

# MR Spectroscopy of GABA and Other Brain Metabolites in Smelters Exposed to Manganese

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## Introduction

Exposure to excessive manganese (Mn) leads to psychological and motor disorders similar to symptoms seen in idiomatic Parkinson disease. Understanding the inherent vulnerability of glutamatergic and GABAergic systems to manganese (Mn) should provide critical insights into the mechanisms of Mn-induced neurotoxicity. We therefore used Magnetic Resonance Spectroscopy (MRS) in this study to determine *in vivo* concentrations of  $\gamma$ -aminobutyric acid (GABA), glutamate (Glu), N-acetylaspartate (NAA), total creatine (tCr) and other brain metabolites in the basal ganglia of a well-established cohort of Mn-exposed smelters from a Mn-Fe alloy manufacturer in China. Because manganese is paramagnetic, its distribution within the CNS can be visualized as hyperintense signal using T1-weighted Magnetic Resonance Imaging (MRI) [1]. Therefore high-resolution 3D whole brain T1-weighted imaging was performed on the same cohort, providing the possibility to visualize the areas with hyperintense signal in high detail.

## Materials and Methods

10 male highly Mn-exposed smelters (average airborne  $\text{MnO}_2 = 1.26 \text{ mg/m}^3$ , mean occupational exposure = 7.8 years) and 10 male age-matched control subjects with no history of Mn exposure (mean airborne Mn exposure =  $0.01 \text{ mg/m}^3$ ) from a Mn-Fe alloy manufacturer in China were recruited for this study. All MRI and MRS scans were performed on a 3T Philips Achieva whole body system. Mn, iron and copper levels in blood and urine were measured in all subjects prior to the MRI scan. A 3D T1-weighted gradient echo sequence (FOV=240x240x150 mm<sup>3</sup>, voxel size=1x1x1.25 mm<sup>3</sup>, 120 slices, SENSE-factor=2, TE/TR= 4.6ms/9.7ms) was used to obtain high-resolution images from the whole brain.

**Pallidal Index:** The pallidal index (PI) was calculated based on (a) the signal ratio between a region of interest (ROI) within the globus pallidus (GP) and white matter in the frontal cortex (PIwm) and (b) the signal ratio between the same ROI in the GP and a ROI in the neck muscle (PImu).

**MRS:** Single voxel 1H spectra (PRESS, TE/TR = 30ms/1500ms, volume of interest (VOI) size = 4 – 8 ml) were obtained from four different brain areas: thalamus, putamen, globus pallidus and frontal cortex. A water reference scan was acquired for each VOI. Concentration ratios of NAA, tCr, choline (Cho), myo-inositol (mI), glutamine+Glu (Glx) and Glu to brain water were obtained by fitting the raw spectroscopy data with LCModel [2], using only values with a Cramer-Rao lower bound (CRLB) < 20% for statistical analysis.

**GABA Editing:** In addition, a MEGA-PRESS pulse sequence was optimized to detect GABA (TE/TR = 68ms/2000ms) [3] in a volume centered on the thalamus, but also containing parts of putamen and globus pallidus ("GABAVOI"=3x3x3 cm<sup>3</sup>). 128 averages were acquired with the MEGA-PRESS editing pulse centered at 1.9 ppm and 128 averages with the pulse centered at 7.6 ppm in an interleaved fashion, but still allowing for phase cycling. GABA concentrations were obtained using LCModel with basis sets generated from density matrix simulations of the MEGA-PRESS sequence with an exact treatment of evolution during the two frequency-selective MEGA inversion pulses, and are corrected for changes in receiver gain.

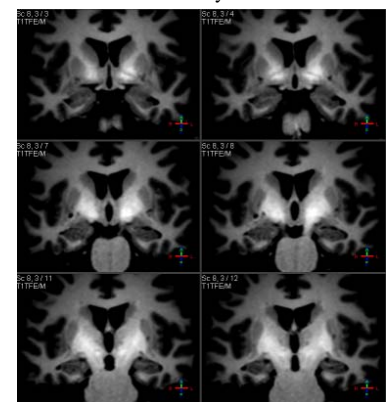


Fig.1: T1-weighted MRI images of a Mn-exposed subject.

## Results and Discussion

**MRI:** 8 out of 10 exposed subjects showed clear T1 shortening in the globus pallidus, but also in the subthalamic nucleus, inferior portions of the putamen, and medial cerebral peduncle (including frontopontine and cerebrospinal fibers of the corticospinal tract and the substantia nigra), revealing Mn deposition in these brain areas (Fig 1). PI<sub>mu</sub> and PI<sub>wm</sub>, based on the 3D T1w-MRI, showed 90% and 70% success in predictability, respectively, in identifying the Mn exposed smelters out of the 20 subjects scanned. This may reflect the fact that Mn has been shown to also accumulate in frontal white matter, making the PI<sub>wm</sub> less sensitive. Both PI values correlated significantly with the years of Mn exposure of the subjects.

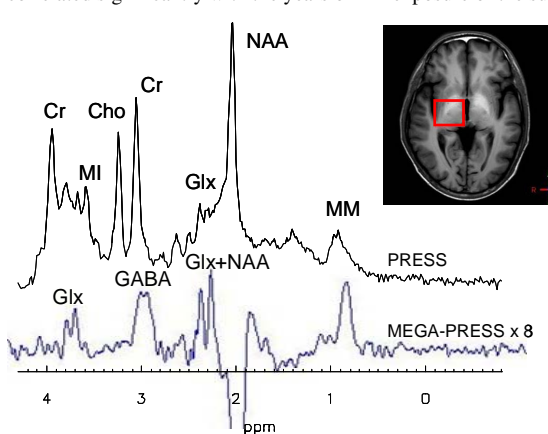


Fig.2: Short TE PRESS and MEGA-PRESS spectrum from a basal ganglia volume (GABAVOI)

**GABA:** A significant increase of GABA/tCr by 46% was found in the basal ganglia region due to Mn exposure ( $p < 0.05$ ). As the GABAVOI contains primarily thalamic tissue, this result is consistent with the hypothesis that an increased GABA concentration in the thalamus causes increased inhibition and thus reduction of thalamic signals to the cortex, which in return explains the hyperkinetic characteristics of manganese symptoms.

**Other metabolites:** The only significant group difference found between exposed and non-exposed subjects was a decrease in NAA/Cr in frontal cortex ( $p < 0.05$ ), similar to the decrease of NAA in parietal cortex found in [4]. While we found a decrease of NAA/Cr in the globus pallidus of the exposed group, this result was not significant, as six out of 20 NAA values had a CRLB > 20% and were thus excluded. No NAA changes were found in thalamus or putamen, consistent with other reports from basal ganglia. Moreover, no significant group differences were found for either Glu or Glx; however, a negative correlation between Glu in the globus pallidus and the Mn concentration in red blood cells was seen over all subjects ( $p < 0.05$ ). In summary, this pilot study demonstrates the feasibility of successfully measuring a variety of metabolites (notably GABA and Glu) in the basal ganglia of highly Mn exposed subjects.

## References:

- [1] Kim Y. et al., Neurotox 1999;20:901-907
- [2] Provencher SW. Magn Reson Med. 1993; 30:672-679.
- [3] Edden RAE et al., Magn Reson Med. 2007;58:1276-82
- [4] Guilarte TR et al., Toxicol Sci 2006; 94:3518