Early disturbance and subsequent reorganization of neuronal connectivity as studied with manganese-enhanced MRI after stroke in rats

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Introduction

Acute loss of sensorimotor function after stroke often partially recovers over time. Loss of function is due to acute neuronal damage, whereas restitution of lost function may be explained by reorganization of neuronal networks. Manganese-enhanced MRI (MEMRI) after intracerebral injection of paramagnetic manganese (Mn^{2+}), a calcium analogue, provides a tool for in vivo neuronal tract tracing [1]. The aim of this study is to characterize temporal alterations in structural connectivity in the sensorimotor network and correlate observed changes in connectivity to functional recovery after stroke in rats.

Materials and Methods

Transient focal cerebral ischemia was induced in adult male wistar rats (250-350g) (n=21) by 90 minutes occlusion of the right middle cerebral artery using the intraluminal suture technique described by Longa et al. [2]. Sham-operated rats (n=7) served as controls. In all rats, a general neurological score [3] was acquired and an adhesive removal test [4] was performed on day 3/4, day 6/7, day 9/10 and every week thereafter to assess functional status. In vivo neuronal tract tracing studies were performed using MEMRI at 2 (n=7), 4 (n=7) or 10 (n=7) weeks after ischemia induction, or at 4 weeks after sham operation (n=7).

Four days prior to manganese-enhanced MRI, 0.2 µl $MnCl_2$ solution was injected into the spared ipsilesional sensorimotor cortex (0.5 mm anterior, 1.5-3.0 mm lateral, and at a depth of 1.5 mm relative to bregma). MRI measurements were performed on a 4.7T horizontal bore spectrometer (Varian instruments (Palo Alto, CA, USA)) at 2 days prior to and at 4 days after $MnCl_2$ injection. Multi-echo, multi-slice T_2-weighted images (TR/TE = 3000/17.5 ms; echo train length = 8; acquisition matrix = 128 x 128; voxel resolution = 0.2 x 0.2 x 1.2 mm^3) were acquired for anatomical details and identification of the ischemic lesion. A saturation recovery, multi-slice T_1-weighted gradient echo sequence with 7 repetition times (TR/TE = 55-3000/18 ms; acquisition matrix = 128 x 128; voxel resolution = 0.2 x 0.2 x 1.2 mm^3) was used to calculate R_1 maps. Manganese-induced R_1 changes were determined in four ipsi- and contralateral regions of interest (ROIs) of the sensorimotor network (sensorimotor cortex (SMCX), caudate putamen (CPu), thalamus (Th) and substantia nigra (SN)) from pre- and post-manganese R_1 maps. One-way ANOVA followed by post hoc Bonferroni correction for pairwise multiple comparisons was used for statistical evaluation and P<0.05 was considered significant**.**

Results

Functional recovery was reflected by significant improvement of neurological score at day 17 and removal time of the sticky tape at day 10 as compared to the first measurement after stroke. Recovery of function stabilized at 2-3 weeks after stroke (Fig 1).

The sensorimotor network was clearly visualized using MEMRI (Fig 2A). Manganese-induced ΔR_1 was reduced in the ipsilateral subcortical sensorimotor network areas at 2 weeks after stroke, particularly in the substantia nigra (Fig 2B). ΔR_1 differences as compared to sham-operated controls were smaller or absent at 4 or 10 weeks after stroke. Additionally, ΔR_1 was significantly increased in the contralateral caudate putamen, thalamus and substantia nigra at 4-10 weeks after stroke (Fig. 2C).

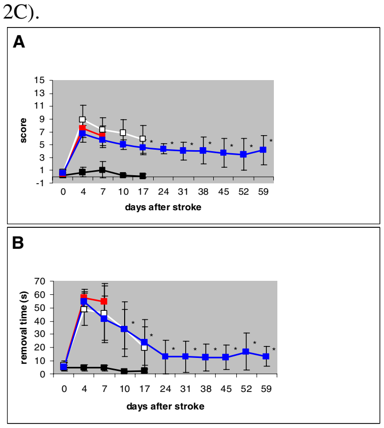

Fig 1. Neurological score ± SD (A) and time of removal of adhesive tape from the left forepaw ± SD (B) in sham-operated rats (■) and in rats subjected to MEMRI at 2 weeks (■), 4 weeks (□) or 10 weeks (■) after stroke. *P<0.05 vs. day 4.

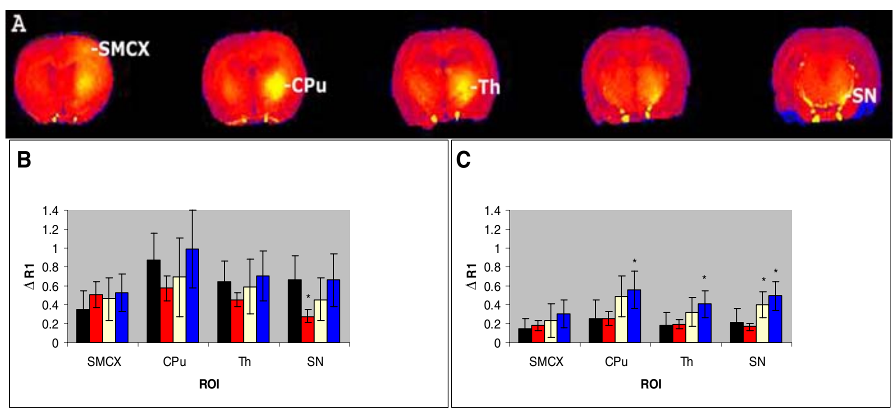

Fig 2. Manganese-induced R_1 changes at four days after $MnCl_2$ injection displayed on a color-coded R_1 map of adjacent coronal brain slices of a sham-operated rat (A). Mean ΔR_1 values ± SD in different ROIs of the ipsilateral (B) and the contralateral (C) hemisphere of sham-operated rats (■) and of rats 2 (■), 4 (□) or 10 (■) weeks after stroke. *P<0.05 vs. sham-operated rats.

Discussion

Reduced manganese-induced R_1 changes in the ipsilateral substantia nigra at two weeks after stroke suggest disturbance of neuronal connectivity, which may be associated with acute loss of sensorimotor function. At 4 and 10 weeks after stroke, when partial functional recovery was observed, our MEMRI data suggest that disturbed connectivity in the ipsilateral sensorimotor network was restored. In addition, interhemispheric connectivity appeared to be increased. In conclusion, mapping temporal changes in cortical connectivity after stroke using MEMRI may provide unique information on structural reorganization of neuronal networks and may give important insights in the underlying mechanisms of functional recovery.

References

1. Pautler RG et al. *Magn Reson Med* 40: 740-748 (1998).
2. Longa EZ et al. *Stroke* 20: 84-91 (1989).
3. Reglödi D et al. *Brain Res Bull* 59: 459-466 (2003).
4. Schallert T et al. *Pharmacol Biochem Behav* 18: 753-759 (1983).

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