Tracing cortico-fugal projections \textit{in vivo} using high-resolution MRI with Mn$^{2+}$ induced contrast.

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The aim of this study was to investigate the potential of \textit{in vivo} MRI detection of Mn$^{2+}$ transport for tracing neuronal projections originating in the sensorimotor cortex in healthy and lesioned rat brains. Special attention was given to the potential of visualizing neuronal sprouting of central nervous system across the midline. Beside the cortico-spinal tract, the cortico-thalamic fibres were also visualized by anterograde Mn$^{2+}$ transport. Interhemispheric connections were found in healthy rat brains but highly enhanced fiber tract contrast connecting both hemispheres was visible 16 weeks after onset of focal photothrombotic cortical injury.

\textbf{Introduction}

In the past neuroanatomical tracing studies\cite{1, 2} revealing insights into neuronal connectivity required fixed tissue for data analysis and therefore, cannot be applied to longitudinal studies. Recently a technique was described, which allows \textit{in vivo} mapping neuronal pathways\cite{3, 4} by application of Mn$^{2+}$, a well known paramagnetic ion acting as T1 MRI contrast agent. Unchelated Mn$^{2+}$ are taken up, analogous to Ca$^{2+}$, by neurons and anterogradely transported as demonstrated with radioactive $^{54}$Mn$^{2+}$ by Sloot and Gramsbergen\cite{5}. Application of this method is of particular interest regarding the recent developments in the field of neuroregeneration. Axonal sprouting after focal brain lesions by inhibition of Nogo-A went together with functional recovery\cite{6}. The aim of this study was to investigate the potential of \textit{in vivo} MRI detection of Mn$^{2+}$ transport for tracing neuronal projections originating in the sensorimotor cortex in healthy and lesioned rat brains. Special attention was given to the potential of visualizing neuronal sprouting of central nervous system across the midline.

\textbf{Methods}

High resolution 3D T1-weighted MR images were acquired by a gradient recalled echo sequence on a 4.7 T Biospec DBX 47/30 spectrometer (Bruker Medical, Fällanden CH). Measuring parameters: TR = 15 ms, TE = 2.5 ms, $\alpha$ = 25°, NA = 8, isotropic resolution 195x195x195 µm$^3$.

Photothrombotic lesion was induced using a slightly modified method described by Watson et al.\cite{7}. 1 µl of 1 M MnCl$_2$ was injected into the forelimb area of the sensorimotor cortex (coordinates: Bregma 1.0 mm anterior, 2.5 mm lateral, 2.0 mm dorsomedial). In lesioned animals the experiments was performed 16 weeks after onset of photothrombic cortical injury. The sensorimotor cortex contralateral to the lesion was chosen for fiber tract tracing. MR image acquisitions were performed 6, 24 and 48 h after MnCl$_2$ infusion.

\textbf{Results and Discussion}

After injecting unchelated MnCl$_2$ into the forelimb area of sensorimotor cortex corticofugal projections could be traced through the internal capsule (ic) to the cerebral peduncle (cp) and the pyramidal tract (py) (Fig 1, 2). At the pyramidal decussation, the trace was lost in all animals. Although the neuronal tract was visible 6 h after MnCl$_2$ injection, best contrast was achieved after 24 – 48 h. Beside the cortico-spinal tract, the cortico-thalamic fibres were also visualized by anterograde Mn$^{2+}$ transport. Cortico-striatal fibres were partially covered by the very high signal near the MnCl$_2$ injection site.

Interhemispheric connections were found in healthy rat brains as slight, diffuse signal enhancement of cortical tissue contralateral to the MnCl$_2$ injection site. Highly enhanced fiber tract contrast connecting both hemispheres was visible 16 weeks after onset of focal photothrombotic cortical injury.

In conclusion our study has shown that we were able to visualize the main descending corticofugal projections and interhemispheric connections by non-invasive MRI after localized injection of MnCl$_2$. The method may bear potential to follow non-invasively gross plastic changes of connectivity in the brain after injury, as indicated by the increase of interhemispheric Mn$^{2+}$ transport after photothrombotic focal injury.

\begin{figure}[h]
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\caption{3D representation and frontal sections of cortico-spinal tract}
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\includegraphics[width=\textwidth]{figures.png}
\caption{Sagittal and frontal sections of the pyramidal tract}
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\caption{Frontal and transversal slice of a lesioned rat brain showing transhemispheric fibre tracts.}
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\textbf{References}
