Virtax: a new software-based stereotaxic tool for brain research

[1]Benno Gesierich, [1]Rosario Canto, [2]Mario Cipone and [1]Luciano Fadiga

[1]*Department of Biomedical Sciences and Advanced Therapies, Section of Human Physiology, University of Ferrara, 44100 Ferrara, Italy*

[2]*Department of Veterinary Clinical Sciences, School of Veterinary Medicine, University of Bologna, 40064 Ozzano Emilia (BO), Italy*

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Contact information:

Luciano Fadiga

Sezione di Fisiologia Umana

Università degli Studi di Ferrara

Via Fossato di Mortara, 17/19

44100 Ferrara

Italy

fdl@unife.it

Tel.: +39-0532-291338

SUMMARY

In neurophysiology, prior to intracranial recording, it is vital to identify the sites of interest inside the brain and to determine their stereotaxic coordinates in individual brains. This is necessary in order to match sites inside the brain with overlying sites on the skull surface (e.g. to implant devices on the skull) or to overlap functional data arising from neural recordings onto individual brains. Here we propose the use of a software tool, which can visualize anatomic 3D models of both the brain surface as well as the external skull surface (derived by both magnetic resonance scans, MRI, and computerized tomography scans, CT). The 3D models can be aligned in stereotaxic coordinates and correlations between them can be figured out. The tool is particularly useful in planning the location of implants (e.g. recording chamber, multielectrode arrays). Moreover it allows to track microelectrode penetrations and to assess the correlation of electrophysiological data with individual brain anatomy in the live subject.

INTRODUCTION

Traditionally, electrophysiological experiments require at their conclusion to precisely determine the anatomic location of the recording sites. The improvement of imaging techniques makes it possible to correlate recording sites with brain anatomy in an alternative way, without the otherwise unavoidable sacrifice of the animal. Imaging techniques, like magnetic resonance imaging (MRI) and computerized tomography (CT) have been primarily developed for diagnosis in human medicine but are now used also in veterinary clinic (Garland et al. 2002; Moissonnier et al. 2002). Consequently, MRI and CT scanners for veterinary use are becoming more and more available. Furthermore, recent technical improvements, like the elimination of image distortion due to scanner non-linearity or the processing of data by fast 3D-rendering algorithms, allow to enhance the quality of the 3D reconstructed organs. These developments make these techniques of more interest for experimental neurophysiology and brain anatomy (Frey et al. 2004).

The possibility of using modern brain imaging in traditional neurophysiology is particularly useful for at least three purposes. Firstly, it makes possible the precise positioning of a recording chamber in living subjects, on the basis of individual anatomy. Secondly, it may allow reconstructing the brain anatomy without the shrinkage, typically occurring during hystological processing. Thirdly, and more importantly, it may allow to precisely localize the recording sites during the experiments. This last point makes possible to adjust the experimental strategy ‘online’, in accordance with the current findings. A linked benefit is the reduction in the number of experimental subjects required, becoming feasible to redirect the exploration in the brain of the same subject on the basis of the results of the ‘online’ anatomical reconstruction.

The use of MRI and CT for localizing structures in the individual monkey's brain (Alvarez-Royo et al. 1991; Maciunas and Galloway 1989; Rebert et al. 1991;Sapolsky et al. 1990;Saunders et al. 1990) allows higher precision when compared to the use of traditional brain atlases of 'standard' brains (Paxinos et al. 2000; Snider and Lee 1961; Szabo and Cowan 1984). This technical improvement reduces the risk of failure the correct positioning of implants (e.g. recording chamber) on the skull. In addition, anatomical imaging techniques could reduce the time necessary to functionally map the exposed brain. They give the experimenter some initial data about the location of anatomical landmarks of the to-be explored brain, often correlating with its functional properties.

As far as we know, there is still the need for a specialized software tool, which facilitates the necessary virtual manipulations in an easy and automatic way. We describe here a software tool allowing the use of the wide ranging possibilities given by modern imaging techniques for brain research, particularly in non-human primates. Our software visualizes individual MRI/CT images and 3D reconstructions of bone/brain structures in parallel. It allows the simulation of electrode penetrations with different angles and targets, creates a database of the coordinates of the penetration sites, and visualizes them on the cortical surface or inside the brain to display neurophysiological functional maps on the live animal. Conversions between the standard stereotaxic space (Horsley and Clarke 1908) and other user-defined coordinate systems (i.e. that of 3D manipulanda used during recordings) is also possible with our system. The software tool 'Virtax' can be freely downloaded together with sample datasets (i.e. MRIs, 3D skull models) from the web site of our laboratory (http:/web.unife.it/progetti/neurolab).

METHODS

Software development

The software 'Virtax' was created using Microsoft DirectX 7 SDK for Visual Basic 6.0 (Microsoft Corporation). The software is compatible with Windows 98, ME, NT, 2000 or XP operating systems and requires DirectX 7 or higher.

Brain imaging and data preprocessing for use in 'Virtax'

MRI (Vet-MR, Esaote, Italy) and CT scans (Picker CT, Picker Inernational, Inc.) were obtained from two *Macaca fascicularis* monkeys. All experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Ferrara, by the Italian Ministry of Health and complied with the European laws on the use of laboratory animals.

MRI data (more frequently in DICOM format) is at first manipulated using MRIcro software (MRIcro, Version 1.39 build4, Chris Rorden, downloadable from www.sph.sc.edu/comd/rorden/mricro.html). In detail, voxels derived from scanning process are first converted in isotropic voxels and the acquired volumes are aligned according to the orbito-meatal stereotaxic space as originally defined by Horsley and Clarke (1908) on the basis of the anatomical landmarks shown by the images set (i.e. external auditory meata and lover margin of the orbits). The resulting coronal brain slices can then be converted by MRIcro into images of a standard graphic format (e.g.: JPG, BMP). This series of images can be opened in one step by 'Virtax' software.

To reconstruct 3D meshes of the inner and outer surface of the skull from CT images we used the public domain software ETDIPS (Mullick et al. 1998, downloadable from www.cc.nih.gov/cip/software/etdips/agreement.html). The 3D mesh derived by

ETDIPS describes both the inner and the outer surface of the skull (see Fig. 1A,B). To make the inner skull surface visible from the outside, the direction of the normal vectors was flipped over by a 3D modeling software (Rhinoceros 2.0, Robert McNeel & Associates, USA). The normal vectors define the front and rear of the single faces composing the mesh. Thus, flipping over the normal vectors of these faces makes the outer skull surface invisible and the inner surface visible. The original mesh and that with the flipped normal vectors, thus, represent the inner and outer skull surface respectively. As the inner surface of the skull is a quite precise cast of the brain surface (see Fig. 1B), it can be used to locate the gyri and even the sulci, as long as precise information about their depth is not crucial. Finally, the two skull surfaces were converted into the DirectX format, compatible with our software.

FIG. 1 APPROXIMATELY HERE

A sample dataset, including MRI and CT images and 3D meshes representing the skull's internal and external surface of one macaque monkey is downloadable from our web site (web.unife.it/progetti/neurolab) together with the executable version of the software. Sample files, including a database of coordinates of electrode penetrations during an electrophysiological experiment are also available. This database was used to create functional cortical maps with our software.

Electrophysiology Database

The sample database downloadable from our web site, was created in our laboratory during the functional characterization of monkey ventral premotor cortex (area F5, Matelli et al, 1985). The surgical procedure, the building of the recording chamber and the preparation of microelectrodes will be the subject of a future publication (Fadiga et al., in preparation). Briefly, in order to allow neuron recordings, head fixation system and recording chamber were implanted on the monkey's skull on the basis of individual bone anatomy as reconstructed by the 'Virtax' software. All implants were built from medical grade titanium by a computer driven milling procedure which produces implantable parts perfectly fitting the bone surface and thus not requiring the use of cement to fix them on the skull. This new implant prevents infections and tissue reactions typical of the acrylic cement-based implants and lasts much longer than the traditional ones (more than four years). The position of the implant on the skull was stereotaxically determined using individual brain imaging data and the 'Virtax' software we present here. During recordings, the behaving monkey was sitting in a restraining chair with its head held in a fixed position. Arms and legs were allowed to move freely. The recording electrode was moved to the desired location, according to the stereotaxic coordinates of the target region. Single neuron recordings were made by using tungsten microelectrodes, insulated by a polyamideimidic enamel (Altana Chemie, Wesel, Germany) with an impedance of 0.15–1.5MΩ (measured at 1 kHz). The electrode penetrated with an angle of 32-40° (with respect to the sagittal plane) in the ventral premotor cortex. It was pushed into place using a hydraulic microdrive (Kopf Instruments, CA, USA; step resolution, 10 μm). The recorded signal was amplified ×10,000 (BAK Electronics, Germantown MD, USA), filtered by a dual variable filter VBF-8 (KEMO Ltd., Backenham, UK)

(bandwidth 300-6000 Hz), digitized (PCI-6071E, National Instruments, USA) at a sampling rate of 10 kHz and stored for further off-line analysis. The acquisition and spike isolation program (Oleyinik et al., in preparation) was made in our laboratory by using the LabView 7 Express software (National Instruments, USA). In addition, the action potentials of single neurons were isolated from the electrical activity online, using a dual voltage-time window discriminator (BAK Electronics, Germantown MD, USA). The original electrical signal or alternatively the isolated action potentials were fed to an Audio monitor (Grass Instruments, USA) to give the experimenter an auditory feedback on the neuron discharge during testing. Criteria and functional characteristics described by Umiltà et al., (2001) were used to distinguish motor and premotor areas as well as regions within area F5 characterized by a high density of neurons exhibiting hand-related activity during goal-directed actions (Umilta et al. 2001). In addition, intracortical microstimulation (train duration, 50-100 ms; pulse duration, 0.2 ms; frequency, 330 Hz; current intensity, 3–40 μA) was undertaken at different depth in the cortex (i.e. each 500 μm intervals), to establish the motor threshold and the motor somatotopy of the recorded site. Current intensity was controlled by measuring the voltage drop across a 10 kΩ resistor in series with the stimulating electrode and by displaying it onto an HM 507 oscilloscope (HAMEG Instruments, Germany). Electrode entry points for penetrations in the cortex were arranged according to a 1 mm side grid.

RESULTS

Rendering 3D meshes of the brain and external skull surfaces

3D meshes representing the monkeys' brain and skull surfaces can be rendered in a 3D Viewport on the left side of the program's main screen (see Fig. 2). Before their

use in ‘Virtax’, the 3D meshes have to be converted into the Microsoft DirectX format. Two 3D meshes can be loaded by the software and alternatively visualized in the 3D Viewport. This possibility allows for a rapid switch between the brain surface and the outer surface of the skull during the visualization of the meshes.

FIG. 2 APPROXIMATELY HERE

Although the software usually works with the orbito-meatal stereotaxic system, as defined by Horsley and Clarke (1908) and based on anatomical landmarks on the skull (the external auditory meata and the inferior orbital rims), the 3D meshes can be aligned also on different skull landmarks. To this end, it is possible to interactively define and subsequently save each alignment data on a specific file. A specific window of the program guides this operation (see Fig. 3A). The user can zoom and rotate the 3D models in the 3D Viewport, in order to reach the best visualization of the region of interest. Furthermore, position and intensity of the virtual light sources in the 3D Viewport can be arranged to obtain an optimal visualization of the rendered surfaces.

FIG. 3 APPROXIMATELY HERE

Displaying coronal images of the brain

A series of coronal MRI/CT images (or even of stereotaxic atlas images, previously scanned and saved in jpeg or bmp image formats) can be uploaded by the program and visualized in the Picture box located on the right of the 3D Viewport (see Fig. 2). Images are selected through a specific window 'Brain Atlas' (Fig. 3B) where metric parameters, characterizing image position and pixel spacing, can be specified. Once determined by the user, these parameters are saved in a configuration file and automatically read by the software during the use of the images set.

A semitransparent plane crossing the mesh in the 3D Viewport defines the location of the brain slice actually displayed in the Picture box. The thickness of the semitransparent plane automatically matches that of the brain slice actually displayed. If no individual MRI of the subject's brain can be acquired, it is convenient to use a standard MRI atlas instead. MRI templates for macaque and baboon monkey have been created by Black, KJ et al. (2001a, 2001b, 2005), and are downloadable for free use within the neuroscience community (http://www.nil.wustl.edu/labs/kevin/ni/cyno/).

Determining the coordinates of brain sites of interest.

Sites of interest can be selected by left mouse clicking on the Picture Box (right) or on the 3D Viewport (left) by taking into account the sulcal and gyral patterns. The selected point is then marked in the 3D Viewport by a small sphere and on the images frame by a small point of the same color. A list of all points produced in this way can be visualized by opening a separate window 'Visualized Points' (see Fig. 4B), which indicates the identity numbers and the 3D coordinates of each point. If desired, selected points can then be stored in a database associated to the program (see below).

The possibility to use a secondary, user-defined, coordinate system may be important if the points are selected as targets for future electrode penetrations or tracer injections. In this case, very often, the electrode or cannula are inserted by a positioning instrument moving according to its own coordinates system. The software 'Virtax' allows the easy conversion of each selected point according to a user-defined coordinate system.

Database functions

The 'Virtax' software can work with an associated database to manage the various stereotaxically defined points. In addition to their coordinates in the used stereotaxic system, other properties, like identity number, color and additional user-defined coordinates can be stored and subsequently visualized for each point (Fig. 4 A,B) These points can be visualized in the 3D Viewport and in the Picture Box to show sites along microelectrode penetrations and to display the properties of some critical locations along the penetrations (e.g. particular neurons).

A list of the saved points can be visualized in a specific 'saved in…' window. A list of points can be created also by a program other than Virtax (e.g. Microsoft Excel, Wordpad, etc.).

Simulation of electrode penetrations

A red line simulating the recording/stimulating electrode or the injecting cannula (virtual electrode) can be visualized both, in the 3D Viewport and in the Picture Box (see left and right upper panels of Fig. 2). The position of the virtual electrode is automatically associated with the latest selected point. By interactively changing the inclination of the virtual electrode it is possible to test and determine the trajectory

most suitable to reach a target point inside the brain. The coordinates of the intersection point between the virtual electrode and the mesh displayed in the 3D Viewport can be calculated by the software 'Virtax'. This helps to determine the site on the skull where implants have to be placed in order to reach the point inside the brain located at the tip of the virtual electrode.

Visualizing functional brain maps

Starting from a set of penetrations, whose location and trajectory has been determined by an external micromanipulator (with respect to an arbitrary coordinate system), it is possible to define and visualize their trajectory in the virtual brain.

FIG. 4 APPROXIMATELY HERE

Points marking either the cortical entrance of various electrode penetrations or the position of specific neurons encountered during the penetration can be used to draw functional brain maps and to project them on the cortical surface (see Fig. 4A). To facilitate the creation of cortical maps, data concerning the microelectrode inclination can be stored in the database described above together with the other characteristics of the stored points. The intersection point of each penetration with the brain surface mesh is automatically calculated by 'Virtax'. Individual points can thus be displayed either in their actual position inside the brain, or projected on the surface of the brain surface mesh.

Software Testing

The Software 'Virtax' was tested in the different phases of an electrophysiology experiment done with two Macaque monkeys. During the planning phase, on the basis of the sulcal pattern, 'Virtax' was used to determine the putative location of the cortical areas F1, F4 and F5 (Matelli et al. 1985), targets of the study. Then Virtax was used to define the region of the skull to be opened and the location of the recording chamber. Spatial accuracy was tested by comparing landmarks on the reconstructed skull with landmarks of the real skull measured in a stereotaxic apparatus (Kopf Instruments, CA, USA). The errors in antero-posterior and medio-lateral dimensions were 1.75% and –0.76% respectively.

Cortical penetrations were performed in the different cortical areas exposed within the recording chamber. Motor, visual and somatosensory properties of the isolated neurons were neurologically tested (see Methods) and intracortical microstimulation was used to determine the motor excitability along the various penetrations. Neurons properties, evoked motor responses and differences in motor threshold were used to determine the borders between the various frontal areas (see Rizzolatti et al, 1988, Gentlucci et al., 1988). An example of a typical F5 motor neuron recorded during Virtax testing and discharging during precision grip is shown in Figure 5.

FIG. 5 APPROXIMATELY HERE

DISCUSSION

The software ‘Virtax’ was used to determine, in individual subjects and on the basis of their brain imaging data, the stereotaxic coordinates of cortical areas of interest and of the overlaying skull. The software has been successfully used to define the location on the skull of the recording chamber and to plan penetration sites for single cell recording and microstimulation. The physiological data about the location of functional cortical areas can be projected onto the cortical surface and referred to the individual sulcal pattern by using ‘Virtax’. Thus, our software was verified to correctly manipulate brain-imaging data allowing their exploitation for planning of electrophysiological experiments in primates as well as for mapping the results onto individual anatomy.

The determination of the site of the skull overlaying a particular brain site, which is the possible target for either neuron recording, or tracer deposition, or electrolytic lesions, is a critical step during experiments in primates. Exploiting the accuracy of individual brain imaging data, ‘Virtax’ allows the determination of the most relevant anatomical features of individual brains, the orientation of the head/brain dataset in either standard or user-defined coordinate system, and thus, provides a determinant aid in planning and conducting neurophysiological experiments in a simple and precise way. The risk of errors in the planning/analysis steps can be minimized by ‘Virtax’ by the presence of several automatic calculations, such as 3D coordinate conversion and electrode penetration angles used during the experiment. Moreover, through the correlation of recording sites with individual brain anatomy, the software allows the visualization of functional brain maps, as assessed by electrophysiology. This is particularly relevant in cases where anatomical studies are not possible or not desired, and makes possible the planning of subsequent experiments in different

cortical areas with a degree of confidence about their location that cannot be achieved on the basis of standard stereotaxic atlases. Finally, the use of the Virtax software allows to significantly reducing the number of animals used in electrophysiology, with the evident benefits arising from this opportunity.

GRANTS

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Figure Legends:

Fig. 1. (A): Anterolateral view of the 3D reconstructed skull of monkey MK1. (B): Internal surface of the reconstructed skull. Note the prints impressed on the bone by the main cortical sulci.

Fig. 2. Main Screen of the software 'Virtax'. The 3D Viewport on the left side is used to display 3D meshes of monkey brain and skull. The red line represents the virtual electrode. In the Picture box on the right side, the coronal brain slice corresponding to the position of the semitransparent yellow plane in the 3D Viewport is continuously upgraded and shown. The command buttons and text-fields are grouped thematically and allow the manipulation of the rendering mode of the 3D model, the selection of the coronal brain slice picture series, the selection of points of interest and the manipulation of the virtual electrode (see below).

Fig. 3. (A): Controls on the 'Align Skull' window allow to change the orientation of the 3D models to the orbito-meatal stereotaxic system. (B): The window 'Select MRI series' allows to select the series of coronal brain slice images to be displayed in the Picture box, as obtained from CT scans, MRI scans or from brain atlases. Metric parameters, characterizing image position and pixel spacing, can be here specified.

Fig. 4. (A): Overlap of a functional brain map onto individual brain anatomy. The small spheres represent the entrance point of several electrode penetrations. Each color indicates a specific functional property of the neurons found during the penetration: red, neurons responding during goal directed hand actions (ventral

premotor area F5); blue, neurons active during reaching movements (ventral premotor area F4); green, low motor threshold (4-10 μA) sites where microstimulation evokes single joint hand movements (primary motor cortex, F1). Penetrations in the bank of the arcuate or of central sulci are here represented by half-colored/half-gray spheres. (B): The list of points corresponding to the electrode entrance points is displayed together with the stereotaxic coordinates in the window 'Visualized Points'.

Fig. 5. Rasters and histogram of a typical F5 motor neuron acquired during a grasping task. The monkey had to open the door of a food-containing box by means of a small handle that could be grasped only by precision grip. After opening, the monkey could reach inside the box and grasp a rewarding piece of food. Rasters (upper part of the panel) correspond to 12 repetitions of the task, with black lines representing single spikes. The histogram was created by summing up the spikes from all trials found in each 20ms bin. The trials are aligned to the moment at which the monkey touched the handle of the door to open the food-containing box (red line). The second peak of the histogram corresponds to the grasping of the food inside the box.