Increased NAA Concentration in the Restless Legs Syndrome: Possible Link to Hypomyelination

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Introduction: Iron deficiency has been known as a contributing factor for restless legs syndrome (RLS). Iron plays a pivotal role in brain physiology as an essential component for proper myelination, as a cofactor for enzymes in neurotransmitter synthesis, lipid and DNA synthesis. Most *in vivo* studies on RLS have been carried out with fMRI and MR relaxation mapping [1-3]. To date, the associated neurochemical changes in the dopaminergic brain structures has not been studied *in vivo* such that the important experimental relationship of iron insufficiency with neurochemical distribution in RLS brains can be established. The aim of present study was to investigate the neurochemical alterations in the RLS brain, specifically in the putamen, using *in vivo* proton MRS. In addition, the involvement of iron in myelin synthesis and insufficient brain iron in RLS led to the hypothesis that hypomyelination could exist in RLS brains. To test the hypothesis, we investigated the expression of myelin-related proteins, including myelin basic protein (MBP), 3'5'-cyclic nucleotide phosphohydrolase (CNPase), and proteolipid protein (PLP).

Methods:

Subjects: 26 RLS patients (53.5 ± 12.2 yrs) and 23 age- and gender-matched controls (48.5 ± 15.1 yrs) were recruited in this study. RLS disease was clinically assessed by the International Restless Legs Syndrome Study Group (IRLSSG) rating scale. All of patients stopped taking RLS medication for at least one week in order to eliminate drug confounding effect on neurochemical distribution.

Data Acquisition: A high resolution T1-weighted image (TR/TE=9.9ms/4.6ms, matrix size=256×256) was acquired on a 3.0T system (Philips Medical) used for placement of the voxel (1.5×1.5×1.5cm^3) in the putamen. A short echo PRESS sequence was used for spectral acquisition (TR/TE= 2000ms/30ms, number of points=1024 and acquisition time=5 minutes). Water suppression was achieved with outer volume suppression.

Quantification: Unsuppressed water signal acquired from the same voxel was used as in internal reference for the quantification and eddy current correction. ^{1}H NMR spectra were analyzed using LCModel software [4]. Metabolites with a Cramer-Rao lower bound (CRLB) <20% was included in the statistical analysis.

Myelin Analysis in Autopsy brains: Cortex tissue was obtained at autopsy from the brains of RLS (n=11) and age- and gender- matched controls (n=11) who lacked any significant neurological history. Myelin was extracted from frozen brain tissue [5] with minor modifications. Equal amounts of total protein (20ug) were analyzed by western blot for quantification of MBP, CNPase and PLP.

Statistics: Two-sample *t*-test was applied for the mean comparison between RLS and control group using SPSS software. Statistical significance was set at a p<0.05.
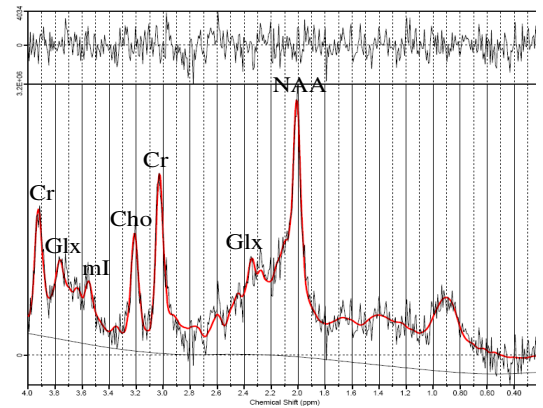
Results: Fig. 1 shows a typical *in vivo* spectrum from the putamen. As shown in Fig. 2, RLS had a trend of higher concentrations of the metbolites compared to the controls (*t*-test, $p < 0.05$). We observed a highly significant increase in NAA in addition to other metabolites including glutamate, choline- and creatine-containing compounds. Fig. 3 shows a significant decrease in the expression of all the three proteins in RLS compared to the controls ($p < 0.05$).

Discussion: It is of interesting to note that the concentrations of NAA-containing compounds were increased in RLS. Since intraneuronal NAA, synthesized in the neuronal mitochondria, is also required for myelin synthesis and supplies acetyl groups for myelin lipid synthesis, the increased NAA level may represent decreased utilization of the compound for myelination. Our previous MRI study found iron deficiency in the putamen of RLS subjects [3]. Accordingly, we hypothesize that insufficient brain iron may cause hypomyelination that, in turn, alters NAA concentration. Analysis of crude myelin from RLS and control autopsy brain tissues also revealed hypomyelination in RLS brains. From these findings, we conclude that iron deficiency significantly associated with the neurophysiology alteration such as neurotransmission (glutamate) and energy status (choline) as well as neuropathology such as hypomyelination (NAA).
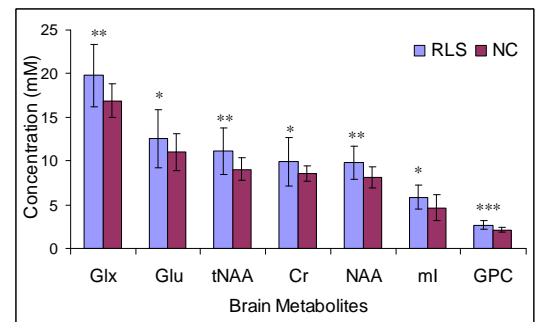

Figure 1 The curve fit of ^{1}H-MRS spectrum from a young control subject using LCModel software. At the top are plotted the residual. Abbreviation: NAA = N-acetylaspartate, Cr = creatine+phosphocreatine, Cho = choline-containing compound, mI = myo-inositol, Glx = glutamate+glutamine

Figure 2 shows significantly decreased concentrations in RLS compared to the controls.*p<0.05,**p<0.01,***p<0.005

Figure 3 shows the expression of MBP, CNPase and PLP in the crude myelin extracted from RLS and control autopsy brain tissue. The expression of the three proteins significantly was decreased in RLS compared to controls (p <0.05)

References:
[1]. Water TC *et al*., 2004. *Sleep Med*. 5, p 401-406   [2]. Unrath A *et al*., 2007. *Mov Disord* 22, p 1751-1756   [3]. Lee BY *et al*., 2006. *Proc. Intl. Soc. Mag. Med*.3840
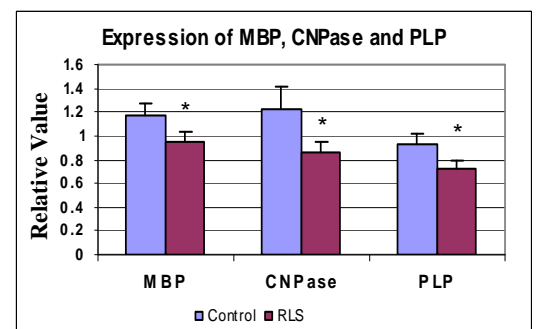[4]. Provencher SW, 1993. *Magn Reson Med* 30, p 672-679   [5]. Norton WT & Poduslo, 1973. *J. Neurochem*. 21, p 749-757