LETTER TO THE EDITOR

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Low-Field vs. High-Field Proton MRS of Mouse Brain In Vivo

In a recent paper on in vivo proton MRS of mouse brain at 9.4 T, Tkáč et al. (1) reported high-quality single-voxel spectra of small volumes, but failed to accomplish a fair comparison with a previous study by Schwarcz et al. (2) at 2.35 T. Instead, the reader was left with the impression that proton MRS of mouse brain at low field strengths is of low value and is generally hampered by problems that are either absent or avoidable at 9.4 T.

Although comparisons of MRI or MRS at different field strengths are usually confounded by a variety of other factors involving differences in radiofrequency coils, acquisition techniques, and postprocessing procedures, physiologic or biochemical quantities (such as metabolite concentrations) should of course be independent measures. Tkáč et al. (1) obtained total creatine (tCr) concentrations in mouse brain in vivo that were "in excellent agreement with the published values" reported by Schwarcz et al. (2). On the other hand, Schwarcz et al. (2) obtained strong interstrain variabilities, whereas Tkáč et al. (1) found only small (though significant) metabolite differences in the strains investigated. Assuming that the low-field study was incorrect, Tkáč et al. (1) offered a list of problems that may explain this discrepancy. In particular, they noted that "the shimming resulted in threefold broader line widths (in ppm) at 2.35 T" and that line widths decreased rather than increased post mortem. They further questioned a high in vivo concentration of glucose (Glc), a nonzero post mortem concentration of Glc, and complained about missing information about taurine (Tau). They concluded that these flaws all "[point] to systematic errors in quantification" in the work of Schwarcz et al. (2). The discrepancies between the two studies can be described as follows:

1) Strain differences. Tkáč et al. (1) compared C57BL/6 mice with CBA and CBA/BL6 mice and found interstrain differences that were "limited to Gln, Glu, Ins, Lac, NAAG, and PE," whereas Schwarcz et al. (2) observed "substantially larger differences in metabolite concentrations between other mouse strains"—more precisely between C57BL/6 mice and BALB/c (tNAA, tCr, Cho, Glc, and Lac), but *not* NMRI mice (except for Glc). In other words, each study compared C57BL/6 mice with *different* strains and reported variable interstrain dependencies. Thus, in contrast to what was suggested by Tkáč et al. (1), both studies were consistent with each other and did not report conflicting results.

2) Spectral resolution. When given in units of Hz rather than in ppm, the line width allows for a direct comparison with the coupling constants of multiplet resonances, which for protons are on the order of 3–7 Hz. In fact, despite a better relative resolution at 9.4 T that facilitates the separation of glutamate and glutamine methylene multiplets, a full width at half maximum (FWHM) of 11–14 Hz (1) is worse than the 6.9–9.3 Hz reported for 2.35 T (2), and is not sufficient to unambiguously distinguish between lactate (Lac) and lipids.

3) Post mortem line width. Tkáč et al. (1) incorrectly stated that Schwarcz et al. (2) observed a decrease of the line width in C57BL/6 mice post mortem. In fact, the FWHM remained unchanged in C57BL/6 and BALB/c mice, and significantly increased for NMRI mice (see Table 2 in Ref. 2).

4) Glucose assignment. Although Tkáč et al. (1) did not identify Glc resonances in their spectra while they left no resonances unassigned (see Fig. 1 of Ref. 1), they reported Glc concentrations of 3–4 μmol/g for different brain regions in C57BL/6 mice. In contrast, low-field spectra concentrate most of the intensity of the Glc protons in a well-defined apparent singlet at 3.43 ppm, which may be readily exploited for accurate quantification by LCModel. Moreover, whereas Schwarcz et al. (2) confirmed the Glc assignment and quantification by its rapid decrease post mortem (see Fig. 3 and Table 2 in Ref. 2), Tkáč et al. (1) did not perform such consistency experiments.

5) Glucose concentrations. Using a correction for CSF partial volumes, Schwarcz et al. (2) reported Glc concentrations of 6.2 ± 2.3 and 5.4 ± 2.7 mM for anesthetized NMRI and BALB/c mice, respectively, and 9.8 ± 3.9 mM for C57BL/6 mice. This latter value was characterized as abnormally high and was suggested to reflect a strain-related shift of the cerebral energy metabolism toward enhanced anerobic glycolysis and/or alterations of glucose transport. This understanding is in line with the well-known vulnerability of the C57BL/6 strain to cerebral ischemia (see references in Ref. 2). It is also consistent with the simultaneous observation of much higher Lac concentrations in C57BL/6 mice (2.8 ± 0.8 mM) than in NMRI (1.9 ± 1.4 mM) and BALB/c mice (1.1 ± 1.0 mM), while the Glc/Lac ratio was found to be similar in all three strains (2).

6) Lactate. In contrast to the results obtained at 2.35 T (see previous section), Tkáč et al. (1) reported in vivo Lac concentrations of 4.5–6.5 μmol/g in the striatum of different strains. Such levels must be considered exceptionally high under normal physiologic conditions. Apart from putative difficulties with anesthesia, lipid suppression, and spatial selectivity of the voxel, it may also be an

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indication of the limited power of LCModel to discriminate Lac and lipid resonances due to the unresolved Lac doublet at 9.4 T.

7) Taurine. Without doubt there is a partial overlap of Glc and Tau resonances in proton spectra at 2.35 T. Nevertheless, it is still possible to quantify Tau by LCModel, particularly if the Tau concentrations are assumed to be as high as 8–13 μmol/g (1). Although not explicitly stated, Schwarcz et al. (2) indeed used a set of 12 metabolite model spectra that included Tau. In line with a 7.0 T study (3), the analysis yielded Tau concentrations of 6.7 ± 3.1, 6.9 ± 1.6, and 6.0 ± 2.3 mM for NMRI, BALB/c, and C57BL/6 mice, respectively. Their Cramer-Rao lower bounds (20–35%) correspond with those for multiple metabolites reported by Tkáč et al. (1).

In summary, it must be concluded that the concentrations of major metabolites in the mouse brain in vivo reported at 9.4 T (1) and 2.35 T (2) are in general agreement, and that both studies observed variations for two different sets of strains. On the other hand, an unjustified assignment of differences in the Glc and Tau concentrations of C57BL/6 mice to apparent limitations of low-field MRS must be rebutted. In fact, the use of high field strengths introduces new problems, such as those caused by the pronounced splitting of strongly spin-coupled resonances and the unavoidable broadening of resonance line widths (in Hz). High fields in conjunction with advanced shim capabilities certainly have their merits, as demonstrated by Tkáč et al. (1) using resolution-enhanced proton MRS of the mouse brain in vivo. Nevertheless, it should be good scientific practice to provide fair comparisons.

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