INTRODUCTION

$^{31}$P MRS has the potential to reveal important information about energy metabolism, cell membrane turnover, and intracellular pH without the need of an invasive intervention. Potential applications include pH imaging as well as imaging of energy and phospholipid metabolism, which can be utilized in cancer and neurological and psychiatric disorders. Nevertheless, the potential of phosphorus MRS is limited by a low intrinsic sensitivity. This translates into low spatial resolutions leading to significant cross-voxel contamination, which reduces the quantification precision and regional specificity.

The spatial resolution can be improved by increasing the static magnetic field strength $B_0$; the dependence of $^{31}$P SNR was estimated by Lu et al to be proportional to $B_0^{1.4}$. In
recent studies of the human brain, nominal spatial resolutions in the order of 1 cm could be achieved for a reduced FOV at 7T, 1.25 to 2 cm for whole brain imaging at 7T, 1.5 cm for whole brain MRSI at 9.4T. At 3T, nominal resolutions are usually in the range of 2.6 cm to 3 cm.

Another contribution to optimal SNR is the design of dedicated RF coils. Therefore, advanced coil designs are becoming more common. Phased array coils provide a higher average SNR at the expense of an inhomogeneous sensitivity profile, which makes comparability and quantification more challenging.

The quantification of metabolite concentrations is a crucial step because it allows comparisons between different facilities, volunteers, and reconstruction/acquisition techniques. Measurements of concentrations for healthy volunteers provide a baseline to estimate the effects of different diseases on metabolite concentrations in the human brain. Previous reports of concentrations estimated with 31P MRS have acquired signals from a relatively large volume determined by the receive field of the coil or by applying outer volume suppression—or studies have focused on only a small number of metabolites with relatively big voxel sizes. Few studies have estimated concentrations from 31P MRSI measurements, and therefore from localized volumes in the human brain. The nominal resolutions of these studies ranged between 6.3 mL and 8 mL acquired at a field strength of 4T.

The aim of the present study was to acquire highly spatially resolved 31P MRSI data of the human brain and to provide a robust quantification protocol to determine metabolite concentrations in gray (GM) and white matter (WM) brain tissue. The current study was performed on a 9.4T human whole-body MR system with a dual-tune 1H/31P RF coil design optimized to maximize SNR and yield a high transmit efficiency for 31P in the human brain. For optimal SNR and data quality, a localized 31P transmit field calibration based on the Bloch-Siegert effect was developed and performed; flip angle and pulse profile of the excitation pulse were optimized; the nuclear Overhauser enhancement (nOe) scheme was implemented and optimized for B0 = 9.4T; and a k-space weighted acquisition scheme was used.

First, the scan protocol optimization and quantitative analysis steps are presented. Afterward, quantitative highly spatially resolved metabolic images of different 31P detectable metabolites are shown. Regression and group-average analysis were performed to estimate the metabolite concentrations of different 31P resonances in GM and WM tissue. Finally, the results are compared to earlier publications, and limitations are discussed.

2 | METHODS

Data were acquired at a Siemens Magnetom 9.4T whole body imaging system (Erlangen, Germany). A double-tuned phased array coil was used with 10 transceiver channels for proton and 8 transceiver channels for phosphorus. The vendor-implemented, image-based second-order B0 shimming was applied.

All studies with volunteers were approved by the local ethic committee. All volunteer data were acquired after written informed consent. Fourteen healthy subjects participated in the study (females: 3, average age: 26.6 y, min: 23 y, max: 31 y). Two volunteers were excluded due to significant motion during the acquisition.

2.1 | Scan protocol optimization

2.1.1 | 31P B1+ calibration

The B1+ calibration of phosphorus was performed with a single voxel image-selected in vivo spectroscopy sequence combined with a Bloch-Siegert pulse. This localized B1+ calibration technique is illustrated in Figure 1. It allowed for a fast (approximately 3 min) calibration of the phosphorus transmit field, which enabled an individual 31P B1+ calibration for each volunteer. The following sequence parameters were used: (80 mm) voxel size, T = 5 s, acquisition delay 0.3 ms, 16 averages, 5 kHz acquisition bandwidth, gradient-modulated offset-independent adiabaticity pulse duration 5 ms (bandwidth 34.3 kHz), 350 μs rectangular excitation pulse, 5 ms Bloch-Siegert pulse, and Bloch-Siegert frequency offset ± 2500 Hz relative to PCr. The transmit voltage was calculated from the phase difference of the PCr resonance for 2 frequency offsets of the Bloch-Siegert pulse (Figure 1B) for each individual volunteer.

2.1.2 | 31P Ernst angle and excitation pulse optimization

The Ernst angle of the MRSI measurement of 25 degrees was calculated for PCr with a T1 relaxation time of 2502 ± 25 ms, which is in the middle range of the T1 range of all 31P metabolites. Different sinc pulses were tested with the aim to find a possibly homogeneous excitation profile over the range of the measured 31P resonances. The highest frequency shift that needs to be covered is 7.52 ppm for α-ATP (α-adenosine triphosphate). The multiple-tested pulse profiles were optimized in phantom measurements. The phantom was matched to the dielectric properties of the human head (length = 18 cm, axes of ellipse = 19 cm × 15 cm, ε = 62.4, σ = 0.54 S/m at 160 MHz).

2.1.3 | NOe sequence block

To optimize SNR, a nOe preparation was applied. nOe can be achieved via different preparation techniques in which...
nOe pulses are applied on the proton frequency of the water resonance prior to excitation and readout. Therefore, the $B_1^+$ profile of the proton channel can have an impact on the detected $^{31}$P signal amplitudes. The goal was to avoid such side effects. Two different nOe techniques were compared in phantom measurements: the transient nOe and the truncated-driven nOe (TOE) (Figure 3). For transient nOe, a single 180-degree pulse was applied on the water proton frequency. Herein, an adiabatic hyperbolic secant pulse was used. The adiabatic pulse was optimized in phantom measurements and Bloch simulations. In the TOE preparation, 24 rectangular saturation (90 degree) pulses with an individual pulse duration of 7 ms and a delay of 3 ms were used. The same phantom was used as for the pulse optimization.

Finally, the quantitative effect of the TOE-based nOe preparation was measured in vivo from a nonlocalized pulse acquire measurement. The same parameters were used as for the $^{31}$P MRSI measurement described below. The nOe measurements were performed on 14 healthy volunteers; 13 of these volunteers were also included in the $^{31}$P MRSI protocol.

2.2 | Data acquisition

2.2.1 | $^{31}$P MRSI

The 3D $^{31}$P MRSI was performed with the following parameters: FOV = (180 x 200 x 180) mm$^3$, (28 x 30 x 13) grid size, (6.4 x 6.7 x 13.8) mm$^3$ voxel size, $T_R = 250$ ms, acquisition delay 1.3 ms, 16 averages, Hanning weighted k-space averaging, 2 ms sinc pulse excitation (4kHz bandwidth and a time-bandwidth-product of 8), 5 kHz acquisition bandwidth, vector size = 512, Ernst-angle excitation with 25 degree, and a nOe preparation using a TOE scheme with 14 rectangular saturation pulses with a duration of 8 ms and a delay of 2 ms. The total measurement time
was 74 min with a nominal voxel size of 0.59 mL. The effective resolution is 2.47 mL (factor of 4.19 estimated as the FWHM of the corresponding sphere). The nOe preparation, and therefore the proton channels, are the major contribution to total SAR leading to an approximate SAR of 90% of the maximal SAR level allowed at the 9.4T Siemens system.

2.2.2 | 1H MRI

For proton $B_1^+$ calibration, actual flip angle imaging was applied with the following parameters: $T_{R1} = 20$ ms, $T_{R2} = 100$ ms, TE = 4 ms, and flip angle 60 degrees. Additionally, a 3D MP2RAGE (spatial resolution (1 mm)$^3$, flip angle = 4 and 7 degrees, TE = 2.27 ms, TI = 750/2100 ms, TR = 6 ms, volume TR = 5.5 s) was acquired for anatomical images and tissue segmentation. $^{32}$ MP2RAGE images were acquired in all volunteers included into the $^{31}$P MRSI protocol.

2.3 | Data analysis and metabolite quantification

2.3.1 | $^{31}$P MRSI reconstruction, postprocessing, and spectral fitting

$^{31}$P MRSI data were reconstructed by a 3D fast Fourier transformation. A correction from the deviation of an ideal Hanning filter was applied. Whitened singular value decomposition coil combination was performed.$^{15,33,34}$ The $^{31}$P MRSI postprocessing steps included: zero-order phase correction and $B_0$ corrections performed by a frequency alignment of the PCr resonance. For the zero-order phase correction, the phase was calculated by maximizing the integral of the real part of the PCr resonance. The first-order phase was fixed to a constant value (1.37 ms), which was determined by the sequence parameters. Supporting Information Figure S1 shows an exemplary fit. The α-, γ-ATP, nicotinamide adenine dinucleotide (NAD+), and UDPG were fitted as doublets.$^{16,36}$

2.3.2 | Quantitative metabolite maps and respective corrections

To calculate quantitative $^{31}$P MRSI metabolic images, different corrections needed to be considered$^1$: pulse profile, $^{31}$P transmit field inhomogeneity ($B_1^+$), $T_1/T_2$ relaxation of the $^{31}$P metabolites, nOe, and $^{31}$P receive field inhomogeneity ($B_2^+$). In the following section, the reasoning behind the applied corrections will be explained.

The pulse profile was optimized such that no correction needed to be applied (see Scan Protocol Optimization section 2.1, Figure 2).

Because the study was performed with an array coil, inhomogeneity of the $^{31}$P transmit field needs to be considered. The effect of this error is $T_1$-dependent because we were using Ernst angle excitation with a very short repetition time ($T_R$) of 250 ms. To estimate the error due to the $^{31}$P $B_1^+$ field inhomogeneity, simulations based on phantom $^{31}$P $B_1^+$ measurements were performed. The same phantom was used as before. The parameters of the underlying $^{31}$P $B_1^+$ measurement are described in an earlier publication.$^{23}$ The phase-sensitive $B_1^+$ imaging sequence used to estimate the $^{31}$P $B_1^+$ distribution was taken from Allen et al.$^{37}$ Based on these phantom derived $^{31}$P $B_1^+$ maps, which are shown in Avdieievich et al. in Figure 6,$^{23}$ the distribution of the flip angle and the corresponding expected measured steady-state magnetization were calculated:

$$M_Z (\alpha, T_1) = \frac{M_0}{1 - \cos (\alpha)} e^{-T_R/T_1} \sin (\alpha).$$

(1)

$T_1$ saturation, as a result of the short repetition time of the $^{31}$P MRSI protocol, was corrected based on the individual metabolites $T_1$ relaxation times.$^{29}$ $T_2^*$ relaxation corrections were not applied. The major component of $T_2^*$ in this study is due to $B_0$ inhomogeneities. This component is identical for all resonances and therefore automatically considered in the presented metabolite images, and concentration as an internal reference standard was applied.

The phase-sensitive $B_1^+$ was optimized such that no correction needed to be applied (see Scan Protocol Optimization section 2.1, Figure 2).

To correct for the signal enhancement due to nOe, the expected effect was measured individually for each volunteer, and the individual value was used for correction. The nOe enhancement was calculated via:

$$\eta = \frac{(A_{nOe} - A_{ref})}{A_{ref}}.$$

(2)

Finally, the receive field $B_2^+$ as well as differences in the loading of the coil between different volunteers were considered. As shown in earlier publications,$^{23}$ the receive field causes significant errors on the signal amplitudes. This issue was addressed using an internal reference. Therefore, the $T_1$ and nOe-corrected γ-ATP signal were used as an internal reference with an assumed concentration of 3 mM.$^{18,21,38}$ To avoid additional statistical errors due to the calculation of the ratio, a low rank approximation was applied to the γ-ATP images before the quantitative maps were calculated$^{19}$:

$$A_k = U_k S_k V.$$

(3)
Hereby, $A_k$ is the low rank approximation of the $\gamma$-ATP metabolic image and $U$, $S$, and $V$ were obtained from singular value decomposition of the full rank metabolic image $A$ of $\gamma$-ATP. $S_k$ is the truncated singular value matrix $S$, which includes only the first $k$ singular values. The optimal rank was calculated via the optimal hard threshold formalism of Gavish and Donoho \(^\text{40}\) (see Supporting Information Figure S2). All metabolite maps are ratio maps to the low rank approximation of the $\gamma$-ATP signal. The low rank approximation was only performed on the underlying $\gamma$-ATP image.

2.3.3 | GM versus WM metabolite concentrations and pH

To calculate the tissue fraction in each MRSI voxel, the MP2RAGE measurement was segmented using the SPM12 algorithm. The fraction of GM, WM, and CSF in each MRSI voxel were then calculated by an affine transformation using an in-house implemented Python tool. Hereby, for each MR2RAGE voxel a tissue type was assigned. Subsequently, these voxels were coregistered to the MRSI grid. The corresponding tissue fraction for each MRSI voxel was calculated by adding all coregistered MP2RAGE voxels, which were assigned to a certain tissue type and divided by the total number of MP2RAGE voxels that belong to the MRSI voxel. Finally, the PSF of the MRSI data was applied to the tissue content matrix.

Using the tissue fractions, an average over voxels with high GM versus WM content of 4 central slices was calculated after correcting for coil sensitivity by scaling with the $\gamma$-ATP amplitude. Voxels were assigned to WM or GM, respectively, if their WM or GM fraction was at least 70%. Only voxels, which were entirely in the brain, were included. Prior to averaging, a frequency alignment was applied using the
frequency shift of PCr. GM and WM metabolite concentrations were calculated from averaged spectra over the selected GM and WM matter voxels for individual volunteers.

In addition to the concentrations of the different metabolites, the tissue type-specific pH value was calculated from the chemical shift of the free phosphate resonance ($P_i$) using the modified Henderson-Hasselbalch equation:

$$\text{pH} = pK + \log \left( \frac{(\delta - \delta_a)}{(\delta_b - \delta)} \right). \tag{4}$$

The equation constants are$^{18}$: $pK = 6.73$, $\delta_a = 3.275$, and $\delta_b = 5.685$. The extracellular as well as the intracellular pH could be estimated for individual volunteers.

As a second step, a regression analysis of the metabolic concentrations against the WM fraction was performed for each individual volunteer. The regression was done in MatLab (MathWorks) with the Curve Fitting Toolbox using the function `LinearLeastSquares`. Here, all voxels with a CSF fraction of over 30% were excluded from the analysis.

2.3.4 | Statistical analysis

To compare concentrations measured from averaged voxel of individual volunteers and regression analysis, Wilcoxon sign rank tests were applied (MatLab R2018a, `signrank`, MathWorks).

3 | RESULTS

3.1 | Excitation pulse optimization

Figure 2 shows the results of the excitation pulse optimization and the simulation of $B_1^+$ effects. A sinc pulse with pulse...
duration of 2 ms and TBP of 8 shows the most homogeneous profile over the relevant ppm range.

Considering $^{31}$P $T_1$ relaxation times and underlying phantom $B_1^+$ distributions, Figure 2 demonstrates that the smallest related error, given as the SD over the shown slice, occurs for PCr because the Ernst angle was optimized for this metabolite (see Scan Protocol Optimization section 2.1). PE has the highest $T_1$ relaxation time ($5130 \pm 516$ ms). The $B_1^+$-related error for PE in the simulation is 4.9%. The highest error occurs for $\alpha$-ATP with approximately 8% as $\alpha$-ATP has the shortest $T_1$ relaxation time ($991 \pm 125$ ms) among all metabolites. $B_1^+$ is considered a minor source of error; therefore, corrections for $B_1^+$ inhomogeneity were neglected.

### 3.2 NOe sequence block

The results of the NOe optimization in phantom measurements are shown in Figure 3. Significant signal dropouts due to the proton $B_1^+$ profile at 9.4T are visible for the transient NOE. The TOE appeared to be more robust to these variations in the proton $B_1^+$ and was therefore applied in the study protocol. The results for the quantitative NOe enhancement factor measurements from $N = 14$ volunteers are also presented in Figure 3. Significant enhancements could be detected for all measured metabolites, including PCr, $\gamma$-/ $\alpha$-adenosine triphosphate (ATP), glycerolphosphorylcholine (GPC), glycerolphosphorylethanolamine (GPE), $P_i$, phosphorylcholine (PC), phosphorylethanolamine (PE), and total nicotinamidphosphate. The mean enhancements $\eta$ were (with SD over all $N = 14$ volunteers): 14.8 ± 0.6 % (PCr), 6.9 ± 1.2 % ($\gamma$-ATP), 4.9 ± 1.7 % ($\alpha$-ATP), 13.9 ± 3.4 % (GPC), 18.2 ± 6.9 % (GPE), 16.8 ± 4.7 % ($P_i$), 16.3 ± 6.8 % (PC), 17.9 ± 5.3 % (PE), and 14 ± 11 % (total nicotinamidphosphate).

### 3.3 $^{31}$P MRSI

Uncorrected $^{31}$P MRS images are shown in Figure 4. PCr and $\gamma$- and $\alpha$-ATP $^{31}$P images are presented with and without brain mask to show the signal contribution of the muscle tissue, which is especially high for metabolites of the energy metabolism.
In Figure 5, the results of a low rank approximation of the γ-ATP reference images as well as corresponding ratio images for PCr and GPC are presented. The ratios between the original PCr and GPC metabolite images and the approximated γ-ATP metabolite maps represent receive sensitivity corrected metabolite images. The first row shows the results using a full rank γ-ATP image. In this case, a rank of 4 was considered optimal according to the hard optimal threshold formalism of Gavish and Donoho\(^\text{40}\) (Supporting Information Figure S2). Specifically regarding PCr, one can see a significant improvement of the anatomical correspondence of the metabolite image after internal referencing.

Figure 6 shows the GM and WM spectra averaged over all dominantly GM/WM voxels and all volunteers as well as respective representative spectra from individual GM and WM voxel of 1 volunteer. In Table 1 and Figure 6, the concentrations of the averaged spectra of individual volunteers for WM and GM (shown in Figure S3) are presented. Significant differences between GM and WM were detected for PCr, α-ATP, GPC, GPE, P\(_i\), and NAD+ and NADH. In addition, the intracellular and extracellular pH appeared to differ significantly between GM and WM.

In Figure 7, the results of the regression analyses are shown for \(N = 11\) volunteers and 6 different resonances: PCr, α-ATP, GPC, GPE, P\(_i\), and PE. In the top row, the regression analysis for a single volunteer is shown. The corresponding \(R^2\)s for these exemplary regression lines are given in the plot. Especially for PCr, α-ATP, GPC, GPE, and PE, the correlations are very consistent between the different volunteers (bottom row). The following \(P\) values were calculated based on the concentrations of pure WM (WM fraction = 1) and pure GM (a WM fraction of zero): \(p(\text{PCr}) < 0.001, p(\alpha-\text{ATP}) < 0.001, p(\text{GPC}) < 0.001, p(\text{GPE}) = 0.002, p(\text{P}_i) = 0.41, \) and \(p(\text{PE}) = 0.007\). Therefore, PCr, α-ATP, GPC, GPE, and PE showed significant differences at a 5% significance level.

Concentration maps for the metabolites with the highest SNR (PCr, α-ATP, GPC, and PE) as well as the corresponding GM and WM fractions for 3 different volunteers are shown in Figure 8. The metabolite maps reflect higher concentrations of PCr and potentially PE in GM. GPC images show a potentially elevated concentration in WM. α-ATP images show no clear GM/WM concentration difference. A zero-filling in k-space to twice the resolution was applied to the images for better visualization. Metabolic maps for GPE and P\(_i\) can be found in Supporting Information Figure S4. Additionally, Figure 8 includes maps for the intracellular pH (pH\(_{\text{int}}\)), which shows higher values in GM compared to WM.

In Figure 9, concentration maps for PCr, GPC, PE, and α-ATP as well as pH\(_{\text{int}}\) images in sagittal, coronal, and transversal direction for one volunteer are shown. The images were zero-filled to twice the resolution.
**DISCUSSION**

### 31P MRSI acquisition methods and metabolite maps

Former publications on 31P MRSI presented metabolic maps of only a very limited number of metabolites, with a relatively low spatial coverage and/or a nominal resolution above 1 cm.7,10,20,42,43 Only Dudley et al reported tissue concentrations for multiple 31P MRS detectable metabolites using 3D MRSI. The nominal spatial resolution was 1.85 cm isotropic.20 With the presented protocol, we were able to present highly spatially resolved, quantitative metabolite images of five 31P MRS-detectable metabolites covering the entire brain at an ultrahigh field strength of 9.4T with an effective resolution of 2.47 mL.

### Metabolite concentrations and pH in GM and WM

Through different analyses, we were able to show significant differences between GM and WM for the intra- and extracellular pH as well as for the concentrations of 9 different metabolites: PCr, α-ATP, GPC, GPE, PC, PE, P_i^int, NADH, and NAD+. In the following section, we will discuss the measured concentrations in comparison to earlier publications.

The corrected metabolic 31P images (Figures 8 and 9) and quantitative analysis of PCr show a lower concentration in WM (WM: 3.02 ± 0.22 mM, GM: 3.54 ± 0.25 mM). For PCr, concentrations of 4.37 ± 0.39 mM were reported for non-localized acquisitions,18 4.27 ± 0.32 mM in the GM dominated visual cortex44 and 2.97 ± 0.11 mM/2.42 ± 0.26 mM,11 3.53 ± 0.33 mM/3.33 ± 0.37 mM,21 6.78 ± 0.76 mM/4.52 ± 0.63 mM,20 and 3.1 ± 0.3 mM/2.9 ± 0.4 mM45 in localized GM and WM. The higher concentrations measured in GM, for example, in Dudley et al,20 most likely arise from muscle tissue contributions. The concentration in muscle tissue is over 6 times higher than the brain tissue concentration with 25.4 ± 2.3 mM.21 An influence of muscle metabolite contributions onto the measured PCr concentrations especially in GM cannot be entirely excluded neither for previous studies nor for this study. However, the effect should decrease with higher spatial resolution and weighted k-space acquisition as presented herein. Differences can also arise from different reference methods as discussed later. Nevertheless, PCr concentrations appear to be consistently higher in GM.

A slightly higher concentration in GM was detected for α-ATP. Earlier publications reported mostly total ATP values. A nonlocalized concentration for α-ATP of 3.09 ± 0.23 mM
was estimated by Ren et al., which is slightly higher than the values estimated from this study, whereas localized concentrations in the parieto-occipital lobe of around 2.5 mM were reported by Jensen et al. Different quantification methods with respect to references standards and correction factors can be a potential reason for differences. Contaminations from muscle tissue are also a potential source of bias (estimated concentration of ATP in muscle is 8.5 ± 1.9 mM).

The difference in concentrations of α-ATP and γ-ATP could potentially arise from additional contributions of other metabolites such as α-ADP, NADH, NAD+, and UDPG to the α-ATP peak. According to literature, these contributions are expected to be small in the healthy human brain. Figure 4 shows that the potential contamination of signal from the surrounding muscle could be higher for α-ATP, which could explain higher measured α-ATP concentrations in GM. As stated earlier, 31P transmit inhomogeneities also has the largest effect on α-ATP, which according to Figure 2 can also lead to a lower signal in GM. In addition, the pulse profile in Figure 2 shows a small decrease in the range of α-ATP, which is a potential explanation for the lower overall α-ATP concentration. The γ-ATP resonance is also broader than α-ATP (Figure 6). It is known that the phosphorus nucleus of γ-ATP exchanges with PCr and Pi (eg, Ren et al.), which could also induce a difference between the estimated γ-ATP and α-ATP concentrations if the linewidth broadening cannot be entirely considered in the fitting model. Further investigations of this discrepancy could be of interest.

For GPC and GPE, higher concentrations were measured in WM, whereas PC and PE seem to have lower concentrations.

**FIGURE 6** Spectral quality and concentrations in WM and GM for multiple 31P metabolites. The upper row shows the summed spectra for dominantly WM and GM voxel over N = 11 volunteers (A) as well as WM and GM MRSI spectra from a single volunteer and single voxels in the occipital lobe (B). A 40 Hz Gaussian time domain filter was applied to the MRSI spectra. All relevant metabolites are indicated in the summed spectra. The second row shows the calculated concentrations from dominantly GM and WM voxels from individual volunteer for 12 different metabolites (C) as well as the differences between GM and WM for individual volunteers (D) with 25th and 75th percentile intervals calculated over N = 11 volunteers. WM, white matter.
in WM. This is also slightly visible in the corresponding metabolic 31P images (Figures 8 and 9). This result was also reported in previous studies.20,42

No differences were detected for inorganic intracellular phosphate (Pi) (Figures 6 and 7, Table 1). In contrast to these findings, Dudley et al.20 reported higher concentrations of Pi in WM. In Zhu et al.,11 concentrations of 0.93 ± 0.11 mM in GM-like and 0.96 ± 0.10 mM in WM-like tissue were reported. In the GM-dominated visual cortex, a concentration of 0.96 ± 0.1 mM was reported by Zhu et al.44 Therefore, Zhu et al also did not detect a difference between GM and WM. The extracellular inorganic phosphate, moreover, showed a higher signal in GM in our study (Table 1 and Figure 6). The potential origin of the extracellular inorganic phosphate resonance is investigated in Ren et al.19 According to Ren et al, contaminations from CSF and peripheral blood could have an influence on the measured concentrations.

For NAD+ and NADH, we measured higher concentrations in GM for the average spectra of individual volunteers. According to Lu et al,36 the NAD+ resonance was fitted as

FIGURE 7  Regression analysis. Shown is the regression analysis of metabolite concentrations over WM fraction for 6 different metabolites. The upper row shows representative data of a single volunteer with corresponding R² values. The lower rows show the regression lines from N = 11 volunteers with median values calculated over all volunteers and 25th and 75th percentile intervals for 0% and 100% WM fraction. Only voxels entirely in the human brain and with less than 30% CSF were included

FIGURE 8  31P metabolic images for 3 different volunteers. Metabolite images with corresponding WM/GM fractions maps are shown of 4 different metabolites of a single slice for 2 different volunteers: PCr, GPC, PE, and α-ATP. The data were zero-filled to twice the original size for better visualization. Also shown are maps of the intracellular pH (pHᵢᵣᵣ): pHᵢᵣᵣ, intracellular pH
FIGURE 9  Corrected in vivo MRSI data: Sagittal, coronal, and transversal metabolic images of 5 different $^3$P metabolites. Shown are the metabolic maps of PCr, $\alpha$-ATP, GPC, and PE as well as the intracellular pH of a single volunteer. For better visualization, the data were zero-filled to twice the original resolution. GPC, glycerolphophorylcholine; PE, phosphorylethanolamine
a duplet and the NADH resonance as a singlet (Supporting Information Figure S1). The reported concentrations can be compared to previous publications at 7T.16-18 De Graaf et al reported concentrations of 0.367 ± 0.078 mM for NAD+ and 0.159 ± 0.034 mM for NADH,16 Ren et al 0.28 ± 0.13 mM for total NAD,18 and Zhu et al 0.30 ± 0.02 mM for NAD+ and 0.06 ± 0.01 mM for NADH.17 All of these studies used nonlocalized sequences. The values from Zhu et al correspond well to the presented results.

UDPG was fitted with 2 peaks following de Graaf et al.16 For UDPG, concentrations of 0.315 ± 0.067 mM16 and 0.08 ± 0.04 mM18 measured at 7T were reported in earlier publications. Nonlocalized sequences were applied. The measured UDPG concentrations correspond well to the later publication. Nevertheless, it is not possible to correct for nOe and T1-weighting; only a limited comparison to existing literature is possible.

Intracellular pH was detected to be significantly higher in GM (Table 1), and respective pH maps revealed these differences clearly (Figure 8). This is consistent with earlier publications.11,20 The same difference could also be detected for extracellular pH. As stated earlier, the measured extracellular pH can be influenced by blood and CSF contaminations.19 Overall, the detected difference between intracellular and extracellular pH is consistent to earlier publications.18 pH can also be detected with CEST imaging.46 Sun et al showed that a lower MTR asym could be detected in WM. This corresponds to a lower pH values and is therefore consistent with our findings.

Overall, studies with a higher number of volunteers as well as reproducibility studies, potentially with different reference standards, would be necessary to further investigate the presented difference and metabolite maps, especially for the low SNR resonances.

4.3 Correction factors and reference standard for quantitative analysis

A number of influence factors that affect the accuracy of the metabolite concentration estimates have been analyzed and partly corrected for. The weighting arising from the pulse profile as well from the transmit field $B_1^+$ distribution of the RF coil were neglected. Therefore, both cause small errors that could have had an influence on the measured concentrations, especially on $\alpha$-ATP. For $^{31}$P $B_1^+$ corrections, corresponding maps from individual volunteers would have been needed for an accurate transmit field correction, whereas spatially resolved $B_1^+$ mapping was only feasible in phantoms due to SNR and scan time constraints. Even if theoretically possible, for example, with the Bloch-Siegert CSI technique26 or by steady-state MRSI flip angle mapping,47 it would have lengthened the in vivo measurement protocol significantly and would also have come with different potential sources of error as motion artifacts or poor SNR. Therefore, we deemed the potential benefits as being relatively small.

The application and corresponding correction of the nOe could also be a source of potential errors for the presented metabolite concentrations. For example, a high variance in nOe between different volunteers was observed in this study. This was also reported in earlier publications.8 The source of this variation should be investigated further in following studies. Another potential source of error due to nOe is the underlying $B_1^+$ transmit field of the proton channels. Especially with higher fields, this effect increases. It could be addressed using $^1$H $B_1^+$ maps for correction. This correction would require numerical simulations of the Solomon equations, which describe the dynamics of the nOe, as well as further measurements of the corresponding dynamic constants. Because the phantom used for testing of the nOe enhancement was matched to the dielectric conditions of the human brain and only minor effects of the underlying $^1$H transmit distribution were detected for the TOE, this correction was considered negligible. The TOE preparation seems to be robust against variations in proton $B_1^+$ if the applied voltage is above a certain threshold.

Overall, the nOe seems to have a positive effect on the repeatability of $^{31}$P MRSI measurements at 7T.8 However, these results cannot directly be transferred to 9.4T. If we compare the nOe enhancement factors measured at 9.4T to measurements at 7T8,9 and 3T,12 we can see that the respective values at 9.4T are usually lower. Especially for $\alpha$- and $\gamma$-ATP, the nOe seems to become very inefficient at higher fields (> 7T) due to an increased contribution of chemical shift anisotropy (CSA)-induced relaxation.1,12 Nevertheless, for most of the resonances an nOe enhancement above 10% of the signal intensity of the respective resonance line was still detected, especially for low signal resonances as GPC/GPE and PE/PC. Because this is substantial, the nOe enhancement was applied herein.

In general, the nOe and T1 relaxation time corrections rely on correction factors measured in a nonlocalized manner. Therefore, respective differences between different tissue types could not be considered. Beside potential differences of relaxation in GM and WM, the contribution from muscle tissue during the estimation of the relaxation times and nOe enhancement factors also can cause errors. Localized measurements of $^{31}$P metabolite T1 relaxation times and nOe enhancement factors would be needed for a more precise analysis.

To correct for the strong receive sensitivity and RF coil-loading dependence of quantitative readouts, $\gamma$-ATP was chosen as the internal reference standard. An assumption made in this context was that the concentration of $\gamma$-ATP is constant at 3 mM in both GM and WM.18,21,38 The assumption of constant $\gamma$-ATP concentrations cannot be tested by our protocol. Nevertheless, we looked at potential correlations.
between tissue fractions and measured γ-ATP signal amplitudes (Supporting Information Figure S5). No significant correlations between WM tissue fraction and γ-ATP signal amplitudes could be detected (P value Wilcoxon signed rank test: 0.57). In addition, if compared to the total phosphate pool as an internal reference, γ-ATP seems to be the better choice. The same regression analysis showed a P value of < .001 for the total phosphate pool.

Aside from potential differences in relaxation times for the γ-ATP resonance between tissue types or intrinsic concentration differences, there could also be errors arising from contaminations from muscle tissue and contribution from other resonances; specifically, the γ-ATP resonance overlaps with β-adenosine diphosphate. Nevertheless, γ-ATP was the preferable choice as an internal reference because ATP concentrations are stable during different brain activation states by various buffer mechanisms, and contribution of ADP is expected to be small in healthy human brain. For the total phosphate pool, a potential issue is the muscle contamination from surrounding muscle tissue for the PCR resonance caused by the point-spread function, as shown in Figure 4. PCR concentrations are significantly higher in muscle tissue compared to brain tissue\(^1\); therefore, they could cause significant errors if used for sensitivity correction/ratio maps. In addition, PCr and P\(_i\) concentrations may not be stable in different brain activation states because they are part of the buffer mechanism that keeps ATP concentrations constant. Another option would be total ATP. Because β-adenosine diphosphate is not detected in the study presented here due to pulse bandwidth limitations and the α-ATP resonance overlaps with other resonances, no improvement in quantification precision is expected from this method.

Potentially, also the assumption of an ATP concentration of 3 mM could be false, for example, Zhu et al\(^4\) reported a total ATP concentration of 2.83 ± 0.16 mM in the visual cortex. Thus far, there is no clear consensus in the literature.

The internal reference standard was applied as a low rank approximation. The low rank approximation was calculated with the optimal hard threshold approach of Gavish and Donoho.\(^40\) As shown in Supporting Information Figure S2, the difference between the corresponding low rank approximation and the original image shows no clear structure. If the chosen rank is too small, the low rank approximation can lead to image artifacts (Supporting Information Figure S5). In addition, potential artifacts from the γ-ATP metabolite images can influence the metabolite images.

An alternative would be an external reference standard as shown by Purvis et al\(^48\) for human liver phosphorus spectra at 7T. Potential sources of error for these methods are the calibration of the reference phantom to the appropriate electromagnetic properties of the volume of interest in the human body.\(^48\) It is known\(^40\) that the conductivity and permittivity differ between GM and WM. Therefore, a homogeneous phantom as an external standard for B\(_1\) correction would most likely also introduce a bias on the measured GM and WM metabolite concentrations because the dielectric properties are difficult to match in vivo conditions. Another problem with external reference standards is the differences in RF coil loading and thus respective impedance changes of the RF coil induced by different head sizes and shapes. This in turn could induce substantial differences in metabolite peak amplitudes and must be corrected. To overcome this problem, the ERETIC method has been previously presented for \(^1H\)\(^5\) and \(^13\)C MRS\(^51\) and could be adapted to \(^31\)P MRS in future.\(^52,53\)

5 | CONCLUSION

With highly spatially resolved quantitative \(^31\)P MRSI at 9.4T, metabolite maps visualizing anatomical differences between GM and WM metabolite concentrations could be derived for high SNR resonances and pH. These highly resolved metabolic maps were achieved with whole brain coverage. In addition, we were able to extract metabolite concentrations in GM and WM for 12 different metabolites from average spectra, indicating the improvement of \(^31\)P MRSI at ultrahigh field. Significant differences between GM and WM could be detected for 9 out of the 12 resonances and pH.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

**FIGURE S1** Summed spectrum for dominantly WM voxels averaged for all N = 11 volunteers (total number of voxels = 2361) with the corresponding fitted resonances for all relevant metabolites and the corresponding residue of the fit

**FIGURE S2** Rank selection and corresponding impact on image quality. In the upper right corner, the original γ-ATP image is shown. Below, one can see the corresponding low rank approximations for different ranks (k = 1, …, 5) as well as the relative difference to the original image (kN) and the relative difference to the next higher rank. In the upper left corner, the corresponding normalized singular values (Snorm) are shown

**FIGURE S3** Summed spectra for dominantly GM and WM voxels for individual volunteers (N = 11). For each volunteer, the number of GM and WM voxels are indicated in the plot

**FIGURE S4** 31P metabolic images of resonances with lower peak amplitudes. Shown are the measured metabolite images of GPE and inorganic phosphate (P_i) for three different spatial directions of one volunteer. The metabolite maps are presented as ratios to a low rank approximated γ-ATP signal to correct for coil sensitivity. In addition, a nOe and T_1 correction was applied. The images are zero-filled to twice the original resolution

**FIGURE S5** Comparison of different internal reference standards. To investigate the assumption of constant γ-ATP concentration over the entire brain and compare this reference technique to another internal reference using the total phosphate pool (total P), regression plots over white matter fraction for N = 11 volunteers are shown for total P (A) and γ-ATP (B). Below, the low rank approximated reference calculated from γ-ATP and total P is shown as well as the corresponding PCR images (C). The last row shows the ratio of the γ-ATP to the total P

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