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Intraindividual variability of striatal ^{1}H-MRS brain metabolite measurements at 3 T[☆]

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Abstract

Purpose: To measure possible positional and diurnal physiological effects on brain metabolites in single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) measurements of the right and left striatum.

Methods: ^{1}H-MRS measurements were performed in 10 healthy adult volunteers using a short echo PRESS sequence (TE=30 ms, TR=3000 ms). Each individual was scanned during both morning and afternoon hours. Regions of interest were right and left striatum. To control for systematic drift in scanner performance, ^{1}H-MRS measurements of a standard phantom solution were also acquired. Statistical analysis was performed using a repeated measures analysis of variance that included three within-subject factors: metabolite (*N*-acetyl-aspartate [NAA] or creatine [Cr]), laterality (left or right caudate) and time (morning or afternoon).

Results: A significant interaction ($P<.016$) between time of day and metabolite levels was observed. Further exploration of this finding revealed a significant difference between morning and afternoon levels of NAA ($P<.044$) but not Cr. In addition, no significant morning-to-afternoon differences were observed for the ^{1}H-MRS phantom measurements.

Conclusions: Systematic variation due to scanner performance does not account for the changes observed in repeated measurements of striatal NAA levels. This difference may be accounted for by either repositioning effects or circadian physiological effects. Further studies are required to learn whether time of day standardization of ^{1}H-MRS acquisitions may contribute to improved reproducibility of measurements.
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1. Introduction

In vivo single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) is now a widely available method on magnetic resonance imaging (MRI) scanners. ^{1}H-MRS can measure levels of several brain metabolites, such as creatine/phosphocreatine (Cr), choline+phosphocholine+glycerophosphocholine [N1] (Cho) and *N*-acetyl-aspartate (NAA). Recently, ^{1}H-MRS studies of common and severe neuropsychiatric disorders (e.g., obsessive–compulsive disorder, schizophrenia, etc.) have reported abnormal metabolite levels in the striatum (caudate and putamen nuclei) [1–4].

The interpretation of striatal ^{1}H-MRS findings, however, remains problematic. Although the majority of ^{1}H-MRS reproducibility studies report overall good to moderate results, poorer results are usually reported for striatal regions [5,6]. To improve reproducibility and reliability of striatal ^{1}H-MRS acquisitions, we need to

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identify factors associated with variability of measurements. To date, however, no systematic attempts have been made to investigate the sources of variation of striatal ^{1}H-MRS measurements.

Recently, several technical and intraindividual factors [7,8] have emerged as possible contributors to variability of striatal ^{1}H-MRS measurements. First, a typical ^{1}H-MRS study involves a single spectral acquisition per patient brain region. Second, low signal-to-noise ratio (SNR) is usually reported for caudate nuclei measurements [6] Third, SNR limitations dictate relatively large voxel sizes with resultant partial volume effects (i.e., coinclusion of white matter and CSF) and greater sensitivity to repositioning effects (i.e., variations in tissue sampling during repeated voxel positioning) [9]. Indeed, ^{1}H-MRS studies of homogeneous brain regions (i.e., parietal white matter) reported higher overall single-voxel reproducibility compared with striatal regions that contained varying ratios of gray matter, white matter and CSF [5]. Lastly, physiological factors may affect striatal MRI measurements [7]. For example, circadian cycles may affect striatal metabolite levels [10]. However, to the best of our knowledge, there are no studies of circadian effects on striatal metabolite measurements.

The current ^{1}H-MRS study directly examined two possible sources of intraindividual variation: interregional variation in voxel positioning (i.e., right vs. left striatal measurements) and time of day effects on metabolite measurements. We hypothesized that both interregional and time of day factors would have significant effects on ^{1}H-MRS metabolite measurements. To test our hypothesis, we performed three separate series of experiments that included morning and afternoon measurements of subjects and a standard phantom solution. Finally, a single-subject study was also performed to directly assess repositioning effects.

2. Materials and methods

All studies were performed at the Imaging Research Center at the Brain–Body Institute, St. Joseph's Healthcare, Hamilton, using a GE short-bore twin-speed 3-T MRI system (General Electric Healthcare, Milwaukee, WI) and a standard quadrature head coil. This study was approved by the Research Ethics Board at St. Joseph's Healthcare and done in compliance with the code of ethics as stated in the Declaration of Helsinki. The study was performed on 10 right-handed healthy adults (6 males and 4 females). To ensure optimal sample homogeneity, all subjects were right handed, nonsmokers and were not taking any medications. All subjects gave informed consent following careful screening for MRI compatibility.

Each subject was scanned twice during the same 24-hour interval. For each participant, one scanning session was performed in the morning (9:00 a.m.±0.7 h) and one in the afternoon (4:00 p.m.±0.8 h). These times of day were chosen because of substantial evidence of morning-to-afternoon differences in human physiology [11–13]. In addition, a recent animal study showed evidence supporting morning-to-afternoon differences in striatal physiology [10]. Subjects were instructed not to drink caffeine-containing beverages before the morning scan and in the hour preceding afternoon scans. To control for possible order effects on metabolite measurements, 7 of the 10 subjects had their first scan during morning hours whereas 3 were first scanned in the afternoon. All scanning sessions were performed by the same MRI technologist (TC).

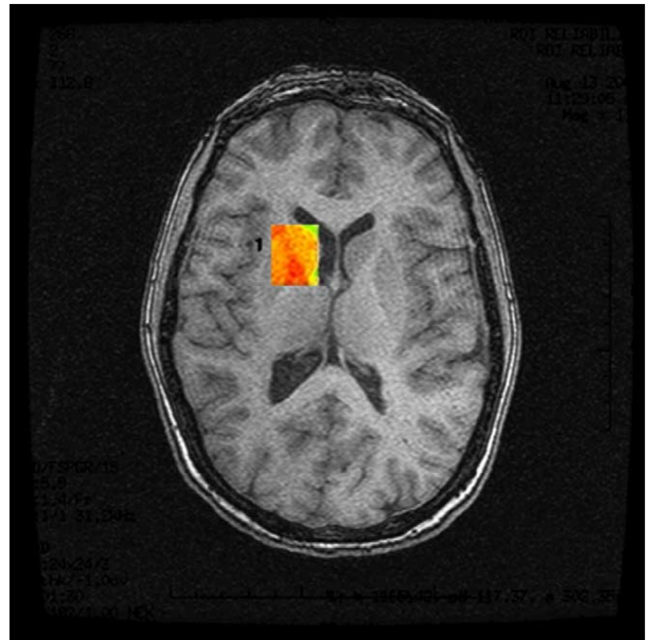

Fig. 1. An AC–PC-oriented axial slice showing a typical striatal ROI, defined by the colored frame in the image.

To position our region of interest (ROI) we used a rigorous anatomical localization protocol. Following head positioning, immobilization and localizer scan, a T1-weighted 3D-FSPGR scan (flip angle=20°, TE=4 ms, TR=10 ms, 22 cm FOV, 120 images 1.5 mm thick, 256×192 matrix, 2NEX) was prescribed parallel to the anterior commissure–posterior commissure (AC–PC) line. Voxels 4.5 cm^3 (1.5×1.5×2.0 cm anterior to posterior) were prescribed for right, then left caudate nuclei. All voxels were placed on an AC–PC-oriented oblique axial slice corresponding to midthalamic level on a midsagittal view (Fig. 1). Single-voxel ^{1}H-MRS spectroscopic measurements were performed using a PRESS (Point RESolved Spectroscopy) sequence (TE=30 ms, TR=3000 ms, 256 acquisitions, 2500 Hz spectral width, 2048 points, duration 14:00 minutes). We chose a longer TR and shorter TE to help minimize T_1- and T_2-associated signal losses and optimize postprocessing quantization [14]. Outer-volume suppression bands contiguous with the PRESS-selected volume were manually placed in all three dimensions.

2.1. Estimation of possible positioning effects

Four consecutive individual scanning sessions of the right striatum were performed on a single patient, a healthy right-handed 32-year-old female. Each of the four sessions included a localizer scan and a 3D-SPGR sequence. Then, a right striatal ROI was positioned using the above anatomic protocol.

2.2. Phantom measurements

To rule out possible systematic daily scanner performance drift we acquired single-voxel ^{1}H-MRS measurements of the standard spherical GE-MRS "Braino" phantom (contains *N*-acetyl-L-aspartic acid [NAA], 12.5 mM; creatine hydrate [Cr], 10 mM; choline chloride [Ch], 3 mM; *myo*-inositol [mI], 7.5 mM; L-glutamic acid [Glu], 12.5 mM; DL-lactic acid [Lac], 5 mM; sodium azide (0.1%); potassium phosphate monobasic [KH_2PO_4], 50 mM; sodium hydroxide [NaOH], 56 mM; and Gd-DPTA [Magnevist], 1 mL/L). A total of 9 morning voxels and 7 afternoon voxels (8:45 a.m.$\pm$0.8 h and 4:20 p.m.$\pm$1.9 h, respectively) were obtained (12 cm^3 centrally placed voxel, 128 acquisitions, TE=30 ms, TR=3000 ms, 256 acquisitions, 2500 Hz spectral width, 2048 points).

2.3. Postprocessing

2.3.1. Spectral analysis

For both in vivo and phantom acquisitions, metabolite quantization was done using LCModel [14], an operator-independent spectral analysis software that obtains approximate maximum likelihood estimates of the metabolite concentrations and their uncertainties. Changes in transmit gain and receiver amplitudes (R1 and R2) were taken into account to obtain consistent absolute metabolite concentrations. LCModel operator was blind to any laterality, time of day or order data. Typical LCModel outputs for phantom and in vivo measurements are displayed in Figs. 2 and 3, respectively.

2.3.2. Voxel segmentation

For the single subject study, analysis of possible positioning effects was performed using AFNI software [11]. ROI were placed on corresponding SPGR images (Fig. 1). Using those coordinates, we were able to create an ROI pixel intensity histogram (Fig. 4). For each acquisition, typical white matter and CSF intensity ranges were identified in the corpus callosum and lateral ventricles, respectively. Gray matter intensity ranges were defined as intensity values

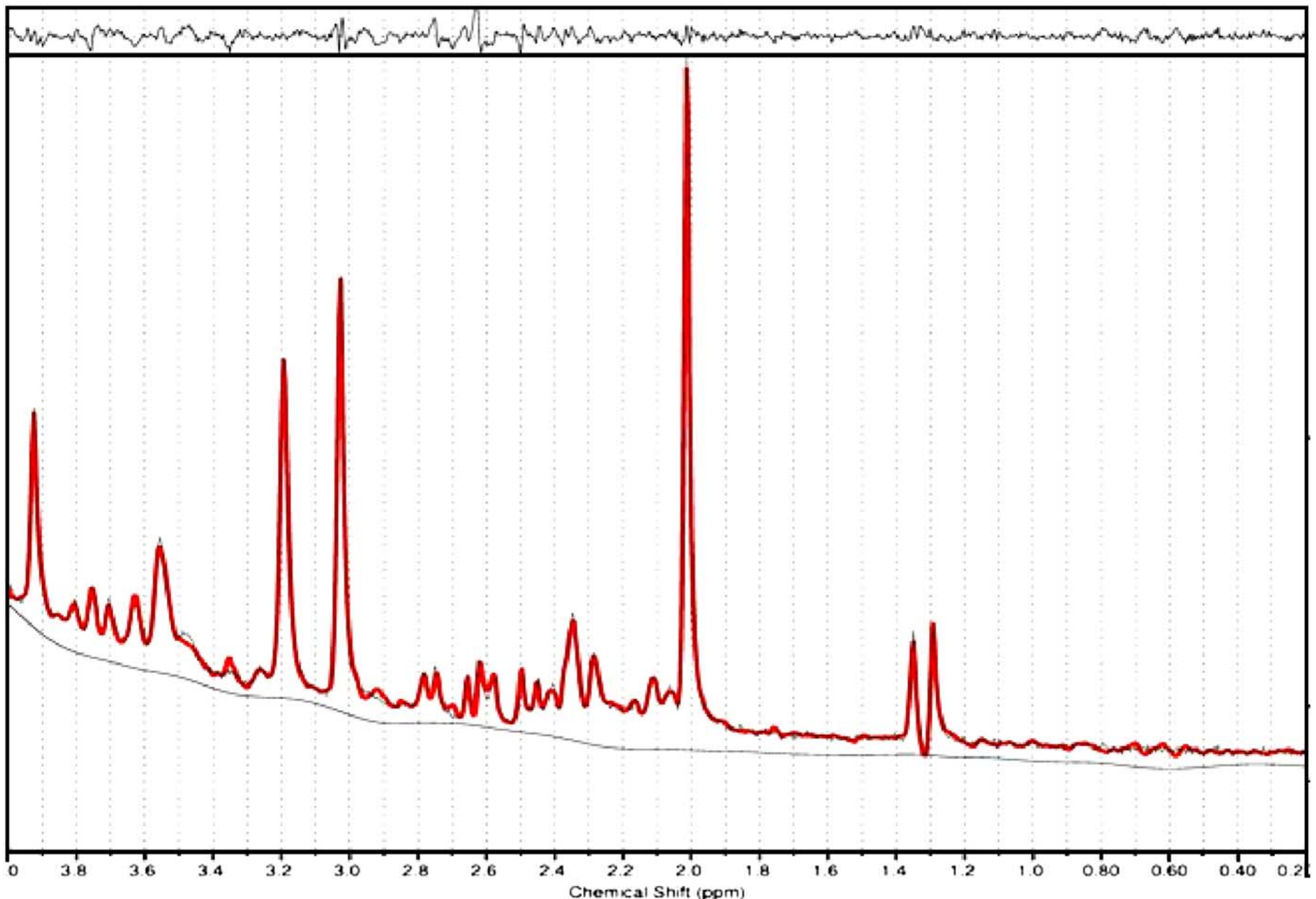

Fig. 2. LCModel analysis of GE MRS ("Braino") phantom (details of contents are noted in the text). The upper panel shows a plot of residuals (i.e., observed–fitted) for the analysis.

below minimal white matter intensities and above maximal CSF intensities. Using those intensity ranges, we segmented each ROI to calculate the number of white matter, gray matter and CSF pixels. Because the number of CSF pixels was negligible (i.e., CSF pixels/total ROI pixels <10%), results are reported as ratios of GM to total ROI pixels (GM/ROI).

2.4. Statistics

Statistical analysis was done using SPSS (PC version 9.0). Coefficients of variance (CV) were calculated for each metabolite and scanning session. All subsequent statistical analysis was performed only for metabolites that had overall CV values ≤20% (i.e., NAA and Cr). Reliability of metabolite measurements was assessed using the intraclass correlation coefficients [12]. To test our hypothesis, we performed a repeated measures ANOVA that included three within-subject factors: metabolite, with 2 levels (Cr and NAA); laterality, with 2 levels (left and right caudate); and time, with 2 levels (morning and afternoon). For the phantom measurements, we used a similar design with two within-subject factors (metabolite, with 2 levels, and time, with 2 levels). Significant interactions were then further explored using paired *t* tests. Finally, assessment of repositioning effects was performed using the range of acquired GM/ROI values.

3. Results

3.1. In vivo study

3.1.1. Reliability of in vivo metabolite measurements

Only NAA and Cr had CV<20%. CVs for morning and afternoon Cr and NAA ranged between 17% and 26% and between 14% and 26%, respectively. Intraclass correlation coefficients for Cr and NAA were 0.78 and 0.69, respectively. No significant order effects were found between subjects that were first scanned in the morning ($n=7$) and those that were first scanned in the afternoon ($n=3$). In addition, a paired *t* test did not reveal any SNR differences between in vivo morning (mean SNR=9.95) and afternoon (mean SNR=10.45) measurements.

3.1.2. Morning and afternoon metabolite measurements

Fig. 5 displays a comparison of morning and afternoon NAA and Cr levels for both right and left striatal nuclei.

3.1.3. Effects of voxel positioning and time of day on metabolite measurements

A repeated measures analysis revealed a significant interaction ($P<.020$) between metabolites and time of day (Table 1). Although a trend for significance was found for

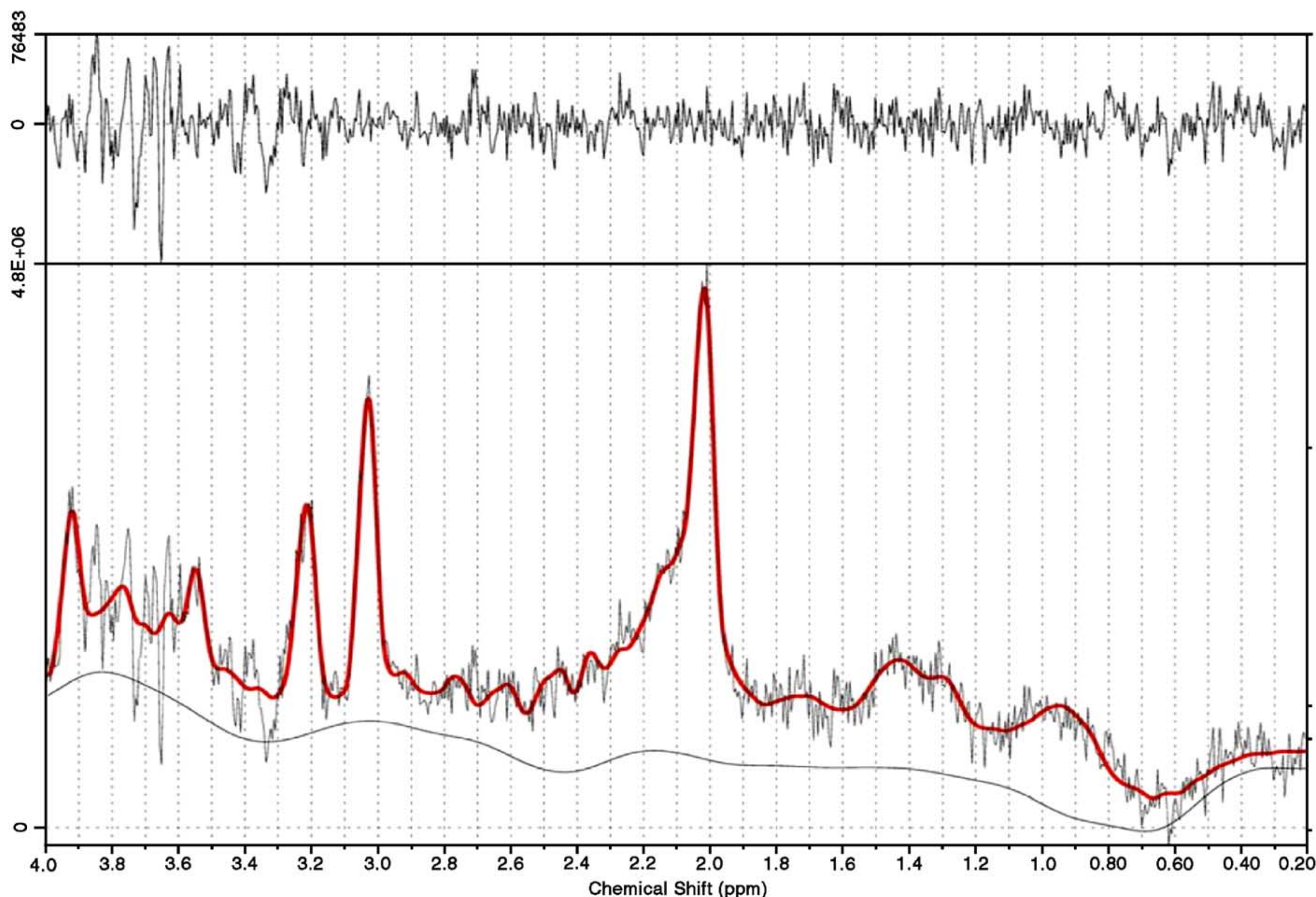

Fig. 3. Typical analysed in vivo brain striatum ^{1}H-MRS spectrum. On the bottom panel the acquired spectrum is shown (without line broadening, hence the more noisy appearance) superimposed with the bold fitted spectrum from LCModel. Residuals (observed–fitted) are presented in the top panel.

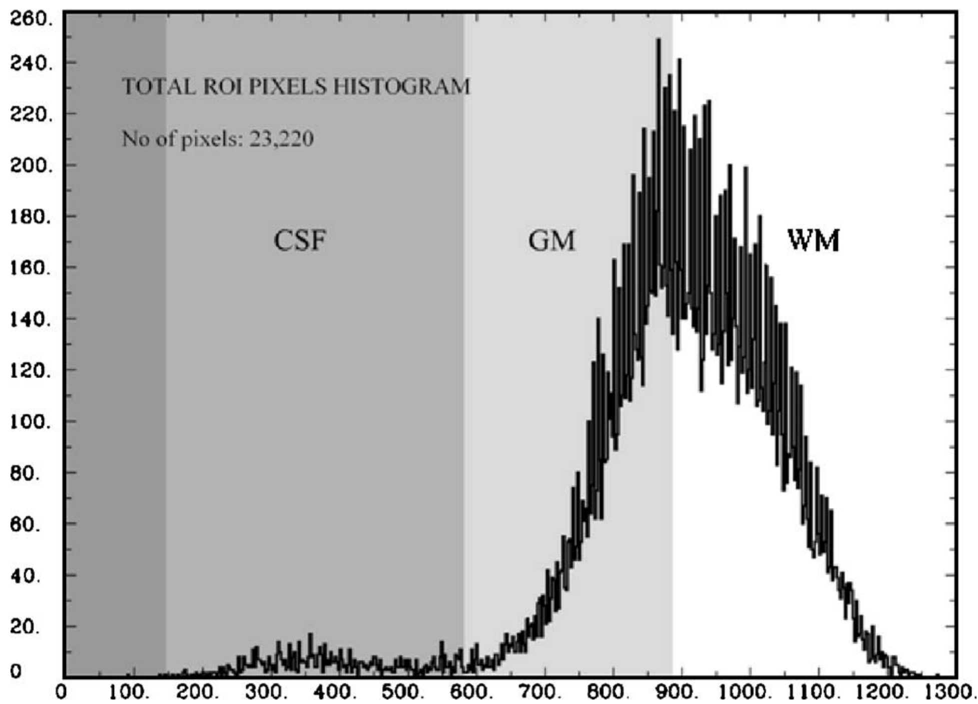

Fig. 4. Pixel intensity histogram from a striatal ROI. Histogram boundaries were chosen based on mean signal intensities calculated from ROIs chosen from definite cortical gray matter (frontal lobe), white matter (internal capsule) and lateral ventricle cerebrospinal fluid. CSF = cerebrospinal fluid fraction; GM = gray matter fraction; WM = white matter fraction.

laterality (P<.052), the Metabolite×Laterality interaction was not significant (P<.641.). Furthermore, paired sample t tests of either morning or afternoon measurements did not demonstrate significant right and left differences in metabolite levels for either Cr or NAA levels.

To further explore the Metabolite×Time of Day interaction, we have performed two independent t tests of Cr and NAA levels according to time of day. For NAA measurements, morning levels were significantly higher (P<.044) than those measured in the afternoon (Figs. 5 and 6). However, no significant changes in Cr levels were observed (Fig. 5). Finally, a repeated measures analysis of NAA to Cr ratios (NAA/Cr) revealed a significant main effect for time (P<.012). No significant effects were observed for either laterality (P<.330) or for the Laterality×Time interaction (P<.715).

3.1.4. Estimation of positioning effects

GM/ROI values for each of the four acquisitions ranged between 0.25 and 0.47 (Table 2).

Estimation of the magnitude of chemical shift effects was performed using paired t tests of the center frequency of each voxel. Significant left–right chemical shift effects were found during morning sessions (P<.05), and a trend was found for afternoon sessions (P<.08). In contrast, no significant chemical shift differences were found for morning and afternoon acquisitions (P<.15 and P<.099 for the right and left striatal nuclei, respectively).

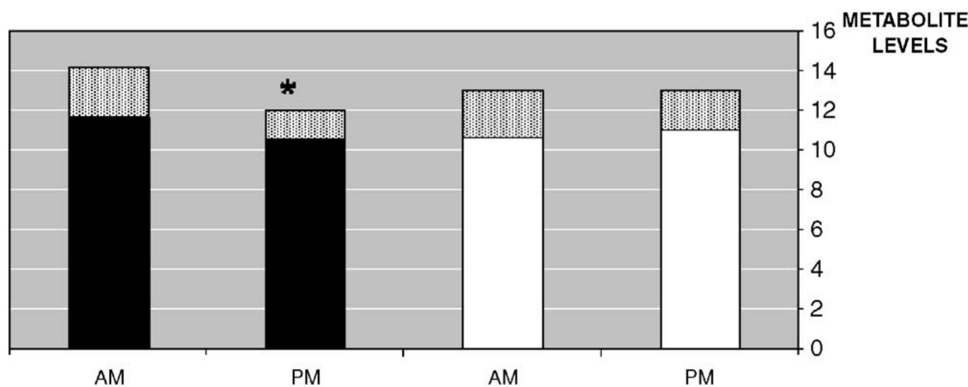

Fig. 5. Morning and afternoon NAA and Cr levels. White = Cr levels; black = NAA levels. *Significant (P<.05) morning – afternoon differences).

Table 1
Repeated measures procedure: NAA and Cr in vivo (Wilks' λ)

Effect	Wilks' λ	*F* value	Error *df*	*P* value
Metabolite	0.909	0.896	9	.369
Laterality	0.642	5.023	9	.052
Time	0.931	0.668	9	.435
Metabolite×Laterality	0.975	0.232	9	.641
Metabolite×Time	0.533	7.897	9	.020*
Laterality×Time	0.964	0.333	9	.578
Metabolite×Laterality×Time	0.998	0.022	9	.885

Repeated measures analysis results for three factors: metabolites (NAA or Cr), laterality (right or left) and time (morning or afternoon).

Table 2
ROI segmentation results of the single-subject experiment

Scan no.	ROIvoxels	WMvoxels	GMvoxels	CSFvoxels	GM/ROI	GM/WM
1	21,930	15,820	5547	380	0.25	0.35
2	23,220	13,162	8967	859	0.39	0.68
3	20,640	13,298	6190	984	0.30	0.47
4	26,910	14,087	12,649	175	0.47	0.89

ROIvoxels = total number of voxels in the ROI; WMvoxels = number of white matter voxels; GMvoxels = number of gray matter voxels; CSFvoxels = number of CSF voxels; GM/ROI = gray matter to ROI voxels ratio; GM/WM = gray matter to white matter voxels ratio.

3.2. Phantom measurements

CV values of phantom measurements were lower than the in vivo values and varied between 2% and 3% for both CRE and NAA. A repeated measure procedure did not reveal a significant main effect of time of day (P<.814) or an interaction between metabolite levels and time of day (P<.899).

4. Discussion

The present study explored possible intraindividual sources of variability in ^{1}H-MRS measurements of striatal nuclei. We hypothesized that both interregional (left vs. right striatal nuclei) and time of day (morning vs. afternoon) factors would have significant effects on variability of metabolite measurements. For both raw NAA and Cr levels, a main interregional effect was found, as well as a significant interaction between metabolite levels and time of day, explained by significantly higher NAA (but not Cr) levels during afternoon scanning sessions (Fig. 5). Further analysis of our data revealed a significant interaction between NAA/Cr values and time of day, with a lack of a significant laterality effects.

Our finding that NAA levels change significantly from morning to afternoon measurements seem to contrast previously published papers. Those studies have demonstrated the long-term stability of Cr and NAA levels [15]. We chose to scan patients at distinct times of day during a much shorter interval. Therefore, it is possible that the detected difference in NAA levels reflects physiological effects on brain NAA levels. That biological effects may be as important as repositioning effects was also supported by a recent study of intra- and interscanner in vivo reproducibility of brain metabolites [7].

Possible circadian mediators of change in metabolite levels include changes in brain temperature, hydration (more pronounced in TE <15 ms) and osmotic regulation. However, temperature changes would probably be associated with nonselective (i.e., similar) time of day effects on both NAA and Cr levels. In contrast, we have found that only NAA levels changed significantly from morning to afternoon.

Recent studies of NAA metabolism suggest that NAA may be associated with brain osmotic regulatory processes [16]. A recent [1-13C] glucose MRS study calculated the rate of NAA synthesis using a steady-state assumption [17]. Based on those findings, others [16] have suggested that the turnover of total brain NAA is around 16.7 h. However, the same study has also suggested that NAA metabolism may be closely coupled with glucose metabolism. Because there are known circadian variations of blood glucose [18,19], the rate of brain NAA synthesis may also vary during the circadian cycle. Therefore, both the hypothesized quasi-circadian NAA turnover cycle and NAA's possible involvement in osmotic regulation processes may support hydration as a possible mediator of circadian effects on NAA levels.

In addition to physiological effects on NAA levels, there also exist alternative explanations for differential morning to

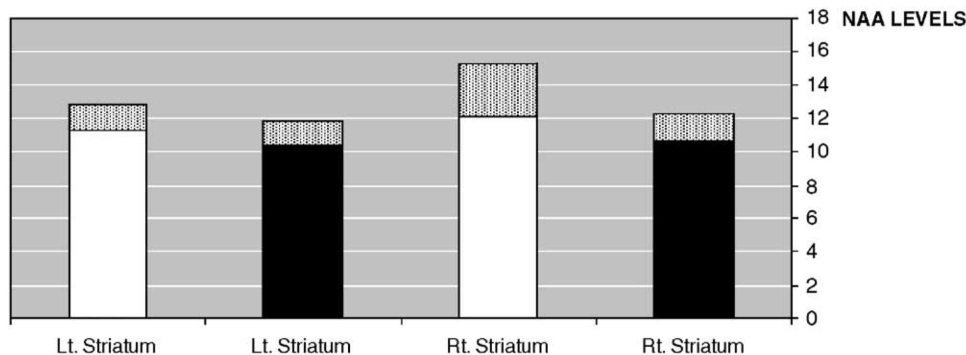

Fig. 6. Morning and afternoon NAA levels from right and left striatal nuclei. White = morning NAA levels; black = afternoon NAA levels.

afternoon changes of metabolite levels. Theoretically, our findings could have resulted from a systematic drift in scanner performance, test–retest effects (i.e., change in metabolite levels during repeated acquisitions), difficulties of measuring metabolite levels in the caudate nuclei or repositioning effects (leading to variable measurement of noncaudate tissues) [6,7,9]. A systematic mechanical drift seems unlikely, given the absence of both time of day effects and metabolite-time interaction effects for phantom measurements. Similarly, test–retest effects on metabolite levels are also unlikely due to the lack of any observed significant order effects.

The measurement of metabolite levels in the caudate nuclei can be challenging due to magnetic field inhomogeneities associated with iron depositions [6,20], relatively low SNR and partial volume effects [6,20]. We have attempted to minimize some of those effects using several measures. First, because striatal iron content increases with advancing age [6], our sample included primarily young adult subjects (age 29.4±9.7 years). Second, the use of a 3-T MRI scanner enabled us to acquire relatively small 4.5 cm^3 voxels, thereby decreasing possible partial volume effects (i.e., the inclusion of non striatal nuclei tissues).

Finally, partial volume effects could also result from known variations of voxel placements between morning and afternoon measurements (a repositioning effect [21]). Although we used relatively small voxels (4.5 cm^3) and image-aided positioning, striatal ROIs included subcortical gray matter (caudate and putamen nuclei) and white matter tissues (internal capsule). This is supported by our single-subject segmentation analysis that showed noticeable variations of gray to white matter ratios following repeated repositioning of a right striatal ROIs. However, repositioning effects were probably less significant for CSF, as results of the single subject segmentation analysis showed that CSF/ROI ratios were always below 5% and did not appear to have an association with time of day of scanning. In addition, comparison of center voxel frequencies failed to reveal any significant morning-to-afternoon differences.

In accordance with previous studies [21–23], we found a significant main effect for laterality. However, our results suggest that laterality effects in striatal measurements can be associated with repositioning effects. First, no laterality effects were found when NAA/Cr ratios were analyzed. Second, the analysis of center voxel frequencies showed significant left–right differences during morning measurements, with a trend towards significance during afternoon measurements. This is likely due, in part, to spatial selectivity. Although current literature and our own findings demonstrate significant laterality effects on metabolite levels, this could be due, in part, to center frequency variation rather than true biological variance.

Several limitations exist on the interpretations of our findings. First, a relatively small sample size was used in this study. However, this sample size is representative of many current imaging projects. Second, due to the preliminary nature of the study, we have only sampled two distinct times of day points. Third, we have focused on a single brain structure. Since we have found significant morning-to-afternoon differences within only a single brain structure, future studies should include more temporal and spatial sampling points.

5. Conclusions

Our findings highlight the importance of studying intraindividual sources of variability of ^{1}H-MRS measurements. Intraindividual sources may be important for the design, implementation and analysis of MRS studies (i.e., standardization of time of day, use of metabolite levels rather than metabolite ratios). Moreover, our findings may point to the importance of differentiating time of day from repositioning effects. Therefore, further studies that focus on circadian brain changes are needed.

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