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THREE-DIMENSIONAL COMPUTER MODEL OF BRAINSTEM RESPIRATORY NEURONAL CIRCUITS - APPLICATION FOR RESEARCH IN RESPIROLOGY

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A b s t r a c t

Methods that had been applied to study central neuronal circuits regulating cough and respiratory reflexes so far rely on recording performed in vivo, ex vivo, micro injecting and lesion methods. Based on the available data it is clear that this network is complicated, multilevel, holarchical, undergoing reconfiguration under afferent inputs. For many students and researchers it is complicated to get a virtual spatial image of these cooperating neuronal populations. The project was aimed to create graphical three-dimensional computer model of the brainstem using environment MATLAB and the matrix algebra to visualize neuron localization within the brainstem. Relevant data for the model had been taken from recent and also former research papers published in particular areas. This model may help scientists to visualize groups of neurons, help them to find targets for microinjecting or lesion studies together with stereotaxic positioning. The model is upgradeable and highly flexible for future use, research and teaching applications in MATLAB environment. MATLAB is a high-level language and interactive environment that enables you to perform computationally intensive tasks faster than with traditional programming languages.

Keywords: respiratory neurons; brainstem; model; MATLAB.

1. INTRODUCTION

Central neuronal circuits of brainstem respiratory neuronal network that generates breathing also significantly contribute to the up-regulation and/or down-regulation of respiration, cough and other respiratory and airway reflexes (1). Modulated reflex output may contribute to coughing in many patients with respiratory diseases and also diseases affecting other organs (GERD – gastroesophageal reflex disease) (2, 3). All methods that had been employed in study of respiratory neuronal circuits suggest that this network is anatomically as well as functionally complex, multilevel, multi-tasking, hierarchical and holarchical, undergoing reconfiguration and plasticity (4, 5, 6).

Respiratory network consists of different neuronal populations (7). They can be classified according to the phase of breathing cycle they are active in, according to the frequency changes of neuron discharge or the position of maximum discharge, synaptic connectivity, localization etc. (7). For many students and researchers it is very difficult just to get a virtual spatial either morphological or functional image of these cooperating neuronal populations.

It is assumed that the brainstem respiratory neuronal network has specific spatial and functional hierarchy and it has capabilities to generate respiratory rhythm at multiple levels (8). This attribute contributes to the adaptation of breathing pattern to different physiological and pathological situations (9). Respiratory neurons are organized in rostrocaudal sections and each of these sections controls specific aspects of respiratory rhythm and pattern formation (6).

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In medulla oblongata there are well defined populations of inspiratory neurons (dorsal respiratory group, intermediate ventral respiratory group), expiratory neurons (Bötzinger complex, caudal ventral respiratory group), and multiple types of neurons (pre-Bötzinger complex of ventral respiratory group, nucleus ambiguus, pontine respiratory group, reticular formation). Experimental sequential rostral to caudal transections through the pontine-medullary respiratory network within an in situ perfused rat brainstem – spinal cord preparation showed capabilities for rapid reconfiguration of the network (8). Thus, sequential reduction of the network leads to the reorganization and new rhytmogenic mechanisms are emerging (6, 9).

The aim of the present project was to create graphical three-dimensional computer model of the brainstem using computer environment MATLAB and the matrix algebra to visualize neuronal localization within the brainstem. The relevant data for the model had been taken from research papers published in the area of neurogenesis of breathing, coughing and other respiratory reflexes.

2. MODEL DEVELOPMENT

Data on neurons and their exact location were taken from stereotaxic atlas and number of relevant scientific papers published in the field of neurophysiology and experimental respirology (10, 11, 12, 13, 14, 15, 16) and these data were processed into tabular form. Recorded neurons were plotted on the coordinates of their location in the brainstem model created by isosurface in the computer environment MATLAB.

2.1 GAINING INFORMATION

Extensive database of experimental data on the location and distribution of respiratory neurons is based mainly on experiments performed on cats. Therefore, the basic graphic input was cytoarchitectonic atlas of a cat brainstem with stereotaxic coordinates provided by Berman (17). Three dimensional model of the brainstem was based on a set of 29 images of the cat brainstem transversal sections.

2.2 PRE-PROCESSING IMAGE DATA AND REDUCTION OF REDUNDANCY

The original RGB images are presented by red (R), green (G) and blue (B) chromaticities divided into separate bands. Useful information in an RGB image is the luminance level of the individual colour zones. To create an isosurface model it is not necessary to perform operations with truecolour or RGB images respectively. Therefore in this case it was possible to convert original images to greyscale images with 256 shades of gray without affecting overall model quality. The original scanned images were converted into greyscale format with 8 bit per pixel using following original MATLAB commands (1), (2), (3):

A=imread(*original_image*); (1)

I=rgb2gray(*A*); (2)

I=imresize(*I*,[256 256]); (3)

MATLAB function „*rgb2gray*" converts RGB format to intensity greyscale format by using weighted summation S_w of R, G and B colour bands (4):

$$S_w = w_1 \times R + w_2 \times G + w_3 \times B; \quad (4)$$

Where w_1, w_2, w_3 are weighting coefficients with values: $w_1 = 0.2989$; $w_2 = 0.5870$;

$w_3 = 0.1140$. Weighting coefficients come from luminance-brightness component Y in the standard YIQ colour model and YIQ model coefficients are calculated on the basis of relative sensitivity of human eye to different colours - red, blue, green (18).

The images were scaled to 256×256 pixels and their colour range was optimized to colour map containing 256 shades of gray to simplify computing of isosurface. Afterwards, to enhance the local contrast of greyscale images, we applied the CLAHE method - contrast-limited adaptive histogram equalization. MATLAB function *„adapthisteq“* with default parameter settings (applying uniform distribution parameter with flat histogram and default number of tiles 8×8) brought satisfying results. The series of 29 pre-processed images defined three-dimensional numeric matrix of image data and to fill in this matrix we used *„imread* function (8) and *for*-loop with control variable k = 1:1:29 so the final volume is contained within an 256×256×29 array.

$$rez(:,:,k) = \mathrm{imread}(I); \qquad (8)$$

Three-dimensional matrix represents volumetric data and it is essential for extracting isosurface data. Before extracting isosurface and to accelerate computing we reduced the number of elements in the volume by retaining every 4th element in the x-direction and every 4th element in the y-direction. Afterwards we smoothed the reduced data by function *„smooth3“* with Gaussian filter. We set the size of convolution kernel to 11×11×11 and standard deviation to 20. Subsequently, the smoothed data were passed directly to the isosurface and patch commands.

2.3 ISOSURFACE

In medical applications, isosurfaces are mostly used to visualize three-dimensional volume data gathered from medical-imaging devices such as CT, MRI and SPECT scanners to visualize internal organs, bones, or other structures (19, 21, 22). In other words, generating polygons to approximate surfaces of organs from 3D volume data is used by clinicians to visualize, manipulate and measure three-dimensional internal structures of patients (21). High speed isosurface rendering algorithms are also part of modern real-time applications helping by virtual surgeries examination of vascular systems and medical training (19, 23).

Because we designed our project in MATLAB, we applied the build-in *„isosurface“* function with its own algorithm for reconstructing the brainstem model and the isosurface was computed from volume data at the isovalue 250. The value was chosen based on the image data histograms for optimal displaying of model. The output structure of the function contains faces, edges and vertices of the isosurface which are necessary for defining the patch command. MATLAB function *„patch“* is used to create filled polygons so the *„patch graphic object“* is composed of one or more polygons. Again, to shorten the computing time we reduced the number of faces to only 10% of the original value without affecting the overall shape of the original object.

MATLAB Guide built-in tool was needed to design easy graphical user interface which allows interacting with the program without text commands. It contains buttons for visualizing the model or individual slides and transections, setting the transparency, zoom and adding data on neurons localization (Fig. 1).

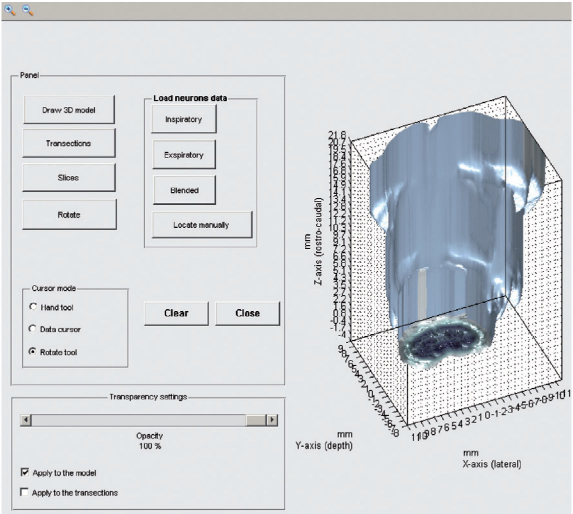

Figure 1: Graphical user interface with visualised 3D model of brainstem. It contains functional buttons for easy and comfortable operations such as drawing of 3D model, rotating the model, zooming, setting transparency, loading database etc.

2.4 STEREOTAXIC COORDINATES

It was necessary to summarize available information on populations of respiratory neurons from numerous studies and measurements and subsequently to select an appropriate uniform system of entry and positioning of individual types of neurons into a table complying with the accurate stereotaxic coordinates (Fig. 2). Therefore, the arbitrary point of the coordinate system is determined by the point where the line of spinal cord midline meets the fourth ventricle bottom and obex.

For example, if we consider neurons in an area bounded by three vectors *x*, *y* and *z*, the volume of this area in our model is approximated by the volume of an irregular prism. If we take into account that the selected area consists also of some extracellular fluid and non-respiratory cells, we can estimate that there can be found about 20% respiratory neurons [24, 25].

A

x1 [mm]	x2 [mm]	y1 [mm]	y2 [mm]	z1 [mm]	z2 [mm]	Number of neurons	Type
3.00	4.50	-2.50	-1.00	3.00	5.50	28	E-AUG
3.00	4.50	-2.50	-1.00	3.00	5.50	21	E-DEC
3.00	4.50	-2.50	-1.00	3.00	5.50	6	E-IE
3.00	4.50	-2.50	-1.00	3.00	5.50	10	EN
3.00	5.00	-1.50	-0.50	-4.00	-2.00	35	E-AUG

B

Figure 2: Neuron localization data (A) Neuron locations database is designed as Microsoft Excel table, so any additional data can be added easily. User defines the coordinates of the area, number of neurons and their type. (B) It is also possible to locate neurons manually and display any desired localization by using an input dialogue window from the graphical user interface.

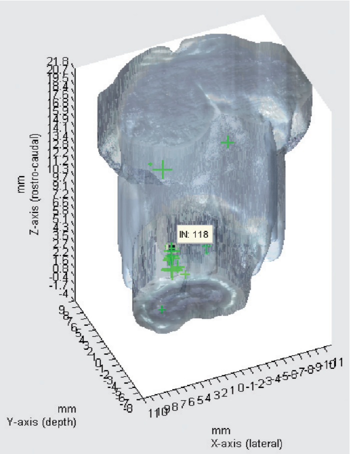

Figure 3: Some of the inspiratory related populations. When clicking on the marker it is possible to see the type and size of population as defined in the database. All data on localisation from inspiratory-related neurons database are marked with a green cross.

When user does not specify the count of registered neurons, our program calculates with the average respiratory neuron diameter of 35.9 μm what determines the size of population displayed [26]. The populations are plotted by function „scatter3" in different colours, marker shape and marker size in respect of the type and count of neurons recorded (Fig. 3).

Tab.1: Marker types used for displaying neuron populations. Primary, there are four types of markers used for visualising neurons population localisation which differ in the shape and the colour as well.

Type of population	Marker
Inspirium related neurons	+
Expirium related neurons	○
Blended types	▽
Manually localized	*

User localises neurons by three coordinates in millimetres considering the distance from the point where the line of spinal cord midline meets the fourth ventricle bottom and obex (Fig. 4).

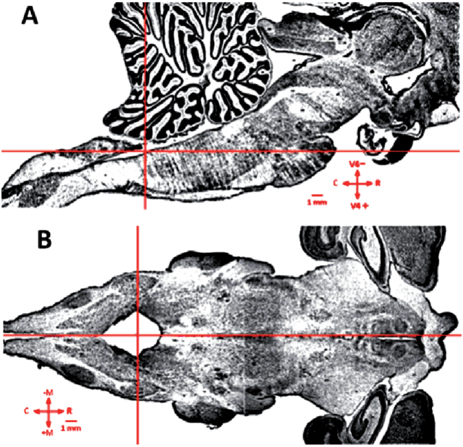

Figure 4: Defining the arbitrary point. (A) Where the obex line meets the bottom of the fourth ventricle and concurrently (B) Where the obex line meets the line of spinal cord midline. C caudal direction, R rostral direction, V4+ below the level of fourth ventricle bottom, V4- above the level of fourth ventricle bottom, M+ to the right of the midline, M- to the left of the midline.

The isosurface model is extracted from numeric matrix with size 256×256×29. Therefore it was necessary to establish a conversion calculation from stereotaxic localisation into matrix element localisation. We considered the proportions from the brainstem atlas – in the lateral direction the width of the interval is 23 millimetres and level of the spinal cord midline is in the distance of 11.5 mm from the beginning of this interval. The depth interval is 21 mm wide and the level of the fourth ventricle bottom is at 15.59 mm from the beginning of this interval. In the rostro-caudal direction the interval width is 25.8 mm and the level of obex is at 5 mm from the beginning of this rostro-caudal interval. The program converts the coordinates given in millimetres to the matrix elements (256×256×29).

Our program allows displaying 3D model and orthogonal transections through volumetric data together in one plot. For displaying the transections we used the MATLAB function *„slice."* For defining the input variables we created an input dialogue where user defines the required slices along the *X, Y* or *Z* axis direction.

3. DISCUSSION

General idea of this work was to create a computer model of brainstem allowing for display localizations of respiratory related neuronal populations. This model is improvable and adjustable for visualization of features of interest. The model had been developed in close cooperation of respiratory researchers and biomedical engineering experts. It represents an integral fusion of knowledge of respiratory neuronal network and a need to visualize its components with the ability to create the model applicable for teaching purposes and research in neuroscience. This work has no ambition to replace neurophysiology animal experiments. It is based on primary data provided by numerous experimental trials (10, 11, 12, 13, 14, 15, 16). The model is capable to summarize data, integrate data from separate sources, point out certain aspects of the data set and provide different level of results simplicity. Three-dimensional brainstem model is useful for students, graduates, and researches in the field of neuroscience, for all those that are dealing with spatio-temporal and functional complexity of neuronal circuits.

The greatest advantage of this model is that it can be easily modified and additional data can be uploaded to the tables. The table database allows simple orientation in registered neuron populations and it can be expanded by adding new data anytime.

New information about neurons, their location and count, their properties including their size, synaptic connections and receptor characteristics, activation and/or inhibition of neurons and their involvement in particular neuronal population or behaviour (e.g. respiratory, cough or sneeze related etc.) can be provided by the database of our model.

Every previously published „database" including atlas or map form (17), tables or drawings, (25) or simply a description of neuronal populations or circuits (27) provided static information of given aspect of the network. Our database can be adjusted and transformed to follow user requirements, e.g. to show additional information about cardiovascular related neuronal populations, trigeminal or some particular afferents target neurons, or provide information related to cellular properties of neurons if this is introduced in the model.

The approach employed in this study could be used to prepare such model of brainstem or possibly other parts of brain and the 3D map of it for other species e.g for guinea pig, or even humans. Guinea pig is nowadays extensively and successfully used in neuroscience (28). Any laboratory animal with stereotaxic coordinates and available data for neuronal localization can be processed that way.

This model may help scientists investigating respiratory neuronal functions to visualize particular groups, neurons and neuronal connections among them, including the targets of microinjections, recordings, particular mediator activation etc. The model is upgradeable and flexible for future use, research and teaching applications in MATLAB environment and is available on request with a guide how to use it.

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