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http://dx.doi.org/10.1016/j.jneumeth.2018.08.028

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PII: S0165-0270(18)30264-4
DOI: https://doi.org/10.1016/j.jneumeth.2018.08.028
Reference: NSM 8100

To appear in: Journal of Neuroscience Methods

Received date: 13-5-2018
Revised date: 30-8-2018
Accepted date: 30-8-2018

Please cite this article as: Andrade P, Ciszek R, Pitkänen A, Ndode-Ekane XE, A web-based application for generating 2D-unfolded cortical maps to analyze the location and extent of cortical lesions following traumatic brain injury in adult rats, Journal of Neuroscience Methods (2018), https://doi.org/10.1016/j.jneumeth.2018.08.028

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A web-based application for generating 2D-unfolded cortical maps to analyze the location and extent of cortical lesions following traumatic brain injury in adult rats

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Highlights

- Novel web based application for rapid generation of cortical unfolded maps in adult rat brain
- Can be used, for example, to unfold cortical areas in histologic sections or MRI slices
- Useful for analysis of lesion location and extent as well as mapping of the location of axon terminals

Abstract

Background: Both the type and severity of functional impairments caused by damage to the cerebral cortex depend on the functional cortical areas and networks affected, as well as the lesion extent and type.

New Method: To accelerate the laborious quantitative analysis of cortical lesion location and size, we created user-friendly software that generates two-dimensional unfolded cortical maps of the lesions in adult rats. The program imports and superimposes simple user-made measurements, e.g., from histologic sections, on a template. The software then quantifies the total lesion area and the area of damage in each cortical
cytoarchitectonic region. To validate the accuracy of the software, we compared computer-generated maps with manually created unfolded maps from 32 rats with lateral fluid-percussion-induced traumatic brain injury.

**Results:** The total area of the cortical lesions varied from 7.37 to 38.45 mm² in the automated analysis and from 7.26 to 38.97 mm² in the manual analysis (p>0.05). The Pearson correlation coefficient between the automated and manual analyses was 0.998 (p<0.001). The mean difference between the automated and manual measurements was 0.50 ± 0.69 mm² (range 0.00-3.30 mm²). The slight differences between the analyses related to human error in positioning the measurements to the anteroposterior coordinates on the template map.

**Comparison with Existing Methods:** Compared with the manual method, the automated method accurately and quickly generates unfolded cortical maps of lesioned cortical areas.

**Conclusions:** Application provides a novel tool for accurately positioning the lesioned area on the cortical mantle and quantifying the lesion area in histologic sections or magnetic resonance images.

**Keywords:** automated, cerebral cortex, cytoarchitectonic region

1. Introduction

Traumatic brain injury (TBI) affects 2.5 million people in Europe and the USA each year (https://www.center-tbi.eu/; http://www.cdc.gov/traumaticbraininjury/). Structural damage to the brain, particularly to the cerebral cortex, is the major contributor to cognitive disabilities (Awad et al., 1991; Chen et al., 2000; Di Paola et al., n.d.; Macciocchi et al., 1998; Perlbarg et al., 2009) and epileptogenesis (Bragin et al., 2016; Gupta et al., 2014). Accurate and reliable assessment of the lesion location and extent is critically important for predicting the outcome in both preclinical and clinical studies.

Approximately 30 years ago, Van Essen and colleagues described the use of cortical unfolded maps for analyzing the different visual cortical areas (Van Essen and Maunsell, 1980). Since the original description, this method has been applied in numerous neuroanatomic studies, including assessment of the location and extent of TBI-induced
cortical lesions (Ekolle Ndode-Ekane et al., 2017). Manual generation of cortical unfolded maps, however, is both laborious and time-consuming.

To accelerate the analysis of cortical lesions, we created a web application that accurately generates a cortical unfolded map from histologic sections of the rat brain, revealing the lesion location and area over the different cytoarchitectonic cortical areas. The application can also be applied, for example, to analyze lesion location in coronal magnetic resonance images.

2. Description of the web-based application

The unfolded cortical map web-based application is implemented using HTML5, JavaScript, and CSS. The Bootstrap front-end framework and jQuery JavaScript library are used to construct the graphical user interface (GUI). Plain PNG images and JSON files are used to store the application’s configuration data.

*Software architecture and region-of-interest mapping.* The application software architecture is divided into a UI layer and analysis layer. The UI layer comprises HTML files, a CSS file, and the unfoldedMapGUI JavaScript module. The unfoldedMapGUI module is decoupled from the rest of the system via a facade pattern implementing the Core module, which resides in the analysis layer. The Core module provides an abstracting interface to the mapping process through which the UI interacts with the rest of the system. The Core module communicates with the Importer and Mapper modules. The Importer module parses the coordinates inputted in different file formats by executing a separate worker thread. The Mapper module performs the actual region of interest (ROI) mapping based on the given inputs via mapping a specific worker thread. In addition to mapping the ROI coordinates, the Mapper module draws the mapped ROIs on the Javascript canvas and exports the mappings as Tagged Image File Format (TIFF) files. The results are stored in memory within the user browser’s JavaScript engine and are lost unless specifically exported from the application by the user (Fig. 1).

*Area calculations.* Each cortical area presented in the template image has a unique color and is stored as a separate area map image. The area map image is used during the area calculations, during which the number of pixels of each color in the area map image within the ROI contour is counted. Using these counts, the ratio of pixels of each color within the contour to the known true counts of pixels of each color can be used to estimate the affected areas. The JSON files define the mapping between the unique
area colors and brain regions, the number of pixels within each area, and the expected widths for each bregma level.

**Software settings.** The software is equipped with an upload button; “Open” (see method for file options and procedure). The import button opens a modal displaying the imported coordinate file and mapping settings. In the mapping setting, the “Type”, which describes the kind of preparation from which the images will be analyzed, is selected. This is can be either histologic sections (Histology) or magnetic resonance (MR) images (MRI). For MRI, slide thickness can be specified. The contour setting enables the line mode (linear or spline). Interpolated mapping mode uses Catmull-Rom spline interpolation to generate a smoothed mapping. It is important to specify the alpha value of the spline and the number of points used in the interpolation to achieve the desired level of smoothing (Fig. 2)

3. Validation of software function

3.1. Materials

**Raw images.** The images were of coronal sections of the entire hemisphere, containing the whole rostrocaudal extent of the cortical lesion (section thickness 30 μm, 1-in-8 series). The image can be in any format that can read by ImageJ or any other image analysis software capable of analyzing length. Here we used thionin-stained coronal sections of 12-wk old adult male Sprague-Dawley rats (Harlan Netherlands B.V., Horst, the Netherlands, 339–405 g on the day of injury) with lateral-fluid percussion–induced TBI [for a detailed description, see Ekolle Ndode-Ekane et al. (2017)]. All animal procedures were approved by the Animal Ethics Committee of the Provincial Government of Southern Finland, and performed in accordance with the guidelines of the European Community Council Directives 2010/63/EU. Animal experiments comply with ARRIVE guidelines.

The template of the unfolded cortical map was generated from the atlas of Paxinos and Watson (2007) as described in detail by Ekolle Ndode-Ekane et al. (2017). Briefly, the plates available in the rat brain atlas were uploaded into ImageJ (v. 1.51w; https://imagej.nih.gov/ij/) as TIFF files. After scaling the program based on the atlas, the length of each cortical region within one hemisphere was measured in each plate using several reference points. The measured lengths were then drawn as a straight line mediolaterally on a scaled PowerPoint sheet. The points marking the beginning and end
of each cortical region were connected to each other to reveal the boundaries of the different cytoarchitectonic cortical areas (for details see Ekolle Ndode-Ekane et al. (2017)).

**Unfolded map web-based application.** Any computer (i.e., Mac, Linux, or Windows-based operating systems) connected to the internet having a browser capable of accessing the URL www.unfoldedmap.org can be used.

3.2. Procedure

3.2.1. Quantification of the lesion area

1. Open ImageJ and set the scale from the Analyze menu. Setting the scale will allow for correct length measurement.
2. Upload the image into ImageJ
3. On the hemisphere ipsilateral to the injury, measure the medial-lateral length of the cortex beginning from the medial reference point (R1) to the lateral reference point (R2), that is, the rhinal fissure (rf). For details about the anatomic reference points, see Ekolle Ndode-Ekane et al. (2017).
4. For each section, measure the length from R1 to the dorsal edge of the lesion (M1), then the length along the lesion (M2), and finally the length from the lateral edge of the lesion to the rf (M3). When the lesion extends laterally beyond the rf, M2 is the length from the dorsal edge of the lesion to the rf, and M3 (negative value) is the length from the rf to the ventral edge of the lesion (Fig. 3).
5. The output of the measurements appears in a log window if you are using ImageJ. This is then copied and pasted to an Excel worksheet.
6. Arrange the Excel so that the data from each anteroposterior coordinate is arranged in four columns. Column 1 is the anteroposterior coordinate, column 2 is length M1, column 3 is length M2, and column 4 is length M3 (see Fig. 3, Supplementary Table 1). These data will appear as Bregma, M1, M2, and M3, respectively, in the import display (Fig. 2A).

3.2.2. Importing data from the Excel file into the Web-based unfolded map application

7. From your browser, open the URL www.unfoldedmap.org.
8. Press the “Open” button and select the Excel file with the data to be mapped. This will open in a display window. Files can be imported as comma separated values (.CSV) or as Excel worksheets (.xls, .xlsx) (Fig. 2).

9. The display will show the data (see step 6).

10. Select the type of preparation. "Histology" for histologic sections or “MRI” for MR images. If MRI is selected, impute the slice thickness.

11. Select the contour type for mapping – linear or spline mode. If spline mode is selected, impute the desired alpha value and the number of points to be used for interpolation to achieve the desired level of smoothing (see software settings above).

12. Select the desired color of the map output.

13. Press the import button to generate the unfolded map and a table showing (a) the percentage (%) of the lesion occupying the total area of a given cytoarchitectonic cortical region and (b) lesion area in mm² (Fig. 4)

14. Save the Table and image by pressing the “Table” and “Image” buttons, respectively.

3.3. Anticipated Results

Cytoarchitectonic location of the cortical lesion. Using this protocol, we were able to show the distribution of the cortical lesion on the cortical mantle following TBI in rats (Fig. 5, Supplementary Fig. 1).

Area of the cortical lesion. With the Web application, we were able to estimate the two-dimensional area of the cortical lesion and the percentage of the lesion in the different subareas of the cortex (Table 1, Supplementary Table 1).
4. Validation

To validate the protocol of the web application for the unfolded map of the cortical lesion, we compared the output from the web application with that of the manual method described earlier by Ekolle Ndode-Ekane et al. (2017). Data from 32 animals with TBI were used for validation.

4.1. Web application vs. the manual method - lesion location on the cortical mantle

When the application-generated 2D-unfolded lesion was superimposed on the lesion generated using the manual method, lesions from both methods co-localized in the same cytoarchitectonic areas (Fig. 5; Supplementary Fig. 1). In 9 of 32 cases, however, there were very slight deviations in the lesion areas between the automated and manual methods. These differences likely related to human errors in the manual method. For example, in the manual method, it is virtually impossible to align the section in the right anteroposterior coordinate with 0.01-mm precision compared with the computerized method. Furthermore, as lines have values, errors are added when the edges of the lines denoting the lesion were connected in the manual method as described by Ekolle Ndode-Ekane et al. (2017). These errors are not present in the Web application method because the calculations are performed at the pixel level.

4.2. Web application vs. the manual method - lesion area

The total area of the cortical lesion varied from 7.37 to 38.45 mm² (19.65±7.48 mm²) in the automated analysis and from 7.26 to 38.97 mm² (20.15±7.85 mm²) in the manual analysis (p>0.05; Table 1). The Pearson correlation coefficient between the automated and manual analysis methods was 0.998 (p<0.001). The mean difference between the automated and manual measurement methods was 0.50 ± 0.69 mm² (range 0.00-3.30 mm²), increasing with bigger lesion areas (correlation with manual measurement 0.646, p<0.001; automated measurement r=0.727, p<0.001). The slight differences between the analyses were related to human error in positioning the measurements to the anteroposterior coordinates on the map.

To further validate the web application protocol, we compared the area of the lesion in the different cortical cytoarchitectonic areas generated by the web application to that estimated by the manual method. As summarized in Supplementary Table 2, the percentage of a given cytoarchitectonic area varied substantially between animals.
[detailed description in Ekolle Ndode-Ekane et al. (2017)]. The difference in the percentage of damage in the automated and manual analyses was typically less than one percentage unit.

5. Discussion

We developed a rapid and accurate web application for localizing cortical lesions and calculating the area on the cortical mantle of the adult rat. Our automated method provides more reliable and consistent data than the manual methods, essentially because it eliminates inter-researcher variability and reduces human error.

For an experienced analyzer (X. N.-E.), the time to measure M1, M2, and M3 in digitized histologic sections of the injured brain (30-μm thick and 150-μm apart; on average, 15 coronal sections containing the lesion), and to enter the numbers on the Excel worksheet takes about 30 minutes. Manual transfer of the measurements to the PowerPoint template, generation of the unfolded cortical map and its export to ImageJ to calculate the areas takes approximately 1 h. The last 1-h stage can be performed substantially faster using the automated method because downloading the datasheet and generating an unfolded map takes less than 1 minute.

In addition to quantifying cortical lesions after TBI, stroke, or other injuries in histologic or immunohistologic staining and MRI (an example in Supplementary Fig. 2), the application can also be used to locate the other anatomical zones as well as distribution of axon terminals, tracer injections, and positions of implanted cannulas or other probes in the cerebral cortex.

Acknowledgements

This study was supported by the Academy of Finland (A.P.) and the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement n°602102 (EPITARGET) (A.P.).
References


Legends to Figures

**Figure 1.** Software architecture. Note that the software is divided into layers: a User Interface (UI) Layer where the graphical interface resides and an Analysis Layer where the calculations and extrapolations are made.
Figure 2. Web application. Marked with arrows are the “Open” button and “Config” button. (A) The “Open” button opens an “import” window that allows the user to import data containing the measurements. In the “import” window, “Bregma” refers to the coronal level from bregma according to rat brain atlas of Paxinos and Watson (2007).
M1, M2, and M3 are the measurements related to the lesion throughout its rostrocaudal extent as specified in Fig. 3. In this case, the lesion extends ventral to the rhinal fissure, M2 is the lesion length to the rhinal fissure, and M3 (negative value) is the length from the rhinal fissure to the ventral edge of the lesion. Measurements can be imported to the web application as an Excel or text file [two examples showing a structure of the Excel file (rats # 5 and #47) are shown in Supplementary Table 1a-b]. By clicking “Histology” or “MRI”, one can choose the data origin. In case of “MRI”, the user is asked to provide the slice thickness. By clicking “Contour”, the user can choose whether the unfolded map should have a linear or spline (smoothened) outline. (B) The “Config” button allows the user to select the color and level of transparency of the region of interest.
Figure 3. Parameter measurements. An example demonstrates the drawing of M1, M2, and M3 in thionin-stained coronal sections of a rat with lateral fluid-percussion–induced traumatic brain injury (rat #47 from Ekolle Ndode-Ekane et al., 2017). Note that the lesion extends below the rhinal fissure (open arrow). Abbreviation: rf, rhinal fissure.
Figure 4. Analysis window. Dashboard (dashed outline) shows the cortical region affected [percentage and area (mm$^2$)]. Left arrow points to the "Table" button that will export the table containing the results from each cytoarchitectonic area and the total lesion area. The right arrow points to the "Image" button that will export a TIFF file of the unfolded map (on the left) containing the lesion area.
**Figure 5.** Four representative unfolded maps (A-D) generated from rats #4, #5, #47, and #32 using the automated web application (orange dashed outline) superimposed on the corresponding manually generated maps (light blue shading). Panel C is from case #47 shown in Fig. 3. Measurements M1, M2, and M3 for cases #5 and #47 are
shown in **Supplementary Table1a-b**. Black arrows point to slight differences between the unfolded maps generated using automated or manual methods. Horizontal line (medio-lateral) tick marks equal 1 mm distance. Vertical line (rostro-caudal) is shown in 0.1 mm accuracy (bregma equals 0).

Figure 5 (2-column fitting)
**Table 1.** Total cortical lesion area in 32 rats with lateral fluid-percussion-induced traumatic brain injury.

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