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

A parametric study of intracortical microstimulation in behaving rats for the development of artificial sensory channels

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Abstract— **In the framework of developing new brain-machine interfaces, many valuable results have been obtained in understanding which features of neural activity can be used in controlling an external device. Somatosensory real-time feedback is crucial for motor planning and for executing "on-line" errors correction during the movement. In people with sensory motor disabilities cortical microstimulation can be used as sensory feedback to elicit an artificial sensation providing the brain with information about the external environment. Even if intracortical microstimulation (ICMS) is broadly used in several experiments, understanding the psychophysics of such artificial sensory channel is still an open issue.**

Here we present the results of a parametric study that aims to define which stimulation parameters are needed to create an artificial sensation. Behaving rats were trained to report by pressing a lever the presence of ICMS delivered through microwire electrodes chronically implanted in the barrel cortex. Psychometric curves obtained by varying pulse amplitude, pulse frequency and train duration, demonstrate that in freely moving animals the perception threshold of microstimulation increased with respect to previous studies with head-restrained rats.

I. Introduction

A crucial issue in developing brain machine interfaces (BMIs) is how to communicate with the brain through artificial sensory channels. This issue is important both for restoring bidirectional communication between the brain and the external world in paralyzed people and for providing sensory feedback (e.g., proprioception) to better control neuroprosthetic devices in motor impaired patients [1-7]. An important tool is represented by intracortical microstimulation (ICMS) that, when applied in the sensory cortex, is able to evoke artificial sensation and therefore is able to enhance or even substitute the natural sensory input. Microstimulation has been used in visual [8-12], auditory [13-16] and somatosensory regions [17-19]. This technique has also been used to explore functional connectivity, the mechanisms that underlie the synaptic transmission [20] and cortical plasticity [21, 22]. The effects produced by ICMS on perception have been successfully used in many experiments, for example to bias monkey's decision in a perceptual task [23] and to substitute a mechanical flutter stimulus in a discriminatory task [18]. The effect of microstimulation on behavior has also been studied in rats for modeling the perceived intensity of stimulation pulse trains with a leaky neural integrator [24].

Even if ICMS is widely studied, the majority of the experiments make use of different electrical stimulation parameters, which mostly depend upon the subjects involved and the features of the microelectrodes utilized. The purpose of this study was to systematically explore the relationship between the perception of ICMS and the electrical parameters defining the pulse train delivered to the barrel cortex of behaving rats, chronically implanted with one microwire array.

Here we present the results of a series of experiments in which we use an operant conditioning paradigm to instruct rats to report, by pressing a lever, the presence of ICMS patterns. We describe the relationship between the behavioral performances in the detection task and the electrical parameters characterizing the pulses of the microstimulation, such as current amplitude, frequency and train duration.

II. Methods

A. Surgery and experimental setup

Experiments were carried out on male Long-Evans rats weighing 350-400g in compliance with the Italian law regarding the care and the use of experimental animals (DL116/92) and approved by the Italian Ministry of Health. Animals were housed in plastic cages and kept in a temperature-controlled room with a 12:12h light/dark cycle. Before starting the behavioral training phase, a microwire array of 16 polyimide insulate tungsten electrodes (50µm wire diameter, Tucker-Davis Technologies, Gainesville, FL) was chronically implanted in the left hemisphere barrel cortex (S1BF). Rats were anaesthetized with a mixture of Zoletil (30mg/kg) and Xylazine (5mg/kg) and to place the microwire array a small craniotomy (2x4mm) was made in the parietal bone to expose the barrel cortex by using the stereotaxic coordinates and, without removing the dura mater, electrodes were inserted using an hydraulic microdrive (Kopf, 2650) at depth between 900 and 1200µm. The correct position of the electrodes was confirmed by observing the neuronal responses to manual deflection of the whiskers. After the recovery period, implanted rats were placed in an operant conditioning test chamber (Med. Associates Inc., 30x20x25cm) equipped with a response lever, a liquid dispenser with a drop receptacle, a house light and an audio speaker. The system is controlled via PCI-Card using ad-hoc software to design the entire experiment.

This work was supported by the BMI project of the Robotics, Brain and Cognitive Sciences Department, Istituto Italiano di Tecnologia, Italy.

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978-1-4577-1787-1/12/$26.00 ©2012 IEEE

During the experiment the microwire array was connected with a multichannel stimulator (STG 2008, Multichannel Systems) synchronized with the events occurring in the operant chamber via TTL triggers.

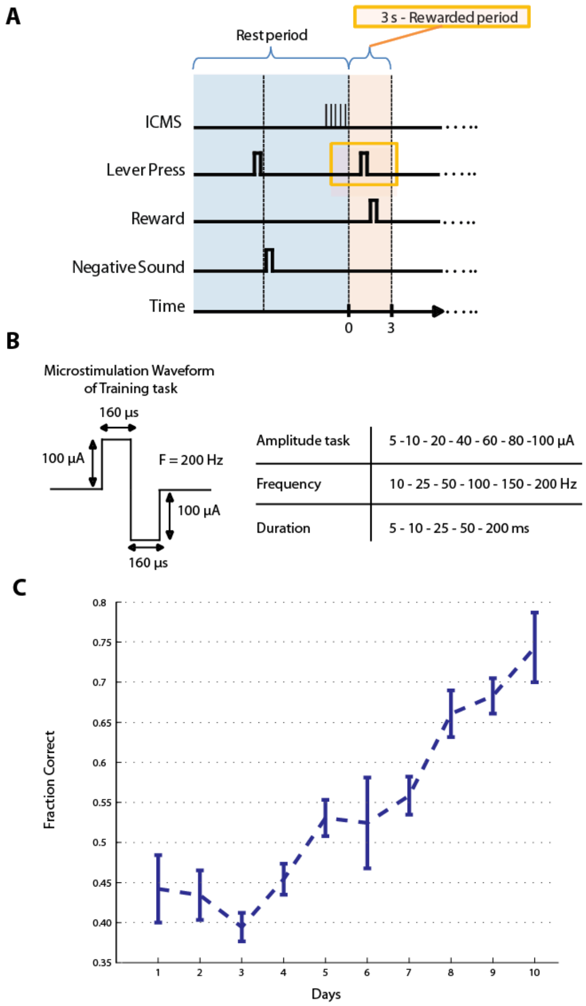

Fig.1. (A) *Experimental design*. Rats were trained to press a lever within 3s (rewarded period) after the stimulation in order to get reward. To start another trial, the lever had not to be pressed during a period randomly lasting between 4 and 12s (rest period), otherwise a negative sound was played and the rest period started over. (B) *Stimulation design.* The parameters used to design the stimulation pulse trains during the training phase (left) and a summary of the values of the stimulation parameters used during the testing phases (right). (C) *Mean Fraction Correct during training phase.* For each rat the training phase took a period of ten days to obtain a performance of 75% of correct trials.

B. *Behavioral task*

Operant conditioning paradigm was used to instruct the rats to press a lever every time an electrical stimulation was delivered through the microelectrodes chronically implanted in the barrel cortex. The behavioral task consisted in delivering an electrical stimulation followed by an interval of time during which, if the rat pressed the lever, a drop of water was released as reward. To do this we defined three time intervals for each trial (Fig. 1.A.): i) a *rest period*, randomly lasting from 4 to 12s, during which the rat has not to press the lever otherwise the time counter starts over, ii) a *rewarded period,* set to 3s, during which a lever press is rewarded with a drop of water, iii) a *drink period* lasting 12s during which the rat can drink after a correct press; an additional lever press during this time is not taken into account. A single trial starts with the rest period followed by the delivery of an electrical stimulation. After the stimulation press lever was rewarded only if occurring within 3s. If this does not occur, a new trial starts over with the rest period. During the experimental period, rats were water restricted with water available 3hours per day and weighted daily before each session.

The experimental design is constituted by the training and the testing phase. During the training phase a single electrical stimulation pulse train was used for all the sessions to instruct the rats to press the lever when stimulation occurred. As soon as the performances crossed the threshold of 75% rewarded presses, the rats started the testing phase in which we added catch trials where the stimulation parameters changed in terms of amplitude (A), frequency (F) or train duration (D).

C. *Electrical microstimulation and experimental design*

During the training phase we delivered a stimulation pulse train that rats are able to detect as demonstrated by previous experiment [2] constituted by 40 rectangular biphasic current pulses (cathodal first, 160µs pulse duration for each phase) delivered at 200Hz with a current intensity of 100µA (Fig. 1B). For each subject the stimulation was delivered through adjacent pairs of electrodes in a bipolar fashion identified according to their capability of evoking a twitched whisker movement.

The testing phase consisted of three behavioral tasks called *amplitude*, *frequency* and *duration tasks*. During each task we randomly varied the corresponding stimulation parameter, using the following ranges: A = [5, 10, 20, 40, 60, 80, 100]μA for current amplitude, F = [10, 25, 50, 100, 150 200]Hz for the frequency, and D = [5, 10, 25, 50, 200]ms for train duration. In all cases there were also control trials without any stimulation delivered that are indicated with the parameter set to 0.

Each task consisted of at least 5 experimental sessions (for each parameter tested) and accounted a number of stimulation trials ranging from 50 to 100. Each session ended after 20 minutes or after 20 correct trials.

D. *Behavioral data analysis*

To study the ability of the subjects to detect the presence of ICMS, the behavioral performances were evaluated for each session as the number of correct lever presses over the total number of first lever press after the stimulation (*Fraction Correct*). In this way we did not take into account the trials in which the rats did not perform any action.

III. RESULTS

A. *Training procedure for detection task*

The experiment presented here started with a training procedure during which the subjects were trained to report the presence of a pattern of ICMS by pressing a lever within a 3s rewarded period, starting after the end of the stimulation. Four rats were chronically implanted with a 16 channel micro-wires array and successfully ended the training procedure. Three of them were able to perform completely the amplitude, frequency and duration task. In Fig. 1.C. we show the improvement of the performances across days in the detection task during the training procedure for the four rats. During the training the electrical parameters were kept unchanged (40 pulses of 100μA delivered at 200Hz) and the number of delivered stimulations changed each day, according to the behavioral performances, from 200 to 330 stimulations per day.

B. Amplitude detection task

The behavioral data presented here were collected from three subjects able to carry out amplitude, frequency and duration detection tasks. During the amplitude detection task, subjects were asked to report the presence of 8 different stimulation patterns randomly delivered. Each stimulation train was set up by 40 pulses delivered at 200 Hz with different current amplitude. We explored the relationship between the behavioral performances and the amplitude of different stimulation patterns observing the mean value and standard error (SEM) of fraction correct (Fig. 2.A) calculated as the number of correct trials over all the trials in which the rats presses a lever after a stimulation. One-way ANOVA was run (Rat1 p<10-5; Rat2 p<10-7; Rat3 p<10-5) and Turkey-Kramer post-hoc test showed that mean performances with stimulation amplitude of 60, 80 and 100μA are significantly different from the chance level represented by the control stimulation pattern (data not shown).

C. Frequency detection task

After testing the ability of the rats to perceive ICMS patterns at different current amplitudes, we set the amplitude to 80μA, the train duration to 200ms and we varied the frequency of the pulses within the train (Fig. 2.B). In this case high performances were reached approximately after the 2^{nd} and the 3^{rd} stimulation frequency (between 20 and 50Hz) reaching a plateau of saturated performances of ≈0.8% .

D. Duration detection task

The last set of experimental sessions was designed to study how the perceived stimulation depends on pulse train duration, while keeping constant amplitude (80μA) and pulse frequency (200Hz). By changing the number of pulses of the stimulation train, we obtained trains lasting 5, 10, 25, 50 and 200ms. Rats were able of successfully reporting the presence ICMS for stimulation train duration of 10ms and they rapidly reached a plateau of ≈0.7 fraction correct for longer durations (Fig. 2.C).

IV. Discussion

Electrical stimulation was first used to identify the motor cortex by Fritsch and Hitzig in 1870. This technique has then been used in a broad type of experiments with several objectives, as understanding the synaptic transmission and plasticity or identifying the functionalities and boundaries of different brain regions. In the last decade, with the development of multi electrode array chronically implanted both to record the neural activity and to deliver electrical pulses in behaving animals, the electrical stimulation gained a new interest between researchers for the development of BMIs.

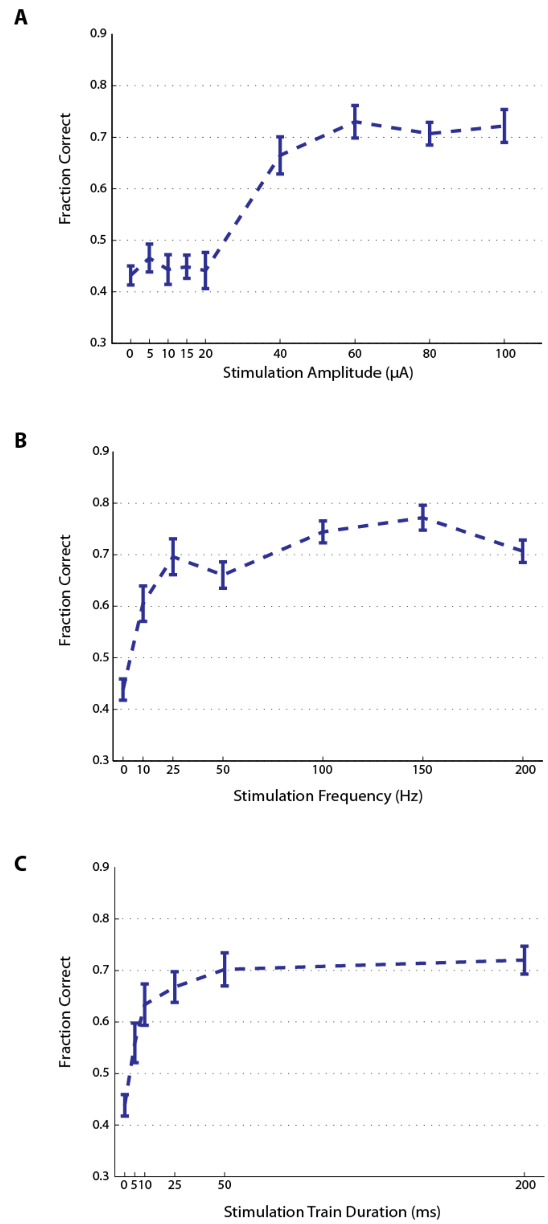

Fig. 2 Mean psychometric curve for three rats showing the relationship between the performances in detecting the stimulation patterns and (A) the stimulation amplitude, (B) the stimulation frequency and (C) the duration of the stimulation trains.

How ICMS affects the brain tissue, how populations of cortical neurons are activated by electrical stimuli and how the microstimulation generate or alter precepts are issues that have been studied by several scientists [2, 21, 22, 24, 25] but are still open issues. Butovas and Schwarz [24] explored the effects of repetitive stimuli on perception using head-restrained rats trained to lick within 0.5s after the stimulation. They changed three parameters (i.e.: stimulus intensity, number of pulses and frequency) designing a psychometric curve for each subject. Extending the work done by Butovas, here we report the results of an experiment in which we tested the capability of freely behaving rats of perceiving the presence of ICMS by pressing a lever within 3s after stimulation.

The goal of this experiment is to explore the effects of stimulation on perception in a more naturalistic environment: the rat is indeed free to move inside the cage without any motor or sensory external constrain. In this experimental condition there is an increase of the neuronal variability due to many factors: i) unlike Butovas' experiment, ICMS occurred while the barrel cortex was in the active state, characterized by high background activity, also due to whisker movements, ii) the surgery procedure to chronically implant a microwire array is a source *per se* of variability inter subjects, iii) the presence of external visual/auditory distracters and the freedom of movement inside the cage allowed rats exploring the environment and grooming during the experiment.

Even if it is difficult to compare results coming from different studies, in this work we report different psychophysical thresholds with respect to previous investigations in terms of pulse amplitude, pulse frequency and train duration. Butovas in his study, using over-trained and immobilized subjects, defined the perception threshold close to the minimal one. These values might reasonably increase in behaving animals, as shown in our work.

In the framework of using ICMS in BMIs as neuroprostheses, it is highly desirable to reduce the tissue damage due to the charge transfer during the stimulation. This can be achieved by looking for the electrical parameters that, at the same time, maximize the information transfer and minimize the charge transfer. Our results demonstrate that in a more naturalistic scenario rats are still able to detect the presence of ICMS delivered in the barrel cortex but the electrical parameters needed to evoke a sensation have to be set while taking into account the ongoing activity of the brain. This statement leads to other questions that have to be addressed: how to determine the internal state of the neural tissue hit by the microstimulation? Which is the relationship between the external electrical stimulation and the internal state in terms of perception? How to tune the parameters according to the ongoing activity?

More work is needed to understand how the electrical stimulation affects the perception before using this technique in developing bidirectional brain machine interface systems that make use of ICMS as sensory feedback. Our results demonstrate that the designed experiment provides new understanding of these open issues and could also be a basis to design new experiments involving multimodal stimulation (e.g., ICMS paired with auditory or visual stimuli) to better understand the mechanism that underlies the perception using such sensory channels.

V. Acknowledgments

We acknowledge Dr. Emma Maggiolini for useful discussion and for technical support during surgery procedures.

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