Roska Tamás Doctoral School of Sciences and Technology
Faculty of Information Technology and Bionics
Pázmány Péter Catholic University

Balázs Gábor Dombovári

IN VIVO VALIDATION AND SOFTWARE CONTROL OF ACTIVE INTRACORTICAL MICROELECTRODES

Theses of the Ph.D Dissertation

Scientific advisor:
István Ulbert

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1 Introduction

Neuroscience is a fascinating research field dealing with the complex nervous systems of animals and humans. During the last decades, remarkable progress has been achieved in explaining how the brain processes and stores information. Most of the neuroscientific researches can only be performed by using any technical devices, like brain imaging technique, which are offering both high spatial and temporal resolution. Nonetheless, recording the electrical activity of the brain with electrophysiological tools is still one of the most widely used methods to investigate the complex spatiotemporal activity patterns of neuronal circuits.

There is also a strong demand from the scientific point of view to record from as many locations from the brain as possible to better understand the large-scale machinery of neural networks, facilitate reproducibility and to characterize interindividual differences. To fulfill the experimental necessities of the mass neural recording demand, usually a limited number (16–32) of recording sites are implemented on a single silicon probe shaft and the probe is physically moved with respect to the brain tissue in order to explore gradually more and more brain areas. Of course, this approach poses the risk of probe breakage, especially in the case of rigid silicon carrier and, most important, tissue damage due to frictional forces and bleeding from the rupture of blood vessels. To overcome the fragility and tissue damage problem and establish large numbers of neuronal recording sites in a relatively wide brain area, we have developed electronic depth control (EDC) devices using MEMS or CMOS technology. These devices resemble a regular silicon probe in shape; however, instead of having only a limited number of recording sites, they have 188 recording contacts occupying a considerably larger proportion of the area of their 4-mm long shaft. Of the 188 possible contacts, eight sites can be electronically selected and routed out to an external amplifier to record neuronal activity simultaneously from the eight sites. Selected sites can be rapidly reconfigured, using an FPGA-based controller on a separate PCB, through the parallel port of a personal computer, allowing the experimenter to record from widespread brain areas without physically moving the device. As part of the system, graphical user interface (GUI), called NeuroSelect, software made it possible to visualize electrode selection and reselect different configurations according to the experimental situation.

The thesis presents the setup, experimental in-vivo studies and corresponding data analysis through the properties of cortical slow oscillation (SO), based on previous research results, successfully demonstrating the

concept of EDC.

Ketamine/xylazine anesthesia we used for the experiments is known to induce SO. The oscillation is roughly in the 1 Hz range; it is clearly composed of two states, the active "up-state" and the silent "down-state". The up-state local field potential (LFP) is positive on the brain surface and negative in deeper layers; it contains nested higher-frequency oscillations (e.g., 5 – 100 Hz) accompanied by cell firing. The down-state LFP is negative on the brain surface and inverts to positivity in the deeper layers, and contains no spectral or cellular firing activity.

The results show the potential of EDC devices to record good-quality LFPs, and single- and multiple-unit activities (SUA and MUA, respectively) in cortical regions during pharmacologically induced cortical SO in animal models. Furthermore, probe system development, including NeuroSelect software for real-time data acquisition and visualization is also an important part of the thesis.

2 Methods

Implantation procedures

Wistar rats (n = 5, weight of 250 - 350 g) were used for the experiments. All procedures were approved by the Animal Care Committee of the Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary. Initial anesthesia was administered through intramuscular injection of a mixture of 37.5 mg/ml ketamine and 5 mg/ml xylazine at 0.2 ml/100 g body weight; temperature was maintained at 37 °C throughout the 1 - 4-h-long recording sessions. The anesthesia was maintained with successive updates of the same drug combination of 0.2 ml/h. Animals were placed in a stereotaxic frame and craniotomy was performed over the trunk region of the primary somatosensory cortex (S1) anterior-posterior: (AP: -1.0 -4.0), medial-lateral: (ML: 1.0 + 4.0), with respect to the bregma.

The EDC probe was attached to a manual microdrive through its mounting PCB and it was slowly (0.1 mm/s) inserted in the S1 trunk region AP: -2.6 mm, ML: 2.5 mm with respect to the bregma driven by hand. The probes usually penetrated the dura and pia mater without bending, breaking and causing significant brain dimpling or visible bleeding. After recording from the trunk region of S1 for 1– 4 h, the probe was withdrawn and the animal was sacrificed.

Neural data recording procedures

Before the implantation, impedance measurements were carried out to confirm the integrity of the probe, using 250 nA, 1 kHz sine wave as testing signal injected into selected recording sites. The measurements were done in Ringer's lactate solution against an Ag/AgCl reference electrode. In all cases, the impedances were measured between 0.5 and 1 MOhm for the functioning electrode sites. The impedance measurements were also repeated while the probe was implanted in the brain, with roughly similar results. The outputs of EDC probe were fed into a high input impedance referential preamplifier (bandwidth of DC-100 kHz, gain = 10). Stainless-steel needle ground and reference electrodes were placed on the left and right side of the craniotomy. Wide bandwidth electrical activity (0.1 - 7000 Hz) with an overall gain of 1000 was recorded (with custom-made filter amplifier) at 20 kHz/channel sampling rate, on eight channels, with 16-bit precision and stored in hard drive. To extract the LFP, the wideband data were fur-

ther band-pass filtered at 0.1 - 500 Hz, 24 dB/oct, zero phase shift. To extract SUA and MUA, the raw data were further band-pass filtered at 500– 5000 Hz, 24 dB/oct, zero phase shift offline using NeuroScan Edit 4.3 software. The recording site selection was sent to the EDC probe through the FPGA-based controller using the NeuroSelect software.

3 Novel scientific results

Thesis group I.

Software control of electronic depth control silicon probes

1.1. I have enabled to the experimenters to control and effectively use the CMOS-based neural probes in neurophysiological experiments through an intuitive tool.

NeuroSelect is a tool for data acquisition system, signal processing and hardware controller of EDC probes. Using this tool, user can select up to 8 preferred electrodes, program the EDC probe, acquire and store the signal from selected electrodes.This tool enables the experimenter to visualize the recorded signals, the spikes, as well as the calculated metrics like the signal-to-noise ratio (SNR) value for each electrode and their relative ordering with respect to each other. NeuroSelect also manages the recording of signals through the data acquisition card, the storage of these signals into European data format (EDF) files, the execution of SNR calculation algorithms, and the steering of electronic circuitry to record from the electrodes when selected manually or semi-automatically.

The software is written in C++ and uses the multiplatform framework wxWidgets. It is developed for Windows, but can be ported to other platform environments. Visual Studio is used as main Integrated Development Environment. Design of the GUI was developed using the DialogBlocks editor. Signal analysis package is developed in C/C++ and uses the OpenMP library in the parallel-processing version. This allows to use all available processors (and cores) of the computer to process multiple signals in parallel. All comments in the whole source code are written using the Doxygen format which simplifies the generation of the documentation. Source code to control the acquisition hardware, i.e., the PCIe 6259 DAQ hardware from National Instruments and to visualize the neural data is generated using LabWindows/CVI from National Instruments. Development of NeuroSelect software is controlled using the version control software Subversion.

Publication related to thesis group I: [I]

Thesis group II.

In vivo electrophysiology with the electronic depth control silicon probe

2.1 I have shown that with the aid of the EDC probe, I was able to detect both states of the SO with high reliability using LFP, MUA/SUA and spectral measures.

LFP recordings were conducted with the EDC probe from the trunk region of the primary somatosensory area (n = 8 penetrations). The time series of SO was characterized by the rhythmic recurrence of positive and negative half-waves in the recorded LFP traces.

Down-states were negative in LFP recording close to the cortical surface and inverted into positivity in the deeper layers (Figure 1B). Multiple unit firing and higher-frequency oscillations were low in all layers of the cortex during down-states (Figure 1D). The frequency spectrum of the oscillation was calculated using Fast-Fourier transformation (FFT) algorithm. The LFP data were cut into 8192-ms-long segments and averaged in the frequency domain using cosine window smoothing. We found that the average peak frequency of the SO was usually in the 1 – 2 Hz range (Figure 1C).

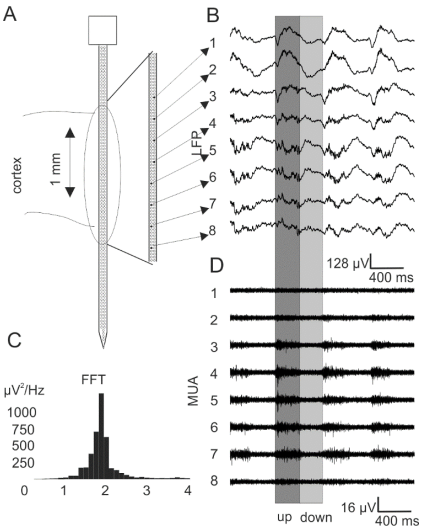

Figure 1: (A) Approximate recording position of the 4-mm long, active probe in the cortex. Close-up of eight roughly equidistant recording locations separated by approximately 300 micron. (B) Example LFP traces from the eight recording locations. Rhythmically recurring positive (dark gray) and negative (light gray) half-waves are highlighted. (C) FFT of the LFP spectrum. (D) Example MUA traces from the eight recording locations. The original raw data were bandpass filtered between 500 and 5000 Hz.

As the EDC probe was implanted under the surveillance of a surgical microscope, we were able to verify the depth of implantation by counting the recording contacts outside of the brain. To evaluate the spatial pattern of the LFP phase inversion, we recorded from eight roughly equidistant locations from the depth of the cortex spreading all layers, separated by approximately 300 micron (Figure 1A). We found a clear LFP phase inversion of the SO in all of our recordings. The phase inversion was usually located between 300 and 600 micron depth measured from the pia mater.

2.2 I have shown that the bimodality of the oscillation using MUA measures can be reliably characterized with the EDC probe, which is also in correspondence with the basic properties of SO and previous findings.

In addition, similar analysis techniques can be implemented on the EDC data as they were used on data obtained by the classic silicon probes. Besides LFP and MUA measures, another characterizing feature of SO is the spectral signature of cortical electrical activity indexed by an LFP spectrogram. Previous investigations showed large cortical oscillatory power in a wide frequency band (10 - 200 Hz) during up-states, while the spectral power was much smaller during down-states. Our findings are in a perfect match with these reports.

2.3 Consistent with prior studies in animals, I have shown with the aid of the EDC probe that the up-state was associated with increased firing and elevated spindle and gamma power during the surface-positive LFP half-wave, while the down-state was characterized by the widespread surface-negative LFP half-wave with decreased firing, and oscillatory activity.

Close to the surface, up-states were characterized by large positive deflections crowned by higher-frequency (spindle and gamma range) LFP oscillations (channels 1 – 3 in Figure 1B), while in the cortical depth, the up-states were negative, and the trough of the wave was also characterized by higher-frequency oscillations. Joint time-frequency analysis was performed on the recorded LFP data using wavelet-based methods. The spectral content of the oscillation was calculated from single sweep LFP waveforms followed by averaging of the resultant individual time-frequency measures. Dividing the wavelet amplitude values with that of a distant baseline (-1000 to -500 ms) in each frequency band gives the relative change of spectral activity in time expressed in dB.

We found that up-states were characterized by increased oscillatory activity mainly in the gamma range (30 – 80 Hz) in all of the layers, while in the down-state the spectral activity was decreased in all layers (Figure 2).

2.4 I have also shown that the EDC probe is capable of recording well-

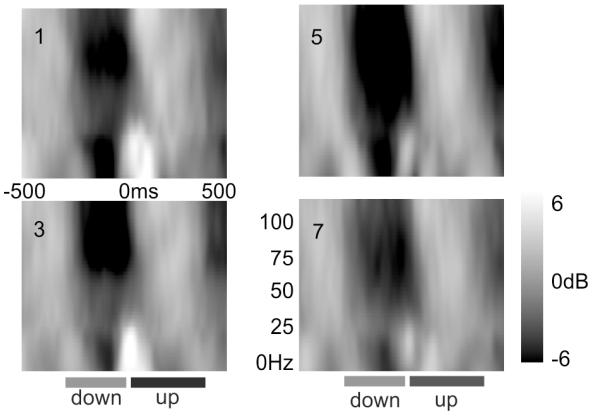

Figure 2: Average time-frequency maps of up-state locked epochs on selected recording channels 1, 3, 5, and 7 separated by approximately 600 micron. See close-up in Figure 1A for distribution of recording channels (1 – 7) along the probe shaft. Increased (light colors) oscillatory activity in the gamma range (30 – 80 Hz) during up-state and decreased (dark colors) spectral activity during down-state in all layers.

sortable single units, and these clustered cells show similar properties as similarly processed records of classic silicon probes.

Putative SUA was analyzed by filtering, threshold detection, and clustering methods using custom-made Matlab software. The wide-band signal was further digitally filtered (500–5000 Hz, zero phase shift, 24 dB/octave) to eliminate low-frequency contamination of the action potential (AP) data (Figure 3A). After threshold recognition at a given channel (mean ± 3 – 5 SD, each channel separately), two representative amplitude values (e.g. peak and trough) were assigned to each unclustered AP waveform. These duplets were projected into the two-dimensional space (Figure 3B) and a competitive expectation-maximization-based algorithm was used for cluster cutting (Figure 3C and D).

If the autocorrelogram (Figure 3E, F and G) of the resulting clusters contained APs within the 2-ms refractory interval, it was reclustered. If reclustering did not yield a clean refractory period, the AP was regarded as originated from multiple cells and omitted from the single cell analysis.

2.5 I have shown that reliable unit detection and search is possible with the EDC system by just switching between the recording channels rather than moving the device in the brain.

To test the temporal stability of the recording system, if a putative

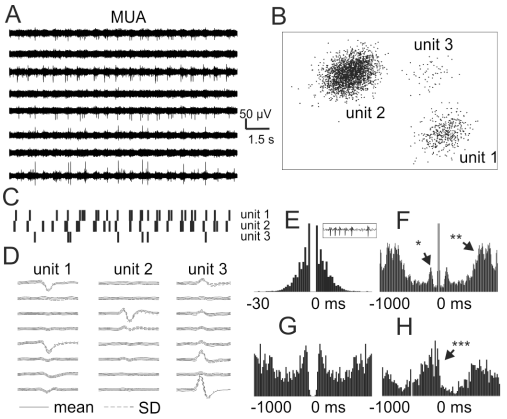

Figure 3: (A) Representative SUA traces. (B) Isolated clusters of three units from A. (C) Raster plots of the three isolated units in B. (D) Mean spike waveforms with SD of the three isolated units in B along the eight recording channels. (E) Autocorrelogram of unit 2 firing. Inset: burst firing of unit 2. (F) Autocorrelogram of unit 2 firing with longer time scale. (*) marks spindle modulation and (**) marks SO modulation of unit firing. (G) Autocorrelogram of unit 1 firing. (H) Cross-correlogram of unit 1 and unit 2 firing. (***) marks SO modulation in the cross-correlogram.

single unit was found at a given site, the probe was configured using the NeuroSelect software to record from a distant location. After usually 5 – 10 min, without moving the device, the probe was reconfigured to the location where the single unit was originally found. In all of the attempts (n = 5), we were able to find the same putative single unit, proving the reliability of the recording site switching software, hardware, and the stability of the probe within the cortex.

Publications related to thesis group II: [II, III]

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Conducting my Ph.D. thesis research within the European FP6 NeuroProbes and FP 7 NeuroSeeker project was a real pleasure that brought me the invaluable experience of working at the fascinating interface of electrophysiology and neuroscience. To investigate and implement control software for neural systems and apply them in in-vivo experiments with possible benefit for humans, although in the far future, was truly inspiring.

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