Brain ischemic lesion visualized by microCT

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Aims
The aim of this study was to determine whether microCT could be used as a method for assessing ischemic lesion size following introduction of middle cerebral artery occlusion in mice.

Method
To stain the brain tissue Omnipaque (GE-HealthCare) contrasting agent was used, while PBS was used as a control. Different dilutions of contrasting agent ranging from 1:2, 1:5, 1:10 and 1:20 were used to determine contrasting properties on non-stroked animals¹. Cerebral ischemia was induced by filament occlusion of the middle cerebral artery (MCAO)². Brains were scanned using 1076 µCT scanner. Images were obtained using 48 kV and 200 µA, which corresponds to a resolution of 18 µm, with a 0.6° rotation step, 0.5 mm aluminum filter and to reduce image noise frame averaging was set to a value of 5. Images were reconstructed using NRecon software, employing range of 0-0.07 on a histogram scale. DataViewer software was used to identically position all the scanned brains with the concurrent saving of transaxial data set.

In order to determine optimal dilution and gray/white matter contrast, standard X-ray attenuation units (Hounsfield units, HU) were calculated. Polypropylene tube of 1.5 mL volume was filled with water and used as a phantom. Phantom object was scanned in the micro-CT device using the same parameters used for brain scanning. To calculate the mean density value of HU for the water phantom CTAn software was used³. In the reconstructed brain images region of interest (ROI) consisting of 10 slices was analyzed in each, white matter (corpus callosum) and grey matter (cortex) and HU were calculated.

To assess ischemic area in the brain, color pallet in CTAn software was changed to Color 1. Within this color scale brain lesion, which had grater water content was clearly visible. Brain lesion area was manually traced and interpolated as a region of interest (ROI) and volume within ROI was calculated.

Results
Best staining properties Omnipaque contrasting agent showed at dilution of 1:2, while brain stained in PBS showed no morphological structures (Figure 1.A). Contrast for gray and white matter as well as the difference between them was calculated and plotted against Omnipaque dilutions (Figure 1.B).
Figure 1. Dilution influences Omnipaque contrasting efficacy (A). Grey/white matter signal was plotted against contrasting agent dilution (B).

When we compared SHAM operated to ischemic brains we observed no difference in grayscale while changing the color pallet revealed ischemic lesion (Figure 2.)

Figure 2. Brain tissue visualized by microCT in two color pallet: grayscale (A, C) and color 1 pallet (B, D). SHAM operated brain (A, B) and ischemic brain (C, D). Lesion extent and boundaries become clearly visible in color pallet 1.
Figure 3. Manually traced area (A) and 3D view (B) of the brain ischemic lesion.

Conclusion
We showed that microCT scanning of the brain can be useful as a method for assessing the volume of ischemic lesion and could possible replace laborious and time consuming histology methods in determining extent of the ischemic event.

References: