Supplemental Material

In Vivo Measurement of Brain GABA Concentrations by Magnetic Resonance Spectroscopy in Smelters Occupationally Exposed to Manganese

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Exposure data

Exposure data for both the smelters (the Mn-exposed group) and the age-matched controls can be found in Supplemental Table 1, including cumulative Mn exposure as well as a number of internal parameters: Mn, Fe, and Cu levels in blood plasma, red blood cells, and urine.

A guide to the GABA-VOI

The concentration of GABA is only on the order of 1-2 mM (de Graaf 2007). A relatively large 26.2 ml voxel (the "GABA-VOI") was therefore used for MEGA-PRESS spectroscopy in the basal ganglia to obtain sufficient signal. With dimensions of 30 mm (AP) × 35 mm (LR) × 25 mm (HF), the GABA-VOI was centered in each subject on the right thalamus, but it also contained the posterior portions of the putamen and globus pallidus, as well as parts of the substantia nigra. The positioning is illustrated in Supplemental Figure 1.

Spectral editing sequence for GABA and quantification of the GABA signal

In recent years the homonuclear MEGA-PRESS J-editing sequence (Mescher et al. 1998) has been adapted for the detection of GABA (Edden and Barker 2007; Waddell et al. 2007). It uses single voxel PRESS localization combined with frequency-selective narrowband editing pulses, which are applied in alternate scans to the C3 GABA multiplet resonance at 1.9 ppm or to a noise region in the spectrum at 7.6 ppm. The difference of the alternate scans results in selective observation of the outer lines of the GABA C4 triplet at 3 ppm and cancellation of the overlapping creatine methyl group resonance. A GABA-optimized MEGA-PRESS sequence (TR = 2000 ms, TE = 68 ms) was used in addition to the short TE single voxel scan to determine possible changes in GABA concentrations in the current study. 128 averages were acquired with the MEGA-PRESS editing pulse centered at 1.9 ppm ("ON spectra") and 128 averages with the pulse centered at 7.6 ppm ("OFF spectra") in an interleaved fashion, but still allowing for phase cycling of the localization pulses to minimize signal contamination from beyond the voxel. The resulting spectra were then corrected for any changes in receiver gain. A typical GABA-edited spectrum can be

seen in Supplemental Figure 2, in comparison to a non-edited short echo-time spectrum from the same volume of interest.

Using LCModel (Provencher 1993) for fitting of *in vivo* ^{1}H spectra is the gold standard in MRS. LCModel fits the spectra as a linear combination of the spectral signature of each metabolite. The spectral signatures, or "basis spectra" of all metabolites constitute the "basis set" and depend on the field strength, sequence parameters, RF pulse shapes and more and must either be measured in phantom solutions or simulated numerically for each used sequence. For fitting the MEGA-PRESS spectra, a basis set was generated from density matrix simulations of the sequence using published values for chemical shifts and J-couplings (Govindaraju et al. 2000), with an exact treatment of metabolite evolution during the two frequency-selective MEGA inversion pulses. [In contrast, the localization pulses were assumed to be hard pulses, a simplification that fails to model the partial cancellation of GABA signal caused by chemical shift misregistration (Edden and Barker 2007; Kaiser et al. 2008)]. Difference basis spectra were obtained by averaging the simulated metabolite response to selective inversion at 1.9 and 7.6 ppm. Because the subtraction was assumed to be perfect, only those metabolites with resonances close to 1.9 ppm were included in the simulations: GABA, glutamate (Glu), glutamine (Gln), glutathione (GSH), and NAA.

A known problem with estimating GABA concentrations from difference spectra is the presence of co-edited macromolecule (MM) signal at 3.0 ppm – a featureless bump that underlies the approximate GABA doublet there. Although LCModel uses the entire GABA difference lineshape in its calculations (including C2 peaks at ~2.22 and ~2.33 ppm), the MM signal at 3.0 ppm is a potential source of error. Two different approaches were used to model its effect. For method 1, nothing special was done – it was assumed that the flexible baseline of LCModel plus a built-in default macromolecular peak ("MM20") with a contribution at 3.0 ppm would fill in the bump. The resulting LCModel fits had satisfyingly small relative standard deviation values ("%SD") for GABA, but the concentrations were probably overestimated. For method 2, an extra Gaussian peak at 3.0 ppm ("MM30") was added to the LCModel calculation to explicitly fit the macromolecular signal. GABA concentrations obtained from method 2 were reduced by roughly a factor of two relative to method 1, but the %SD values were larger, reflecting the inherent uncertainty of fitting one experimental feature with two different functions.

GABA also co-edits with homocarnosine, a dipeptide composed of GABA and histidine (Öz et al. 2006; Waddell et al. 2007). Because the spectra of these two molecules around 2.0-3.0 ppm are expected to be similar, MEGA-PRESS editing tricks are unlikely to resolve them. Thus our "GABA" values are actually always GABA + homocarnosine. The latter is estimated to contribute 30% to the total GABA signal in the occipital cortex (Rothman et al. 1997) and roughly half of that amount in the substantia nigra (Öz et al. 2006).

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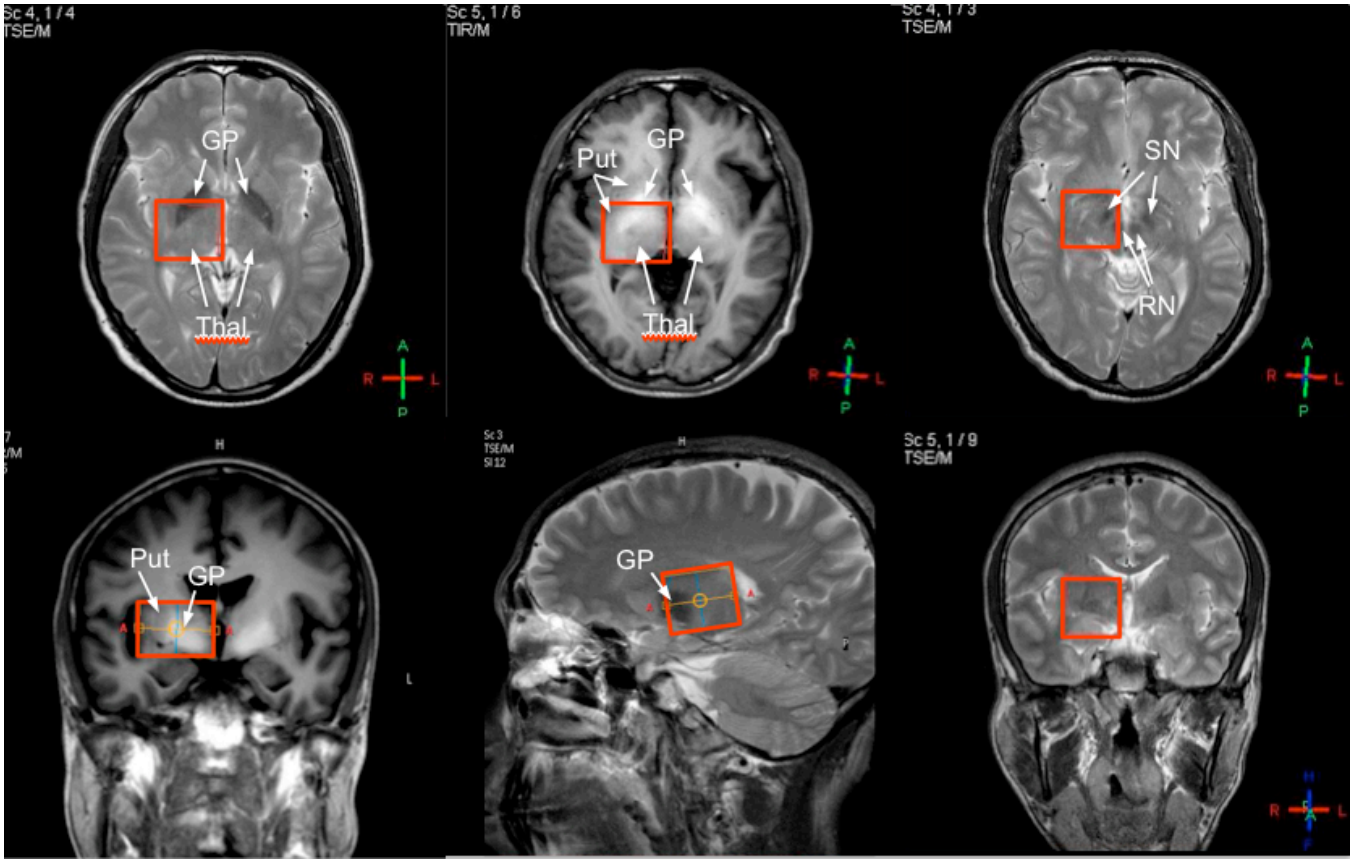
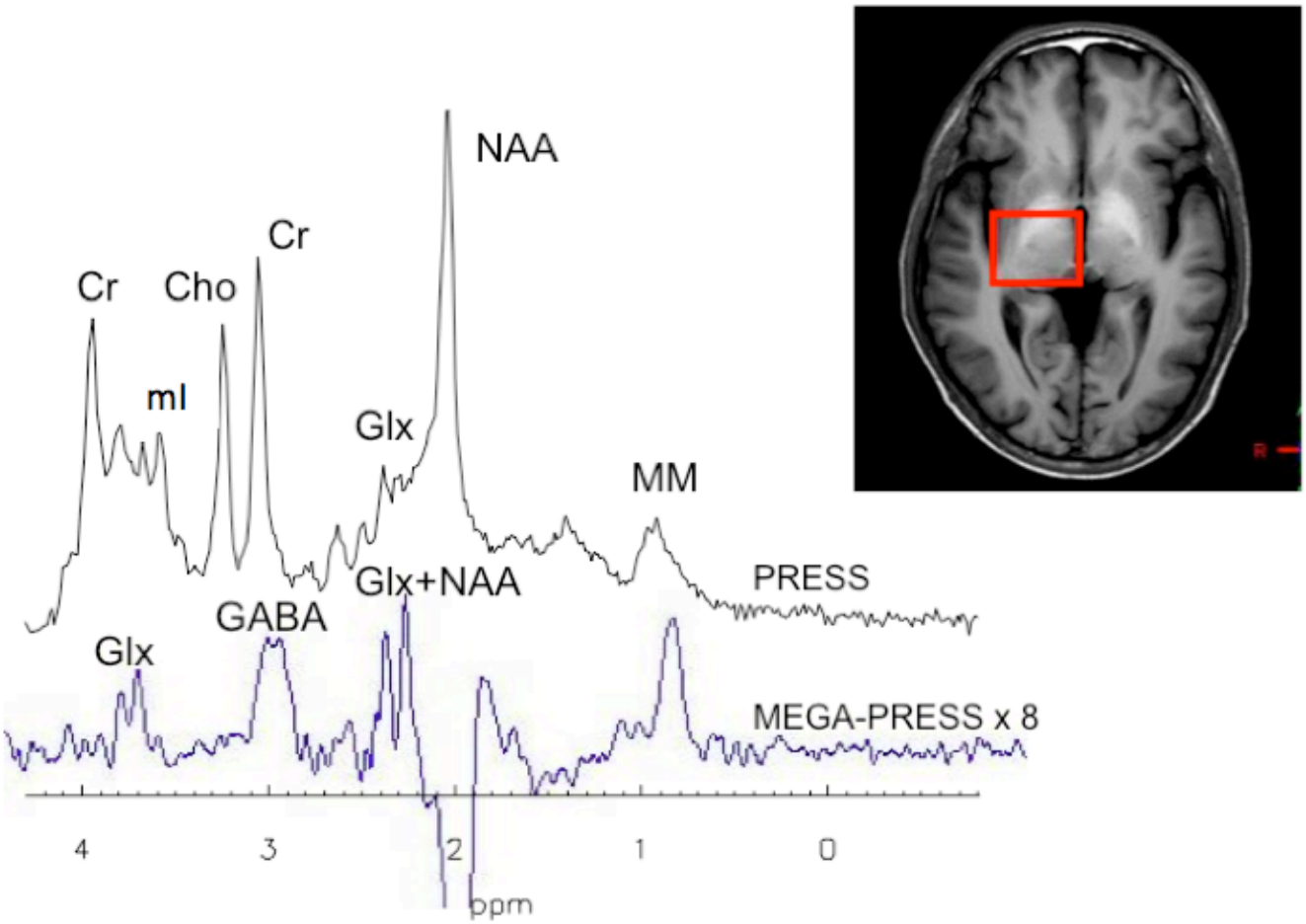


Supplemental Figure 1: Brain areas included in the GABA-VOI: Thal = Thalamus, GP = Globus Pallidus, Put = Putamen, SN = Substantia Nigra, RN = Red Nucleus



Supplemental Figure 2: Short TE PRESS spectrum (top) and GABA edited MEGA-PRESS spectrum (bottom, 8-fold enlarged along the y-axis) from the GABA-VOI delineated in the anatomical image. The short TE spectrum allows for quantification of the metabolites NAA, Cho, Cr, Glx (= Glu+Gln), and mI plus macromolecules and lipids (MM), whereas the MEGA-PRESS spectrum features the resonances of GABA, Glx and an inverted NAA peak.

Supplemental Table 1: Exposure data

Subject	Age (yr)	Mn Exposure (yr)	Cumulative Mn exposure (mg)	MnP[a] (ng/mL)	FeP[a] (g/mL)	CuP[a] (g/mL)	MnRBC[b] (ng/mL)	FeRBC[b] (g/mL)	CuRBC[b] (g/mL)	MnU[c] (ng/mL)	FeU[c] (ng/mL)	CuU[c] (ng/mL)
Smelters												
MN01	42	12	5505	1.1	0.998	0.69	40.4	761	0.533	1.42	9	5
MN02	45	6	2753	2.1	2.322	0.865	13.3	683	0.4	1.08	9	8
MN03	41	1	459	4	2.15	0.164	61.3	716	1.02	8.02	14	24
MN04	40	3	1376	1.4	1.503	0.891	17	659	0.422	0.587	14	23
MN05	38	3	1376	4.4	1.023	0.699	45.2	608	0.442	1.32	5	31
MN06	42	10	4588	1.5	1.229	0.599	22.5	608	0.409	0.372	9	33
MN07	36	1	459	4.8	1.021	0.664	28.4	663	0.441	0.329	5	19
MN08	38	12	5505	4.5	0.719	0.823	25.8	784	0.479	0.488	9	24
MN09	40	12	5505	2.4	1.02	0.596	80.1	610	1.18	1.09	9	10
MN10	45	7	2753	2.1	1.418	0.563	46.4	768	0.484	2.25	10	31
Controls												
MN11	46	n/a	n/a	3.1	0.901	0.749	21.2	329	0.315	1.33	5	5
MN12	41	n/a	n/a	0.5	0.921	0.502	22.4	691	0.454	0.155	9	4
MN13	42	1	31	3.9	0.791	1.134	24.3	626	0.344	1.26	10	5
MN14	41	0.5	15	6.5	0.718	0.53	29.8	929	0.73	1.1	10	5
MN15	37	2	61	3.1	0.974	0.894	31.9	696	1.372	2.14	29	5
MN16	54	5	153	0	0.68	0.605	23.2	701	0.516	2.08	34	24
MN17	57	3	92	2.9	0.895	0.866	17	684	0.462	3.17	9	18
MN18	42	3	92	2.5	1.4	0.964	14.8	644	0.363	0.478	9	5
MN19	37	5	153	3.6	1.569	0.616	81.7	721	0.632	1.56	9	8
MN20	37	3	92	7.3	1.305	0.547	105	699	0.73	0.375	5	19
Mean Exp	41±3	6.7±4.6	3028±2099	2.8±1.4	1.3±0.5	0.7±0.2	38±21	686±68	0.6±0.3	1.7±2.3	9±3	21±10
Mean Ctrl	43±7	2.8±1.6	86±50	3.3±2.3	1.0±0.3	0.7±0.2	37±31	672±146	0.6±0.3	1.4±0.9	13±10	10±8
p (t-test)			0.001									0.013

[a] MnP, FeP, CuP — Manganese, iron, copper in blood plasma
[b] MnRBC, FeRBC, CuRBC — Manganese, iron, copper in red blood cells
[c] MnU, FeU, CuU — Manganese, iron, copper in urine