# Enhanced functional and structural connectivity in the contralesional hemisphere after unilateral stroke in rats: A combined resting-state fMRI and MEMRI study

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## Introduction

Functional and structural MRI have provided improved insights into ipsi- and contralesional reorganization of neuronal networks in relation to functional recovery after unilateral focal cerebral ischemia in animal models.[1] Recent manganese-enhanced (MEMRI)[2] and resting-state functional MRI (rs-fMRI) studies in rats[3] have demonstrated initial loss and subsequent restoration of structural and functional connectivity between the ipsi- and contralesional hemisphere, which may be associated with changes in functional status after stroke. However, the relationship between post-stroke functional and structural connectivity changes remains largely unknown. The goal of this combined rs-fMRI and MEMRI study was to test our hypothesis that improved intrahemispheric functional connectivity in the contralesional sensorimotor cortex is associated with enhanced neuroanatomical connectivity at chronic stages after unilateral stroke in rats.

## Methods

Experimental stroke was induced by transient (90 minutes) intraluminal occlusion of the right middle cerebral artery (tMCA-O) in male adult Sprague Dawley rats (n=10). Sensorimotor function was measured longitudinally by scoring neurological deficiency and adhesive removal (in seconds) from the affected forelimb.[2] Structural MRI and rs-fMRI measurements were acquired before and at 3, 7, 21, 49 and 70 days after tMCA-O and, except for the pre-stroke measurement, at the same time-points in age-matched controls (n=10) on a 4.7 T horizontal bore Varian MR system. Multi-echo multi-slice $T_2$-weighted MRI (TR/TE = 3600/15 ms; echo train length = 12; 256×128 matrix; 19 coronal slices; 0.25×0.25×1.0 $mm^3$ voxels) and gradient echo 3D (GE3D) MRI (TR/TE = 6/2.58 ms; flip angle = 40°; 256×128×128 matrix; 0.23×0.31×0.31 $mm^3$ voxels) were conducted to determine ischemic lesion size and location, and for registration purposes, respectively. Then, for at least 10 minutes, end-tidal isoflurane was reduced to 1%, followed by 10 minutes of rs-fMRI with a $T_2$*-weighted gradient echo EPI sequence (TR/TE = 500/19 ms; flip angle = 35°; 64×64 matrix; 7 coronal slices; 0.5×0.5×1.5 $mm^3$ voxels, 1200 BOLD images). At 10 weeks after stroke, $T_1$-weighted MRI (Look-Locker gradient echo; TR/TE = 5000/3.4 ms; TI = 10 ms; TRimage = 8 ms (N=24); flip angle = 10°; data matrix = 64×64 matrix; 25 coronal slices; 0.5×0.5×0.5 $mm^3$) was included for baseline measurement of pre-manganese tissue $R_1$. For this MEMRI part of the study, only animals with both subcortical and cortical tissue damage at 3 days post-stroke were included (n=5) as well as seven control animals. At 72 days after tMCA-O, rats received an injection (90 nL) of $MnCl_2$ (0.1 M in PBS) in the left, contralesional primary motor cortex. $T_1$-weighted MRI and GE3D MRI were repeated at day 1, 3 and 7 after $MnCl_2$ injection.

Pre-processing included spatial smoothing with an isotropic Gaussian kernel of 1.0-mm FWHM, rigid-body motion correction and linear regression against i) rigid-body realignment parameters, ii) signals from the internal capsule and lateral ventricles and iii) global mean signal. Subsequently, low-frequency BOLD fluctuations were obtained from the functional time-series using a band-pass filter ($0.01 < f < 0.08$ Hz). GE3D images were registered non-rigidly to a reference image that was matched to a 3D model of a rat brain atlas.[4] Bilateral sensorimotor cortex regions-of-interest (ROIs), i.e. the primary and secondary motor cortex (M1, M2), forelimb region of the primary somatosensory cortex (S1FL), and the secondary somatosensory cortex (S2) were projected from the atlas onto the functional time-series and the $R_1$ maps. Functional connectivity was measured as the correlation coefficient r between low-frequency BOLD fluctuations and Fisher-transformed according to $z' = \ln((1+r)/(1-r))/2$. Manganese-induced $\Delta R_1$ was quantified in the above-mentioned ROIs from the pre- and post-manganese $R_1$ maps. Linear mixed model analysis with fixed effects, 'group', 'time' and 'group×time', and random effect, 'rat', was performed for statistical comparison of functional connectivity or $\Delta R_1$ over time and between control and stroke groups. Post hoc Sidak testing was used to correct for multiple comparisons. Pearson product moment correlation was performed to test for correlation between $\Delta R_1$ at 1 day post-injection and intrahemispheric functional connectivity with the contralesional sensorimotor cortex areas M2, S1fl and S2 for both stroke and control animals at the 10-weeks time-point. $P < 0.05$ was considered significant.

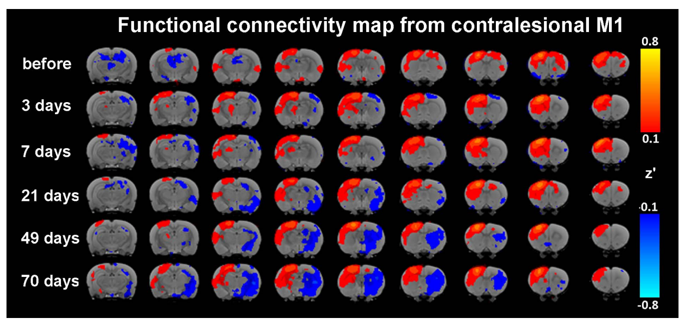


*Fig.1. Functional connectivity map with left, contralesional M1 as seed region overlaid on a $T_2$-weighted multislice anatomical rat brain template before, and at 3, 7, 21, 49 and 70 days after 90-minutes right MCAO (n=10).*

## Results

Figure 1 shows the temporal pattern of functional connectivity with left, contralesional M1 after t-MCAO. Before stroke, clear interhemispheric connectivity existed between M1 and the contralateral primary sensorimotor cortex. After stroke this interhemispheric functional connectivity was significantly reduced between contralesional M1 and intact, ipsilesional S1FL as compared to baseline and the control group. However, at 3, 7 and 10 weeks after stroke significant increases of intrahemispheric functional connectivity of contralesional M1 with M2, S1FL and S2 were found compared to the control group.

Manganese-induced $\Delta R_1$ in the contralesional M2, S1FL and S2 at 1 day after injection in contralesional M1 was significantly increased in stroke animals as compared to controls, in line with the increase in functional connectivity between these regions (figure 2). $\Delta R_1$ and functional connectivity with M1 in these ROIs correlated significantly for all animals (stroke and control group) ($r = 0.43$, $P = 0.009$).

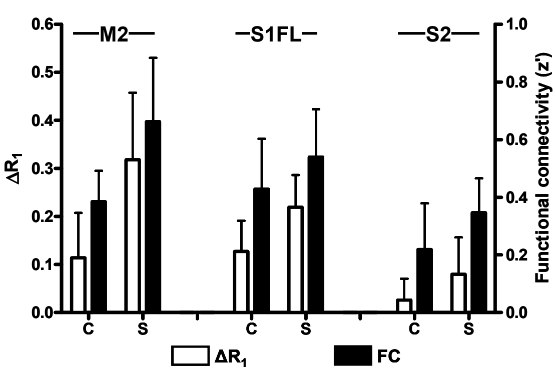


*Fig.2. Manganese-induced $\Delta R_1$ in contralesional M2, S1FL and S2 at 1 day after injection in M1, and corresponding functional connectivity (FC) between contralesional M1 and M2, S1FL and S2 for both control (=C) (n=7) and stroke (=S) (n=5) groups.*

## Discussion

This study shows a clear correspondence between functional connectivity, as measured with rs-fMRI, and neuroanatomical connectivity, as measured with MEMRI, in control and post-stroke rat brain. We detected an increase in structural and functional connectivity in the contralesional sensorimotor network connectivity at 10 weeks after tMCA-O. The improved structural and functional connectivity in the contralesional hemisphere could be reflective of post-stroke synaptogenesis and dendritic growth.[5] The detection of contralesional remodeling with rs-fMRI and MEMRI emphasizes the potential of these techniques to assess functional and structural reorganization of in vivo neuronal networks after stroke.

**References:** 1. van der Zijden JP et al., Textbook of in vivo imaging in vertebrates, Chichester: John Wiley & Sons, 2007: 239-41. 2. van der Zijden JP et al., J Cereb Blood Flow Metab 2008;28:832-40. 3. van der Marel K. et al., Proc. Intl Soc Mag Reson Med 2009;17:p698. 4. Paxinos G and Watson C, The rat brain in stereotaxic coordinates, Elsevier: Academic Press; 2005. 5. Jones TA et al., Brain Res 1996;733:142-8.