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A Comparative Study of Automatic Approaches for Preclinical MRI-based Brain Segmentation in the Developing Rat*

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Abstract

Accurate pre-clinical study reporting requires validated processing tools to increase data reproducibility within and between laboratories. Segmentation of rodent brain from non-brain tissue is an important first step in preclinical imaging pipelines for which well validated tools are still under development. The current study aims to clarify the best approach to automatic brain extraction for studies in the immature rat. Skull stripping modules from AFNI, PCNN-3D, and RATS software packages were assessed for their ability to accurately segment brain from non-brain by comparison to manual segmentation. Comparison was performed using Dice coefficient of similarity. Results showed that the RATS package outperformed the others by including a lower percentage of false positive, non-brain voxels in the brain mask. However, AFNI resulted in a lower percentage of false negative voxels. Although the automatic approaches for brain segmentation significantly facilitate the data stream process, the current study findings suggest that the task of rodent brain segmentation from T2 weighted MRI needs to be accompanied by a supervised quality control step when developmental brain imaging studies were targeted.

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I. INTRODUCTION

Magnetic resonance imaging (MRI) represents an important intersection of engineering and medical science that has led to important discoveries in the fields of neuroscience and biology. MRI continues to be a major modality in both clinical and pre-clinical settings, most notably due to its high spatial resolution, and varied contrast mechanisms for probing tissue microstructure and function. However, its use as an indirect readout of cellular activity and its relatively low temporal resolution compared to other modalities are potential limitations in the proper interpretation of MRI data [1], [2]. Anatomical imaging of brain using MRI has been cited amongst the most accurate techniques to detect structural abnormalities that arise in the diseased brain [3]–[9]. The accuracy of image analysis pipelines for functional MRI (fMRI) as well as Diffusion Weighted Imaging (DWI) and associated next level interpretations in the form of connectivity networks [10]–[14], are primarily dependent on a basic step in segmenting the acquired image volume to brain and non-brain tissues to generate a brain-specific mask. Significant advances have been made toward automating this process of brain extraction in the clinical research arena with neuroimaging software packages such as AFNI [15], [16], Freesurfer [17], FSL [18], and SPM [19]. Previous studies have reported the existence of both randomized and systematic bias in brain segmentation tasks via automatic routines within the variety of software packages [20]–[22], with the bias depending on the demographic characteristics of the population in which brain segmentation is performed. Far less progress has been made toward the development of automated brain extraction algorithms that can cope with the more rectangular shape of the rodent brain. However, there are some significant exceptions [23]–[25] which we utilize in the current study.

Fast and accurate delineation of brain from non-brain structures is required to enhance the reproducibility and speed of preclinical image analysis and this will ultimately benefit preclinical drug trial data acquisition as well as basic research. The gold standard solution to extract brain masks from high resolution imaging data in preclinical studies has been largely limited to manual segmentation, a tedious and time-consuming process. The current study aims to assess the reliability and accuracy of existing, publicly available rodent brain extraction techniques for structural MRI data obtained from the developmentally immature rat. The selection criteria for inclusion of techniques into the study were constrained to software being publicly available to the research community, and performing the brain extraction task in a fully unsupervised fashion. The skull stripping program from AFNI [26], optimized for rat brain, a neural network based approach [23], and the RATS package [27] were selected and studied for their ability to estimate brain masks from T2-weighted, MRI volumetric data acquired from post-natal day 23 rat pups and requiring minimal operator intervention to perform the task. Herein we report on the selected unsupervised routines for rodent brain extraction along with a manual segmentation protocol used as the gold standard, present the statistical comparison of the estimated mask volumes, and provide suggestions for possible future directions.

II. METHODS

T2-weighted, anatomical imaging data were acquired from 23-day-old, Sprague-Dawley rat pups (n=41) using a Bruker Biospin 7.0 Tesla spectrometer with the following sequence parameters:

Rapid Acquisition with Relaxation Enhancement (RARE) recording method, RARE factor 8, Single repetition single echo with TE/TR of 14/6021 ms, 180° flip angle. All image volumes were corrected for bias field due to inhomogeneity in the B1 field using a nonparametric, non-uniform normalization retrospective algorithm [28], as shown in Figure 1.

A. Manual Reference Brain Segmentation

Reference brain mask construction was manually performed for all subjects by an operator who has been previously trained on a large and diverse population for brain masking task [S.S.] No specific subject order was considered. Manual segmentations were performed over the time course of two months imposing no time limitation for the completion of the process. Anterior landmarks used for outlining the brain include the olfactory bulb (when available in the MRI field of view) as well as the brain stem in the ventral posterior part of the brain.

B. Automatic Brain Segmentation

Preprocessed T2-weighted image volumes of all animals were also subjected to automatic brain mask construction using software packages developed and optimized for rodent brain extraction tasks. A set of default parameters were chosen when required by the routines, but no manual intervention was used in the acquisition of the brain masks. The AFNI routine for skull stripping (3dSkullStrip) with the “-rat” option [26], MATLAB (www.mathworks.com) based implementation of pulse coupled neural network (PCNN-3D) with preset rat brain size [23], and layered optimal graph image segmentation of multiple objects and surfaces (LOGISMOS) from the RATS package [25] [27] were evaluated for their performance in automatic segmentation of developing rat brain from nonbrain tissues.

D. Standard Space Registration

All acquired brain volumetric masks from either manually-outlined reference or automatic estimation protocols, explained in sections B and C, were warped to an in-house developed rat brain template using FSL tool “flirt” [29], [30] applying the registration matrix with six degree of freedom derived from warping the brain-extracted anatomical image volumes.

E. Reliability Analysis of Automatic Tools

Group-wide reliability assessment of brain volumetric masks generated via automatic routines (section C) was performed by calculating the *Dice Coefficient of Similarity* (D_a), as followed

$$D_a = \frac{2|V_r \cap V_a|}{|V_r| + |V_a|} \quad (1)$$

$$a \in \{AFNI, PCNN3D, RATS\}$$

where V_r is the animal subjects' reference segmented brain volumetric mask, and V_a represents the associated volumetric mask from the automatic approaches (*AFNI*, *PCNN-3D*, and *RATS* software packages). $|\cdot|$ represents the number of embedded voxels in a volume and $\cap$ presents the intersection symbol. The percentage of false positive and false negative voxels were calculated assuming the manually-drawn, reference estimation as the ground truth.

III. RESULTS AND DISCUSSION

Figure 1. shows a three-dimensional rendering of the mean volumetric masks from the segmented brains, calculated using manual and automatic segmentation approaches. Visual inspection of the masks compared to the reference manual mask highlighted an existing inaccuracy in the proper segmentation of the brain tissue that varied depending on the automatic routine used. Pair-wise inspection of the outer edge of the group mean volumetric masks created from the manual and automatic approaches provided evidence of location dependency of segmentation errors, as shown in Figure 2 by the superimposed mask outlines. The *AFNI* routine showed greater deficits in segmenting the brain tissues over the posterior brain regions, especially in the brainstem, while the *PCNN-3D* method showed poor performance in anterior brain regions (Figure 2). This indicates that there is a brain regional dependency on pipeline performance for rodent brain segmentation which requires further investigation.

When the methods were compared using the Dice coefficient of similarity, the *RATS* method outperformed the other two automatic approaches, reporting a larger Dice coefficient of similarity ($Mean \pm SD$, 0.91 ± 0.05) compared to 0.89 ± 0.03 and 0.81 ± 0.05 , respectively for *AFNI* and *PCNN-3D* routines. Figure. 3 shows the distributions of Dice coefficient of similarity, percentage of false positive and percentage of false negatives in brain segmentation following automatic approaches.

RATS also outperformed the other software packages in terms of the number of false positive voxels (4.96 ± 3.32), when compared to *AFNI* (11.48 ± 5.28) and *PCNN-3D* (16.55 ± 3.32). In terms of percentage of false negative in brain segmentation, However, *AFNI* produced a lower quantity of false negative, brain segmented voxels (10.11 ± 4.89) whereas *RATS* (13.28 ± 8.08) and *PCNN-3D* (19.86 ± 8.25) were found to miss larger numbers of brain voxels. Although the automatic approaches for brain segmentation significantly enhance the speed of the data analysis pipeline, the findings in the current study suggest that the task of brain segmentation from T2-weighted MRI requires supervised quality control when developing brains are being studied.

IV. CONCLUSION

Fully automated pipelines (AFNI, PCNN3D, RATS) for the rodent brain extraction task in pre-clinical imaging studies were evaluated. Findings suggested that the task requires a set level of quality control and visual inspection.

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REFERENCES

- [1]. Sargolzaei S, "Impact of Accelerometry and Spirography Data Analysis of Essential Tremor on BOLD-f MRI Data Interpretation," 2012.
- [2]. Sargolzaei S, "Intracranial Volume Estimation and Graph Theoretical Analysis of Brain Functional Connectivity Networks," FIU Electron. Theses Diss, Mar. 2015.
- [3]. Davenport ND, Lim KO, and Sponheim SR, "White matter abnormalities associated with military PTSD in the context of blast TBI.," Hum. Brain Mapp, vol. 36, no. 3, pp. 1053–64, Mar. 2015. [PubMed: 25387950]
- [4]. Geuze E, Vermetten E, and Bremner JD, "MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed.," Mol. Psychiatry, vol. 10, no. 2, pp. 147–59, Feb. 2005. [PubMed: 15340353]
- [5]. Sonuga-Barke EJS et al., "Child-to-adult neurodevelopmental and mental health trajectories after early life deprivation: the young adult follow-up of the longitudinal English and Romanian Adoptees study," Lancet, Feb. 2017.
- [6]. Loewenstein DA et al., "Recovery from Proactive Semantic Interference and MRI Volume: A Replication and Extension Study.," J. Alzheimers. Dis, vol. 59, no. 1, pp. 131–139, Jul. 2017. [PubMed: 28598850]
- [7]. Dennis EL et al., "Diverging white matter trajectories in children after traumatic brain injury: The RAPBI study.," Neurology, vol. 88, no. 15, pp. 1392–1399, Apr. 2017. [PubMed: 28298549]
- [8]. Harris NG, Verley DR, Gutman BA, and Sutton RL, "Bidirectional changes in fractional anisotropy after experiment TBI: Disorganization and reorganization?," Neuroimage, vol. 133, pp. 129–143, 2016. [PubMed: 26975556]
- [9]. Doshi J, Erus G, Ou Y, Gaonkar B, and Davatzikos C, "Multi-Atlas Skull-Stripping," Acad. Radiol, vol. 20, pp. 1566–1576, 2013. [PubMed: 24200484]
- [10]. Sargolzaei S, Cabrerizo M, Goryawala M, Eddin AS, and Adjouadi M, "Functional Connectivity Network based on Graph Analysis of Scalp EEG for Epileptic Classification," Signal Process. Med. Biol. Symp, pp. 1–4, 2013.
- [11]. Eddin AS et al., "The effects of pediatric epilepsy on a language connectome," Hum. Brain Mapp, vol. 35, no. 12, pp. 5996–6010, 2014. [PubMed: 25082062]
- [12]. Sargolzaei S, Cabrerizo M, Sargolzaei A, Noei S, and Adjouadi M, "Epilepsy, a Cyberattack on Brains? Networked Control System," in 2016 15th IEEE International Conference on Machine Learning and Applications (ICMLA), 2016, pp. 622–625.
- [13]. Eddin AS, Wang J, Sargolzaei S, Gaillard WD, and Adjouadi M, "ICA-based connectivity on brain networks using fMRI," in 2013 6th International IEEE/EMBS Conference on Neural Engineering (NER), 2013, pp. 391–394.
- [14]. Ziaei A, Yeganeh H, Faez K, and Sargolzaei S, "A novel approach for contrast enhancement in biomedical images based on histogram equalization," Biomed. Eng. Informatics New Dev. Futur. - Proc. 1st Int. Conf. Biomed. Eng. Informatics, BMEI 2008, vol. 1, pp. 855–858, 2008.
- [15]. Cox RW, "AFNI: what a long strange trip it's been.," Neuroimage, vol. 62, no. 2, pp. 743–7, Aug. 2012. [PubMed: 21889996]

- [16]. Cox RW, "AFNI: software for analysis and visualization of functional magnetic resonance neuroimages.," Comput. Biomed. Res, vol. 29, no. 3, pp. 162–73, Jun. 1996. [PubMed: 8812068]
- [17]. Fischl B, Sereno MI, and Dale AM, "Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system.," Neuroimage, vol. 9, no. 2, pp. 195–207, Feb. 1999. [PubMed: 9931269]
- [18]. Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, and Smith SM, "FSL.," Neuroimage, vol. 62, no. 2, pp. 782–790, Aug. 2012. [PubMed: 21979382]
- [19]. K. J. (Karl J. Friston, Ashburner J, Kiebel S, Nichols T, and Penny WD, Statistical parametric mapping : the analysis of functional brain images. Elsevier/Academic Press, 2007.
- [20]. Sargolzaei S et al., "A practical guideline for intracranial volume estimation in patients with Alzheimer's disease," BMC Bioinformatics, vol. 16, no. 7, 2015.
- [21]. Sargolzaei S et al., "Estimating Intracranial Volume in Brain Research: An Evaluation of Methods," Neuroinformatics, vol. 13, no. 4, 2015.
- [22]. Sargolzaei S. Comparative reliability analysis of publicly available software packages for automatic intracranial volume estimation; 2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBC; 2014. 2014
- [23]. Chou N, Jiarong Wu J Bai Bingren, Anqi Qiu, and Kai-Hsiang Chuang, "Robust Automatic Rodent Brain Extraction Using 3-D Pulse-Coupled Neural Networks (PCNN)," IEEE Trans. Image Process, vol. 20, no. 9, pp. 2554–2564, Sep. 2011. [PubMed: 21411404]
- [24]. Cox RW, "AFNI: What a long strange trip it's been," Neuroimage, vol. 62, no. 2, pp. 743–747, Aug. 2012. [PubMed: 21889996]
- [25]. Yin Yin Y, Xiangmin Zhang X, Williams R, Xiaodong Wu X, Anderson DD, and Sonka M, "LOGISMOS—Layered Optimal Graph Image Segmentation of Multiple Objects and Surfaces: Cartilage Segmentation in the Knee Joint," IEEE Trans. Med. Imaging, vol. 29, no. 12, pp. 2023–2037, Dec. 2010. [PubMed: 20643602]
- [26]. Taylor PA and Saad ZS, "FATCAT: (an efficient) Functional and Tractographic Connectivity Analysis Toolbox.," Brain Connect, vol. 3, no. 5, pp. 523–35, Oct. 2013. [PubMed: 23980912]
- [27]. Oguz I, Zhang H, Rumple A, and Sonka M, "RATS: Rapid Automatic Tissue Segmentation in rodent brain MRI.," J. Neurosci. Methods, vol. 221, pp. 175–82, Jan. 2014. [PubMed: 24140478]
- [28]. Tustison NJ et al., "N4ITK: improved N3 bias correction.," IEEE Trans. Med. Imaging, vol. 29, no. 6, pp. 1310–20, Jun. 2010. [PubMed: 20378467]
- [29]. Jenkinson M and Smith S, "A global optimisation method for robust affine registration of brain images.," Med. Image Anal, vol. 5, no. 2, pp. 143–56, Jun. 2001. [PubMed: 11516708]
- [30]. Jenkinson M, Bannister P, Brady M, and Smith S, "Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images," Neuroimage, vol. 17, no. 2, pp. 825–841, 2002. [PubMed: 12377157]

Figure 1.

Study protocol in estimating the brain masks via manual (reference) estimation compared with automatic approaches. T2-weighted anatomical scan of 41 Sprague-Dawley rat pups were acquired on post-natal day 23. Image preprocessing stage included nonparametric non-uniform correction for the existence of inhomogeneity in B1 magnetic field. Manual estimation of brain was guided by visual localization of an anterior landmark (olfactory bulb) and a posterior landmark (brainstem) (*This landmark is mostly found to be located on the posterior end of olfactory bulb in rodent imaging studies).

Preprocessed MRI volumes were also subjected to brain segmentation via automatic approaches (AFNI, PCNN-3D, and RATS). Group mean volumetric mask were constructed and color coded as a representation of segmentation output across the study population.

Figure 2.

Representation of the outer edge of the intersected volumetric mask from the study population for the reference segmentation of the brain (red line) along with automatic approaches AFNI (blue line), PCNN-3D (green line), RATS (yellow line) overlaid on the template brain.

Figure 3.

Distribution of calculated Dice coefficients of similarity for the study population for as an index of similarity among the reference brain segmentation and the volumetric masks generated from automatic approaches (AFNI, PCNN-3D, RATS). (b) Calculated percentage of missing brain voxels from either automatic approaches for rodent brain segmentation task, and (c) calculated percentage of falsely annotated voxels across each method.