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Semi-automated method for estimating lesion volumes

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HIGHLIGHTS

- We present a method to estimate and visualize lesion volume.
- The method provides higher accuracy than the hemisphere comparison method.
- The method provides similar accuracy in lesion volume size estimation, but provides higher accuracy in the lesion localization.
- Tools are provided for graphic representation of group averaged lesion volume.

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ABSTRACT

Accurately measuring the volume of tissue damage in experimental lesion models is crucial to adequately control for the extent and location of the lesion, variables that can dramatically bias the outcome of preclinical studies. Many of the current commonly used techniques for this assessment, such as measuring the lesion volume with primitive software macros and plotting the lesion location manually using atlases, are time-consuming and offer limited precision. Here we present an easy to use semi-automated computational method for determining lesion volume and location, designed to increase precision and reduce the manual labor required. We compared this novel method to currently used methods and demonstrate that this tool is comparable or superior to current techniques in terms of precision and has distinct advantages with respect to user interface, labor intensiveness and quality of data presentation.

1. Introduction

The assessment of lesion volume (LV) and lesion location is pivotal in both clinical and experimental settings. Clinically, this practice is used for many purposes, including prognostic assessment of stroke (Merino et al., 2007; Rivers et al., 2006), multiple sclerosis (Bagnato et al., 2011; Zivadinov et al., 2012), traumatic brain injury (Darling et al., 2011; Di Stefano et al., 2000), and atrophy of the hippocampus in epileptic patients (Wieshmann et al., 1997). In research, LV is often the most important control variable when assessing the efficacy of novel interventions for post-stroke neurorehabilitation, including emerging biological therapies (Chen et al., 2001), electrical stimulation (Adkins-Muir and Jones, 2003; Brown et al., 2003; Kleim et al., 2003), and physical therapy (Wolf et al., 2006). Unfortunately, current methods to assess LV either lack precision or require extensive labor. Given the critical relevance of LV and the need for frequent measurements of this variable in the course of preclinical experimentation, it is important to develop a method that is both precise and time efficient. Here we report a novel semi-automated method for estimation of LV and compare its precision and efficiency to currently used methods.

Commonly employed techniques for LV measurement include adapted use of imaging software (i.e. Adobe Photoshop CS ©, ImageJ) to manually outline the lesion with a tracing mechanism, such as the free-form or edge-detection selector tools (Di Stefano et al., 2000; Lee et al., 1996; Zivadinov et al., 2012). The metric area is then calculated based on the resolution of the image. A ruler or object of known size can be included in all images or in an index figure in order to establish a firm correlation between metric distance and pixels for the specific imaging modality (i.e. flat bed slide scanning) (Machado et al., 2009). There are several variations to this approach. The choice of imaging methodologies represents a compromise between data accuracy and time efficiency. The higher-precision lesion selection tools tend to require

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extensive user input. In these cases, the total time required to measure stroke volume for an entire experiment can become prohibitive. Computational algorithms and macros, often custom built to reduce user effort, may undermine the precision of area estimation. For example, hemispheric comparison, though less manual and therefore less time-consuming, is a low-precision method and can be particularly prone to error, especially when lesion sizes are small.

Methods employed once areas of cross-sections are measured must represent the actual volume. Although Simpson's rule (Lee et al., 1996) is frequently used to estimate the volume size from slices in parallel planes, it does not provide a method for visual reconstruction of three-dimensional volumes. Moreover, the volume reconstruction by cylindrical approximation (Goldberg et al., 1995) is crude and non-continuous.

In order to facilitate a time-efficient and accurate means of determining LV, we devised semi-automated software that calculates the area of multiple cross sections of the lesion and then creates a three-dimensional model of the LV, named Serial Lesion Image Computed Estimation (SLICE). The LV determination process is facilitated by a custom-designed MATLAB® interface that cycles through the images, calculates LVs, reconstructs the lesion in 3D space, and stores the results for analysis. Furthermore, it also has a novel feature for projecting lesion volumes onto a rodent stereotactic atlas (Paxinos and Watson, 1998) saving the user considerable time in creating lesion-representative figures.

2. Materials and methods

Methods for measuring the stroke volume were compared utilizing histology from the brain of rats that had undergone cortical ischemia induced by intracortical injections of endothelin. Since the true size of a natural lesion cannot be calculated with absolute accuracy – all methods aim at best estimating the size of a lesion – we also utilized two hundred computer generated images with artificially created lesions to compare the methods. The animal experiments were performed using male Sprague–Dawley rats (250–350 g). The animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved animal facility in a climate controlled environment that included a 12-h light/dark cycle and free access to water. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Cleveland Clinic.

2.1. Endothelin injections

Anesthesia and stereotactic procedures were performed as previously described (Baker et al., 2010; Machado et al., 2009). Anesthesia was initiated in a chamber saturated with isoflurane and then maintained under mechanical ventilation. The rat was positioned on a stereotactic frame (David Kopf, Tujunga, CA) and fixed at the external auditory canals and maxilla. A midline incision was opened over the calvaria. Three bur holes were created for injecting one dose of endothelin-1 (800 pmol each) at the following coordinates in relation to bregma (depth 2.3 mm): (1) AP = −1.0 mm, ML = 2.5 mm, (2) AP = +1.0 mm, ML = 2.5 mm and (3) AP = +3.0 mm, ML = 2.5 mm (Windle et al., 2006). Animals were monitored during recovery from anesthesia, with food and water provided ad libitum. Pain was alleviated post-operatively with buprenorphine (0.05 mg/kg twice daily) subcutaneously. Animals were sacrificed after 7 weeks.

2.2. Histology and image preparation

All rats were transcardially perfused with 0.1 M phosphate-buffered saline (PBS) followed by 500 ml of 4% paraformaldehyde in PBS. The tissue was then sent for histological processing (Neuroscience Associates, Knoxville, TN) and prepared according to the following protocol (modified from Neuroscience Associates):

1. Brains were treated with 20% glycerol + 2% dimethylsulfoxide.
2. Brains were then fixed in agelatin matrix (MultiBrain Technology™, NeuroScience Associates).
3. The block was cured and quickly frozen (−70 °C isopentane chilled with crushed dry ice) and placed on the freezing stage of a sliding microtome (AO-860; American Optical, Buffalo, NY).
4. The block was sectioned in the coronal plane at 40 μm and sequentially collected into a 4 × 6 array of containers with Antigen Preserve solution (50% ethylene glycol, 49% PBS pH 7.0, 1% polyvinyl pyrrolidone).
5. One of every 24th section was slice mounted. Therefore, the inter-slice distance was 24 × 40 μm = 960 μm.
6. Each of the large sections cut from the block was a composite holding individual sections from each of the brains embedded in the block so that uniformity of staining was achieved across treatment groups (MultiBrain technology).
7. Sections were placed onto gelatinized slides for Nissl staining.
8. Sections were dehydrated through alcohols prior to defatting in a chloroform/ether/alcohol solution and rehydrated and stained with 0.05% Thionine/0.08 M acetate buffer, pH 4.5.
9. Following deionized water rinses, the sections were differentiated in 95% alcohol/acetic acid, dehydrated in a standard alcohol series, cleared in xylenes, and coverslipped.

Histological sections were scanned to a computer at 2400 dpi with multiple coronal cerebral sections on each slide. Images containing the lesion area were then cropped and organized into folders by animal.

2.3. Description of programs used

The estimation of lesion volumes is divided into three stages. The first stage is the measurement of the lesion area in each slice and the second stage is the measurement of the lesion volume using the interpolation of the lesion area. In the final stage, lesion volumes and their percentile in relation to the whole group were overlaid onto the rat brain atlas.

2.3.1. Lesion area measurement

There are two traditional methods for estimating lesion area. One is the hemisphere comparison method and the other is the tracing method. In the hemisphere comparison method, the lesion area is estimated by the difference between the hemispheres. The hemisphere comparison method assumes symmetry of the brain after histological preparation of the lesioned tissue and absence of major slicing artifacts. This method may be ineffective, particularly for sections that may have asymmetry caused by damage during slice preparation and different shrinkage rates in fixation solutions (Fig. 1). In contrast, with the tracing method, the user delineates the perimeter of the desired region based on the expected perimeter of a non-lesioned brain and the corresponding boundary coordinates are determined and stored by the program. A commonly used tracing software tool is the free-form selector tool or Lasso tool available in Adobe® Photoshop® CS; however other software packages can be used such as ImageJ and GIMP. Once regions of the lesion have been traced, the numerical value for the number of pixels in the selection are obtained and manually entered into a spreadsheet. From these values, the areas of each selection are calculated by dividing the number of pixels per unit area. However, in cases where lesion size is large and missing a substantial cortical boundary, the investigator must arbitrarily estimate the original boundary from the preserved boundary. This limitation is