has failed.

The chronic recording over a decade implies that even longer time periods are likely. The design of the electrode tip is crucial to providing a firm attachment to the neuropil to ensure longevity. This design allows the neuropil to grow into and through the tip and thus anchors it in place. A second important feature is the strain relief provided by coiling the delicate 2-mil gold wires. After the neurites have grown into the tip, they become myelinated and electrically active, with stability occurring at about 3 months. Examples of functional single unit activity at 4 years and 9 years after implantation are described above and provide strong evidence that this neural interface is reliable. After implantation in December 2004, data have been obtained on a continuing basis. For example, data obtained in 2005, 2006, and 2007 indicate that half the English 39 phonemes can be identified as confirmed by using multiple decoding paradigms (Brumberg et al. 2011); data in 2009 indicate that vowel production can be produced using a linear discriminant analysis decoding paradigm and controlled by the subject (Guenther et al. 2009); data recorded in 2008, 2009, and 2010 describe the effect of emotional state on background firing rates (Kennedy 2011); data recorded in 2007, 2008, and 2009 indicate that pure tones are associated with synchronized firing of single units, but not multi-units (Kennedy et al. 2011); data collected over the 2008 and 2009 periods indicate that vocalization onset could be detected by analyzing the pattern of activation in the low beta frequency range (Sarmah and Kennedy 2013); and conditioning of unit firings was undertaken in 2013, an example of which is described above. Such single-unit conditioning was originally successfully demonstrated in 1973 by Fetz and Baker in monkeys (Fetz and...
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Baker 1973). These authors demonstrated that monkeys could control firing rates of more than one unit by increasing the firing of one and decreasing the firing of the other. This is broadly similar to the data presented in Figure 15.4d and e and is the first such demonstration in humans of reciprocal conditioning.

So where does that leave the field of brain–machine/computer interfacing? First and most obvious, the field must overcome the fear of implantation, just as the cardiovascular field overcame the fear of cardiac pacemaker implantations. In experienced hands, brain surgery is safe. It has the usual risks of bleeding, infection, seizures, and damage to the underlying brain. In the case of electrode implantation, any damage is done to the functional cortex that is not being used because of the paralysis or loss of output. With modern sterilization techniques and careful surgical technique, the questions of infection and hemorrhage are minimized. Seizures can be controlled with medication. So the question becomes which electrode to implant? For short-term usage, the tine-type electrodes are excellent choices, but for long-term implantation, the data show that their signals do not persist and their utility ends (Ward et al. 2009; Simeral et al. 2011; Hochberg et al. 2011; Perge et al. 2013). Therefore, the neurotrophic electrode or some variation thereof is the choice for long term survival of the signal. The aim is at least 50 years of recording. The disadvantages of the neurotrophic electrode are that it is bulky and damages the cortex. However, the cortex is not useful anyway because the individual is paralyzed or locked-in so the damage is not relevant especially since function can be obtained from the implanted cortex. This electrode has been studied in rats (Kennedy 1989) and has been found to record from a radius at least 600 μm of cortex, thus covering a relatively wide area. In fact, in the most recent implantation (Kennedy et al. 2016), four electrodes were implanted 6 mm apart with no ill effects long term. However, the principle of using trophic factors to ensure a long-lasting tight binding between the neuropil and the electrodes is mandatory for long-term signal stability and functionality. The present results strongly imply that the most reliable neural interface can be produced by growing the neuropil into the electrode rather than by inserting the electrode into the neuropil.

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**GLOSSARY**

**Cross-correlations:** The firing rate of one single unit is correlated with the firing rates of all the others. If some or all of the other units fire at the same time as the single unit fires, then they are related.

**Ensemble:** Units act together like a crowd, for example, clapping a speaker all together.

**Histogram:** A bar graph with bar heights indicating the frequency of a variable, in our case firing rate, depicted over time.

**Impedance:** The resistance of the tip of the electrode when measured using an AC versus a DC source.

**Interspike interval histogram:** The time between firings of the units is plotted as a histogram over time.

**Microns:** One micron is one-thousandth of a meter.

**Mil:** There are 39.37 mils in 1 mm; 1 mm = 0.0254 mils.

**Neurites:** A general term for any outgrowth from a neuron. When a neurite becomes myelinated, it is called an axon or dendrite depending on its location with respect to the neuronal body.

**Neuropil:** The neural tissue that makes up the brain and consists of neurons, axons, dendrites, glial cells, and interneurons. It also includes blood vessels such as capillaries.

**Polarization and after polarization:** Polarization refers to the abrupt change in membrane potential that generates the spike, and “after polarization” refers to the brief period after the main spike.

**Refractory period:** A short period (less than 1 ms usually) during which the neuron will not fire (depolarize).

**Spikes:** This term refers to action potentials that are discharges from neurons or axons that exceed a baseline membrane potential that results in a sudden sharp increase in the potential, and hence the (somewhat slang) term “spike” or action potential.

**Tines:** Tiny, sharp needles that penetrate the brain and made of a metal (platinum or iridium for example) that is insulated except at its tip. The impedance of the de-insulated tip is of the order of a few tens of ohms or a mohm (rarely more).

**Trophic factors:** Substances such as nerve growth factor, ciliary nerve growth factor, and so on, attract and nourish neurites and neurons.
ACKNOWLEDGMENTS

Roy A.E. Bakay, Rush Presbyterian Medical Center, Chicago, IL, USA, is posthumously acknowledged as being a key person in the early and most recent study. Thanks also to the many people who participated over the years in these studies: Frank Guenther, Boston University, Boston, MA, USA; Jonathan Brumberg, 1450 Jayhawk Boulevard, Lawrence, KS, USA; and Edward Joe Wright, formerly at Neural Signals Inc. Grateful thanks to the subject and his parents for their enthusiastic participation and support.

Funded by NIDCD grant R44DC007050, NINDS grant R44NS36913, and funds from Community Neurological Clinic.

INSTITUTIONAL REVIEW BOARD APPROVAL

All studies were approved by the institutional review board of Neural Signals Inc.; Gwinnett Medical Center, Lawrenceville, GA; and Emory University, Atlanta, GA (earlier studies). Studies were carried out in accordance with the Helsinki declarations.

FEDERAL DRUG ADMINISTRATION APPROVAL

Study was approved by the FDA G960032.

CONFLICTS OF INTEREST

P.R. Kennedy owns 98% and D. Andreasen owns 2% of the stock of Neural Signals Inc.