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Quantification of Metabolic Alterations of Dorsolateral Pre-Frontal Cortex in Depression SD Rat by MR Spectroscopy

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Abstract : **Purpose:** Contrary to the human study, it has rarely investigated metabolic alterations in the dorsolateral prefrontal cortex (DLPFC) of depressed rats versus age- and sex-matched controls using proton magnetic resonance spectroscopy (MRS). Thus, the purpose of this research was to verify the feasibility of metabolic differences between the normal rat and the depression model rat.
Materials and Methods: A homogeneous group of 20 SD male rats was used for MRI and in vivo 1H MRS. To induce a depressed status in SD rats, we performed the forced swimming test (FST). Using image-guide, water suppressed in vivo 1H MRS with 4.7 T MRI/MRS system, NAA/Cr and Cho/Cr ratios were mainly measured between depressed rats and normal subjects.
Results: In depressed rats, increased Cho/Cr ratio was measured versus control subjects. However, no significant group effect for NAA/Cr was observed between case-control pairs.
Discussion and Conclusions: The present 1H MRS study shows significant brain metabolic alterations of dorsolateral prefrontal cortex with experimental depressed status of SD rat induced by FST compared to normal subjects. This result provides new evidence that in vivo 1H MRS may be a useful modality for detecting localized functional neurochemical markers alterations in left DLPFC in SD rats.
Key words : ^{1}H magnetic resonance spectroscopy (MRS), Forced swimming test (FST), Depression, Dorsolateral prefrontal cortex (DLPFC)

INTRODUCTION

Neuroimaging techniques have significantly developed in the last two decades and have helped to diagnose specific brain regions possibly complicated in the pathophysiology of

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psychiatric patients.[1] It is well-known that the dorsolateral prefrontal cortex (DLPFC) is an important area of a neuroanatomical circuit mediating various components or symptoms of major depressive disorder (MDD) [2-4] and playing a key role in cognitive and executive brain function.[5] The majority of functional brain-imaging studies in depressed patients showed reduction in blood flow, metabolism and volumes in the DLPFC [6-8] which could worsen with a longer length of illness.[9,10] Structural brain imaging also revealed increased numbers of deep white matter lesions in the frontal lobe [11] and an altered frontal lobe volume [12,13] even in childhood-onset major depression.[14]

Apart from utilizing neuroimaging techniques, the measurement of neurotransmitter concentrations in specific brain areas is a promising opportunity offered by proton magnetic resonance spectroscopy. Metabolites such as N-acetyl aspartate (NAA), a putative marker of neuron functionality, creatine and phosphocreatine (Cr) as measures of the energy metabolism, choline-containing compounds (Cho) as markers of the membrane turnover, myoinositol (mI), glutamate (Glu) and gamma-aminobutyric acid (GABA) can be non-invasively assessed by 1H MRS. Recently, Kumar and coworkers showed that major mood disorder (MDD) had increased mI/Cr and Cho/Cr levels in the frontal white matter to age and gender matched controls.[15] Except Cho/Cr, significantly lower NAA/PCr+Cr ratio in the left DLPFC was investigated in the chronic than in the less chronically depression patients and healthy controls. The low levels of NAA/PCr+Cr ratio in the left DLPFC of unipolar patients who had been more chronically ill suggest a potential role for illness chronicity neuronal abnormalities in the DLPFC in unipolar patients.[16]

However, contrary to the human study, it has been rarely performed on the animal model of depression by MRS although there are many kinds of animal depression models such as forced swimming test (FST),[17] tail suspension test [18] and olfactory bulbectomy (OB) model.[19,20] Briefly, FST is based on the observation that a rat, when forced to swim in a situation from which there is no escape, will, after an initial period of vigorous activity, eventually cease to move altogether making only those movement necessary to keep its head above water.[17] The TST model is similar to the FST model except for suspending the tail from a lever instead of swimming to trigger depressive feeling. The concept of the OB model is different from the FST and TST on that the OB model is based on assuming depression is caused by neuronal regulatory deficits.

Originally all the methods were invented to search for the effect of new antidepressant drug as a pre-clinical screening test. However, it was seldom performed MRS in the depression models contrary to human depression patients in our knowledge. Thus, the purpose of this research was to find the metabolic alteration patterns between the normal subjects and the depression model rats corresponding to the human studies. We hypothesized that levels of Cho/Cr would be significantly higher in the DLPFC in the FST model compared with control group. These hypotheses were based on published papers of metabolite ratio alteration in DLPFC in human studies with depressed patients.

MATERIAL AND METHOD

Animals and Anesthesia

Naïve male Sprague-Dawley (Charles River) rats weighting 160 ~ 180g were used. Totally 20 rats were investigated and were divided into 2 group normal (n=10) and the depression model (n=10). The animals were housed under conditions of controlled temperature (23 ± 2 ^{0}C) and illumination (14h light / 10h darkness commenced 18:00). Food and water were freely accessible. Animal anesthesia was induced by inhalation of isoflurane with 4 - 6% concentration in a 5:5 mixture of N_2O and O_2 and was maintained by inhalation of isoflurane with 1.5-2% concentration in a 5:5 mixture of N_2O and O_2. For MRI / MRS animals were placed in a prone position on a palate holder equipped with an adjustable nose cone. The rectal temperature was maintained at 35^{0}C by heated water blankets positioned around the body and warm air circulation. Also, observing of respiratory cycle and heart rate was performed using monitoring unit. MR experiments were performed between 09:00 am and 6:00 pm.

All animal treatment and procedures were conducted according to the institutional animal care and ethical committee of our university.

Forced swimming test

Each of rats was placed into a vertical glass cylinder (height: 40 cm, diameter: 18 cm) containing 25 cm of water maintained 25 ^{0}C and left 15min. After 15 min in the cylinder, rat

was removed and allowed to dry before being returned to its cages. 24h later the rats were plunged again in the cylinder for 5 min. The water was replaced between each test.

1H MRI / MRS procedure

Water-suppressed hydrogen magnetic resonance imaging/spectroscopy was conducted on 4.7 T Bruker scanner (BioSpec, Ettlingen, Germany) using a standard quadrature head coil. A scout image was first obtained to verify subject position and image quality. T2-weighted MR images were obtained using rapid acquisition with relaxation enhancement (RARE) sequence (TR = 5000 ms, TE = 22 ms, slice thickness = 1.0 mm, NEX = 1, matrix size = 256 x 192) to place the voxel accurately. Magnetic resonance spectra were acquired using a point resolved spectroscopy (PRESS) pulse sequence (TR = 3000 ms, TE = 20 ms, 512 acquisitions, 2048 complex data points, voxel dimensions = 3.5 x 3.5 x 3.5 mm^3, acquisition time of 25 min) to minimize T1 and T2 relaxation effect within an acceptable examination time. The position of the voxel was visually adjusted in the left prefrontal regions, predominantly in DLPFC. Shimming was performed by fast automatic shimming technique by mapping along projections (FASTMAP) [21] for each voxel of (3.5 x 3.5 x 3.5 mm^3) positioned in the DLPFC. Variable power RF pulses with optimized relaxation delay (VAPOR) was used for water suppression and manually adjusted by controlling the transmit gain.

Data analysis

Acquired data were analyzed by using TOPSPIN. Post-processing involved the exponentional line broadening of 2 Hz, Fourier transformation, and zero/first – order phase correction, and baseline correcting of the transformed spectrum. The water - suppressed time domain data were analyzed between 1.0 ppm and 4.0 ppm without further T1 and T2 correction. Resonance peak assignments of major neurometabolites at in vivo 1H MRS of this experiment were NAA at 2.0 ppm, Glx at 2.23 ~ 2.44 ppm, Cr at 3.0 ppm and Cho at 3.2 ppm. The quantitative expression of results was evaluated as ratios to Cr resonance at 3.0 ppm, maintaining accuracy that minimizes errors that caused by variations in magnetic field homogeneity and tissue volume.[22, 23] Any resulting spectra which resulted from inappropriate signal-to-noise ratio (SNR), outer volume suppression (OVS), water suppress-

ion, distorted baseline, severe phase distortion due to the heavy eddy current as well as subject motion during data acquisition were regarded as unusable and excluded from data analysis.

Statistics

Statistical analysis was performed by using SPSS (Windows Version 13.0, SPSS Inc., Chicago, IL). The data were analyzed with independent sample *t*-tests for comparison of the two groups' data, depression model rats induced by FST and normal subjects; P values <0.05 were considered significant to account for the multiple comparisons.

RESULTS

Fig. 1 shows a well-defined voxel position in the dorsolateral prefrontal cortex on T2-weighted transverse MR image obtained by a depression model rat. Based on MR image, there is no difference between two groups indicating the fact that detecting the variations of neurotransmitters on MR images is difficult.

The typical MR spectra obtained from the depressed SD rats and the controls are shown in Fig. 2. A flat baseline of the *in vivo* spectrum and a high SNR were acquired. As can be seen in Table 1, the Cho / Cr ratio was significantly higher in the depressed SD rat group (N=10) than in the normal control group (N = 10) (mean ± SD = 0.87 ± 0.61 and 0.78 ± 0.56, respectively, p = 0.032). However, no significant differences of the NAA / Cr ratio were observed between the two groups (mean ± SD = 2.41 ± 0.16 and 2.30 ± 0.19, respectively, p = 0.641). Fig. 3 shows the comparison of the NAA / Cr and Cho / Cr brain metabolite ratios between the depressed SD rat group and the normal controls.

DISCUSSION

We undertook the challenging task of comprehensively analyzing a spectral database of rat brain of depressed model. With the advancement of *in vivo* 1H MRS, it has become

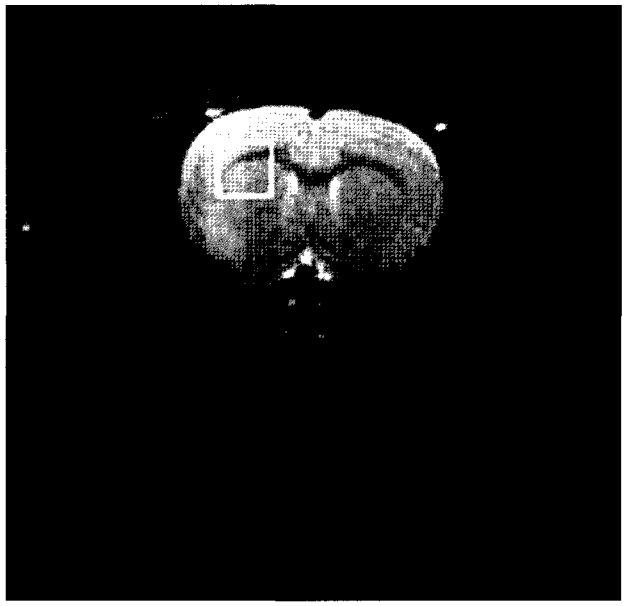


Fig. 1. T2-WT transverse MR image of a depressed rat showing voxel in the left dorsolateral prefrontal cortex. The pink line represents the location of the volume of interest (3.5 x 3.5 x 3.5 mm^3) in the left dorsolateral prefrontal cortex.

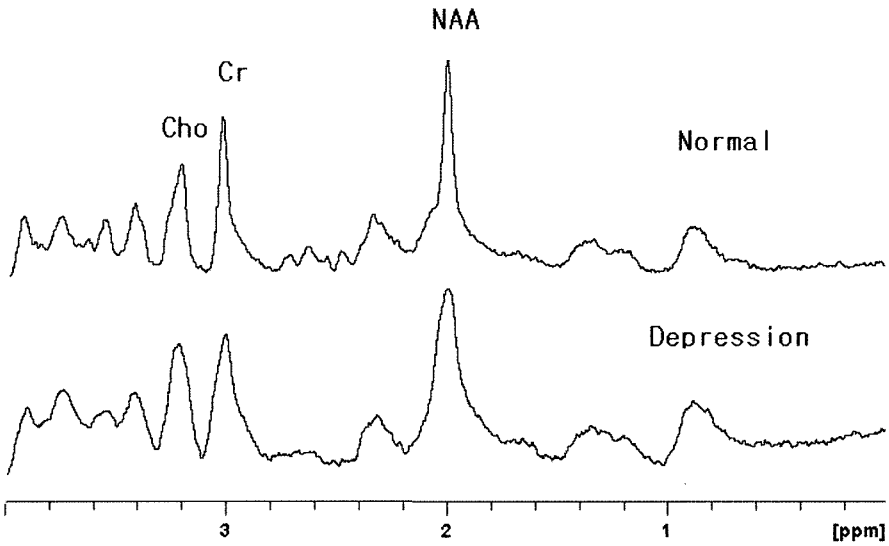
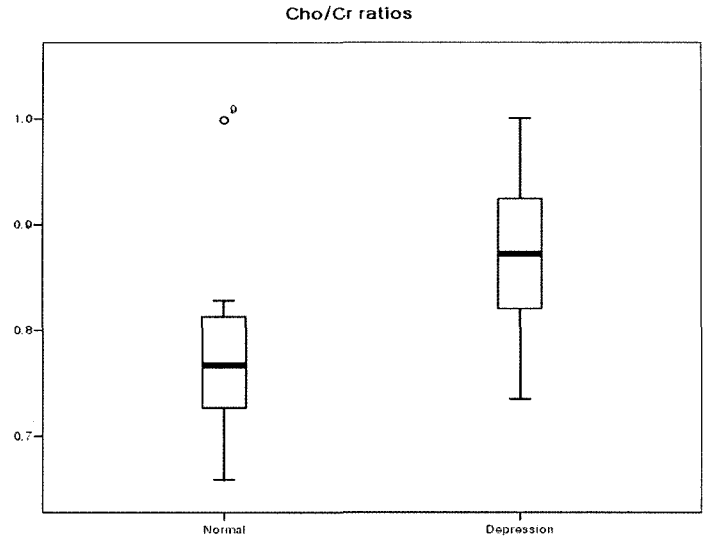
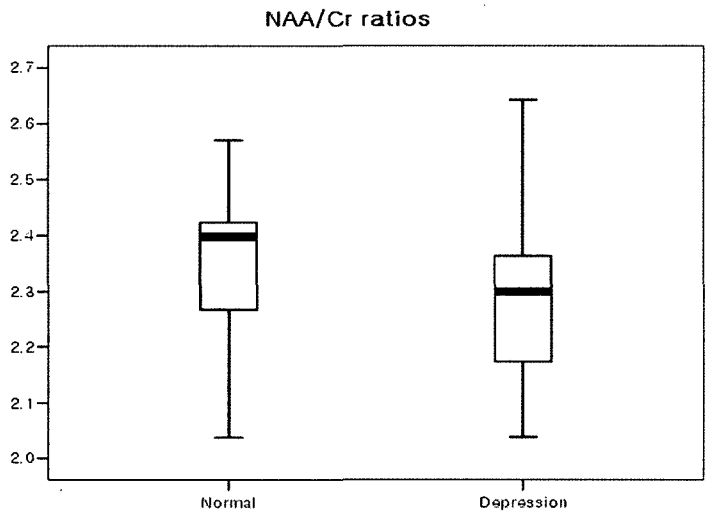


Fig. 2. Representative proton magnetic resonance spectroscopy spectra of the left dorsolateral prefrontal cortex from the normal SD rat brain (lower spectrum) and the brain after FST. Compared to the normal brain, a marked increment in the Cho signal intensity is noted in the depression model rat brain.



(A)



(B)

Fig. 3. Left dorsolateral prefrontal cortex Cho/Cr and NAA/Cr ratios in 10 control subjects compared to 10 depressed rats. Depressed rats had significantly increased Cho/Cr ratio than normal subjects (A), but no differences in NAA/Cr ratio was shown between two groups (B).

Table 1. Comparison of MRS metabolite ratio between normal subjects and depression model rats induced by FST.

	Normal	Depression	P value
NAA/Cr	2.41±0.16	2.30±0.19	0.641
Cho/Cr	0.87±0.61	0.78±0.56	0.032

NAA = N-acetyl-aspartate, Cr = creatine, Cho = choline

possible to directly study the cerebral imtermediary metabolism of animal brain tissue. By suppressing the water signal, different metabolites that are present in very small quantities may be detected by measuring chemical shift. This metabolic information can be helpful for psychiatric diseases diagnosis which is difficult in MRI because these didn't have focal brain pathology.

For the interpretation of the 1H MRS spectrum of the brain, it is necessary to understand the biochemical role of the different compounds. It is unfortunate that the function of some of the main compounds observed in the 1H MRS of the human brain is not very well understood. This is particular case for the NAA resonance, which happens to be the largest signal in the normal human or animal brain spectra. It accounts for approximately 85 % of the proton signal of N-acetyl group whereas NAAG accounts for the remaining 15 % .[24] It is accepted as a neuronal and axonal marker whose physiological role is not yet very clear.[25] It has been shown to increase during brain development after birth and in childhood and to decrease in old age.[26-29] Regardless of the precise biochemical role of NAA, a relative decrease of this compound is commonly considered to be an indication for neuronal loss. Increased NAA is only found in children with Canavan's disease.[30] NAA is unequivocally specific to neurons and a decrease of NAA in MRS has been shown to be equivalent to neuronal loss.

The major peak at 3.0 ppm is from the CH_3 group of creatine and phosphocreatine (Cr + PCr), normally referred to as total creatine. The role of creatine and phosphocreatine is much better understood. These compounds play a role in high-energy metabolism. Total

creatine has been considered to be stable enough to be used as an internal reference in reporting relative concentrations of other brain metabolite, but recent findings suggest that this assumption should be used with care.[31]

The 1H MRS Cho signal comprises a diversity of choline-containing compounds, such as phosphorylcholine, glycerolphosphocholine, and acetylcholine [32, 33] which are involved in pathway of phospholipid synthesis and degradation, thereby reflecting membrane synthesis and degradation. It is also revealed that Cho plays a critical role in signal transduction in major depressive disorder (MDD).[15, 34] Indirect evidence from several pharmacological studies suggests that choline may have a "depressogenic" effect on the central nerve system (CNS).[35] Previous MRS studies have focused on basal ganglia and yielded opposing results. Charles and coworkers described the increased Cho / Cr ratio in the basal ganglia of drug-free patients with depression.[36] In contrast, Renshaw and coworkers found left basal ganglia Cho / Cr reduction in unmedicated depressed patients that were more pronounced in fluoxetine, selective serotonin reuptake inhibitor (SSRI), responder than in the nonresponders.[37] Also no variations for Cho levels were reported in another 1H MRS report investigating the left DLPFC in major depressive episode, unipolar with melancholic features.[38] One of the reasons concerning the discrepancies among these studies may be generated in the patients subjects involved. Charles and coworkers studied seven patients with late-life major depression (mean age = 71.14), whereas Renshaw and coworkers investigated 41 major depression patients (mean age $\pm$ S.D = 39 $\pm$ 10).

Our results revealed localized increased DLPFC Cho / Cr in depressed rat models compared to age- and gender-matched control subjects. However, we recognize that our methods have a limitation on the generalizability of its findings. To make it successful, we feel that future research should proceed in several directions. First, contrary to the human study, relatively small rat brain volume size may not guarantee the accuracy of our voxel position. To minimize this potential problem, we pay close attention to place voxel on the epicenter of DLFPC while we selectively excited of a restricted voxel that excludes areas of high lipid concentration or on spatial saturation of external lipids such as those from the scalp and orbital region. However, to verify the reproducibility of voxel position, voxel composition has to be analyzed by image segmentation into cerebrospinal cord (CSF), white and grey matter.[39] Second, while most of the FST experiments are subjected to male rat,

depression is more frequently taken women than men. So, it is essential to include female rat to observe the effect of sex difference. Third, the animal studies, both before and after taking antidepressants, will be needed to verify the extent to which the increased Cho / Cr ratio is, in fact, mood state dependent. Fourth, recent review of the literature suggests that left hemispheric lesions may be more commonly associated with depression, whereas right-sided lesions are more common in mania.[40] As similar results, right DLPFC had not shown any differences in major peaks such as NAA, Cho and Cr both absolute and relative quantification.[41, 42] Therefore, right DLPFC Cho / Cr also has to be measured to compare with the human cases. And, increased left DLPFC Cho / Cr, alternatively, may be represented an epiphenomenon or even a compensatory response to illness. To prove exact reason for increased Cho / Cr, further MRS studies in other brain regions are essential.

CONCLUSION

In conclusion, we demonstrated significantly increased Cho / Cr in DLPFC of the FST model compared with the normal subjects by using 1H MRS. This finding is in corresponded with the previously reported human study results indicating the variation of neurochemical alteration. However, to verify the exact mechanism of depression, additional studies combining 1H MRS with neuroimaging and other neurochemical studies are required to further elucidate the biological basis of depression occurring in a rat depression model.

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