Comparison of linear combination modeling strategies for GABA-edited MRS at 3T

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Abstract

Purpose
J-difference-edited spectroscopy is a valuable approach for the in vivo detection of \( \gamma \)-aminobutyric acid (GABA) with MRS. A recent expert consensus article recommends linear combination modeling (LCM) of edited MRS, but does not give specific details of implementation. This study explores different modeling strategies to adapt LCM for GABA-edited MRS.

Methods
62 medial parietal lobe GABA-edited MEGA-PRESS spectra from a recent 3T multi-site study were modeled using 102 different strategies combining six different approaches to account for co-edited macromolecules, three modeling ranges, three baseline knot spacings, and using basis sets with or without homocarnosine. The resulting GABA and GABA+ estimates (quantified relative to total creatine), the residuals at different ranges, SDs and CVs, and Akaike information criteria, were used to evaluate the models’ performance.

Results
Significantly different GABA+ and GABA estimates were found when a well-parameterized MM\(_{3\text{co}}\) basis function was included in the model. The mean GABA estimates were significantly lower when modeling MM, while the CVs were similar. A sparser spline knot spacing led to lower variation in the GABA and GABA+ estimates and a narrower modeling range – only including the signals of interest – did not substantially improve or degrade modeling performance. Additionally, results suggest that LCM can separate GABA and the underlying co-edited MM\(_{3\text{co}}\). Incorporating homocarnosine into the modeling did not significantly improve variance in GABA+ estimates.

Conclusion
GABA-edited MRS is best quantified by LCM with a well-parameterized co-edited MM\(_{3\text{co}}\) basis function with a constraint to the non-overlapped MM\(_{0.93}\) in combination with a sparse spline knot spacing and a modeling range between 0.5 and 4 ppm.
Introduction

A recent expert consensus paper recommended that linear combination modeling (LCM) should be used for the quantification of edited MRS data, stating that standard fitting approaches originally optimized for short-TE MRS should be adapted for edited MRS. Further, it was recommended that quantum-mechanical simulations should be used to confirm the co-edited profile of all metabolites in the edited spectrum, and contributions from macromolecule (MM) signals should be specified. Despite these recommendations, little detail was given regarding several unique features of edited spectra, and how they should be appropriately modeled. These features include:

1) The MEGA-PRESS experiment is well-known to co-edit MM signals with coupled spins at 1.7 and 3 ppm, causing substantial contamination of the edited GABA signal, and forcing researchers to report the composite measure GABA+MM (GABA+)\(^1\). Because the co-edited MM signal is poorly characterized, there is currently no consensus or recommendation on how to appropriately account for it during spectral modeling. Instead, the most widely used analysis algorithms implement entirely different strategies to fit the composite 3-ppm signal. For example, the Gannet software uses a single Gaussian model\(^2\), while a double-Gaussian is used in Tarquin\(^3\), and LCModel\(^4\) defaults to a basis set that only includes the GABA basis function.

2) Another co-edited compound contributing to the 3 ppm signal is homocarnosine (HCar), a dipeptide of GABA and histidine. While the 3 ppm multiplets of GABA and homocarnosine are separated by just 0.05 ppm (which are therefore unlikely to be successfully separated), inclusion of a homocarnosine basis function may be warranted based on its reported concentration in vivo (~0.5 mmol/kg\(^5\), compared to ~1-2 mmol/kg for GABA), but it has not been investigated whether doing so has a stabilizing or destabilizing effect on the modeling\(^6\).

3) Unedited spectra are typically modeled over a restricted frequency-domain range covering the visible upfield peaks, including macromolecular and lipid resonances between 0 and 1 ppm, but usually avoiding the water suppression window above ~4 ppm. The choice of frequency-domain modeling range for edited spectra is less obvious. Since...
the main advantage of spectral editing is the isolation of a single target resonance, modeling signals outside the immediate surrounding of the target may dilute the resolving power of editing. On the other hand, increasing the modeling range may offer useful constraints to stabilize the solution of the modeling problem. The difference is highlighted by the different strategies encountered in common software tools – while the Gannet software fits the GABA-edited difference spectrum over a narrow range (only including the 3-ppm GABA+ and 3.75 ppm glutamate and glutamine peaks), the LCModel recommendation is to include the strong co-edited signals from glutamate (Glu), glutamine (Gln), glutathione (GSH), N-acetylaspartylglutamate (NAAG), N-acetylaspartate (NAA), which heavily overlap with GABA around 2.25 ppm. The effects of limiting the modeling range have not been assessed systematically to date.

4) Linear combination modeling methods commonly include terms to account for smooth baseline curvature, usually parametrized from cubic B-spline or polynomial functions, or by smoothing residuals. The flexibility of the baseline model substantially affects metabolite estimates from unedited spectra\(^7\); while baseline terms are necessary to account for e.g. lipid contamination, poor water suppression etc., they are potential sources of overfitting if awarded too many degrees of freedom. Baseline modeling may have an even greater influence when modeling difference spectra, since only \textit{co-edited} lipid and MM signals contribute to the smooth background variation. Importantly, the co-edited MM background of the GABA-edited difference spectrum has not been appropriately characterized (e.g., through metabolite-nulled acquisition), suggesting that the choice of baseline flexibility can drastically influence modeling results through two highly susceptible regions of the spectrum. First, in the absence of an appropriate model for the co-edited broad MM signal at 3 ppm, this signal may be absorbed into the baseline depending on its flexibility. Second, strong MM and lipid signals in the region between 0.5 and 2.5 ppm may be affected by the 1.9 ppm editing pulse (either directly through saturation or indirectly through coupling), likely leading to an unknown, but substantial, MM contribution in this spectral region\(^8,9\). This is especially important considering that the co-edited signals from NAA, NAAG, Glu, Gln, and GSH overlap with GABA in this region. Overly rigid baselines may provide insufficient flexibility to capture these signals, in turn compromising the accuracy of the estimation of the co-edited metabolites.
The aim of this study was to evaluate different strategies for linear combination modeling of GABA-edited MEGA-PRESS difference spectra, and to establish initial ‘best practices’ substantiating the recommendations of the expert consensus on spectral editing. To this end, different approaches to account for co-edited MM signals, various modeling ranges and baseline knot spacings, as well as the inclusion of homocarnosine were compared. In the absence of a ‘gold standard’, the performance of each modeling strategy was assessed by comparing descriptive statistics of the metabolite estimates, calculating the Akaike information criteria, and assessing the fit residuals.
Methods

Study participants & data acquisition

In this study, 62 publicly available GABA-edited MEGA-PRESS datasets originating from 7 sites from a recent 3T multi-center study\textsuperscript{10} were analyzed (see Supplementary Material 1 for subject list). All datasets were acquired on Philips 3T scanners with the following acquisition parameters: TR/TE = 2000/68 ms; 320 excitations (10m 40s scan time); 2 kHz spectral width; 2000 samples; 27-ml cubic voxel volume in the medial parietal lobe. For the edit-ON transients, the editing pulses with 15 ms pulse duration and 82.5 Hz inversion bandwidth (FHWM) were applied at a frequency of 1.9 ppm to refocus the coupling evolution of the GABA spin system. For the edit-OFF transients, the editing pulses were applied at a frequency of 7.5 ppm. Edit-ON and edit-OFF transients were acquired in alternating order. An additional water reference scan was acquired for each dataset using interleaved water referencing \textsuperscript{11}, i.e. one excitation with water suppression and editing pulses deactivated every 40 water-suppressed excitations (total of 8 averages).

Data pre-processing

Data were analyzed in MATLAB using Osprey\textsuperscript{12,13}, a recently published open-source MRS analysis toolbox. Raw data were eddy-current-corrected \textsuperscript{14} based on the water reference, and individual transients were aligned separately within the edit-ON and edit-OFF conditions using the robust spectral registration algorithm\textsuperscript{15}. Averaged edit-ON and edit-OFF spectra were aligned by optimizing relative frequency and phase such that the water signal in the difference spectrum was minimized. The final difference spectra for quantification were generated by subtracting the edit-OFF from the edit-ON spectra. Finally, any residual water signal was removed with a Hankel singular value decomposition (HSVD) filter\textsuperscript{16}.

Basis set

The basis set used for modeling was generated from a fully localized 2D density-matrix simulation implemented in a MATLAB based simulation toolbox FID-A \textsuperscript{17}, using vendor-specific refocusing pulse shape and duration, sequence timings, and phase cycling. It contains 17 metabolite basis functions (ascorbate, aspartate, creatine (Cr), negative creatine methylene (-CrCH\textsubscript{2}),...
GABA, glycerophosphocholine, GSH, Gln, Glu, water, myo-inositol, lactate, NAA, NAAG, phosphocholine, phosphocreatine (PCr), phosphoethanolamine, scyllo-inositol, and taurine) and 8 Gaussian MM and lipid resonances (MM0.94, MM1.22, MM1.43, MM1.70, MM2.05, Lip09, Lip13, Lip20, defined as described in the LCModel software manual18) for the edit-OFF spectrum.

For the difference spectrum, MM0.94 and the co-edited macromolecular signal at 3 ppm (MM3co) were parametrized as Gaussian basis functions (MM0.94: 3-proton signal; chemical shift 0.915 ppm, full-width at half-maximum (FWHM) 11 Hz; MM3co: 2-proton signal; chemical shift 3 ppm; FWHM 14 Hz). The MM0.94 amplitude was defined as described in the LCModel software manual. The MM3co amplitude was defined under the assumption of a pseudo-doublet GABA signal at 3 ppm and the MM3co contribution to the 3-ppm GABA peak to be around 50%1,6,8,19. The optimum FWHM used to parametrize the MM3co basis function was determined to be 14 Hz by fitting the mean difference spectrum of all datasets with a composite GABA+ basis function (GABA + MM3co) with varying FWHM (between 1 and 20 Hz). The parameterized Gaussian MM3co basis function was integrated into the modeling process using different assumptions and constraints described in the following paragraphs.

Linear combination modeling of GABA-edited difference spectra

Osprey’s frequency-domain linear combination model was used to determine the metabolite estimates. Model parameters include metabolite basis function amplitudes, frequency shifts, zero/first order phase correction, Gaussian and Lorentzian linebroadening, and cubic spline baseline coefficients. All parameters are determined by Levenberg-Marquardt20,21 non-linear least-squares optimization, using a non-negative least-squares (NNLS) fit22–24 to determine the metabolite amplitudes and baseline coefficients at each iteration of the non-linear optimization. Amplitude ratio soft constraints are imposed on MM and lipid amplitudes, as well as selected pairs of
metabolite amplitudes, as defined in the LCModel manual\textsuperscript{4,18}. The strength factor of the amplitude ratio soft constraint $\lambda$ is set to 0.05 by default.

**Figure 1** – Different linear combination modeling strategies for GABA-edited spectra. (A) Different co-edited MM$\textsubscript{3co}$ modeling approaches derived from a Gaussian function at 3.0 ppm (B) All combinations of basis set composition, modeling range, spline knot spacing, and MM$\textsubscript{3co}$ modeling leading to 102 different modeling strategies.

A range of modeling strategies for the GABA-edited difference spectrum was included in this study, covering various aspects of the modeling process (Figure 1). The different parametrizations and soft constraints to account for the co-edited MM$\textsubscript{3co}$ signal are shown in Figure 1A. All possible combinations for the modeling strategies: i) inclusion of homocarnosine in the basis set; ii) different parametrizations and soft constraints to account for the co-edited MM$\textsubscript{3co}$ signal; iii) different modeling ranges and iv) different baseline spline knot spacings (Figure 1B). Each modeling aspect is described in detail below:
Co-edited macromolecule models

Seven different strategies to model the GABA-edited difference spectrum were implemented (Figure 1A). The trivial approach – not accounting for the co-edited signal MM$_{3co}$ at all – is labeled none. The other six modeling strategies all include a dedicated parametrized Gaussian MM$_{3co}$ basis function. This basis function is given different degrees of freedom in the different strategies, e.g. hard- or soft-constrained relative to the amplitude of the GABA or the MM$_{0.94}$ basis functions, and with a fixed or free width. Here, strategies with fewer degrees of freedom reflect frequently the made assumptions that the GABA-to-MM ratio (and the MM background itself) are relatively stable across subjects, while strategies with more degrees of freedom or soft constraints relax these assumptions:

- The GABA$_{\text{hard}}$ model uses a single composite GABA+MM basis function by adding the GABA and MM$_{3co}$ (FWHM = 14 Hz) basis functions with a fixed 1:1 amplitude ratio. The 1:1 ratio reflects the widely used empirical assumption that 50% of the 3-ppm signal in a conventional GABA-edited difference spectrum can be attributed to co-edited macromolecules$^6$.

- The GABA$_{\text{soft}}$ model uses separate GABA and MM$_{3co}$ (FWHM = 14 Hz) basis functions, and imposes a soft constraint on the ration of the amplitudes of both basis functions during the optimization (1:1 ratio).

- The Gauss$_{\text{fixed}}$ model uses separate GABA and MM$_{3co}$ (fixed FWHM = 14 Hz) basis functions. No further constraints are imposed. This means possible changes in the contributions to the 3-ppm GABA peak are modeled.

- The Gauss$_{\text{free}}$ model uses separate GABA and MM$_{3co}$ basis functions. In contrast to the Gauss$_{\text{fixed}}$ model, the FWHM of the Gaussian MM$_{3co}$ signal is represented by an additional model parameter. This means that the MM$_{3co}$ basis function itself is not static, but dynamically modified during optimization.

- The MM$_{0.94}$$_{\text{hard}}$ model uses separate GABA and MM basis functions. The MM$_{3co}$ basis function is replaced by a composite MM$_{0.94}$ + MM$_{3co}$ basis function (i.e., the fixed MM$_{0.94}$ (fixed FWHM = 11 Hz) and MM$_{3co}$ (fixed FWHM = 14 Hz) basis functions are added in a 3:2 ratio).
The MM09.soft model uses separate GABA, MM$_{0.94}$ and MM$_{3co}$ basis functions. In contrast to the MM09.hard model, soft constraints enforce a $\sim$3:2 amplitude ratio for the MM$_{0.94}$ and MM$_{3co}$ amplitudes during optimization.

The models Gauss$_{fixed}$, MM09.hard and MM09.soft correspond to models previously investigated using the LCModel software$^{25}$ and the amplitude assumptions were derived empirically.

Varying the modeling range and baseline knot spacing

Two aspects of linear combination modeling are suggested to have a considerable influence on metabolite estimates$^{7,26}$. First, the choice of the modeling range, i.e., the frequency interval that defines the part of the frequency-domain spectrum that is considered to calculate the least-squares difference between model and data. Second, the baseline knot spacing, i.e., the frequency difference between two adjacent knots of the cubic spline basis that is used to approximate the smooth baseline.

Three different modeling range scenarios were considered, reflecting common choices in the literature and widely used software tools: a) a wide modeling range typically used to analyze unedited spectra, including all signals in the GABA-edited difference spectrum (0.5 to 4 ppm – “wide”); b) an intermediate modeling range excluding signals below 1.9 ppm (e.g. co-edited lipids and macromolecules), but including strong co-edited signals from NAA, NAAG, Glu, Gln, and GSH (1.85 and 4.1 ppm, “intermediate”), comparable to the range recommended in LCModel; and c) a narrow modeling range only including the co-edited signals from GABA+ and Glx (2.79 – 4.2 ppm, "narrow"), the default modeling range in Gannet$^2$.

Three spline knot spacings were included in the analysis, with 0.4 ppm being the default Osprey option, shown to create reproducible and comparable metabolite estimates for conventional MRS$^27$, as well as sparser (0.55 ppm) and denser (0.25 ppm) spline knot spacings.
Including homocarnosine in the basis set

To assess the effects of including homocarnosine in the linear combination model, we repeated all analysis steps with two different basis sets: the default Osprey basis set with and without an additional HCar basis function. Chemical shift and scalar coupling parameters describing the HCar spin system were taken from literature. Combining the various MM$_{3co}$ models (5 + 2 that were used for the wide modeling range only), modeling ranges (3), baseline spline knot spacings (3), and basis sets (2), a total of 102 different modeling strategies were investigated in this study. All models were implemented in Osprey and are available on GitHub.

Quantification, visualization, and statistics

Quantification

For the basis set without homocarnosine, GABA refers to the model amplitude estimate for the GABA basis function, which is of course only available for the modeling strategies with separate basis functions for GABA and MM$_{3co}$ (none, GABA$_{soft}$, Gauss$_{fixed}$, Gauss$_{free}$, MM09$_{soft}$). GABA+ refers to the sum of the amplitude estimates for GABA and MM$_{3co}$ (GABA$_{soft}$, Gauss$_{fixed}$, Gauss$_{free}$, MM09$_{hard}$, MM09$_{soft}$) or the amplitude estimate for the composite basis function including both MM and GABA (GABA$_{hard}$), and is therefore calculated for all strategies with an explicit MM$_{3co}$ model. The GABA amplitude for the `none` strategy is reported at GABA+.

For the basis set that included homocarnosine (HCar), the difference in GABA and MM$_{3co}$ estimates between the modeling strategies with and without HCar ($\Delta$GABA and $\Delta$MM$_{3co}$, respectively) were investigated to evaluate whether the inclusion of HCar has a systematic effect on the estimation of those signals with which it overlaps. edit-OFF spectrum over the wide modeling range with a spline knot spacing of 0.4 ppm. Differences in GABA(+)/tCr between modeling strategies are therefore only related to the modeling of the difference spectra, but not to the reference compound modeling. No further tissue or relaxation corrections were applied.

Further, the relative contributions of MM$_{3co}$ to the GABA+ estimate and the relative contributions of HCar to the sum of GABA+ and HCar estimate were calculated.
Visualization

The modeling performance and systematic characteristics of each modeling strategy were visually assessed through the mean data, mean fit, mean residual, and mean models of GABA+, GABA, MM$_{3co}$, HC$_r$ (if included) and the baseline, i.e., averaged across all datasets.

The metabolite estimate distributions were visualized as violin plots including boxplots with median, 25th/75th quartile ranges, and smoothed distributions to identify systematic differences between modeling strategies. In addition, the mean value of the models without a co-edited MM model was added as a horizontal line. Bar plots were created to visualize quality metrics, including the standard deviation if appropriate. All plots were generated with R$^{28}$ (Version 3.6.1) in RStudio (Version 1.2.5019, RStudio Inc.) using SpecVis$^{27,29}$, an open-source package to visualize linear combination modeling results with the ggplot2 package$^{30}$. All scripts and results are publicly available$^{31}$.

Statistics

Significant differences in the mean and the variance of the GABA, GABA+, and MM$_{3co}$ estimates were assessed between all modeling strategies. The statistical tests were set up as paired without any further inference. Differences of variances were tested with Fligner-Killeen’s test, with a post-hoc pair-wise Bonferroni-corrected Fligner-Killeen’s test. The means were compared with an ANOVA or a Welch’s ANOVA, depending on whether variances were different or not. Post-hoc analysis was performed with a paired t-test with equal or non-equal variances, respectively.

Model evaluation criteria

The performance of each modeling strategy was evaluated in different ways, including the impact of the different modeling strategies on the GABA, GABA+, and MM$_{3co}$ estimates, as well as several quality measures:

1) Visual inspection: Mean model, residual, and baseline were assessed for characteristic features.

2) Range fit quality: the difference between the maximum and minimum of the residual was determined, and then normalized by the noise level$^{26}$ (calculated as the standard
deviation of the noise between -2 and 0 ppm). This was done over the entire modeling range of the difference spectrum and termed residual\_range.

3) 3-ppm peak fit quality: Similar to the second criterion, the residual was calculated over the range of 3.027 ± 0.15 ppm to assess the fit quality of the 3-ppm GABA peak and termed residual\_3ppm.

4) Consistency of metabolite estimates: Coefficients of variation for all metabolite estimates (GABA/tCr, GABA+/tCr) were calculated for each modeling strategy.

5) Akaike Information Criterion (AIC): The Akaike information criterion \(^{32}\), which takes the number of model parameters into account, is defined as follows:

\[
AIC_i = -2 \log(SSE_i) + 2K_i
\]

Here, \(SSE_i\) is the sum of squared error/residual of modeling strategy \(i\), and \(K_i\) is the number of free model parameters for that strategy. Soft constraint model parameters were included with a value of 0.5. Lower \(AIC_i\) values indicate a more appropriate model. Subsequently, \(\Delta AIC_i\) scores were calculated as the difference of \(AIC_i\) of modeling strategy \(i\) and the model with the lowest \(AIC_{min}\):

\[
\Delta AIC_i = AIC_i - AIC_{min}
\]
Results

All 62 datasets were successfully processed and modeled with all 102 modeling strategies. No data were excluded from further analysis.

Summary and visual inspection of the modeling results

Figure 2 – Mean modeling results for all modeling strategies without homocarnosine. A substantial structured residual is apparent at 3 ppm if no MM modeling strategy is included. All three modeling ranges (columns), three spline knot spacings (rows), and MM$_{3co}$ model (color-coded) are presented with mean residuals and fits, as well as the GABA+, GABA, MM$_{3co}$, and spline baseline models. The mean data is included in black. The dashed lines indicate the range...
of the residual across one row and the arrows indicate the maximum of a specific modeling range and spline knot spacing (color-coded).

**Figure 2** shows the mean modeling results for all modeling strategies without homocarnosine. Not including MM$_{3co}$ leads to a substantial structured residual around 3 ppm for all knot spacings and modeling ranges. In contrast, all modeling strategies with MM$_{3co}$ appear to reflect the line-shape of the 3-ppm signal more accurately, with very similar results for the complete fit (metabolites, MMs, and baseline) and the individual components. Modeling strategies with the intermediate and wide modeling range further show strong residuals around 2 ppm, suggesting slightly inaccurate lineshape modeling of the methyl singlets from NAA and NAAG, or inaccurate modeling of co-edited MM signals in this region. Structured residuals appear also in the region of the 3.75 ppm Glx signals, although they are much less pronounced in strategies with the narrow modeling range, suggesting that including the 2.25 ppm multiplets (and underlying baseline fluctuation) has a considerable impact on phase estimation.

In general, the residuals are consistent between different MM$_{3co}$ models for any given knot spacing and modeling range. Notably, residuals tend to be smaller for denser knot spacing and narrower modeling range.

Mean GABA models agree well between all strategies with a separate MM$_{3co}$ model. The GABA$_{hard}$ strategy appears to produce a larger signal as its GABA basis function includes the MM$_{3co}$ signal, but does not model it separately, while the strategies that do so produce comparable mean MM$_{3co}$ models.

The mean baseline is consistently flatter around 3 ppm for modeling strategies with an explicit MM$_{3co}$ model, while absorbing substantially more signal for the ‘none’ approach without an MM model. This behavior is particularly obvious for the dense knot spacing (0.25 ppm) over the wide modeling range. Baseline curvature generally increases for denser knot spacings around 2.2 ppm for the intermediate and wide range.
### Metabolite level distribution

<table>
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<th>Modeling range</th>
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<th>Wide</th>
<th>Knot spacing</th>
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**Figure 3** – Distribution and coefficients of variation (CVs) of GABA+ estimates for all modeling strategies. Including a MM<sub>3co</sub> model significantly increases the mean estimates for all modeling strategies, while giving similar or reduced CVs. All three modeling ranges (column) and three spline knot spacings (within each column), and co-edited MM models (color-coded) are presented. Distributions are shown as half-violins (smoothed distribution), box plots with median, interquartile range, and 25<sup>th</sup>/75<sup>th</sup> quartile. CVs are summarized as bar plots.

**Figure 3** shows distributions and coefficients of variation (CVs) of the GABA+ estimates for all modeling strategies. GABA+ estimates are significantly higher than GABA-only estimates of the ‘none’ modeling strategy for all modeling ranges and knot spacings, supporting the notion from **Figure 2** that not including an MM model leaves a considerable fraction of the edited 3-ppm signal unmodeled, resulting in substantial residuals or increased baseline amplitudes flexion. Notably, CVs for the strategies including MM models are comparable or reduced.

All modeling strategies with MM<sub>3co</sub> model return comparable mean estimates and CVs within the same knot spacing. In addition, sparser knot spacing leads to lower CVs. The intermediate modeling range does not appear to perform more consistently than both other modeling ranges.
Model evaluation

Figure 4 – Evaluation of all modeling strategies. Including an MM_{3co} model reduces the 3-ppm residual by ~30% without significant impact on the ΔAIC. All three modeling ranges (column) and three spline knot spacings (within each column), and co-edited MM models (color-coded) are presented. Bar plots represent mean values; SD is indicated by whiskers where appropriate.

Figure 4 summarizes the metrics used for model evaluation. The residual over the modeled frequency range (residual_{range}) is lowest for the narrow modeling range. For the intermediate and wide modeling ranges, residual_{range} is substantially higher, largely driven by the 2-ppm region (see also Figure 2). Consequentially, residual_{range} is comparable between MM modeling strategies for a given knot spacing.

The residual around the GABA+ peak (residual_{3ppm}) is consistently reduced by up to 30% if a MM_{3co} model is included, in line with the reduction of structured residual in Figure 2. This effect is less pronounced for the dense knot spacing (0.25 ppm), indicating that a flexible baseline is to some degree capable of accounting for otherwise unmodeled MM signal. Together, these findings again support the notion that omitting an explicit MM_{3co} model does not capture the whole edited 3-ppm signal, which remains unmodeled (in the residual) or gets partially absorbed by the baseline or interpreted incorrectly as GABA signal.

The strategy with the lowest AIC is the GABA_{hard} model with the narrow modeling range and sparse knot spacing, reflecting the low number of model parameters: there is no separate basis
function for MM, and the lowest possible number of splines. The ΔAIC (the difference between the lowest AIC and the individual model’s AIC) consequently increases for larger modeling ranges, as more splines are included. Similarly, ΔAIC increases for denser knot spacings, and in fact, this increase is much stronger compared to the resulting reduction in both residual measures, suggesting that the increased flexibility and reduction of the residual does not justify the greater number of model parameters.

For any given knot spacing and modeling range, ΔAIC are comparable between MM3co models, with moderate increases when more parameters are estimated. Together with its low CV for GABA+, the ΔAIC for the MM09hard model over the wide modeling range with sparse knot spacing (ΔAIC = 15.4) indicates a good performance of this particular model without introducing overfitting.
Separation of GABA and MM$_{3co}$

Figure 5 - Distribution of GABA and MM$_{3co}$ estimates and the relative contribution of GABA to GABA+ for all modeling strategies. All three modeling ranges (column) and three spline knot spacings (within each column), and MM$_{3co}$ models (color-coded) are presented. Distributions are shown as half-violins (smoothed distribution), box plots with median, interquartile range, and 25th/75th quartile. CVs are summarized as bar plots.

Figure 5 shows the distributions and CVs of the separate GABA and MM$_{3co}$ estimates of all modeling strategies. Including a separate MM$_{3co}$ basis function significantly decreases GABA estimates, suggesting that not doing so may lead to GABA overestimation, as MM signal is mistakenly modeled as GABA. As was seen for the composite GABA+ estimates in Figure 3, sparser knot spacing appears to stabilize modeling, leading to lower CVs of GABA. This becomes especially obvious for the wide modeling range, where GABA CVs exceed 50% for dense knot spacing.

MM$_{3co}$ estimates are stable across the different knot spacings, suggesting that the different parametrizations accurately account for most of the co-edited MM signal at 3 ppm.

The GABA$_{soft}$ model, in combination with a wide modeling range and 0.55 ppm knot spacing, exhibits the lowest CV for GABA (12.9%). However, the MM09$_{hard}$ model in combination with
the same knot spacing and modeling range has comparable GABA CVs (17.3%). The corresponding MM₃₀ CVs were 22.6% (GABA₀) and 16.2% (MM₉), respectively. One might argue that it is beneficial to opt for the MM₉ model, since the MM₀₉₄ peak provides an ‘external’, non-overlapped reference anchor for the expected MM₃₀ peak – the MM landscape is thought to be relatively stable across subjects, at least in the absence of pathology.

Furthermore, the MM₉ model does not impose any amplitude assumptions or constraints on GABA. Supplementary Material 2 reports the mean and SDs of the GABA+, GABA, and MM₃₀ estimates. Significant differences between the mean or the SD compared to the corresponding model omitting co-edited MMs are indicated bold.
Figure 6 – Impact of including homocarnosine in the basis set. The directionality of the correlation indicates that HCar absorbs GABA signal specifically for the intermediate and wide modeling range and absorbs MM$_{3co}$ signal for all modeling ranges. Correlation analysis between the differences between GABA/MM$_{3co}$ estimates with and without HCar in the basis set and the HCar estimates. All three modeling ranges (column) and three spline knot spacings (within each column) were investigated. Pearson’s correlation was calculated for each MM$_{3co}$ model (color-coded).

Finally, Figure 6 shows the impact of including HCar into the basis set with the difference in GABA and MM$_{3co}$ estimates between the modeling strategies with and without HCar ($\Delta$GABA and $\Delta$MM$_{3co}$, respectively). Interestingly, clear differences in the systematic effects of HCar are evident between the modeling ranges:

For the narrow modeling range (Figure 6 A), HCar estimates correlate positively with $\Delta$GABA, but the correlation is only substantial for strategies with a separate MM basis function. For precisely these strategies, HCar estimates correlate negatively with $\Delta$MM$_{3co}$. These observations suggest that HCar is likely to account for MM$_{3co}$ in the narrow modeling range. In contrast, HCar and $\Delta$GABA correlate negatively for most strategies in the intermediate and wide modeling ranges (Figure 6 B and C). The negative correlations between HCar and $\Delta$MM$_{3co}$ and are notably weaker for these modeling ranges, indicating that HCar is more likely to account for GABA signal instead of MM.

This behavior can possibly be explained by the HCar signal shape for each modeling range (Supplementary Material 3). For the narrow modeling range, the HCar basis function offers the model an additional degree of freedom to account for deviations of the actual edited 3-ppm signal from pure GABA and the symmetric Gaussian MM3co component, as no resonances below 2.78 ppm are considered. As a result, HCar shows a high correlation with the difference in MM$_{3co}$. For the intermediate and wide range, the HCar basis function resembles the GABA basis function since other resonances are included, effectively coupling GABA and HCar estimates to each other. Perhaps unsurprisingly, HCar estimates are significantly higher for ’none’ modeling strategy, and are substantially lower for more flexible baselines, supporting the notion that HCar rather serves as a substitute for an explicit MM signal, in particular if the baseline cannot absorb the latter (Supplementary Material 3). Within a given knot spacing and modeling range, HCar estimates are comparable between different MM$_{3co}$ models, a behavior observed for GABA estimates as well.
The GABA+ + homocarnosine estimates show a slight increase compared to the GABA+ estimates without HCar (Supplementary Material 4). For the ‘none’ model, stronger changes occur as HCar accounts for MM signal (see also Figure 6). There was no improvement in the CVs observed when including HCar in the model. The relative contribution of HCar to GABA+ ranged between 2.2% and 19.1% for modeling strategies with an MM$_{3c0}$ basis function and between 18% and 36% for the ‘none’ model.
The application of linear combination modeling to edited difference spectra is neither straightforward nor intuitive. The conceptual advantage of spectral editing arises from isolating a resolved target resonance, i.e. reducing the overlap of the target metabolite with other signals, as well as the number of signals in the spectrum in general. LCM, on the other hand, benefits from maximizing the use of prior knowledge to solve the spectral modeling problem, i.e. using all available information for meaningful constraint, including from overlapping signals. The specific case of GABA-edited MRS at 3T poses unique and unresolved challenges. Firstly, a compromise must be drawn between maximizing the prior knowledge by increasing the modeling range and reducing the impact of co-edited and unwanted signals. Secondly, an appropriate parametrization of poorly characterized co-edited signals must be found, and possible interactions with the target metabolite GABA must be evaluated. Thirdly, effects of baseline modeling must be studied, again a consequence of the macromolecular background signal in the GABA-edited difference spectrum not being determined to this date. In this study, a total of 102 linear combination modeling strategies were compared for GABA-edited difference spectra, each with different modeling ranges, parametrizations of co-edited signals, and baseline model flexibility. The key findings are:

- Including a dedicated basis function for co-edited MM improves fit residuals, reduces CVs of GABA and GABA+ estimates, and avoids overestimation of GABA.
- Reducing the modeling range does not substantially stabilize or destabilize modeling, while removing potentially valuable information (MM<sub>0.93</sub> and 2-ppm NAA peak) from the optimization.
- Sparser baseline spline knot spacing leads, on average, to the lowest CV across all modeling ranges.
- GABA and MM<sub>3co</sub> show a stable separability by linear combination modeling, and stable MM<sub>3co</sub> estimates indicate appropriate parametrization within each M<sub>3co</sub> parametrization.

There is surprisingly little systematic investigation into linear combination modeling of GABA-edited difference spectra. To the best of our knowledge, there is only one conference abstract...
studying MM parametrization in GABA-edited MRS with the LCModel software\textsuperscript{25}. The results from this preliminary investigation indicate that including a specific MM basis function significantly reduces GABA estimates, which our findings confirm.

Although the substantial contribution of broad MM signals to the 3-ppm peak in the GABA-edited spectrum is widely known\textsuperscript{1,33}, it is rarely explicitly addressed in linear combination modeling. Instead, it is assumed that either an incomplete model (without explicit MM term) will still provide an accurate GABA estimate, or that baseline modeling will account for the MM signal. The current results provide evidence that including an appropriately parametrized MM model is a preferrable and easily implemented strategy, reducing the residual over the 3-ppm signal range by up to 30%, with similar or lower CVs for GABA+.

The different MM models in this study were based on certain assumptions, including the relative contribution of MM\textsubscript{3co} to the 3-ppm GABA peak to be around 50%\textsuperscript{1,6,8,19}. Levels of MM\textsubscript{0.93} have been found to be stable across the whole brain\textsuperscript{34} and are thought to be stable across healthy subjects. Under these assumptions, the MM\textsubscript{09} hard model with a rigid amplitude coupling between MM\textsubscript{3co} and the non-overlapped MM\textsubscript{0.93} peak is a suitable strategy, supported by favorable CVs and \textDelta AIC. Further studies need to be performed to investigate the distribution and correlation between MM\textsubscript{0.93} and MM\textsubscript{3co} in the brain.

Unedited MRSI data measured at 7T indicates significant differences between white and gray matter for several macromolecules in the healthy brain\textsuperscript{34}. Changes in the MM concentrations during disease may also affect the relative contribution to the 3-ppm peak, and therefore render models with prior amplitude assumptions inaccurate. If there is reason to expect strong fluctuations of MM\textsubscript{3co}, a modeling strategy with fewer assumptions about amplitude ratios between the metabolite of interest GABA or the MM\textsubscript{0.93} signal and the MM\textsubscript{3co} signal is preferable to the
MM09\text{hard} strategy. Here, the Gauss\text{free} and Gauss\text{fixed} strategies could be used to account for changes in the MM$_{3co}$ contribution more freely, as their mean estimates of GABA and GABA$+$ were in good agreement with the more constrained approaches, although they led to increased CVs and $\Delta$AICs. In addition, the less-constrained models are available for model-based investigations of changes in MM$_{3co}$ due to age$^{35}$ or disease, and may offer a parametrization of frequency-drift-related effects on the co-edited MM signal$^{1,8,36}$. Another potential way to model the co-edited MM signal is to include lysine in the simulated basis set, as it has been identified as the potential source of the signal$^6$, although this approach would require appropriate broadening and incorporation of chemical shift and coupling values from protein databases$^{37}$.

Overall, results did not differ drastically between modeling ranges, although it is noteworthy that the effects of baseline flexibility were less pronounced for the narrow modeling range, likely because the complex interaction of the overlapping 2.25 ppm GABA and Glx signals with the underlying baselines is omitted. Furthermore, there was no evidence that the intermediate modeling range, which is proposed in the LCModel manual$^{18}$ to avoid frequently occurring co-edited lipid signals, improved quantification substantially compared to both other modeling ranges, although it should be mentioned that this particular dataset did not suffer from severe lipid contamination. Taken together, the choice of modeling range does not impact quantitative results as substantially as the inclusion of an MM model.

Baseline models are included in most LCM algorithms to account for signals not otherwise modeled, e.g. residual water tails or unparametrized macromolecules and lipids. Compared to conventional short-TE spectra, water and non-co-edited MMs are removed upon subtraction in the GABA-edited spectrum, which is therefore frequently modeled with a stiffer baseline$^{4,18}$. Our results show that sparser knot spacing (0.55 ppm) leads to lower CVs in metabolite estimates. A more flexible baseline (0.25 ppm) improves local and global residuals, but not enough to justify the additional model parameters (as per the AICs). More importantly, an overly flexible baseline may absorb edited signal, although it appeared that it did not do so excessively even for the 0.25-ppm strategies. The exception was the ‘none’ model, where the baseline was the only available part of the model to take up signal, underlining the inadequacy of the default LCModel approach. Taken together, a relatively rigid baseline with a parametrized MM basis function is preferable.
for LCM of GABA-edited spectra. A caveat to this recommendation is the observation of structural baseline fluctuations underneath the 2.25 ppm signals from GABA, Glx, GSH, NAA and NAAG, particularly for the 0.25 ppm knot spacing. These were observed previously\textsuperscript{25}, and are likely signals from un-parametrized MMs directly and indirectly affected by the editing pulse. Rigid baselines may force a wrong metabolite model in that region and interfere with accurate estimation of GABA and Glx. In fact, the structural Glx residual at 3.75 ppm suggests a systematic misestimation of the Glx phase, likely driven by the 2.25 ppm signals. While beyond the scope of this investigation, it is conceivable that more informed parametrization (or, ideally, direct measurement) of this unexplored MM background may benefit the modeling of the entire difference spectrum. Alternatively, hitherto unexplored approaches with variable baseline knot spacing may be worth investigating.

The HCar molecule has a GABA moiety with similar chemical shifts and is therefore co-edited. Evidence regarding in-vivo HCar levels in the human brain is inconclusive – early work determined HCar levels to be 0.5 mM\textsuperscript{5} (compared to ~1 mM for GABA), while a recent hybrid upfield/downfield inversion-recovery method determined the HCar/GABA ratio as 17\%\textsuperscript{38}. Therefore, we tested the impact of adding HCar to the basis set without additional constraints. Including HCar systematically affected GABA and MM\textsubscript{3co} estimates, in a way that strongly depended on the choice of modeling range. HCar estimates themselves ranged from 2.2\% to 19.1\% of the GABA+ signal, depending strongly on the degree of baseline flexibility. The results suggest that the overlap between the three model terms (HCar, GABA, MM\textsubscript{3co}) is too substantial for reliable three-way separation, particularly in the presence of a highly flexible baseline. A minor increase in “GABA+ plus HCar” estimates compared to GABA+ estimates was observed and the inclusion of HCar did not substantially improve the CVs. Additionally, the disagreement between the model and the data at 2.9 ppm indicates that a simple unconstrained addition of HCar to the modeling is not justified.

A limitation of this study is the high spectral quality (SNR, linewidth, no apparent subtraction artefacts, or lipid contaminations) of the dataset analyzed. We did not investigate model parametrizations of movement or drift, which may introduce systematic changes to the co-edited MM signal. While our results suggest that using the wide modeling range with a rigid baseline is
beneficial, strong co-edited lipid signals are likely to not be modeled appropriately, and the intermediate modeling range may be more suitable. Further studies of the possible impact of changes in spectral quality need to be performed to validate the modeling strategies under suboptimal conditions.
Conclusion

This study proposed and compared different modeling strategies for LCM of GABA+-edited difference spectra from a multi-site MEGA-PRESS dataset. Introducing a parametrized model for co-edited macromolecules reduces fit residuals, while maintaining low coefficients of variation of GABA+ estimates. Under certain conditions, it was found that GABA and co-edited MM are separated in a stable way. A rigid baseline was found to be beneficial, while using a narrower modeling range did not significantly improve the modeling. The overall modeling results suggest that GABA-edited data are reliably modeled with an adequately parametrized MM$_{\text{co}}$ model, constrained by the non-overlapped 0.93-ppm MM resonance, in combination with a full modeling range and sparse knot spacing. Incorporating homocarnosine into the modeling did not significantly improve the GABA+ estimates and did not allow for a stable separation of GABA and HCar.
566 **References**


Supplementary Material

Supplementary Material 1 – List of included subjects. All datasets are available at https://www.nitrc.org/projects/biggaba/

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Supplementary Material 2 – GABA+, GABA and MM_{3co} mean and SDs for all modeling strategies (ratios to tCr). Significant differences between the corresponding model and the ‘none’ are indicated in bold. Differences in MM_{3co} are compared between the corresponding model and the GABA_{soft} model.
<table>
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<th>MM3co spacing (ppm)</th>
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Supplementary Material 3 – Mean modeling results and homocarnosine estimates for all modeling strategies with homocarnosine. A substantial structured residual is visible at 3 ppm if for all modeling strategies and for the narrow and intermediate modeling range the homocarnosine concentrations are significantly lower compared to omitting the co-edited MM, especially for knot spacings <= 0.4 ppm. All three modeling ranges (columns), three spline knot
spacings (rows), and MM\textsubscript{3co} model (color-coded) are presented with mean residuals and fits, as well as the GABA, MM\textsubscript{3co}, homocarnosine (HCar) and spline baseline models. The mean data is included in black. The dashed lines indicate the range of the residual across one row and the arrows indicate the maximum of a specific modeling range and spline knot spacing (color-coded).

Supplementary Material 4 - Distribution of GABA+ plus HCar and HCar estimates and the relative contribution of HCar to GABA+ plus HCar for all modeling strategies. All three modeling ranges (column) and three spline knot spacings (within each column), and MM\textsubscript{3co} models (color-coded) are presented. Distributions are shown as half-violins (smoothed...
distribution), box plots with median, interquartile range, and 25th/75th quartile. CVs are summarized as bar plots.
Declaration of competing interests

The authors have nothing to declare.
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CRediT authorship contribution statement

A

Co-edited MM model implementations

GABA<sub>hard</sub>

GABA + MM<sub>3co</sub> 1:1 amplitude ratio

add MM<sub>3co</sub> with fixed FWHM

MM<sub>09</sub> + MM<sub>3co</sub> 3:2 amplitude ratio

Basis function (MM<sub>3co</sub>)

1H area 3 ppm Gaussian 14 Hz FWHM

GABA<sub>soft</sub>

soft constraint GABA & MM<sub>3co</sub> 1:1 amplitude ratio

add MM<sub>3co</sub> with free FWHM model parameter

GMM<sub>09</sub> soft

soft constraint MM<sub>09</sub> & MM<sub>3co</sub>

B

Combination of modeling strategies

basis set modeling range spline MM<sub>3co</sub> model

basis set without homocarnosine

narrow intermediate wide

0.55 ppm 0.40 ppm 0.25 ppm

none GABA<sub>hard</sub> GABA<sub>soft</sub> Gauss<sub>fixed</sub> Gauss<sub>free</sub> MM<sub>09</sub> hard MM<sub>09</sub> soft

basis set with homocarnosine

2.78-4.2 ppm 1.85-4.1 ppm 0.5-4 ppm

HCar

= 102 strategies