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Original contribution

Performance of MRS in metabolic profiling of the lumbar spinal cord in rat and mice

Saeedeh Amirmohseni, Lydia Wachsmuth, Nathalie Just, Cornelius Faber *

Department of Clinical Radiology, University Hospital Muenster, Germany

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ABSTRACT

Object: In vivo magnetic resonance spectroscopy (MRS) of the rodent spinal cord (SC) is technically challenging. We investigated the feasibility of MRS in the SC of both rat and mice, by comparing the spectral characteristics. We assessed possible species dependent differences in the suitability for non-invasive metabolite monitoring in the SC.

Materials and methods: MR spectra using a STEAM sequence were acquired from a rectangular voxel in lumbar SC of rats and mice, after a two-step shim procedure.

Results: In addition to total choline (tCho) and total creatine (tCr), seven and eleven metabolites were reliably detected in rats and mice, respectively. No significant differences were observed in metabolite concentrations or spectral characteristics between species.

Conclusion: Identification and quantification of major metabolites including the neurotransmitters γ-aminobutyric acid (GABA) and glycine (Gly) in the SC was successful in both rat and mice showing that investigation of SC neurochemical profiles is feasible in both species.

1. Introduction

The neurochemical profile of the spinal cord (SC) may serve as biomarker for a number of pathologies that are related to alterations in concentrations of metabolites or neurotransmitters. Excitatory and inhibitory neurotransmitters such as glutamate (Glu), γ-aminobutyric acid (GABA) and glycine (Gly) have concentrations in the millimolar range and can be studied non-invasively by proton magnetic resonance spectroscopy (^{1}H MRS) in mouse or rat models. In the rodent brain, the neurochemical profile including at least 20 metabolites has been measured with a high spectral resolution provided by short-echo time sequences at high field strengths [1–3]. Though vastly employed in brain, MRS in rodent models of SC disease is rarely conducted [4–9], since low spectral resolution remains an unsolved issue in the SC, due to physiological motion or to strong local susceptibility variations at the interface of soft tissue and air or bone. Furthermore, models of disease often involve surgical interventions [10], which introduce further inhomogeneities, necessitating robust B_0 shimming procedures to achieve sufficient spectral resolution. Efficient shimming methods such as FASTMAP (Fast, Automatic Shimming Technique by Mapping along Projections), FLATNESS (Five Linear Acquisitions for up to Third-order, Noniterative, Efficient Slice Shimming) or DYNAMITE (DYNAmic Multi-coil TEchnique) [11–14] have enabled drastic improvements of the spectral quality in the rodent brain. Yet, efficient minimization of the B_0 inhomogeneity in SC, which is fundamental for biochemical investigations of disease progression and treatment outcome using MRS, remains challenging.

Several models of SC injury (SCI) including traumatic, inflammatory and ischemic injury have been established in both rat and mice [15–17], and have been investigated by MRI non-invasively [5,18–20]. While rat models provide similar physiology to humans [21], a larger size, and easier handling for surgical interventions, mouse models are available with genetic modifications mimicking a large number of pathologies [22]. Possible differences in suitability for non-invasive monitoring of neurotransmitter levels in the SC, due to physiological differences between the two species or due to technical challenges, have not been addressed systematically to date. In this work, we assessed the neurochemical profiles of the lumbar SC (L1) of naïve rats and mice. To this end, it was essential to use an efficient shim protocol

Abbreviations: MRS, magnetic resonance spectroscopy; SC, spinal cord; tCho, total choline; tCr, total creatine; Glu, glutamate; GABA, γ-aminobutyric acid; Gly, glycine; SCI, spinal cord injury; L1, first lumbar vertebrate; CRLB, Cramer–Rao lower bounds; Ala, alanine; PCr, phospho-creatine; Gln, glutamine; GSH, glutathione; Ins, inositol; Lac, lactate; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; SNR, signal to noise ratio; OVS, outer volume suppression.

* Corresponding author at: Department of Clinical Radiology, University Hospital Muenster, Albert-Schweitzer-Campus 1, Building A16, 48149, Muenster, Germany. Tel.: +49 251 83 57608; fax: +49 251 83 52067.
E-mail address: faberc@uni-muenster.de (C. Faber).

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to achieve sufficient spectral resolution and signal to noise ratio (SNR) for a reliable detection of neurotransmitters.

2. Materials and methods

All experiments were carried out according to the German Tierschutzgesetz and were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz of Nordrhein-Westfalen, Germany.

2.1. Animals

Three groups of *Sprague–Dawley* rats and one group of *C57BL/6* mice were included. To establish a shim procedure in the SC, adult male rats were used ($n = 6, 311 \pm 26$ g) and spectra were acquired to validate the shim procedure ($n = 5, 296 \pm 16$ g). Species-specific differences were assessed comparing adult female rats ($n = 5, 286 \pm 42$ g) and adult female mice ($n = 6, 28 \pm 2$ g). To assess possible gender differences in the different groups used for shim validation and SC MRS in rats, the spectra from the adult male and female rat groups were compared. Animals were kept under the standard conditions of 12 h light–dark cycle with ad libitum access to food and water.

MRI examinations were performed under isoflurane anesthesia (1.5–2% isoflurane in compressed air/O_2 80:20, 1 L/min). Body temperature and respiration rate, for gating, were monitored (SA Instruments, Inc., Stony Brook, NY).

2.2. MRI measurements

Measurements were performed on a Bruker BioSpec 94/20 small animal MR scanner (Bruker BioSpin GmbH, Ettlingen, Germany) operating at 400 MHz. For mice, a quadrature transmit/receive Cryoprobe™ (Bruker) with two coil elements (20×27 mm^2 and 10 mm radius) was used with animals placed in prone position, with the end of the rib cage (the level of lumbar SC) positioned in the center of the coil. For rats, a 10-mm planar surface coil in combination with a transmit volume coil was used. To minimize respiratory motion, rats were positioned supine with the coil beneath them on the cradle. To further restrict motion in L1, careful fixation of the abdominal part of animals was performed.

Anatomical images for MRS voxel positioning were acquired using an Intragate FLASH sequence in multiple orientations (TR/TE/flip angle: 84/1.8 ms/30°; number of repetitions: 10; slice thickness: 0.75 mm, number of slices: 8; spatial resolution for mice/rat: 97/176 μm).

2.3. Shim procedure

Second-order respiration-triggered shimming using FASTMAP [11] (TR: 800 ms; BW: 7000 Hz; acquisition size: 128) was performed in all groups following a two-step procedure: First, within a cubic voxel placed at the center of L1 inside the SC (voxel volume, 5.8 ± 0.61 μl), and second, in a larger voxel covering the whole L1 (voxel volume, 106.1 ± 19.59 μl) (Fig. 1). Since FASTMAP performs iteratively, better initial conditions (after shimming in a smaller voxel) may improve the outcome. The results of this two-step procedure were compared in a group of six rats to those of a one-step shimming procedure using only a single large voxel, by comparing the full width at half maximum (FWHM) of the tissue waterline, as well as the FASTMAP-specific parameters including B_0 field variations along the FASTMAP projections (Δf) and evolution time. Evolution time is an extra echo delay in a stimulated echo sequence, during which magnetization evolves. Traditionally, in most conventional shimming strategies, B_0 field variations are deduced from the phase differences estimated during this evolution time. To avoid artifacts, multiple evolution times are used with a higher sensitivity for optimizing B_0 homogeneity at longer evolution times. The possibility of acquiring data at longer evolution times usually represents increased B_0 homogeneity.

2.4. MRS measurements

MRS voxels were positioned in the SC at the level of the L1 vertebra. The acquisition volume ranged from 18.0–25.9 μl in rats and from 3.6–5.8 μl in mice, depending on the individual size of the SC. A respiration-triggered Stimulated Echo Acquisition Mode (STEAM) sequence was used (TR/TE: 2160/3 ms; spectral points: 2048; spectral width: 4.8 kHz) with 1000–1500 averages, depending on the voxel size. At the start of the sequence, a reference water signal was acquired for calculation of metabolite concentrations. MRS acquisitions lasted between 43 and 59 min.

2.5. Data analysis

MRS data were analyzed using Linear Combination of Model spectra (LCModel) with a basis set of 19 simulated metabolite spectra (including Gly) for short TE at 9.4 T. Metabolites with Cramer–Rao lower bounds (CRLB) ≤25% were considered reliable. The spectra were referenced to the NAA peak, and the metabolite ratios were calculated

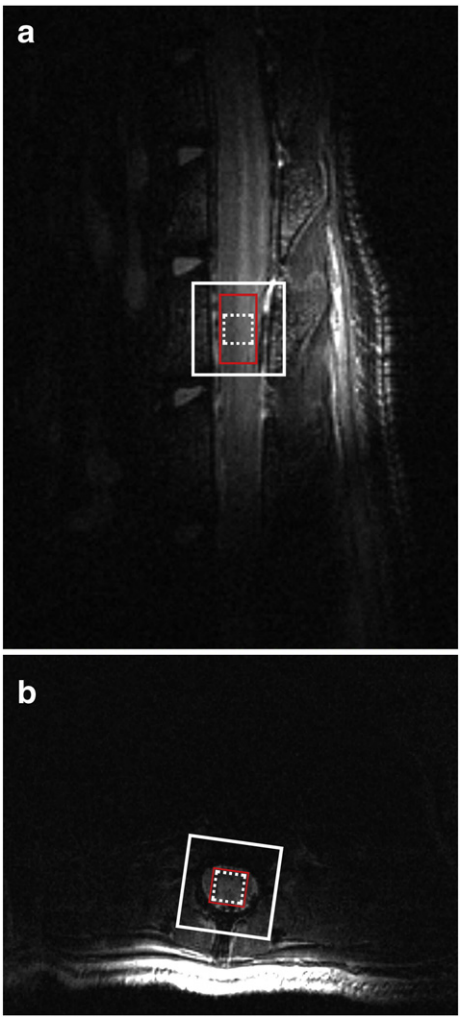

Fig. 1. Schematic view of the shimming and MRS voxels in rat. FASTMAP shimming was performed in two steps: first, within a small cubic shim voxel defined at the center of L1 (dotted white line, $1.8 \times 1.8 \times 1.8$ mm^3), and second, within a larger cubic voxel covering the SC at L1 vertebra level (solid white line, $4.8 \times 4.8 \times 4.8$ mm^3). MRS voxel was localized within the L1 (red line, $2.1 \times 2.2 \times 4.5$ mm^3).