The use of high-resolution MRI to evaluate brain injury in newborn mouse.

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INTRODUCTION: The neonatal brain is highly susceptible to apoptotic activation in response to hypoxia-ischemia (H-I) (1). To better understand this process, it is useful study newborn mice, particularly since transgenic strains are available. However, these animals typically weigh only 3 to 4 g on postnatal day 7 (P7). As a result, high spatial resolution is necessary. One approach to obtaining the increased signal-to-noise ratio required for high spatial resolution is to image at high field strength (11.74 T), though water/fat frequency shift artifact is much more prominent at high field strength. We were able to eliminate this artifact through the application of water/fat-selective excitation pulses (3). In this study, we acquired high-resolution, fat-suppressed, T_2-weighted (T2W) images on neonatal mice following H-I at P7.

ANIMALS AND NEONATAL H-I: P7 C57BL/6 mice were anesthetized with halothane and the left carotid artery was permanently ligated by cauterization. After a 1-hour recovery, the pups were place for 45 minutes in a hypoxia chamber kept at 37° C through which 8% oxygen (balance nitrogen) flowed. They were returned to the dam until MRI acquisition.

MAGNETIC RESONANCE IMAGING: MR images were acquired on a Varian UNITY*INOVA* MRI system (Palo Alto, CA, USA) with an 11.74 Tesla, 26-cm clear bore diameter horizontal Magnex Scientific (Oxford, UK) magnet equipped with an 8-cm-inner diameter gradient insert assembly (maximum gradient strength = 120 G/cm). Mouse pups were stabilized and kept anesthetized (isoflurane/O_2 0.4% ~ 1.0%) in a custom-designed mouse holder. Respiratory rate and body temperature were monitored and kept stable throughout the experiment. A quadrature rf Litzcage coil with a 15-mm diameter (Doty Scientific, Columbia, SC, USA) was employed for rf transmission and reception. T2W images were acquired using a multislice spin-echo sequence which incorporated binomial-series frequency-selective excitation 3°-15°-30°-30°-15°-3° plus spoiling gradient (3) in front of each slice-selection loop. The time delay between two adjacent pulses was set to 298.7 μs at 11.74 Tesla B_0 field so that during each delay a 180° phase shift was generated between water and fat magnetization. A group of seven P7 pups were imaged at 6, 12, 24, and 48 hours post H-I with the following parameters: TR 4 s, TE 80 ms, 4 averages, resolution of 59 × 59 × 250 μm, and acquisition time 1 hour. Animals were sacrificed at P14 for histology. In order to test the modified sequence, phantom and control animal experiments were conducted with and without the fat-saturation implementation using the following T2W parameters: TR 4s and TE 80 ms. The phantom was made with a 3-mm diameter outer glass tube filled with deionized water and inside which a 1-mm diameter inner glass tube filled with vegetable oil.

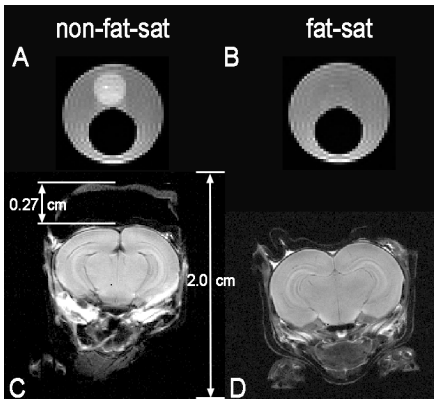

Figure 1 T2W images of a water/oil phantom (A-B) and two dead P7 control mice (C-D), with (B, D) and without (A, C) fat-saturation excitation pulses. Note the chemical shift artifact in C.

RESULTS AND DISCUSSION: Figure 1 shows T2W images, with and without fat-suppression (frequency encoding direction up-down). The bright circle in Figure 1A is shifted signal from vegetable oil. In Figure 1B, this signal was eliminated. Figures 1 C and D were acquired on two dead P7 control mice respectively. The chemical-shift distance of fat (Δx) is restricted by B_0, water/fat chemical shift (σ_{wf}) and applied readout gradient strength (G_{RO}) according to the following relationship: $\Delta x = (\sigma_{wf} \times B_0)/G_{RO}$ (4). This value was as large as 0.27 cm (Figure 1C) with our imaging parameters.

Imaging abnormalities were present as early as 6 hours after hypoxia. T_2 hyperintensity was evident in the striatum (Figure 2 A-C) and hippocampus (Figure 2 D-F) ipsilateral to carotid ligation. This acute injury detected by T2W imaging corresponded very well with the tissue loss analysis made at 7 days post H-I (data not shown). Figure 2 also demonstrates the evolution of tissue injury in the mice within 48 hours after H-I (only data obtained at 6, 12, and 24 hours are shown). Interestingly, we found that the full extent of injury was apparent on T2W images within 24 hours after hypoxia for all animals that are studied. One pup in the group was severely injured in cortex in addition to the common injury found in other animals (Figure 3).

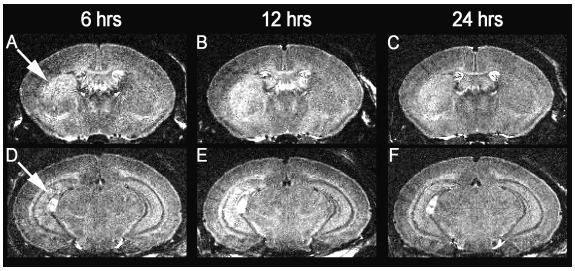

Figure 2 T2W images acquired at 6, 12, and 24 hours post H-I on a P7 mouse. Injury was found in the straitum (A-C) and hippocampus (D-F)

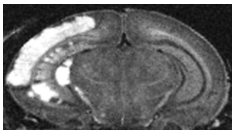

Figure 3 T2W image of a special case with injury in cortex and hippocampus

CONCLUSIONS: Our results demonstrate very early injury in the neonatal mouse brain following H-I. Surprisingly, T2W MR imaging shows the full extent of injury at 24 hours. In older animals, the evolution of changes on T2W imaging typically is slower (5). Binomial-series frequency-selective excitation pulses were incorporated into a conventional spin-echo imaging sequence to selectively saturate fat signal and hence suppress chemical-shift artifacts. Studies utilizing other MR techniques, such as diffusion tensor imaging, to evaluate the early cellular-scale CNS structural changes *in vivo* after H-I, are currently underway.

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