In Vivo Quantitative Tract Tracing with Manganese Enhanced MRI

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Introduction

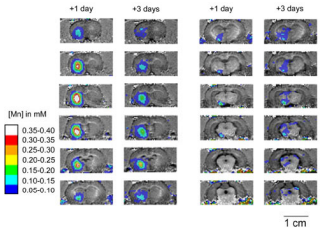
Studies of neural connectivity have contributed greatly to our understanding of the brain, enabling a qualitative understanding of the functional organization of different neural systems. Quantitative analysis of projections offers a more rigorous analysis of neural connectivity differences associated with normal development and aging, behavioral manipulations (e.g. learning, dominance changes), pathological rewiring associated with diseases and disease models, and sex and species differences. Attempts to quantitate connections have used a number of different methods involving histological sections, all of which require sacrificing the animal. The existence of an in vivo method of analysis would be very helpful for this sort of analysis. To this end, the recent development of *in vivo* tract tracing with injection of $MnCl_2$ coupled with magnetic resonance imaging (MRI) has been an enormous step forward. This technique depends on the anterograde transport of Mn in the central nervous system, as well as its paramagnetic properties, which results in the enhancement on T1-weighted images. In addition, because the change in the longitudinal relaxation rate (R1) is proportionate to the concentration of Mn, it is possible to quantitate the amount of Mn transported to different targets.

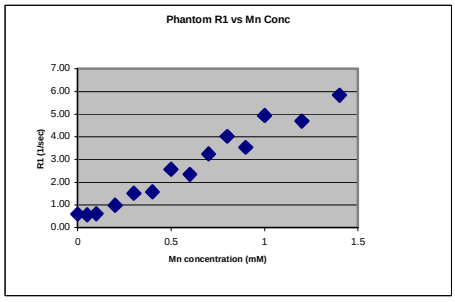
Methods

We anesthetized eight 300-450g male Sprague-Dawley rats with 50 mg/kg of pentobarbital and injected 200nl of 100 mM $MnCl_2$ unilaterally or bilaterally into the striatum (CPu). Some of these animals had also received unilateral injections of 6-hydroxydopamine into the substantia nigra (5% solution of 6-OHDA (Sigma) in 0.9% NaCl with 0.1% ascorbic acid infused unilaterally into the substantia nigra through a 0.5 µl Hamilton microsyringe). Before the infusion, and one and three or four days after the infusion, the animal underwent MRI scanning.

We used a Varian 4.7T/33 MRI scanner with a surface coil positioned on the animal's head using a plastic stereotactic head holder to place the center of the surface coil directly over the interaural line. A 3D gradient echo inversion recovery sequence was used with a TR of 5s, Te of 2.3 ms and TI's of 250ms, 500ms, 1000ms, 1500ms and 3000ms. We obtained T1 times using the method of Bluml and colleagues implemented in a Matlab® program. FOV was 4.8x4.8x1.2 mm, and the data matrix was 128x128x32 to give the voxel size of 0.375 mm x 0.375mm x 0.375 mm.

The 3D T1 maps were loaded into Analyze® version 5.0 software and converted to R1 maps, registered in 3 dimensions with rigid transformations and smoothed with a median filter using a 3x3x3 kernel. We then subtracted the baseline R1 map from each the day 1 and day 4 post-injection maps. These R1 difference maps were then divided by the slope obtained from the standards curve to obtain maps of Mn concentration. The standards curve was derived from scanning phantoms consisting of several 3 cm lengths of plastic tubing containing $MnCl_2$ (Sigma) at concentrations ranging from 0 to 1.5 mM in 0.9% NaCl at 37ºC. The same sequences were used for the phantom curves except the TR was 10s.



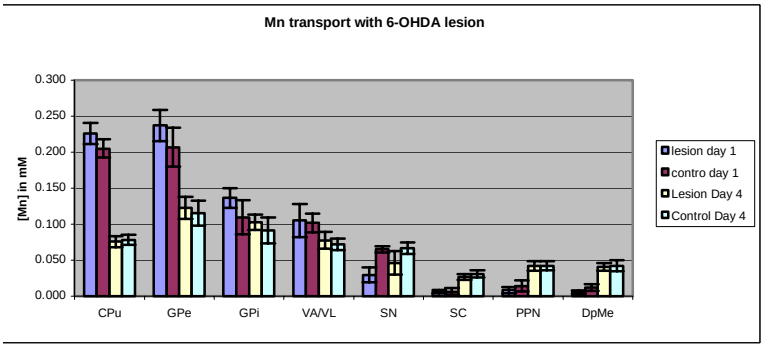


Results and Discussions

The standards curve (Figure on the right) revealed a linear relationship between R1 and Mn concentration. The slope of the curve was 3.94 sec^{-1}/mM Mn.

Mn transport was present at day 1 in the globus pallidus, and substantia nigra. At 3 days, there was further transport to the ventral anterior thalamus, lateral habenula, superior colliculus (SC), deep mesencephalic gray (DpMe), and pedunculopontine tegmentum (PPN). These projections were almost exclusively ipsilateral. This is consistent with literature reports of the first and second order projections of the striatum.

To assess the impact of changes in neuronal firing rate on Mn transport, we looked at the effects of unilateral lesions of the dopaminergic system. Several lines of evidence suggest that such lesions produce increases in firing rate in neurons projecting to the external segment of the globus pallidus (Gpe) and decreases in neurons projecting to the substantia nigra (SN) and internal segment of the globus pallidus (GPi). Thus, we would expect parallel changes in the amount of Mn transported to these structures on the lesioned side.



There were lower Mn concentrations in the lesioned SN as expected but this may have been confounded by the direct effect of the lesion there. Otherwise there were no differences between the injected side and the lesion side. This suggests that Mn transport in this setting is largely unaffected by neuronal firing rates.

Overall, we have demonstrated the feasibility of quantitative measurement of neuronal connectivity using Mn transport and its invariance with neuronal firing.

Acknowledgement : This work was supported by the STC Program of the NSF under Agreement No. IBN-9876754, NIH grant RO1EB002009, Georgia Research Alliance. We also acknowledge the technical assistance of Marcelia Maddox.

Reference: S. Bluml, R. Schad, B. Stepanow, W. Lorenz, 1993, MRM 30:289-295; Pautler RG, Koretsky AP, 2002,Neuroimage, 16:441-448