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Fast Volumetric Spatial-Spectral MR Imaging of Hyperpolarized ^{13}C -Labeled Compounds using Multiple Echo 3D bSSFP

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Abstract

PURPOSE—The goal of this work was to develop a fast 3D chemical shift imaging technique for the non-invasive measurement of hyperpolarized ^{13}C -labeled substrates and metabolic products at low concentration.

MATERIALS AND METHODS—Multiple echo 3D balanced steady state MR imaging (ME-3DbSSFP) was performed in vitro on a syringe containing hyperpolarized [1,3,3- $^2\text{H}_3$; 1- ^{13}C]2-hydroxyethylpropionate (HEP) adjacent to a ^{13}C -enriched acetate phantom, and in vivo on a rat before and after IV injection of hyperpolarized HEP at 1.5 T. Chemical shift images of the hyperpolarized HEP were derived from the multiple echo data by Fourier transformation along the echoes on a voxel by voxel basis for each slice of the 3D data set.

RESULTS—ME-3DbSSFP imaging was able to provide chemical shift images of hyperpolarized HEP in vivo, and in a rat with isotropic 7 mm spatial resolution, 93 Hz spectral resolution and 16 second temporal resolution for a period greater than 45 seconds.

CONCLUSION—Multiple echo 3D bSSFP imaging can provide chemical shift images of hyperpolarized ^{13}C -labeled compounds in vivo with relatively high spatial resolution and moderate spectral resolution. The increased signal-to-noise ratio (SNR) of this 3D technique will enable the detection of hyperpolarized ^{13}C -labeled metabolites at lower concentrations as compared to a 2D technique.

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Keywords

hyperpolarized ^{13}C ; chemical shift imaging; balanced steady state free precession imaging; spectroscopic imaging

INTRODUCTION

An important goal of molecular imaging is the development of non-invasive techniques for measuring in vivo metabolism. Magnetic resonance spectroscopy (MRS) has demonstrated the ability to monitor aerobic and anaerobic metabolism using isotopically enriched ^{13}C -glucose and pyruvate substrates in brain [1,2], heart [3], and animal tumor models [4–7]. The non-radioactive ^{13}C -labeled substrates are chemically equivalent to naturally occurring ^{12}C -substrates, and therefore undergo the same metabolic reactions. This powerful tool for non-invasively monitoring metabolism in vivo has not been widely applied to the characterization of human cancers in vivo due to the low MR sensitivity necessitating long imaging times. Administration of a large dose of a ^{13}C -enriched substrate to increase the MR sensitivity would disrupt the endogenous metabolism defeating the purpose of using the ^{13}C -enriched substrate to probe the metabolic state of the system.

The low MR sensitivity of ^{13}C -enriched substrate metabolite imaging and spectroscopy is currently being addressed by the development of hyperpolarization methods to significantly increase the MR signal obtained from ^{13}C -labeled substrates. PASADENA (parahydrogen and synthesis allows dramatically enhanced nuclear alignment)[8–11], and DNP (dynamic nuclear polarization)[12–14] methods are able to increase the MR signal of ^{13}C -labeled compounds by factors of 20,000 or more. This increase in ^{13}C polarization directly translates into increased MR signal of both substrates and metabolites since the ^{13}C nuclei retain their hyperpolarization while undergoing chemical reactions. The increased signal of hyperpolarized ^{13}C -labeled metabolic substrates has begun to allow non-invasive MR detection of the original ^{13}C -labeled substrate and metabolic products at millimolar levels using fast chemical shift imaging [15,16].

The main limitation of MR imaging and spectroscopy of hyperpolarized ^{13}C -labeled molecules is useful lifetime of the hyperpolarized spins as the spin system reverts from the hyperpolarized state back to Boltzmann polarization with characteristic relaxation time constant T_1 . The T_1 relaxation times for this decay are on the order of 20 to 60 seconds for most biologically relevant ^{13}C -substrates, which limits the detection time to the order of several T_1 times ($\approx 4T_1$ or 80 to 240 seconds). Furthermore, the T_1 relaxation time of the hyperpolarized metabolic product may be different from that of the hyperpolarized substrate due to a different local chemical environment. Therefore, the key requirements for metabolic imaging of hyperpolarized substrates are fast delivery and uptake by the target tissue (tumor), rapid conversion of the substrate to its metabolites, and MR imaging times which are short relative to both the T_1 of the substrate and the conversion rate of substrate to product. These requirements are the driving force behind the development of fast MR pulse sequences which are able to provide both spectral (chemical shift) and spatial (image) information.

The chemical-shift induced amplitude modulation of the MR signal for gradient echo imaging was first reported by Wehrli et al. [17]. They noted that this modulation could be used to generate fat and water images by performing the Fourier transform along the echoes in a multiple gradient echo image acquisition. Recently Wieben et al. [18] proposed a fast method for acquiring both the spatial and spectral information in one scan using a multiple-echo 2D balanced steady state free precession (bSSFP) imaging technique. The Nyquist

frequency (F_N) for this technique is determined by the echo spacing, Δt , where $F_N = 1/(2\Delta t)$, and the spectral resolution (Δf) is determined by $\Delta f = 1/(N_E \Delta t)$, where N_E is the number of acquired echoes. The multiple-echo 2D bSSFP technique is fast, has relatively high spatial resolution, and is able to adjust the number of echoes (N_E) and the echo spacing (Δt) to provide the desired spectral resolution.

Previous multiple gradient echo chemical shift bSSFP imaging was performed in a single slice acquisition mode [17,18,21]. We have implemented three-dimensional multiple gradient echo balanced steady state free precession (ME-3DbSSFP) imaging in order to observe hyperpolarized ^{13}C -labeled substrates and products at lower concentrations due to the increased signal-to-noise ratio (SNR) of the 3D acquisition, and to provide multiple contiguous slice chemical shift imaging in order to measure the MR signals of the substrates and products at more than one slice in the subject. The ME-3DbSSFP technique presented here is conceptually similar to echo planar spectroscopic imaging (EPSI) [19,20], however, with much shorter 3D spatial and 1D chemical shift image acquisition time.

MATERIALS AND METHODS

PASADENA Hyperpolarization

The instrumentation and polarization transfer technique necessary for generating hyperpolarized ^{13}C molecules is described in detail by Golman et al. [9], Johannesson et al. [10], and Bhattacharya et al. [11]. Briefly, the unsaturated PASADENA precursor molecule undergoes hydrogenation by parahydrogen gas in the presence of a rhodium catalyst, producing a molecule with high proton spin order. This proton spin polarization is then transferred to the ^{13}C atoms at a low magnetic field using tailored RF pulses transmitted at both the ^1H and ^{13}C Larmor frequencies. The rhodium catalyst enables the transfer of the parahydrogen protons as a unit onto the precursor molecule, thus maintaining the spin order of the protons immediately after hydrogenation. The chemistry takes place at an elevated temperature (60 °C) and pressure (10 bar) in a reactor vessel where a solution containing both precursor and catalyst (pH:7.8) is injected into an atmosphere of parahydrogen gas. The goal is to achieve this reaction in a timescale which is small (≈ 4 s) compared to spin lattice relaxation times. Relaxation losses are minimized by proton irradiation of the substrate in the reactor prior to polarization transfer, which traps the singlet state. The PASADENA precursor for the experiments presented below was [1,3,3- 2H_3 ; 1- ^{13}C]2-ethylacrylate, synthesized in collaboration with Isotech, OH, USA, which becomes [1,3,3- 2H_3 ; 1- ^{13}C]2-hydroxyethylpropionate (HEP) upon hydrogenation during the PASADENA hyperpolarization process as shown in Figure 1.

The PASADENA polarizer was provided under loan agreement between Huntington Medical Research Institutes and General Electric Healthcare established by Dr. Klaes Golman, Ms. Marivi Mendizabal and Dr. J-H Ardenkjaer-Larsen.

^{13}C and ^1H MR Imaging

All ^{13}C and ^1H imaging was performed on a 1.5 T General Electric Signa MR scanner (GE Healthcare, Milwaukee, WI) operating with version 9.1 software. The imaging was performed using a dual purpose RF coil designed and built in our laboratory incorporating two single-turn 12 cm diameter proton surface coils arranged in quadrature configuration which overlap a two-turn transmit/receive 11 cm diameter circular ^{13}C surface coil at an angle of 45° minimizing the mutual inductance between the proton and carbon RF coils [22].

The MR scanner was equipped with a broadband RF amplifier and special broadband excite and receive hardware enabling non-proton imaging and spectroscopy. The manufacturer's standard 3D balanced steady state free precession pulse sequence was modified to allow

multi-nuclear and multiple gradient echo imaging (ME-3DbSSFP). A diagram of the ME-3DbSSFP pulse sequence is shown in Figure 2 for a four echo acquisition. Steady state free precession was established by performing 100 excitations without data collection prior to acquiring the first 3D data set of the sequential 3D acquisitions.

In Vitro

In vitro spectral imaging experiment: A total of 100 in vitro assays were performed over the course of 12 months. A phantom consisting of a sphere containing 4.4 M ^{13}C -acetate (AC) and a syringe containing 50 mM of HEP hyperpolarized using the PASADENA technique [11] were placed in the ^{13}C surface coil. ME-3DbSSFP imaging was performed in the coronal plane using a $16 \times 64 \times 64$ matrix with isotropic 7 mm spatial resolution, with a bandwidth of ± 31.25 kHz (976 Hz/pixel). Eight gradient echoes were acquired with an echo spacing of 1.34 ms which, following Fourier transform along the echoes, provided eight spatial-spectral images with 93 Hz spectral separation with a Nyquist frequency of 372 Hz. The time to acquire a full 8 echo 16 slice 3D data set using a TR of 14.5 ms was 16.3 seconds. A flip angle α of 60° was chosen based upon our estimate of the T_2/T_1 ratio of HEP in aqueous solution. The RF flip angle was calibrated using the stimulated echo method of Perman et al. [23].

In Vivo Experiment

Thirty in vivo experiments were performed in male Wistar rats weighing 250 – 350 g. The animal preparation and maintenance procedure for MR imaging was approved by the Institutional Animal Care Committee. A rat was anesthetized and an indwelling catheter was placed in the right jugular vein. The Teflon catheter (PP10) was of a length sufficient to exit via the dorsal skin fold where it was fixed in a ‘backpack’ until required. At that time, the animal was re-anesthetized and the catheter extended to permit injection at a distance of 30 cm within the magnet bore. The rat was positioned supine on the ^{13}C surface coil. A sphere containing 4.4 M ^{13}C -acetate was placed next to the animal to serve as a chemical shift and ^{13}C concentration reference standard. ME-3DbSSFP ^{13}C MR imaging was performed in the coronal plane immediately following injection of 1 ml solution containing 50 mM of hyperpolarized HEP (total injected = 50 micromoles HEP; estimated final blood concentration = 1 – 5 mM). The acquisition matrix was $16 \times 64 \times 64$ providing isotropic 7 mm spatial resolution, BW = ± 31.25 kHz (976 Hz/pixel), 8 gradient echoes with echo spacing of 1.34 ms, giving a sampled and reconstructed spectral resolution of 93 Hz and a Nyquist frequency of 372 Hz. The time to acquire a full 8 echo 16 slice 3D data set was 16.3 seconds using a TR of 14.5 ms. A flip angle α of 60° was used based upon the assumption of similar in vitro and in vivo T_2/T_1 ratio for HEP.

RESULTS

In Vitro

Selected chemical shift images derived from ME-3DbSSFP ^{13}C MR imaging of a syringe containing 50 mM HEP hyperpolarized using the PASADENA technique together with the ^{13}C -acetate (AC) reference are shown in Figure 3. The resonant frequency was set on the ^{13}C -acetate resonance which occurs at 181.9 ppm and is represented by the 0 Hz chemical shift image. The HEP resonance peak appears in the -93 Hz chemical shift image, as expected, since this resonance occurs at 176.1 ppm and is 96 Hz downfield from the ^{13}C -acetate resonance.

In Vivo

Rapid, sequential coronal ME-3DbSSFP ^{13}C MR imaging (16 slices, 8 echoes) was performed on a rat before and immediately following IV injection of 1 ml saline containing 50 mM of hyperpolarized HEP. First echo images ($\text{TE} = 2.1$ ms) of the first ME-3DbSSFP acquisition of slices 4 through 6 are shown in Figure 4. The bright sphere in slices 5 and 6 (arrows) is the ^{13}C -acetate reference phantom. The ^{13}C MR signal from the hyperpolarized HEP within the rat is present in both slices 4 and 5, but with greater signal intensity in slice 4. The ^{13}C MR signal from the ^{13}C -acetate reference occurs primarily in slice 5 and to a lesser degree in slice 6. Note the decrease in the MR signal for the ^{13}C -acetate reference phantom in slice 6 due to the increased distance from the ^{13}C RF coil.

Selected chemical shift images calculated from the first ME-3DbSSFP image acquisition are shown in Figure 5 for slices 4 and 5. These images have isotropic 7 mm spatial and 93 Hz spectral resolution. The ^{13}C MR signal of the hyperpolarized HEP in the rat only appears in the -93 Hz chemical shift images of slices 4 and 5. The ^{13}C MR signal of the ^{13}C -acetate reference sphere is only present in the 0 Hz spectral images of slices 5 and 6 (not shown). The spatial distribution of the hyperpolarized HEP in the rat for the -93 Hz chemical shift images of slices 4 and 5 is the same as that seen in the first echo spatial images of slices 4 and 5 shown in Figure 4.

The MR signal of the hyperpolarized HEP in the rat is plotted as a function of time in Figure 6 for regions-of-interest (ROI) selected in the -93 Hz chemical shift images of slices 4 and 5. The MR signal of the hyperpolarized HEP is significantly above background signal level 45 seconds following IV administration, with both ROIs exhibiting similar MR signal decay. The peak MR signal was significantly higher for slice 4 as compared to slice 5 as noted in Figure 4. Based upon the data in this figure, assuming a T_1 of 48 seconds, a 10 second delay between the end of the hyperpolarization process and IV injection into the rat, and a 1 to 10 dilution as the agent reaches the brain, we estimate a 1600 fold increase in the ^{13}C -HEP MR signal due to hyperpolarization for this experiment.

The relationship between the spatial distribution of the hyperpolarized ^{13}C -HEP MR signal and the rat anatomy can be seen in Figure 7, where the -93 Hz spatial-spectral images of slices 4 and 5 have been overlaid onto the corresponding proton images taken at the same location with the same field of view. Based on the injection route via the jugular vein and the orientation of animals within the RF coil, the majority of the hyperpolarized ^{13}C -HEP MR signal appears to be confined to the cephalic region of the rat.

DISCUSSION

With the advent of hyperpolarized ^{13}C metabolic imaging, there is increasing need for hardware and software adjustments to clinical and small animal MR scanners which will enable optimal signal capture. In this paper we discuss optimization of fast ^{13}C imaging, with chemical shift selectivity and its implementation on a 1.5 T clinical scanner, with broadband capability, previously employed for in vivo human ^{13}C MRS studies. Recently, several approaches have been proposed to speed up the acquisition of spatial-spectroscopic information from hyperpolarized ^{13}C -labeled compounds. They have in common that prior knowledge is used in order to shorten acquisition time while maintaining high spatial resolution. Reeder et al. [24] have proposed an EPSI based method with echo-planar readout with flyback gradients in order to have all readout gradients at the same polarity. Levin et al. [25] have proposed to implement an EPSI-like readout into a spiral k-space trajectory in order to further improve temporal resolution. We demonstrate successful implementation of ^{13}C ME-3DbSSFP and its application on a small laboratory animal. The reagent chosen for this study with PASADENA was HEP, a somewhat toxic, and not metabolizable reagent

suited to the special requirements of this hyperpolarization method. However, a similar approach with non-toxic and metabolizable PASADENA reagents (e.g. ^{13}C -glucose, ^{13}C -choline) is expected to yield in vivo results similar to those demonstrated with an alternative hyperpolarization technology, dynamic nuclear polarization (DNP), where the imaging reagent was ^{13}C -pyruvate [16]. Indeed, the method described here is likely to be universally applicable to any hyperpolarization technique for which the product has a T_1 of the order of tens of seconds.

The advantages and disadvantages of 2D multiple echo bSSFP chemical shift imaging have been previously discussed by Leupold et. al. [21]. The advantage of a multiple echo technique vs. 2D chemical shift imaging (2DCSI) is an increase in spatial resolution at the expense of spectral resolution. One possible disadvantage of the multiple echo bSSFP technique is the increased sensitivity to chemical shift artifacts. 2DCSI is only sensitive to chemical shift artifacts (spatial displacement) during slice localization since the spatial encoding is accomplished by phase encoding. Whereas multiple echo bSSFP chemical shift imaging is sensitive to chemical shift artifact during slice localization and readout. Therefore, care must be taken to use a readout bandwidth resulting in a Hertz per pixel greater than the chemical shift range of the data in order to avoid these artifacts. This condition was satisfied in our experiments by using a readout bandwidth corresponding to 976 Hz/pixel. Remaining inconsistencies due to the alternating readout gradient can be corrected according to the proposals of Lu et al. [26] and Brodsky et al. [27]. The displayed experiments did not include a correction for field inhomogeneities, as the reconstruction of the relatively small sample proved to be stable enough after careful shimming. It is useful to note that susceptibility artifacts scale with the resonant frequency, such that the same degree of artifact would be present in a ^{13}C image acquired at $TE = 14.5$ ms and a ^1H image acquired at $TE = 3.6$. A correction for field inhomogeneities may be necessary for in vivo applications and could be performed by the application of the iterative approach as proposed by Reeder et al. [24].

The advantages of a 3D over a 2D multiple echo bSSFP technique are the ability to measure spatial and spectral MR changes in more than one slice, and an increase in the signal-to-noise ratio (SNR) of the MR signals given by $(N_S)^{1/2}$, where N_S is the number of slices acquired in the 3D data set. Note, however, that when using the minimum repetition time increasing the number of slices acquired in a multiple echo 2D bSSFP acquisition decreases the temporal resolution, increasing overall imaging time without a concomitant $(N_S)^{1/2}$ increase in the SNR. The disadvantage of the 3D as compared to the 2D multiple echo bSSFP technique is the longer imaging time resulting from the additional “ N_S ” phase encodings in the slice direction. Although the imaging time is increased, the data shown in Figure 6 indicates that, even for the low degree of hyperpolarization achieved in this particular experiment, the MR signal is significantly above background for about 45 seconds allowing the acquisition of at least 3 time points following IV injection of the hyperpolarized ^{13}C -labeled substrate. We have recently achieved a much greater increase ($> 30,000$ fold) in the ^{13}C MR signal of hyperpolarized HEP through further optimization of the spin order transfer process [28]. In addition to optimizing the degree of hyperpolarization, we are also working on the application of an asymmetric partial Fourier data acquisition technique in order to provide a significant ($\approx 50\%$) decrease in the time to acquire a ME-3DbSSFP data set while maintaining both spatial and spectral resolution [29]. We estimate that using an asymmetric partial-Fourier ME-3DbSSFP technique together with an RF coil optimized for imaging a rat or mouse and a hyperpolarization increase in the ^{13}C MR signal of 30,000 to 50,000 fold should provide sufficient SNR to monitor in vivo metabolism of ^{13}C -labeled substrates at 10 to 100 micromolar levels with isotropic 7 mm spatial and 10 second temporal resolution.

When using the Fourier transform to reconstruct the chemical shift information, it is important to have a priori knowledge of the substrate and resultant metabolic product chemical shifts (Δf_{MP}) in order to select an echo spacing and number of echoes such that the ratio of $\Delta f_{MP}/\Delta f$ is as close to an integer as possible, thereby minimizing partial volume effects in the chemical shift images. For example, the frequency differences of acetone and peanut oil vs. water are 130 and 235 Hz, respectively, acquiring eight echoes with an echo spacing of 1.6 ms yields a frequency resolution of 78 Hz which places the peanut oil an optimal 3.0 (235/78) chemical shift images away from water, but places the acetone a suboptimal 1.67 (130/78) chemical shift images away from water, dividing the acetone MR signal between the -78 and -156 chemical shift images. The choice of echo spacing can be difficult and is typically constrained by the gradient strength and slew rate of a particular MR scanner, and increasing the number of echoes increases the sensitivity to susceptibility artifacts due to the longer TR. This technique may be most useful for metabolic products whose chemical shifts are significantly different (≥ 30 Hz) from the substrates due to this hardware limitation. Matrix inversion [30,31], an alternate reconstruction technique, also requires a priori information concerning the number and relative frequencies of the resonant peaks to be reconstructed but allows more freedom in the choice of echo spacing and total number of echoes to be collected.

Although the hyperpolarization signal of HEP is not renewable, the short TR of 14.6 ms together with the long T_1 and T_2 relaxation of the hyperpolarized HEP spins enables a pseudo steady-state, whereby the spin populations created by the fast excitations ($TR \ll T_2$) are all equally affected by the T_1 decay of the hyperpolarized spin population. The T_1 decay of the hyperpolarized spins is very small over several TR periods and acts as a blurring function, multiplying the image data by a Lorentzian function whose width is inversely proportional to the image acquisition time for images acquired using linear (+gradient maximum to -gradient minimum) phase encoding. The maximum MR signal for balanced steady state imaging is a function of the flip angle α and the ratio of T_2/T_1 [32]. Estimating the $T_2 = 8$ s from our multiple echo data and measuring a T_1 of 46 ± 0.5 s in a subsequent experiment, we find the ratio of T_2/T_1 for HEP in aqueous solution to be approximately 0.17. The optimal flip angle for this T_2/T_1 ratio is approximately 48° yielding a maximum signal of 22 %, indicating that our chosen $\alpha = 60^\circ$ was incorrect due to overestimating the T_2 of aqueous HEP. Also, since the MR signal for balanced steady state imaging is relatively independent of TR over the range of 5 to 50 ms and mainly dependent upon the T_2/T_1 ratio, and since the T_2/T_1 ratios of most biological tissues do not change significantly with pathology (T_1 and T_2 both increase), the ME-3DbSSFP chemical shift imaging technique may provide metabolic ratios that are much less sensitive to partial saturation effects as compared to 2DCSI imaging.

In conclusion, ME- 3DbSSFP MR imaging provides volumetric chemical shift images of hyperpolarized ^{13}C -labeled substrates in vivo at increased SNR as compared to multiple echo 2D bSSFP imaging. The increased SNR of this 3D technique should provide increased sensitivity for measuring the MR signals of hyperpolarized substrates and metabolites with high spatial resolution and minimal T_1 dependence.

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2-Hydroxyethylacrylate

pH2

2-Hydroxyethylpropionate

FIGURE 1.

The [1-¹³C]2-hydroxyethylacrylate precursor molecule undergoes hydrogenation during the PASADENA hyperpolarization process to form hyperpolarized [1-¹³C]2-hydroxyethylpropionate.

FIGURE 2.

Diagram of the multiple echo 3D balanced steady state free precession pulse sequence (ME-3DbSSFP) depicting the acquisition of four gradient echoes with ΔTE echo spacing.

FIGURE 3. Selected ME-3DbSSFP chemical shift images of 50 mM hyperpolarized [1- ^{13}C]2-hydroxyethylpropionate (HEP) and the 4.4 M ^{13}C -acetate (AC) reference sphere.

FIGURE 4.

Slices 4–6 selected from the first 16 slice ME-3DbSSFP acquisition of a rat injected with 1 ml of 50 mM hyperpolarized [1- ^{13}C]2-hydroxy-ethylpropionate (HEP). The bright sphere in the upper right (arrow) is the 4.4 M ^{13}C -acetate (AC) reference phantom.

FIGURE 5.

Selected ME-3DbSSFP chemical shift images calculated from the multiple echo data of slices 4 and 5. The spatial distribution of the hyperpolarized $[1-^{13}\text{C}]2$ -hydroxyethylpropionate in the rat is clearly seen in the -93 Hz spatial-spectral image. Note, the 4.4 M ^{13}C -acetate reference sphere is present in the 0 Hz spatial-spectral image of slice 5 (arrow) but not in slice 4.

FIGURE 6.

The ^{13}C MR signal of the 4.4 M ^{13}C -acetate (AC) reference and regions-of-interest selected from the hyperpolarized -93 Hz $[1-^{13}\text{C}]2\text{-hydroxyethylpropionate}$ (HEP) resonances within the rat.

FIGURE 7.

The -93 Hz chemical shift images of slices 4 and 5 overlaid on proton images taken at the same location with the same field of view. The majority of the hyperpolarized [1- ^{13}C]2-hydroxyethylpropionate (HEP) MR signal appears in the cephalic region of the rat.