# Detection of GABA, Aspartate and Glutathione in the Human Spinal Cord

Andreas Hock[1,2], Bertram Wilm[1], Giorgia Zandomeneghi[3], Garyfalia Ampanozi[4], Sabine Franckenberg[4], Nicola De Zanche[5], Jurek Nordmeyer-Maßner[1], Spyros S. Kollias[6], Thomas Kraemer[4], Michael Thali[4], Matthias Ernst[3], and Anke Henning[1,7]

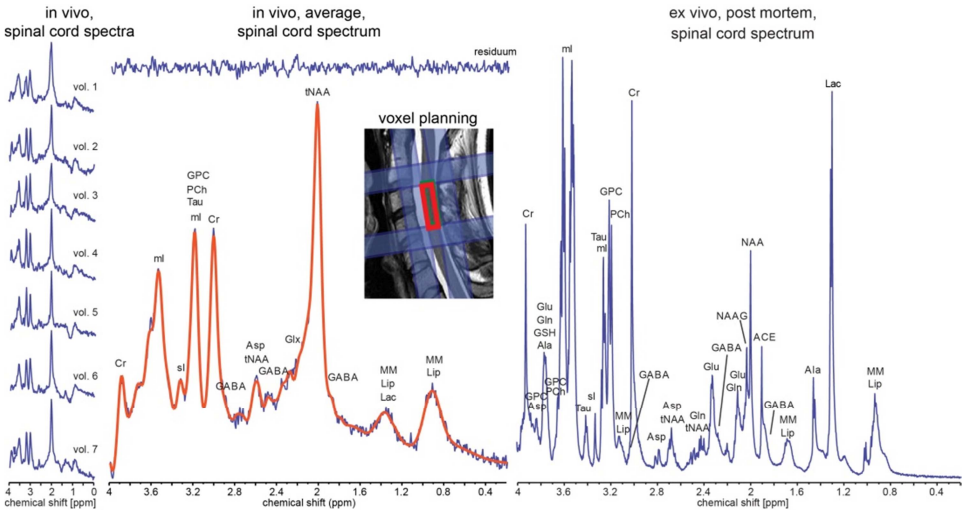
*[1]Institute for Biomedical Engineering, University and ETH Zurich, Zurich, Zurich, Switzerland, [2]Department of Psychiatry, Psychotherapy and Psychosomatics Hospital of Psychiatry, University of Zurich, Zurich, Zurich, Switzerland, [3]Physical Chemistry, ETH Zurich, Zurich, Zurich, Switzerland, [4]Institute of Forensic Medicine, University of Zurich, Zurich, Zurich, Switzerland, [5]Department of Medical Physics, Cross Cancer Institute and University of Alberta, Edmonton, Alberta, Canada, [6]Institute of Neuroradiology, University Hospital of Zurich, Zurich, Zurich, Switzerland, [7]Institute for Biological Cybernetics, Max Planck Institute, Tuebingen, Baden Württemberg, Germany*

**Introduction:** Spinal cord proton MR spectroscopy (MRS) provides insight into the spine-specific metabolism and is therefore a promising candidate to improve diagnoses, therapy planning and monitoring in the clinical routine. To date only the detection of the most prominent metabolite resonances has been reported in the healthy spinal cord: N-acetyl aspartate (NAA) (1), creatine (Cr) (1), choline (Cho) (1), myo-inositol (mI) (2), glutamate/glutamine (Glx) (2) and scyllo inositol (sI) (3). An increased number of detectable metabolites would improve the chances of finding predictive markers for disease progression or therapy response in various pathologies. However, additional markers are not easily accessible since the small diameter of the spinal cord leads to an inherently low signal-to-noise ratio (SNR) in these measurements. Additionally, the strong susceptibility changes around the cord as well as pulsatile CSF flow result in spectral artefacts (4) which can mislead data interpretation. Therefore, conclusions about concentrations of additional metabolites can only be drawn on the basis of high-quality spectral data from reproducible and robust measurements. **The aim of this investigation** was to further improve the spectral quality to allow for the determination of an extended metabolite profile of the spinal cord. Therefore, in vivo MRS data was acquired with specially designed motion and instability correction methods for the application to the spinal cord (3,5) as well as with a custom-made, neck coil array (6). In addition, for the first time, the findings were cross-validated with those of a human cadaveric spinal cord sample extracted from a human cadaver using high resolution, magic angle spinning NMR measurements enabling a qualitative indication of the presence of the metabolite markers found in vivo.

**Methods:** ECG-triggered, non-water suppressed PRESS localized inner-volume saturated MRS (3) (3 T Achieva, Philips Healthcare, Best, TE/TR = 30/2000 ms, voxel size = 1.2 ml, 512 averages/volunteer, 2000 Hz band width) was performed at cervical level C3-4 (Figure) in the spinal cord of seven healthy volunteers. ECG-triggered, higher order, projection-based $B_0$ shimming (5) was used to obtain narrow line width and good SNR. To further improve the SNR, the spinal cord of the volunteers was measured with a dedicated, custom-made, three element coil array, placed close to the neck (6). To further reduce the line width in individual spectra the high water peak of non-water suppressed MRS spectra was used for frequency and phase alignment of single acquisitions prior to spectral averaging. In addition, frequency and phase correction was also used for coherent averaging of data from different volunteers. Individual and averaged group spectra were quantified as in (3). Metabolite concentrations based on fitting results (LC Model (7)) with Cramer-Rao lower bounds (CRLBs) lower than 50% were considered in the quantitative analysis, and ratios of Cr were calculated. To validate the in vivo findings, a spinal cord sample at the level of C 3-4 of an approximate volume of 1 $cm^3$ was extracted from an autopsied corpse without any known trauma or other pathologies of the spinal cord. The extraction was performed at the local university's Institute of Forensic Medicine and was approved by the local ethics committee. The sample was measured with high resolution, magic angle spinning NMR spectroscopy according the protocol published by Beckonert et al. (8) using a Bruker TOPSPIN 600 MHz (14 T) (Bruker Corporation, Billerica, MA, USA) spectrometer. Acquisition parameters: sample temperature t = 278 K, rotation frequency $f_r$ = 2788 Hz, transmitter frequency $f_t$= 600.13 MHz, spectral width = 12019.3 Hz, Carr-Purcell-Meiboom-Gill sequence consisting of 32 refocusing pulses ($d_w$ = 1ms), TE= 2ms, TR=4s).

**Results and Discussion:** Compared to previous spinal cord studies (4), the spectral quality of all individual (SNR: 13.4 ± 2.3, line width: 7.8 ± 2.1 Hz) and group (SNR: 31, line width: 8.8 Hz) measurements was greatly improved. In addition, the figure shows all individual spectra proving that the method is robust and the spectral quality is reproducible. The table shows metabolite over Cr ratios, CRLBs and the number of volunteers in which the metabolite could be fitted (CRLB<50%). The high data quality allows a reliable (CRLB<25%) identification of Cr, NAA, mI, Cho, sI and Glx in individual spectra from all volunteers. Furthermore, an identification of GSH in all, Asp in four, Lac and GABA in three volunteers was possible with CRLBs below 50%. The group average spectra allow a reliable detection (CRLB<25%) of Cr, NAA, mI, Cho, sI, Glx, GSH, Asp, and GABA and results are in very good accordance with the individual measurements. Individual Lac levels may vary significantly, but the average concentration of Lac in healthy volunteers might be too small for reliable detection. The superior spectral resolution, achievable ex vivo, proved the qualitative existence of the in vivo quantified metabolites in spinal cord tissue, but methodological differences and disintegration processes (e.g. increased Lac and ACE, reduced NAA (9)) complicate the quantitative comparison of metabolite concentrations. Nevertheless, resonances of Cr, NAA, mI, NAAG, GPC, PCh, Tau, sI, Gln, Glu, GSH, Asp, Lac, Ala, ACE and GABA can be identified clearly ex vivo. By comparing the in vivo spinal cord metabolite concentrations with in vivo measured brain estimates derived from (10) increased mI/Cr and sI/Cr can be observed (Table). In addition, Asp/Cr is potentially increased and GABA/Cr may be decreased. The reason for the different metabolite profile of the spinal cord compared to the brain may be its distinct tissue composition and physiology.

**Conclusion:** The identification of metabolites such as Asp, Lac, GSH, and GABA, in the spinal cord ex vivo suggests that the quantification in vivo is feasible, provided that the spectral quality is sufficient (e.g. using group analyses). In diseases such as multiple sclerosis identified alterations of these metabolites could stimulate the development of novel and individual treatment concepts.




| *table 1* | | *NAA/Cr* | *mI/Cr* | *Cho/Cr* | *sI/Cr* | *Glx/Cr* | *GSH/Cr* | *Asp/Cr* | *GABA/Cr* | *Lac/Cr* |
|---|---|---|---|---|---|---|---|---|---|---|
| spinal cord (individual) | conc/Cr | 1.2±0.1 | 3.2±0.6 | 0.4±0.1 | 0.2±0.1 | 2.4±0.5 | 0.3±0.2 | 0.5±0.2 | 0.4±0.1 | 0.3±0.1 |
| | valid # | 7 | 7 | 7 | 7 | 7 | 7 | 4 | 3 | 3 |
| | CRLB [%] | 6±2 | 6±1 | 7±1 | 13±4 | 15±4 | 30±9 | 31±12 | 39±9 | 24±4 |
| spinal cord (group) | conc/Cr | 1.0 | 2.8 | 0.4 | 0.2 | 2.1 | 0.1 | 0.6 | 0.4 | |
| | valid # | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - |
| | CRLB [%] | 2 | 4 | 3 | 7 | 8 | 21 | 15 | 22 | |
| brain Cr ratios, (min-max) | | 0.8-3.78 | 0.36-1.59 | 0.08-0.49 | 0.03-0.12 | 0.85-3.59 | 0.19-0.39 | 0.09-0.27 | 0.52-2.11 | 0.05-0.11 |

1. Cooke F. J. et al., Magn Reson Med 2004;51(6):1122-1128.
2. Henning A. et al., Magn Reson Med 2008;59(6):1250-1258.
3. Hock A. et al., Magn Reson Med 2013;69(5):1253-1260.
4. Hock A. et al., Am J Neuroradiol 2012.
5. Hock A. et al., NMR Biomed 2013;26(3):329-335.
6. Wilm B. et al., Proc Intl Soc Mag Reson Med, Berlin, Germany 2007;3537.
7. Provencher S. W. Magn Reson Med 1993;30(6):672-679.
8. Beckonert O. et al., nature protocols 2010;5(6):1019-1032.
9. Ith M. et al., Magnetic Resonance in Medicine 2002;48(5):915-920.
10. Govindaraju V. et al., NMR Biomed 2000;13(3):129-153.