NEURONAL SIGNAL DESCRIPTION AFTER CHRONIC STAINLESS STEEL MICROELECTRODE ARRAY IMPLANTS IN MARMOSETS

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Abstract: A main challenge in the development of invasive brain machine interfaces is the designing of electrodes that provide reliable neural sampling throughout the life of an individual. A first step in accomplishing that involves the characterization of signal after chronic implant of different types of electrodes. In this study, stainless steel microelectrodes were implanted in 2 marmoset monkeys. The total number, the firing rate and amplitude of the recorded units in 4 periods throughout the experiment (1, 4, 14 and 27 weeks after implantation) was assessed and compared. There was a great increase in the first 4 weeks after implantation followed by a continuous decrease in the total number of recorded neurons. In addition, the firing rate of the remaining units and the peak-to-peak amplitude of the signal did not decline over time. Together, these findings indicate that stainless steel electrodes can be reliably used to record neuronal activity in marmosets for at least 27 weeks.

Keywords: stainless steel microelectrodes, chronic recordings, brain-machine interfaces, common marmosets

Introduction

Brain-machine interfaces (BMIs) are functional interfaces between the brain and other devices designed to restore motor function after injury or neurodegenerative diseases. These interfaces work through the acquisition and processing of brain activity. BMIs can use either non-invasive or invasive methods for acquiring the neural signal. Noninvasive techniques, such as electroencephalogram (EEG), are able to capture the neural signal outside the cranium and therefore can be readily used in any patient, without previous surgery. This signal reflects the sum of several neuron activity, having a relatively good temporal but a poor spatial resolution [1]. Invasive techniques, such as microelectrode implants, require surgical intervention but allow the recording of single units activity with higher spatial and temporal resolution [2]. A main challenge in the development of invasive BMIs is the designing of electrodes that provide reliable neural sampling for long periods, preferentially throughout the life of an individual. Previous studies have shown that usually after a few months the quality of the signal decreases considerably and eventually ceases completely [3]–[5]. Such decrease may be related to abiotic factors, such as electrode corrosion or oxidation [6], [7], or to the interaction of the brain tissue with the electrodes. It has been shown, for example, that there is an inflammatory response following implantation in the tissue surrounding the electrodes that progressively encapsulate them [5], [8], [9]. Different kinds of microelectrodes may interact with brain tissue in different ways, leading to differences in the quality of the signal recorded over extended periods. The full characterization of signal quality and reliability over time with different electrode designs and materials is therefore essential for the future development of long lasting microelectrodes. To date, most of the available data come from studies in rodents [6], [10], [11]. As non-human primates are phylogenetically closer to humans, the interaction between their brain tissue and microelectrode implants are more likely to resemble the ones that would probably occur in the human brain. In this study, the neuronal signal after stainless steel microelectrode arrays implantation in 2 marmosets (*Callithrix jacchus*) was assessed over 27 weeks.

Materials and Methods

Subjects – Two adult male marmoset monkeys were used in this study. All procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were previously approved by the Alberto Santos Dumont Association for Research Support Ethics Commitee for animal use.

Microelectrodes – In each animal, 2 microelectrode arrays with ~~30–~~27 channels each were symmetrically implanted, one in each brain hemisphere. These arrays were made of stainless steel microwires (diameter: 50 μm) separated from each other by 300 μm. They were designed to simultaneously record 6 brain areas: primary motor cortex (M1), putamen (PUT), primary somatosensory cortex (S1), ventrolateral thalamic nucleus (VL), ventroposterior lateral thalamic nucleus (VPL) and subthalamic nucleus (STN) (Fig. 1, Table 1).

Recording Procedures – The recording sessions started one week after the implantation. These sessions were made during 27 weeks and were performed while the animals were awake and freely moving in an open field (45 x 45 x 45 cm). Single-unit activity (spikes) was acquired by an OmniPlex D Neural Data Acquisition System (Plexon Inc., TX, USA). The signal was amplified, high-pass filtered with a cut-off frequency of 600 Hz and digitized at 16 bits and 40 kHz in 800 μs

epochs around samples that crossed a threshold determined by the experimenter. Waveforms with a signal-to-noise ratio greater than 2.5 were sorted online. Later, offline analysis (Offline Sorter v3.3.2, Plexon Inc., TX, USA) was performed to validate online sorting, according to two main criteria: stereotypy of waveform shapes (determined by a waveform template and principal component analysis) and inter-spike interval (<0.1% of inter-spike intervals smaller than 1ms).

Table 1: Coordinates (relative to interaural line) of the recording target areas.

Area	AP (mm)	ML(mm)	DV(mm)
M1	10.0	6.5	14.4
PUT	8.5	6.5	11.5
S1	8.0	5.2	15.6
VL	5.5	3.7	10.0
VPL	4.5	3.7	7.3
STN	5.5	3.7	7.3

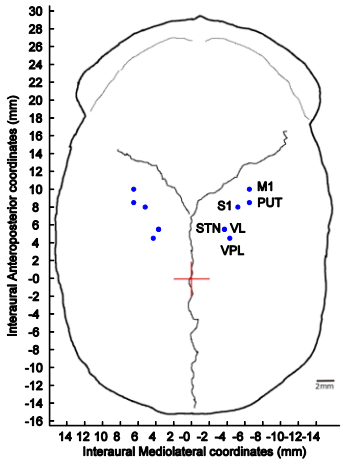
Signal Analysis – The total number, the mean firing rate and the peak-to-peak amplitude of units sorted in the recording sessions from the 1st, 4th, 14th and 27th weeks after microelectrode implantation were determined and compared. The mean firing rate (spikes/s) of each neuron was obtained by dividing the number of spikes by the total time of a recording session. Statistical analyses were performed for the brain areas in which at least 3 neurons were recorded in at least 3 of the analyzed periods. The peak-to-peak amplitude was determined by the difference between the highest and lowest values of the representative waveform of each sorted unit. All comparisons were performed by Friedman test. The significance criterion was set at $p<0.05$. Post hoc analysis with Wilcoxon signed-rank tests were conducted with a Bonferroni correction applied, resulting in a significance level set at $p<0.0083$.

Results

Units – There was an initial rise followed by a reduction in the number of recorded units (Fig.2). In both animals, the total number of neurons 4 weeks after implantation doubled (animal K, from 6 to 14 units) or tripled (animal D, from 15 to 48 units) compared to the first week. In the subsequent weeks, the number of neurons continuously decreased. By the end of the 27th week, there was a decrease of 92.8% (animal K) and 66.7% (animal D) in the total number of units compared to the 4th week (Fig. 2).

Firing Rate – Despite the changes in number of recorded units over time, there were no significant differences in the mean firing rate over time in each brain region analyzed: VL, STN and VPL for animal K (Fig. 3a) and M1, Put, S1, VL and STN for animal D (Fig. 3b). Fig. 3c shows the mean firing rate of the units over time in all recorded brain areas of animal D.

(a)



(b)

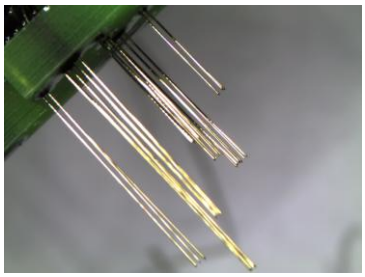


Figure 1: (a) Schematic of a marmoset cranium (top view) with the anteroposterior (AP) and medio-lateral (ML) coordinates of the targeted areas shown in blue. (b) Example of a stainless steel microelectrode array.

Mean Amplitude – The overall peak-to-peak amplitude of the neuronal signal was relatively stable across weeks. In animal K, the amplitude did not vary significantly over time (Fig. 4a). In animal D the mean amplitude was significantly lower in the first week, increasing after the 4th week after implantation [$\chi^2(3)=28.040$, $p<0.001$] and remaining relatively stable in the subsequent weeks (Fig. 4b). Fig. 4c depicts an example of the amplitude of an unit recorded in the M1 of animal D over weeks.

Discussion

In the present study, stainless steel microelectrodes were implanted in 2 marmoset monkeys. The neuronal activity was recorded during 27 weeks and the quality of the signal, expressed by the total number, the mean firing rate and the amplitude of recorded units over time, was determined in the 1st, 4th, 14th and 27th weeks after implantation.

There was an increase in the first 4 weeks after implantation followed by a continuous decrease in the number of recorded neurons. Neuronal signals, however, were still detected after 27 weeks of the surgery. This pattern has been previously described in studies using a variety of microelectrodes [6],[11], [12] and is thought to reflect inflammatory response to the

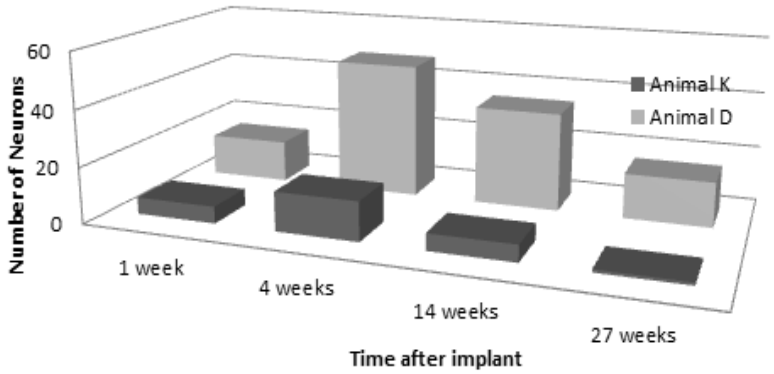


Figure 2: Number of recorded units in each animal (K and D) along weeks.

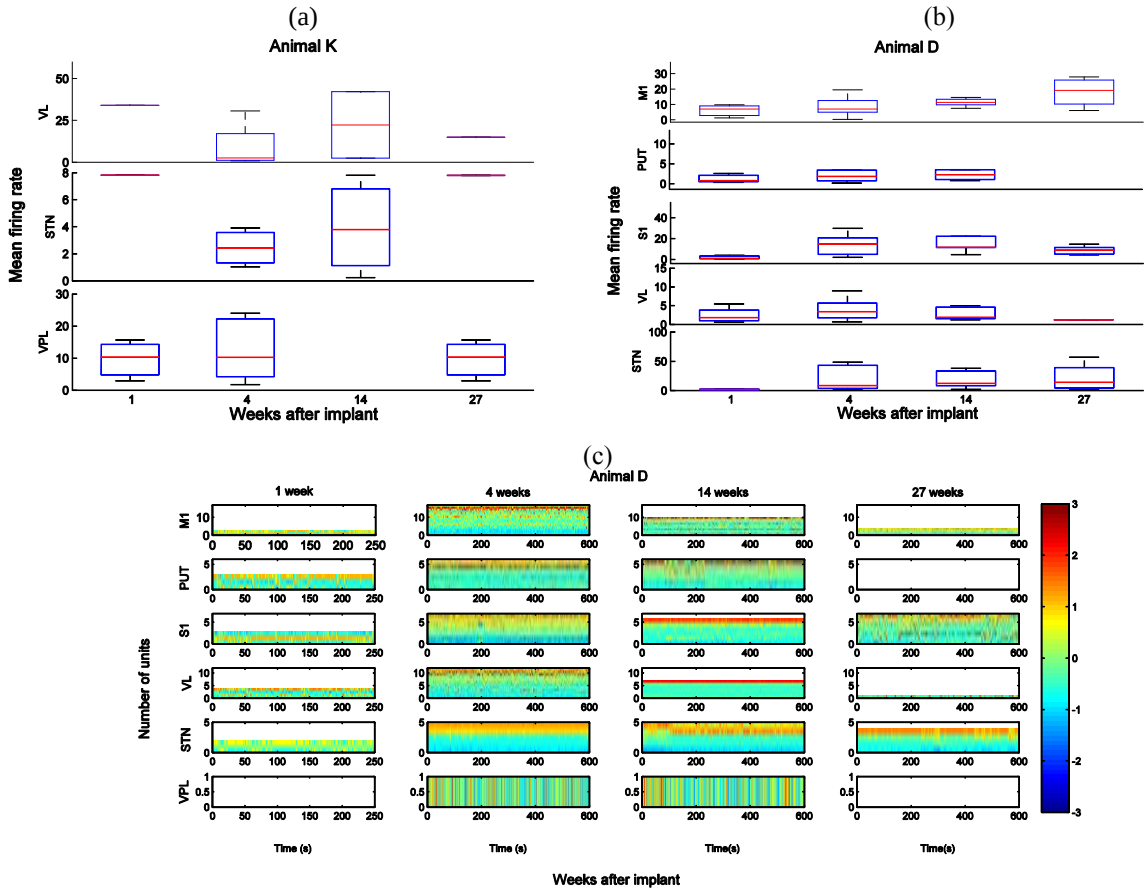


Figure 3. Neuronal firing rates of the units recorded throughout the experiment. Box plots of firing rates of all units of VL, STN and VPL of animal K (a) and all units of M1, PUT, S1, VL and STN of animal D (b). In (c) z-scored firing rates of each brain area during recording sessions in 1st, 4th, 14th and 27th weeks after implantation.

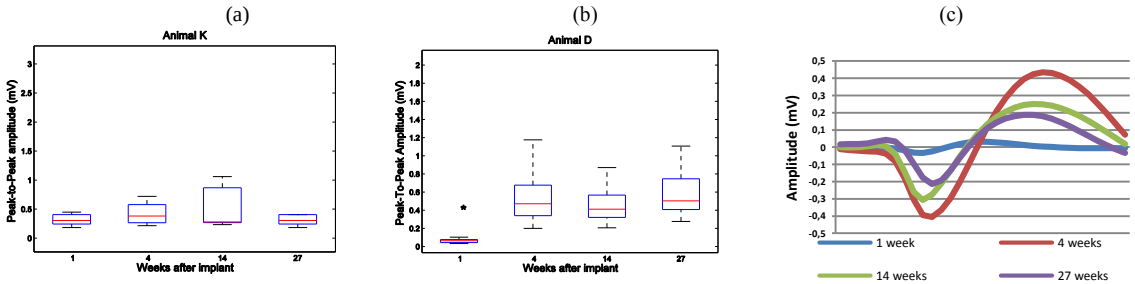


Figure 4: (a and b) Peak-to-Peak amplitude of units recorded in each animal over implantation time *($p<0.001$). (c) Sample waveform recorded from the same channel in M1 across weeks in animal D.

presence of electrodes in the brain [8], [13]. After the implantation, the defense cells of the nervous system (mainly microglia and astrocites) are locally activated and are progressively deposited around the electrodes, encapsulating them [14]. The decrease in number of recorded units may also reflect neuronal death, which can be induced by microglial activation [8]. Some studies, however, suggest that the inflammatory response of the brain tissue has no correlation with signal quality and that other factors, such as intraparenchimal bleeding or electrode deterioration may better account for decreases in it [10]. Future histological analyses of the brain tissue of the 2 monkeys used in the present study may shed some light into this discussion.

In contrast with number of recorded units, the firing rate of the remaining units did not vary across weeks. Similarly, the peak-to-peak amplitude did not decrease over time. The only significant variation was in the peak-to-peak amplitude of animal D: it was significantly lower in the first week post implantation. Together, these data suggest that the quality of the signal that could still be recorded throughout the experiment did not substantially decrease. Similar results were previously described for silicon [5] and platinum/Iridium arrays [6]. Another study, however, found that the mean firing rate of neurons recorded with tungsten electrodes decreased over time [11]. Such differences might be related to differences in microelectrode material~~, design~~ and manufacturing.

Altogether, the findings of the present study indicate that, despite decreases in number of units over time, chronically implanted stainless steel electrodes can be used to reliably record the neuronal signal in marmosets for at least 27 weeks. Further studies are necessary to determine the causes of the reduction in the number of neurons recorded and how differences in electrode manufacturing or in the surgery procedures may affect the quality of the signal recorded throughout longer periods.

Acknowledgments

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