Automated Computational Analysis of Neonatal Hypoxic Injury and Implanted Therapeutic Neuronal Stem Cells

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Introduction: Neonatal hypoxic ischemic injury (HII; 2-4/1000 births) remains a devastating cause of brain injury [1]. Recent studies have shown that neuronal stem cell (NSC) based therapies reduce HII volume in adult stroke [2] and neonatal HII animal models [3]. Prior to clinical applications, translational research in animal models is needed to assess NSC safety and efficacy. Non-invasive MRI has proven to be effective in stratifying HII severity in neonates based on lesion volumes [4] for selecting candidates [5] and sites of NSC implantation [3], and for visually monitoring NSC migration and proliferation towards and inside ischemic tissues up to 58wks after NSC implementation [6]. But manual quantification of the HII lesion and NSC activity is subjective, irreproducible, and error-prone. For better understanding of NSC therapeutics in HII, we need automated, objective, rapid and robust computational methods [7]. In this study we developed a computational method, Hierarchical Region Splitting (HRS) that adaptively detects and quantifies HII and NSCs from MRI, and extracts information that is virtually impossible to address manually, such as degree of ischemic tissue salvageability; relation of NSC migration/proliferation with the lesion location and their interactions.

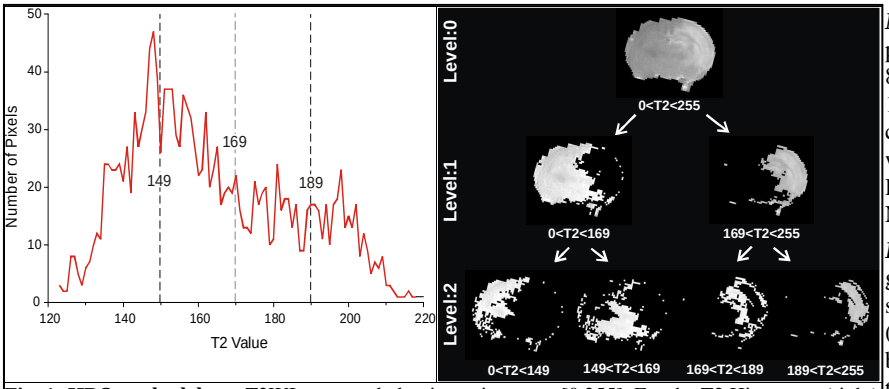

Fig. 1: HRS methodology. T2WI are rescaled to intensity range [0,255]. For the T2 Histogram (right) of the Level 0 image (left), HRS method fits the best possible bimodal distribution and detects the valley at T2 = 169 as the threshold to split the histogram and the level 0 image. This splitting is repeated (next level thresholds are T2 = 149 and 189) to form the HRS tree (left). Sub-images in the right are rescaled for visualization. Note, only part of the complete HRS tree is shown.

Materials and Methods: We have induced HII in 10d rat pups by unilateral common carotid artery occlusion with 8% hypoxia for 90mins (Rice-Vannucci Model (RVM)). 1d later, 500K human NSCs were implanted into the contralateral ventricle and T2 weighted images (T2WI) were collected at 1, 4, 7, 14, and 28d after implantation. HRS automatically analyzed T2WI to quantify HII and NSCs.

Results: HRS splits the MR images recursively to generate a binary tree-like structure (Fig. 1) where each sub-image contains different uniform image regions (normal brain tissue, injured brain tissue, or stem cells) because intensity variance of the regions decreases down the tree structure. The key steps of HRS are as follows (see Fig. 1). *(1) Rescaling:* Sometimes MR (e.g., T2WI) values are distributed within a narrow range of large numbers. So MR values are rescaled to an image intensity range [0, 255] to reduce computational complexity and robustness to contrast variation. The conversion factors are stored to map the automatically derived results back to the T2 values. *(2) Deriving histogram:* The histogram or signal spectrum of the MRI is computed. *(3) Computing adaptive segmentation threshold:* A method similar to Otsu's method [8] is used to model the histogram as a bimodal distribution with two distinct and distant peaks. The valley between them is decided as an adaptive threshold to split the image into two sub-image regions that are relatively uniform in intensity. Each peak is a region with minimum intra-region MRI variance and maximum inter-region MRI variances. *(4) Recursive bimodal segmentation:* The method in step (3) is recursively used to split each of the regions obtained from step (3) to generate a tree-like hierarchical data structure (Fig. 1). Each segmented region at any level is the region of the MRI data within two threshold values. *(5) Criteria for stopping segmentation:* Recursive splitting is continued until individual sub-regions have uniform MRI intensity. This is decided by three factors: (a) small regions are unlikely to be partitioned into different brain regions; (b) low standard deviation means regional MRI intensities are uniform; and (c) low kurtosis value of the histogram means the peak is too distinct to be modeled as a bimodal distribution.

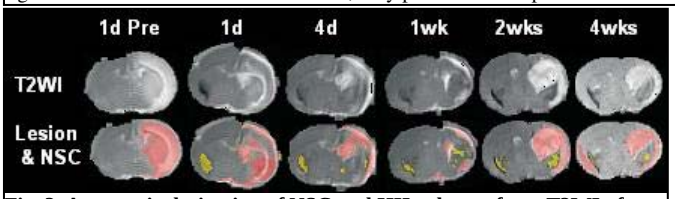

Fig. 2. Automatic derivation of NSC and HII volumes from T2WI after RVM. The bottom row superimposes the lesion (red) and NSC (yellow) regions detected by HRS onto the T2WI.

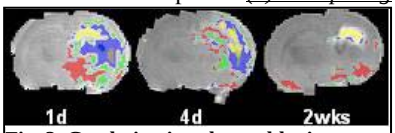

Fig. 3. Gradation in salvageable tissues inside lesion. Same brain slice at different time points. Mean T2 intensities: Red (199 ± 2), Green (208 ± 3), Blue (217 ± 3), and Yellow (231 ± 6).

The mean values of the sub-images in the HRS tree (considering from top to bottom) are compared with two approximate thresholds, *meanTh1* and *meanTh2*, to automatically detect the lesion and NSCs respectively. When the mean intensity is greater (lesser) than *meanTh1* (*meanTh2*), the region is classified as HII lesion (NSCs). The HRS sub-tree below the lesion or NSCs provides tissue salvageability inside HII or cell density inside NSC regions respectively for spatiotemporal activity monitoring. HRS could effectively detect lesion (*meanTh1* = 180) and NSC (*meanTh2* = 80) regions and identify their volume and shape variation over space and time (Fig. 2). Using the HRS sub-trees below the lesion sub-image, we could detect significant salvageability gradations inside the lesion (Fig. 3) and cell densities inside NSC regions (not shown here) and their variation over time.

Discussions and Conclusions: HRS could detect lesion and NSCs effectively, 100 times faster (manual: 3 hrs; HRS: 15 secs). It non-invasively provides much detailed information on lesion and NSCs that can be correlated later for understanding stem cell therapeutics in brain ischemic injury. Though developed for HII in T2WI, the HRS based computational method is generic to quantify other injuries detectable in different MRI modalities.

References:

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