An optimized approach for long-term single neurons recordings in behaving monkeys

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Introduction and background

Electrophysiological recording of single neurons activity is a fundamental tool to investigate brain functions. However, due to the complexity of the equipment and the time-consuming nature of the methodology, single-unit recordings in behaving primates still present several problems. Three major technical difficulties have been addressed by our recent work:

A. The **pre-surgical selection** of the **target brain region** where to implant the recording chamber and the ***in vivo* localization of microelectrode penetrations**;

B. The **stability of the implant**, including the **head fixation system**, on the skull over a long period of time;

C. The **fast and accurate signal processing** and the **automatic on-line classification** of spikes. In this work we present our technological approaches to these methodological issues.

A. Pre-surgical selection of the target brain region

1. In order to define the target area for recordings and to place the recording chamber on the skull, monkeys were submitted to CT and MRI scans.

Then, a 3D reconstruction from CT images of the skull and the brain was performed by multi-dimensional volume visualization and analysis software (ETDIPS, free online).

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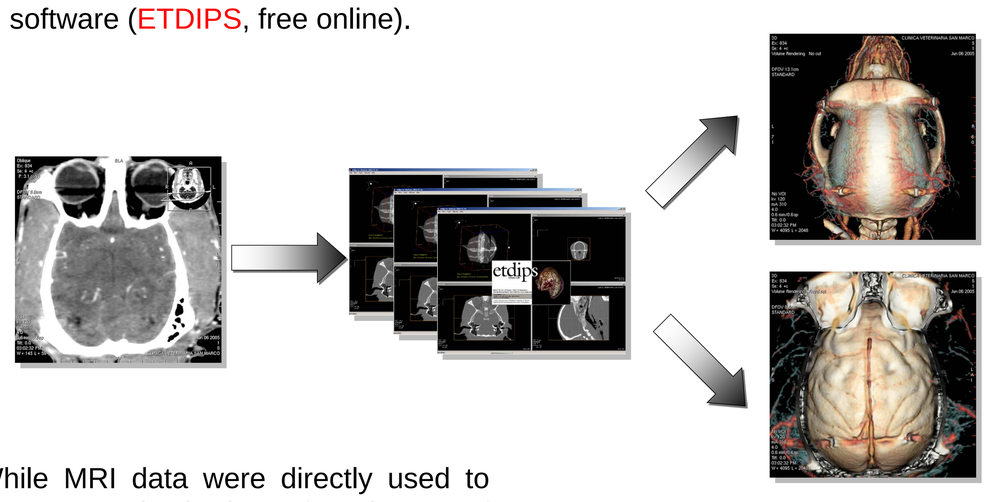
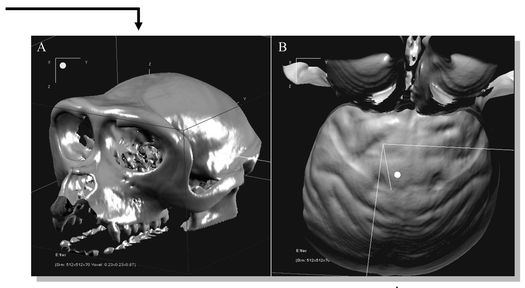
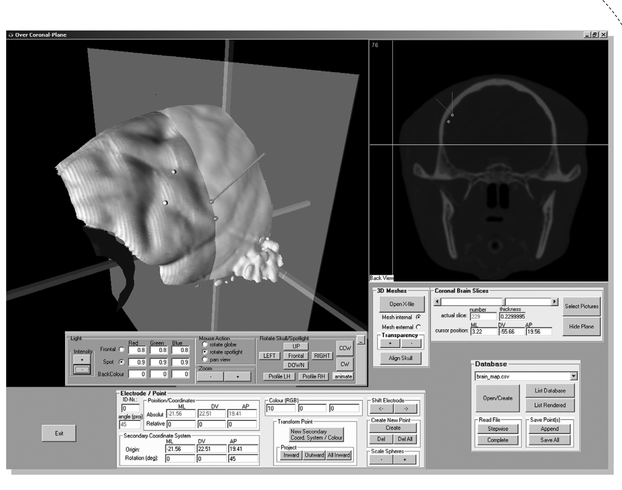


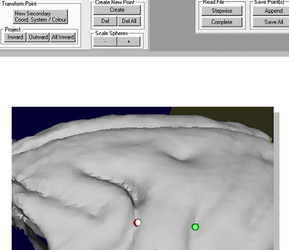
While MRI data were directly used to reconstruct the brain surface in one of the two monkeys, in another animal the cortical surface was indirectly rendered by using CT data. In this case, the inner skull surface **(fragment A)** was made visible from the outside by flipping the direction of the normal vectors by using a 3D modeling software (Rhinoceros 2.0). The inner surface of the skull, being a fairly precise cast of the brain surface, was thus used to localize the cortical gyri and sulci **(fragment B)**.



3. After these procedures, the monkey “virtual” head was aligned to the traditional stereotaxic coordinate system according to the orbito-meatal stereotaxic plane. Stereotaxic coordinates were thus used to determine the location of the cortical target areas by means of a specially designed software created in our lab (‘Virtax’).



After the surgical implantation of the recording chamber and of the head fixation system, ‘Virtax’ software was used during the experiment to plan the points and the appropriate angles of microelectrode penetrations.



B. The design and stability of the implant and the head fixation system on the skull

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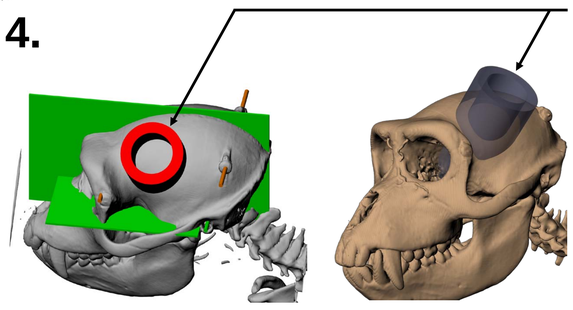
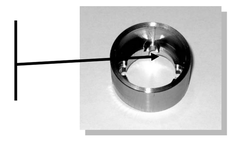
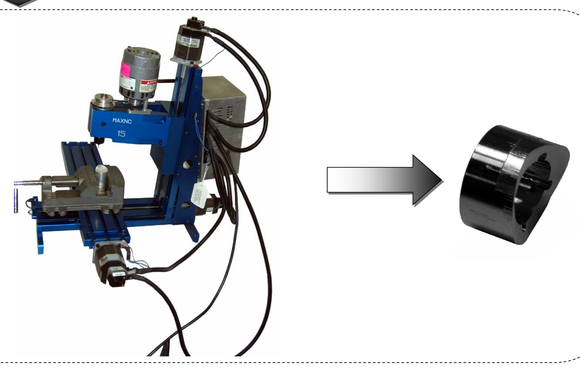


Our recording chambers were made of medical grade titanium and were perfectly fitting the skull surface curvature to maximize the contact surface. For this purpose, the part adjacent to the skull was cut out from the digital 3D mesh of outer skull surface by using the ‘Rhinoceros 2.0’ software.

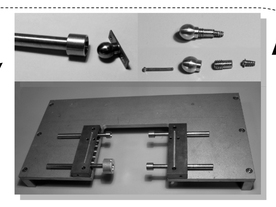
5. On the inferior border inside the chamber a small rim was left to bear four titanium screws used to fix the chamber on the skull.

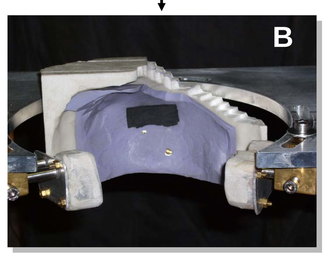


6. The complete virtual model of recording chamber was further converted by MillWizard software (© 2000 Delcam plc., Birmingham, UK) into G-code exploitable by computer-driven 3D milling machine MAXNC 15 (MAXNC inc., Arizona, USA).

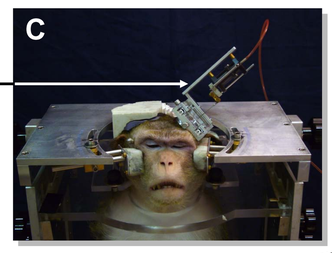


7. The head fixation system was the most critical part of our implant. After several attempts to use titanium head holders implanted on the skull **(fragment A)**, we decided to build a non-invasive head holder restraining the head by means of shell-like structure **(fragment B)**.



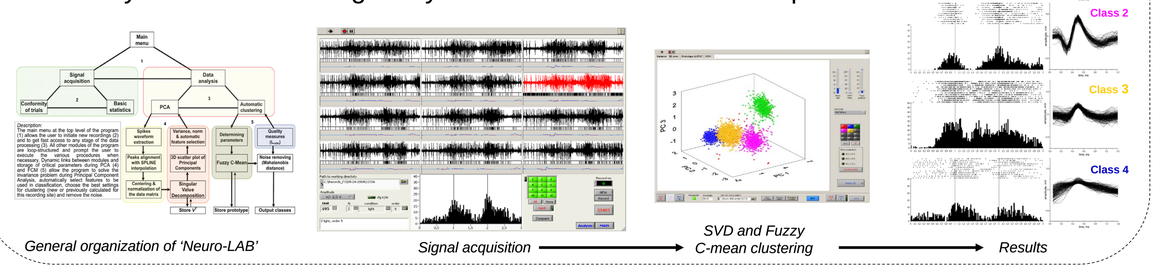


This system allows some small head movements, thus forcing us to directly mount the microelectrode holder to the chamber **(fragment C).**



C. The signal processing and the automatic on-line classification of spikes

8. As for signal acquisition and processing we have designed a new software, ‘Neuro-LAB’, performing fast and accurate single neurons isolation on the basis of Singular Value Decomposition of the data matrix containing spike shapes and following Fuzzy C-Mean clustering analysis in multidimensional PCs space.



The possibility to perform supervised classification after unsupervised clustering analysis together with automatic feature selection, determination the number of classes, the inclusion of new quantitative parameters of the cluster quality, allows on-line separation of units. This is the most innovative part of our procedure. All the program was entirely written in LabVIEW Express 7 (National Instruments, USA) and evaluated during polyspikes recordings from motor and premotor cortices of two Macaque monkeys.

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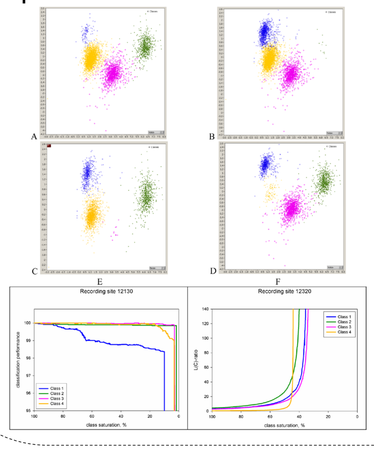

The comparative experimental evaluation of data sets classification by manipulating with clusters size, aiming at illustrating its performance and reliability. This technique was previously introduced in the context of PCA and performed well when one neuron at a time was removed, and a model was still determined on the remaining samples. Then the predicted class membership of the object left out was tested, but the proportion was still assigned to correct groups. Thus, to illustrate the stability of our FCM-classifier against the size of the cluster, we performed the following experiment on data that had already been correctly clustered. Beginning with first class we repeatedly removed at random one spike from the class, undergoing the resting PCs with classification procedure and recomputed validity index L_{ratio}. Fragments ***A*** to ***D*** show diminishing clusters 1 to 4, correspondingly, and display the last possible correct classification after which the classifier failed to associate membership values to existing data.

The level of errors in classification (false matching) for all clusters during mentioned tests is shown in fragment ***E***. Changes of validity index L_{ratio} being recalculated each time when selected cluster diminished in size is presented in fragment ***F***. Besides the other original meanings of L_{ratio} value as index of cluster quality, it may prove that starting from the moment of inflection on the graph, the FCM clustering algorithm in its standard application will never segment the space correctly due to unequal cluster sizes.

In contrast, in our application the classification is possible with minimal and acceptable level of false matching. The number of false matching spikes is not exceeding 2% even when the cluster is dramatically modified in terms of size (e.g. decreased more than 10 times), while L_{ratio} value starts failing as soon as the cluster size (saturation) reaches approximately 50% of the original number of spikes.

Conclusion

The presented methodological improvements allow a better planning and conducting of extracellular electrophysiology in primates, increasing the reliability of single-unit recordings and significantly reducing the time spent in the preparatory phases of the experiments.

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