In Vivo MRI of Neuronal Connections in the Macaque Monkey.

B. Prause[1], K.S. Saleem[2], J. Pauls[1], M. Augath[1], T. Trinath[1], T. Hashikawa[2] and N.K. Logothetis[1]
1 Max-Planck Institute for Biological Cybernetics, Spemannstr.38, 72076 Tübingen, Germany;
2 Riken Brain Science Institute, 2-1 Hirosawa, Wakoshi, Saitama 351-0198, Japan

We report high-resolution (0.5mm) in-vivo Mn^{2+} tracer studies of neuronal connections in the macaque monkey. Time-series of T1-weighted volume images up to 18 days reveal that manganese ions are transported anterogradely across several synapses, from the striatum through GP and SN into thalamic nuclei. In two cases the differential distribution of Mn^{2+} enhanced signal in GPe, GPi and SN after 24 and 45 hours was confirmed histologically using WGA-HRP injections into the same locations. The signal returns to pre-injection levels after four weeks with no discernible toxic effects.

Introduction Neuronal connectivity has been mainly studied by means of histological techniques, including anterograde and retrograde tracer injections, degeneration studies, and immunohistochemistry, and to a lesser extent by physiological studies, including those of cross correlation, optical imaging, stimulation, or inactivation. Using MRI, Paulter et al. [1] found that Mn^{2+} is transported anterogradely through axons and subsequently transsynaptically. Our study was focused on the output connections of the striatum in macaque monkeys, specifically the striato-pallidal and striato-nigral projections in-vivo, because these projections are well established by standard anatomical tracing methods.

Methods Three rhesus monkeys were injected between 0.1 and 1.0 µl of 0.8 mmol/ml solution $MnCl_2$ into the right head of the caudate nucleus and left putamen in two of the animals (cases 1 and 3), and vice versa in one animal (case 2). In a fourth monkey (case 4) we injected 0.1 µl into the left orbitofrontal cortex area13. Cases 1 and 2 received a focal injection of the antero- and retrograde tracer WGA-HRP into the same stereotaxic coordinates where the $MnCl_2$ was injected, after scanning for 24 and 45 hours, respectively. Segmented T1 weighted 3D-MDEFT [2] scans of the whole brain with τ=800ms, TR=21ms, TE=4ms at an isotropic linear resolution of 500 µm were obtained on a vertical 4.7T/40cm scanner. Angiographies of arterial blood flow in the brain were obtained after each MDEFT, using a Gradient-Echo Time-Of-Flight method (TR=22ms, TE=9ms) with the same voxel resolution as the anatomy scans. Cases 1 and 2 were scanned continuously under anesthesia for 24 and 45 hours. Cases 3 and 4 were scanned every two to three days for 8 and 18 days post-injection, respectively.

Results For each study all images were coregistered and intensity normalized using the first scan in the series as reference. The angiographies were used to subtract major blood vessels from the image volumes. We performed unpaired t-tests between groups of images acquired early and late during the studies, and used a single threshold ($P < 0.02$) to generated maps of significant signal increases. For all identified regions we obtained the average signal intensity and standard deviation as a function of time (Fig. 3).

Signal intensity changes reach above 100% in GP, up to 50% in SN and 40% in thalamic nuclei between 2 and 6 days post injection. Peak signal intensities are delayed in the thalamic areas VA/Vl and habenular nucleus compared to GP and SN, indicating that the Mn^{2+} is transported into the thalamus via GP and SN, thus crossing at least two synapses. Fig.1 shows the identical localization of Mn^{2+} and WGA-HRP tracers along characteristic fiber bundles in GPe and GPi that are anterogradely connected to the injection sites. Fig. 2 shows the signal changes in GP over the course of 18 days.

Case 4 shows strong signal increases in the ventral part of the caudate nucleus and the putamen (VS), the ventral pallidum (VP) and SN. VP and SN are known to receive projections from VS [3], indicating that Mn^{2+} ions cross at least two synapses between orbitofrontal cortex area 13 and SN/VP.

Case 3 and 4 were monitored for several weeks following injections, and showed no outward signs of neurological damage. In both cases signal returned of pre-injection levels after four weeks. This shows that Mn^{2+} can be used in vivo to study neuronal connections emanating from localized sites, which can be used in conjunction with fMRI or electrophysiology to elucidate detailed connectivity.

References 1. Paulter R et al, MRM 1998, 740. 2. Lee JH et al, MRM 1995, 308. 3. Haber SN et al, J. Neurosci 2000, 2369.

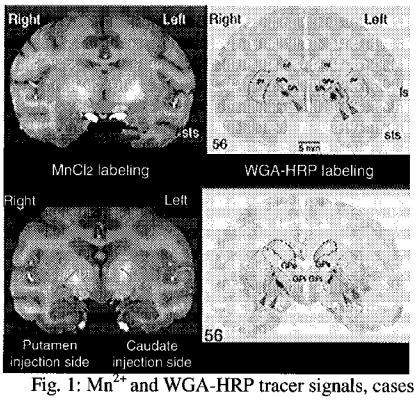

Fig. 1: Mn^{2+} and WGA-HRP tracer signals, cases 1 and 2

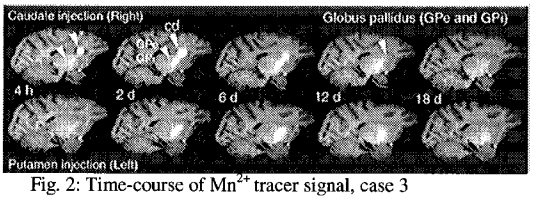

Fig. 2: Time-course of Mn^{2+} tracer signal, case 3

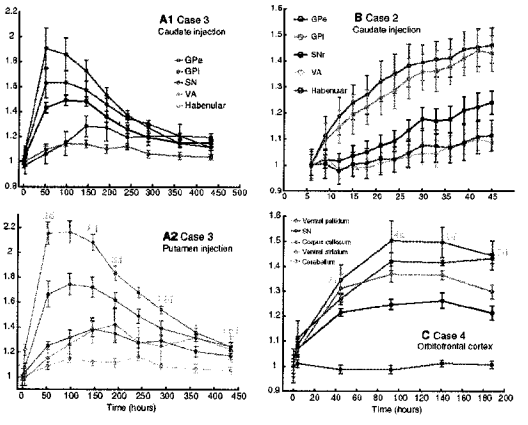

Fig 3: Time-course of signal intensity changes