Morphological and Functional Characterization of a New Model of Hypoxic-Ischemic Encephalopathy in Neonatal Rat using a Bilateral Carotid Artery Occlusion

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Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) is increasingly reliant on magnetic resonance imaging (MRI) for diagnosis, however technical limitations have prevented the establishment of a suitable animal model to appropriately study this condition. Whereas neonatal HIE clinically presents with global diffuse injury, the most popular rat pup models (Rice-Vannucci & transient filament MCAO) induce a focal unilateral injury. The bilateral carotid artery occlusion (BCAO) model has been shown to induce bilateral injury in animals aged five days or younger[1]. If adapted for use at an age that developmentally correlates with a term neonate, the BCAO model could show promise in providing an animal model that more closely resembles the clinical condition of neonatal HIE. We now report on new imaging correlates, injury stratification schema, and extended behavioral assessment of BCAO in a 10d old rat pup.

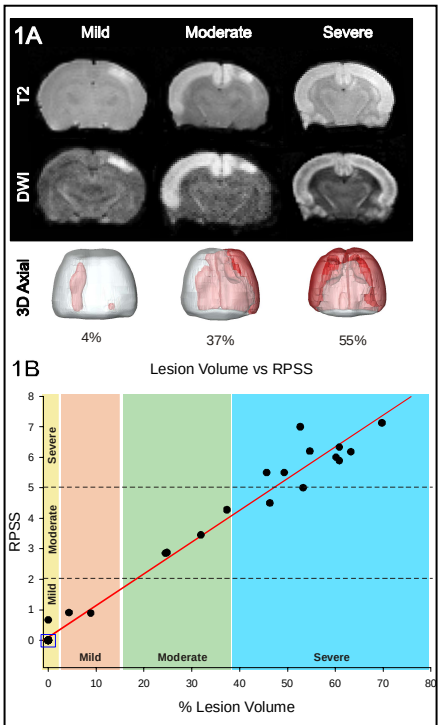

Fig. 1: A) T2 and DWI assist in discrimination between injury severities at 1 day. Mild injuries were often only cortical and unilateral, while moderate injury involved bilateral portions of the cortex. The severe injury phenotype included virtually the entire cortex and affected subcortical regions (basal ganglia, thalamus). Hippocampal involvement was infrequent. 3D volumetric reconstructions (axial plane, dorsal view) confirmed the assessment**.** B) The RPSS strongly correlated (R2=0.98, p<0.0001) with 3D T2WI MRI lesion volume. Mild injury is defined as RPSS<2 & 3Dvol<15%; moderate injury as 5<RPSS>2 & 15%<3Dvol>35%; and severe injury as RPSS>5 & 3Dvol>35%.

Methods

BCAO: Neonatal hypoxia ischemia was induced in 10 day old rat pups using bilateral carotid ligation/8% hypoxia (n=16, BCAO model) similar to that previously published[1]. Hypoxia time ranged from 2-15 minutes to produce a gradation of injury that will later allow for candidate selection for future therapeutic treatment. Neuroimaging and Analysis: BCAO pups were imaged using diffusion weighted imaging (DWI) and T2WI to monitor ischemic development at 24 hrs and 28 days in an 11.7 T and 4.7 T scanners (Bruker, Bruker Biospin). Analysis consisted of 3D volumetric reconstructions of whole brain volume and ischemic tissue volume using Amira (Mercury Computer Systems) software. Using the 24 hr imaging data, we developed a scoring system (rat pup scoring system; RPSS) that evaluated lesion appearance as a severity indicator (Fig. 1). Histology: After the first imaging time point animals were processed to confirm lesion size using cresyl violet. Neurobehavioral Testing: The accelerating rotarod (Columbus Instruments), activity test, and water maze were used to test for sensorimotor coordination, general activity levels, and learning and memory using protocols previously published[2] (Fig. 2).

Results

Ischemic injury was readily observed in all 16 animals on DWI and T2WI (Fig. 1A). The RPSS was developed as an animal correlate to the clinically used Barkovich score in order to differentiate animals into mild, moderate, and severe injury groups (Fig. 1). The average MRI-derived lesion volumes were 4.4±2.5%, 35.4±4.4%, and 57.0±2.2% of the total brain volume for mild (n=3), moderate (n=4), and severe groups (n=9). Histology confirmed lesion volume. Moderately-injured rats exhibed profound deficits on the water maze spatial navigation test (p<0.002), but eventually learned the task (Fig. 2). Severely-injured rats were incapable of learning the task (p<0.00001). Moderate levels of injury also produced a significant decrease in activity levels during a 30-minute open field test (p<0.018).

Conclusion

Our findings demonstrate that the BCAO is an excellent model of bilateral neonatal HIE in the term infant as it produces diffuse global injury. The BCAO can be manipulated to produce a gradation of injury with appropriate accompanying behavioral deficits that will be necessary in the selection of candidates for future therapeutic treatment. Finally, MRI can be used to monitor the evolution of HIE injury progression and will be important for evaluation of therapeutic interventions.

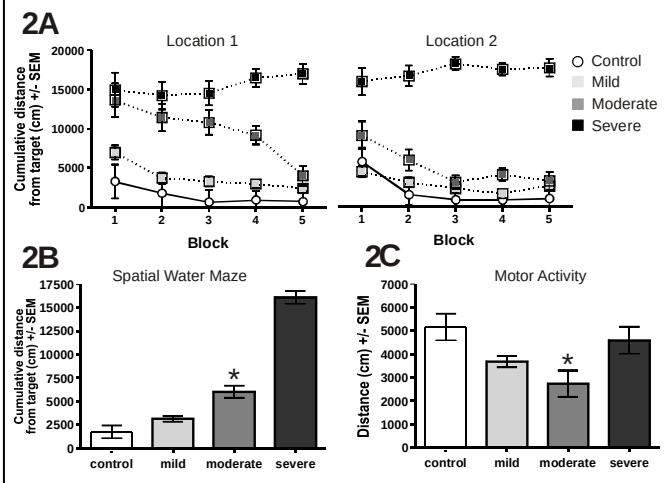

Fig. 2: The learning ability of BCAO rats was tested in both the visible escape platform (cued learning) and submerged escape platform (spatial learning) water maze tasks. A and B show performance on the spatial learning task in which animals learned two different spatial locations. The distance from the rat to the escape platform was measured five times every second and accumulated until the rat found the platform. A higher score thus reflects worse per-formance. Panel C shows a significant decrease in motor activity between moderate and control animals on the open field test.

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