Comparison of four $^{31}$P single-voxel MRS sequences in the human brain at 9.4 T

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**Purpose:** In this study, different single-voxel localization sequences were implemented and systematically compared for the first time for phosphorous MRS ($^{31}$P-MRS) in the human brain at 9.4 T.

**Methods:** Two multishot sequences, image-selected in vivo spectroscopy (ISIS) and a conventional slice-selective excitation combined with localization by adiabatic selective refocusing (semiLASER) variant of the spin-echo full intensity–acquired localized spectroscopy (SPECIAL-semiLASER), and two single-shot sequences, semiLASER and stimulated echo acquisition mode (STEAM), were implemented and optimized for $^{31}$P-MRS in the human brain at 9.4 T. Pulses and coil setup were optimized, localization accuracy was tested in phantom experiments, and absolute SNR of the sequences was compared in vivo. The SNR per unit time (SNR/t) was derived and compared for all four sequences and verified experimentally for ISIS in two different voxel sizes ($3 \times 3 \times 3 \text{ cm}^3$, $5 \times 5 \times 5 \text{ cm}^3$, 10-minute measurement time). Metabolite signals obtained with ISIS were quantified. The possible spectral quality in vivo acquired in clinically feasible time (3:30 minutes, $3 \times 3 \times 3 \text{ cm}^3$) was explored for two different coil setups.

**Results:** All evaluated sequences performed with good localization accuracy in phantom experiments and provided well-resolved spectra in vivo. However, ISIS has the lowest chemical shift displacement error, the best localization accuracy, the highest SNR/t for most metabolites, provides metabolite concentrations comparable to literature values, and is the only one of the sequences that allows for the detection of the whole $^{31}$P spectrum, including β–adenosine triphosphate, with the used setup. The SNR/t of STEAM is comparable to the SNR/t of ISIS. The semiLASER and SPECIAL-semiLASER sequences provide good results for metabolites with long $T_2$. 

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1 | INTRODUCTION

Phosphorus MRS (\(^{31}\)P-MRS) is a powerful tool to detect in vivo brain metabolites noninvasively. This includes phosphomonoesters (PMEs) (ie, phosphoethanolamine and phosphocholine), free inorganic phosphate, phosphodiesters (ie, glycerophosphoethanolamine and glycerophosphocholine), phosphocreatine (PCr), adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide (NAD\(^+\), NADH).\(^{1-3}\) These metabolites play key roles in energy and membrane metabolism. In addition, biologically relevant parameters, such as intracellular and extracellular pH and intracellular Mg\(^{2+}\) concentration, can be extracted.\(^{1,4}\) Therefore, \(^{31}\)P-MRS offers a wide range of potential clinical diagnostic applications in the human brain, for pathologies such as schizophrenia,\(^{5,6}\) bipolar disorder,\(^{7,8}\) Parkinson’s disease,\(^{9-11}\) epilepsy,\(^{12,13}\) or cancer.\(^{14,15}\) So far, few clinical studies using \(^{31}\)P-MRS have been performed due to challenges including low spatial resolution and long measurement times. This shortcoming can be mitigated using ultrahigh magnetic field (UHF) strength (≥ 7 T). Higher magnetic-field strengths enhance the spectral dispersion and signal intensity.\(^{16,17}\) Moreover, the faster T\(_1\) relaxation of most \(^{31}\)P metabolite resonances at higher field strength increases SNR per unit time (SNR/t) further.\(^{18,19}\) These enhancements lead to higher temporal and spatial resolution. As a drawback of UHF, the transmit and receive field homogeneity decreases, while RF energy deposition in the tissue as defined by the specific absorption rate (SAR) increases.

To provide clinically relevant information about physiologic function, accurate spatial localization is a fundamental requirement. Choosing a localization sequence depends on the research question or application. Magnetic resonance spectroscopic imaging is a multivoxel technique that allows investigation of the spatial distribution of metabolites. It is often used to study large, heterogeneous lesions, such as in brain tumors.\(^{14,15}\) However, some brain regions, such as posterior fossa, or frontal lobes are not very amenable to MRSI.\(^{20}\) Moreover, the acquisition time is quite long, a homogeneous main magnetic field over the entire volume of interest (VOI) is required, and the spatial resolution is compromised by significant partial-volume errors resulting from the Fourier transform–based reconstruction.\(^{21,22}\) When the research question only addresses a small homogeneous VOI, single-voxel localization might be preferred. Single-voxel techniques offer higher localization accuracy and provide spectra with a high SNR in a short scan time and a good field homogeneity over the small shimmed volume.\(^{20}\) Due to the high-quality spectra, metabolite quantification is more precise and accurate than in MRSI.\(^{23}\)

Until now, 16 \(^{31}\)P-MRS studies have been reported for human brain applications at UHF, from which nine present surface coil localized pulse-acquired data\(^{16,18,24-30}\) and six present MRSI data.\(^{14,15,17,25,31,32}\) So far, only one study has used a single-voxel localization technique in the human brain at UHF (7 T) for \(^{31}\)P acquisition (ie, image-selected in vivo spectroscopy [ISIS]),\(^{33}\) but no quantitative results were presented. The ISIS technique was shown to be a robust sequence that can be used to acquire the whole \(^{31}\)P spectrum.\(^{33,34}\) However, ISIS is a multishot sequence that is prone to motion-induced artifacts.\(^{21}\) To acquire MR spectra from uncooperative patients, or when measuring dynamic processes, single-shot sequences with a higher temporal resolution are an alternative. However, single-shot \(^{31}\)P sequences, such as stimulated echo acquisition mode (STEAM) or conventional slice-selective excitation combined with localization by adiabatic selective refocusing (semiLASER), have only been applied in skeletal muscle at UHF.\(^{35,36}\)

This work presents the first systematic comparison of \(^{31}\)P single-voxel localization (ie, STEAM, semiLASER, ISIS, and a semiLASER variant of spin-echo full intensity–acquired localized [SPECIAL] spectroscopy),\(^{37}\) which was newly implemented for \(^{31}\)P-MRS. It analyzes the pulse profiles, chemical shift displacement errors, and the localization performance in phantom experiments. Moreover, it presents the first localized \(^{31}\)P spectra acquired at 9.4 T in the healthy human brain, compares absolute SNR and SNR/t, and reports metabolite concentrations.

2 | METHODS

2.1 | Hardware setup

All experiments were conducted on a 9.4T whole-body MRI scanner (Siemens, Erlangen, Germany) using a home-built double-tuned 20-loop \(^{31}\)P/\(^{1}\)H head array.\(^{38}\) The array consisted of 16 transceiver surface loops (eight loops at each frequency) circumscribing the head. All 16 loops were placed on the surface of the same tight-fit holder at the same distance to the sample, ensuring high transmit and receive performance at both frequencies. In
addition, the array had two pairs (\(^{31}\)P and \(^{1}\)H) of “vertical” loops placed at the superior location of the head. During transmission, we used two different modes to drive the \(^{31}\)P array. In the first mode (i.e., “volume coil” or circularly polarized mode), we distributed power equally among eight transceiver loops with a 45° phase increment. In this mode, the \(^{31}\)P array produced a \(B_1^+\) of 27 \(\mu\)T/\(\sqrt{\text{kw}}\) in the center of an elliptical phantom (length: 17 cm; axes of ellipse: 18 \(\times\) 15 cm; \(\varepsilon\): 62.4; \(\sigma\): 0.54 S/m at 160 MHz). To increase \(B_1^+\) for \(^{31}\)P single-voxel spectroscopy locally, the \(^{31}\)P array can also be driven in the “surface coil” mode. In this mode, the entire RF power was applied only to the three bottom surface coil elements using an unbalanced three-way Wilkinson power splitter, similarly as previously described.\(^{39}\) In this case, we used a phase shift of 90° between adjacent loops. Also similarly to the previous work,\(^{39}\) we distributed power unequally among the three loops (i.e., 50% of the power was applied to the central loop, and 25% of the power to each of the two adjacent ones) (Figure 1). As a result, maximum experimentally measured \(B_1^+\) in a phantom could be increased to 44 \(\mu\)T/\(\sqrt{\text{kw}}\).

Before conducting in vivo measurements, SAR simulations were performed for the visual cortex according to our institutional safety procedure.\(^{40}\) This surface coil mode could also be used to increase \(B_1^+\) in other brain regions, such as in the prefrontal cortex, by driving coil elements adjacent to the region of interest.

2.2 | Pulse design

The three different pulse types of the different localization sequences used in this study were numerically simulated with in-house-written MATLAB (MathWorks, Natick, MA) tools based on the Bloch equations\(^{41}\) to find optimal parameter sets. All pulses were simulated at 0 ppm as well as over a ±8 ppm chemical shift range at 9.4 T (equal to ±1.3 kHz), which covers all metabolites between PMEs and \(\alpha\)-ATP. Simulations of pulse profiles over a chemical shift range of ±16 ppm at 9.4 T (equal to ±2.6 kHz), which would also cover \(\beta\)-ATP, are shown in Supporting Information Figure S1. Pulses were simulated for \(B_1^+\) of 40 \(\mu\)T and 60 \(\mu\)T to reflect the average \(B_1^+\) of the two different coil setups in vivo.

For slice-selective excitation, Hamming-windowed sinc pulses with a duration of 1.5 ms and a flip angle of about 90° (estimated from phantom \(B_1^+\) maps) were used. The time-bandwidth products were 4.5 (corresponding to four zero-crossings of amplitude modulation) for \(B_1^+\) of 40 \(\mu\)T, and 6.0 (corresponding to six zero-crossings of amplitude modulation) for \(B_1^+\) of 60 \(\mu\)T, resulting in an excitation bandwidth of 3 kHz and 4.3 kHz, respectively (Figure 2A), independent of voxel dimensions.

For nonlocalized excitation with a target flip angle of 90°, a block-shaped pulse with a duration of 330 \(\mu\)s (bandwidth of 3.4 kHz) was used for a \(B_1^+\) of 40 \(\mu\)T, while a duration of only 250 \(\mu\)s (bandwidth of 4.5 kHz) was possible for a \(B_1^+\) of 60 \(\mu\)T (Figure 2D).

Gradient-modulated offset-independent adiabatic (GOIA)\(^{42}\) pulses were used for inversion and refocusing. WURST waveforms of 16th-order and 4th-order were used for RF field and gradient modulation, respectively.\(^{43,44}\) This pulse is subsequently referred to as GOIA-W(16,4). In our measurements, the gradient strength was fixed to 25 mT/m, the gradient modulation factor to 0.9, and the ramp times to 1 ms. To obtain adiabaticity for our two different coil setups and the two different voxel sizes used, a pulse duration of 8 ms was chosen for a \(B_1^+\) of 40 \(\mu\)T, and 5 ms for a \(B_1^+\) of 60 \(\mu\)T. This resulted in a bandwidth of 12.9 kHz for a voxel length of 3 cm, and a bandwidth of 21.4 kHz for a voxel length of 5 cm (Figure 2B,C). It is important to note that with our implementation, the GOIA inversion bandwidth was dependent on the slice thickness when the gradient strength of the pulse was

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**FIGURE 1** A, CST Studio Suite 2017 electromagnetic simulation model of the phosphorous (\(^{31}\)P) part of the double-tuned \(^{31}\)P/\(^{1}\)H array loaded by the “Duke” voxel model from the “Virtual Family” (ITIS, Switzerland). The three bottom surface coil elements used in the surface coil mode for transmission are marked in red. B, Simulated transversal \(B_1^+\) map obtained using the \(^{31}\)P surface coil mode of the array (only three surface loops for transmission) loaded by the Duke voxel model. C, Measured SNR map obtained using the \(^{31}\)P array with all 10 receive loops loaded by the elliptical phantom, as described in detail in Avdievich et al.\(^{38}\) Dotted lines present the chosen voxel positions of the two different voxel sizes 3 \(\times\) 3 \(\times\) 3 cm\(^3\) and 5 \(\times\) 5 \(\times\) 5 cm\(^3\).
kept constant. For this reason, GOIA pulses were simulated for both used voxel dimensions (3 cm and 5 cm).

Outer volume saturation (OVS) bands consist of a sinc-shaped amplitude modulation with a time-bandwidth product of 10.0, a duration of 2.56 ms, and an excitation bandwidth of 3.9 kHz.

2.3 | Sequence design

Two multishot and two single-shot single-voxel localization schemes were compared for $^{31}$P-MRS. Three-dimensional localized ISIS requires multiples of eight encoding steps with different combinations of slice-selective inversion pulses.21,45 Here, three GOIA-W(16,4) pulses were incorporated for slice selection, along with all three directions before a block-shaped excitation pulse.33 (Figure 3A).

The SPECIAL-semiLASER sequence,37,46,47 which needs only multiples of two encoding steps for 3D-localization, is a combination of a one-dimensional ISIS sequence and a slice-selective adiabatic spin-echo sequence (Figure 3B). A GOIA-W(16,4) pulse was implemented for slice-selective inversion in the coronal dimension and was followed by a Hamming-windowed sinc excitation pulse, which provided
slice selection in the transversal dimension. This was complemented by slice selection in the sagittal dimension using a pair of GOIA-W(16,4) pulses.

A semiLASER sequence \(^{48,49}\) was implemented with a Hamming-windowed sinc excitation pulse in transversal direction, while two pairs of GOIA-W(16,4) pulses were used for refocusing in the coronal and sagittal direction (Figure 3C).

The STEAM \(^{50}\) sequence consisted of three slice-selective Hamming-windowed sinc pulses. Crusher gradients had a strength of 25 mT/m and durations of 3 ms after the first and third slice-selective pulse each, and 6 ms in the mixing time. Before localization, several OVS bands of 30-mm thickness were implemented. A crusher was added after each OVS pulse (Figure 3D). For the other three sequences, no crusher gradients apart from inherent gradient ramps were implemented. All sequences were carried out without phase cycling.

### 2.4 Phantom measurements

Localization performance of all sequences was tested on a two-compartment phantom filled with equally concentrated phosphate buffer solution (100 mmol/L). Sugar and NaCl (1135 g /33 g per 1000 g of H\(_2\)O) were added to mimic a coil load similar to in vivo experiments. \(^{51}\) The inner cuboid-shaped compartment (inner size, 48 × 48 × 19 mm\(^3\); thickness of the side walls, 1 mm; top and bottom thickness, 1.5 mm) was placed in the center of a bigger compartment (cylinder length, 14 cm; elliptical footprint, 17 × 14 cm), which filled the whole coil. The pH in the two compartments was adjusted to 5.0 and 8.0 by mixing different concentration ratios of KH\(_2\)PO\(_4\) and K\(_2\)HPO\(_4\), which resulted in a chemical-shift difference of 2.2 ppm.

All measurements were carried out with an acquisition bandwidth of 10 kHz, 4096 complex sampling points, TR = 7.5 seconds, eight preparation scans, 32 averages, and 5-minute measurement time. The ISIS, SPECIAL-semiLASER, semiLASER, and STEAM sequences used TEs of 0.425 ms, 17 ms, 30 ms, and 10 ms, respectively; the mixing time for STEAM was set to 10 ms, and six OVS bands were placed close around the VOI. Localized spectra of the inner compartment (VOI = 48 × 48 × 19 mm\(^3\)) and a bigger VOI (88 × 88 × 59 mm\(^3\)), including most of the outer compartment plus the inner compartment, were acquired with each of the sequences to calculate selection efficiency, suppression efficiency, and contamination, as suggested by Keevil et al. \(^{52}\) (Figure 4A). Voxels were placed using 2D-FLASH images acquired in three orientations. B\(_0\) shimming was performed for all measurements using the Siemens second-order shimming interface.

### 2.5 In vivo measurements

In total, data from 14 healthy volunteers were acquired. All studies were approved by the local ethics committee, and written informed consent was obtained from all volunteers before the examination. In vivo scan times varied between 35 minutes and 2 hours for the different measurements. The study was well tolerated by all volunteers. For all in vivo
measurements, the acquisition bandwidth was set to 10 kHz, and 4096 complex sampling points were acquired.

The SNR was compared for all four sequences in a VOI of 5 × 5 × 5 cm³ placed in the occipital brain region in 4 volunteers (26 ± 2.5 years, 1 female, total measurement time of 2 hours) using the surface coil mode. A TR of 25 seconds (≥ 5 × T₁) was chosen to guarantee that the inverted magnetization was completely recovered before the next excitation. For ISIS, the SNR efficiency was compared for VOIs of 5 × 5 × 5 cm³ and 3 × 3 × 3 cm³ using the surface coil mode.

Sixty-four averages were acquired for ISIS (TE = 0.425 ms), SPECIAL-semiLASER (TE = 17 ms), semiLASER (TE = 30 ms), and STEAM (TE/TM = 10/10 ms). For STEAM localized measurements, five outer-volume suppression bands covering the skull lipids and muscles were applied (see insets in Figure 5).

For ISIS, the SNR efficiency was compared for VOIs of 5 × 5 × 5 cm³ and 3 × 3 × 3 cm³ using the surface coil mode.
The measurement time per acquisition was set to 10 minutes. Eight volunteers (28 ± 2.8 years, 4 females, total measurement time of 35 minutes) were measured for quantitative analysis with ISIS (TE/TR = 0.425/5000 ms, 120 averages). For PCr with $T_1$ of 2.5 seconds, the chosen flip angle of 90° is close to the Ernst angle of 82° for TR of 5 seconds.

To demonstrate the high spectral quality achievable with ISIS at 9.4 T in short measurement time, ISIS localized spectra (TR = 5000 ms, 120 averages, and 40 averages) were acquired in 10:10 minutes and three spectra in a row in 3:30 minutes, each using a VOI of $3 \times 3 \times 3$ cm$^3$ in 1 volunteer using the surface coil mode (TE = 0.425 ms, 28 years, female, total measurement time of 35 minutes) as well as the volume coil mode (TE = 0.465 ms, 31 years, male, total measurement time of 35 minutes). While all other presented spectra are non-filtered, these spectra were exponentially filtered by 15 Hz.

### 2.6 Data analysis

Raw data were processed using an in-house written MATLAB tool. The processing steps included averaging, strong Gaussian

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**FIGURE 5** Comparison of absolute $^{31}$P SNR measured with a TR of 25 seconds (acquisition time of 26:40 minutes for each spectrum) in a $5 \times 5 \times 5$ cm$^3$ voxel from the back of the head (inset) with the surface coil mode with ISIS (TE 0.425 ms) (A), SPECIAL-semiLASER (TE 17 ms) (B), semiLASER (TE 30 ms) (C), and STEAM (TE/TM 10/10 ms) (D) localization. The spectra are representative spectra from 1 volunteer. For representation, ISIS data were first-order phase-corrected, and seven missing points at the beginning of the FID were predicted using the MATLAB autoregressive all-pole model parameters Yule-Walker method with a model order of 18. First-order phase-uncorrected ISIS spectra are shown in Supporting Information Figure S2. The inset shows the voxel placement and the positioning of the five OVS bands, which were used in STEAM localization. The color boxes illustrate the CSDEs between phosphocreatine (PCr) and phosphoethanolamine (PE) ($\Delta f = 6.76$ ppm) for the different sequences. It has to be taken into account that the CSDE effect in STEAM measurements is reduced by OVS bands. Abbreviations: ATP, adenosine triphosphate; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; $P_{\text{extra}}$, extracellular inorganic phosphate; $P_{\text{intra}}$, intracellular inorganic phosphate; tNAD, NADH and NAD+.
filtering with a decay rate of 30 Hz before calculating coil combination weights with singular value decomposition \(^{53}\) algorithm based on PCR and applying calculated weights to nonfiltered data, zero-order phase correction, shifting PCR to 0 ppm, and finally truncation of the FID (after 80 ms for measurements with volume coil mode, after 90 ms for measurements with surface coil mode; 409 ms were originally acquired) with subsequent zero-filling to 4096 points. Only for display purposes, ISIS data were first-order phase-corrected, and missing points at the beginning of the FID were predicted using the MATLAB autoregressive all-pole model parameters Yule-Walker method \(^{54}\) (Supporting Information Figure S2).

The FWHM of the peaks, as well as SNR, were determined. The SNR was calculated in the frequency domain as the ratio between the metabolite peak heights and the spectral noise between +15 ppm and +30 ppm. The SNR/t was calculated using equations given in de Graaf, \(^{4}\) using measured T1 and T2 relaxation times. \(^{19,55}\) For a fair SNR comparison, TR was adjusted for every sequence matching a SAR value of approximately 80%.

In vivo spectra of ISIS were quantified using an in-house written MATLAB implementation of AMARES. \(^{56}\) To remove the fast-relaxing macromolecular contributions to the baseline of ISIS spectra, the first two data points of the FIDs were removed before fitting (resulting in a theoretical TE of 0.625 ms), as discussed in the Supporting Information and illustrated in Supporting Information Figure S3. For fitting, spectral prior knowledge about phosphoethanolamine, phosphocholine, extracellular inorganic phosphate, intracellular inorganic phosphate, glycerophosphoethanolamine, glycerophosphocholine, PCr, γ-ATP, α-ATP, tNAD (NADH and NAD+), and β-ATP was applied. Prior knowledge contained soft constraints on phase, amplitude, chemical shift, and line widths, as indicated in Supporting Information Table S1. For the line shape, the model function used in AMARES was first optimized to match the Voigt line shape of each measured peak, and the mean Voigt line shape of all peaks was used for the final fit. Cramer-Rao lower bounds were determined by calculating the inverse Fisher information matrix and the SD of the measurement noise from the last 900 points of the nontruncated FIDs. \(^{4}\) Obtained peak areas were corrected for T1 and T2 relaxation, \(^{19,55}\) but not for pulse profile distortions and chemical shift displacement. An internal γ-ATP reference of 3 mM was used to convert fitted peak areas to 31P metabolite concentrations. \(^{18,24,57}\) The calculated concentrations from the two different voxel sizes were compared for statistically significant differences with a non-parametric Wilcoxon signed-rank test for paired data (\(\alpha = 0.05\)).

### 3 | RESULTS

#### 3.1 | Sequence performance

For 31P-MRS at 9.4 T, metabolites from PME to α-ATP resonate within a spectral range of ±8 ppm. For sinc pulses with a bandwidth of 3 kHz, this results in a chemical-shift displacement error (CSDE) of ±43.2%, and for sinc pulses with a bandwidth of 4.3 kHz in a respective CSDE of ±29.9%. Because pulses should be as short as possible to ensure short TEs, the time-bandwidth product of the used pulses was only 4.5 for the volume coil mode and 6.0 for the surface coil mode, which resulted in transition bandwidths (defined between 5% and 95% of the maximum magnetization) of 1.4 kHz for the 3-kHz broad pulse, and 1.3 kHz for the 4.3-kHz broad pulse, as demonstrated in simulated slice profiles in Figure 2A. For Hamming-windowed sinc pulses, the excitation bandwidth did not change for different slice thicknesses.

For GOIA-W(16,4) pulses, slice profiles for the two different voxel lengths (3 cm and 5 cm) were simulated separately, as the inversion bandwidth changed as the gradient strength was kept constant (Figure 2B,C). Simulations revealed an inversion bandwidth of 12.9 kHz for a 3-cm slice thickness, and 21.4 kHz for a 5-cm slice thickness. The slice profiles show narrow transition bands with little signal dropout around half maximum, which enabled accurate slice selection. For both $B_1^+\text{ strengths, on-resonance slice profiles were characterized by 100% inversion. Off-resonant profiles at ± 8 ppm displayed slight smearing at the edges due to the time-varying gradient waveform.}^{58}$ The CSDEs between PME and α-ATP are ±10% for a slice thickness of 3 cm, and ±6.1% for a slice thickness of 5 cm. This results in an absolute CSDE of ±3 mm, independent of the voxel length.

Using nonselective block-shaped excitation pulses, the shorter pulse at $B_1^+\text{ of 60 µT excited the whole spectral range of ±8 ppm (1.3 kHz), while the signal intensity dropped to 85% of the ideal intensity at a chemical shift of 8 ppm using the longer pulse at $B_1^+\text{ of 40 µT (Figure 2D).}$

The vendor implemented OVS bands with an excitation bandwidth of 3.9 kHz experienced a CSDE between PME and α-ATP of ±33% for the spectral range of ±8 ppm.

The localization performance of all four sequences, as assessed in phantom experiments, shows a similar performance among sequences, with ISIS having the highest selection efficiency and STEAM having the lowest, as seen in Figure 4 and Table 1.

#### 3.2 | In vivo measurements

In vivo spectra of all four localization sequences obtained from the absolute SNR measurements in a $5 \times 5 \times 5 \text{ cm}^3$ VOI in the occipital brain region using the surface coil mode are shown in Figure 5. Corresponding SNR and FWHM are listed in Table 2, and metabolite concentrations of fully relaxed ISIS spectra are given in Supporting Information Table S2. The SNR of all metabolites decreased with increasing TE. The SNR of STEAM acquisitions was lower in comparison to the other sequences, even though TE is shorter than...
in SPECIAL-semiLASER and semiLASER. The FWHM of PCr is comparable for all sequences (about 11 Hz).

Calculated SNR per unit time for TR = 25 seconds, as well as for TR values adjusted for each sequence to SAR values of approximately 80%, are given in Table 3. Absolute SNR as well as SNR/t is highest for ISIS localized spectra. For metabolites with short T2 relaxation times, SNR/t of STEAM is comparable to SNR/t of ISIS.

Spectra and corresponding AMARES fits of SNR efficiency measurements acquired with the surface coil mode in the occipital lobe of the human brain using ISIS are shown in Figure 6A.B. Measured SNR/t for 5 × 5 × 5 cm3 VOI increased by a factor of 3.96 ± 0.83 by reducing TR from 5 × T1 to 5 seconds. The SNR of the 3 × 3 × 3 cm3 VOI was, on average, 55% lower than for the 5 × 5 × 5 cm3 VOI for all metabolites.

AMARES quantification results for unfiltered ISIS data acquired with the surface coil mode are reported in Figure 6D for the two VOIs 5 × 5 × 5 cm3 and 3 × 3 × 3 cm3. Calculated mean SNR, mean concentrations, and mean Cramer-Rao lower bounds and their respective SDs are given in Supporting Information Table S3. Concentrations of phosphoethanolamine and α-ATP obtained for the smaller VOI were significantly higher (P < .05) than in the bigger VOI.

A demonstration of the spectral quality after 15-Hz exponential filtering that can be obtained in 10:10-minute and 3:30-minute measurement time for a 3 × 3 × 3 cm3 VOI in the occipital brain using the surface coil as well as in the center of the brain using the volume coil mode is shown in Figure 7. The spectra are scaled to the same noise level, so they become easily comparable. In Supporting Information Figure S4, the same spectra are shown scaled to the same noise level, but different axis intercepts are displayed to be able to clearly see the metabolite peaks. To demonstrate the stability of the spectral quality of ISIS spectra measured in 3:30 minutes, a measurement series of three 31P spectra acquired consecutively in 1 volunteer is presented in Supporting Information Figure S5.

### DISCUSSION

In this study, the two multishot localization sequences ISIS and SPECIAL-semiLASER, as well as two single-shot localization sequences semiLASER and STEAM, were implemented for 31P-MRS in the human brain at 9.4 T. To the best of our knowledge, the applicability of SPECIAL-semiLASER for 31P-MRS was demonstrated for the first time. A detailed evaluation of RF pulse and sequence performance was conducted. It was shown that B1+ could be increased by focusing the power to a specific region, which substantially improved pulse performance and SNR. The study presents the first single-voxel localized 31P spectra, SNR comparison, and quantification results of the human brain at 9.4 T.

The large frequency dispersion of phosphate resonances in vivo results in excellent spectral resolution, but also demands pulses with high spectral bandwidths. Simulations show that at UHF, higher B1+ and longer pulse durations than at lower fields are needed to achieve sufficiently large excitation and inversion bandwidths to cover the whole spectrum, and to reduce CSDEs. In contrast, pulse durations should be as short as possible to reduce TE of the localization sequences and to increase SNR. Therefore, in this study, adiabatic GOIA-W(16,4) inversion pulses were used in ISIS, semiLASER, and SPECIAL-semiLASER sequences, which showed an excellent inversion profile and limited the CSDE to a maximum of ±10% for a 3 × 3 × 3 cm3 VOI, or ±6.1% for a 5 × 5 × 5 cm3 VOI for a ±8-ppm spectral range at 9.4 T due to the high achievable inversion bandwidth. It is challenging to also cover β-ATP with a chemical shift of −16.26 ppm when the transmit frequency is set to PCr. Adiabaticity for GOIA pulses is hard to reach at −16.26 ppm, which leads to substantial ripples in the stopband at this offset (Supporting Information Figure S1). Substantially longer GOIA pulse durations would be necessary to overcome this problem at a given B1+ field strength, but this would also lead to higher SAR. Furthermore, the limited bandwidth of the block-shaped excitation pulse used in ISIS led to a strongly

|            | ISIS    | SPECIAL-semiLASER | semiLASER | STEAM  |
|------------|---------|--------------------|-----------|--------|
| Selectivity (%) | 95.0    | 93.4               | 93.4      | 87.4   |
| Suppression (%)  | 98.7    | 98.5               | 98.6      | 98.1   |
| Contamination (%) | 9.0     | 9.3                | 9.0       | 11.7   |
| CSDE (x, y, z; ± 2.2 ppm, volume coil mode) | ±1.7%, ±1.7%, ±1.7%, ±1.7% | ±1.7%, ±1.7%, ±1.7%, ±1.7% | ±11.8%, ±11.8%, ±11.8%, ±11.8% |

**Note:** The selection efficiency, suppression efficiency, and contamination were calculated according to equations given in Keevil et al. The CSDEs are given in all three directions for the used pulses for a 48 × 48 × 19 mm3 voxel and the chemical shift of 2.2 ppm between the two compartments of the phantom. The CSDEs of in vivo measurements (3 × 3 × 3 cm3 and 5 × 5 × 5 cm3) are shown in Figure 2 for the two different coil setups.
attenuated β-ATP peak, which can only be overcome by higher $B_1^+$ fields. Therefore, when information about Mg$^{2+}$ concentration shall be extracted, the transmit frequency should be set between PCr and β-ATP. Nevertheless, in this study, the ISIS sequence allows for the detection of β-ATP. In contrast to ISIS, the Hamming-windowed slice-selective sinc excitation pulses used in semi-LASER, SPECIAL-semiLASER, and STEAM had broader transition bands and caused exceedingly higher CSDEs due to the limited available excitation bandwidth. To achieve higher bandwidths for slice-selective excitation pulses, substantially higher $B_1^+$ would be necessary, which is not easily possible considering constraints of power and SAR. It is also possible to achieve higher excitation bandwidths at the same $B_1^+$ using frequency-modulated asymmetric excitation pulses and longer pulse durations, but slice profiles of these pulses are more susceptible to $B_1^+$ offsets.37 Another possibility to obtain higher excitation bandwidths, and, at the same time, more uniform nutation, is the use of adiabatic half-passage pulses.39-61 However, at UHF, their application is severely limited by the total RF energy

### Table 2

| Metabolite | ISIS | SPECIAL-semiLASER | semiLASER | STEAM |
|------------|------|-------------------|-----------|-------|
| PE         | SNR  | 20.3 ± 1.0        | 12.8 ± 3.8| 11.4 ± 3.4 | 8.1 ± 0.8 |
|            | FWHM (Hz) | 19.1 ± 1.1 | 20.1 ± 3.1 | 20.8 ± 5.5 | 19.0 ± 5.2 |
| PC         | SNR  | 6.4 ± 1.0         | 4.8 ± 1.1 | 4.4 ± 2.1 | 3.6 ± 0.4 |
|            | FWHM (Hz) | 20.6 ± 4.2 | 28.4 ± 12.0 | 18.8 ± 12.7 | 51.9 ± 16.9 |
| $P_{\text{intra}}$ | SNR | 9.7 ± 0.9 | 8.4 ± 1.5 | 6.3 ± 1.6 | 4.4 ± 1.0 |
|            | FWHM (Hz) | 28.1 ± 2.4 | 28.4 ± 6.4 | 33.1 ± 5.9 | 33.4 ± 7.3 |
| GPE        | SNR  | 14.2 ± 1.2        | 11.2 ± 2.7| 11.0 ± 4.0 | 5.8 ± 1.2 |
|            | FWHM (Hz) | 18.9 ± 3.2 | 20.8 ± 0.6 | 18.3 ± 2.1 | 20.7 ± 7.7 |
| GPC        | SNR  | 22.0 ± 0.8        | 16.9 ± 3.6| 16.5 ± 4.1 | 8.0 ± 0.8 |
|            | FWHM (Hz) | 17.4 ± 1.1 | 19.7 ± 1.5 | 19.8 ± 2.3 | 19.4 ± 1.8 |
| PCr        | SNR  | 148.6 ± 7.7       | 107.0 ± 20.4| 99.9 ± 18.6 | 57.1 ± 1.9 |
|            | FWHM (Hz) | 10.7 ± 0.3 | 11.2 ± 0.8 | 10.8 ± 0.4 | 11.0 ± 0.2 |
| $\gamma$-ATP | SNR | 19.3 ± 0.9 | 7.7 ± 0.8 | — | 6.2 ± 0.5 |
|            | FWHM (Hz) | 50.4 ± 3.3 | 46.0 ± 9.2 | — | 48.1 ± 25.6 |
| $\alpha$-ATP | SNR | 19.8 ± 1.4 | 8.7 ± 1.2 | — | 6.8 ± 2.8 |
|            | FWHM (Hz) | 36.5 ± 0.7 | 47.0 ± 12.3 | — | 33.7 ± 3.4 |

Note: The ISIS metabolite concentrations are given in Supporting Information Table S2.

### Table 3

| SNR/t (s) | ISIS | SPECIAL-semiLASER | semiLASER | STEAM, five OVS bands |
|----------|------|-------------------|-----------|---------------------|
|          | TR = 25 seconds | TR = 5 seconds | TR = 25 seconds | TR = 6.5 seconds | TR = 25 seconds | TR = 10 seconds | TR = 25 seconds | TR = 1.5 seconds |
| PE       | 0.76 | 2.39              | 0.48      | 1.34               | 0.43   | 0.92                | 0.30        | 1.29           |
| PC       | 0.24 | 0.97              | 0.18      | 0.61               | 0.17   | 0.40                | 0.14        | 0.88           |
| $P_{\text{intra}}$ | 0.36 | 1.43              | 0.32      | 1.05               | 0.24   | 0.56                | 0.17        | 1.02           |
| GPE      | 0.53 | 1.86              | 0.42      | 1.28               | 0.41   | 0.94                | 0.22        | 1.10           |
| GPC      | 0.83 | 2.86              | 0.64      | 1.91               | 0.62   | 1.40                | 0.30        | 1.49           |
| PCr      | 5.57 | 24.09             | 4.01      | 14.28              | 3.75   | 9.19                | 2.14        | 16.09          |
| $\gamma$-ATP | 0.72 | 3.50              | 0.29      | 1.10               | 0.17   | 0.42                | 0.23        | 2.49           |
| $\alpha$-ATP | 0.74 | 3.69              | 0.33      | 1.25               | 0.15   | 0.38                | 0.26        | 3.31           |
| tNAD     | 0.20 | 0.96              | 0.16      | 0.61               | 0.09   | 0.23                | 0.15        | 1.61           |

Note: The SNR/t for TR = 25 seconds was calculated from mean SNR values measured for a 5 × 5 × 5 cm$^3$ voxel placed in the occipital lobe using the surface coil mode, as given in Figure 5 and Table 2. On that basis, the best possible SNR/t per sequence for TR values that match a SAR value of approximately 80% were calculated according to equations given in de Graaf.4
deposition, which would result in high TR and lower SNR/t efficiency.

Pulse performance directly relates to localization performance of the sequences. The localization performance and contamination of ISIS using GOIA-W(16,4) and a rectangular excitation pulse are comparable to previously performed studies at 7 T\textsuperscript{31,33} and to results obtained at 1.5 T\textsuperscript{62} using frequency offset–corrected inversion pulses,\textsuperscript{63} which have similar properties as the GOIA-W(16,4) pulses used in our study. Selectivity of SPECIAL-semiLASER and semiLASER was slightly lower, as the sinc pulse used for excitation in transversal direction has a worse selection profile and a higher CSDE. For the same reason, selectivity of STEAM, which uses three sinc pulses, is lower than for the other sequences. Moreover, the vendor-implemented OVS bands, which reduce contamination, are sinc-Gauss pulses as well and therefore experienced a substantial CSDE, thus affecting the magnetization within the selected voxel due to an inaccurate slice profile. The vendor-implemented OVS bands need to be replaced by high-quality saturation pulses with high bandwidth, low transition bandwidth, and T\textsubscript{1} and B\textsuperscript{T1} insensitivity.\textsuperscript{64,65} This, in turn, would enable inner-volume localization schemes to mitigate the CSDE of slice-selective excitation pulses. The influence of motion on localization performance was not evaluated in this study.

All four sequences provided well-resolved spectra when measuring a VOI of 5 × 5 × 5 cm\textsuperscript{3} with a TR of 25 seconds. The experimentally measured reduction in absolute SNR for SPECIAL-semiLASER and semiLASER in comparison to ISIS corresponds to T\textsubscript{2} relaxation losses\textsuperscript{17,32} due to longer minimum TEs. In addition to T\textsubscript{2} losses, the signal amplitude of ATP is reduced in spin-echo sequences due to J-evolution during TE.\textsuperscript{35} As a result, in this study, ATP was not reliably detectable in semiLASER with a long TE. For STEAM, the SNR loss, which is inherent to stimulated echo sampling, was the highest for all metabolites.\textsuperscript{21} It was shown that SNR of the spin echo–based semiLASER technique was not substantially compromised by the longer TE due to the spin-locking effect.\textsuperscript{60} This gain in SNR between semiLASER and STEAM, as well as the comparable SNR of SPECIAL-semiLASER and semiLASER, has also been reported in 1H-MRS studies at 7 T\textsuperscript{79} and 9.4 T\textsuperscript{39} Although an absolute SNR benefit from spin echo–based sequences over STEAM could be observed for metabolites with long T\textsubscript{2}, short TE sequences like

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**FIGURE 6** Comparison of representative 31P ISIS spectra (TE/TR = 0.625/5000 ms) and AMARES fits from optimal SNR efficiency measurements with the surface coil mode for 5 × 5 × 5 cm\textsuperscript{3} (A) and 3 × 3 × 3 cm\textsuperscript{3} (B) VOIs measured in the occipital brain region. For representation, ISIS data and corresponding AMARES fits were first-order phase-corrected. C, Voxel placement in sagittal and transversal directions. D, Relaxation-corrected AMARES quantification results for nine metabolites from spectra acquired from 8 healthy volunteers for both voxel sizes (5 × 5 × 5 cm\textsuperscript{3} and 3 × 3 × 3 cm\textsuperscript{3}). Concentration values are given in mM with reference to γ-ATP as an internal reference. The central mark in each box indicates the median; the bottom and top edges denote the 25th and 75th percentiles, respectively. The error bars correspond to the SD over all measured subjects. Statistically significant differences between concentrations with P < .05 are displayed with red asterisks.

Abbreviations: ISO, isotropic; PC, phosphocholine
STEAM are superior for $^{31}$P-MRS when metabolites with very short $T_2$ relaxation times, such as ATP or NAD, are of interest. Also, because SAR is much lower for STEAM than for the adiabatic sequences, the SNR/$t$ of STEAM is higher than the SNR/$t$ of SPECIAL-semiLASER and much higher than that of the SAR-intensive semiLASER. The SNR/$t$ of STEAM is comparable to SNR/$t$ of ISIS for metabolites with very short $T_2$ relaxation times, but offers a better time resolution. Even though the time resolution of STEAM is higher than the time resolution of ISIS, for studies in which localization accuracy and CSDE are of high importance, or the whole $^{31}$P spectrum is of interest, ISIS is superior to STEAM.

To study, for example, only PCr or pH, semiLASER and SPECIAL-semiLASER with a higher localization accuracy than STEAM, but less motion artifact sensitivity than ISIS, might be useful alternatives. To further improve localization accuracy, implementation of phase cycling should be taken into consideration. Moreover, the $B_1^+$ inhomogeneity of the surface coil mode might influence pulse performance further inside the brain. These two reasons reduce the effective voxel size of the $5 \times 5 \times 5 \text{ cm}^3$ VOI, which results in a lower SNR difference than theoretically calculated.

The acquisition of $3 \times 3 \times 3 \text{ cm}^3$ ISIS spectra in only 3:30 minutes demonstrates a high spectral quality for both coil setups after filtering data exponentially by 15 Hz. Noise appears to be higher in spectra acquired with the volume coil. There are several reasons for this. First, in the surface coil mode, the use of only three coil elements for transmission (but all 10 elements for reception) with appropriate phase and power distribution maximized $B_1^+$, which enabled shorter pulses and a slightly shorter TE ($0.425$-ms surface coil vs $0.465$-ms volume coil). Furthermore, the SNR in volume coil mode–acquired spectra was reduced in comparison to surface coil mode spectra, because the selected VOI was closer to the coil, where the receive sensitivity of the coil is higher, and contamination from remote tissue was reduced. In addition, voxels in the volume coil measurements touched the ventricle, so CSF content in these voxels was higher than in voxels measured with the surface coil. Because CSF only contains glucose, lactate, and free phosphate, the effective VOI in $^{31}$P volume coil measurements was smaller than in surface coil measurements. Therefore, to achieve a higher spectral quality, the SNR/$t$ increase of ISIS by a factor of $3.96 \pm 0.83$ by reducing TR from 25 seconds to 5 seconds matches the theoretically calculated increase of PCr by a factor of 4.32.

When measuring with the surface coil mode, the SNR of the $3 \times 3 \times 3 \text{ cm}^3$ VOI was only 55% lower than for the $5 \times 5 \times 5 \text{ cm}^3$ VOI for all metabolites, whereas the volume is 78.4% smaller. This occurs as a result of voxel positioning. The voxel was positioned as close to the skull as possible, independent of the voxel size (see insets in Figure 6). However, the SNR of the coil is higher the closer to the periphery (see Figure 1C). Moreover, the $B_1^+$ inhomogeneity of the surface coil mode might influence pulse performance further inside the brain. Therefore, to achieve a higher spectral quality, it is recommended to use the surface coil mode for $^{31}$P-MRS studies.
spectral quality when measuring with the volume coil mode, a larger VOI needs to be chosen, or more averages need to be acquired, resulting in longer measurement times. A drawback of the surface coil mode is the increased $B^+_1$ inhomogeneity in comparison to the volume coil mode. This leads to non-uniform nutation angles for conventional pulses and incomplete inversion for adiabatic pulses when $B^+_1$ is too low to reach adiabaticity. This causes a reduction in sensitivity and increased contamination due to $T_1$ smearing for multishot sequences under the condition of short TRs, especially for bigger VOIs that reach far into the brain where $B^+_1$ is strongly attenuated. To reduce this problem, the sequences should be made completely adiabatic. Moreover, for ISIS, it was shown that $T_1$ smearing can be eliminated by extending the eight-step inversion scheme to a 36-step scheme with optimized ordering of the inversion pulses.

Comparing ISIS spectra acquired in 3:30 minutes using the surface coil mode at 9.4 T with spectra presented in a previous 7T study, a slightly higher frequency resolution can be seen. However, $\beta$-ATP was reduced compared with 7 T, as the bandwidth of the block pulse needs to be higher to cover the whole spectrum, which could not be achieved in our experimental setup.

Because the spectral quality of ISIS data measured with the surface coil mode is high in this study, it would be possible to measure metabolite concentrations from even smaller voxels, such as $2 \times 2 \times 2$ cm$^3$, which would increase the spatial selectivity in clinical applications. However, SNR is proportional to the selected volume and the square root of the number of averages, so SNR would decrease by a factor of 3.4 when keeping the measurement time constant. To obtain the same SNR by reducing the voxel size from $3 \times 3 \times 3$ cm$^3$ to $2 \times 2 \times 2$ cm$^3$, the number of averages should theoretically be increased by a factor of 11. Because the SNR is substantially lower when measuring with the volume coil setup, it might become difficult to obtain good quality spectra when reducing the VOI and keeping the acquisition time constant.

Outer volume saturation localization, in which magnetization outside the VOI is eliminated by OVS bands before the unaffect ed magnetization inside the VOI is excited by a $90^\circ$ pulse, would be an alternative to ISIS. However, to eliminate the magnetization along all three axes around the VOI, at least six OVS bands are necessary. To make this method as robust as ISIS against $B^+_1$ field inhomogeneities, the conventional OVS pulses need to be replaced by adiabatic pulses, such as in BISTRO pulse trains. This would be more SAR-demanding than ISIS, which increases the minimum TR and, therefore, decreases SNR/t.

In addition to single-voxel and OVS localization, MRSI is used. This technique produces spatial metabolite maps, which is advantageous for the examination of large heterogeneous tissue, such as in tumors. However, the acquisition takes longer, the spatial resolution is compromised by significant partial volume errors, and signal intensity nonuniformities (especially at UHF) need to be corrected when processing the data, and, together with the lower SNR, limit the possibility for accurate and precise quantification.

Most metabolite concentrations obtained in this single-voxel study are comparable to those previously measured at 7 T with surface coil localization. The measured integral ratio of $P_{Cr}/\gamma$-ATP of 1.6 in ISIS localized measurements is slightly higher than the reported integral ratios of $P_{Cr}/\gamma$-ATP between 1.1 and 1.5 measured at 7 T in the occipital brain region using surface coil localization or 3D chemical shift imaging. For tNAD, our measured concentrations are much higher than previous 7T data suggest.

There may be various reasons for differences in metabolite concentrations between our data and literature values. First, there might be systematic differences in the spectral fitting routines. Even though the TE used in ISIS acquisitions is short, it has to be taken into account that the signal amplitude of ATP (which was used to normalize all metabolite concentrations) might be slightly underestimated, as no J-evolution model was implemented in the fitting routine. In addition, spectral fit results are very sensitive to baseline issues, as shown in the Supporting Information. Reasons for the higher tNAD concentration measured in this study, as compared with 7T data, might be that the SNR of tNAD is very low, so it was fitted as one broad peak, whereas in the mentioned literature, NADH and NAD$^+$ were fitted separately. Also, in this study, no uridine diphosphate glucose could be fitted, as the SNR was too low. However, uridine diphosphate glucose has a diphosphate group that overlaps with NAD, thereby increasing our determined NAD concentration value. Moreover, the $T_1$ and $T_2$ relaxation times measured at 9.4 T and used for relaxation correction could be inaccurate, resulting in slightly inaccurate concentration values. Our results may also be affected by the CSDE of the pulses, which influences the localization accuracy. Furthermore, the relatively large voxels placed very close to the skull to exploit the highest possible $B^+_1$ of the surface coil mode might have led to contamination from extracranial muscles in the neck area, which would increase the PCr concentration.

Concentration differences between the two different voxel sizes might also be a result of voxel placement. Because the voxels were positioned as close to the skull as possible, independent of the voxel size, the tissue-volume fractions are different. The smaller voxel contains primarily gray matter, white matter and CSF, while the bigger voxel also contains cerebellum. It was already shown in $^1$H spectroscopy that metabolite concentrations vary regionally. A recent $^{31}$P study also presents slightly different metabolite concentrations of white and gray matter tissue, which might explain the measured concentration differences between the two different chosen voxel sizes in this study.
5 | CONCLUSIONS

The four localization sequences, ISIS, SPECIAL-semiLASER, semiLASER and STEAM, were implemented and optimized for $^{31}$P-MRS in the human brain at 9.4 T. The individual steps were optimization of the RF coil setup, simulation and optimization of the RF pulses, and a systematic comparison of the CSDEs, investigation of the localization accuracy in phantoms, and measurement of absolute SNR in in vivo experiments. It was found that ISIS provides the highest localization accuracy with a high SNR/t. Moreover, it is the only sequence that allows for the detection of the whole $^{31}$P spectrum with the used setup. It was demonstrated that high spectral quality can be acquired in a clinically feasible time using ISIS (3:30 minutes, $3 \times 3 \times 3$ cm$^3$).

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Individual spectra of 8 healthy volunteers, as well as spectra summed over all volunteers, were quantified, and SNR was determined. The SNR of spectra summed over all volunteers was divided by $\sqrt{8}$ for easier comparison to the SNR of the individual spectra. In ISIS spectra acquired with a $3 \times 3 \times 3$ cm$^3$ voxel size, no extracellular inorganic phosphate could be reliably fitted in per-volunteer spectra.