MRS in mouse models of neurodegenerative diseases

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Enormous progress has been made in the last decade towards elucidating the pathogenesis of human neurodegenerative diseases. The successful characterization of pathogenic mutations in several inherited diseases together with genetic engineering enabled the creation of transgenic and knock-in mouse models of human neurodegenerative diseases, such as Huntington's disease (HD), spinocerebellar ataxia (SCA1), early-onset familial Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (1-4). These mouse models require advanced diagnostic methods to characterize the disease progression and the effectiveness of therapies. In vivo MR spectroscopy (MRS) has the potential to become an invaluable method for a non-invasive monitoring of brain neurochemistry in longitudinal studies using mouse models (5,6). However, ^{1}H MRS of the mouse brain is technically rather challenging due to the small size of the brain and a strong B_0 inhomogeneity induced in the brain by air/tissue interfaces. The aim of this article is to provide some recommendations to maximize the information content extractable from MRS and increase the reliability of neurochemical data.

Neurochemical profiles of various mouse brain regions are significantly different (7), therefore small volumes of interest (VOI = 5 – 10 μL) are necessary to minimize the partial volume effect and to increase the measurement reproducibility and specificity. To keep the total duration of measurement in reasonable limits, high magnetic fields are preferable to compensate for reduced SNR from small VOI by an increased sensitivity at high fields.

Increased chemical shift dispersion at high fields is extremely important to resolve overlapped resonances and to simplify strongly coupled spin systems. However, efficient minimization of B_0 inhomogeneity (shimming) is essential to take advantage of increased chemical shift dispersion and to increase the spectral resolution. Successful shimming requires an efficient shimming method and shim system (coils and drivers) strong enough to compensate for the field gradient induced in the brain. These local field gradients are scaled with B_0 and are not linear, therefore strong 2nd-order shims are required. Shim strengths up to 2000 Hz/cm^2 (47.0 μT/cm^2) for XZ, YZ, Z2 and 1000 Hz/cm^2 (23.5 μT/cm^2) for 2XY and X2Y2 are recommended for mouse spectroscopy at 9.4 T (7). Higher field strengths require even stronger shims. Shimming can be efficiently performed by mapping along projections using the FASTMAP method (8,9) or by 3D B_0 mapping (10). A water linewidth of 10 – 12 Hz is achievable at 9.4T in most brain regions except cerebellum, in which lines are intrinsically broader.

Short echo time (TE) localization pulse sequences are desirable to maximize the information content in ^{1}H MR spectra. There are two reasons for using a short TE. First, is to minimize the J-modulation of coupled spin systems, which is the majority of all MR detectable brain metabolites. Secondly, to reduce the signal loss caused by T_2 relaxation. T_2 relaxation times are significantly shorter at high fields and in addition, it is rather difficult to quantify them in coupled spin systems under in vivo conditions. Therefore spectroscopic pulse sequences with ultra-short TE and long TR (~fully relaxed spectra) is the best choice for metabolite quantification, by eliminating unknown T_1 and T_2 values. Short TE is necessary, but not the only requirement needed to acquire ^{1}H MR spectra suitable to provide broad biochemical information from the selected VOI in the mouse brain. Localization performance of the sequence, i.e. the efficiency of the sequence to detect only the signal from the selected VOI and to suppress all

coherences originating from outside of VOI, is especially important for quantification of metabolites beyond NAA and Cr. In general, RF pulses used for the slice selection do not have an ideal excitation or rephasing profile, but this drawback can be suppressed by using double-localization, e.g., STEAM combined with OVS (7,11), or by applying the rephasing RF pulse twice for the same slice, such as in LASER sequence (12). Poor localization can be easily recognized by the out-of-phase signal of subcutaneous lipids around 1.5 ppm, which overlaps with four signals of fast relaxing macromolecules (0.9 – 1.7 ppm).

Efficient water suppression is very important to eliminate all unwanted residual water signals, which can overlap with metabolite signals near the water resonance and cause baseline distortions. Total elimination of the residual water signal is possible using VAPOR water suppression (11). This type of the water suppression is very robust; all parameters are automatically set based on a local B_1 calibration and therefore do not require any operator input.

The selection of an optimal RF coil can substantially improve SNR. It is true that volume RF coil provide higher quality of MR images of the whole mouse brain due to homogeneous B_1 field. On the other hand, linear or quadrature surface coils (of 8 – 15 mm loop size) provide much higher SNR from brain regions close to the RF coil, which is advantageous for the MRS of a mouse brain to maximize the SNR acquired from very small VOIs. However, the B_1 field of these coils is spatially inhomogeneous, which must be taken into account for the pulse sequence design. An optimal selection of the RF coil depends on how deep is the brain region of interest, the number of brain regions of interest, and how far those brain regions are from each other, etc. As a rule of thumb, a small RF coil as close as possible to the studied brain region is the best option.

The purpose of ^{1}H MRS is the reliable quantification of metabolites in selected brain region. Even with increased chemical shift dispersion at high fields and optimal shimming, metabolite spectra can still be highly overlapped. This means that the quantification of brain metabolites require sophisticated processing methods to resolve them. Currently two different types of fitting analysis working in the frequency domain (LCModel (13,14)) and time domain (MRUI (15)) are commonly used. For meaningful fitting results, both methods require prior knowledge

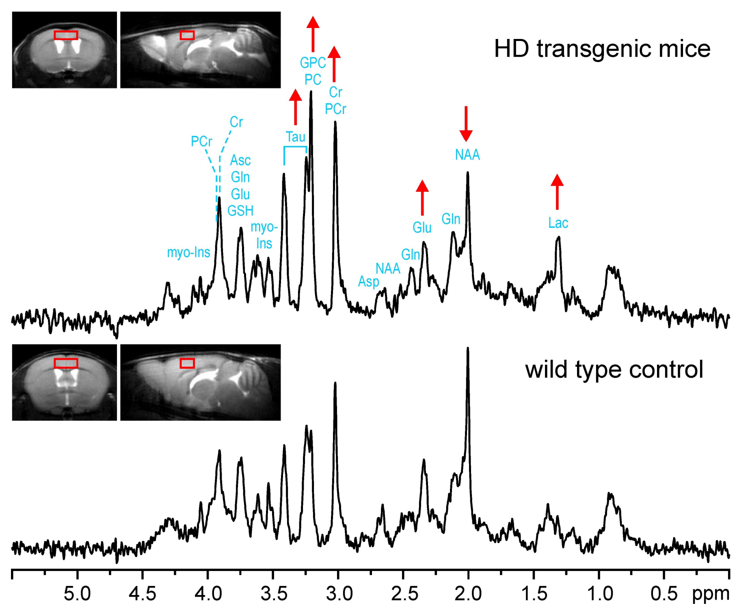

Fig.1 In vivo ^{1}H MR spectra measured from the cerebral cortex of HD R6/2 mouse and WT control. STEAM, TE = 2 ms, TR = 5 s, NT = 240. Processing: Gaussian multiplication (gf = 0.15), FT, zero-order phase correction, no water removal or baseline correction were applied.

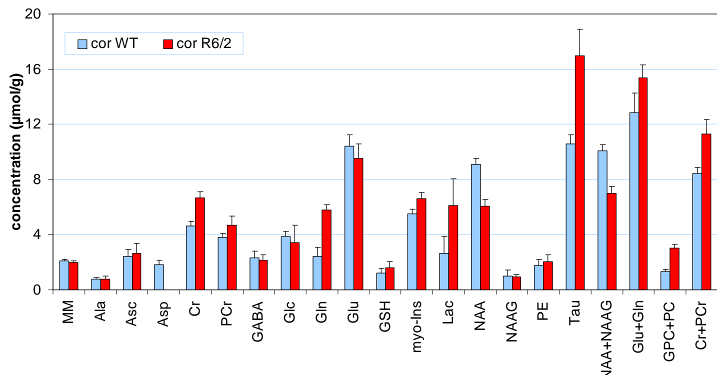

Fig. 2 Neurochemical profiles of Huntington's disease R6/2 transgenic mice and WT controls (n = 8) measured from the cerebral cortex at 15 weeks of age. Error bars indicate SD.

containing information about spectral features of each detectable brain metabolite. Short TE spectra have substantial signal contribution from fast relaxing macromolecules (predominantly proteins), therefore it is beneficial to include a macromolecule spectrum in LCModel basis set. Number of metabolites that can be reliably quantified (Cramer-Rao lower bound CRLB < 25%) at 9.4T depends on spectral resolution and SNR. Under the assumption of perfect shimming, reliable quantification of at least fifteen brain metabolites is feasible from VOIs as small as 5 μL and the acquisition time less than 20 min.

Metabolite ratios can be used as markers for neurochemical changes; however, metabolite concentrations are necessary for deriving an interpretation of neuropathological disorders on molecular level. Different methods have been developed for “absolute” metabolite quantification using internal or external references. The quantification method based on unsuppressed water signal as an internal reference requires information about the tissue water content, which requires an in vitro assessment. But once the water content in a specific tissue is determined then referencing using an internal water signal is simple, robust and highly reproducible. If CSF content is not negligible and is expected to be included in the selected VOI then decomposition of the VOI water signal into a tissue and a CFS component has to be applied (TE dependence).

The non-invasive nature of MRS is ideal for longitudinal studies of disease progression in transgenic mouse models, which requires repeated measurements of a transgenic and a control group of mice. A high level of automatic parameter adjustment can significantly reduce the time requirements for studying one animal and consequently increase the throughput of MRS study. Based on our experience, two hours per mouse is sufficient time to start anesthesia, position the mouse in the holder, tune the RF coil, acquire scout MRI, high-resolution multislice images, adjust spectroscopic parameters and acquire ^{1}H MR spectra with a sufficient SNR from two different brain regions as small as 5 μL. Ultra-short TE STEAM spectra from the cerebral cortex of a 15 week old HD transgenic mouse and a wild type (WT) control are shown in Fig.1 to illustrate the spectral quality. Sixteen metabolites were consistently quantified from these MR spectra. Highly significant differences between neurochemical profiles of HD transgenic mice and WT controls can be readily observed (Fig. 2). In vivo ^{1}H MRS has been successfully used to study transgenic and knock-in mouse models of Huntington’s (16-18) and Alzheimer disease (19). These examples demonstrate the potential of ^{1}H MRS in studies of neurodegenerative diseases using transgenic and knock-in mouse models.

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