34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

Analyzing neuronal activation with macroelectrode vs. microelectrode array stimulation

Sharanya Arcot Desai, Claire-Anne Gutekunst, Steve M. Potter and Robert E. Gross

Abstract—**Deep Brain Stimulation (DBS) has provided remarkable relief to patients with brain disorders. Traditionally, DBS is performed through a single macroelectrode implanted at a specific deep brain structure (like the subthalamic nucleus for Parkinson's disease). Despite its great success, little is known about its mechanisms of action. We propose that using several microelectrodes for stimulation, instead of a single macroelectrode, may provide advantages including reduced tissue damage and increased brain area activated. We compare the area of brain affected by macroelectrode and microelectrode arrays implanted in rat hippocampus using stimulation-induced c-Fos expression and immunohistochemistry.**

I. INTRODUCTION

Deep Brain Stimulation (DBS) has relieved a significant number of patients from clinical symptoms of brain disorders including Parkinson's, tremor, dystonia, depression, OCD, and others[1-3]. Additionally, early stage clinical trials are underway for using DBS for diseases like temporal lobe epilepsy where ablative procedures may not be an option due to risk to memory[4].

Typically in DBS, a linear array of four cylindrical contacts (1.27 mm in diameter) is implanted at a specific deep brain structure determined using techniques such as magnetic resonance imaging or intraoperative microelectrode recording[5, 6]. A lead connected to the implanted electrodes provides stimulation pulses delivered by an IPG (implanted pulse generator) implanted subcutaneously in the clavicle or the abdomen of the patient[7]. Despite its tremendous success in alleviating the clinical symptoms of an array of neurological disorders, a lot remains unknown about the underlying mechanisms of action of DBS[8].

* This work was supported by the CURE Epilepsy Foundation and NSF EFRI COPN Grant (0836017)
Sharanya Arcot Desai is with the Laboratory for Neuroengineering, Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332 USA (e-mail:adsharanya@gatech.edu).
Claire-Anne Gutekunst is with the Department of Neurological Surgery, Emory University School of Medicine, Atlanta, GA 30322 USA (e-mail: cguteku@emory.edu).
Steve M. Potter is with the Laboratory for Neuroengineering, Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332 USA (e-mail: steve.potter@bme.gatech.edu).
Robert E Gross is with the Department of Neurological Surgery, Emory University School of Medicine, and Atlanta, GA 30322 and the Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332 USA (e-mail: rgross@emory.edu)

A number of simulation studies using finite element analysis methods have provided some insight into the volume of tissue activated in DBS[9-11], but all these need validation in living neuronal tissue. Directly studying the effects of electrical stimulation on the extent/radius of neuronal tissue activation and number of neurons activated will help gain understanding into the basic DBS mechanisms.

Additionally, using an array of microelectrodes[12, 13] (each of which has a diameter of few tens of micrometers as opposed to a single macroelectrode with diameter >500 μm) for stimulation may provide certain advantages over macroelectrode stimulation. These may include reduced tissue damage caused by implantation and increased net neuronal activation. We compared the area of brain tissue activated by microelectrodes and macroelectrodes with the goal of developing new stimulation techniques and devices that could be more effective than traditional macroelectrode DBS.

Histed et. al[14], used 2-photon Ca^{2+}imaging to study cortical activation patterns with varying amounts of current injection. This technique provides excellent spatio-temporal details of neuronal activation, but is not yet feasible in deep brain structures like the thalamus or the hippocampus.

C-Fos is an immediate early gene whose expression indicates recent neuronal activity. Expression of c-Fos in neurons has been used extensively for studying neural populations that respond to various types of stimuli including DBS[15]. We were interested in studying differences in neuronal activation caused by stimulation using a macroelectrode and a microelectrode array. For this, we quantified the number and distribution of c-Fos immunoreactive neurons following electrical stimulation with the two types of electrodes in the hippocampus of rats.

II. METHODS

12 adult male Sprague Dawley rats were used for this study. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Emory University Institutional Animal Care and Use Committee.

A. Rodent surgery:

Rats were anesthetized with 1.5-3% inhaled isoflurane. A craniotomy was made over the right dorsal hippocampus in all rats. In 6 rats, a single macroelectrode (150 μm diameter; Plastics One Inc.) was implanted in the CA3 cell layer. In the other 6 rats a microelectrode array (MEA) with 16 electrodes (each electrode with 33 μm diameter; TDT, FL) was implanted with 8 electrodes targeted at the CA1 and 8 electrodes targeted at the CA3 cell layers. The

microelectrode array has a row separation of 1 mm and the electrodes within each row were separated by 175 µm. A skull screw drilled over the left cortex served as ground (see [12]for details on the surgery method).

B. *Electrical Stimulation:*

Our lab's custom-built electrophysiology suite, NeuroRighter was used for providing electrical stimulation[16]. 25 Hz ±1 V biphasic square pulses with 400 µs per phase width was delivered continuously for 4 hours in 4 rats implanted with the single macroelectrode and 4 rats implanted with the MEA. In the case of the MEA, the 25 Hz was delivered synchronously across all electrodes. The remaining 4 rats (2 with macroelectrode and 2 with MEA) did not receive any stimulation and served as unstimulated controls. Both the macroelectrode and the MEA electrodes were insulated except at the tip.

C. *Immunohistochemistry and cell counting:*

Following stimulation, each rat was deeply anesthetized with a lethal dose of Euthasol (130 mg/kg), injected intraperitoneally, and then perfused intracardially with 0.9% NaCl, followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline at pH 7.2 (PBS) for 15 min at a rate of 20 ml per min. Brains were removed and cryoprotected in 30% sucrose at 4°C, and the region spanning the entire electrode sectioned in the horizontal plane at 50 µm thickness using a freezing microtome, collected in series of 4 in 4% paraformaldehyde PBS, and rinsed in PBS.

To identify the number and identity of cells activated by the electrical stimulation, we performed double immunofluorescence labeling for the immediate early gene, c-Fos, and the neuronal marker, NeuN. Free-floating sections were rinsed in PBS, blocked in 5% normal donkey serum (NDS) and 0.1% Triton-X for 30 min and rinsed in PBS. After rinses in PBS, sections were incubated overnight at 4°C in rabbit anti-cfos (1:5000; Calbiochem) and mouse anti NeuN (1:1000; Millipore) in PBS containing 1% NDS. Sections were rinsed in PBS and incubated in Alexa 594-conjugated donkey anti-rabbit (1:1000; Jackson Immunoresearch) and Alexa 488-conjugated donkey anti-mouse (1:1000; Jackson Immunoresearch) in 1% NDS for 1 hr. Additionally, all sections were counterstained by incubation with the nuclear dye DAPI (Molecular Probes Inc., Eugene, OR) that labels all cell nuclei. Sections were rinsed with PBS, then mounted on glass slides with Fluoromont-G mounting medium (SouthernBiotech) for fluorescence microscopy. For some sections, nuclear counterstaining was obtained by a short incubation of the sections in PBS containing bis-benzimide (Molecular Probes Inc., Eugene, OR) before mounting. For each double-label experiment, controls included omission of one or both primary antibodies. Sections were visualized using a Nikon eclipse E400 microscope equipped with 4 fluorescent cubes, a monochrome and color digital camera and Nikon BR software (Nikon Instruments Inc, Melville, NY). For each brain at least 2 series were stained and images corresponding to the tip of the electrodes were used for counting. For each section, c-Fos+/NeuN+ and c-Fos+/NeuN- cells surrounding the electrode track were counted using ImageJ and compared between the various stimulation treatments. Test for significance was performed by using standard Student's t-test.

III. RESULTS

1. *Number of neurons activated*

Cells expressing c-Fos were counted in all 12 animals and were classified as neuronal or glial depending on the presence or absence of the neuronal marker NeuN. Figure 1 shows representative sections near the tip of the electrodes from 3 different rats. Row 1 contains both NeuN (neuronal marker: pink) and c-Fos (activation marker: blue) staining while row 2 shows only c-Fos staining of the same tissue. Only a fraction of the NeuN+ cells are c-Fos+ indicating the

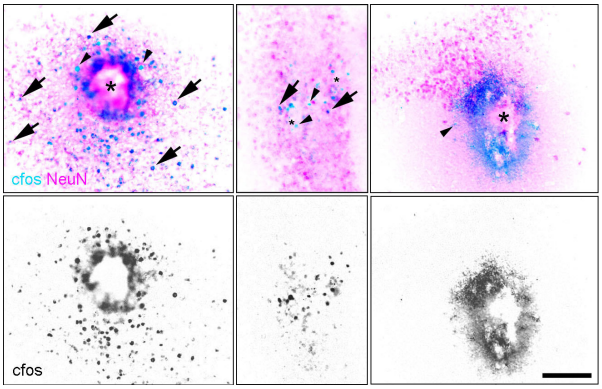

Figure 1.C-Fos and NeuN stained sections. Arrows: examples of c-Fos+/NeuN+ cells, arrowheads: examples of c-Fos+/NeuN- cells. Stars mark electrode track. Scale bar: 100 µm.

selective nature of stimulation. Cells with c-Fos+ and NeuN- indicate the presence of glial activity in response to implantation and stimulation. Autofluorescence from tissue damage was observed at the electrode tissue interface as described in [17, 18].

In all cases, only those electrodes that ended within the CA1 or the CA3 cell layers were taken into account for counting. In the macroelectrode implanted rats, electrodes ended in the cell layer in all six rats, whereas with the microelectrodes, we had a total of 21 electrodes that ended within the cell layer across the 6 microelectrode array-implanted rats, with atleast 3 in each rat. A neuron activated between any two microelectrodes was assigned to the closer electrode. Figure 2 shows the average number of cells that are c-Fos+/NeuN+ (active neurons) and c-Fos+/NeuN- (active glia) counted for each type of electrode stimulation. On an average, stimulation with the macroelectrodes activated 21.5 neurons on sections at the electrode tip (n = 4 macroelectrodes in 4 rats) whereas, a single microelectrode stimulation activated

5.8 neurons on sections at the electrode tip (n = 15 microelectrodes in 4 rats). The unstimulated controls on the other hand, had fewer than 0.5 neurons per section activated at the electrode tip (n = 2 macroelectrodes in 2 rats and n = 6 microelectrodes in 2 rats). Anatomically similar regions of the opposite hippocampus in both types of stimulated brains showed c-Fos expression comparable to the unstimulated case, confirming that the c-Fos expressed is indeed in response to stimulation and also showing that the effect of stimulation was contained within the stimulated hippocampus. Glial activation did not show significant differences between the four types of implantation, although the mean number of activated glia is higher in the stimulated rats compared to control rats (implanted but unstimulated).

Using the volume of a cylinder, $V = \pi r^2 h$, we know that the single microelectrode (r = 16.5 µm, h = 3.4 mm implanted depth) has a volume equal to 4.8% that of a single macroelectrode (r = 75 µm, h = 3.4 mm). The volume of

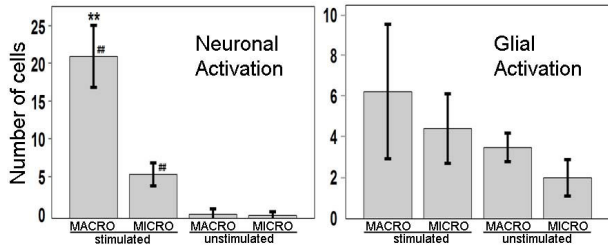

Figure 2. Neuronal and glial excitation caused by macro and micro electrode stimulation in the hippocampus. ** p<0.001 with respect to microstim.# p<0.01 with respect to control.

each electrode can be estimated as the volume of brain tissue damage caused by its implantation in the brain (the diameters of visible tissue damage with both the electrode types were comparable to their actual diameters (see Fig. 1) which justifies equating the two volumes).Thus, it would take ~21 microelectrodes to cause tissue damage comparable to a single macroelectrode. By extrapolating the results found in Fig. 2, it can be seen that the total number of neurons activated by 21 microelectrodes implanted in the CA1 or the CA3 cell layers would be more than 5 times the number activated by a single macroelectrode (both while causing the same amount of brain tissue damage).

2. *Distribution of neuronal activation.*

We used Scholl analysis to assess the distribution of neurons activated by each type of stimulation: Circles with radius in increments of 25 µm were drawn around the electrode tracks with the 1st circle starting at the edge of the visible hole caused by the implantation of the electrodes. The diameter of the 1st circle drawn matched well with the actual diameter of the electrode in every case. Cells with c-Fos+/NeuN+ and with c-Fos+/NeuN- were counted between adjacent circles (Fig.3). Counting was done until we encountered an annulus with no c-Fos+/NeuN+ cells.

Since the microelectrodes were 142 µm apart (edge to edge within a row), only two completely non-overlapping and a third 'slightly-overlapping' circle could be drawn around each of them. When circles overlapped, a line drawn midway between the consecutive microelectrodes was used to help assign c-Fos+/NeuN+ cells to the closest microelectrode (Fig 3B).Since >90% of activated neurons with the microelectrodes belonged within the 1st three 25 µm radius circles, this method of counting did not skew results.

As can be seen from Fig. 4, both the macroelectrode and the

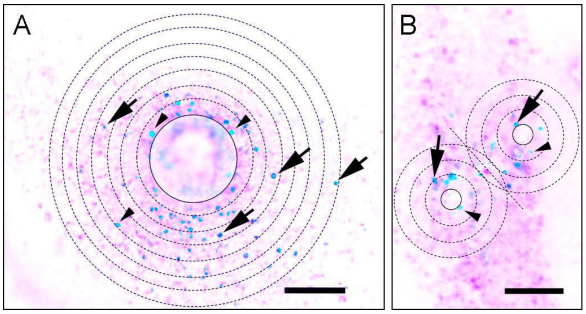

Figure 3. (A) Seven 25µm circles are drawn around a macroelectrode for counting. (B) Three 25µm circles are drawn around three microelectrodes for counting. Arrows: examples of c-Fos+/NeuN+ (purple dots), Arrowheads: examples of c-Fos+/NeuN- (blue dots). Scale bar: 100 µm.

microelectrode activate decreasing numbers of neurons with increasing distance from the electrode edge, with a single macroelectrode activating a significantly higher number of neurons than a single macroelectrode within each of the 25 µm circle. The farthest neuron activated by a single

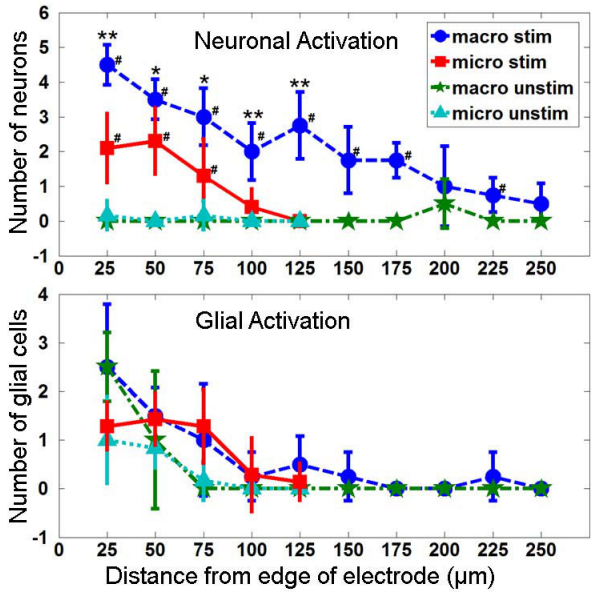

Figure 4. Distribution of activated neurons (c-Fos+/NeuN+) and glial cells (c-Fos+/NeuN-) in 25 µm radius increments around the electrodes.**p<0.001, *p<0.05 with respect to micro stim; #p<0.01 with respect to corresponding controls (unstim).

macroelectrode is within 250 µm from the edge of the electrode tip, whereas for microelectrodes, it is within 100

μm. It should be noted here that Fig. 4 shows neuronal excitation distribution caused by one microelectrode only (averaged across 15 microelectrodes in 4 animals that received stimulation). But using several microelectrodes (such as in a microelectrode array) spaced sufficiently close or far apart from each other as the need may be, the activation distribution may be changed. The glial activation distribution did not show significant differences between the four cases. Additional staining for specific glial types (microglia, astrocytes, etc.) would be necessary to further understand the response of the different types of glia to implantation and stimulation.

IV. DISCUSSION

Voltage-controlled ±1 V stimulation from a single macroelectrode (impedance 1.5kΩ at 1 kHz) activates more neurons within a larger radius than a single microelectrode (impedance 25 kΩ at 1 kHz). However, note that the single macroelectrode would also draw more current when compared to a single microelectrode because of its lower impedance. For this study we chose 25 Hz biphasic square pulses for stimulation. It should be noted that by altering the stimulation frequency, pulse width, or waveform, a different number and distribution of neurons maybe activated[19].

By quantifying the number and stimulation radius of the neurons that get activated by using macroelectrode and microelectrode stimulation in the hippocampus, we hope to gain a better understanding of the underlying mechanisms of action of DBS. Since microelectrode stimulation has a smaller activation radius, it could be used in cases where the stimulation target is small, avoiding neurons that lie outside the target zone, thus minimizing side effects of stimulation[20]. Several microelectrodes placed sufficiently close to each other may also be used to increase the number of neurons activated within a given target, while still causing lesser tissue damage than a single macroelectrode. For human DBS electrodes, which are typically 1.27 mm in diameter, this difference will be much greater than with the small (150 μm) DBS electrodes used here.

V. CONCLUSION

Through this study we have quantified the number of neurons and the activation radius with macroelectrode and microelectrode stimulation. Macroelectrodes have a larger activation radius and greater number of neurons activated when compared to a single microelectrode. However, several microelectrodes can be used to activate more neurons over larger stimulation areas while still causing less tissue damage when compared to a single macroelectrode.

ACKNOWLEDGMENT

We would like to acknowledge help provided by Temitope Adeyeye with tissue slicing and staining and Amit Parekh, Alexandria Harrison and Jonathan Joe with imaging and counting.

REFERENCES

[1] H. S. Mayberg, A. M. Lozano, V. Voon, H. E. McNeely, D. Seminowicz, C. Hamani, J. M. Schwalb, and S. H. Kennedy, "Deep brain stimulation for treatment-resistant depression," *Neuron,* vol. 45, pp. 651-60, Mar 3 2005.

[2] A. L. Benabid, G. Deuschl, A. E. Lang, K. E. Lyons, and A. R. Rezai, "Deep brain stimulation for Parkinson's disease," *Mov Disord,* vol. 21 Suppl 14, pp. S168-70, Jun 2006.

[3] J. K. Krauss, J. Yianni, T. J. Loher, and T. Z. Aziz, "Deep brain stimulation for dystonia," *J Clin Neurophysiol,* vol. 21, pp. 18-30, Jan-Feb 2004.

[4] A. L. Velasco, F. Velasco, M. Velasco, D. Trejo, G. Castro, and J. D. Carrillo-Ruiz, "Electrical stimulation of the hippocampal epileptic foci for seizure control: a double-blind, long-term follow-up study," *Epilepsia,* vol. 48, pp. 1895-903, Oct 2007.

[5] A. Machado, A. R. Rezai, B. H. Kopell, R. E. Gross, A. D. Sharan, and A. L. Benabid, "Deep brain stimulation for Parkinson's disease: surgical technique and perioperative management," *Mov Disord,* vol. 21 Suppl 14, pp. S247-58, Jun 2006.

[6] R. E. Gross, P. Krack, M. C. Rodriguez-Oroz, A. R. Rezai, and A. L. Benabid, "Electrophysiological mapping for the implantation of deep brain stimulators for Parkinson's disease and tremor," *Mov Disord,* vol. 21 Suppl 14, pp. S259-83, Jun 2006.

[7] C. R. Butson and C. C. McIntyre, "Differences among implanted pulse generator waveforms cause variations in the neural response to deep brain stimulation," *Clin Neurophysiol,* vol. 118, pp. 1889-94, Aug 2007.

[8] J. S. Perlmutter and J. W. Mink, "Deep brain stimulation," *Annu Rev Neurosci,* vol. 29, pp. 229-57, 2006.

[9] C. C. McIntyre, S. Mori, D. L. Sherman, N. V. Thakor, and J. L. Vitek, "Electric field and stimulating influence generated by deep brain stimulation of the subthalamic nucleus," *Clin Neurophysiol,* vol. 115, pp. 589-95, Mar 2004.

[10] C. C. McIntyre, W. M. Grill, D. L. Sherman, and N. V. Thakor, "Cellular effects of deep brain stimulation: model-based analysis of activation and inhibition," *J Neurophysiol,* vol. 91, pp. 1457-69, Apr 2004.

[11] C. R. Butson, S. E. Cooper, J. M. Henderson, and C. C. McIntyre, "Patient-specific analysis of the volume of tissue activated during deep brain stimulation," *Neuroimage,* vol. 34, pp. 661-70, Jan 15 2007.

[12] S. A. Desai, J. D. Rolston, L. Guo, and S. M. Potter, "Improving impedance of implantable microwire multi-electrode arrays by ultrasonic electroplating of durable platinum black," *Front Neuroengineering,* vol. 3, p. 5, 2010.

[13] J. D. Rolston, N. G. Laxpati, C. A. Gutekunst, S. M. Potter, and R. E. Gross, "Spontaneous and evoked high-frequency oscillations in the tetanus toxin model of epilepsy," *Epilepsia,* vol. 51, pp. 2289-96, Nov 2010.

[14] M. H. Histed, V. Bonin, and R. C. Reid, "Direct activation of sparse, distributed populations of cortical neurons by electrical microstimulation," *Neuron,* vol. 63, pp. 508-22, Aug 27 2009.

[15] T. Schulte, S. Brecht, T. Herdegen, M. Illert, H. M. Mehdorn, and W. Hamel, "Induction of immediate early gene expression by high-frequency stimulation of the subthalamic nucleus in rats," *Neuroscience,* vol. 138, pp. 1377-85, 2006.

[16] J. D. Rolston, R. E. Gross, and S. M. Potter, "A low-cost multielectrode system for data acquisition enabling real-time closed-loop processing with rapid recovery from stimulation artifacts," *Front Neuroeng,* vol. 2, p. 12, 2009.

[17] K. A. Potter, J. S. Simon, B. Velagapudi, and J. R. Capadona, "Reduction of autofluorescence at the microelectrode-cortical tissue interface improves antibody detection," *J Neurosci Methods,* vol. 203, pp. 96-105, Jan 15 2012.

[18] G. C. McConnell, H. D. Rees, A. I. Levey, C. A. Gutekunst, R. E. Gross, and R. V. Bellamkonda, "Implanted neural electrodes cause chronic, local inflammation that is correlated with local neurodegeneration," *J Neural Eng,* vol. 6, p. 056003, Oct 2009.

[19] D. A. Wagenaar, J. Pine, and S. M. Potter, "Effective parameters for stimulation of dissociated cultures using multi-electrode arrays," *Journal of Neuroscience Methods,* vol. 138, pp. 27-37, 2004.

[20] A. Beric, P. J. Kelly, A. Rezai, D. Sterio, A. Mogilner, M. Zonenshayn, and B. Kopell, "Complications of deep brain stimulation surgery," *Stereotact Funct Neurosurg,* vol. 77, pp. 73-8, 2001.