MEMRI provides morphological correlate of functional deficits observed by fMRI in experimental stroke

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Introduction: Normal cortical activity is often affected by structural alterations of neuronal circuitry after stroke. Brain infarcts with lesions, apparently closely similar based on anatomical MR imaging, can show very different behavior concerning the existence or severity of functional deficits. Thus, a morphological correlate of functional deficits is important to allow early distinction between permanent and transient loss of function after stroke.
Manganese-Enhanced MRI (MEMRI) has been utilized to describe architecture of neuronal circuits. As antero- or retrograde track tracing techniques are useful to elucidate intact neuronal pathways and connectivities between cerebral nuclei, we have investigated whether MEMRI provides functional information on cortico-thalamic connectivity. Validation has been achieved by co-injection of manganese with the invasive axonal tracer fluorogold, analyzed on tissue sections following the in vivo MEMRI experiments. It was our aim to demonstrate that brain activity after stroke depends on the preservation of certain brain circuits using Manganese-Enhanced MRI and fluorescent retrograde tracers.
Methods: Brain ischemia was induced by transient (60 minutes) occlusion of the middle cerebral artery in male Wistar rats (300-350g). After 6 months, animals were divided in 2 groups depending on recovery of somatosensory cortical activity, measured by fMRI. MR experiments were conducted on a 7T scanner (Bruker BioSpin, Ettlingen, Germany), equipped with 20 cm wide actively shielded gradients (200 mT/m) and using a custom-built 12 cm Helmholtz coil (transmission) and a 2.5 cm surface coil (detection), both actively decoupled. Spin-echo EPI images were acquired (TE=30ms; TR=3s; BW=150 kHz; 5 consecutive slices of 2mm; 400µm in-plane resolution). Functional activation imaging was achieved with BOLD contrast by electrical stimulation (2.0 mA; 3 Hz; 0.3 ms) of both forepaws alternatively. Statistical parametric activation maps were constructed with the software STIMULATE using a paired Student's t test ($p < 0.01$). MEMRI was performed injecting a mixture of 100nL $MnCl_2$ 0.1M plus 100nL fluorogold into the primary somatosensory cortex, ipsilateral to the lesion. T1-weighted images were acquired 30min, 24h, and 76h after Mn application. Animals were then perfusion fixated and brains were cryoprotected. Coronal tissue sections were used to detect fluorogold fluorescence.
Results: Animals with recovered cortical activity (group 1) demonstrated clear manganese enhancement along the cortico-thalamic pathway at 24 hours after injection. In animals of group 2 (no BOLD fMRI activity detectable) Mn^{2+} remained at the injection site without any detectable transport to the thalamus. Both groups showed fluorogold fluorescence in the somatosensory cortex, while only animals of group 1 showed fluorescence in the thalamus (Fig. 1). Other brain regions such as corpus callosum and contralateral somatosensory cortex showed fluorescent fibres and neurons in both groups, indicating the cortico-cortical transcallosal connections
Conclusions: The neuronal retrograde tracer fluorogold provides invasive information about anatomical connectivity. Manganese enhanced MRI reflects this connectivity on a large morphological level, and thus serves as a morphological correlate providing essential information about potential brain activity through the intactness of these fibres. Thus, recovery of functional deficits may be predictable through the morphological correlate of MEMRI demonstrating the structural intactness of nuclei connections.

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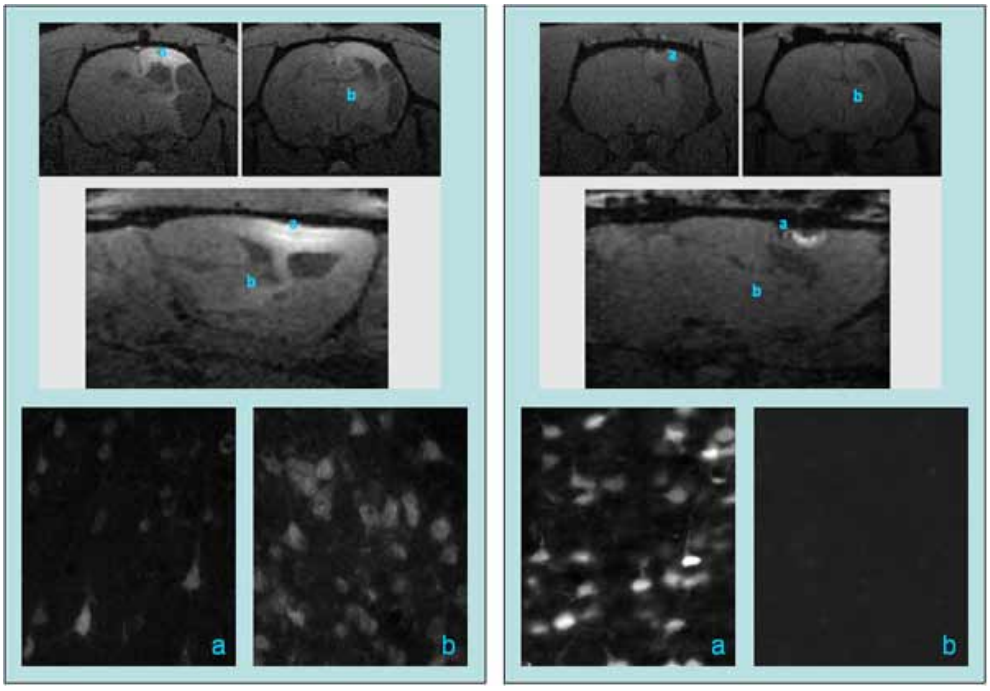

Fig. 1: T1-weighted images 24 h after manganese injection in the somatosensory cortex. Left panel: Manganese was detectable in the cortex (a) and along the cortico-thalamic pathway and the thalamus (b). Fluorescence images of the same regions (a and b), 76 hours after injection. Right panel: Manganese was only detectable in the cortex but was not transported to the thalamus. Fluorescence was detectable only in the cortical region (a) but not in the thalamus (b).