

Two-Voxel Spectroscopy With Dynamic B₀ Shimming and Flip Angle Adjustment at 7 Tesla in the Human Motor Cortex

Author names:

Clark Lemke¹

Aaron Hess²

Stuart Clare¹

Velicia Bachtiar¹

Charlotte Stagg¹

Peter Jezzard¹

Uzay Emir¹

Affiliations:

¹ Oxford Centre for Functional MRI of the Brain, University of Oxford, Oxford, Oxfordshire,
United Kingdom

² Oxford Centre for Clinical Magnetic Resonance Research, University of Oxford, Oxford,
Oxfordshire, United Kingdom

Corresponding Author:

Uzay Emir, Oxford Centre for Functional MRI of the Brain, University of Oxford, John Radcliffe
Hospital, Oxford, Oxfordshire, United Kingdom, OX3 9DU. uzay.emir@ndcn.ox.ac.uk. Telephone:

+44 (0) 1865 222769 Fax: +44 (0) 1865 222717

Word Count: 4266

Abbreviations

AFI – Actual flip angle imaging

Ala – Alanine

Asc – Ascorbate/Vitamin C

Asp – Aspartate

Cr – Creatine

CRLB – Cramér–Rao lower bound

CSDE – Chemical shift displacement error

CSF – Cerebrospinal fluid

DSU – Dynamic shim updating

FASTMAP – Fast, automatic shimming technique by mapping along projections

FWHM – Full width at half-maximum

HLSVD - Hankel-Lanczos singular value decomposition

GABA - γ -aminobutyric acid

Glc – Glucose

Glu – Glutamate

GM – Grey matter

GPC – Glycerophosphocholine

GRESHIM – Gradient echo shimming

GSH – Glutathione

Lac – Lactate

PRESS – Point resolved spectroscopy

M1 – Primary motor cortex

MP-RAGE – Magnetization prepared 180 degrees radio-frequency pulses and rapid gradient-echo sampling

MRS - Magnetic resonance spectroscopy

MRSI – Magnetic resonance spectroscopic imaging

myo-Ins – Myo-inositol

NAA – N-acetylaspartate

NAAG – N-acetylaspartylglutamate

OVS – Outer volume suppression

PCho – Phosphocholine

PE – Phosphoethanolamine

RF – Radiofrequency

RTE – Relative transmit efficiency

SAR – Specific absorption rate

semi-LASER – Semi-localized by adiabatic selective refocusing spectroscopy

SNR – Signal to noise ratio

STEAM – Stimulated echo acquisition mode

scyllo-Ins - *Scyllo*-inositol

Tau – Taurine

tCho – Total Choline

tCr – Total Creatine

UHF – Ultra high field

VAPOR – Variable power RF pulses with relaxation delays

VOI – Volume of Interest

WM – White matter

Abstract (max. 300 words)

The aim of this study was to acquire high-quality *in vivo* ^1H spectra concurrently from two voxels at ultra-high field (7 Tesla) without specialized hardware. To this end, an acquisition scheme was developed in which first order shims and flip angles are dynamically updated to acquire spectra from both of the brain's motor cortices in an alternating fashion. To validate this acquisition scheme, separate, static, single voxel acquisitions were also performed for comparison. Six subjects were examined using semi-LASER spectroscopy at 7 T. Barium titanate pads were used to increase the extent of the effective transmit field (B_1^+). Spectra were obtained from the hand area of both motor cortices for both acquisition schemes. LCModel was used to determine neurochemical profiles in order to examine variations between acquisition schemes and volumes of interest. The dynamic two-voxel acquisition protocol produced water linewidths (full width half maximum between 11.6 and 12.8 Hz) and signal-to-noise ratios similar to static single voxel measurements. The concentrations of 13 individual and 3 combined metabolites with Cramer-Rao Lower Bounds below 30% were reliably detected for both acquisition schemes and agreed well with previous spectroscopy and histology studies. The results show that high spectral quality from two voxels can be acquired concurrently without specialized hardware. This practical technique can be applied to many neuroscience applications.

Introduction

Significant benefits of ultra-high field (UHF, $7\text{ T} \leq$) magnetic resonance (MR) have been demonstrated in magnetic resonance spectroscopy (MRS) leading to considerable gains in signal-to-noise ratio (SNR) and spectral resolution (1). MRS at UHF enables the accurate quantification of numerous metabolites from small volumes-of-interest (VOIs). For example, concentrations of up to 18 neurochemicals can be quantified from selected regions in the human brain at 7 T (2) as SNR is

increased approximately two-fold at 7 T relative to 3 T in the occipital cortex (3). Despite this potential, employing spectroscopy in the human brain at 7 T involves multiple challenges. First, the required RF power increases quadratically with static field (B_0) strength (4). Together with increased inhomogeneity of the transmit magnetic fields (B_1^+) at UHF, this leads to the necessity for increased RF pulse durations with smaller bandwidths, introducing a large chemical shift displacement error (CSDE) in the spectra. This problem is further worsened as standard volume coils provide higher B_1^+ in the centre of the human head (e.g., putamen, substantia nigra and caudate) compared with peripheral voxels (e.g., frontal, occipital and motor cortices) (4). Secondly, inhomogeneities in the B_0 field created due to differences in magnetic susceptibility are exacerbated at UHF since the magnitude of the B_0 shift for any given difference in magnetic susceptibility is proportional to the static field (5). Although efficient minimization of the B_0 inhomogeneity at standard field strengths (≤ 3 T) is mitigated with 1st and 2nd order shims via field-map based shimming techniques, it becomes extremely difficult with standard hardware at UHF resulting in a significant decrease in chemical shift dispersion (6).

To acquire adequate MRS data despite the increased B_0 inhomogeneity at UHF, it is necessary to use an approach that allows a closer focus on the volume of interest (VOI) and an optimized shim set for this region. Two common methods to measure B_0 and optimize the local shim sets are FASTMAP and B_0 field map techniques (for example GRE-SHIM). The FASTMAP (7) method generates 1D profiles which are used to estimate the required shim currents to achieve a uniform B_0 field. GRE-SHIM acquires a B_0 field map using a gradient-echo images with 2 different echo times in order to calculate the required shim currents (8). Additionally, a number of innovative shimming methods have been developed: diamagnetic (9) and paramagnetic passive shims (10), dynamic shimming (11,12) and unconventional shim hardware (13). Unfortunately, most of these techniques require modification of the standard hardware (e.g. customized coils or gradients).

Problems resulting from limitations in available B_1^+ magnitude can be avoided by utilizing various innovative excitation and refocusing RF pulse designs or multiple transmit array coils (B_1^+ shimming) (14,15). However, employing these new RF pulse designs and/or coil schemes is not trivial due to the increased energy deposition of the RF and these techniques also necessitate the modification of standard hardware (e.g. customized coils or amplifiers). Recently, it has been suggested that placing dielectric pads between the subject's head and coil can focus and manipulate the B_1^+ to suit specific needs (16). Dielectric pads have the advantage of not requiring software or hardware modification, are simple to fabricate and recent studies have shown that dielectric pads do not produce higher local energy deposition (17).

Single voxel MRS is generally limited to a small volume of interest whereas magnetic resonance spectroscopic imaging (MRSI) acquires metabolic profiles over large regions of the brain. However, single voxel MRS has the advantage that spectra can be temporally binned, enabling functional MRS. With single voxel MRS it is straightforward to obtain a water reference and mitigation of B_1^+ and B_0 inhomogeneities is made much easier in the small VOIs. Furthermore, one can adapt single voxel localization sequences such as STEAM (22) or semi-LASER (23,24) to multiple regions by dynamic shim updating (DSU) for each region and by acquiring spectra in an interleaved fashion. This approach maintains the precision and relative simplicity of single voxel techniques but enables multiple regions to be studied concurrently.

Ernst *et al.* first demonstrated this technique for two voxels at 2 T using PRESS (25). They dynamically shimmed the first order gradients and tilted the volume selection gradients in order to avoid mutual saturation of the two voxels and greatly increase scan efficiency. Théberge *et al.* used STEAM at 4 T to acquire signal from 2 voxels in a single TR (2.6 s) by employing DSU of the first order shims and decreased the experimental time by approximately 50% (26). Koch *et al.* recently used semi-LASER at 4 T to acquire signal from 4 voxels in a single TR (2.5 s) by employing DSU for both the first and second order shims (27). They implemented a specialized B_0 compensation

and shim pre-emphasis (28) system to correct for temporal effects associated with eddy currents generated by changing second order shim currents (29). Their results showed a significant improvement in spectral linewidth compared with a static global shim resulting in high quality spectra in all 4 voxels. While there are a number of high quality MRSI studies at 7 T (26–28), there is currently no implementation of DSU for single voxel spectroscopy at 7 T without multiple transmit coils.

Here we present a practical acquisition strategy to acquire high quality spectra at 7 T from two VOIs by combining DSU of first order shims (in a manner as first outlined by Ernst et al. (25)) generated by regular gradient systems with dielectric pads. As dielectric pads may not produce a uniform B_1^+ between the VOIs, VOI specific flip angles may be required to maintain high quality spectra for each voxel. To test the feasibility of this technique at 7 T, spectra were acquired from voxels centered on the hand knob of the motor cortices (M1) bilaterally (29). In order to compare the effectiveness of this acquisition strategy, isolated static single voxel experiments were also performed from each M1 separately.

Experimental

Subjects

Six healthy volunteers [5 male; aged 25 ± 3 years; 2 left handed] participated in this study after giving informed consent under an institutionally agreed technical development protocol.

Dielectric Pad

A dielectric pad containing barium titanate (BaTiO_3) of approximately 5 mm thickness and $11 \times 11 \text{ cm}^2$ was prepared by heat-sealing within polypropylene. BaTiO_3 powder (99%, Sigma Aldrich, UK) was suspended in deuterium oxide (99%, Sigma Aldrich, UK) until the powder became saturated with a mass-to-mass ratio of 4:1. The pad was positioned on top of each subject's

head covering the scalp over both motor cortices for the duration of the experiment. A previous study has shown that placement of dielectric pads in this way increases B_1^+ efficiency while leading to no increase in peak local SAR (30).

MR Protocol

All measurements were performed on an 83-cm bore, 7 T Siemens MAGNETOM scanner (Siemens, Erlangen) equipped with a 60 cm (inner diameter) gradient coil (maximum amplitude 70 mT/m; maximum slew rate 200 mT/m/ms) and a Nova Medical 1 channel transmit, 32-channel receive array head-coil (Nova Medical, Wilmington, USA). The second-order shim coils in the gradient system had a maximum strength of 7.04 mT/m² at 20 A for ZX, ZY, XY, X^2+Y^2 and 11.0 mT/m² at 20 A for Z^2 . In order to verify that adequate and normalized transmit power had been achieved in the regions of interest with the dielectric pads, B_1 maps were acquired using the actual flip angle imaging (AFI) sequence (31) (field of view, 240×240 mm²; TR1/2 = 6/30 ms; TE = 2.58 ms; slice thickness 2.5 mm; non-selective flip angle 60°, scan time 3 mins). To aid in selecting the regions of interest, structural 3D T₁-weighted images were acquired with an MP-RAGE sequence (32) (field of view, 192×192 mm²; TR = 2.2 s; TE = 2.82 ms; slice thickness 1 mm; slices 96; non-selective inversion; scan time 3 mins) for all subjects.

Shimming

In order to compare the effectiveness of DSU at UHF, spectra were acquired from two voxels under two conditions: (i) DSU, and (ii) static single voxel spectroscopy. For the DSU acquisitions, B_0 shimming was performed in a two-step process. First, GRE-SHIM (8) (field of view, 384×384 mm²; TR = 600 ms; TE1/2 = 2.04/4.08 ms; slice thickness 4 mm; flip angle 15°; slices 64; scan time 45 s) was used to determine the optimal second order shim currents (Z^2 , ZX, ZY, XY, X^2+Y^2). The second order shim currents were optimized for the total volume encompassing the two voxels ($80 \times 20 \times 20$ mm³). The second step involved determining optimized

first order shims (X, Y, Z) for each voxel using FASTMAP with 6 projections for 1st order shims (7) – while keeping the second order shims static. Prior to acquisition, excitation, water suppression and OVS flip angles were calculated for each voxel. Specific parameters and shim values for both voxels were then entered into the system prior to the start of acquisition. The sequence was programed to alternate all the relevant parameters between each transient with a short delay (10 ms) included for synchronization of the sequence and shim updates. For the static single voxel acquisitions, B₀ shimming for all shim coils was performed using FASTMAP optimized for each individual voxel. This was performed over four sequential FASTMAP acquisitions – two to determine the 2nd order shims and 2 to determine the first order shims. Fig. 1 illustrates the difference between DSU and static acquisitions.

MR Spectroscopy

All spectra were localized using semi-LASER as described by Oz and Tkac (23) as this method has been shown to be robust against B₁-inhomogenities and has a low CSDE at 7 T (15). The duration of the asymmetric slice-selective excitation pulse was 4.2 ms with bandwidth 3.70 kHz and the duration of the adiabatic slice selective refocusing pulses was 4.5 ms with bandwidth 5.27 kHz (33). Crushing gradients were applied before and after the refocusing pulses as well as between each acquisition to suppress unwanted coherences. All spectra were acquired with VAPOR (34) water suppression (pulse duration = 35 ms) and 8 outer volume suppression pulses (pulse duration = 4.6 ms) as performed previously (33). 64 averages with an echo time of 30 ms and repetition time of 7 s were acquired for both DSU and static acquisition schemes. 7T has unique challenges for multi voxel MRS and, as a result, we have chosen to excite two voxels independently and in separate TR's. As a result, scan times of 15 minutes were required for both acquisition schemes, however our intention is to acquire interleaved spectra to enable both locations to be temporally resolved simultaneously. Signal was acquired from two voxels without angulation

located in the left and right motor cortex with a voxel size of $20 \times 20 \times 20 \text{ mm}^3$ and average distance of 40 mm apart.

Data Processing and Metabolite Quantification

The unsuppressed water signal acquired from the same VOI was used to remove residual eddy current effects and to reconstruct the phased array spectra. This included weighting the spectra based on the sensitivity of each receive element at the VOI and correcting for the different constant phase shift terms of the complex spectra prior to summation. Single scan spectra summed from 32 channels were corrected for frequency and phase variations induced by subject motion and then signal averaged. Finally any residual water signal was removed by using a Hankel-Lanczos singular value decomposition (HLSVD) (35) filter before LCModel (36) analysis.

Metabolites were quantified using LCModel. The model spectra of alanine (Ala), aspartate (Asp), ascorbate/vitamin C (Asc), glycerophosphocholine (GPC), phosphocholine (PCho), creatine (Cr), phosphocreatine (PCr), GABA, glucose (Glc), glutamine (Gln), Glu, glutathione (GSH), *myo*-inositol (*myo*-Ins), Lac, N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), scyllo-inositol (scyllo-Ins) and taurine (Tau) were generated based on previously reported chemical shifts and coupling constants (37,38) by VeSPA Project (Versatile Simulation, Pulses and Analysis) (39). *In-vivo* acquired macromolecule spectra from the occipital cortex were included in the model. LCModel analysis was performed on all spectra within the chemical shift range 0.5 to 4.2 ppm. Metabolite concentrations were obtained relative to an unsuppressed water spectrum acquired from the same VOI assuming a water content of 71% (40). Only metabolites quantified with Cramér–Rao lower bound (CRLB) $\leq 30\%$ in at least four of six subjects were included in the final neurochemical profile. In the case of a high correlation between two metabolites ($r < -0.5$) being measured, the sum was used instead e.g. total creatine (tCr), total choline (tCho), Glu+Gln and Glc+Tau. FMRIB's Automated Segmentation Tool (FAST) (41) was used to correct the structural images for B_1 inhomogeneity and calculate the relative quantities of

grey matter, white matter and cerebrospinal fluid (CSF) within the voxels before correcting metabolite concentrations by the fraction of the voxel containing CSF. Finally corrections were made for grey matter (GM) / white matter (WM) differences between subjects by employing the method outlined by Dou et al. (42). The corrected metabolite concentrations were obtained from a linear regression between uncorrected metabolite concentrations and corresponding GM percentages across both VOIs and subjects.

Paired two-tailed *t*-tests were performed to examine differences in linewidths between VOIs and acquisition techniques. To assess the agreement between dynamic and static acquisition schemes, a Bland Altman analysis (43) was performed on each reported metabolite. For all subjects, in each VOI, for each metabolite, the difference (in $\mu\text{mol/g}$) between acquisition techniques is calculated. If a normal distribution is assumed, 95% of the differences will fall between ± 1.96 standard deviations of the mean difference (referred to as the confidence interval). As CRLBs express a theoretical low limit on the standard deviation of a metabolite, we compared the static CRLBs with the standard deviation of the Bland-Altman differences between acquisition techniques in order to assess if the DSU scheme can be used interchangeably with static acquisitions.

Results

B₁ Optimization

In order to confirm the efficacy of the dielectric pad at increasing local B₁⁺ sensitivity in the VOIs, B₁ maps were acquired with and without the pad present (Fig. 2). The B₁⁺ maps established that the dielectric pad increased the B₁⁺ in the VOIs for all subjects. The relative transmit efficiency (RTE) or ratio of the B₁⁺ maps with and without dielectric pads averaged over each voxel – was 1.2 ± 0.1 for the left VOI and 1.3 ± 0.1 for the right VOI. This resulted in transmit reference voltages in the left and right motor cortices of 213 ± 42 V and 216 ± 23 V respectively.

Spectral Quality

Fig. 3 shows the average spectra from both methods in all six subjects, it shows that spectra with high SNR and resolution were obtained from the two VOIs with both DSU and static measurements. Water suppression was effective and virtually identical for both techniques leading to a residual water signal smaller than the major metabolite peaks for both regions. In addition, the double localization accomplished by semi-LASER and OVS eliminated signals from outside the voxels resulting in artifact-free spectra with a flat baseline. Table 1 shows linewidths and SNR achieved with the two methods. It shows a marginal increase in linewidths (0.8 Hz) and drop in SNR compared to a static shim. These differences were not significant (paired t-test, 2-tailed, p-value > 0.1). The measured linewidths were similar to those reported previously (30).

Neurochemical Profiles

The high spectral quality enabled the quantification of a neurochemical profile consisting of 13 individual and 3 combined metabolites. The mean concentrations of metabolites that met our criteria for reliable quantification are shown in Fig. 4. Both the sum and individual values are reported for NAA and NAAG because these metabolites could be reliably distinguished in both regions with correlation coefficients between -0.43 and -0.48 (Fig. 5). A table summarizing the Bland-Altman analysis of the agreement between dynamic and static acquisition methods is found in Table 2. The differences between acquisition schemes for all metabolites (except Lac) resulted in variations similar to the static CRLBs suggesting a good agreement.

Discussion

This study demonstrates that high-quality semi-LASER spectra can be obtained from two VOIs in an interleaved mode within a single session at 7 T using a single channel transmit, 32 channel receive coil, and dielectric pads. We have shown that, by dynamically updating first order shims in multiple regions, excellent spectra from bilateral motor cortex VOIs are achievable at 7 T. In addition, neurochemical profiles from these spectra contained 13 individual and 3 combined

metabolites and were consistent with the biochemical literature (see below). Notably, this study was accomplished without B_1^+ shimming and/or second order or higher B_0 shim manipulations thereby allowing facilities not equipped with multiple channel transmit or other specialized hardware to acquire high-quality spectra in multiple brain regions concurrently. As less than a dozen of the currently more than 60 7 T research centers (44) have access to B_1^+ shimming in the human brain and/or pre-emphasis of higher order shim coils, a more pragmatic approach may be desirable for most centers.

It has been shown that the electromagnetic wave behavior of B_1 for volume coils at 7 T inside a human head is non-uniform and that the bright center region results in B_1^+ degradation in the motor cortex (4). Therefore, in order to achieve sufficient B_1 (23 μ T) for MRS localization in the bilateral hand areas of the motor cortex, the B_1^+ field was optimized by proper placement of dielectric pads. As illustrated in Fig. 2, barium titanate dielectric pads result in slightly increased B_1^+ inhomogeneity within the voxels. While this is not desirable, increasing B_1^+ efficiency outweighs the increased inhomogeneity (45). The dielectric pad provided satisfactory B_1^+ for spectroscopy at 7 T in the motor cortex. The RTE achieved is similar to previous MRS studies in the hand area of the motor cortex at 7 T: transceiver array coils driven by multichannel transmit system (local B_1^+ shimming) achieved an RTE of 1.31 ± 0.1 (46) and an ROI and coil setup very similar to ours determined a peak RTE of 1.5 (30). As dielectric pads result in a B_1^+ ‘hot spot’ and decreased efficiency away from the pad, acquisition from voxels that are further apart than performed in this study may not be feasible (*i.e.* one voxel near the front and one near the back).

For this study, the semi-LASER sequence was chosen over STEAM or PRESS as the adiabatic refocusing pulses provide high B_1^+ insensitivity, a desirable slice selection profile and a minimal chemical shift displacement error (CSDE) (23,24). Previous multi-voxel MRS studies borrowed elements of multislice imaging where multiple voxels are excited in a single TR (25,26). Adiabatic inversion pulses of semi-LASER are SAR intensive and, as a result, only a single voxel

can be excited every 3.5 s. Consequently, the technique presented here provides temporal interleaving rather than a decrease in total acquisition duration. Therefore, this technique supports functional studies that require temporal resolution on the order of minutes and spectra from more than one brain region and for 7 T research centers that do not have access to specialized shim equipment, this technique presents a practical solution. Using a long TR resulted in a sufficient recovery time to avoid mutual saturation of voxels. Previously reported values for water T_1 in GM, WM and CSF at 7 T suggest an average T_1 in our VOIs of 1.63 s, therefore we would expect approximately 99% of our magnetization to return to equilibrium by the end of each TR (47,48).

To benefit from chemical shift dispersion at UHF, efficient B_0 shimming is very important. In the authors experience it was found that imaging based shimming methods (GRE-SHIM) are well suited to large volumes of interest, and FASTMAP to small VOIs and while this has never been systemically investigated, it has been shown that using FASTMAP results in a water resonance shift and increased line broadening towards the periphery of the shimming (49). However, our protocol of GRE-SHIM requires all shim currents to be determined together whereas FASTMAP allows optimization of first or second order shims separately. Therefore, in this study, a hybrid GRE-SHIM/FASTMAP shimming approach was used for DSU.

The spectral quality achieved (high SNR, excellent localization performance, efficient water suppression and a distortion-free baseline) allowed reliable quantification of 13 individual and 3 combined metabolites with LCModel analysis. The CRLBs for the dominant metabolites (e.g. NAA, tCr, tCho, mIns, Glu) were below 5% and were comparable with those obtained previously at 7 T in the motor cortex (46). The CRLBs for all other metabolites were below 30% (supplementary Table 1). The Bland-Altman analysis revealed an excellent agreement in the neurochemical profiles between DSU and static acquisition schemes. For Lac, the large agreement error may be explained by the presence of overlapping lipid peaks that result in large differences in LCModel outputs. While there are no significant hemispheric or acquisition differences for individual metabolites,

there are a few trends. The static technique reports lower NAA+NAAG and Cr+PCr for both VOIs – though the difference is relatively small. These tendencies may be caused by subtle differences in linewidths between VOIs or acquisition schemes – which could give rise to an unpredictable bias in LCModel outputs (50). Line broadening of up to 4 Hz was performed on the raw spectra prior to LCModel to check if linewidth differences were an issue but we found that line broadening had virtually no effect on the resulting neurochemical profile (results not shown).

In order to obtain accurate absolute quantification of metabolites partial volume effects must be included – otherwise artificial deviations in metabolite concentrations may be overlooked. The most commonly employed correction method scales uncorrected metabolite values by the CSF percentage in the VOI - as no relevant metabolites (apart from Gln) exist in the CSF (51). This method does not include differences in the relative amounts of GM to WM and, used on its own, can lead to less accurate quantification of certain metabolites (52,53). Regrettably there is currently no consensus on the best method to correct for relative amounts GM or WM and there are a variety of techniques employed (54,55). We chose the use the method of Dou et al. (42) as the only assumption made is that metabolite concentrations are linearly dependent on tissue composition.

Our neurochemical profile findings from the human primary motor cortices were in agreement with previous postmortem studies of individuals, whereas previous *in-vivo* MRS studies differ slightly from those reported here. For instance, the absolute NAA (9.3-9.7 $\mu\text{mol/g}$), NAAG (2.0-2.3 $\mu\text{mol/g}$), Glu (6.0-6.4 $\mu\text{mol/g}$) and Asp (1.1-1.6 $\mu\text{mol/g}$) concentrations quantified from the 8 ml VOIs were similar to those measured from postmortem human brain (NAA 7.4-9.4 $\mu\text{mol/g}$, NAAG 1.4-1.6 $\mu\text{mol/g}$, Glu 6.0-7.0 $\mu\text{mol/g}$, Asp 1.3-1.6 $\mu\text{mol/g}$) (56). However, the GABA (1.2-1.5 $\mu\text{mol/g}$) and the Gln (2.6-2.7 $\mu\text{mol/g}$) concentrations reported here are lower than from postmortem human brains (GABA 1.97 $\mu\text{mol/g}$ (57), Gln 4.5-4.9 $\mu\text{mol/g}$) though this discrepancy is almost certainly due to an increase of GABA (58) and Gln (59) in the postmortem human brain. The slight difference observed between the concentrations reported in this study and those reported

in previous MRS studies is partly due to the fact that the concentrations we report were not corrected for T_2 values of individual metabolites. Additionally, differences in relative amounts of GM to WM also contributed to this difference since the VOIs used in this study contain more GM (0.30 compared with 0.19) and less WM (0.63 compared with 0.73) (46).

This study does not include DSU of the higher order shims as the rapid altering of the unshielded and uncompensated higher order shims generates eddy currents that result in strong field variations that can take 3-10 seconds to disappear (60,61). As the majority of the 7-second TR is spent waiting (due to SAR limitations), it may be possible to include DSU of the higher order shims without requiring pre-emphasis. This would improve linewidths and may be required for areas of the brain that are more difficult to shim (*i.e.* near the nasal cavity) but would require investigating the eddy current decay time on a system specific basis.

This technique lends itself to functional spectroscopy applications requiring high spectral resolution in multiple brain regions. Acquiring data from more than one region in an interleaved fashion is vital in allowing the study of any intervention that modulates the brain with a timescale of minutes to hours and could reasonably be expected to involve more than one brain region. Three immediate applications might be as follows: [1] To study the neurochemistry of pharmacological interventions, where the temporal dynamics of induced changes in neurotransmitters might well be expected to be different across the brain, and this information would be very important in understanding the brain's response (62). [2] To examine the response to short-term stimuli, such as a tonic pain stimulus, where multiple brain regions would be expected to respond, but in different directions and with different temporal dynamics. Understanding these responses, and therefore how the brain regions might interact, would be very important in understanding the mechanisms underlying the cortical pain response, for example (63). [3] To study neurotransmitter changes during plasticity-induction, for example learning or brain stimulation. In the context of the motor

system, for example, it is well known that plasticity does not only occur in the directly involved network node (usually primary motor cortex (M1)), but rather occurs between and within the directly involved network node and the rest of the functional network. Understanding the time courses of changes occurring at these remote sites, and how they interact with the M1 is of vital importance in understanding plasticity and learning in humans (64,65).

In conclusion, the present study provides an effective method for acquiring high-quality spectra at UHF in multiple brain regions concurrently within a single session – without additional specialized hardware. The outlined acquisition scheme acquired neurochemical profiles from both motor cortices with accurate quantification of 13 individual and 3 combined metabolites.

Acknowledgements

The authors thank Wouter Teeuwisse and Andrew Webb for providing the dielectric pads.

References

1. Uğurbil K, Adriany G, Andersen P, Chen W, Garwood M, Gruetter R, Henry P-G, Kim S-G, Lieu H, Tkac I, Vaughan T, Van De Moortele P-F, Yacoub E, Zhu X-H. Ultrahigh field magnetic resonance imaging and spectroscopy. *Magn. Reson. Imaging* 2003; 21(10): 1263–1281.
2. Emir UE, Auerbach EJ, Moortele P-FVD, Marjańska M, Uğurbil K, Terpstra M, Tkáč I, Öz G. Regional neurochemical profiles in the human brain measured by ¹H MRS at 7 T using local B1 shimming. *NMR Biomed.* 2012; 25(1): 152–160.
3. Mekanle R, Mlynárik V, Gambarota G, Hergt M, Krueger G, Gruetter R. MR spectroscopy of the human brain with enhanced signal intensity at ultrashort echo times on a clinical platform at 3T and 7T. *Magn. Reson. Med.* 2009; 61(6): 1279–1285.
4. Vaughan JT, Garwood M, Collins CM, Liu W, DelaBarre L, Adriany G, Andersen P, Mekanle H, Goebel R, Smith MB, Ugurbil K. 7T vs. 4T: RF power, homogeneity, and signal-to-noise comparison in head images. *Magn. Reson. Med.* 2001; 46(1): 24–30.

5. Hennig J, Speck O. High-Field MR Imaging. Springer: Berlin, Germany; 2011. 261 p.
6. Koch KM, Rothman DL, de Graaf RA. Optimization of static magnetic field homogeneity in the human and animal brain in vivo. *Prog. Nucl. Magn. Reson. Spectrosc.* 2009; 54(2): 69–96.
7. Gruetter R, Tkáč I. Field mapping without reference scan using asymmetric echo-planar techniques. *Magn. Reson. Med.* 2000; 43(2): 319–323.
8. Shah S, Kellman P, Greiser A, Weale P, Zuehlsdorff S, Jerecic R. Rapid Fieldmap Estimation for Cardiac Shimming. *Proceedings of the 17th Annual Meeting ISMRM Honolulu, Hawaii, USA; 2009; 565.*
9. Wilson JL, Jenkinson M, Jezzard P. Optimization of static field homogeneity in human brain using diamagnetic passive shims. *Magn. Reson. Med.* 2002; 48(5): 906–914.
10. Juchem C, Muller-Bierl B, Schick F, Logothetis NK, Pfeuffer J. Combined passive and active shimming for in vivo MR spectroscopy at high magnetic fields. *J. Magn. Reson.* 2006; 183(2): 278–289.
11. Morrell G, Spielman D. Dynamic shimming for multi-slice magnetic resonance imaging. *Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med.* 1997; 38(3): 477–483.
12. Blamire AM, Rothman DL, Nixon T. Dynamic shim updating: a new approach towards optimized whole brain shimming. *Magn. Reson. Med.* 1996; 36(1): 159–165.
13. Juchem C, Nixon TW, McIntyre S, Rothman DL, de Graaf RA. Magnetic field homogenization of the human prefrontal cortex with a set of localized electrical coils. *Magn. Reson. Med.* 2010; 63(1): 171–180.
14. Van de Moortele P-F, Akgun C, Adriany G, Moeller S, Ritter J, Collins CM, Smith MB, Vaughan JT, Ugurbil K. B1 destructive interferences and spatial phase patterns at 7 T with a head transceiver array coil. *Magn. Reson. Med.* 2005; 54(6): 1503–1518.
15. Boer VO, van Lier ALHMW, Hoogduin JM, Wijnen JP, Luijten PR, Klomp DWJ. 7-T (1) H MRS with adiabatic refocusing at short TE using radiofrequency focusing with a dual-channel volume transmit coil. *NMR Biomed.* 2011; 24(9): 1038–1046.
16. Teeuwisse WM, Brink WM, Haines KN, Webb AG. Simulations of high permittivity materials for 7 T neuroimaging and evaluation of a new barium titanate-based dielectric. *Magn. Reson. Med.* 2012; 67(4): 912–918.

17. Snaar JEM, Teeuwisse WM, Versluis MJ, van Buchem MA, Kan HE, Smith NB, Webb AG. Improvements in high-field localized MRS of the medial temporal lobe in humans using new deformable high-dielectric materials. *NMR Biomed.* 2011; 24(7): 873–879.
18. Frahm J, Merboldt K-D, Hänicke W. Localized proton spectroscopy using stimulated echoes. *J. Magn. Reson.* 1969 1987; 72(3): 502–508.
19. Öz G, Tkáč I. Short-echo, single-shot, full-intensity proton magnetic resonance spectroscopy for neurochemical profiling at 4 T: Validation in the cerebellum and brainstem. *Magn. Reson. Med.* 2011; 65(4): 901–910.
20. Scheenen TWJ, Klomp DWJ, Wijnen JP, Heerschap A. Short echo time 1H-MRSI of the human brain at 3T with minimal chemical shift displacement errors using adiabatic refocusing pulses. *Magn. Reson. Med.* 2008; 59(1): 1–6.
21. Ernst T, Hennig J. Double-volume 1H spectroscopy with interleaved acquisitions using tilted gradients. *Magn. Reson. Med.* 1991; 20(1): 27–35.
22. Théberge J, Menon RS, Williamson PC, Drost DJ. Implementation issues of multivoxel STEAM-localized 1H spectroscopy. *Magn. Reson. Med.* 2005; 53(3): 713–718.
23. Koch KM, Sacolick LI, Nixon TW, McIntyre S, Rothman DL, de Graaf RA. Dynamically shimmed multivoxel 1H magnetic resonance spectroscopy and multislice magnetic resonance spectroscopic imaging of the human brain. *Magn. Reson. Med.* 2007; 57(3): 587–591.
24. Koch KM, McIntyre S, Nixon TW, Rothman DL, de Graaf RA. Dynamic shim updating on the human brain. *J. Magn. Reson.* 2006; 180(2): 286–296.
25. De Graaf RA, Brown PB, McIntyre S, Rothman DL, Nixon TW. Dynamic shim updating (DSU) for multislice signal acquisition. *Magn. Reson. Med.* 2003; 49(3): 409–416.
26. Henning A, Fuchs A, Murdoch JB, Boesiger P. Slice-selective FID acquisition, localized by outer volume suppression (FIDLOVS) for (1)H-MRSI of the human brain at 7 T with minimal signal loss. *NMR Biomed.* 2009; 22(7): 683–696.
27. Avdievich NI, Pan JW, Baehring JM, Spencer DD, Hetherington HP. Short echo spectroscopic imaging of the human brain at 7T using transceiver arrays. *Magn. Reson. Med.* 2009; 62(1): 17–25.

28. Boer VO, Klomp DWJ, Juchem C, Luijten PR, de Graaf RA. Multislice ^1H MRSI of the human brain at 7 T using dynamic B_0 and B_1 shimming. *Magn. Reson. Med.* 2012; 68(3): 662–670.
29. Yousry TA, Schmid UD, Alkadhi H, Schmidt D, Peraud A, Buettner A, Winkler P. Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. *Brain J. Neurol.* 1997; 120 (Pt 1)141–157.
30. Schaller B, Xin L, O'Brien K, Magill AW, Gruetter R. Are glutamate and lactate increases ubiquitous to physiological activation? A ^1H functional MR spectroscopy study during motor activation in human brain at 7 Tesla. *NeuroImage* 2014; 93, Part 1138–145.
31. Yarnykh VL. Actual flip-angle imaging in the pulsed steady state: a method for rapid three-dimensional mapping of the transmitted radiofrequency field. *Magn. Reson. Med.* 2007; 57(1): 192–200.
32. Brant-Zawadzki M, Gillan GD, Nitz WR. MP RAGE: a three-dimensional, T1-weighted, gradient-echo sequence--initial experience in the brain. *Radiology* 1992; 182(3): 769–775.
33. Van de Bank BL, Emir UE, Boer VO, van Asten JJA, Maas MC, Wijnen JP, Kan HE, Oz G, Klomp DWJ, Scheenen TWJ. Multi-center reproducibility of neurochemical profiles in the human brain at 7 T. *NMR Biomed.* 2015; n/a – n/a.
34. Tkác I, Starcuk Z, Choi IY, Gruetter R. In vivo ^1H NMR spectroscopy of rat brain at 1 ms echo time. *Magn. Reson. Med.* 1999; 41(4): 649–656.
35. Cabanes E, Confort-Gouny S, Le Fur Y, Simond G, Cozzzone PJ. Optimization of residual water signal removal by HLSVD on simulated short echo time proton MR spectra of the human brain. *J. Magn. Reson.* 2001; 150(2): 116–125.
36. Provencher SW. Automatic quantitation of localized in vivo ^1H spectra with LCModel. *NMR Biomed.* 2001; 14(4): 260–264.
37. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed.* 2000; 13(3): 129–153.
38. Tkác I. Refinement of simulated basis set for LCModel analysis. *Proceedings of the 16th Annual Meeting ISMRM Toronto, Canada; 2008; 1624.*
39. Soher BJ, Semanchuk P, Todd D, Steinberg J, Young K. Vespa: Integrated applications for RF pulse design, spectral simulation and MRS data analysis. *Proceedings of the 19th Annual Conference ISMRM Quebec, Canada; 2011; 1410.*

40. Whittall KP, MacKay AL, Graeb DA, Nugent RA, Li DK, Paty DW. In vivo measurement of T2 distributions and water contents in normal human brain. *Magn. Reson. Med.* 1997; 37(1): 34–43.
41. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage* 2002; 17(2): 825–841.
42. Dou W, Palomero-Gallagher N, Tol M-J van, Kaufmann J, Zhong K, Bernstein H-G, Heinze H-J, Speck O, Walter M. Systematic Regional Variations of GABA, Glutamine, and Glutamate Concentrations Follow Receptor Fingerprints of Human Cingulate Cortex. *J. Neurosci.* 2013; 33(31): 12698–12704.
43. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat. Methods Med. Res.* 1999; 8(2): 135–160.
44. Plantinga BR, Temel Y, Roebroek A, Uludağ K, Ivanov D, Kuijf ML, ter Haar Romenij BM. Ultra-high field magnetic resonance imaging of the basal ganglia and related structures. *Front. Hum. Neurosci.* 2014; 8876.
45. Emir UE, Auerbach EJ, Moortele P-FVD, Marjańska M, Uğurbil K, Terpstra M, Tkáč I, Öz G. Regional neurochemical profiles in the human brain measured by ¹H MRS at 7 T using local B1 shimming. *NMR Biomed.* 2012; 25(1): 152–160.
46. Marjańska M, Auerbach EJ, Valabrègue R, Van de Moortele P-F, Adriany G, Garwood M. Localized ¹H NMR spectroscopy in different regions of human brain in vivo at 7 T: T2 relaxation times and concentrations of cerebral metabolites. *NMR Biomed.* 2012; 25(2): 332–339.
47. Rooney WD, Johnson G, Li X, Cohen ER, Kim S-G, Ugurbil K, Springer CS Jr. Magnetic field and tissue dependencies of human brain longitudinal ¹H₂O relaxation in vivo. *Magn. Reson. Med.* 2007; 57(2): 308–318.
48. Xin L, Schaller B, Mlynarik V, Lu H, Gruetter R. Proton T1 relaxation times of metabolites in human occipital white and gray matter at 7 T. *Magn. Reson. Med.* 2013; 69(4): 931–936.
49. Shen J, Rothman DL, Hetherington HP, Pan JW. Linear projection method for automatic slice shimming. *Magn. Reson. Med.* 1999; 42(6): 1082–1088.
50. Near J, Andersson J, Maron E, Mekle R, Gruetter R, Cowen P, Jezard P. Unedited in vivo detection and quantification of γ -aminobutyric acid in the occipital cortex using short-TE MRS at 3 T. *NMR Biomed.* 2013; 26(11): 1353–1362.

51. Lynch J, Peeling J, Auty A, Sutherland GR. Nuclear magnetic resonance study of cerebrospinal fluid from patients with multiple sclerosis. *Can. J. Neurol. Sci.* 1993; 20(3): 194–198.
52. McLean MA, Woermann FG, Barker GJ, Duncan JS. Quantitative analysis of short echo time 1H-MRSI of cerebral gray and white matter. *Magn. Reson. Med.* 2000; 44(3): 401–411.
53. Hetherington HP, Mason GF, Pan JW, Ponder SL, Vaughan JT, Twieg DB, Pohost GM. Evaluation of cerebral gray and white matter metabolite differences by spectroscopic imaging at 4.1T. *Magn. Reson. Med.* 1994; 32(5): 565–571.
54. Schuff N, Ezekiel F, Gamst AC, Amend DL, Capizzano AA, Maudsley AA, Weiner MW. Region and Tissue Differences of Metabolites in Normally Aged Brain Using Multislice 1H Magnetic Resonance Spectroscopic Imaging. *Magn. Reson. Med.* 2001; 45(5): 899–907.
55. Gasparovic C, Yeo R, Mannell M, Ling J, Elgie R, Phillips J, Doezenia D, Mayer AR. Neurometabolite Concentrations in Gray and White Matter in Mild Traumatic Brain Injury: An 1H-Magnetic Resonance Spectroscopy Study. *J. Neurotrauma* 2009; 26(10): 1635–1643.
56. Tsai G, Stauch-Slusher B, Sim L, Hedreen JC, Rothstein JD, Kuncl R, Coyle JT. Reductions in acidic amino acids and N-acetylaspartylglutamate in amyotrophic lateral sclerosis CNS. *Brain Res.* 1991; 556(1): 151–156.
57. Banay-Schwartz M, Palkovits M, Lajtha A. Heterogeneous distribution of functionally important amino acids in brain areas of adult and aging humans. *Neurochem. Res.* 1993; 18(4): 417–423.
58. Van der Heyden J a. M, Korf J. Regional Levels of Gaba in the Brain: Rapid Semiautomated Assay and Prevention of Postmortem Increase by 3-Mercapto-Propionic Acid. *J. Neurochem.* 1978; 31(1): 197–203.
59. Finkbeiner WE, Ursell PC, (M.D.) RLD. *Autopsy Pathology: A Manual and Atlas.* Saunders Elsevier: Philadelphia, PA; 2009. 377 p.
60. Sengupta S, Welch EB, Zhao Y, Foxall D, Starewicz P, Anderson AW, Gore JC, Avison MJ. Dynamic B0 shimming at 7 Tesla. *Magn. Reson. Imaging* 2011; 29(4): 483–496.
61. Juchem C, Nixon TW, Diduch P, Rothman DL, Starewicz P, de Graaf RA. Dynamic Shimming of the Human Brain at 7 Tesla. *Concepts Magn. Reson. Part B Magn. Reson. Eng.* 2010; 37B(3): 116–128.

62. Poels EMP, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR. Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. *Mol. Psychiatry* 2014; 19(1): 20–29.
63. Lee MC, Tracey I. Imaging pain: a potent means for investigating pain mechanisms in patients. *Br. J. Anaesth.* 2013; 111(1): 64–72.
64. Bachtar V, Stagg CJ. The role of inhibition in human motor cortical plasticity. *Neuroscience* 2014; 27893–104.
65. Stagg CJ. Magnetic Resonance Spectroscopy as a tool to study the role of GABA in motor-cortical plasticity. *NeuroImage* 2014; 8619–27.

Figure Legends

Figure 1 – Acquisition scheme for ^1H spectroscopy. Upper: Static single-voxel sequence. Lower: Interleaved DSU scheme. $S_{\text{left}} S_{\text{right}}$ – signal localization and acquisition for left and right VOIs, n – number of acquisition averages.

Figure 2 – Acquired B_1^+ field maps without (left) and with (center) the barium titanate dielectric pad placed on subject's head. Ratio of images (right) shows a B_1^+ increase in both VOIs.

Figure 3 – Average *in vivo* ^1H semi-LASER spectra ($\text{TR} = 7 \text{ s}$; $\text{TE} = 30 \text{ ms}$; Averages = 64) from all six subjects from the two VOIs using both acquisition schemes at 7 T. The shaded grey area is the standard deviation. Note the high spectral quality and reproducibility in all conditions.

Figure 4 - Neurochemical profile (above) and corresponding CRLBs (below) from both VOIs and acquisition schemes determined by LCModel fitting of semi-LASER spectra acquired at 7 T. Note that no significant difference ($p\text{-value} \geq 0.5$) between conditions was determined for any metabolite concentrations. Error bars are intersubject standard deviation.

Figure 5 - Mean correlation coefficient matrices between the metabolite concentrations for both VOIs and techniques determined by LCModel. Nonzero off-diagonal elements of the correlation coefficient matrices indicate the level of covariance between the fitting results for the corresponding two metabolites.

Table 1 - Water signal linewidths, methyl resonance tCr linewidths, SNR_{NAA} , and tissue content for both VOIs using both acquisition methods. Values reported are mean \pm standard deviation of all

spectra and linewidths refer to FWHM. SNR_{NAA} is calculated using the maximum height of the signal of NAA methyl resonance. Note that no significant difference (p -value ≥ 0.5) between both VOIs and acquisition methods was determined for linewidths, SNR or tissue content.

Table 2 – The 95% confidence interval of agreement between static and DSU acquisition schemes ($\mu\text{mol/g}$) and variation (standard deviation of differences divided by mean concentration) measured using the Bland-Altman method for both left and right VOIs. The CRLBs for the static condition are also included to assess the agreement strength.