Reproducibility and effect of tissue composition on cerebellar γ-aminobutyric acid (GABA) MRS in an elderly population

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MRS provides a valuable tool for the non-invasive detection of brain γ-aminobutyric acid (GABA) in vivo. GABAergic dysfunction has been observed in the aging cerebellum. The study of cerebellar GABA changes is of considerable interest in understanding certain age-related motor disorders. However, little is known about the reproducibility of GABA MRS in an aged population. Therefore, this study aimed to explore the feasibility and reproducibility of GABA MRS in the aged cerebellum at 3.0 T and to examine the effect of differing tissue composition on GABA measurements. MRI and \textsuperscript{1}H MRS examinations were performed on 10 healthy elderly volunteers (mean age, 75.2 ± 6.5 years) using a 3.0-T Siemens Tim Trio scanner. Among them, five subjects were scanned twice to assess the short-term reproducibility. The MEGA-PRESS (Mescher–Garwood point-resolved spectroscopy) J-editing sequence was used for GABA detection in two volumes of interest (VOIs) in the left and right cerebellar dentate. MRS data processing and quantification were performed with LCModel 6.3-0L using two separate basis sets, generated from density matrix simulations using published values for chemical shifts and J couplings. Raw metabolite levels from LCModel outputs were corrected for cerebrospinal fluid contamination and relaxation. GABA-edited spectra yielded robust and stable GABA measurements with averaged intra-individual coefficients of variation for corrected GABA+ between 4.0 ± 2.8% and 13.4 ± 6.3%, and inter-individual coefficients of variation between 12.6% and 24.2%. In addition, there was a significant correlation between GABA+ obtained with the two LCModel basis sets. Overall, our results demonstrated the feasibility and reproducibility of cerebellar GABA-edited MRS at 3.0 T in an elderly population. This information might be helpful for studies using this technique to study GABA changes in normal or diseased aging brain, e.g. for power calculations and the interpretation of longitudinal observations. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: MRS; γ-aminobutyric acid (GABA); aging brain; reproducibility; partial volume correction

INTRODUCTION

The precise balance between excitatory and inhibitory neurotransmitter systems is critical for the maintenance of the function of the central nervous system. Inhibition is mediated by γ-aminobutyric acid (GABA) through actions on both ionotropic and metabotropic receptors. In the developing nervous system, GABA plays a key role in the modulation of neural progenitor proliferation, cell migration and circuit formation (1). In the normal aging brain, a decrease in GABAergic parameters, such as reduced GABA current amplitude and loss in GABA-immunopositive neurons, is known to affect brain functions, proliferation, cell migration and circuit formation (1).
such as visual acuity and orientation sensitivity (2–5). Moreover, disturbances of the GABA neurotransmitter system have been implicated in many neurological and psychiatric diseases, including epilepsy (6), mood and anxiety disorders (7,8), sleep disorders (9), schizophrenia (10), autism (11) and essential tremor (12). An understanding of in vivo GABA changes would provide important insights into the mechanism of age-related brain function deficits and the role of GABA in various disease models, as well as aid in potential treatment evaluations.

Currently, MRS provides a valuable tool for the non-invasive detection of brain metabolites in vivo. Although it has proven to be difficult to measure in vivo GABA reliably because of its low concentration and spectral overlap with other more abundant metabolites, several methods have been adapted to successfully measure GABA, the most frequently used of which is the MEGA-PRESS (Mescher–Garwood point-resolved spectroscopy) sequence (13–15). Indeed, MRS studies have shown GABA perturbation in the above-mentioned neurological and psychiatric diseases (16–21). Several groups have also reported age-associated decreases in brain GABA levels in humans and rodents (22,23). In addition, reduced cortical GABA has been demonstrated in elderly patients with mild cognitive impairment (24). A better understanding of the reproducibility of the technique in an aged population would aid in the association of these changes with motor, sensory and cognitive deficits.

Several studies have reported within-session, short-term and long-term reproducibility of brain GABA MRS measurements, with coefficients of variation (CVs) differing across studies, but generally less than 20%, depending on the brain region, acquisition parameters and spectral fitting tools (25–32). However, all of these studies recruited a young subject population (mean age of these eight studies was 29 years). The aging brain is known to suffer from substantial structural and neurochemical changes compared with the young brain (33). Losses in brain tissue volume as a result of age-related atrophy also differ substantially between white matter (WM), gray matter (GM) and cerebrospinal fluid (CSF) (34). Therefore, the results obtained from the young population may not be generalized to an elderly population. The assessment of the reproducibility of GABA MRS measurements and the effects of tissue composition in an elderly population might provide new and relevant information for studies exploring GABA changes in normal and diseased aging brain regions. For example, one region of interest is the cerebellum, which not only shows age-related volumetric change, but is also involved in age-related motor deficits, learning function and processing speed decline (33,35–37). GABAergic dysfunction has been observed in the aging cerebellum and seems to be of great interest in age-related motor and cognitive deficits (38–40). One of the potential targets is to study essential tremor, the most common cause of tremor in humans. Increased $^{11}$C-flumazenil binding at GABAergic receptor sites has been reported by a positron emission tomography study (38), suggesting the potential involvement of GABA activity in essential tremor.

The aims of the current analyses were: (1) to explore the feasibility and reproducibility of GABA-edited spectroscopy in the aged cerebellum at 3.0 T; and (2) to examine the effect of tissue composition on GABA measurements.

**MATERIALS AND METHODS**

**Subjects**

Ten healthy elderly volunteers (three males, seven females; mean age ± standard deviation (SD), 75.2 ± 6.5 years; range, 65–86 years) were recruited as part of a longitudinal study of brain GABA in patients with essential tremor and in age-matched healthy elderly volunteers. All healthy elderly volunteers underwent a structured clinical interview to rule out any neurological or psychiatric diseases, as well as a detailed videotaped neurological examination that was reviewed by a senior movement disorder neurologist (EDL) to rule out the presence of essential tremor, Parkinson’s disease or other disorders of involuntary movement. None of the volunteers was taking medication that could increase brain GABA concentration (e.g. clonazepam, primidone) or had a history of heavy exposure to ethanol, as previously defined (41). To assess short-term, intra-individual reproducibility, five healthy volunteers were scanned twice at various time intervals ranging from 2 to 28 days (mean ± SD, 10.8 ± 8.9 days). The study protocol was reviewed and approved by the Human Subjects Institutional Review Board at Columbia University, Yale University, Purdue University and Weill Cornell Medical College. Written informed consent was obtained from each subject prior to participation in the study.

**In vivo MRI/MRS measurements**

MRI and $^1$H MRS examinations were performed on a 3.0-T Siemens Tim Trio scanner (Siemens Healthcare, Erlangen, Germany), equipped with a 32-channel head coil. Fast T$_1$-weighted images were acquired in all three orientations to ensure the exact localization of the MRS volumes of interest (VOIs). Short-TE $^1$H spectra [PRESS localization; TR/TE = 1500/30 ms; CHESS (chemical shift selective) water suppression] were acquired in four VOIs: left and right cerebellar cortex (both 15 mm × 15 mm × 25 mm, 128 averages), and left and right cerebellar dentate nucleus (both 25 mm × 25 mm × 25 mm, 128 averages) (Fig. 1). For all of the VOIs, a reference spectrum was acquired without water suppression. These reference spectra were then used for phase and frequency correction of the corresponding water-suppressed spectra. FASTESTMAP shimming (IPR#577; Siemens Healthcare) was performed before each voxel measurement to achieve water linewidths of <20 Hz (42). The MEGA-PRESS J-editing sequence was used for GABA detection (TR/TE = 1500/68 ms) (13,14) in two of the four VOIs described above. MEGA-PRESS spectra were only acquired in the right and left dentate nucleus, given the fact that this is the level at which the Purkinje cells release their GABA into the synaptic cleft (43); 196 averages were acquired with the spectrally selective editing pulse centered at 1.9 ppm (edit-on) and 196 averages with the pulse centered at 7.5 ppm (edit-off) in an interleaved fashion. The resulting difference spectrum contains a GABA peak at 3.0 ppm, which also includes contributions from co-edited macromolecules and homocarnosine, a dipetide consisting of GABA and histidine. Therefore, the signal is referred to as GABA+. In order to determine voxel tissue composition, high-resolution MPRAGE (magnetization-prepared rapid acquisition gradient echo) images were acquired [TR/TE/TI = 2300/2.91/900 ms; flip angle, 9°; bandwidth, 240 Hz/pixel; voxel size, 1.0 mm × 1.0 mm × 1.2 mm; GRAPPA (generalized autocalibrating partially parallel acquisition) = 2]. Every effort was made to ensure that the subjects were as comfortable as possible in the scanner. The dentate nucleus was clearly identified on the T$_2$-weighted images on both axial and coronal planes. The GABA voxel was placed on the axial plane such that the entire dentate nucleus was included, whilst minimizing the contributions from vascular and CSF compartments. The voxel was then confirmed to be completely within the cerebellum on the coronal plane. The voxel placed in the cerebellar cortex was positioned in the posterior...
cortex of the axial plane and in the superior cortex of the coronal plane. The voxel was angled to follow the edge of the cerebellar cortex in both axial and coronal planes. Placement was then confirmed to be completely contained within the cerebellum on the sagittal plane. The use of this prescription allowed for reproducible placement of voxels across subjects and in repeat scans.

Data processing and analysis

MRS data processing and quantification were performed with LCModel 6.3-0L (44), fitting each spectrum as a weighted linear combination of basis spectra from individual metabolites. For the short-TE spectra, a basis set of in vitro spectra from individual metabolite solutions was used. For the MEGA-PRESS spectra, basis sets were generated from density matrix simulations of the sequence using two sets of published values for chemical shifts and \( J \) couplings from Govindaraju et al. (45) and Kaiser et al. (46), with an exact treatment of metabolite evolution during the two frequency-selective MEGA inversion pulses. These two sets of chemical shift and \( J \)-coupling values (Table 1), one from an early publication reporting multiple metabolites and one from a later publication for refining GABA values, have been used extensively in GABA spectroscopy studies to date. Therefore, we chose to analyze our MEGA-PRESS spectra with two separate basis sets that employed both sets of values, and to further test their relationship. The two basis sets are denoted as ‘Govindaraju basis set’ and ‘Kaiser basis set’, or abbreviated as ‘G basis set’ and ‘K basis set’, respectively. Difference basis spectra were obtained by subtracting the simulated metabolite response to selective inversion at 7.5 ppm from that at 1.9 ppm.

The full width at half-maximum (FWHM) and signal-to-noise ratio (S/N) were checked to ensure consistent spectral quality. Two sets of short-TE spectra from the cerebellar cortex VOIs were excluded from further analysis because of insufficient water suppression. LCModel fitting %SD values for our metabolites of interest – creatine (Cr), N-acetylaspartate (NAA) and glutamate (Glu) – were lower than 10% and, for GABA+, lower than 20%.

In order to compare results with previously published reports, the ratios of GABA+/Cr and NAA were also calculated. Although raw GABA+/NAA values were given directly by LCModel outputs, raw GABA+ values were derived from raw water-scaled GABA+ output values, multiplied by a water scaling factor. Cr levels were obtained from spectra with the MEGA-PRESS editing pulse centered at 7.5 ppm, in order to calculate the ratios of GABA+/Cr. To determine the tissue composition of the voxels, MPRAGE images were segmented into GM, WM and CSF using an in-house MATLAB 2013a (MathWorks Inc., Natick, MA, USA) code, incorporated with statistical parametric mapping (SPM8, Wellcome Department of Imaging Neuroscience, London, UK). One segmentation example of a dentate nucleus voxel is shown.

Table 1. Chemical shifts and \( J \)-coupling constants from two previous publications used for the generation of \( \gamma \)-aminobutyric acid (GABA) (NH\(_2\)-CH\(_2\)-CH\(_2\)-CH\(_2\)-COOH) basis sets

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>( J ) coupling (Hz)</th>
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<tr>
<td>( ^2\text{CH}_2 )</td>
<td>( ^3\text{CH}_2 )</td>
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in Fig. 2. Metabolite levels corrected for CSF and relaxation were obtained using the following equation (47,48):

\[
M_{\text{cor}} = \left( M_{\text{raw}} \right) \times \frac{43300 \times f_{\text{GM}} + 35880 \times f_{\text{WM}} + 55556 \times f_{\text{CSF}}}{35880} \times \frac{1}{1 - f_{\text{CSF}}} \times \frac{\text{VIS}_W}{\exp \left( -\frac{\text{TE}_{\text{raw}}}{T_{1,\text{CSF}}} \right)} \times \frac{1}{\exp \left( -\frac{\text{TE}}{T_{2,m}} \right)}
\]

where \( M_{\text{cor}} \) is the corrected value, \( M_{\text{raw}} \) is the uncorrected value, \( \text{VIS}_W \) is a correcting factor for MR water visibility (0.65), \( T_{2,m} \) is the \( T_2 \) relaxation time of the metabolite, and \( f_{\text{GM}}, f_{\text{WM}} \) and \( f_{\text{CSF}} \) are the fractions of GM, WM and CSF within the voxel, respectively. The term \( \frac{43300 \times f_{\text{GM}} + 35880 \times f_{\text{WM}} + 55556 \times f_{\text{CSF}}}{35880} \) is included to adjust for voxel water concentration, where the LCModel default water concentration is 35880 mM CSF contamination is corrected for by the term \( \frac{1}{1 - f_{\text{CSF}}} \). LCModel estimates \( T_1 \) relaxation correction through \( f = \frac{1}{1 - \exp \left( -\frac{\text{TE}}{T_{1,m}} \right)} \) when water scaling is used. Therefore, no additional correction for \( T_1 \) relaxation was performed. The \( T_2 \) values of GABA, Cr, NAA, Glu and water were chosen to be 88, 154, 259, 181 and 95 ms, respectively (49–51). For GABA, raw output from the difference spectrum should be multiplied by the FCALIB factor, which can be obtained from the .PRINT file from the edit-off spectrum analysis to estimate water scaled concentration. Corrected metabolite ratios were calculated from corrected GABA+ divided by corrected Cr and corrected NAA, respectively. Both raw and corrected levels are reported.

**Statistics**

Results from a total of 30 GABA-edited spectra from the cerebellar dentate, 30 short-TE spectra from the cerebellar dentate and 28 from the cerebellar cortex were analyzed. Statistical analyses were performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). All metabolites of interest were found to be normally distributed, as assessed by the Shapiro–Wilk test (\( p > 0.05 \)). Descriptive values are reported (mean, SD). CVs for each of the five individuals who underwent two scans were calculated and averaged as an estimate of intra-individual reproducibility, whereas CVs for all subjects were calculated as an estimate of inter-individual reproducibility for each metabolite of interest. Metabolite levels were compared between left and right corresponding VOIs using Student’s t-tests. Pearson’s correlation was used to estimate the relationship between combined left and right corrected GABA+ versus raw GABA+, GABA+/Cr and GABA+/NAA measurements. The correlations between combined left and right NAA and Glu in cerebellar dentate and cortex were also determined, respectively, in order to compare with one previous publication, which found a significant correlation in young subjects (52). In order to compare the performance of the two basis sets, pairwise Wilcoxon signed rank tests were used to test the mean values of all raw and corrected GABA+ measurements, and the corresponding inter- and intra-individual CVs between, using the two basis sets. Bonferroni correction was employed to correct for multiple comparisons.

**RESULTS**

Figure 1 shows representative VOI placements for right cerebellar cortex and dentate, as well as pairs of right cerebellar GABA-edited difference spectra from five subjects. GABA+ measurements (GABA+, GABA+/Cr and GABA+/NAA), including raw and corrected values, using two sets of LCModel analysis, are reported in Table 2. Intra-individual CVs for the repeated GABA+ measurements (raw and corrected GABA+, GABA+/Cr and GABA+/NAA) range from 4.0 ± 2.8% to 13.9 ± 6.9%, whereas inter-individual CVs range from 12.6% to 27.0%. Overall correction for CSF contamination and relaxation did not significantly improve the CVs. No difference was observed between left and right cerebellar dentate GABA+ levels. Corrected Cr, NAA and Glu levels obtained from the short-TE spectra in all four VOIs are shown in Table 3. Intra-individual CVs for these corrected metabolites were lower than 5.5 ± 0.5%, whereas inter-individual CVs were lower than 15.4%. NAA levels were significantly higher in the left than in the right cerebellar cortex (\( p < 0.001 \)). In addition, there was a significant correlation between NAA and Glu in the cerebellar cortex (\( R = 0.699, p < 0.01 \)), but not in the dentate.

Figure 3 shows the individual corrected GABA+ levels in the left and right cerebellar dentate from all subjects using two LCModel basis sets, with five subjects scanned twice to assess the short-term reproducibility. Using the Kaiser basis set, corrected GABA+ correlated significantly with raw GABA+, GABA+/Cr and GABA+/NAA (\( R = 0.985, 0.869 \) and 0.857, respectively; all \( p \) values < 0.001) (Fig. 4). Using the Govindaraju basis set, the corrected GABA+ levels also correlated significantly with raw GABA+, GABA+/Cr and GABA+/NAA (\( R = 0.965, 0.729 \) and 0.737, respectively; all \( p \) values < 0.001). Corrected GABA+ values using the Kaiser basis set and the Govindaraju basis set were significantly different (\( p < 0.01 \)). However, they showed a significant correlation (\( R = 0.858, p < 0.001 \)). For all raw and corrected measurements, the use of the Kaiser basis set yielded smaller GABA values and higher inter-individual CVs than the use of the Govindaraju basis set, as shown by pairwise Wilcoxon signed rank tests (both \( p < 0.01 \)). No significant difference was seen in intra-individual CVs between the two basis sets. Table 4 shows the percentages of tissue compositions of all four VOIs.
Table 2. Raw and corrected mean GABA+ levels in institutional units obtained from GABA-edited spectra using LCModel with two basis sets (abbreviated as K basis set and G basis set), and corresponding intra-individual and inter-individual coefficients of variation (CVs)

<table>
<thead>
<tr>
<th></th>
<th>Left cerebellar dentate</th>
<th>Right cerebellar dentate</th>
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<tbody>
<tr>
<td></td>
<td>K basis set</td>
<td>G basis set</td>
</tr>
<tr>
<td></td>
<td>Level</td>
<td>Intra-individual CV (%)</td>
</tr>
<tr>
<td>Raw GABA+</td>
<td>1.64 ± 0.39</td>
<td>12.0 ± 3.7</td>
</tr>
<tr>
<td>Corrected GABA+</td>
<td>1.05 ± 0.25</td>
<td>11.9 ± 4.1</td>
</tr>
<tr>
<td>Raw GABA+/Cr</td>
<td>0.17 ± 0.04</td>
<td>13.9 ± 6.9</td>
</tr>
<tr>
<td>Corrected GABA+/Cr</td>
<td>0.22 ± 0.05</td>
<td>13.4 ± 6.3</td>
</tr>
<tr>
<td>Raw GABA+/NAA</td>
<td>0.15 ± 0.03</td>
<td>9.4 ± 7.3</td>
</tr>
<tr>
<td>Corrected GABA+/NAA</td>
<td>0.24 ± 0.05</td>
<td>11.5 ± 4.2</td>
</tr>
</tbody>
</table>

Cr, creatine; GABA, γ-aminobutyric acid; G basis set, Govindaraju basis set; K basis set, Kaiser basis set; NAA, N-acetylaspartate.

Table 3. Raw and corrected mean metabolite levels in institutional units obtained from short-TE spectra using LCModel from all subjects, and corresponding intra-individual and inter-individual coefficients of variation (CVs)

<table>
<thead>
<tr>
<th></th>
<th>Left cerebellar dentate</th>
<th>Right cerebellar dentate</th>
<th>Left cerebellar cortex</th>
<th>Right cerebellar cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level</td>
<td>Intra-individual CV (%)</td>
<td>Inter-individual CV (%)</td>
<td>Level</td>
</tr>
<tr>
<td>Raw Cr</td>
<td>7.00 ± 0.77</td>
<td>2.8 ± 2.1</td>
<td>10.9</td>
<td>6.95 ± 0.62</td>
</tr>
<tr>
<td>Corrected Cr</td>
<td>4.87 ± 0.61</td>
<td>3.0 ± 1.7</td>
<td>12.6</td>
<td>4.65 ± 0.50</td>
</tr>
<tr>
<td>Raw NAA</td>
<td>6.79 ± 0.32</td>
<td>1.3 ± 0.6</td>
<td>4.7</td>
<td>6.97 ± 0.47</td>
</tr>
<tr>
<td>Corrected NAA</td>
<td>4.35 ± 0.17</td>
<td>1.3 ± 0.5</td>
<td>4.0</td>
<td>4.31 ± 0.33</td>
</tr>
<tr>
<td>Corrected NAA/Cr</td>
<td>0.91 ± 0.11</td>
<td>2.1 ± 2.5</td>
<td>12.1</td>
<td>0.93 ± 0.09</td>
</tr>
<tr>
<td>Raw Glu</td>
<td>5.53 ± 0.79</td>
<td>3.7 ± 2.9</td>
<td>14.3</td>
<td>5.99 ± 0.93</td>
</tr>
<tr>
<td>Corrected Glu</td>
<td>3.73 ± 0.50</td>
<td>3.4 ± 2.4</td>
<td>13.5</td>
<td>3.89 ± 0.60</td>
</tr>
</tbody>
</table>

Cr, creatine; Glu, glutamate; NAA, N-acetylaspartate. *p < 0.01 when comparing left versus right cerebellar cortex.
DISCUSSION

Our results demonstrate the feasibility of cerebellar GABA-edited spectroscopy at 3.0 T in an elderly population with a mean age of 75.2 years. In general, the use of the Kaiser basis set yielded smaller GABA+ values than the use of the Govindaraju basis set, which should be a result of the fact that Kaiser et al. considered realistic experimental issues, such as volume selection, radiofrequency pulse shapes, editing efficiency, etc., leading to significant signal loss compared with ideal conditions. No significant difference in intra-individual CVs was observed between the two basis sets. However, inter-individual CVs were higher using the Kaiser basis set. This could be a result of mean GABA+ values being lower using the Kaiser basis set, resulting in a smaller denominator for the calculation of the inter-individual CVs, and thus higher CVs (corrected GABA+ (mean ± SD): 1.10 ± 0.23 for the Kaiser basis set and 1.46 ± 0.21 for the Govindaraju basis set).

Two recent studies have reported GABA-edited spectra in the cerebellar vermis and left cerebellum in younger healthy populations (mean age, 24.6 and 43.5 years; GABA+/Cr, 0.23 ± 0.06 and 0.21 ± 0.09, respectively) (52,53). Our GABA+/Cr levels were found to be in a similar range: in the left cerebellum, the corrected GABA+/Cr value was 0.22 ± 0.05 using the Kaiser basis set and 0.30 ± 0.04 using the Govindaraju basis set, whereas averaged left and right cerebellar GABA+/Cr was 0.23 ± 0.05 using the Kaiser basis set and 0.31 ± 0.05 using the Govindaraju basis set. In the literature, no change or increased Cr was found (54,55), whereas no change or decreased GABA was reported in older subjects (22–24,56). GABA+/Cr values calculated with the Kaiser basis set were more similar to the reported GABA/Cr values in the younger populations, whereas the GABA+/Cr levels calculated with the Govindaraju basis set were slightly higher than those in the younger populations. If using literature values as the gold standard, this finding may reflect the fact that Kaiser et al. refined their GABA chemical shifts and J-coupling values relative to Govindaraju et al. However, it is unknown what chemical shifts and/or coupling constants were used for the reported values. Therefore, direct comparison may be problematic. The current study suggests that we need to be aware of the different outcomes when using these basis sets. It is important for the authors to report how their basis sets were simulated, and comparisons with literature values must take this difference into account. Nevertheless, it is worth mentioning that there was a significant correlation between the GABA levels obtained from both basis sets.

The corrected NAA levels in our study were lower than the corrected Cr levels in all four VOIs. Guerrini et al. (57) have reported corrected NAA/Cr = 0.79 ± 0.17 in a VOI that included the superior cerebellar vermis in 29 healthy subjects (age, 37 ± 11 years). Our mean corrected NAA/Cr was 0.73 ± 0.09 in the cerebellar cortex, a similar VOI to theirs. In addition, mean raw NAA/Cr can be derived as 1.08 in the cerebellum of elderly subjects from Zahr et al. (54), whereas our mean raw NAA/Cr was 1.00 ± 0.11 in a similar VOI in the dentate nucleus. The currently reported intra- and inter-individual CVs of cerebellar NAA and Cr are also in close agreement with previous reports (58,59). It is worth noting here that different investigators, and hence

No significant difference was observed between the left and right cortical or dentate voxel composition.
different studies, may use different acquisition parameters, voxel sizes/placements and spectral quantification tools. Thus, absolute values are difficult to compare directly with each other; even metabolite ratios may differ slightly without real physiological cause.

The asymmetry between left and right cerebellar cortical NAA levels is in line with two previous reports on higher NAA in the left prefrontal cortex and left thalamus from healthy subjects (60,61). Indeed, a greater neuronal density was observed in the left than in the right hemisphere in the literature (62,63). The current correlation between NAA and Glu in the cerebellar cortex, but not in the dentate, is in general agreement with Waddell et al. (52), because their cerebellar vermis VOI contained more cortical regions.

The reproducibility studies on GABA MRS in younger adult populations reported intra-individual CVs for GABA+ to be between 3.5% and 20.4%, whereas inter-individual CVs were between 9.1% and 38%, depending on different brain regions, acquisition and analysis techniques (25–32). It is important to mention that three of these reports appear not to have corrected for CSF contamination; however, they did not necessarily have higher CVs. Despite being conducted in an elderly study population, our corrected GABA+ showed similar reproducibility results (mean intra-individual CVs between 4.0% and 13.4% and inter-individual CVs between 12.6% and 24.2%). Although corrected for inter-individual differences in neuronal density and atrophy, CSF- and relaxation-corrected GABA+ levels still correlated significantly with raw GABA+, GABA+/Cr and GABA+/NAA in the dentate nucleus calculated with both Kaiser and Govindaraju basis sets in this elderly population. Correction for CSF and relaxation did not improve the reproducibility results, which may be a result of the very small CSF volume in the voxel (<5.3% for both dentate VOIs) and the large difference in the measured GABA+ values among these differently aged subjects (between 65 and 86 years). Nevertheless, correction is still critical and must be considered as an important factor to control in the study of older subjects, who may have volume loss and atrophy in the region of interest.

One major limitation of the current MEGA-PRESS sequence is macromolecular contamination of the GABA signal at 3.0 ppm. This could be corrected by placing the editing pulses at 1.9 and 1.5 ppm in order to symmetrically suppress macromolecules at 3.0 ppm (64), but often this results in substantial suppression of GABA as a result of insufficient selectivity of the editing pulse at 3.0 T. This technique is also impractical in an aged population, who probably have decreased GABA levels. Alternatively, the contamination problem could be addressed using other sequences for acquisition or by explicitly modeling the macromolecules in the LCModel basis set in post-processing procedures (65,66). Nevertheless, the current study chose to use the original MEGA-PRESS sequence and direct LCModel analysis to evaluate GABA reproducibility, because of the availability and practicability of this approach, especially in a clinical study. Our results must therefore be interpreted as showing the short-term reproducibility of measurement of the sum of GABA, homocarnosine and co-edited macromolecules in the cerebellum of an elderly population. In addition, our basis sets were created assuming ideal pulses; therefore, they actually correspond to the center point in the voxel. Consequently, the resulting quantification would slightly overestimate the true metabolite levels as a result of spatial variation in excitation, refocusing and chemical shift misregistration.

**CONCLUSIONS**

In summary, our results demonstrate the feasibility of cerebellar GABA-edited MRS at 3.0 T in an elderly population. All GABA measurements yielded reproducible estimates using the MEGA-PRESS sequence, with the approach using the Kaiser basis set yielding quantification results of GABA+/Cr that were most consistent with literature reports. With a small CSF percentage in the dentate VOIs and a large range in age amongst the elderly subjects (approximately 20 years), correction for CSF contamination and relaxation did not improve the reproducibility results. Corrected GABA+ levels correlated significantly with uncorrected GABA+ measurements. These results might be helpful for studies using this technique to examine GABA changes in normal or diseased aging brain, e.g. for power calculation and the interpretation of longitudinal observations.

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