Characterization of the 6-OHDA Model of Parkinson's disease using Manganese-enhanced MRI

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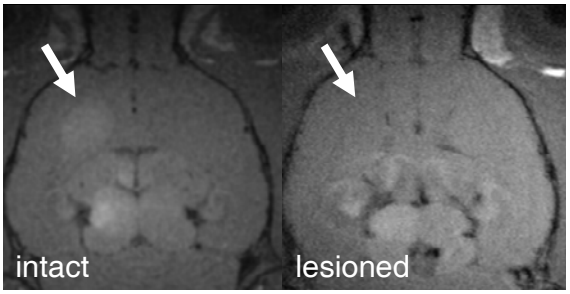
Objective.
Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra. The consecutive loss of dopamine in their main projection area, the striatum, causes the neurological symptoms of the disease. 6-Hydroxydopamine (6-OHDA) has a specific neurotoxic effect on dopaminergic neurons. In a well characterized animal model of PD, local injection of 6-OHDA into the striatum of rats leads to the retrograde degeneration of the dopaminergic nigrostriatal tract. The 6-OHDA model is routinely used to study neuroprotective or regenerative treatment strategies in a preclinical setting. Location and size of the lesion before and after experimental treatments are usually measured histologically by the quantification of dopaminergic neurons, which requires sacrificing the animals. In the present study, we investigated the use of *in vivo* Manganese-enhanced Magnetic Resonance Imaging (MEMRI) to characterize and quantify 6-OHDA lesions repetitively over time in the living rat.

Methods.
Adult male Sprague-Dawley rats received stereotactical injections of 6-OHDA into the right striatum, and saline injections of equal volume into the left striatum as a negative control. MR imaging was performed 24 hrs after the lesion, followed by weekly imaging over 7 weeks. Prior to each imaging session, some rats were injected intraperitoneally with 30 mg/kg $MnCl_2$ as a contrast agent. Some rats were stereotactically injected with 100 mM $MnCl_2$ into the substantia nigra 2 weeks after 6-OHDA lesioning for neuronal tract tracing. MR imaging was performed on a horizontal 7T Bruker Avance scanner with the brain centered in a 72/25 mm transmit/receive coil ensemble. Fast spin echo (FSE) 1 mm multi slice axial images were acquired through the brain (matrix 256^2, NA = 8, echoes = 8, TR/TE = 3500/14.15 ms, FOV = 3.2 cm). An identical set of FSE images with T_1 weighting (TR/TE = 355/10.25 ms) as well as 8 T_2 weighted axial images (TR/TE = 3500/20 ms, 8 echoes, FOV=3.2 cm, matrix 128^2) were acquired. A 2-D multi slice look-locker sequence (1) with 20 point samples along the inversion recovery curve was used to calculate T_1. Quantitative T_1 and T_2 maps were calculated using custom written software in MATLAB (Mathworks Inc., Natick, MA) taking into account the perturbation caused by flip angle in determining T_1.

Results.
T_2 weighted quantitative MR images of rat striatum over time following 6-OHDA lesion showed significant T_2 elongation 24 hrs after lesion compared to cortex (2.51-fold; $p<0.01$; 60 µg 6-OHDA), while saline injection showed no effect. At later time points post-lesion this effect was markedly reduced. Systemic application of $MnCl_2$ as a contrast agent led to T_1 shortening in lesioned striatum for up to 7 weeks after the lesion, indicating a persistent lesion of the blood-brain barrier. More extensive lesions (60 µg 6-OHDA) showed more pronounced effects than partial ones (20 µg 6-OHDA).
To trace the dopaminergic nigrostriatal pathway *in vivo*, we injected $MnCl_2$ into the substantia nigra of intact rats. This led to the transport of Mn^{2+} into the striatum, measured by a shortening in T_1 relaxation time (**see figure**). This signal peaked at 12-24 hrs post injection into substantia nigra and was still detectable after 36 hrs. No tracing occurred in rats with a lesioned dopaminergic pathway, indicating that only functionally intact neurons promote the transport of Mn^{2+} along the nigrostriatal tract (**see figure**).



Horizontal T_1-weighted MR-images of rat brains with intact (left) or lesioned (right) nigrostriatal pathway 24 hrs after injection of $MnCl_2$ into the right substantia nigra. Arrows point to right striatum. No tract tracing to the striatum occurred in the lesioned rat.

Conclusion.
MEMRI using $MnCl_2$ as a contrast agent can detect minor lesions of the blood-brain barrier in the striatum caused by 6-OHDA. Moreover, the ability of $MnCl_2$ to trace functionally intact neuronal pathways can be used to identify rats that have a degenerated nigrostriatal pathway. These techniques allow a non-invasive monitoring of neuroprotective or regenerative treatments in the living experimental animal over time, and can therefore facilitate the preclinical development of novel therapies.

Reference.
1. Chuang KH, Koretsky AP. Improved neuronal tract tracing using MEMRI with fast T_1 mapping. Magn. Reson. Med. 55(3):604-11, 2006.