Segmentation and calculation of stroke- and manganese-enhanced volumes after reconstruction of the injured hemisphere in the Hypoxic-Ischemic Neonatal Rat Brain

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Introduction: Hypoxic-Ischemic injury (HI) to the premature brain may result in a prolonged period (weeks) of delayed neuronal death[1]. Manganese-enhanced MRI (MEMRI) can be used to follow this process of delayed cell death in vivo, as previously shown[2,3]. In order to follow the delayed cell death after HI, repeated measurements of volumes of brain hemispheres, cysts and manganese enhancement are necessary. However, on T_1 weighted images the border between cysts and the surrounding cranial tissue can be difficult to determine by automatic segmentation procedures, especially in the young pups. Here we present a semi-automatic method for reconstructing the boundary of the injured hemisphere enabling calculation of absolute and relative stroke- and manganese enhanced (ME) volumes for studying delayed neuronal death in the neonatal rat brain.

Materials and Methods: Animal experiments where conducted in accordance with Guidelines set by the Norwegian Ethics Committee for Animal Research and were approved by the responsible governmental authority. *Animals:* 7 day old (P7) Wistar rats (Scanbur, Norway AS) were used. 5 pups were sham-operated while 7 pups had their right carotid artery ligated under Isoflurane anesthesia (4% induction, 2% maintenance), followed by hypoxia (8% O_2) at 36ºC for 75min - resulting in a hypoxic-ischemic (HI) injury to the right brain hemisphere[4,5]. 6 hrs after the hypoxia and 24hrs before the 6 weeks scan, $MnCl_2$ (# 7773-01-5, Sigma-Aldrich Inc., St. Louis, USA) (100mM, 40mg/kg) was injected intraperitoneally to all pups.

MEMRI was performed 1 week (w) and 6w after HI (day 0) using a 7T magnet (Biospec 70/20 AS, Bruker Biospin MRI, Ettlingen, Germany) with water-cooled (BGA-12, 400 mT/m) gradients with a 72mm volume resonator for RF transmission and an actively decoupled mouse head surface coil for RF reception. During scanning the anaesthetized (2% isoflurane in 30% O_2, 70% N_2) pups lay prone in dedicated water heated mouse/rat beds (Bruker Biospin MRI). A 3D data set was obtained using a T_1-weighted gradient echo FLASH sequence with flip-angle = 30º, TR = 12ms, TE = 3.0ms. For the 1 week scan the FOV = 20×20×17.5mm and acquisition matrix = 128×96×84 giving an acquired resolution of 156×208×208 μm^3. Zero-filled to 128×128×112, the interpolated resolution was 156µm isotropic. Acquisition time was 25min with 16 averages. For the 6 weeks scan the FOV = 30x35x25mm and acquisition matrix was 172x150x108 giving an acquired resolution of 174×233×231 μm^3. Zero-filled to 172x200x144, the interpolated resolution was 174µm isotropic. Acquisition time was 52min with 16 averages. The spatially inhomogeneous sensitivity of the surface-coil was corrected for using two low resolution scans with 3D T1-weighted FLASH sequences with matrix size 32×32×32, but otherwise the same scan parameters as the high-resolution scans at each time-point Acquisition-time was 2min for each scan. The first scan used the surface coil for reception, whereas the second scan used the volume-coil for reception.

Data analysis: The corrected 3D dataset was used. The left and right intracranial volume was divided by a least square fitted mid-sagittal plane based on manually placed landmarks in the 3D volume. The intracranial volume of the non-injured hemisphere was segmented using 6-connected region growing and flipped around this mid-sagittal plane. This defined the intracranial boundaries for the injured hemisphere. To correct for asymmetries of the brain hemispheres caused by injury, a sagittal plane dividing the two brain hemispheres was fitted, the non-injured brain hemisphere was segmented and flipped around this new plane using the same procedure as described above. The reconstructed injured brain hemisphere was defined to be the result of the latter procedure being inside the intracranial boundaries for the injured hemisphere. To validate the reconstruction, animals from the Sham-group were used and the relative difference between the volume of the reconstructed and segmented hemisphere was calculated. Segmentation of ME and cystic volumes in the HI group was done by 6-connected region growing after a threshold based on the signal in a ROI placed in the non-injured hemisphere. A t-test on the relative difference between the segmented and reconstructed hemispheres with 5% significance level was conducted, using SPSS 14.0

Figure 1

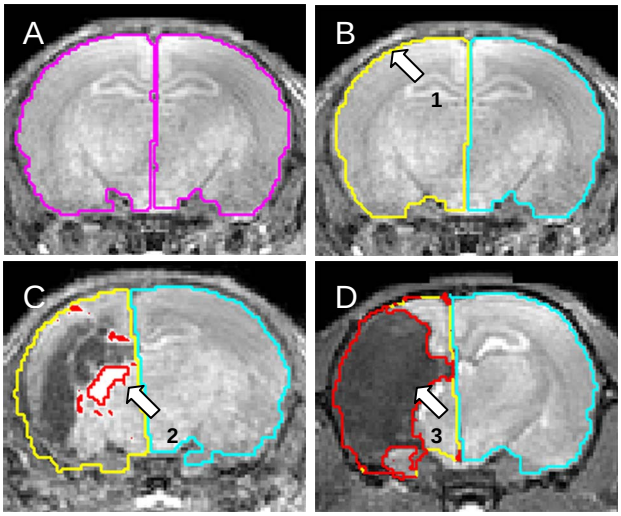

Figure 1: A: 3D Segmented left and right hemispheres of a 6 week old rat with no HI injury. **B:** Same as A, but with reconstructed right hemisphere (yellow [1]) based on the segmented left side. **C**: 3D segmented ME volume (red [2]) 1 week after HI. **D:** 3D segmented cystic volume (red [3]) 6 weeks after HI. Note how the segmented volume is restricted by the reconstructed hemisphere.

Results and Discussion: The animals from the Sham+Mn group did not develop HI and the segmented and reconstructed brain hemispheres corresponded well (Figure 1 A,B). A non-significant mean difference of 1.3% between the segmented and reconstructed hemisphere volumes (n=5 (1w)+n=4 (6w)) was detected, p = 0.951, 95% confidence interval CI = (-4.58, 4.84) %.

In the HI+Mn group the mean ME volume at 1w was 6.2% (SD=1.6%) of the reconstructed brain volume (Figure 1C). The cystic volume at this time was 45.7% (SD=8.9%). Six weeks after HI the cystic volume had increased to 62.6% (SD=8.3%).

In conclusion, we have developed a semi-automatic method for reconstructing and segmenting the HI injured hemisphere in the neonatal rat brain enabling calculation of absolute and relative stroke- and ME volumes. This enables in vivo longitudinal study of HI injury development and associated manganese enhancement. The method promises to be useful in the studies of effect of different treatments in the hypoxic-ischemic animal model.

References:

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